EFFECTS OF -3 POLYUNSATURATED FATTY ACIDS SUPPLEMENTATION ON LIVER FUNCTION AND SERUM APELIN-13 IN TYPE II DIABETIC RATS

By

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ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) is a chronic liver condition complicating type II diabetes. Insulin resistance (IR) plays a vital role in the pathogenesis of fatty liver in diabetes mellitus. Interestingly, both ω-3 polyunsaturated fatty acids (ω-3 PUFA) and apelin-13 have controversial relationship to IR and liver function. Objective: To demonstrate the effect of ω-3 PUFA on serum apelin-13 and its association to liver function in type II diabetic rats. Material and methods: This study was conducted on 40 of adult male albino rats divided into 3 groups: Control group (A) in which rats fed normal chow, type II diabetic group (B) in which type II diabetes was induced by feeding the rats HFD for 2 weeks followed by a single intraperitoneal injection of streptozotocin (35 mg/kg BW), and type II diabetic treated group (C) in which rats treated with ω-3 PUFA (500mg/kg/day; orally) for 4 weeks after induction of diabetes. Results: There was a significant elevation in serum apelin-13, glucose, HOMA-IR, ALT, AST, plasma prothrombin and fibrinogen accompanied by significant decrease in serum insulin and albumin in group (B) when compared with control group. Treatment with PUFA in group (C) improved glucolipid metabolic parameters with significant reduction in serum apelin-13, ALT and AST. Linear regression analysis test showed that apelin-13 has no predictive value to the histological changes of liver injury in group B. Conclusion: Treatment of diabetic rats with ω-3 PUFA improved insulin resistance, liver enzymes and decreased serum apelin-13 level. However, apelin-13 cannot be used as a non invasive laboratory marker to distinguish the severity of liver injury in type II diabetic rats.

Key words: Apelin, insulin resistance, fatty liver.

INTRODUCTION

Diabetes mellitus type II (DM II) is considered as one of the most important health problems worldwide (Siddiqui et al., 2013). The incidence of DM II is reaching epidemic levels worldwide which could be attributed to the rising in obesity rates and sedentary lifestyle. By 2030, it is predicted that 439 million adults will have diabetes mellitus (Arnason et al., 2017).

NAFLD is one of the most common disorders that its prevalence has been increasing worldwide (Younossi et al., 2011). Despite its high prevalence, the pathogenesis of NAFLD is not yet fully understood. It could be attributed to its pathophysiologic links to obesity, insulin
resistance (IR) and DM II (Stefan and H?ring, 2011).

Interestingly, obesity and DM II are the most common causes of the IR state due to expansion of body fat stores with caloric excess, alterations in lipid metabolism together with inflammation in adipose tissue and ectopic sites of fat deposition leading to post-receptor abnormalities in insulin signaling pathways (Birkenfeld and Shulman, 2014).

Moreover, disturbances in adipokine profiles have also been associated with inadequate lipid control (Kaplon-Cieślicka et al., 2015). It was suggested to participate in the development of systemic low-grade inflammation, IR and metabolic syndrome (MS) (Dunmore and Brown, 2013).

Apelin, an adipocytokine, is synthesized as a 77 amino acid prepropeptide that is cleaved into several active isoforms, with apelin-13 is the final active product, the most potent isoform, and more resistant to enzymatic cleavage (Maguire et al., 2009).

It is widely expressed in heart, brain, lung, kidney, the gastrointestinal tract, pancreatic cells, endothelial cells and hepatic parenchymal, Kupffer and stellate (Kwon et al., 2013), exerting a large number of physiological actions; including regulation of fluid homeostasis, cardiovascular, immune, and gastrointestinal functions (Luan et al., 2012).

Moreover, apelin has been shown to regulate glucose homeostasis especially in skeletal muscle (Dray et al., 2008). However, whether apelin has important functions in liver disease is still under investigation (Xinrui et al., 2017).

