Relationship between Serum Glypican-4 Level and Metabolic Parameters in Experimentally-Induced Non-Alcoholic Fatty Liver Disease in Adult Male Albino Rats

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Abstract

Background: Glypican-4 (GPC4) is a novel adipokine that affects insulin signaling, and adipocyte differentiation. Serum GPC4 level was associated with obesity-related parameters such as hyperglycaemia, Insulin Resistance (IR), and elevated liver enzymes aspartate Amino-Transferase (AST), and alanine Amino-Transferase (ALT).

Non-Alcoholic Fatty Liver Disease (NAFLD) has emerged as one of the manifestations of metabolic syndrome. It is usually associated with IR, dyslipidemia, and elevated AST & ALT levels.

Few studies were performed to elucidate the role of GPC4 in the development of NAFLD in human, and its relation to other metabolic parameters. But, its exact role hasn’t been clarified yet.

Aim of Study: To estimate serum level of GPC4 in NAFLD induced by high fat diet in adult male albino rats, and to examine its relationship to other metabolic parameters.

Material and Methods: A total number of 60 healthy adult male local strain albino rats were divided into 2 main groups: Control group (I) (n=30) were fed on standard chow, and was further subdivided according to the period of their nourishment on standard chow into 3 equal subgroups (n=10), subgroup (IА), for 4 weeks, subgroup (IB), for 12 weeks, and subgroup (IC), for 24 weeks High Fat Diet (HFD) fed group (II) (n=30) were fed on high fat chow, and was further subdivided according to the period of their nourishment on HFD into 3 equal subgroups (n=10), subgroup (IIA), for 4 weeks, subgroup (IIB), for 12 weeks, and subgroup (IIC), for 24 weeks. Body Mass Index (BMI), Abdominal Circumference (AC) were measured. Serum GPC4, glucose, insulin, othomeostasis model assessment (HOMA-IR)-index, Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), AST, ALT, and C-Reactive Protein (CRP) were estimated. Liver histopathology was studied.

Results: GPC4 was positively correlated with BMI, AC, TC, TG, LDL, AST, ALT, and CRP in all HFD-fed rats groups. Concerning, IR and HOMA-IR, it was found that, GPC4 was positively correlated with both of them in HFD-fed rats for 4 weeks and 12 weeks groups, however, no correlations were reported between GPC4 and IR & HOMA-IR in HFD-fed rats for 24 weeks group.

Conclusion: Serum GPC4 level was increased in HFD-fed rats for 4 weeks and 12 weeks groups as a compensatory mechanism to overcome IR, and was reduced in HFD-fed rats for 24 weeks group due to failure of compensation as a result of marked progress of IR.

Key Words: Glypican 4 – Non-alcoholic fatty liver – Rats.

Introduction

GPC4 is an adipokine released from adipose tissue, and related to glycosylphosphatidylinositol-anchored heparin sulfate proteoglycans family [1].

Gesta et al., [2] demonstrated that GPC4 is differentially expressed in visceral and subcutaneous adipose tissue, and its expression in human white adipose tissue is highly correlated with BMI and WHR. It was found that GPC4 can interact with, and regulate insulin receptor activation which in turn enhances insulin signaling and adipocyte differentiation. So, it is closely related to metabolic functions [3].

NAFLD is a common chronic liver condition caused by a build-up of fat in the liver, and it is usually seen in overweight and obese individuals. NAFLD represents a spectrum of pathology ranging from simple steatosis, to Nonalcoholic Steatohepatitis (NASH), to the most danger stage fibrosis and cirrhosis [4]. So, the early stage of NAFLD doesn’t usually cause any harm, but if it gets worse, it may lead to serious liver damage including cirrhosis [5].

It has been shown that NAFLD is strongly associated with the features of metabolic syndrome such as hyperglycemia, IR, dyslipidemia, and
A total number of 60 healthy adult male local strain albino rats weighing 180-220gm were obtained from the Animal House of Faculty of Veterinary Medicine, Zagazig University. Animals were saved in steel wire cages, (40 X 28 X 18cm), 5 rats per cage, under hygienic conditions in the Animal House of Faculty of Medicine, Zagazig University. This study was performed from May 2018 to December 2018. All animals received care in accordance with the guide to the care and use of experimental animals of Institute of Laboratory Animal Resources [10]. All rats had free access to water and commercial rat standard chow, and were further subdivided according to the period of their feeding on commercial rat standard chow into three equal subgroups (n=10); subgroup (IA): For 4 weeks, subgroup (IB): For 12 weeks, and subgroup (IC): For 24 weeks [11].

