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SERUM NESFATIN-1 LEVELS IN RAT MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a frequent progressive disorder manifested by fat accumulation in the liver and usually related to obesity and insulin resistance, but its pathogenesis is still uncertain. Nesfatin-1 is a polypeptide derived from nucleobindin-2 and involved in regulation of food intake and glucose homeostasis. The relationship between nesfatin-1 and NAFLD is still controversial.

Objective: To evaluate serum levels of nesfatin-1 in NAFLD model induced by high fat diet (HFD) in male albino rats.

Material and methods: Forty eight male adult albino rats were divided into four equal groups: 2 control groups that were fed ordinary diet for 4 weeks (group IA) and 12 weeks (group IB), and 2 HFD groups that were fed HFD for 4 weeks (group IIA) and 12 weeks (group IIB). In all groups, abdominal circumference, body weight, serum levels of nesfatin-1, insulin, glucose, C-reactive protein (CRP), lipid profile parameters, and liver enzymes (ALT & AST) were measured. BMI and HOMA-IR were calculated, and isolated liver tissues were examined histopathologically.

Results: After 4-week and 12-week-HFD feeding, the rats developed simple steatosis and steatohepatitis, respectively. These were proved by the progressive rise of liver enzymes and the histopathological findings. Besides, there was a significant progressive rise in BMI, HOMA-IR, serum levels of nesfatin-1, glucose, insulin, CRP, and all lipid profile parameters except high density lipoprotein that significantly decreased in HFD groups in comparison to control groups. Moreover, nesfatin-1 correlated positively with all measured parameters in HFD groups except for HDL that showed negative correlation with nesfatin-1.

Conclusion: Serum levels of nesfatin-1 increased in NAFLD rat model induced by HFD. This rise may be attributed to feeding rats with HFD, hyperglycemia or may compensate for the inflammation and disturbed metabolism.

Key words: NAFLD, nesfatin-1, lipid profile, HOMA-IR.

INTRODUCTION

NAFLD, defined by the presence of fat in the liver in absence of alcohol consumption, is a wide clinico-pathological disease that ranges from simple steatosis to steatohepatitis through to fibrosis and even cirrhosis **(Targher et**

al., 2016). A strong association was reported between NAFLD and metabolic disturbances such as insulin resistance, dyslipidemia, diabetes mellitus, and central abdominal obesity **(Katsiki et al., 2016)**. NAFLD was considered as an independent risk factor for cardiovascular diseases, and liver-related and extra hepatic-related mortalities **(Dunn et al., 2008 and Soderberg et al., 2010)**. The increased prevalence of NAFLD might be attributed to the increased frequency of obesity and diabetes in the general population **(L?pez-Vel?zquez et al., 2014)**.

Nesfatin-1 is an 82–amino acid polypeptide that is widely expressed in the brain and several peripheral tissues **(Stengel et al., 2009)**. Several effects of nesfatin-1 were reported including anorexia and reduction of body weight **(Stengel and Taché, 2010)**, regulation of glucose and lipid metabolism and insulin sensitivity **(Zhang et al., 2012 and Shimizu & Osaki, 2013)**, in addition to the anti-inflammatory and anti-apoptotic properties **(? zsavc?et al., 2011)**.

Few and contradictory data were found about the relationship between circulating levels of nesfatin-1 and NAFLD. While **Basar et al. (2012)** reported reduced serum levels of nesfatin-1 in patients having NAFLD, another study revealed increased plasma levels of nesfatin-1 in rat model of NAFLD **(Wu et al., 2016)**.

The aim of the present work was to evaluate serum levels of nesfatin-1 in NAFLD model induced in adult male albino rats by HFD and to find out the possible correlation to some glycemic, metabolic and inflammatory parameters.

MATERIAL AND METHODS

The study was carried out on 48 adult healthy male albino rats of a local strain weighing 180-200 g that were purchased from the animal house, Faculty of Veterinary Medicine, Zagazig University. Rats were kept at room temperature, housed 4 animals per cage in steel wire cages (50 cm x 60 cm x 60 cm) under hygienic conditions, kept on a normal light/dark cycle, had free access to water and received care along the lines of the national health guidelines. The experimental procedures were applied in the animal house, Faculty of Medicine, Zagazig University and were accepted by the Institutional Research Board, Faculty of Medicine, Zagazig University.

After acclimation for one week, the rats were divided into 2 major equal groups: **Control group (I)** (n=24); that was subdivided equally into 2 subgroups **(IA and IB)**; where rats were fed standard diet (12.6 kJ/g; 5% fat, 18 % protein and 77% carbohydrate) for 4 weeks and 12 weeks, respectively, and **HFD-fed group (II)** (n=24): that was subdivided equally into 2 subgroups **(IIA and IIB)**; where rats were fed HFD (23.4 kJ/g; 58 % fat, 18% protein and 24% carbohydrate) for 4 weeks (for induction of steatosis) and 12 weeks (for induction of non-alcoholic steatohepatitis;

NASH), respectively **(Zhang et al., 2010)**.

The following anthropometric parameters were measured: **Body weight (BW)** was recorded at the start and the end of the experiment, **rat nose to anus length** was measured according to **Warnick et al. (1983)** and **abdominal circumference (AC)** was measured according to **Gerbaix et al. (2010)**. **BMI index** was calculated according to **Novelli et al. (2007)** equation [body weight (g)/length^2 (cm^2)]. Obesity was considered when BMI exceeds 0.68 gm/cm^2.