Interestingly, data on the prevalence of NAFLD are limited by lack of an accurate non-invasive screening tool (Chao et al., 2016). Also, scoring systems based on laboratory parameters are insufficient to distinguish between the different stages of the disease (Duvnjak et al., 2007).

Additionally, multiple studies have shown beneficial effects of omega-3 polyunsaturated fatty acids (ω-3 PUFA), the essential fatty acids α-linolenic acid and eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) on human lipid metabolism, insulin sensitivity and inflammatory response in obesity (Ellulu et al., 2015 and Biniaa et al., 2017).

Because of the association between IR and pathophysiology of liver injury in DM II, therapeutic interventions aiming at improving insulin sensitivity may be a promising approach to treat this injury (Verrijken et al., 2011).

However, data supporting the impact of ω-3 PUFA on adipokine profile, glucose homeostasis, insulin sensitivity, and lipid metabolism in DM II are still limited and inconsistent (Poreba et al., 2017).

Since ω-3 PUFA and apelin share common metabolic effects, the aim of this work was to test the effects of ω-3 PUFA treatment on liver function and serum apelin-13 level, the relationship between serum apelin-13 and liver function, and the possibility of using its levels as a predictor of histological grades of liver injury in rat model of DM II.
MATERIALS AND METHODS

A total number of 30 healthy adult male wistar albino rats weighing 185-220 g were obtained from the animal house of Faculty of Veterinary Medicine- Zagazig University. Animals were kept in steel cages (40cm x 28cm x 18cm - 3 rats/ cage). They were housed at room temperature in natural dark/light cycle and received food and water ad libitum. After one week of acclimation, the animals were divided into 2 groups:

- Group (A) Control group (n=10): In which rats fed normal chow (18% protein, 77% carbohydrate and 5% fat; 12.6 kJ/g) (Faculty of Agriculture-Zagazig University) all through the study duration (6 weeks).

Diabetic type II group (n=30): In which DM II was induced by feeding the rats high fat diet (HFD) (58% fat, 25% protein and 17% carbohydrate; 23.4 kJ/g). On day 14, rats were given a single intraperitoneal injection of streptozotocin (STZ) (Sigma-Aldrich, USA) (35 mg/kg) in citrate buffer, pH 4, while control rats were injected with a corresponding volume of citrate buffer.

Rats with persistent blood glucose levels higher than 250 mg/dl after STZ administration were considered diabetic rats (Srinivasan et al., 2005) and included in the study. Diabetic rats were kept on HFD feeding all through the study and subdivided into 2 equal subgroups:

- Group (B) non treated DM II group.
- Group (C) treated by ω-3 PUFA (500 mg/kg body weight/orally by gavage) (SEDICO- Egypt) for further 4 weeks (Ghadge et al., 2016).

Saline was administered to the control and diabetic non treated groups.

Body weights of all animals were monitored weekly.

Calculating BMI index: It equals body weight (g) / length\(^2\) (cm\(^2\)). This index can be used as an indicator of obesity where the cutoff value of obesity BMI is more than 0.68 g/cm\(^2\) (Novelli et al., 2007).

A. Blood collection: Blood samples (6 ml/rat) were obtained at the end of the experimental period after an overnight fasting from sinus orbitus vein of each rat after ether inhalation. Two ml of the blood was collected in a plastic centrifuge tube containing 3.2% sodium citrate solution (0.1 ml/0.9 ml blood). Plasma was separated by centrifugation of blood immediately at 1258 r.p.m for 10 min. and used for determination of prothrombin and fibrinogen levels. The remaining amount was allowed to clot at room temperature before centrifuging at 3000 rpm for 15 minutes. The serum was stored at -20\(^\circ\) C.

