Research Article

Effect of Peptide Tyrosine Tyrosine (PYY_{3-36}) on High Fat Diet Induced Obesity and its Metabolic Hazards in Adult Albino Rats.

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Abstract
Aim of work: The role of pancreatic polypeptide family including Neuropeptide Y (NPY), and Peptide Tyrosine Tyrosine (PYY) in obesity development and its metabolic changes is controversial due to multiple receptor involvement. Therefore, this study was designed to investigate the effect of a potent Y2 receptor agonist; Peptide Tyrosine Tyrosine (PYY_{3-36}) on high fat diet (HFD) induced obesity in adult albino rats of both sexes. **Materials and Methods:** forty two adult albino rats were divided into 6 equal groups: Controlmale (kept on standard diet), HFD male, HFD male +PYY treated, Control female (kept on standard diet), HFD female and HFD female + PYY treated groups. PYY_{3-36} was administered (50µg\ kg) by intraperitoneal injection twice daily during the 5th week of high fat diet protocol to rats of the treated groups. Blood samples were collected for measurement of lipid profile, glucose and leptin. Body weight, lee index and daily food intake were also measured. The brain was removed and the hypothalamus was isolated for determination of NPY concentration. Peritoneal omental fat was removed as the whole gastrocolicomentum fat (GCOF) and weighed. **Results:** Consumption of HFD resulted in a significant increase in food intake in the first and second weeks then decreased significantly at the end of the study in the HFD groups as compared with control groups. This was accompanied with a significantly higher body weight, lee index, serum glucose, leptin levels and weight of (GCOF) with a significant decrease in hypothalamic NPY concentration. These were associated with a state of dyslipidemia. Peripheral administration of PYY_{3-36} with HFD decreased food intake, body weight, lee index, GCOF weight, hypothalamic NPY, plasma leptin and glucose. ***In conclusion***, PYY_{3-36}; the potent Y2 receptor agonist could prevent partially HFD induced obesity. Gender differences revealed that males developed higher measures of obesity with more adverse metabolic effects.

Key Words: Peptide Tyrosine Tyrosine, obesity, high fat diet, leptin, glucose and Neuropeptide Y.

Introduction
Obesity is a pathological condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems[(1)].

Body weight depends on the balance between energy intake and energy consumption. Correlation between the macronutrient contents of the diet that reflect the nutritional status and the body fat storesare the bases of the glucostatic, lipostatic and aminostatic hypotheses that control energy intake and expenditure. The brain regulates energy homeostasis in response to signals from both adipose tissue and the gastrointestinal tract[(2)].

The pancreatic polypeptide family contributing to the mechanisms of energy balance includes peptide tyrosine tyrosine (PYY), neuropeptide Y (NPY) and pancreatic polypeptide (PP). They are all 36 amino acid peptides with terminal tyrosine residues coded by the letter Y, found in different locations throughout the gastrointestinal tract and nervous system, and

possess different biological actions[3]. The pancreatic polypeptide family binds to a family of receptors that were originally characterized as NPY receptors. There are four NPY receptors in humans and rodents designated as Y1, Y2, Y4, and Y5. An additional receptor identified as Y6 is found in mice and rabbits[4].

The NPY is one of the most abundant and widely distributed peptides in the central nervous system (CNS) of both rodents and humans. Within the hypothalamus, NPY plays an essential role in the control of food intake and body weight; being orexigenic in most studies. Furthermore, its synthesis and release is a subject of neuro endo crinalregulation[5].

Peptide tyrosine tyrosine (PYY), on the other hand is released from the L cells of the gastrointestinal tract, with increasing tissue concentrations found in the more distal portions[6].In the circulation, it exists in two major forms: PYY_{1-36} and $PYY_{3–36}$. PYY_{1-36} is rapidly proteolyzed by the enzyme Dipeptidyl-peptidase IV. The cleaved product; $PYY_{3–36}$, is bioactive. PYY binds to members of the Y receptor family. PYY_{1-36} is a potent agonist of both Y1 and Y2 receptors, whereas PYY_{3-36} is a potent Y2 receptor agonist. PYY is able to cross the blood brain barrier (BBB) by transmembrane diffusion from the circulation[4].

The pancreatic polypeptide (PP) is post-prandially secreted from the endocrine F cells of the pancreatic islets. While peripheral administration decreased food intake and body weight in obese rodents, its central administration produced opposite effects. Y4 and Y5 receptors mediate the peripheral effect, but the central receptors or mechanism is unclear[6].

The role of PYY in the regulation of food intake is controversial: peripheral $PYY_{3–36}$ administration inhibits food intake, reduces weight gain and improves glycemic control in rats and human[7]. Other studies observed that acute and chronic administration of $PYY_{3–36}$ in rats either increased or did not change food intake and body weight[8].Due to these discrepancies, this study was performed using the rat model of high fat diet (HFD) obesity.

Our work aimed to study the effect of intraperitoneal injection of $PYY_{3–36}$on HFD induced obesity in adult albino rats with its metabolic abnormalities during the dynamic phase of obesity development. Measuring the central NPY concentration during this study will reflect its contributory role trying to answer the question: "Is there a relation between leptin, lipid profile, PYY, NPY, food intake and obesity development in this rat model or not?"

Materials and methods

I-Animals

Forty two adult albino rats (Sprague dawley strain) of both sexes (21 males and 21 females) were used. Their weight ranged between 150-180 grams at the beginning of this study. Rats were housed in stainless steel mesh bottomed cages offering individual housing. They were housed at room temperature with natural light\dark cycles for one week for acclimatization to lab conditions. Rats were fed a standard diet of commercial rat chow and tap water *ad libitum* until the time of the experiment. During the acclimatization period, daily food intake was measured to know the mean daily food intake per rat. All the procedures followed with the rats were in accordance with our institutional guidelines. The protocol was ethically approved by The Laboratory Animals Maintenance and Usage Committee of Faculty of Medicine in Minia University.

The rats were randomized into the following groups (7 rats each):

Male groups were divided into:

1-Control male group (CM): in which rats were fed a commercially available standard diet and left for 5 weeks without treatment.

2-High fat diet male group (HFDM group): in which rats were fed high fat diet (HFD) for 5 weeks.

3- High fat diet male treated group (HFDM +PYY):in which rats were fed a HFD.The animals were accustomed to the injection procedure by intraperitoneal injection with saline (0.5 ml /rat) for 2 days before $PYY_{3–36}$ administration [9].Then each

rat received PYY_{3-36} (Sigma Aldrich, USA) at a dose level of 50µg per kg, by intra-peritoneal injection twice daily during the 5th week. It was prepared by dissolving it in saline solution[10].