Blood collection: Rats were anesthetized using ether at the end of the experiment after overnight fasting, and blood was obtained from all rats by decapitation and collected in plastic centrifuge tubes. Blood was centrifuged for 15 minutes at 3000 r.p.m. and serum was separated and stored at -20℃ until used for measurement of the following parameters: **Nesfatin-1 levels** were measured using rat Nesfatin-1 ELISA Kits (Shanghai Sunred biological technology, China) according to **Basar et al. (2012)**. **Glucose levels** were measured using enzymatic (GOD-PAP)-liquizyme glucose kits (Biotechnology, Egypt), according to **Tietz (1995)**. **Insulin levels** were measured using KAP1251-INS-EASIA (Enzyme Amplified Sensitivity Immunoassay) rat Kits (BioSource Europe S.A., Belgium) according to **Temple et al. (1992)**. Calculation of **the homeostasis model assessment of insulin resistance (HOMA-IR)** was done using the following equation: [fasting serum insulin (µIU/mL) x fasting serum glucose (mg/dl)/405] according to **Matthews et al. (1985)**. **Total cholesterol (TC) levels** were measured using rat total cholesterol kits (BioSource, Europe S.A), according to **Tietz (1995)**. **Triglycerides (TG) levels** were measured using triglycerides ESPAS SL kits A (Elttech S.A., Lyon, France) according to **Naito (1989)**. **High density lipoproteins (HDL) levels** were measured by using rat HDL kits (Catalog Number: 2011-11-0255, Shanghai Sunred biological technology, China) according to **Nauk et al. (1997)**. **Low density lipoproteins (LDL) levels** were calculated using the following equation: LDL = TC-HDL-(TG/5) according to **Friedwald et al. (1972)**. **Very low density lipoproteins (VLDL) levels** were calculated according to the equation: VLDL = TG/5 **(Tietz, 1995)**. **Alanine aminotransferase (ALT)** and **Aspartate aminotransferase (AST)** levels were measured using rat ALT and AST ELISA kits (Catalog Number: 2011-11-0595, Shanghai Sunred biological technology, China), according to **Rec (1970)**. **C-reactive protein (CRP) levels** were measured according to **Kimberly et al. (2003)** using rat immuno assay kits (Monobind Inc Lake Forest, USA).

Histopathological examination of liver: The isolated liver tissues were fixed for 48-60 hours in 10% buffered formalin solution, then processed through ethyl alcohol and xylene series, and after that embedded in paraffin blocks. Sections

with 5μm thickness were made from liver specimens, and then stained with hematoxylin and eosin according to **Altunkaynak (2005)**. The stained samples were evaluated using light microscope.

Statistical analysis: Results were presented as mean ± SD. Statistical analysis was performed using SPSS program, version 19 (SPSS Inc., Chicago, IL, USA). One way Analysis Of Variance (ANOVA) followed by LSD post hoc test was used to compare statistical differences between the groups. Besides, Pearson's correlation was used to analyze correlations between serum levels of nesfatin-1 and the measured parameters. P value <0.05 was considered significant for all performed statistical tests.

RESULTS

There was a statistically significant and progressive rise in final BW, BMI and AC in 4-week-HFD group and 12-week-HFD group (IIA and IIB) in comparison to their time-corresponding control groups (IA and IB). There was also a statistically significant and progressive elevation in serum levels of glucose, insulin and HOMA-IR index in HFD groups compared with their time-matching control groups. Concerning the lipid profile parameters, there was a significant progressive increase in serum levels of TC, TG, LDL and VLDL, while there was a significant progressive decrease in serum HDL levels in HFD groups in comparison to their time-equivalent control groups. As regard the liver enzymes, there was a statistically significant and progressive increase in serum levels of ALT and AST in the HFD groups in comparison to their time-matching control groups. Moreover, there was a significant progressive increase in the serum levels of the inflammatory marker; CRP in HFD groups compared with control groups. Serum nesfatin-1 levels significantly increased in 4-week-HFD group and more markedly increased in 12-week-HFD group in comparison to their time-matching control groups. No statistically significant changes were found in serum levels of all measured parameters in 12-week control group compared with 4-week control group except for the final body weight (Table 1).

Statistically significant positive correlations were found between nesfatin-1 and all measured parameters in 4-week and 12-week-HFD groups, except HDL which showed a significant negative correlation with nesfatin-1. No significant correlations were found between serum levels of nesfatin-1 and the other parameters in control groups (Table 2).

Histopathological examination of isolated rat liver tissues revealed steatosis in the form of fat deposition in hepatocytes in group IIA, and steatohepatitis in the form of fat deposition in hepatocytes surrounded by aggregates of chronic inflammatory cells indicating inflammation in group IIB (Fig. 1).

Table (1): Comparison of all measured parameters in all studied groups (Mean ± SD).

Groups / Parameters	Group IA	Group IIA	Group IB	Group IIB
Final BW (g)	**226.27±8.09**	**319.68±8.62[a]**	**246.07±10.16[a,b]**	**409.81±10.10[a,b,c]**
Final BMI (gm/cm^2)	**0.54±0.03**	**0.76±0.06[a]**	**0.56±0.04[b]**	**0.94±0.07[a,b,c]**
Final AC (cm)	**15.18±0.71**	**18.21±0.98[a]**	**15.73±0.71[b]**	**21.41±0.78[a,b,c]**
Nesfatin-1 (ng/ml)	**3.13±0.46**	**4.01±0.63[a]**	**3.03±0.35[b]**	**5.54±0.49[a,b,c]**
Glucose (mg/dl)	**84.45±5.70**	**146.05±5.60[a]**	**86.47±5.15[b]**	**218.63±9.41[a,b,c]**
Insulin (µIU/ml)	**20.02±1.45**	**31.33±3.00[a]**	**20.03±1.23[b]**	**41.01±2.14[a,b,c]**
HOMA-IR	**4.18±0.39**	**11.29±1.12[a]**	**4.28±0.39[b]**	**22.13±1.45[a,b,c]**
TC (mg/dl)	**74.94±5.29**	**127.22±7.93[a]**	**76.94±5.63[b]**	**164.10±5.92[a,b,c]**
TG (mg/dl)	**43.49±5.23**	**77.30±7.91[a]**	**47.53±3.92[b]**	**108.72±8.55[a,b,c]**
HDL (mg/dl)	**43.12±3.06**	**34.10±3.54[a]**	**43.13±2.95[b]**	**26.65±2.39[a,b,c]**
LDL (mg/dl)	**23.12±4.87**	**77.66±8.26[a]**	**24.31±7.42[b]**	**115.71±6.78[a,b,c]**
VLDL (mg/dl)	**8.70±1.05**	**15.46±1.58[a]**	**9.51±0.78[b]**	**21.74±1.71[a,b,c]**
ALT (U/L)	**39.37±3.70**	**86.90±7.07[a]**	**40.33±4.66[b]**	**130.19±6.39[a,b,c]**
AST (U/L)	**145.48±7.37**	**161.09±6.33[a]**	**142.96±4.52[b]**	**184.13±7.72[a,b,c]**
CRP (mg/L)	**0.034±0.01**	**0.084±0.01[a]**	**0.038±0.01[b]**	**0.116±0.01[a,b,c]**