Calculation of homeostasis model assessment of insulin resistance (HOMA-IR): The following equation was used; (insulin (U/mL) x glucose (mg/dl) /405 - Sun et al., 2007). 4. Serum total cholesterol (TC) level: Using rat cholesterol ELISA kit (BioSource Europe S.A.-Rue de l’Industrie, Nivelles-Belgium; 8-B- 1400 - Tietz et al., 1995)

5. Serum triglycerides (TG) level: Using rat triglycerides ELISA kit (BioSource Europe S.A.-Rue de l’Industrie, Nivelles-Belgium; 8-C- 1150 - Fossati, 1982).

6. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were assayed Spectrophotometrically using rat ALT & AST ELISA kit (ABCAM, USA; ab105134 and SIGMA-Aldich, USA; MAK055-1KT; respectively - Vassault, 1983).

7. Serum Albumin by spectrophotometric techniques using albumin kits (Spinreact de Mexico, S.A. de C.V. - Ramezani et al., 2012).


C. Histopathological examination of liver:

- Livers were removed, then fixed in 10% buffered formalin solution for duration of 48h. After this, tissue samples were processed through ethyl alcohol and xylene series, and embedded in paraffine blocks. Liver specimens were sectioned (5?m thick), then stained with hematoxylin and eosin (H&E) (Altunkaynak, 2005).

- A blind expert pathologist evaluated the stained samples using Light microscope with camera attachment and scored them. The histological scoring of NAFLD followed the NAFLD Activity Score (NAS) proposed by The Pathological Committee of the NASH Clinical Research Network (Kleiner et al., 2005).

The score was composed of steatosis (0 = <5%, 1 = 5% – 33%, 2 = 34% –66%, 3 = >66%), lobular inflammation (0 = no foci, 1 = <2 foci per 200 × field, 2 = 2–4 foci per 200 × field, 3 = >4 foci per 200 × field), and ballooning (0 = none, 1 = rare or few, 2 = many or prominent). Fibrosis staging was recorded as following criteria: 0 = none, 1 = perisinusoidal or periportal fibrosis, 2 = perisinusoidal and portal/periportal fibrosis, 3 = bridging fibrosis and 4 = cirrhosis. The score of (NAS ≥ 5 was defined as NASH, 2 < NAS <5 was defined as borderline and NAS ≤ 2 was defined as simple steatosis) (Zhao et al., 2004).

Statistical analysis: The results were presented as the mean ± SD. The statistical significance of differences between groups was determined by ANOVA using SPSS version 18 for windows (StatSoft, Inc. USA) followed by post hoc test. The correlations between parameters were analyzed using Pearsons correlation. The predictive value of apelin-13, ALT and AST to liver histological changes in diabetes II group was analyzed using multiple linear regression analysis. P values <0.05 were considered to be significant.
RESULTS

A. BMI (Table1): Although control rats gradually gained weight, rats received HFD followed by STZ injection were heavier than the animals in the control group during first 2 weeks after induction of DM (group B). However, during the experiment, BMI of diabetic rats was reduced and there was a non significant difference in BMI between all groups at the end of the experiment.

B. Changes in serum glucose, insulin levels and HOMA-IR (Table 1): The fasting blood glucose concentrations and HOMA-IR of diabetic rats were significantly higher than the normal control rats. After treatment with ω-3PUFA for 4 weeks, the fasting blood glucose level was significantly lower than the diabetic group, but still significantly high when compared with those of control group.

However, the fasting blood insulin levels of diabetic rats (group B) and rats treated with ω-3PUFA (group C) were significantly lower than the normal control rats with no significant difference between both group (B) and (C). In addition, there was a non significant change in HOMA-IR in treated group when compared with control group.

C. Serum TC and TG levels were significantly higher in the diabetic groups than the normal control rats. After treatment with ω-3PUFA, these parameters significantly decreased when compared with the diabetic group. However, there was a significant increase in these parameters in the same group when compared to control group (Table 1).