Female groups were divided into:

1-Control female group (CF): in which rats were fed a commercially available standard diet and left for 5 weeks without treatment.

2-High fat diet female group (HFDF group): in which rats were fed high fat diet (HFD) for 5 weeks.

3- High fat diet female treated group (HFDF +PYY): in which rats were fed a HFD. Rats received the same previous PYY treatment regimen as (HFDM +PYY)group.

II -Diet protocol

The composition of standard diet (g/kg diet) was according to the formula of [11].It contained (Fat 5% [corn oil 5%], carbo-hydrates 65% [corn starch 15% and sucrose 50%], proteins 20.3% [casein 20% and DL-Methionine 0.3%], fiber 5%, salt mixture 3.7%, and vitamin mixture 1%) and provided 3.0 kcal/g of diet. The HFD contained (fat 46% [corn oil 25.5%, and beef tallow 20.5%],carbohydrates 24% [corn starch 6% and sucrose 18%], proteins 20.3% [casein 20%and DL-Methionine 0.3%], fiber 5%, salt mixture 3.7%, and vitamin mixture 1%) and provided 4.6 kcal/g of diet[12].Rats were allowed HFD for 5 weeks[13], during which food intake was measured daily and body weight was measured every week.Standard and HFD dietswere purchased from El-Gomhoria Company, Cairo, Egypt, and preserved at 4°C until used.

III-Lee index:

Lee index is used to determine obesity in rats using weight and naso-anal length. It was measured at the beginning of the study, at the end of the 4th week and at the end of the 5th week. Lee index was calculated for each rat according to the following formula:

Cube root of body weight (g) X 10 / nasoanal length (mm)

Rats with Lee index ≥ 0.3 were considered obese[14].

Rats were sacrificed after an overnight fast by decapitation and blood samples were collected, allowed to clot at room temperature, and then centrifuged at 3000 rpm for 15 min. in a cooling centrifuge (Hettich centrifuge). The serum layer was then withdrawn into identified eppendorf tubes and stored at - 20°C till the time of assay.

IV- The weight of gastrocolicomentum:

Rats were opened *via* ventral abdominal incision. Peritoneal omentaladipose tissue was removed by lifting the intestines and cutting the fat free, starting at the distal end close to the appendix, the whole gastrocolicomentum wasweighed [15].

V- Biochemical analysis

Total cholesterol (TC), triglycerides (TGs), low density lipoprotein (LDL-c), high density lipoprotein (HDL-c) and glucose were determined by enzymatic colorimetric methods, using kits purchased from Bio-diagnostic, EGYPT using spectrophoto-meter **(Spectronic 2000, BAUSCH & LOMB**). Leptinconcentration was deter-mined by enzyme-linked immunosorbent assay (**CUSABIO, CHINA**) using ELISA apparatus **(SLT-SPECTRA,Salzpurg).** The methods followed the instruction manual of the manufacturer.

VI- Analysis of brain homogenates:

The heads were dissected, then the brain was removed and the hypothalamus was isolated, weighed, and homogenized by ultrasonicate homogenizer **(4710 series, Chicago)** in 500 µL of 0.5 mol/L acetic acid (Prepared by mixing 28.62 ml glacial acetic acid (El-Nasr pharmaceutical chemical company, Egypt) completed to 1000 ml by adding distilled water) then the homogenate was boiled for 15 min followed by centrifugation at3,000 rpm for 10 min at 4°C. The supernatant was collected and stored at −80°C until use [16].Determination of Neuropeptide Y (NPY) concentration was done by enzyme-linked immunosorbent assay **(Sigma Aldrich USA -Cat No RAB0387).**

VII- Statistical Analysis

Statistical analysis was performed using Graph pad Prism 5 software and significant difference between groups was done by one-way ANOVA followed by Tukey-

Kramar post hoc test for multiple comparisons with a value of $P \leq 0.05$ considered statistically significant

Results

1. Changes in body weight, Lee index and food intake in different groups:

The means of the initial body weight were insignificant among the different groups at the beginning of the study. In control rats, the body weight at the end of 5th week was significantly higher as compared to the initial values as shown in *figure (1-A, B).*

HFD caused a significant higher body weight in both male and female groups from the second week till the end of the study as compared to control groups. Injection of PYY_{3-36} caused a significant lower body weight as compared to HFD groups as shown in *figure (1-A, B) and table (1)*

In all groups(control, HFD and HFD treated groups), the body weight in female was significantly lower as compared to male groups at the end of the 4th and 5th weeks; *table (1).*control rats had a Lee index less than 0.3 at the end of the experiment and were considered non obese. All rats fed with HFD had a Lee index higher than 0.3 at the end of the 4th week and 5th week and were considered obese. The lee index was significantly higher in HFD groups as compared to control groups. Injection of PYY_{3-36} during the 5th week did not decrease the Lee index below 0.3 and rats were still obese, but the Lee index in HFD treated groups was significantly lower as compared to HFD groups and insignificant as compared to control groups as shown in *table (1).* Also, in HFD groups, the lee index was significantly lower in female as compared to male groups only at the end of the 5th week.

The HFD groups showed a significant higher food intake from the control groups in the first and second week, and then lowered to a significant level in the fourth and fifth weeks. Injection of PYY_{3-36} caused a significant lower food intake as compared to the control and HFD groupsas shown in *figure (2-A, B).* There was a significant lower food intake in control female as compared to control male groups at the end of the 4th and 5th weeks; table *(1).*

Table (1): changes in body weight, Lee index and food intake in different groups:

