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Sexual Dimorphism in Serum Kisspeptin Level in Experimentally Induced Non Alcoholic Fatty Liver Disease in Adult Albino Rats

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Abstract

Background: non-alcoholic fatty liver disease (NAFLD) pathophysiology is not properly understood, studies have demonstrated gender differences in prevalence of NAFLD and severity of the disease. Insulin resistance (IR) is well known to play a key role in NAFLD pathogenesis. kisspeptin neuropeptide (KISS1) is main regulator of reproduction. It is thought that kisspeptin has a metabolic role outside its function in reproduction, its association with insulin resistance and its effects on insulin secretion have conflicting results, in addition, Kisspeptin signaling has a different effect on metabolism in male and female rats.

Objective: To clarify a possible sexual dimorphism in the interaction between kisspeptin and pathogenesis of high fat diet (HFD) induced NAFLD in adult male and female albino rats. Material and methods: 20 male and 20 female adult albino rats were used. The animals were divided to 4 equal subgroups: group (Ia): control male rats; group (Ib): HFD male rats; group (IIa): control female rats; group (IIb): HFD female rats. In all groups, BMI, kisspeptin serum levels, glucose, insulin (with calculation of HOMA-IR), lipid profile, glucagon, ALT, AST, LH, FSH, progesterone, estradiol (E2) and testosterone were measured and histopathological liver injury scoring was made.

Results: HFD male and female groups (group Ib and group IIb respectively) showed significant increase in BMI, serum glucose, insulin levels, HOMA-IR index, glucagon, TC, TG, LDL, AST, ALT levels with significant decrease in serum HDL in the same groups. However, the percentage of changes in these parameters was higher in male than female rats. Histopathological examination of HFD groups revealed a non- alcoholic steatohepatitis in group Ib and border line steatosis in group IIb with significantly higher hepatic injury score in group Ib in comparison to group IIb. Serum kisspeptin increased significantly in HFD male group Ib, while it decreased significantly in HFD female group IIb relative to their controls. Moreover group Ib showed a significant increase in serum estradiol, significant decrease in serum testosterone and non-significant change in serum progesterone, LH and FSH. In group IIb, a significant increase in serum

progesterone level was found, while a non-significant change was found in serum estradiol, testosterone, LH and FSH. Conclusions: The present study suggested that kisspeptin may play a role in the pathogenesis of NAFLD in male rather than in female rats.

Keywords: NAFLD, Sexual dimorphism, Kisspeptin, Insulin resistance

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the obesity related disorders [1]. It is characterized by liver injury from simple steatosis to steatohepatitis which can cause fibrosis and cirrhosis [2]. It can also cause hepatic carcinoma [3]. Insulin resistance (IR) has a central effect in the pathogenesis of NAFLD, as strong evidence demonstrates that, in NAFLD patients, insulin does not decrease lipolysis to the same degree that it does in healthy individuals [4]. Previous reports have demonstrated that, resistance of hormone-sensitive lipase to suppressive effect of insulin in insulin-resistant states is the predominant defect responsible for the increased flux of fatty acid from adipose tissue to the liver and stored as triglycride (TG) in the liver [5]. TG can be stored as droplets of lipid in hepatocytes and act as a predisposing factor to hepatic steatosis [5]. Once hepatic steatosis occurs, it proceeds to steatohepatitis (NASH) by inflammation, dysfunction of mitochondria, oxidative stress caused by reactive oxygen species, lipid oxidation resulting in hepatocyte damage and fibrosis [6]. kisspeptin (KISS1) is main regulator of reproduction [7,8]. Kisspeptin affects the reproductive axis by directly stimulating gonadotropin-releasing hormone (GnRH) neurons [9]. There are two major kisspeptin neurones: those in hypothalamic anteroventral periventricular nucleus (AVPV) which are responsible for the luteinizing hormone (LH) surge by mediating positive feedback effect of estradiol (E2), and the arcuate kisspeptin population which mediate steroid negative feedback actions on LH secretion [10,11]. Although kisspeptin is expressed in many areas of the brain [12], it is also present in some peripheral tissues [13, 14] including metabolic tissues like adipose, hepatic and pancreatic tissue [15] which indicates that kisspeptin has other functions outside reproduction. Changes in metabolic signals or energy status affect reproduction and kisspeptin levels [16]. In addition, effect of kisspeptin on insulin secretion has controversial results. While some researchers stated that kisspeptin inhibits insulin secretion [17, 18], others reported that kisspeptin stimulates insulin secretion [14, 19]. Moreover, in polycystic ovary syndrome, plasma kisspeptin levels were correlated negatively with insulin resistance [20]. However, another study reported no correlation between kisspeptin concentrations and index of insulin resistance [21]. Furthermore, Kisspeptin signaling has a sexual dimorphic effect on metabolism, and glucose homeostasis [22]. From the above data, it is worth saying that kisspeptin may have a role in the pathogenesis of NAFLD, and this role may be different among male and female as NAFLD is a sexually dimorphic condition.