For 24 weeks to produce steatosis, subgroup (IIB): For 12 weeks to produce NASH, and subgroup (IIC): For 24 weeks to produce cirrhosis [13].

Few researchers examined the role of GPC4 in the pathophysiology of NAFLD as an example of the manifestations of metabolic syndrome in human, and they studied the relation of GPC4 to other metabolic parameters such as IR, lipid profile, and liver enzymes ALT & AST [8], which are the biomarkers of NAFLD [9].

So, this work was designed to evaluate the level of serum GPC4 in NAFLD induced by high fat diet in adult male albino rats. And, to clear up the relationship between GPC4 and other metabolic parameters related to NAFLD in rats.

Material and Methods

A total number of 60 healthy adult male local strain albino rats weighing 180-220gm were obtained from the Animal House of Faculty of Veterinary Medicine, Zagazig University. Animals were saved in steel wire cages, (40 X 28 X 18cm), 5 rats per cage, under hygienic conditions in the Animal House of Faculty of Medicine, Zagazig University. This study was performed from May 2018 to December 2018. All animals received care in accordance with the guide to the care and use of experimental animals of Institute of Laboratory Animal Resources [10]. All rats had free access to water and commercial rat standard chow that was formed of 62.8% carbohydrates, 11.4% fat, and 25.8% protein [11]. Rats were kept at comfortable temperature (20-24ºC) and were maintained on a normal light/dark cycle [12]. After one week of acclimatization the animals were randomly divided into two main groups:

• Control group (I): (n=30), in which rats were fed on commercial rat standard chow, and were further subdivided according to the period of their feeding on the standard chow into three equal subgroups (n=10); subgroup (IA): For 4 weeks, subgroup (IB): For 12 weeks, and subgroup (IC): For 24 weeks [11].

• HFD fed group (II): (n=30), in which rats were fed on High-Fat (HF) chow that was formed of 24% carbohydrate, 58.0% fat, and 18% protein (23.4 kJ/g) and were further subdivided according to the period of their feeding on HF chow into three equal subgroups (n=10); subgroup (IIA): For 4 weeks to produce steatosis, subgroup (IIB): For 12 weeks to produce NASH, and subgroup (IIC): For 24 weeks to produce cirrhosis [13].

For 24 weeks to produce steatosis, subgroup (IIB): For 12 weeks to produce NASH, and subgroup (IIC): For 24 weeks to produce cirrhosis [13].

(Standard and HFD chows were purchased from the Faculty of Agriculture, Zagazig University).

Anthropometric measures:

Calculation of BMI: (g/cm²): The weights and lengths were measured at the end of the experiment, and immediately before they were sacrificed for calculation of the BMI. It can be calculated by dividing body weight (g)/length² (cm²). Weight was measured by using a digital scale in grams according to Nascimento et al., [14], and nose to anus length was measured in cm. according to Novelli et al., [15].

Measuring of Abdominal Circumference (AC): Rats were put in the ventral position and waist circumference was measured from the largest region of the rat's abdomen using a plastic non-resilient measuring tape [16].

Collection of blood samples: Rats were fasted overnight, and then were sacrificed at the end of the experiment using light ether anesthesia. Blood samples (6-8ml/rat) were obtained by decapitation of animals between 9-11 a.m. The collected blood was permitted to be clotted for 2 hours at room temperature before being centrifuged at 3000r.p.m. for 15 minutes. The supernatant serum was pipetted off using fine tipped automatic pipettes and stored at –20ºC until be analysed. Repeated freezing and thawing was avoided [17].

Biochemical analysis:

- Estimation of Serum glypican-4 level was done by using Rat Glypican-4 Enzyme-Linked Immunoassay (ELIZA) kit according to Aboulmaleki and Heidarianpour [18] Catalog MBS726386 with detection range from: 0.5-10ng/ml and sensitivity: 0.1ng/ml from MyBioSource, Inc. San Diego, CA 92195-3308, USA.

- Estimation of serum glucose levels according to Tietz et al., [19] and serum insulin levels by Enzyme-Linked Immunosorbent Assay (ELISA) according to Temple et al., [20].

- HOMA-IR was calculated as follows: [HOMA-IR] = Fasting serum glucose (mg/dl) X fasting serum insulin (µIU/ml)/405 according to Matthews et al., [21].