Table (1): BMI, apelin and gluco-lipid metabolic parameters in all groups.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>GROUPS</th>
<th>GROUP(A) ( (n=10) )</th>
<th>GROUP(B) ( (n=10) )</th>
<th>GROUP (C) ( (n=10) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (g/cm²)</td>
<td></td>
<td>0.48±0.09</td>
<td>0.54±0.1</td>
<td>0.53±0.11</td>
</tr>
<tr>
<td>Serum glucose (mg/dl)</td>
<td></td>
<td>83.5±11.6</td>
<td>319. ±32.9*ã</td>
<td>170.2±21.2*ã</td>
</tr>
<tr>
<td>Serum insulin (µIU/ml)</td>
<td></td>
<td>19.7±2.6</td>
<td>11.5±1.4*ã</td>
<td>11.1±1.1*ã</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td></td>
<td>4.1*ã</td>
<td>9.1*ã</td>
<td>4.6*ã</td>
</tr>
<tr>
<td>TC(mg/dl)</td>
<td></td>
<td>86.3±15.2</td>
<td>187.5±11*ã</td>
<td>109.8±8.5*ã</td>
</tr>
<tr>
<td>TG(mg/dl)</td>
<td></td>
<td>61.9±5.8</td>
<td>170.4±10.8*ã</td>
<td>82.7±12.3*ã</td>
</tr>
<tr>
<td>Apelin (pg/ml)</td>
<td></td>
<td>18.4±1.7</td>
<td>39.5±1.5*ã</td>
<td>29.4±4.5*ã</td>
</tr>
</tbody>
</table>
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D. Serum ALT and AST showed significant increase in diabetic group (B) when compared with control group and diabetic group treated with ω-3PUFA. Moreover, both parameters were still significantly high in diabetic treated group when compared with control group. Additionally, plasma prothrombin and fibrinogen levels showed significant increase in both diabetic group and diabetic group treated with ω-3PUFA when compared with control group with no significant difference between both groups regarding the same parameters. However, our results showed a significant decrease in serum albumin level in both group (B) and group (C) when compared with control group. Additionally, its level was insignificantly changed after treatment with ω-3PUFA in comparison to diabetic rats (Table 2).

Table (2): Liver function tests in all groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GROUP (A) (n=10)</th>
<th>GROUP (B) (n=10)</th>
<th>GROUP (C) (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>48.3±5.9</td>
<td>142.8±10.4*</td>
<td>105.3±8.5#a</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>141.5±6.4</td>
<td>191.9±10.5*</td>
<td>153.3±5#a</td>
</tr>
<tr>
<td>Albumin (gm/dl)</td>
<td>4.6±0.7</td>
<td>2.9±0.6*</td>
<td>3±0.3*</td>
</tr>
<tr>
<td>Prothrombin (pmol/L)</td>
<td>92.5±8.1</td>
<td>135.1±8*</td>
<td>192±3.4*</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>230±10.3</td>
<td>341.6±11.3*</td>
<td>317.8±31.8*</td>
</tr>
<tr>
<td>NAS score</td>
<td>0</td>
<td>3.2±0.9*</td>
<td>1.4±0.5*</td>
</tr>
</tbody>
</table>

E. Apelin-13 (Table1) showed a significant increase in both group (B) and group (C) when compared with control group. However, its level in group (C) was significantly decreased in comparison with diabetic group (B). Interestingly, there was a significant positive correlation between serum apelin and glucose, insulin, HOMA-IR, TC and TG (Table 3). Additionally, there was a significant positive correlation between apelin and liver function tests in group (B): ALT (fig 1), AST (fig 2), albumin (fig 3), prothrombin (fig 4) and fibrinogen levels (fig 5). Furthermore, there was a significant positive correlation between apelin and NAS score in the same group (fig 6).

Table (3): Correlation coefficient between gluco-lipid metabolic parameters and serum apelin level in group (B).