The aim of this study is to compare kisspeptin level in male and female rats with (HFD) induced NAFLD in relation to different metabolic parameters and sex hormones.

2. Materials and Methods

This study was conducted from May 2017 to January 2018 on, 20 male and 20 female adult albino rats (150-180 g), obtained from animal house, Veterinary Medicine Faculty , Zagazig University, and were housed at room temperature with natural dark/light cycle and received food and water. After one week of acclimatization, the animals were divided in to 4 equal subgroups: group (Ia): control male rats; group (Ib): NAFLD male rats; group (IIa): control female rats; group (IIb): NAFLD female rats. Rats in control groups were fed a standard mixed commercial rat laboratory chow (25.8 % protein, 59.7% carbohydrate and 14.5% fat), while rats in NAFLD groups were fed (HFD) (9.7% protein, 37.7% carbohydrate and 53% fat) for 90 days [23]. Diet was obtained from Department of Nutrition, Veterinary Medicine Faculty, Zagazig University. The experimental protocol used in this study was according to the guiding data for the use of research

animals and were approved by the Institutional Research Board of Faculty of Medicine, Zagazig University.

Anthropometric measures: Measuring body weight: The animal was put in closed plastic container and weighed one day before the experiment and at the last day. The results were recorded for each labeled rat. Measuring rat length: by metal ruler, nose to anus length was measured. Calculating BMI index: Body mass index (BMI) equals body weight (g)/ length2 (cm2), this index can be used as an indicator of obesity where the cut off value of obesity BMI is more than 0.68 g/cm2 [24].

Blood collection: scarification of animals at the end of experimental period under light ether anesthesia after overnight fasting, and blood samples were obtained by decapitation of all rats, blood samples from female rats obtained during estrus stage which was determined by vaginal smear, blood was collected in clean centrifuge tubes and leaved to clot, then centrifuged at 3000 r.p.m. for 15 minutes The supernatant serum was pipetted off using fine tipped automatic pipettes and stored deep frozen at -20 until assayed [25]. Biochemical assay: Serum kisspeptin levels were analyzed by a commercial enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals Inc., Burlingame, California, USA). [21]. Serum glucose level: According to Tietz et al. [26] using glucose enzymatic (GOD-PAP)-liquizyme Kits (Biotechnology, Egypt). Serum insulin level: according to Temple et al. [27], by a solid phase enzyme amplified sensitivity immunoassay. HOMA-IR was assessed by homeostasis model assessment (where HOMA= fasting serum insulin (μIU/mL) x [fasting serum glucose (mmol/L)/22.5] [28, 29]. Glucagon levels: by glucagon ELISA kit (Cat. No: Rab 0202; Sigma AldichChemieGmBH, St.Louis, USA) [30]. Serum testosterone level: according to Tietz et al. [26] using rat testosterone ELISA kit: (Catalog Number: 2011-11-5126, shanghai, China). Serum follicular stimulating hormone (FSH) level: according to Robertson [31] using rat follicle-Stimulating Hormone (FSH) ELISA kit (Catalog Number: 2011-11-0183, shanghai, China). Serum Luteinizing hormone (LH) level: according to Robertson [31] using rat luteinizing hormone (LH) ELISA kit (Catalog Number: 2011-11-0180, China). Estradiol (E2) and Progesterone level: according to March et al. [32], using ELISA rat kits: BC-1029 and BC-1115, respectively, BioCheck Inc 323 Vintage Park , CA 94404. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), were analyzed by an automatic biochemical analyzer using standard enzymatic techniques [33]. Serum total cholesterol (TC) level: according to Tietz et al. [26] using rat cholesterol enzyme-linked immunosorbent assay kit, (BioSource Europe S.A.-Rue de l' Industrie, 8-B-1400 Nivelles-Belgium). Serum triglycerides (TG) level: using rat triglycerides enzyme-linked immunesorbent assay kit, (BioSource Europe S.A.-Rue de l' Industrie, 8-C- 1150 Nivelles- Belgium according to Fossati and Prencipe [34]. Serum high density lipoproteins (HDL) levels: according to Albers et al. [35] using rat HDL- cholesterol enzyme-linked immunosorbent assay kit, (BioSource Europe S.A.-Rue de l ' Industrie, 8-A- 1340 Nivelles-Belgium). Serum low density lipoproteins (LDL) levels: according to Friedwald et al [36], LDL was calculated as follows: LDL=TC-HDL-TG/5.