([a]): significant versus group IA, ([b]): significant versus group IIA, ([c]): significant versus group IB.

Table (2): Correlation of serum levels of nesfatin-1 with the measured parameters in all studied groups.

Groups / Parameters	Group IA	Group IIA	Group IB	Group IIB
	r	**r**	**r**	**r**
Final BW (g)	0.506	0.790**	0.238	0.942***
Final BMI (gm/cm^2)	0.060	0.817**	0.116	0.789**
Final AC (cm)	0.132	0.838**	0.067	0.687*
Glucose (mg/dl)	0.440	0.675*	0.135	0.927***
Insulin (µIU/ml)	0.316	0.644*	0.322	0.936***
HOMA-IR	0.179	0.739**	0.029	0.849***
TC (mg/dl)	0.106	0.754**	0.394	0.781**
TG (mg/dl)	0.167	0.696*	0.238	0.665*
HDL (mg/dl)	-0.407	-0.704*	-0.104	-0.917***
LDL (mg/dl)	0.188	0.897***	0.368	0.823**
VLDL (mg/dl)	0.414	0.735**	0.239	0.821**
ALT (U/L)	0.203	0.820**	0.522	0.834**
AST (U/L)	0.079	0.811**	0.428	0.784**
CRP (mg/L)	0.463	0.700*	0.238	0.832**

(r): correlation versus nesfatin-1, (*): significant ($P<0.05$), (**): significant ($P<0.01$), (***): significant ($P<0.001$).