Groups / Parameters	CM	HFDM	HFDM+PYY	CF	HFDF	HFDF+PYY
Body weight (g)						
Initial	188.7 ± 2.3	187.2 ± 2.4	187.5 ±2.5	186±3.7	187.5±1.6	185.7±3.1
After 4 weeks	225± 1.08[1]	236.2± 0.7[a1]	237± 1.2[a1]	217.5± 0.8[c1]	227.5± 1.6[a c1]	228.5±1.5[a c1]
After 5 weeks	227.7 ± 1.1[1]	238± 0.5[ac1]	232 ± 0.7[abc12]	219.2±0.4[c1]	229.7±1.3[ac1]	224.2±0.4[abc12]
Lee index						
Initial	0.294±0.001	0.293±0.001	0.292±0.001	0.291±0.001	0.285±0.002	0.290±0.004
After 4 weeks	0.296±0.002	0.317±0.007[a]	0.314±0.005[a]	0.295±0.002	0.309±0.002[a]	0.309±0.002[a]
After 5 weeks	0.297±0.001	0.318±0.001[a]	0.303±0.003[b]	0.295±0.001	0.309±0.002[ac]	0.3002±0.003[b]
Food intake (g/day)						
After 1 week	14.4±0.52	19.8±0.64[a]	19.6±0.38[a]	14.04±0.61	19.9±0.44[a]	19.8±0.32[a]
After 4 weeks	15.9±0.35	13.14±0.55[a1]	13.12±0.59[a1]	13.2±0.24[c]	12±0.49[a1]	12.5±0.16[a1]
After 5 weeks	15.08±0.32	13.28±0.78[a1]	9.6±0.47[ab12]	13.2±0.35[c]	12.2±0.33[a1]	9.02±0.39[ab12]

CM= control male, CF= control female, HFDM=high fat diet male, HFDF = high fat diet female, PYY= Peptide YY_{3-36} during 5th week. Data are expressed as mean ± S.E.M. of 7 rats in each group. a: Significant from corresponding control group, b: Significant from corresponding HFD group, c: Significant from corresponding male groups respectively,[1]: Significant frominitial values, [2]: Significant from4th week, $P < 0.05$.

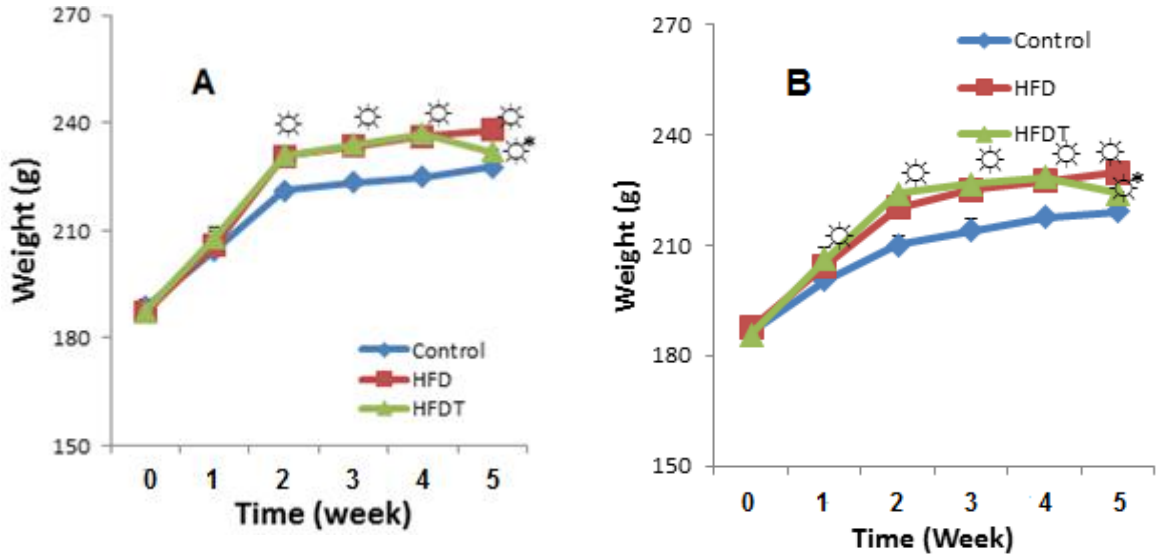

Figure 1: Time course changes in body weight (g) in male HFD groups (A) andfemale HFD groups (B)

HFD: high fat diet, HFDT: high fat diet treatedwith Peptide YY_{3-36} during 5th week. * Significant from HFD group; ☼ Significant from control group.Values are expressed as mean ± SEM of 7 rats in each group.

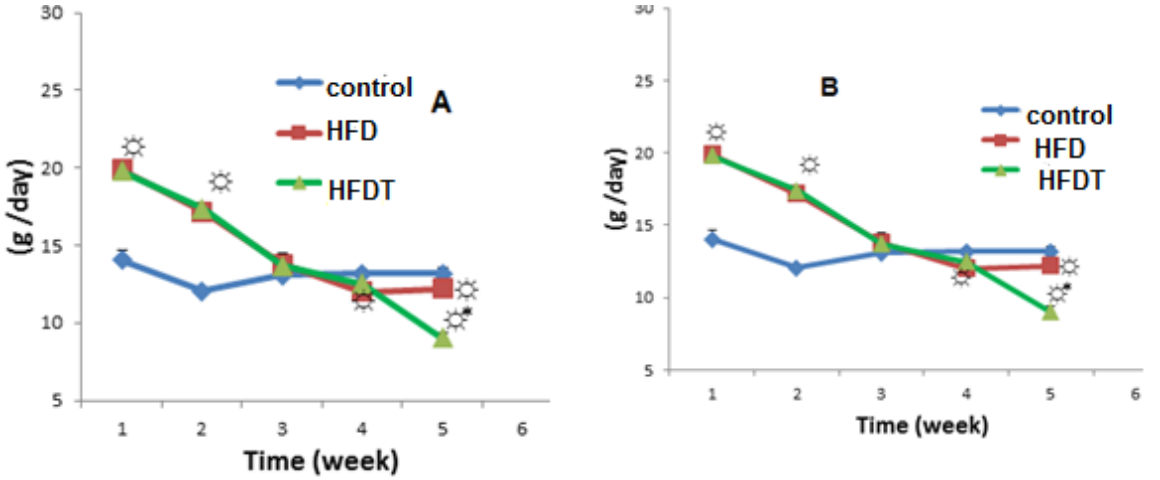

Figure 2 : Time course changes in food intake (g/day) in male groups (A) and in female groups (B)

HFD: high fat diet, HFDT: high fat diet treatedwith Peptide YY_{3-36} during 5th week.* Significant from HFD group, ☼ Significant from control group.Values are expressed as mean ± SEM of 7 rats in each group.

2- Changes in weight of the gastrocolicomentum (GCOF) in the different studied groups:

The weight of the GCOF was significantly higher in HFD groups as compared to the control groups. Injection of PYY_{3-36} caused a significant lower weight of the GCOF as compared to the HFD groups with no significant change from control groups. The weight of GCOF was significantly lower in females than males in all groups including control, HFD and HFD treated groups*(figure 3)*.