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Parameters</th>
<th>Glucose</th>
<th>Insulin</th>
<th>HOMA</th>
<th>TC</th>
<th>TG</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0.797**</td>
<td>0.633*</td>
<td>0.860**</td>
<td>0.761*</td>
<td>0.914***</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>p&lt;0.01</td>
<td>p&lt;0.05</td>
<td>P&lt;0.01</td>
<td>p&lt;0.05</td>
<td>p&lt;0.001</td>
</tr>
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</table>
Liver histopathology: Type II DM could significantly induce liver histological changes in the form of diffuse micro-vesicular fatty changes in the cytoplasm in liver cells with disturbed liver architecture (liver disarray) and mononuclear inflammatory cells infiltration (fig 8) accompanied by significant elevation in NAS score when compared with control group (Table 2). However, in
rats treated with ω-3PUFA there was micro-vesicular fatty changes and a macro-vesicle with no inflammatory cells infiltration indicating improvement in the histological changes (fig 9) accompanied by significant decrease in NAS score when compared with diabetic treated group (Table 2).

Figure (7): Microscopic picture of isolated rat liver tissue in group (A) showing normal liver tissue with normal architecture, normal hepatocyte, and normal central vein (H&E, x400).

Figure (8): Microscopic picture of isolated rat liver tissue in group (B) showing [i] diffuse micro-vesicular fatty change in cytoplasm of hepatocytes with disturbed liver architecture and [ii] mononuclear cells infiltration (H&E, x400).

Figure (9): Microscopic picture of isolated rat liver tissue in group (C) showing [i] micro-vesicular fatty changes and [ii] a macro-vesicle indicating some improvement (H&E, x400).

G. Multiple regression analysis: It was applied for the data in diabetic group (group B) with NAS score as the dependent variable and apelin-13, ALT and AST levels as the independent variables, the analysis revealed a non significant association between NAS score and the mentioned independent variables (Table 4).

Table (4): Multiple linear regression among diabetic rats (group B) with NAS score the dependent variable.

<table>
<thead>
<tr>
<th></th>
<th>NAS score (β coefficient)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apelin</td>
<td>0.117</td>
</tr>
<tr>
<td>ALT</td>
<td>0.099</td>
</tr>
<tr>
<td>AST</td>
<td>0.753</td>
</tr>
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DISCUSSION

In the current study, there was a significant increase in serum blood glucose level and HOMA-IR accompanied by a significant decrease in serum insulin level in group B when compared to control group. This was in line with the findings of Zhang et al. (2008) who demonstrated feeding rats a high-fat diet promoted the development of IR and hyperglycemia, the key symptoms of prediabetic state, and also reported that injection of low-dose of STZ induces a gradual impairment of insulin secretion, which is similar to the natural progression course of DM II in humans (Liu et al., 2013).

As regard to liver function, our results showed a significant increase in serum ALT, AST, prothrombin and fibrinogen levels in the same group when compared to control which was in accordance to the results of Mathur et al. (2016) who demonstrated chronic mild elevation of transaminases are frequently found in type II diabetic patients which indicates the concentration of hepatic intracellular enzyme that has leaked into the circulation. These are used as primary markers for hepatocellular injury (Zhang et al., 2010).

The reason behind the elevation of these enzymes in cases of DM II could be due to direct hepatotoxic effect of excess fatty acid and TG accumulated within hepatocytes (Han et al., 2012). Other potential explanations for elevated transaminases in insulin-resistant states include oxidative stress from reactive lipid peroxidation, peroxisomal β-oxidation, and recruited inflammatory cells which contributes to hepatocellular injury (Foster et al., 2013).

Moreover, the histopathological examination in the present work confirmed hepatic cell injury by presence of histological findings ranging from fat accumulation in hepatocytes without inflammation, to hepatic steatosis with a necroinflammatory component with significant increase in NAS score in the same group when compared to control group.

Additionally, Dhule and Gawali (2014) reported that the significant elevation in prothrombin and fibrinogen levels in type II diabetic patients could contribute to thrombotic risk in those patients. This observation can be explained by activation of the coagulative cascade by hyperglycemia (Stegenga et al., 2008), thus increasing thrombin formation and fibrinogen degradation products which, in turn, may stimulate hepatic fibrinogen synthesis (Dhule and Gawali, 2014).