- Estimation of lipid profile as follows: Total serum cholesterol levels: According to Allain et al., [22], serum TG levels: According to Naito et al., [23], serum HDL levels according to Warnick et al., [24], and serum LDL levels was calculated according to Friedewald et al., [25] as follows: LDL = TC - HDL - TG/5 (kits for estimation of serum glucose, insulin, cholesterol, TG and HDL levels.
were purchased from Biosource Europe S.A. Belgium).

- Estimation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels: According to Rec. [26] (kits for estimation of ALT & AST were purchased from Shanghai Sunred biological technology, China).

- Estimation of serum C-Reactive Protein (CRP): According to Kimberly et al., [27] by using rat Immuno-enzymometric assay kits, (MonobindInc Lake Forest, Ca 92630, USA).

Histopathological examination of liver:
- All removed livers were fixed in 10% buffered formalin solution for a period of 48-60 hours. then, tissue samples were treated with ethyl alcohol and xylene series, and installed in paraffine blocks. Liver specimens were sectioned (5 µm thick), then stained with hematoxylin and eosin

- An expert pathologist examined the stained samples blindly using light microscope with camera attachment to demonstrate the various pathological stages of NAFLD from simple steatosis to NASH to cirrhosis according to Kleiner et al., [29].

Statistical analysis: The data gained in this current-work were expressed as mean ± SD for quantitative variables and statistically analyzed according to the methods described by Kirkwood, [30]. The statistical analysis is done by using SPSS program (version 20 for windows) (SPSS Inc. Chicago, IL, USA).

ANOVA (Post hoc) test was used to compare means among more than two groups.

p-value <0.05 was considered statistically significant.

Correlation coefficient (r): Pearson’s correlation analysis was performed to illustrate the relationships between serum GPC4 and the studied metabolic parameters among different groups. Pearson’s correlation was considered significant at p-values <0.05.

Results

Histopathological findings:

Normal hepatic tissue from the control groups (IA, IB, IC) showing normal morphology of central vein and hepatic lobules (H & E: X400) Figs. (1-3). While fat vacuoles were observed in group (IIA) representing simple steatosis (H & E: X400) Fig. (4), and steatohepatitis evidenced by the presence of inflammatory cells with fat vacuoles and fibrosis were seen in group (IIB) (H & E: X400) Fig. (5). In addition, the liver tissue in group (IIC), showing the presence of fat vacuoles along with cirrhotic nodules indicating the occurrence of cirrhosis (H & E: X400) Fig. (6).

Anthropometric parameters, serum GPC4 level, and metabolic parameters (Table 1):

This study revealed that in group IIA (HFD-fed rats for 4 weeks) there were significant increase in BMI, AC, serum levels of GPC4, glucose, insulin, HOMA-IR value, TC, TG, LDL, ALT, AST, and CRP when compared to their control group IA, (p-value: <0.001 for all). While there was significant reduction of serum HDL level in group IIA when compared to its level in group IA (p<0.01).

In addition, there were significant increase in BMI, AC, serum levels of GPC4, glucose, insulin, HOMA-IR value, TC, TG, LDL, ALT, AST, and CRP in group IIB (HFD-fed rats for 12 weeks) when compared to their control group IB, (p-value: <0.001 for all). While there was significant reduction of serum HDL level in group IIB when compared to its level in group IB (p<0.001).

Moreover, in group IIC (HFD-fed rats for 24 weeks) there were significant increase in BMI, AC, serum levels of glucose, insulin, HOMA-IR value, TC, TG, LDL, ALT, AST, and CRP when compared to their control group IC, (p-value: <0.001 for all respectively). While there were significant reduction of serum GPC4, and HDL levels in group IIC when compared to their levels level in group IC (p-value: <0.01, <0.001 respectively).

It has been shown that, in group IIB, BMI, AC, serum levels of GPC4, glucose, insulin, HOMA-IR value, TC, TG, LDL, ALT, AST, and CRP were significantly elevated when compared to their values and levels in group IIA (p-value: <0.001 for all). While there was no significant change of serum HDL level in group IIB when compared to its level in group IIA (p>0.05).