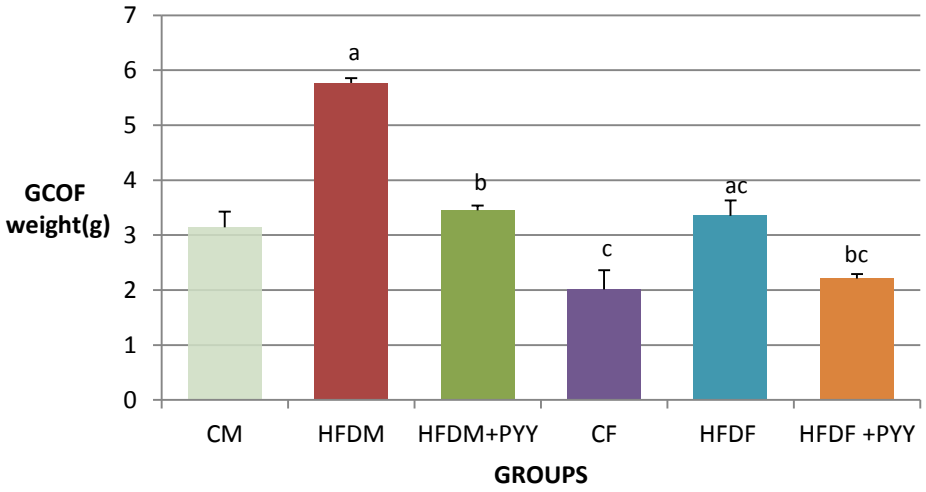

Figure (3): The weight of the gastrocolic omentum (GCOF) in different groups.
CM= control male, CF= control female, HFDM=high fat diet male, HFDF = high fat diet female, PYY= Peptide YY_{3-36} during 5th week.a: Significant from corresponding control group, b: Significant from corresponding HFD group, c: Significant from corresponding male groups respectively, $P < 0.05$. Values are expressed as mean ± SEM of 7 rats in each group.

3-Changes in Serum glucose, leptin and lipid profile in different studied groups (table2):

In comparison to the control groups, HFD groups showed higher serum levels of TC, LDL-c and TGs associated with a signi-

ficant lower serum level of HDL-c. Injection of PYY_{3-36} caused insignificant effect on serum lipid profile as compared to the HFD groups;the levels of TC, LDL-c and TGs remained significantly higher as compared to control groups associated with a significant lower serum level of HDL-c.

Serum glucose and leptin levels were significantly higher in HFD groups as compared to corresponding control groups. On the other hand, injection of PYY_{3-36} caused a significant lower serum glucose and leptin levels in HFD groups as compared to non treated groups; however, the levels were still significantly higher than the control groups

In HFD and HFD treated groups; the serum glucose level was significantly lower in female as compared to male groups. Serum leptin was significantly lower in females as compared to males in control and HFD groups. In control groups, the serum HDL-c was significantly higher in female as compared to male groups, but the serum levels of TC and TGs were significantly lower in females as compared to males in either HFD or HFD treated groups.

Table 2: Serum glucose, leptin and lipid profile concentrations in the different studied groups.

Groups / Parameters	CM	HFD M	HFDM+PYY	CF	HFDF	HFDF+PYY
TC (mg/dl)	133.1 ± 4.5	156.9± 1.3 [a]	153± 3.3 [a]	134.6 ± 1.6	140± 0.7[ac]	139.3± 1.1 [ac]
TGs(mg/dl)	96.8 ± 0.6	167.4± 2.7 [a]	166.2± 2.1 [a]	97.1± 1.1	128 ±3.5[ac]	127.6± 5.8 [ac]
HDL-c(mg/dl)	46.7 ± 0.3	39.1± 0.1 [a]	38.9± 0.1 [a]	55.3 ± 0.6[c]	39.6± 0.2 [a]	39.1± 0.1 [a]
LDL-c(mg/dl)	66.8 ± 1.05	74.7± 1.5 [a]	73.07± 1.8 [a]	66.3 ± 1	72.9± 1.6 [a]	69.9± 1.1 [a]
Serum glucose (mg/dl)	70.8 ± 2.3	123.2± 1.3[a]	113.5± 3 [a b]	66.5 ± 1.7[c]	106± 1.8 [ac]	95.2± 3.03 [a bc]
Serum leptin (ng/ml)	10.8 ± 0.1	27.7± 0.7 [a]	13.22± 0.3 [a b]	5.4 ± 0.09[c]	23.1± 0.8[ac]	12.6± 0.4 [a b]

CM= control male, CF= control female, HFDM=high fat diet male, HFDF = high fat diet female, PYY= Peptide YY_{3-36} during 5th week. Data are expressed as mean ± S.E.M. of 7 rats in each group. a: Significant from corresponding control group, b: Significant from corresponding HFD group, c: Significant from corresponding male groups respectively,$P < 0.05$.

4- Hypothalamic neuropeptide Y (NPY) concentrations in the different studied groups:

The hypothalamic NPY was significantly lower in HFD groups as compared to control groups. Injection of PYY_{3-36} caused a significant lower hypothalamic NPY level in both HFD groups as compared to both control and non treated groups. Hypothalamic NPY concentration was significantly lower in female as compared to male groups in control groups as shown in *(figure 4)*.

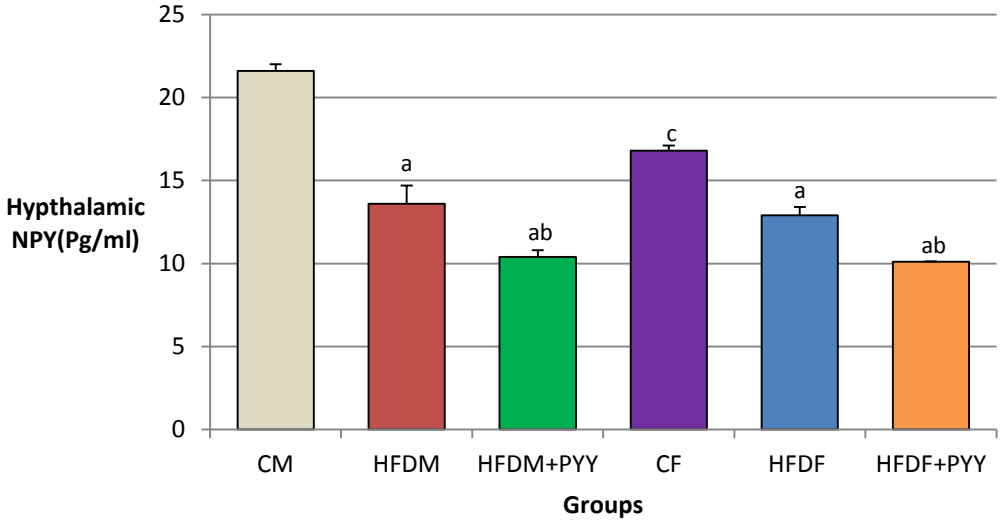

Figure (4): Hypothalamic neuropeptide Y (NPY) concentrations in the different studied groups.