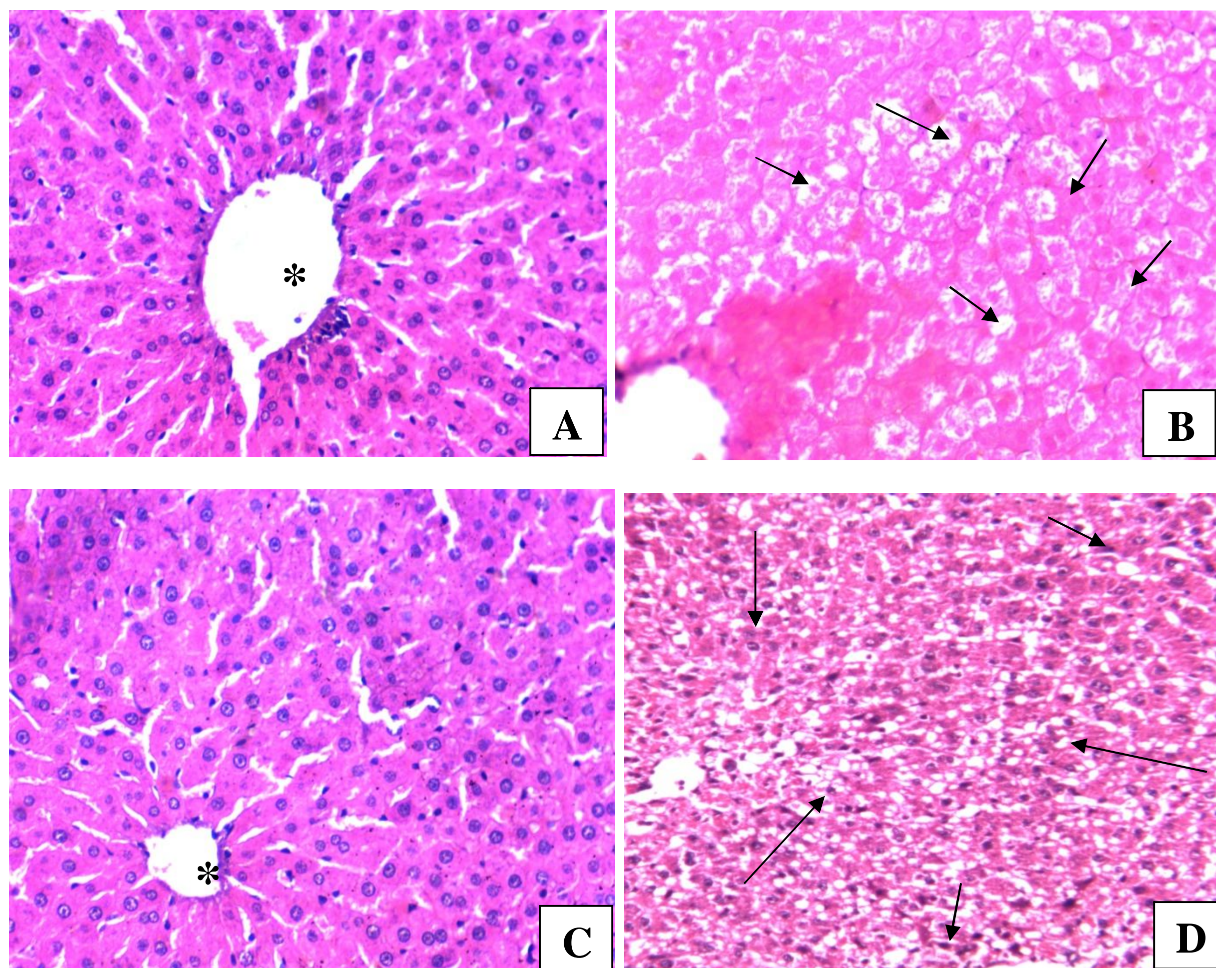

Figure (1): Photomicrographs of isolated rat liver tissues stained with Hematoxylin and Eosin: (**A**): Normal liver tissues formed of normal-sized central vein (*) surrounded by rows and cords of normal hepatocytes with central nuclei and abundant eosinophilic cytoplasm in group IA. (**B**): Marked fatty change of hepatocytes (↑) with central nuclei and clear cytoplasm in group IIA indicating steatosis. (**C**): Normal liver tissue with normal-sized central vein (*) surrounded by normal hepatocytes with central nuclei and abundant eosinophilic cytoplasm in groups IB. (**D**): Hepatocytes with fat infiltration and clear cytoplasm surrounded by aggregates of chronic inflammatory cells (↑) in group IIB.
(x 400).

DISCUSSION

In the present study, the induction of obesity model in rats using HFD for 4 and 12-week duration was confirmed by the significant progressive rise in the final body weight, BMI, HOMA-IR index and serum levels of glucose, insulin and lipid profile parameters except HDL that significantly decreased in HFD groups compared with control groups. These findings involved many signs of the metabolic syndrome specially insulin resistance (IR), type II diabetes mellitus (DM) and dyslipidemia **(Han and Lean, 2016)** and are consistent with those of other studies **(Eisinger et al., 2014 and Yu et al., 2014).**

In addition, the successful induction of NAFLD rat model in the present study was established by the histopathological examination of isolated liver tissues that revealed fatty infiltration in group IIA indicating simple steatosis and chronic inflammatory cell infiltration in group IIB indicating steatohepatitis. These findings were accompanied by a significant time-dependant increase in serum levels of ALT and AST in HFD groups. Consistent with our findings, several studies reported the successful induction of hepatic steatosis and steatohepatitis in rats using HFD **(Freitas et al., 2016 and Wu et al., 2016)**.

The occurrence of hepatic steatosis in our study can be explained by the down regulating effect of HFD on hepatic LDL receptors which resulted in decreased hepatic LDL clearance, prolongation of plasma half-life of VLDL and LDL and consequently steatosis **(Bieghs et al., 2012)**. Another explanation may be through the occurrence of IR which was established in the present study based on finding a significant rise in glucose and insulin serum levels and HOMA-IR index. Peripheral IR was reported to induce hepatic steatosis through decreasing the suppressing effect of insulin on glucose production in the liver which cause deterioration of peripheral IR and initiate lipogenesis in the liver **(Gastaldelli et al., 2007)**. Moreover, IR was found to inhibit β-oxidation of free fatty acids leading to accumulation of hepatic lipids **(Postic and Girard, 2008)**. Furthermore, the anti-lipolytic action of insulin in adipose tissue was reported to be impaired by IR leading to an increased release of free fatty acids, which disturbs lipid metabolism and consequently may induce steatosis **(Gaggini et al., 2013)**. The increase of plasma free fatty acids levels as a consequence of obesity or HFD-feeding was found to induce IR and low-grade inflammation **(Mantzaris et al., 2011).** Additionally, the in-vivo studies showed that saturated fatty acids participate in generation of IR, hepatic steatosis and activation of pro-inflammatory M1 macrophages through activation of the c-Jun terminal kinase (JNK) **(Gadang et al., 2013)**. Other in vitro studies demonstrated that palmitate triggered oxidative stress in hepatocytes endoplasmatic reticulum **(Leamy et al., 2014)** and activated macrophages inducing inflammation **(Snodgrass et al., 2013)**. Also, ceramides, which are synthesized from long-chain saturated fatty acids in the endoplasmatic reticulum of hepatocytes, were reported to be involved in hepatic insulin resistance and to have lipotoxic effect on pancreatic cells **(Ussher et al., 2010)**. Furthermore, it was demonstrated that insulin triggers de novo lipogenesis and glyceroneogenesis pathways **(Saponaro et al., 2015)** which were found to be increased in NAFLD contributing to the development of hepatic steatosis **(Hyotylainen et al., 2016)**.

Compared with control groups, the present study demonstrated a significant rise in serum nesfatin-1 levels in HFD groups that increased with the increase in the duration of HFD in male albino rats. Additionally, positive significant correlations were found between serum levels of nesfatin-1 and all measured parameters with the exception of HDL which showed negative correlation in both HFD groups. In agreement with our results, the study by **Wu et al. (2016)** demonstrated increased plasma nesfatin-1 levels in NAFLD model induced by HFD

for 4 weeks in rats. On the contrary to our findings, it was reported that serum levels of nesfatin-1 in patients with NAFLD significantly reduced in comparison to healthy controls, significantly reduced in obese subjects compared with non-obese subjects, and significantly reduced in insulin-resistant subjects compared with insulin-sensitive ones (**Basar et al., 2012**).