Moreover, dyslipidemia occurring in MS supports activation of coagulation factor VII (Grant, 2007), and inhibits protein C system in endothelial cells which may be a mechanism for the prothrombotic state in this case (Yuanyuanhttp://heart.bmj.com/content/9 6/Suppl_3/A61.2 - aff-1 et al., 2010).

Our results showed a significant decrease in albumin in group (B) when compared with control group. This finding was explained by decrease in hepatic synthesis of albumin (Venkataramana et al., 2013) and/ or increased urinary excretion of albumin (Jin et al., 2015). Furthermore, albumin has antioxidant properties (Taverna et al., 2013), and is...
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the major antioxidant in plasma which is continuously exposed to oxidative stress (Vlassopoulos et al., 2013). So, its level was expected to increase as a defense mechanism against increased oxidative stress in diabetic cases.

However, glycation and oxidative stress cause albumin modifications, impairing its antioxidant properties (Otagiri and Chuang, 2009), and causing its main chain to be fragmented increasing its catabolism (Medina-Navarro et al., 2014).

Regarding body weight, there was a non significant change in BMI between all groups at the end of the study despite the HFD in both diabetic groups as it could also be attributed to the decrease of insulin in the diabetic rats resulting in muscle wasting (Li et al., 2009). Also, it was reported that omega-3 fatty acid has anti-obesity effect, attenuating body weight gain and reducing fat mass (Bertrand et al., 2013).

However, Ugbaja et al. (2013) claimed that ω-3 fatty acid can delay muscle wasting induced by insulin deficiency causing weight gain in HFD fed rats. This controversy could be due to differences in the composition of the diet between studies.

Interestingly, our results showed a significant increase in serum apelin-13 level with a significant positive correlation between apelin-13 and HOMA-IR, ALT, AST, NAS score, albumin, prothrombin and fibrinogen levels in group B when compared to control group which was in line with those of Cavallo et al. (2012) who demonstrated that serum apelin concentrations are higher in type II diabetic patients than in healthy control subjects.

Moreover, Habchi et al. (2014) showed that apelin concentrations were higher in diabetic patients independently of increased BMI suggesting that obesity is not the main determinant of plasma apelin levels in those patients. This is in line with our results that showed a non significant change in BMI between groups. They also suggested that increased apelin level could have a compensatory role in a trial to reduce IR and to improve impaired insulin-secretion.

It was demonstrated that apelin improved insulin sensitivity in insulin-resistant obese mice (Yue et al., 2010 and Habchi et al., 2014) by increasing the glucose uptake in skeletal muscle (Dray et al., 2008). So, Castan-Laurell et al. (2012) suggested that apelin could be considered as a possible target in the treatment of DM II.

In disagreement with our results, Zhang et al. (2009) have found a lower apelin level in type II diabetic patients. This controversy could be explained by the difference in species or dietetic state or sample size between studies.

A recent clinical investigation reported that serum apelin-36 was associated with histological and hemodynamic states of chronic liver diseases (Lim et al., 2016).

Additionally, Drougard et al. (2014) reported that hypothalamic apelin regulated hepatic glucose metabolism in mice fed a HFD.

Moreover, in human NAFLD, plasma apelin levels were higher than that in healthy individuals (Ercin et al., 2010), and Sagiroglu et al. (2014) reported that
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Exogenous apelin administration alleviated hepatic ischemia reperfusion injury in rats. These findings suggest that apelin is involved in the process of liver regeneration (Lv et al., 2017) which is in agreement with our results that showed a significant positive correlation between apelin-13 and both liver enzymes and histological grading in group B.

The association between apelin and liver function in the present study could be explained by the ability of apelin to decrease the TG content in adipose tissue and the weight of different fat depots in HFD fed mice (Yamamoto et al., 2011) through adenosine mono-phosphate kinase (AMPK) activation (Attane et al., 2012).