CM= control male, CF= control female, HFDM=high fat diet male, HFDF = high fat diet female, PYY= Peptide YY_{3-36} during 5th week.a: Significant from corresponding control group, b: Significant from corresponding HFD group, c: Significant from corresponding male groups respectively,$P < 0.05$. Values are expressed as mean ± S.E.M. of 7 rats in each group.

Discussion

Obesity is a pathological condition in which excess body fat has accumulated. A number of factors can contribute to obesity, including lack of physical activity, high caloric intake, genetics and certain medications[17].

The brain receives hormonal, neural, and metabolic signals to regulate body energy status. The brain must modulate appetite, and the core of appetite regulation lies in the gut-brain axis[18].

The results of the present study revealed that HFD caused a significant higher food intake in the first and second weeks then lowered significantly at the end of the fourth and fifth weeks in HFD groups as compared to control groups. These results agree with other previous studies[19, 20, 21].This increased food intake with HDF at first and second weeks may be due high palatability of fat[22].

Various mechanisms have been suggested for an increase in satiety signaling accompanying HFD consumption at the end of the fourth and fifth weeks. These include: (1) The interaction of nutrients, particularly fat, with receptors in the small intestine results in inhibition of gastric emptying, which serves to prolong gastric distension and regulate the rate at which nutrients enter the small intestine[23]; (2) secretion of satiety hormones (cholecystokinin, peptide YY (PYY) and glucagon-like peptide-1[24]; (3) decreased hypothalamic NPY concentration as found in the present study and by previous results [25,26].

Within the hypothalamus, NPY plays an essential role in the control of food intake and body weight[5]. Some studies have shown that NPY drives the animal to overeat, which can lead to obesity after central infusion[27]. No effects were observed after its peripheral infusion[28].The orexigenic effect of NPY is mediated through Y1 and Y5 receptors[29].The reduction in hypothalamic NPY; the orexigenic transmitter in high fat fed rats, perhaps in an attempt to restore energy balance, may be due to the inhibitory effect of leptin. Leptin; the adipokine homone significantly increased in sera of HFD groups of this study secondary to increased visceral fat mass marked by the significant increase in GCO weight and it has been found that both hyperleptinemia and hyperglycemia could act as feedback suppressors of NPY in the hypothalamus[30].However, despite the reduced hypothalamic NPY level, the high-fat fed animals developed obesity, suggesting other hypo-thalamic mechanisms to be involved in the development and progression of diet induced obesity**.** Certain research reported that the hypothalamic NPY mRNA and protein content may increase or remain unchanged in diet induced obesity, and these alterations of hypothalamic NPY gene expression were shown to be dependent on the genetic background of the different strains of rodents[31].

HFD induced a significantly higher body weight and Lee index in the present work. These effects are compatible with the results of previous reports[32, 33].Several mechanisms have been suggested for this obesigenic effect.la Fleur et al.,.[20] ascribed this effect to HFD induced hyperphagia, however, the reduced food intake at the end of this study makes this explanation not reliable in the presence of the high obesity measures. HFD induced obesity in our study could be related to the high caloric value of diet irrespective of its amount according to prevoius studies[34,35].HFD could also lower the rate of oxidation of fatty acids and increase lipogenesis leading to increased fat accumulation[36, 37] and it was evident in the present study by increased GCOF weight**.** Hyperleptinemia; the consequence of adipo-sity is in agreement with other researchers[26,38],however, the increased leptin failed to decrease body weight as leptin alone couldn't fight adiposity in the presence of continuous HFD consumption but only decreases food intake and prevents further gain in body weight[39].

HFD groups showed a significant higher serum glucose level compared to control a group which is in agreement with previous research[37].However, another study found that serum glucose levels were not modified by HFD feeding[40]**.** The hyperglycemia

found in the present work could be explained bythe increased insulin resistance secondary to hyperleptinemia induced by HFD. The ability of insulin to stimulate glucose transport and metabolism in adipocytes and skeletal muscle is impaired resulting in peripheral insulin resistance and hyperglycaemia[41].Pro-inflammatory cytokines; like TNF-α released from visceral fat which increased in HFD fed rats of the present study could have a down regulating effect on glucose transporters specially glucose transporter 4 (GLUT 4) decreasing peripheral glucose uptake and metabolism according to previous research[42].

In the present work, ingestion of HFD resulted in significant higher serum levels of TC, TGs and LDL-c associated with a significant lower serum level of HDL-c. This result is in agreement with Zhukovaet al. [43]. Several studies have explained the dislipidemic effect of HFD; Shah et al. [44] have ascribed it to increased absorption of fat from the small intestine following the intake of HFD. Excess *de novo* hepatic triglyceride synthesis and accumulation leading to hypertriglyceridemia may be the major cause of the other lipid abnormalities since it will lead to delayed clearance of the TG-rich lipoproteins and formation of small dense LDL according to[41, 45].On the other hand, with HFD hepatic lipase hydrolyzes the TG and phospholipids of HDL resulting in smaller HDL particle that can be filtered and degraded in the kidney, leading to a reduction in HDL-c [46].

The reduction of food intake with PYY_{3-36} administration in both HFD groups as found in the present study is in agreement with other studies[47, 48]. PYY is able to cross the blood brain barrier (BBB) by trans-membrane diffusion from the circulation[49]. PYY_{3-36} has been shown to exert the inhibition on food intake through an Y2-receptor dependent manner. Y2 receptors are abundantly expressed on NPY neurons in the arcuate nucleus (ARC) of the hypothalamus. Mittapalli and Roberts[47]reported that the anorexigenic actions of $PYY_{3–36}$ were abolished in Y2 knockout mice and blocked by Y2 antagonist. Y2 receptors primarily act as presynaptic autoreceptors modulating endogenous NPY release. In particular, PYY inhibits NPY neurons and reduces hypothalamic NPY mRNA and /or protein content as found in the present results and by previous research[50]. In addition to its central effect, $PYY_{3–36}$ may transmit satiety signals to the brain in part *via* the vagal afferent pathway through afferent vagal Y2 receptor stimulation; such effect was found to be attenuated in vagotomizedrats[51].