The present study could not determine the precise reason underlying the increased nesfatin-1 levels in NAFLD rat model. Nesfatin-1 was considered as anorexigenic factor that regulates food intake and gastrointestinal functions (**Stengel & Taché, 2011 and Stengel et al., 2011**). **Stengel et al. (2009)** reported the increase in circulating and gastric nesfatin-1 after reducing food intake. Another study revealed that food intake was restricted when nesfatin-1 was injected intraperitoneally (**Shimizu et al., 2009**). Moreover, **Atsuchi et al. (2010)** reported that central nesfatin-1 administration reduced food intake. Also, the study by **Garc?a-Galiano et al. (2010)** demonstrated that intracerebro-ventricular injection of nesfatin-1 caused dose-dependent decrease in food intake in adult rats and the long-standing administration of nesfatin-1 led to decrease in body weight. Therefore, the primary stimulus for the increase in nesfatin-1 levels in our study may be feeding the rats with high-fat diet.

Our results revealed that nesfatin-1 correlated positively with BMI in both HFD groups. This finding was consistent with the results of other studies (**Saldanha et al., 2012 and Zhang et al., 2012**). In contrast, other studies found that nesfatin-1 correlated negatively with BMI (**Tsuchiya et al., 2010 and Basar et al., 2012).**

The current study found that nesfatin-1 correlated positively with glucose, insulin and HOMA-IR index in both HFD groups. Consistent with our findings, **Zhang et al. (2012)** reported the increase in plasma levels of nesfatin-1 in patients with type II DM. Additionally, the studies by **Foo et al. (2010)** and **Gonzalez et al. (2011a)** revealed the expression of nesfatin-1 in rat islet beta cells of pancreas and reported the stimulation of its secretion by high glucose in vitro. Furthermore, it was found that nesfatin-1 improved insulin secretion stimulated by glucose in mouse and rat pancreatic β cells (**Gonzalez et al., 2011b; Nakata et al., 2011 and Gonzalez et al., 2012**). Moreover, **Li et al. (2012)** found that basal nesfatin-1 levels significantly increased by intravenous infusion of glucose in healthy adults. Therefore, the present study suggested that hyperglycemia may be another explanation for the increased nesfatin-1 levels in rat model of NAFLD induced by HFD. In contrast to the previous reports, another study reported that nesfatin-1 correlated negatively with fasting blood glucose and insulin resistance (**Basar et al., 2012).**

Our results revealed that nesfatin-1 levels correlated positively with liver enzymes (ALT and AST) and the inflammatory marker (CRP) in both HFD groups. Conversely, the study by **Basar et al. (2012)** showed that nesfatin-1 did not correlate with liver enzymes in patients with NAFLD. Our study suggested that the association of increased nesfatin-1 levels with NAFLD may be a compensa-

tion for the disturbed glucose and lipid metabolism in rat model of NAFLD. This hypothesis was supported by the study of **Su et al. (2009)** which demonstrated that administration of nesfatin-1 to hyperglycemic rats produced anti-hyperglycemic effect that was attributed to its inhibitory effect on hepatic production of glucose by modulating glycogen synthesis and gluconeogenesis. Additionally, it was found that nesfatin-1 inhibited glucose production in the liver when injected centrally by diminishing the production of the phosphoenolpyruvate carboxykinase enzyme (**Yang et al., 2012).**

The present study also, suggested that the increased nesfatin-1 levels in NAFLD rat model might be a compensation for the inflammation present in steatohepatitis through its anti-apoptotic and anti-inflammatory properties. Consistent with this suggestion, it was reported that nesfatin-1 inhibited neutrophil infiltration and inflammatory responses that depend on nuclear factor kappa-B and reducing neuronal cell apoptosis mediated by caspase-3 after traumatic brain injury in rats (**O¨zsavci et al., 2011 and Tang et al., 2012**).

CONCLUSION

The present study revealed that feeding rats with HFD for 4 and 12 consecutive weeks could successfully induce steatosis and steatohepatitis, respectively in rats that was indicated by obesity, disturbed glucose and lipid metabolism and liver dysfunction. Also, our results demonstrated a progressive time-dependent increase in serum levels of nesfatin-1 that correlated positively with the anthropometric parameters, glycemic control parameters, liver enzymes, CRP and lipid profile parameters, apart from HDL that correlated negatively with nesfatin-1 in both HFD groups. The increase in serum nesfatin-1 levels might be attributed to feeding rats with HFD or hyperglycemia or may be a compensatory mechanism to improve the metabolic and liver dysfunction through its anorexigenic, anti-hyperglycemic and anti-inflammatory effects. Further studies are required to elucidate nesfatin-1 role in the pathogenesis of NAFLD and to explore the potential therapeutic role of nesfatin-1 in NAFLD.

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REFERENCES

1. **Altunkaynak Z (2005):** Effects of high fat diet induced obesity on female rat livers (A histochemical study). Eur J Gen Med., 2(3): 100-109.

2. **Atsuchi K, Asakawa A, Ushikai M, Ataka K, Tsai M, Koyama K, Sato Y, Kato I, Fujimiya M and Inui A (2010):** Centrally administered nesfatin-1 inhibits feeding behaviour and gastroduodenal motility in mice. Neuroreport., 27;21:1008–1011.

3. **Basar ? , Akbal E, K?klü S, Koçak E, Tuna Y, Ekiz F, Gültuna S, Ilmaz FM and Aydog?an T (2012):** A novel appetite peptide, nesfatin-1 in patients with non-alcoholic fatty liver disease. Scand Clin Lab Invest., 72: 479–483.

4. **Bieghs V, Van Gorp PJ, Wouters K, Hendrikx T, Gijbels MJ, van Bilsen M, Bakker J, Binder CJ, Lütjohann D, Staels B, Hofker MH and Shiri-Sverdlov R (2012):** LDL receptor knock-out mice are a physiological model particularly vulnerable to study the onset of inflammation in non-

alcoholic fatty liver disease. PLoS One, 7: e30668.