Liver histopathology: All removed livers were fixed in 10% buffered formalin solution for duration of 48-60 hours. After this, tissue samples were processed through ethyl alcohol and xylene series, and put in paraffine blocks. Liver specimens were cut (5μm thick), then stained with hematoxylin and eosin [37]. An expert pathologist evaluated the stained samples in a blind fashion using Light microscope with camera attachment and scored them. The histopathological scoring of NAFLD followed the NAFLD Activity Score (NAS) [38]. The score is steatosis (0 = <5%, 1 = 5% - 33%, 2 = 34% - 66%, 3 = >66%), lobular inflammation (0 = no foci, 1 = <2 foci, 2 = 2 - 4 foci, 3 = >4 foci), and ballooning (0 = none, 1 = rare or few, 2 =many or prominent). Fibrosis stages: 0 = none, 1 = perisinusoidal or periportal fibrosis, 2 = perisinusoidal and portal/periportal fibrosis, 3 = bridging fibrosis and 4 = cirrhosis. The score of (NAS $\geqslant$ 5 was defined as non alcoholic steatohepatitis (NASH), 2 < NAS <5 was defined as borderline and NAS $\leqslant$ 2 was defined as simple steatosis), cirrhosis was defined as grade 4 fibrosis plus other features of NAS [39].

Statistical analysis: The data presented as mean ± SD and analyzed statistically by using SPSS program (version 18 for windows) (SPSS Inc. Chicago, IL, USA). Independent T test was used to compare means of groups. The correlations between parameters were analyzed using Pearson's correlation. P values < 0.05 considered significant.

3. Results

In group Ib (HFD-fed male rats), there was a significant increase in BMI, serum glucose, insulin levels, HOMA-IR index, TC, TG, LDL, AST, ALT levels in comparison to control (P<0.001). However, there was a significant decrease in serum HDL in the same group (P<0.01) [Table 1]. We found a significant increase in serum kisspeptin and estradiol (P<0.01), while there was a significant decrease in serum testosterone (P<0.01). However, there was non-significant change in serum FSH, LH and progesterone in comparison to control (p>0.05) [Table 2].

In group IIb (HFD-fed female rats), there was a significant increase in serum glucose, TG, LDL (P<0.001) and in BMI, HOMA-IR index, TC, AST, ALT levels (P<0.01) and in serum insulin (P<0.05) in comparison to control. However, there was a significant decrease in serum HDL in the same group (P<0.05) ,the percentage of change in these parameters in group IIb in relation to its control group IIa was lower than percentage of change in the same parameters in group Ib in relation to its control group Ia [Table 1].

In addition, in group IIb (HFD-fed female rats), there was a significant decrease in serum kisspeptin (P<0.001), while there was a significant increase in serum progesterone (P<0.05). However, there was non-significant change in serum FSH, LH, testosterone and estradiol in comparison to control (p>0.05) [Table 2].