Both the central and peripheral mechanism could explain the decreased food intake, body weight, gastrocolicomental fat and serum leptin found in this study with improved insulin sensitivity indicated by the significant lowering of blood glucose level produced by $PYY_{3–36}$ treatment and agrees with the previous results[47]. Diminished food intake in response to PYY_{3-36} treatment may lower the insulin: glucagon ratio, increase lipolysis and decrease *de novo*lipogenesis[52]. However, the lee index though significantly decreased from non treated HFD groups, yet it did not decrease below 0.3 and rats were still borderline obese indicating that $PYY_{3–36}$ only could not reverse completely HFD induced obesity in the absence of diet regimen, or the duration of treatment has to be extended and this will be a subject for future study.

Contradictory results to our study showed that acute and chronic administration of $PYY_{3–36}$ in rats either increase or did not change food intake and body weight [10].Another study found that intraperitoneal injection of PYY_{3-36} in mice produces an acute anorexigenic effect during the light phase followed by a delayed orexigenic effect during the dark phase[53].Oliveira et al.,.[54] reported that PYY_{3-36} produced insignificant effect on serum leptin. This controverse may be caused by different experimental protocols or animal strains used.

Gender differences were observed in the present study with male rats showing higher hypothalamic NPY concentrations probably due to the stimulatory effect of testosterone on both NPY synthesis and release according to previous result[55]. This could have contributed to the higher food intake, higher body weight, higherGCOF weight,

higher serum leptin, with decreased insulin sensitivity marked by a significantly higher serum glucose level in male than female rats and is compatible with the previous result[56].

The increased body weight in male than female rats reflected an increased abdominal fat weight and it was evident in the present study by the increased GCOF weight and this was compatible with the previous studies[57,58]. Other studies documented that this sex difference in body weight might be attributed to the anti-obese effects of estrogen in the female rats [59].In females, estrogens increase in response to HFD which could be protective as the high fat diet increased extra-gonadal sources of estrogens[56].

Higher body weight and more visceral fat accumulation in males could explain the development of secondary complications as hyperglycemia and dyslipidemia[56]. The mechanism of increased HDL-c in female control rats may be due to the protective effect of estrogen as found by previous research[59]. The adipocytes of visceral fat tissue are more lipolytically active than subcutaneous adipocytes and thus release more free fatty acids (FFAs) in plasma. FFAs *per se* are among the most important products of the visceral adipocyte to cause insulin resistance and hence the metabolic syndrome [60].

Controversial studies showed that food intake was equal in rats of both sexes[61], orhigher in males than females with no differences in weight in both sexes [62]. Another study reported that there was no significant difference in serum level of lipid profile and leptin between male and female control rats[38, 63] .This converse may be caused by different experimental protocols or animal strains studied.

In conclusion, HFD produces a restraining feedback effect on hypothalamic food intake by suppressing NPY concentration to overcome obesity. This effect is mostly through Y2 receptors. Intraperitoneal administration of PYY_{3-36}; the potent Y2 receptor agonist could prevent partially HFD induced obesity as well as its metabolic hazards in adult albino rats of both sexes by further suppressing hypothalamic NPY. Gender differences revealed that male rats have higher hypothalamic NPYconcentrations with higher food intake and body weight probably due to the male hormones. When subjected to HFD, males developed higher measures of obesity with more adverse metabolic effects than females probably due to the protective effects of female hormones.

References

1. Steckling F, Lima F, Farinha J, Dos Santos D, AntunesSoares F. Obesity, Inflammation and Aerobic Physical Exercise. Ann Sports Med Res. 2015; 2: 1017.
2. Buhmann H, le Roux C, Bueter M. The gut-brain axis in obesity. Best Pract Res ClinGastroenterol. 2014; 28: 559-571.
3. Zhang L, Bijker M, Herzog H. The neuropeptide Y system: pathophysiological and therapeutic implications in obesity and cancer. PharmacolTher. 2011; 131: 91-113.
4. Pedragosa-Badia X, Stichel J, Beck-Sickinger A. Neuropeptide Y receptors: how to get subtype selectivity. Front Endocrinol. 2013; 4: 5.
5. Bi S, Kim Y,Zheng F. Dorsomedial hypothalamic NPY and energy balance control. Neuropeptides. 2012; 46: 309-314.
6. Perry B, Wang Y. Appetite regulation and weight control: the role of gut hormones. Nutr Diabetes. 2012; 2: e26.
7. Suzuki K, Simpson K, Minnion J, Shillito J, Bloom S. The role of gut hormones and the hypothalamus in appetite regulation. Endocr J. 2010; 57: 359-372.
8. Tschöp M. PYY3-36 as an anti-obesity drug target. Obes Rev. 2005; 4: 307-322.
9. Batterham L, Cowley A, Small G, Herzog H, Cohen A, Dakin L, Wren M, Brynes E, Low J, Ghatei A, Cone D,. Bloom R.2003; Gut hormone PYY3-36 physiologically inhibits food intake. Nature, 418: 650-654

10. Boggiano M; Chandler P, Oswald K, Rodgers R, Blundell J, Ishii Y, Beattie A, Holch P, Allison D, Schindler M, Arndt K, Rudolf K, Mark M, Schoelch C, Joost H, Klaus S, Thöne-Reineke C, Benoit S, Seeley R, Beck-Sickinger A, Koglin N, Raun K, Madsen K, Wulff B, Stidsen C, Birringer M, Kreuzer O, Deng X, Whitcomb D, Halem H, Taylor J, Dong J, Datta R, Culler M, Ortmann S, Castañeda T, Tschöp M. PYY3-36 as an anti-obesity drug target Obes Rev. 2005; 6: 307-322.