5. **Dunn W, Xu R, Wingard DL, Rogers C, Angulo P, Younossi ZM and Schwimmer JB (2008):** Suspected nonalcoholic fatty liver disease and mortality risk in a population-based cohort study. Am J gastroenterol., 9: 2263–2271.

6. **Eisinger K, Liebisch G, Schmitz G, Aslanidis C, Krautbauer A and Buechler C (2014):** Lipidomic Analysis of Serum from High Fat Diet Induced Obese Mice. Int J Mol Sci., 15(2): 2991–3002.

7. **Foo KS, Brauner H, Ostenson CG and Broberger C (2010):** Nucleobindin-2/nesfatin in the endocrine pancreas: distribution and relationship to glycaemic state. J Endocrinol., 204: 255-263.

8. **Freitas RB, Novaes RD, Gonçalves RV, Mendonça BG, Santos EC, Ribeiro AQ, Lima LM, Fietto LG, Peluzio Mdo C and Leite JP (2016):** Euterpe edulis Extract but Not Oil Enhances Antioxidant Defenses and Protects against Nonalcoholic Fatty Liver Disease Induced by a High-Fat Diet in Rats. Oxid Med Cell Longev., 2016: 8173876.

9. **Friedwald WT, Levy RI and Fredrickson DS (1972):** Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem., 18: 499-502.

10. **Gadang V, Kohli R, Myronovych A, Hui DY, Perez-Tilve D and Jaeschke A (2013):** MLK3 promotes metabolic dysfunction induced by saturated fatty acid-enriched diet. Am J Physiol Endocrinol Metab., 305: E549–E556.

11. **Gaggini M, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E and Gastaldelli A (2013):** Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. Nutrients, 10; 5(5): 1544-1560.

12. **Garc?a-Galiano D, Navarro VM, Roa J, Ruiz-Pino F, S?nchez-Garrido MA, Pineda R, Castellano JM, Romero M, Aguilar E, Gayt?n F, Diéguez C, Pinilla L and Tena-Sempere M (2010):** The Anorexigenic Neuropeptide, Nesfatin-1, Is Indispensable for Normal Puberty Onset in the Female Rat. J Neurosci., 30 (23): 7783-7792.

13. **Gastaldelli A, Cusi K, Pettiti M, Hardies J, Miyazaki Y, Berria R, Buzzigoli E, Sironi AM, Cersosimo E, Ferrannini E and Defronzo RA (2007):** Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. Gastroenterology, 133, 496–506.

14. **Gerbaix M, Metz L, Ringot E and Courteix D (2010):** Visceral fat mass determination in rodent: validation of dual energy X-ray absorptiometry and anthropometric techniques in fat and lean rats. Lipids Health Dis., 9:140.

15. **Gonzalez R, Perry RL, Gao X, Gaidhu MP, Tsushima RG, Ceddia RB and Unniappan S (2011a):** Nutrient responsive nesfatin-1 regulates energy balance and induces glucose-stimulated insulin secretion in rats. Endocrinol., 152: 3628-3637.

16. **Gonzalez R, Reingold BK, Gao X, Gaidhu MP, Tsushima RG and Unniappan S (2011b)**: Nesfatin-1 exerts a direct, glucose-dependent insulinotropic action on mouse islet beta- and MIN6 cells. Endocrinol., 208: 9-16.

17. **Gonzalez R, Shepperd E, Thiruppugazh V, Lohan S, Grey C, Chang JP and Unniappan S (2012):** Nesfatin-1 Regulates the Hypothalamo-Pituitary-Ovarian Axis of Fish. Biol. Reprod., 4: 87-84.

18. **Han TS and Lean EJ (2016):** A clinical perspective of obesity, metabolic syndrome and cardiovascular disease. JRSM Cardiovasc Dis., 5: 1-13.

19. **Hyotylainen T, Jerby L, Petaja EM, Mattila I, Jantti S, Auvinen P, Gastaldelli A, Yki-Jarvinen H, Ruppin E and Oresic M (2016):** Genome-scale study reveals reduced metabolic adaptability in patients with non-alcoholic fatty liver disease. Nat Commun., 7: 8994.

20. **Katsiki N, Mikhailidis DP and Mantzoros CS (2016):** Non-alcoholic fatty liver disease and dyslipidemia: an update. Metabolism, 65: 1109 ‑ 1123.

21. **Kimberly MM, Vesper HW, Caudill SP, Cooper GR, Rifai N, Dati F and Myers GL (2003):** Standardization of immunoassay for

measurement of high-sensitivity C reactive protein phase 1: Evaluation of secondary reference materials. Clin Chem., 49: 611–616.

22. **Leamy AK, Egnatchik RA, Shiota M, Ivanova PT, Myers DS, Brown HA and Young JD (2014):** Enhanced synthesis of saturated phospholipids is associated with ER stress and lipotoxicity in palmitate treated hepatic cells. J Lipid Res., 55: 1478–1488.

23. **Li Z, Xu G, Li Y, Zhao J, Mulholland MW and Zhang W (2012):** mTOR-dependent modulation of gastric nesfatin-1/ NUCB2. Cell Physiol Biochem., 29: 493-500.

24. **L?pez-Vel?zquez JA, Silva-Vidal KV, Ponciano-Rodr?guez G, Ch?vez-Tapia NC, Arrese M, Uribe M and Méndez-S?nchez N (2014):** The prevalence of nonalcoholic fatty liver disease in the Americas. Ann Hepatol., 13(2): 166-178.

25. **Mantzaris MD, Tsianos EV and Galaris D (2011):** Interruption of triacylglycerol synthesis in the endoplasmic reticulum is the initiating event for saturated fatty acid-induced lipotoxicity in liver cells. FEBS J., 278: 519–530.

26. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC (1985):** Homeostasis model assessment: insulin resistance and ?-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, 28: 412-419.

27. **Naito HK (1989):** Triglycerides in clinical chemistry: theory, analysis and correlation. Second edition by Kaplan LA and Pesce AJ. (U.S.A.), P. 997.

28. **Nakata M, Manaka K, Yamamoto S, Mori M and Yada T (2011)**: Nesfatin-1 enhances glucose-induced insulin secretion by promoting Ca^{2+} influx through L-type channels in mouse islet beta-cells. Endocr J., 58: 305-313.

29. **Nauk M, Marz W and Jarausch J (1997):** Multicenter evaluation of homogenous assay for HDL-Cholesterol without sample pretreatment. Clin Chem., 43(9): 1622-1629.

30. **Novelli E, Diniz Y, Galhardi C, Ebaid G, Rodrigues H, Mani F, Fernandes A, Cicogna A and Novelli Filho J (2007):** Anthropometrical parameters and markers of obesity in rats. Lab Anim., 41: 111–119.

31. **? zsavc? D, Er¸sahin M, S¸ener A, ? zakpinar ? B, Toklu HZ, Akak ?n D, S¸ener G ? zsavc? D, Er¸sahin M, S¸ener A, ? zakpinar ? B, Toklu HZ, Akak ?n D, S¸ener G and Ye g?en BC (2011):** The novel function of nesfatin-1 as an anti-inflammatory and antiapoptotic peptide in subarachnoid hemorrhage-induced oxidative brain damage in rats. Neurosurgery, 68: 1699 –1708.

32. **Postic C and Girard J (2008):** Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. J Clin Invest., 118: 829–838.

33. **Rec JS (1970):** Estimation of serum ALT. J Clin Biochem., 8: 658-662.

34. **Saldanha JF, Carrero JJ, Lobo JC, Stockler-Pinto MB, Leal VO, Calixto A, Geloneze B and Mafra D (2012):** The newly identifi ed anorexigenic adipokine nesfatin-1 in hemodialysis patients: are there associations with food intake, body composition and inflammation? Regul Pept., 173:82 – 85.

35. **Saponaro C, Gaggini M, Carli F and Gastaldelli A (2015):** The subtle balance between lipolysis and lipogenesis: A critical point in metabolic homeostasis. Nutrients, 7: 9453–9474.

36. **Shimizu H, Oh-I S, Hashimoto K, Nakata M, Yamamoto S, Yoshida N, Eguchi H, Kato I, Inoue K, Satoh T, Okada S, Yamada M, Yada T and Mori M (2009):** Peripheral administration of nesfatin-1 reduces food intake in mice: the leptin independent mechanism. Endocrinol., 150: 662–671.

37. **Shimizu H and Osaki A (2013):** Nesfatin/nucleobindin-2 (NUCB2) and glucose homeostasis. Curr Hypertens Rev., 9: 270-273.

38. **Snodgrass RG, Huang S, Choi IW, Rutledge JC and Hwang DH (2013):** Inflammasome-mediated secretion of IL-1β in human monocytes through TLR2 activation; modulation by dietary fatty acids. J Immunol., 191: 4337–4347.

39. **Soderberg C, St?l P, Askling J, Glaumann H, Lindberg G, Marmur J, Hultcrantz R**

(2010): Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. Hepatology, 2: 595–602.

40. **Stengel A, Goebel M and Taché Y (2011):** Nesfatin-1: a novel inhibitory regulator of food intake and body weight. Obes Rev., 12: 261–271.

41. **Stengel A, Goebel M, Yakubov I, Wang L, Witcher D, Coskun T, Taché Y, Sachs G and Lambrecht NW (2009):** Identification and characterization of nesfatin-1 immunoreactivity in endocrine cell types of the rat gastric oxyntic mucosa. Endocrinol., 150: 232 – 238.

42. **Stengel A and Taché Y (2010):** Nesfatin-1–role as possible new potent regulator of food intake. Regul Pept., 163: 18 – 23.

43. **Stengel A and Taché Y (2011):** Minireview: nesfatin-1--an emerging new player in the brain-gut, endocrine, and metabolic axis. Endocrinol., 152: 4033 – 4038.

44. **Su Y, Zhang J, Tang Y, Bi F and Liu JN (2009):** The novel function of nesfatin-1: anti-hyperglycemia. Biochem Biophys Res Commun., 391: 1039–1042.

45. **Tang CH, Fu XJ, Xu XL, Wei XJ and Pan HS (2012)**: The anti-inflammatory and anti-apoptotic effects of nesfatin-1 in the traumatic rat brain. Peptides, 36: 39-45.

46. **Targher G, Byrne CD, Lonardo A, Zoppini G and Barbui C (2016):** Nonalcoholic fatty liver disease and risk of incident cardiovascular disease: a metaanalysis. J Hepatol., 65: 589-600.

47. **Temple RC, Clark PM and Hales CN (1992):** Measurement of insulin secretion in type 2 diabetes: problems and pitfalls. Diabet Med., 9: 503-512.

48. **Tietz NW (1995):** Clinical guide to laboratory tests, 3rd ED., W.B. Saunders, Co., Philadelphia, pp. 509-512.

49. **Tsuchiya T, Shimizu H, Yamada M, Osaki A, Oh-I S, Ariyama Y, Takahashi H, Okada S, Hashimoto K, Satoh T, Kojima M and Mori M (2010):** Fasting concentrations of nesfatin-1 are negatively correlated with body mass index in non-obese males. Clin Endocrinol [Oxf]., 73: 484–490.

50. **Ussher JR, Koves TR, Cadete VJ, Zhang L, Jaswal JS, Swyrd SJ, Lopaschuk DG, Proctor SD, Keung W, Muoio DM and Lopaschuk GD (2010):** Inhibition of de novo ceramide synthesis reverses diet-induced insulin resistance and enhances whole-body oxygen consumption. Diabetes, 59: 2453–2464.