Regarding correlation of kisspeptin with metabolic parameters and sex hormones, in group Ib, Serum kisspeptin showed a significant positive correlation with BMI (P<0.05), serum glucose (P<0.05) glucagon (P<0.05), TC (P<0.05), TG (P<0.01), LDL (p<0.01), AST (P<0.05) and ALT (p<0.01). On the other hand, in group IIb, serum kisspeptin didn't correlate with any of measured parameters (p>0.05).

Hepatic histopathological examination revealed normal liver tissue with normal architecture,normal hepatocyte and normal central vein in both groups Ia (control male rats) [Fig 1] and IIa (control female rats) [Fig 3], HFD induced non- alcoholic steatohepatitis in group Ib and border line steatosis in group IIb. There was a significant increase in hepatic injury score in group Ib (6.2 ± 0.77) in comparison to group IIb (3.8 ± 0.51) (p<0.001) with a significant positive correlation between kisspeptin level and hepatic injury score in group Ib (p<0.05), but there is no significant correlation between kisspeptin level and hepatic injury score in group IIb (p>0.05) [Fig 2, 4 & Table 3].

Table 1: Metabolic profile of all studied groups

GROUPS / PARAMETERS	Group Ia	Group Ib	Group IIa	Group IIb
Body Mass Index(g/cm²)	0.58±0.06 r= + 0.524	0.81±0.07 [a] *** r = +0 .915 *	0.47±0.04 r= + 0.502	0.61±0.07 [b] ** r = +0.544
% of change		39%		29%
Glucose(mg/dl)	93±6.6 r= + 0.482	188±3.1 [a] *** r = + 0.752*	87±2.5 r= + 0.321	133 ± 4.2 [b]*** r =+0.452
% of change		102%		52%
Insulin (µIU/ml)	11.93 ± 1.77 r= - 0.569	21.33 ± 6.4 [a] *** r = + 0.115	5.02 ± 0.91 r= - 0.423	8.6± 0.2 [b] * r= +0.399
% of change		90%		71%
HOMA-IR	2,5±0.07 r= + 0.422	9,7±1.03 [a] *** r = + 0.299	1.07±0.25 r= + 0.358	2.62±0.7 [b] ** r = +0.402

% of change		288%		144%
Glucagon (ng/ml)	200 ±2.62 r= + 0.602	320± 2.3 [a] *** r = + 0.847*	110± 3.4 r= + 0.558	150± 3.5 [b] ** r = +0.531
% of change		60%		36%
Total Cholesterol (mg/dl)	89.27±10.62 r= + 0.342	152±20.23 [a] *** r= + 0.892*	77.66±13.1 r= + 0.228	105.5±11.08** r = +0.366
% of change		70%		36%
Total Triglycride (mg/dl)	65.50±15.35 r= + 0.381	140.83±11.59[a]*** r = + 0.978**	64.16±4.13 r= + 0.407	103.01±8.91[b]*** r = +0.344
% of change		155%		60%
Low Density Lipoprotein (mg/dl)	35±8.66 r= + 0.264	93±12.14 [a] *** r = + 0.971**	26.96±12.45 r= + 0.298	52.79±15.11[b]*** r = +0.422
% of change		165%		100 %
High Density Lipoprotein (mg/dl)	41.83±0.89 r= - 0.455	31.56±1.57 [a] ** r = - 0.521	38.54±1.42 r= - 0.289	33. 85±2.43 [b] * r = - 0.366
% of change		25%		11.5%
Serum Aspartate Aminotransferase (AST) (u/ l)	48.55±1.9 r= +0.393	78.24 ±2.37 [a] *** r = + 0.811*	55±1.22 r= + 0.311	68.2±0.3 [b] ** r = +0.410
% of change		61%		23%
Serum Alanine Aminotransferase (ALT) (u/ l)	24.8±1.07 r=+0.412	44.27 ±0.96 [a] *** r = + 0.829**	18±0.48 r= + 0.388	30. 2±0.3 [b] ** r = + 0.399
% of change		83%		66%

Group Ia (control male rats), Group Ib (HFD-fed male rats), Group IIa(control female rats), Group IIb (HFD-fed female rats) a= vs group Ia; b= vs group IIa; r =correlation coefficient versus kisspeptin level; *= significant (P<0.05); **= significant (P<0.01); ***= significant (P<0.001)