11. Davidson T, Monnot A, Neal A,Martin A, Horton J, Zheng W. The effects of a high-energy diet on hippo-campal-dependent discrimination performance and blood-brain barrier integrity differ for diet-induced obese and diet-resistant rats. PhysiolBehav. 2012; 107: 26-33.
12. Woods S, Seeley R, Rushing P, D'Alessio D, Tso P. A controlled high fat diet induces an obese syndrome in rats. J Nutr. 2003; 133: 1081-1087.
13. Cheon W, Kim M. Effect of a five-week high-fat diet on serum-lipid composition and LPL mRNA expression in rat skeletal muscles. J ExercNutrBiochem. 2013; 17: 49-55
14. Campos G., Volpato I., Calderon M., Rudge, Damasceno D. Effect of obesity on rat reproduction and on development of their adult offspring. K.E. Braz J Med Biol Res. 2008; 41: 122-125.
15. Caesar R, Drevon C. Pancreatic contamination of mesenteric adipose tissue samples can be avoided by adjusted dissection procedures. J Lipid Res. 2008; 49: 12-55
16. Lee R, Rains T, Tovar-Palacio C, Beverly J, Shay N. Zinc deficiency increases hypothalamic neuropeptide Y and neuropeptide Y mRNA levels and does not block neuropeptide Y-induced feeding in rats. J Nutr. 1998; 128: 1218-1223.
17. Sahoo K, Sahoo B, Choudhury A, Sofi N, Kumar R, Bhadoria A. Childhood obesity: causes and consequences. J Family Med Prim Care. 2015; 4: 187-192.
18. Jennings J, Ung R, Resendez S, Stamatakis A, Taylor J, Huang J, Veleta K, Kantak P, Aita M, Shilling-Scrivo K, Ramakrishnan C, Deisseroth K, Otte S, StuberG.Visualizing hypothalamic network dynamics for appetitive and consummatorybehaviors. Cell. 2015; 160: 516-527.
19. Oliveira T, Leandro C, de Jesus Deiró T, dos Santos Perez G, da França Silva D, Druzian J,; Couto R, Barreto-Medeiros J. A perinatal palatable high-fat diet increases food intake and promotes hypercholesterolemia in adult rats. Lipids. 2011; 46: 1071-1074.
20. la Fleur S, Luijendijk M, van der Zwaal E, Brans M, Adan R The snacking rat as model of human obesity: effects of a free-choice high-fat high-sugar diet on meal patterns. Int J Obes (Lond). 2014; 38: 643-649.
21. Miller C, Morton H, Cooney P, Winters T, Ramseur K, Rayalam S, Della-Fera M, Baile C, Brown L. Acute exposure to high-fat diets increases hepatic expression of genes related to cell repair and remodeling in female rats. Nutr Res. 2014; 34: 85-93.
22. Abdoul-Azize S, Selvakumar S, Sadou H, Besnard P, Khan N. Ca^{2+} signaling in taste bud cells and spontaneous preference for fat: unresolved roles of CD36 and GPR120. Biochimie. 2014; 96: 8-13.
23. Little T,Feinle-Bisset C. Effects of dietary fat on appetite and energy intake in health and obesity--oral and gastrointestinal sensory contributions. PhysiolBehav. 2011; 104: 613-620.
24. Duca F, Covasa M. Current and emerging concepts on the role of peripheral signals in the control of food intake and development of obesity. Br J Nutr. 2012; 108: 778-793.
25. Morris M, Chen H, Watts R, Shulkes A, Cameron-Smith D. Brain neuro-peptide Y and CCK and peripheral adipokine receptors: temporal response in obesity induced by palatable diet. Int J Obes (Lond). 2008; 32: 249-258.
26. Briggs D, Lockie S, Benzler J, Wu Q, Stark R, Reichenbach A, Hoy A, Lemus M, Coleman H, Parkington H, Tups A, Andrews Z. Evidence that diet-induced hyperleptinemia, but not hypothalamic gliosis, causes ghrelin resistance in NPY/AgRP neurons of

male mice. Endocrinol. 2014; 155: 2411-2422.
27. Teubner B, Keen-Rhinehart E,Bartness T. Third ventricular coinje-ction of subthreshold doses of NPY and AgRP stimulate food hoarding and intake and neural activation. Am J PhysiolRegulIntegr Comp Physiol. 2012; 302: R37-48.
28. Kamiji M, Inui A. Neuropeptide y receptor selective ligands in the treatment of obesity. Endocr Rev. 2007; 28: 664-684.
29. Brothers S, Wahlestedt C. Therapeutic potential of neuropeptide Y (NPY) receptor ligands. EMBO Mol Med. 2010; 2: 429-439.
30. Kohno D, Yada T. Arcuate NPY neurons sense and integrate peripheral metabolic signals to control feeding. Neuropeptides. 2012; 46: 315-319.
31. Zammaretti F, Panzica G, Eva C. Sex-dependent regulation of hypothalamic neuropeptide Y-Y1 receptor gene expression in moderate/high fat, high-energy diet-fed mice. J Physiol. 2007; 583: 445-454.
32. Abu M, Samat S, Kamarapani N, Nor Hussein F, Wan Ismail W, Hassan H. Tinosporacrispa Ameliorates Insulin Resistance Induced by High Fat Diet in Wistar Rats..Evid Based Complement Alternat Med. 2015. Published online.
33. Wei X, Sun B, Chen K, Lv B, Luo X, Yan J. Ghrelin signaling in the ventral tegmental area mediates both reward-based feeding and fasting-induced hyperphagia on high-fat diet. Neuroscience. 2015; 2 9. pii: S0306-4522(15)00433-9.
34. Yepuru M, Eswaraka J, Kearbey J, Barrett C, Raghow S, Veverka K, Miller D, Dalton J, Narayanan R. Estrogen receptor-{beta}-selective ligands alleviate high-fat diet- and ovariectomy-induced obesity in mice. J Biol Chem. 2010; 285: 31292-31303.
35. Talukdar S, Oh da Y, Bandyopadhyay G, Li D, Xu J, McNelisJ, Lu M, Li P, Yan Q, Zhu Y, Ofrecio J, Lin M, Brenner M,Olefsky J. Neutrophils mediate insulin resistance in mice fed a high fat diet through secreted elastase. Nat. Med. 2012; 18: 1407-1412.
36. Miras A, Seyfried F, Phinikaridou A, Andia M, Christakis I, Spector A, Botnar R, le Roux C. Rats fed diets with different energy contribution from fat do not differ in adiposity. Obes Facts. 2014; 7: 302-310.
37. Liu M, Montgomery M, Fiveash C, Osborne B, Cooney G, Bell-Anderson K, Turner N. PPARα-independent actions of omega-3 PUFAs contribute to their beneficial effects on adiposity and glucose homeostasis. Sci Rep. 2014; 4: 5538.
38. Choi J, Liu H,, Choi D, Oh T, Mukherjee R, and Yun J. Profiling of gender-specific rat plasma proteins associated with susceptibility or resistance to diet-induced obesity. Proteomics J. 2012; 75:1386-400.
39. Borer K. Counter regulation of insulin by leptin as keycomponent of autonomic regulation of body weight. World J.Diabetes. 2014; 5: 606-629.
40. Estrany M, Proenza A, Lladó I, Gianotti M. Isocaloric intake of a high-fat diet modifies adiposity and lipid handling in a sex dependent manner in rats. Lipids Health Dis. 2011; 10: 52.
41. Kumar P, Bhandari U, Jamadagni S. Fenugreek Seed Extract Inhibit Fat Accumulation and Ameliorates Dyslipidemia inHigh Fat Diet-Induced Obese Rats. Biomed Res Int. 2014, 2014: 606021
42. Karpe F, Dickmann J, Frayn K. Fatty acids, obesity, and insulin resistance: Time for a revaluation, Diabetes. 2011; 60:2 441-2449.
43. Zhukova N, Novgorodtseva T, Denisenko Y. Effect ofthe prolonged high-fat diet on the fattyacid metabolism in rat blood and liver. Lipids Health Dis. 2014; 13: 49.
44. Shah S, Shah G, Singh S, Gohil P, Chauhan K, Shah K,Chorawala M. Effect of piperine in the regulation ofobesity-induced dyslipidemia in high-fat diet rats. Indian J.Pharmacol. 2011; 43: 296–299.
45. Klop B, Elte J, Cabezas M. Dyslipidemia in obesity: mechanisms and potential targets. Nutrients. 2013; 5:1218-1240.
46. Upadhyay K. Review Article Emerging Risk Biomarkers in Cardiovascular