Moreover, chronic apelin treatment has also been shown to increase fatty acid and glucose oxidation in a model of obesity-related decline of cardiac function (Alfarano et al., 2015). In addition, apelin treatment increases mitochondrial biogenesis in skeletal muscle (Attane et al., 2012), and cardiomyocytes (Alfarano et al., 2015).

However, after doing linear regression analysis test, our results showed a non significant association between apelin-13 and liver function tests which could be explained by indirect effect of apelin-13 on liver function through improving lipid and glucose metabolism. This finding can be supported by the observation of Pope et al. (2012) who reported that the liver is not a major target of apelin since APJ (apelin receptor) is weakly expressed in liver.

However, treatment of diabetic rats with ω-3PUFA (group C) showed a significant decrease in HOMA-IR and serum glucose, apelin-13, TC, TG, ALT and AST when compared with diabetic non treated rats. However, these parameters significantly increased when compared to control group except for HOMA-IR which showed a non significant change when compared with control group. Additionally, there were a non significant change in insulin, prothrombin, fibrinogen or albumin levels compared with group B, indicating improvement in gluco-lipid metabolic disturbances induced by DM II. However, liver function was still partially impaired.

Our results were in line with those of some murine models of IR which have shown beneficial effects of ω-3 fatty acids in prevention or reversal of IR and by having insulin sensitizing actions in adipose tissue and liver (Lombardo et al., 2007 and Gonzalez-Periz et al., 2009).

The protective effects of ω-3 PUFA cannot be explained by modifying adiposity; as there was a non significant change in BMI between groups in this work. However, the possible explanation could be the ability of ω-3 PUFA to increase the mRNA expression of insulin-stimulated glucose transporter-4 (GLUT4), insulin receptor substrate-1 (IRS1), phosphatidylinositol (PI) 3-kinase activity, and glycogen synthase-1 (GYS1) in skeletal muscle causing enhanced glucose utilization (Lanza et al., 2013), and insulin signaling in HFD-fed mice (Le Foll et al., 2007).

It was suggested that insulin sensitizing effect of ω-3 PUFA to be tissue specific on muscle, liver, and adipose tissue (Lalia and Lanza, 2016).

Inconsistent with our results, other studies suggested that ω-3 fatty acids
cannot improve insulin sensitivity in human studies (Poudyal et al., 2011), or fa/fa Zucker rats, a rat model of IR (Oh et al., 2014). However, this inconsistency could be explained by species differences, differences in the dose, and/or the duration of treatment.

Interestingly, Weylandt et al. (2015) found that ω-3 fatty acids exerted hypcholesterolemic effect which could be through inhibition of key enzymes responsible for cholesterol synthesis and transfer such as 3-hydroxy-3-methylglutaryl reductase and cholesterol acyltransferase (Jump, 2008).

In addition, they lower triglycerides by increasing glucose flux to glycogen, decreasing triglycerides synthesis and increasing mitochondrial β-oxidation, an effect that is mediated partially by peroxisome proliferator-activated receptor-α (PPAR-α) activation (Weylandt et al., 2015).

Moreover, our study demonstrated a decrease in liver injury after treatment with ω-3 fatty acids which was proved by lowered liver enzymes and improved histological finding in group (C), which can be explained by its insulin sensitizing effect and its antioxidant activity as ω-3 fatty acid can up-regulate expression of the antioxidant enzymes and molecules, and also down-regulate the genes associated with the production of reactive oxygen species (Garman et al., 2009), resulting in an increased resistance to lipid peroxidation (Ugbaja et al., 2013).

However, failure to improve liver protein levels in this study could be attributed to the short duration of the study or to the small sample size. Moreover, the decrease in serum apelin level in this group in comparison to group (B) may be due to the improvement in insulin sensitivity, blood lipid and liver enzymes under the effect of ω-3 fatty acids treatment, or undefined regulatory factors that could affect serum apelin-13 level.