Table 2: Hormonal profile of all studied groups

GROUPS / PARAMETERS	Group Ia	Group Ib	Group IIa	Group IIb
Kisspeptin (ng\ml)	1.5± 0.13	2.1 ±0.08**	2.4 ±0.3	1.6 ±0,2***
FollicleStimulating Hormone (ng/ml)	8±0.05 r= + 0.499	8.7±0.03 r= + 0.554	17.8 ±0.12 r= + 0.433	18±1.36 r= + 0.624
Luteinizing Hormone (ng/ml)	2.2±0.08 r= + 0.318	3 ±0.13 r = + 0.395	3.5±0.3 r= + 0.485	4±0.18 r = + 0.522
Testosterone (ng/ml)	3.13±0.34 r= + 0.425	1.74±0.39[a] ** r = + 0.477	0.96±0.6 r= + 0.492	0.99±1.2 r =+ 0.514
Estradiol (pg/ml)	5.14 ±1.36 r= + 0.559	11.4 ±3.24[a] ** r = 0.580	18.8 ±5.2 r= + 0.548	20.6±2.6 r = + 0.599
progesterone (ng/ml)	2.3±0.3 r= + 0.449	2.5 ±0.4 r = + 0.488	9.6±0.6 r= + 0.450	14.2±0.3 [b] * r = + 0.582

Group Ia (control male rats), Group Ib (HFD-fed male rats), Group IIa(control female rats), Group IIb (HFD-fed female rats) a= vs group Ia; b= vs group IIa; r =correlation coefficient versus kisspeptin level;*= significant (P<0.05); **= significant (P<0.01); ***= significant (P<0.001)

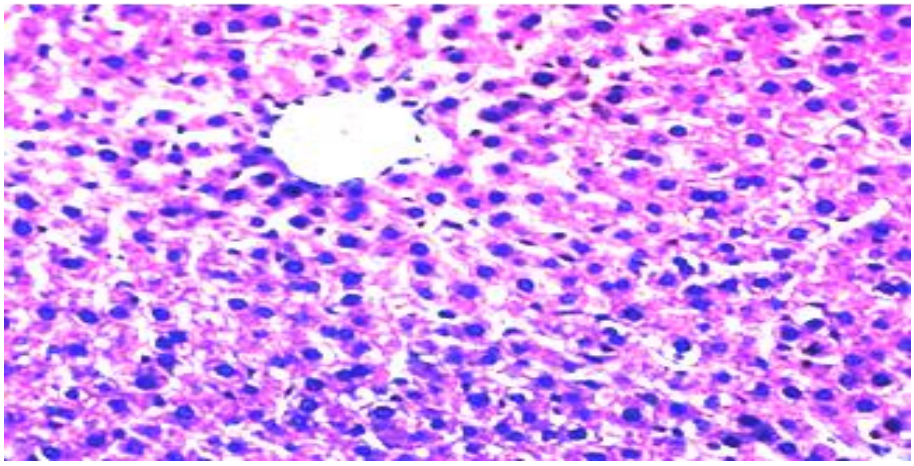

Fig 1: Photomicroscopic picture of isolated liver tissue in group Ia, stained with H & E and viewed under high power magnification x400 showing normal liver tissue with normal architecture, normal hepatocyte, and normal central vein