Also, in group IIC, there were significant increase in BMI, AC, serum levels of glucose, insulin, HOMA-IR value, TC, TG, LDL, ALT, AST, and CRP when compared to group IIA, (p-value: <0.001 for all). While there were significant reduction of serum levels of HDL, and GPC4 in group IIC when compared to their levels in group IIA (p-value: <0.01, <0.001 respectively).
In addition, there were significant increase in BMI, AC, serum levels of glucose, insulin, HOMA-IR value, TC, TG, LDL, ALT, AST, and CRP, in group IIC when compared to group IIB, \( p \)-value: <0.001 for all). While there were significant reduction of serum levels of HDL, and GPC4 in group IIC when compared to their levels in group IIB \( p \)-value: <0.01, <0.001 respectively.

**Correlations between serum GPC4 level and all studied parameters (Table 2):** This study recorded significant positive correlations between serum GPC4 level and each of BMI, AC, TC, TG, LDL, ALT, AST, and CRP in the three HFD-fed rats.
Table (1): Anthropometric parameters, serum GPC4 level, and metabolic parameters among different studied groups.

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<tbody>
<tr>
<td>BMI (g/cm²)</td>
<td>0.45±0.08</td>
<td>0.68±0.011***a</td>
<td>0.52±0.25</td>
<td>0.86±0.023***b</td>
<td>0.96±0.039***c</td>
<td>0.03±0.002</td>
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<tr>
<td>AC (cm)</td>
<td>18.3±0.40</td>
<td>21.4±0.36***a</td>
<td>17.8±0.42</td>
<td>22.6±0.31***b</td>
<td>24.9±0.12***c</td>
<td>0.03±0.002</td>
</tr>
<tr>
<td>Glypican4 (ng/ml)</td>
<td>2.06±0.23</td>
<td>2.97±0.36***a</td>
<td>2.05±0.16</td>
<td>3.54±0.17***b</td>
<td>1.74±0.17***c</td>
<td>0.03±0.002</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>82.9±0.72</td>
<td>149±1.14***a</td>
<td>84.8±0.79</td>
<td>224.8±1.17***b</td>
<td>2.82±0.083</td>
<td>0.03±0.002</td>
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<tr>
<td>Insulin (µU/ml)</td>
<td>19.6±0.83</td>
<td>33.9±0.91***a</td>
<td>19.5±0.86</td>
<td>40.3±0.89***b</td>
<td>19.6±1.01</td>
<td>0.03±0.002</td>
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<tr>
<td>HOMA-IR</td>
<td>4.7±0.47</td>
<td>12.5±3.7***a</td>
<td>4.3±0.32</td>
<td>23.2±4.5***b</td>
<td>4.0±0.16</td>
<td>0.03±0.002</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>60.6±2.4</td>
<td>94.0±2.8***a</td>
<td>74.8±8.9</td>
<td>101.5±6.7***b</td>
<td>74.2±8.7</td>
<td>0.03±0.002</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>50.2±2.2</td>
<td>90.9±4.1***a</td>
<td>46.3±3.1</td>
<td>133.1±9.3***b</td>
<td>55.7±5.0</td>
<td>0.03±0.002</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>26.3±3.2</td>
<td>78.7±4.2***a</td>
<td>27.3±3.0</td>
<td>118.3±3.2***b</td>
<td>23.0±3.1</td>
<td>0.03±0.002</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>28.6±1.7</td>
<td>24.6±3.6***a</td>
<td>29.8±1.8</td>
<td>24.7±2.4***b</td>
<td>27.3±2.2</td>
<td>0.03±0.002</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>41.3±2.3</td>
<td>100.6±2.5***a</td>
<td>48.3±2.1</td>
<td>132.6±2.3***b</td>
<td>50.7±6.4</td>
<td>0.03±0.002</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>142.9±2.1</td>
<td>177.7±1.9***a</td>
<td>150.1±2.1</td>
<td>186.3±1.0***b</td>
<td>151.2±1.1</td>
<td>0.03±0.002</td>
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<tr>
<td>CRP (mg/l)</td>
<td>0.03±0.002</td>
<td>0.08±0.001***a</td>
<td>0.03±0.001</td>
<td>0.11±0.002***b</td>
<td>0.04±0.001</td>
<td>0.18±0.001</td>
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a = vs group IA.  b = vs group IB.  c = vs group IC.