Diseases and Disorders. J Lip. 2015; 2015: 50.
47. Mittapalli K, Roberts E. Ligands of the neuropeptide Y Y2 receptor. BMCL Digest. 2014; 24: 430-441.
48. Henry K, Elfers C, Burke R, Chepurny O, Holz G, Blevins J, Roth C, Doyle R. Vitamin B12 Conjugation of Peptide-YY3-36 Decreases Food Intake Compared to Native Peptide-YY3-36 Upon Subcutaneous Administration in Male rats. Endocrinol. 2015; 156: 1739-1749
49. Smitka K, Papezova H, Vondra K, Hill M, Hainer V, Nedvidkova J. The role of "mixed" orexigenic and anorexigenic signals and autoantibodies reacting with appetite-regulating neuropeptides and peptides of the adipose tissue-gut-brain axis: relevance to food intake and nutritional status in patients with anorexia nervosa and bulimia nervosa. Int J Endocrinol. 2013:483145. Published online.
50. Yulyaningsih E, Zhang L, Herzog H, Sainsbury A. NPY receptors as potential targets for anti-obesity drug development. Br J Pharmacol. 2011; 163:1170-1202.
51. Koda S; Date Y; Murakami N; Shimbara T; Hanada T; Toshinai K; Niijima A; Furuya M; Inomata N; Osuye K. andNakazato M. (2005): The role of the vagal nerve in peripheral PYY3-36-induced feeding reduction in rats. Endocrinology, 146(5):2369-2375.
52. Chelikani P, HaverA, Reidelberger R. Intermittent intraperitoneal infusion of peptide YY(3-36) reduces daily food intake and adiposity in obese rats. Am J PhysiolRegulIntegr Comp Physiol. 2007; 293: R39-R46.
53. Parkinson J, Dhillo W, Small C, Chaudhri O, Bewick G, Pritchard I, Moore S, Ghatei M, Bloom S. PYY3-36 injection in mice produces an acute anorexigenic effect followed by a delayed orexigenic effect not observed with other anorexigenic gut hormones. Am J PhysiolEndocrinolMetab. 2008; 294: E698-E708.
54. Oliveira K, Paula G, Costa-e-Sousa R, Souza L, Moraes D, Curty F, Pazos-Moura C. Peptide YY (PYY)3-36 modulates thyrotropin secretion in rats. J Endocrinol. 2006; 191:459-463.
55. Rugarn O, Hammar M, Theodorsson A, Theodorsson E, Stenfors C. Sex differences in neuropeptide distribution in the rat brain. Peptides 1999; 20: 81-86.
56. Fuente-Martín e, Argente-Arizón P, Ros P, Argente J, Chowen J. Sex differences in adipose tissue. It is not only a question of quantity and distribution. Adipocyte. 2013; 2: 128–134.
57. Hernández-Bautista R, Alarcón-Aguilar F, Del C Escobar-Villanueva M, Almanza-Pérez J, Merino-Aguilar H, Fainstein M, López-Diazguerrero N. Biochemical alterations during the obese-aging process in female and male monosodium glutamate (MSG)-treated mice.Int J Mol Sci. 2014; 15: 11473-11494.
58. Fukushima A, Hagiwara H, Fujioka H, Kimura F, Akema T, Funabashi T. Sex differences in feeding behavior in rats: the relationship with neuronal activation in the hypothalamus. Front Neurosci. 2015; 9: 88.
59. Zhu L, Zou F, Yang Y, Xu P, Saito K, Othrell Hinton A Jr, Yan X, Ding H, Wu Q, Fukuda M, Sun Z, Tong Q, Xu Y.(2015): Estrogens prevent metabolic dysfunctions induced by circadian disruptions in female mice.Endocrinol. 2015; 156: 2114-2123.
60. Ma X, Lee P, Chisholm D, James D. Control of adipocyte differentiation in different fat depots; implications for pathophysiology or therapy. Front Endocrinol (Lausanne). 2015; 6:1.
61. Hwang L, Wang C, Li T, Chang S, Lin L, Chen C, Chen C, Liang K, Ho I, Yang W, Chiou L. Sex differences in high-fat diet-induced obesity, metabolic alterations and learning, and synaptic plasticity deficits in mice. Obesity. 2010; 18: 463-469.
62. Ohta T, Katsuda Y, Miyajima K, Sasase T, Kimura S, Tong B, Yamada T. Gender differences in metabolic disorders and related diseases in Spontaneously Diabetic Torii-Lepr(fa) rats. J Diabetes Res. 2014; 2014:841957.
63. Zareian P, Karimi M, Dorneyani G. The comparison of the effects of

acute swimming stress on plasma corti-costerone and leptin concentration in male and female rats.Acta Med Iran. 2011; 249: 28