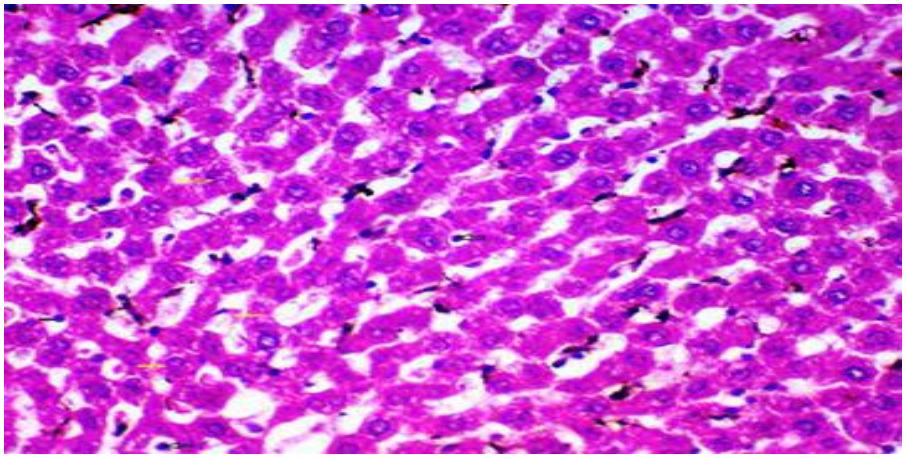

Fig 2:Photomicroscopic picture of isolated liver tissue in group Ib, stained with H & E and viewed under high power magnification x400 showing liver with non alcoholic steatohepatitis (NASH); fatty infiltration, foci of inflammatory infiltration and ballooning degeneration

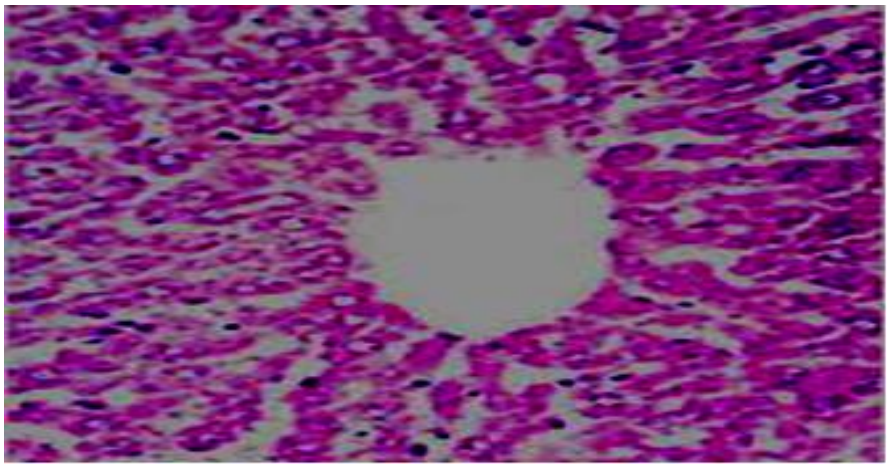

Fig 3: Photomicroscopic picture of isolated liver tissue in group IIa, stained with H & E and viewed under high power magnification x400 showing normal liver tissue with normal architecture, normal hepatocyte and normal central vein

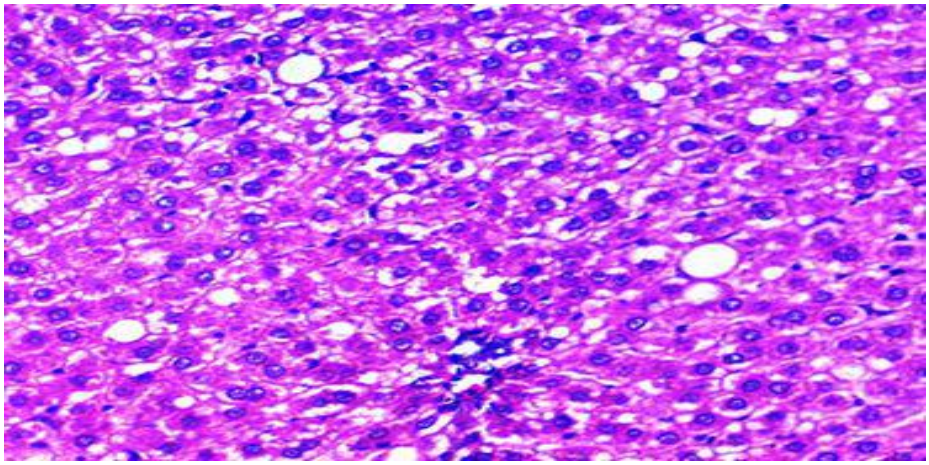

Fig 4: Photomicroscopic picture of isolated liver tissue in group IIb, stained with H & E and viewed under high power magnification x400 showing liver with border line steatosis; fatty deposition and mild inflammation