Group IIA (r=0.987***, p<0.01, r=0.983***, p<0.01, r=0.947***, p<0.01, r=0.839***, p<0.01, r=0.691*, p<0.05, r=0.656, p<0.05, r=0.686*, p<0.05, r=0.623*, p<0.05 respectively), group IIB (r=0.974***, p<0.01, r=0.939***, p<0.01, r=0.949***, p<0.01, r=0.877***, p<0.01, r=0.645*, p<0.05, r=0.885**, p<0.01, r=0.871**, p<0.01, r=0.641* p<0.05 respectively), and group IIC (r=0.970***, p<0.01, r=0.966***, p<0.01, r=0.965***, p<0.01, r=0.876**, p<0.01, r=0.844** p<0.01, r=0.826**, p<0.01, r=0.847**, p<0.01, r=0.881***, p<0.01 respectively).

However, no significant correlations were reported between serum GPC4 level and serum glucose or HDL in the three HFD-groups: IIA (r=0.271IS, p>0.05, r=0.298IS, p>0.05 respectively), IIB (r=0.153IS, p<0.05, r=0.341IS, p>0.05 respectively), and IIC (r=0.221IS, p<0.05, r=0.247IS, p>0.05 respectively).

Concerning serum insulin level, and HOMA-IR, it has been found that, serum GPC4 level was positively correlated with insulin, and HOMA-IR in group IIA (r=0.848**, p<0.01, r=0.856**, p<0.01 respectively), and IIB (r=0.866**, p<0.01, r=0.867**, p<0.01 respectively). However, no significant correlation was reported between GPC4 and either serum insulin or HOMA-IR in group IIC (r=0.374IS, p>0.05, r=0.412IS, p<0.05 respectively).

No significant correlations were recorded between GPC4 and any of the studied parameters in the control groups (IA, IB, IC).

Table (2): Correlations between serum GPC4 level and all studied parameters among different studied.

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<tr>
<td>BMI (gm/cm²)</td>
<td>0.216IS</td>
<td>0.987***</td>
<td>0.311IS</td>
<td>0.974***</td>
<td>0.218IS</td>
<td>0.970***</td>
</tr>
<tr>
<td>AC (cm)</td>
<td>0.311IS</td>
<td>0.983***</td>
<td>0.305IS</td>
<td>0.939***</td>
<td>0.305IS</td>
<td>0.966***</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>0.113IS</td>
<td>0.271L</td>
<td>0.235IS</td>
<td>0.153IS</td>
<td>0.138IS</td>
<td>0.317IS</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>0.124IS</td>
<td>0.848**</td>
<td>0.144IS</td>
<td>0.866**</td>
<td>0.315IS</td>
<td>0.421IS</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.305IS</td>
<td>0.856**</td>
<td>0.305IS</td>
<td>0.867**</td>
<td>0.315IS</td>
<td>0.421IS</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>0.338IS</td>
<td>0.947***</td>
<td>0.319IS</td>
<td>0.949***</td>
<td>0.213IS</td>
<td>0.965***</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>0.137IS</td>
<td>0.839**</td>
<td>0.126IS</td>
<td>0.877**</td>
<td>0.426IS</td>
<td>0.876**</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>0.215IS</td>
<td>0.691*</td>
<td>0.178IS</td>
<td>0.645*</td>
<td>0.115IS</td>
<td>0.844**</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>0.195IS</td>
<td>0.298L</td>
<td>0.183IS</td>
<td>0.341IS</td>
<td>0.025IS</td>
<td>0.274IS</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>0.119IS</td>
<td>0.656*</td>
<td>0.117IS</td>
<td>0.885**</td>
<td>0.114IS</td>
<td>0.826**</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>0.201IS</td>
<td>0.686*</td>
<td>0.046IS</td>
<td>0.871**</td>
<td>0.275IS</td>
<td>0.847**</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.203IS</td>
<td>0.623</td>
<td>0.104IS</td>
<td>0.641*</td>
<td>0.033IS</td>
<td>0.881**</td>
</tr>
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* : Significant (p<0.05).  ** : Significant (p<0.01).  *** : Significant (p<0.001).
Discussion

Studies were performed to investigate the relationship of serum GPC4 and obesity-related metabolic parameters such as BMI, fasting insulin, IR, AST and ALT \[8,31,32\].

NAFLD is considered as one of the manifestations of obesity-related metabolic disorders and is usually associated with IR, and elevated serum levels of AST and ALT which are its biomarkers \[33\].

Few studies were designed to find the link between serum GPC4 and NAFLD \[8\]. So, interestingly, this was the first study that has been performed to clarify the relationship of serum GPC4 level with different metabolic parameters in NAFLD in an animal model.

The current work observed that there was a significant progressive increase in BMI, AC, serum glucose and insulin levels, HOMA-IR values, TC, TG, LDL, and CRP serum levels, in HFD-fed groups. Also, there was a significant reduction in HDL levels in the same groups.