In contrast to our results, Berta et al. (2010) and Wang et al. (2012) found no differences between apelin and APJ gene expression in adipose tissue between HFD- and HFD-treated with EPA mice, although protein levels were not measured. However, Bertrand et al. (2013) claimed that EPA, increased APJ expression in muscle and adipose tissues and increased serum apelin production. However, neither of these studies was on diabetic rats, all were on obese prediabetic mice. Furthermore, there may be a difference in the measured apelin isoform between our study and the other studies.

CONCLUSION

Treatment of diabetic rats with ω-3 PUFA led to a decrease in serum apelin-13, HOMA-IR, blood lipid and liver enzymes which matched with the improved liver injury. However, it could not restore normal levels of plasma proteins. Considering the multivariate regression analysis, apelin-13 does not seem to be a suitable diagnostic marker in predicting the severity of liver injury in type II diabetic rats.

Further studies on larger number of type II diabetic cases are recommended to investigate cellular and molecular mechanisms that ω-3 PUFA could act through to affect liver function and apelin-
13 level, and if the change in apelin-13 level is a cause or a consequence of the disease.

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خلفية البحث: يعتبر الكبد الدهني غير الكحولي من أمراض الكبد المزمنة المصاحبة لمرض السكري من النوع الثاني (السكري 2). وتتبع مقاومة الجسم للإنсулыون دورا هاما في ظواهر المرضية لأمراض الكبد في حالات السكري. و من الملاحظ أنه يوجد تناقضات في علاقة الأحماض الدهنية غير المشبعة أو ميجا-3 والألياف في الأبحاث السابقة.

الهدف من البحث: صمم هذا البحث لدراسة تأثير العلاج بالأحماض الدهنية غير المشبعة أو ميجا-3 على مستوي الأليلين-13 في مصل الدم و علاقته بحئية الكبد في ذكور الجرذان البيضاء المصاحبة بمرض السكري 2.

مواد و طرق البحث: أجريت هذه الدراسة على ثلاثين من ذكور الجرذان البيضاء البالغة، وقد قسمت إلى ثلاثة مجموعات متساوية:

- المجموعة الأولي (الضبابية): فيها تغذت الجرذان على الغذاء المعتاد، والمجموعة الثانية: فيها تم إعداد مرض السكري 2 بتغذية الجرذان بغذاء عالي الدهن لمدة أسبوعين ثم حقن الجرذان بجرعه واحدة من الاسترتيستوسين (35 مجم/كج) والمجموعة الثالثة: فيها تم معالجة الجرذان بالأحماض الدهنية غير المشبعة أو ميجا-3 لمدة 4 أسابيع بعد إحداث مرض السكري 2.

النتائج: وجد ارتفاع ذو دلالة إحصائية في مستويات الأليلين-13 والجلوكوز ومعيار المقاومة للإنسولين والكوليسترول والدهون الثلاثية و إنزيمات الكبد والبروتورامين والفيبرينوجين وصاصبه تقص ذو دلالة إحصائية في مستويات الإنسولين والأليبيومين عند مقارنتها بالمجموعة الضابطة. وقد أثبتت هذه الدراسة وجود تحسن في القيم خاصة في نسبة الكرد والدهون مصحوبة بنفس ذاته في الفئران في مجموعات الدهن-13 و إنزيمات الكبد في المجموعة الثالثة. وقد أثبتت تحليل التحديد الخطبي المتعدد أن التغير في مستوي الأليلين في مصل الدم ليس له قيمة تنبؤية بالنسبة للتغيرات الهاضمة لإصابة الكبد.

الاستنتاج: علاج الجرذان البيضاء المصاب بإصابة الكبد من النوع الثاني بالأحماض الدهنية غير المشبعة أو ميجا-3، أدى إلى تحسن في مقاومة الجسم للإنسولين وصاصبه تقص في مستوي الأليلين-13. كما أن التغير في مستوي الأليلين لا يمكن الإعتماد عليه كمؤشر معملي يعبر عن مستوى إصابة الكبد في هذه الحالات.