Table 3: Histopathological scoring of liver injury induced by HFD

GROUPS	Group Ia	Group Ib	Group IIa	Group IIb
Hepatic injury score	0	6.2±0.77 ^{a***} r = 0.833*	0	3.8 ±0.51 $^{a\ b***}$ r = 0.484

Group Ia (control male rats), Group Ib (HFD-fed male rats), Group IIa(control female rats), Group IIb (HFD-fed female rats) a= vs group Ia; b= vs group Ib; r =correlation coefficient versus kisspeptin level; *=significant (P<0.05); **=significant (P<0.01); ***=significant (P<0.001);

4. Discussion

The current study indicates that high-fat diet induced NAFLD in group Ib and IIb. In both groups HFD caused obesity as the rise in total cholesterol, LDL and triglycerides was associated with significant increase in body mass index. Moreover, it increased serum glucose, serum insulin and insulin resistance. These results are in line with those of Sjoholm et al. [40] and Eisinger, et al. [41] who noticed that HFD can cause development of many features of metabolic syndrome, disturbance in lipid metabolism and increased insulin resistance lead to development of NAFLD[4] , the percentage of increase in these parameters was higher in male than in female, this data is in line with the results of Estrany et al [42-43] and Nickelson et al, [44] who stated that females tend to be less susceptible to many of the deleterious effects of HFD intake. In addition, plasma ALT, AST levels were increased, these results agree with those of Kurek et al. [45] who reported that rats with NAFLD were characterized by significantly elevated serum ALT and AST, which is a sign of a liver lesion, these manifestations were confirmed by histopathological changes in the form of development of NASH in HFD fed male group indicated by fatty infiltration and foci of inflammatory infiltration represented by spotty necrosis, and ballooning degeneration. On the other hand, hepatic histopathological examination in HFD fed female groups showed border line steatosis in the form of macrovesicular fatty infiltration with mild inflammation but without cellular necrosis and accompanied by less increase in ALT and AST compared with male group, these finding are in accordance with those of Kamada et al. [46] and Yatsuji et al. [47].

Kisspeptin is expressed in a number of brain areas that do not contain GnRH [15], as well as in several peripheral tissues each of which might contribute to circulating kisspeptin [48, 49], such as liver, pancreas and adipose tissue [13] placenta, pituitary gland, testis and ovary [50, 51, 52]. In the current study, we found a sexually dimorphic difference in kisspeptin level, as high-fat diet (HFD)-induced obese male rats showed increased serum kisspeptin level as compared to their control and was correlated positively and significantly with BMI, serum glucose, TC, TG, and LDL levels, these findings are supported by those of Zhu et al.

[53] who noticed that kisspeptin was correlated positively with BMI, , TC, TG, and LDL levels and Andreozzi et al. [21] who reported that kisspeptin was correlated positively with serum glucose. The increased serum kisspeptin level might be due to elevation of serum glucagon, it was shown that in HFD induced obesity, glucagon stimulates hepatic expression of kisspeptin which affect pancreatic B cell to suppress insulin secretion [18].

Furthermore, kisspeptin inhibits glucose-stimulated insulin secretion from cultured islets at nanomolar concentrations [54]. In addition, increased liver kisspeptin leads to reduced insulin-suppressing effect on hepatic glucose production that contributes to hepatic lipogenesis [55], and reduced insulin-suppressing effect on lipolysis [4]. It is interesting that, kisspeptin was reported to increase lipolysis and enhanced expression of hormone sensitive lipase in isolated rat adipocytes [56]. Because of increased lipolysis of peripheral fats stored in white adipose tissue that flow to the liver as plasma non-esterified fatty acids and transformed to triglycride (TG) [5]. TG can then be stored as droplets of lipids in hepatocytes and act as a risk factor for development of hepatic steatosis [6]. In addition, increased lipolysis leads to FFAs increase, this impair insulin signaling pathway and result in insulin resistance [57]. Furthermore, Kisspeptin increased cholesterol [58] which causes liver inflammation in mice susceptible to NASH [59]. Moreover, kisspeptin stimulated mRNA expression of tumor necrosis factor α (TNF α), interleukin-6 (IL-6) and monocyte chemotactic protein-1 (MCP-1) [60], secretion of these pro-inflammatory mediators lead to hepatic inflammation and predisposed to hepatic steatohepatitis, as those cytokines activate several serine kinases, including I κ B kinase (IKK) [61], this kinase was shown to inhibit insulin action by stimulating the phosphorylation of serine residues of the insulin signaling pathway [62].