51. **Warnick GR, Benderson V and Albers N (1983):** Selected methods. Clin Chem., 10: 91-99.

52. **Wu R, Chen Z, Xu Y, Ge J and Lü X (2016):** Change in plasma nesfatin-1 concentration within high-fat diet induced nonalcoholic fatty liver disease rat models. Wei Sheng Yan Jiu., 45(3): 452-457.

53. **Yang M, Zhang Z, Wang C, Li K, Li S, Boden G, Li L and Yang G (2012):** Nesfatin-1 action in the brain increases insulin sensitivity through Akt/AMPK/TORC2 pathway in diet-induced insulin resistance. Diabetes, 61: 1959–1968.

54. **Yu RQ, Wu XY, Zhou X, Zhu J and Ma LY (2014):** Cyanidin-3-glucoside attenuates body weight gain, serum lipid concentrations and insulin resistance in high-fat diet-induced obese rats. Zhongguo Dang Dai Er Ke Za Zhi., 16(5): 534-538.

55. **Zhang X, Yang J, Guo Y, Ye H, Yu C, Xu C, Xu L, Wu S, Sun W, Wei H, Gao X, Zhu Y, Qian X, Jiang Y, Li Y and He F (2010):** Functional proteomic analysis of nonalcoholic fatty liver disease in rat models: enoyl-coenzyme a hydratase down-regulation exacerbates hepatic steatosis. Hepatology, 51(4): 1190-1199.

56. **Zhang Z, Li L, Yang M, Liu H, Boden G and Yang G (2012):** Increased plasma levels of nesfatin-1 in patients with newly diagnosed type 2 diabetes mellitus. Exp Clin Endocrinol Diabet., 120: 91-95.

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خلفية البحث: يعتبر مرض الكبد الدهني غير الكحولي من اضطرابات الكبد الشائعة والذى يتميز بتراكم الدهون فى الكبد، ويرتبط غالبا مع السمنة ومقاومة الإنسولين، ولكن آلية حدوث المرض لا تزال غير واضحة حتى الآن. أما النسفاتين-1 فيعتبر من عديدات الببتيد التى تلعب دورا في تنظيم معدل تناول الغذاء ومستوى الجلوكوز فى الدم. ولا تزال العلاقة بين النسفاتين-1 ومرض الكبد الدهني غير الكحولي مثارا للجدل.

الهدف من البحث: أجريت هذه الدراسة لتقييم مستويات النسفاتين -1 في مصل دم ذكور الجرذان البيضاء البالغة المحدث بها نموذج مرض الكبد الدهنى غير الكحولى بإستخدام غذاء عالى الدهن.

مواد وطرق البحث: أجريت هذه الدراسة على 48 من ذكور الجرذان البيضاء البالغة والتى قسمت كما يلى:

- مجموعتين ضابطتين (IA and IB): (12 جرذاً لكل منهما)؛ حيث تم تغذية الجرذان بالغذاء المعتاد لمدة 4 أسابيع و12 أسبوعا، على التوالي.
- مجموعتين مغذيتين بغذاء عالى الدهن (IIA and IIB): (12 جرذاً لكل منهما)؛ حيث تم تغذية الجرذان بغذاء عالى الدهن لمدة 4 أسابيع و12 أسبوعا، على التوالي.

وقد تم قياس وزن ومحيط الجسم، ومستويات النسفاتين -1، والإنسولين، والجلوكوز، ومعامل الإلتهاب، ومعاملات مستوى الدهون، وانزيمات الكبد فى مصل دم الجرذان فى كل المجموعات، وتم حساب مؤشر كتلة الجسم ومعامل مقاومة الإنسولين، وتم إجراء فحص مجهري لأنسجة الكبد المعزولة.

النتائج: أسفرت الدراسة عن النتائج التالية:

حدوث تشحم للكبد بعد 4 أسابيع وإلتهاب كبدى دهنى بعد 12 أسبوعا من تغذية الجرذان بغذاء عالى الدهن، وتم إثبات ذلك من خلال الفحص الخلوى لأنسجة الكبد، والإرتفاع التدريجي لإنزيمات الكبد. أيضا، وحدوث زيادة تدريجية ذات دلالة إحصائية في مؤشر كتلة الجسم، ومعامل مقاومة الإنسولين، ومستويات النسفاتين -1، والجلوكوز، والإنسولين، و ومعامل الإلتهاب، ومعاملات مستوي

الدهون بإستثناء البروتينات الدهنية عالية الكثافة والتى أظهرت إنخفاضا ذا دلالة إحصائية فى مصل دم الجرذان فى المجموعتين التى تم تغذيتهما بغذاء عالى الدهن بالمقارنة بالمجموعتين الضابطتين. علاوة على ذلك، فقد كان هناك إرتباطا إيجابيا ذا دلالة إحصائية بين مستويات النسفاتين-1 وجميع القياسات السابقة في المجموعتين التى تم تغذيتهما بغذاء عالى الدهن بإستثناء البروتينات الدهنية عالية الكثافة حيث إرتبطت إرتباطا عكسيا ذا دلالة إحصائية بالنسفانتين-1.

الإستنتاج: نستخلص من هذه الدراسة أن هناك زيادة فى مستويات النسفاتين -1 في مصل دم الجرذان المحدث بها تجريبيا نموذج مرض الكبد الدهنى الغير كحولى، وهذه الزيادة ربما تعزى إلى تغذية الجرذان بالغذاء عالى الدهن، أو ربما إلى زيادة مستوى الجلوكوز فى الدم، أو من الممكن أن تكون لتعويض الإلتهاب والتمثيل الغذائي المضطرب.