Kisspeptin is a vital component for regulation of GnRH secretion. As such, it has been a focus for the central pathway responsible for conveying homeostatic information to GnRH neurons given its potent role in stimulating the HPG axis. Kisspeptin neurons in the arcuate nucleus (ARC) are associated with the negative feedback regulation of GnRH, in a variety of species including humans [63] and rats [64], this effect is mediated by estrogenic receptor (ER α) [65] and progesterone receptor [66] in females, and by contributions from both ER α and androgenic receptor in males [67].

Another possible cause that may contribute to increased kisspeptin level in obese male rats is increased Kisspeptin mRNA expression in the hypothalamic (ARC) nucleus as reported by many investigators [13, 68, 69]. Obesity was associated with decrease in serum total testosterone (T) and increases in estradiol (E2) levels [70, 71], which concurs with our results. These inverse changes in testosterone and estradiol levels may be a consequence of the rise in aromatase levels expressed at high levels in visceral fat and males have more aromatase activity than females [72]. The increased kisspeptin in the HFD male group could be explained by decreased testosterone [66], because kisspeptin expression in the ARC is inhibited by increased sex steroids [66]. However, inspite of negative feedback between estrogen and kisspeptin, the increased estrogen reported in HFD male group may be unable to cause decrease in kisspeptin expression in the ARC, this conflict might be explained by a previous finding that, HFD increased palmitic acid (PA) in male mice CNS, which cause inflammation and reduced estrogen receptor number in hypothalamic neurons and ARC [73]. Moreover, adipose tissue may contribute to increased serum kisspeptin in obese male rats as a positive correlation between Kiss mRNA in visceral adipose tissue and BMI was reported [74].

On the contrary, the current study found decreased serum kisspeptin in (HFD)-induced obese female rats, as compared to their control and there was no significant correlation with BMI or any of the metabolic parameters which concurs with those of Rafique & Latif [75] and Yerlikaya et al. [76] who stated that kisspeptin was not correlated with BMI. The decreased serum kisspeptin might be due to decreased hypothalamic Kiss mRNA and the number of kisspeptin-immunoreactive cells in HFD-induced obese female rats [68, 77]. Moreover, the high progesterone level stated in this study may provide inhibitory effect on kisspeptin secretion in ARC as reported by Goodman et al. [78] and they stated that, local antagonism of receptors of progesterone in the ARC interfered with the action of progesterone in reducing kisspeptin level.

Moreover, it was reported that, in HFD-induced obese female rats, ovarian kisspeptin was down-regulated during proestrus and estrus stages of rat reproductive cycle. More specifically, the immunoreactivity of kisspeptin was greatly reduced in theca cells in the antral and preovulatory follicles [79]. Furthermore, in HFD induced obesity in Wistar rats, a decrease in Kiss1 mRNA was found in subcutaneous fat which is more predominant in female [80, 81,13]. All these factors may participate in decreased serum kisspeptin in (HFD)-induced obese female rats observed in this study. It is interesting that elevation of serum glucagon observed in HFD induced obese female rats which was reported to stimulate hepatic expression of kisspeptin [18] might not be effective enough to increase serum kisspeptin in female rats, as a sex difference in serum glucagon concentration was reported to be approximately two fold higher in normal-weight or obese male than in normal-weight or obese female [82].

5.Conclusion

HFD induced steatohepatits in obese male rats associated with an increase in serum kisspeptin level that was correlated with metabolic parameters which predispose to the disease, while in obese female rats, HFD induced only border line steatosis associated with a decrease in serum kisspeptin level which indicates a possible role for kisspeptin in the pathogenesis on NAFLD in male rather than in female rat.

Declaration of interest statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

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