Many criteria of metabolic syndrome (metS) such as excess body fat around the waist, hyperglycemia, Insulin Resistance (IR), and dyslipidemia were present in this model according to Timpson et al., \[34\] and You-Min et al., \[35\] classification. Also, these findings are in agreement with those of other studies \[36,37\].

In addition, the histopathological examination of the liver according to Kleiner et al., \[29\]; illustrated: (I) Simple steatosis in HFD-fed rats for 4 weeks (group IIA) that was evidenced by the presence of fat vacuoles with no-inflammatory lesion, (II) NASH in HFD-fed rats for 12 weeks (group IIB) that was evidenced by occurrence of fibrosis and the presence of inflammatory cells, (III) Cirrhosis in rats fed HFD for 24 weeks (group IIC) that was evidenced by loss of normal hepatic morphology and the appearance of cirrhotic nodules, Figs. (4-6). These histopathological findings were accompanied with a significant increase in ALT and AST serum levels in a duration dependent manner.

These results are in agreement with those of Svegliati-Baroni et al., \[13\] and Zhang et al., \[38\] who found that, HFD effectively induced hepatic steatosis and steatohepatitis in rats.

The peripheral IR observed in this present study among the HFD-fed rats groups can induce hepatic steatosis, indicating the presence of IR in liver \[39\]. As, IR impairs insulin suppressing effect on hepatic glucose production, and on lipolysis resulting in enhancing the release of FFA from adipose tissue that will disturb lipid metabolism and this aggravate peripheral IR and contributes to the development of NAFLD \[40,41\].

Moreover, IR and hyperinsulinemia are usually closely related to the inflammatory reactions, and the release of inflammatory mediators as CRP that accompanied advanced stages of the disease \[42\]. Also, obesity-associated inflammatory conditions evokes the adipocytes to release inflammatory mediators that worsen the inflammatory process \[43\].

The results of this study showed that, there were significant increase in BMI, WC, serum levels of glucose, insulin, HOMA-IR, TG, TC, LDL, ALT, AST and CRP in all high fat diet groups (IIA, IIB, IIC) when compared to their control groups (IA, IB, IC respectively). And, there were significant increase in all the previous data in groups IIB and IIC when compared to group IIA, and also, in group IIC when compared to group IIB.

Concerning serum GPC4 level, it increased in HFD groups (IIA, IIB) when compared to their controls (IA, IB) respectively. However, serum GPC4 level significantly decreased in group IIC, when compared to its control group IC.

In addition, this study reported significant positive correlations of serum GPC4 level with each of BMI, WC, serum levels of glucose, insulin, HOMA-IR, TG, TC, LDL, ALT, AST and CRP in all high fat diet groups (IIA, IIB, IIC).

Moreover, significant positive correlations were recorded between serum GPC4 level and each of serum insulin level and HOMA-IR in groups IIA and IIB. However, serum GPC4 level was not correlated with serum insulin level or HOMA-IR in group IIC.

In addition, no significant correlations were reported between serum GPC4 level and serum glucose or HDL levels in all the HFD groups (IIA, IIB, and IIC).

These results were in accordance with, the findings of Zhu et al., \[31\], who observed that serum levels of GPC4 increased in obese patients with insulin resistance and recorded positive correlations between serum GPC4 levels and each of BMI, fasting serum insulin level, HOMA-IR, TC, LDL, ALT, and AST levels, while no correlation was found with fasting serum glucose. However, they
reported no correlation of GPC4 levels with TG, and negative correlation with HDL.

Leelalertlauw et al., [44] found that serum GPC4 levels increased with increasing the degrees of obesity in children, and were significantly positively correlated with BMI, WHR, TC, LDL, ALT, and AST for the same age-group. And, at the same time, they reported no correlations between serum GPC4 level and serum glucose level through different conditions of glucose metabolism. But, Leelalertlauw et al., [44] differed with this study, in that, they did not find any correlations of serum GPC4 level with insulin sensitivity and β-cell function indices in obese children.

Moreover, previous studies coincide with these findings, and illustrated that serum GPC4 levels elevated in overweight and obese than non-obese individuals, and reported significant positive correlations with BMI, WHR, serum insulin level, and HOMA-IR in over-weight and obese individuals [3,45].

A study performed by Ussar et al., [3] on mice and humans showed that in the markedly obese mice (ob/ob mice) used as a model for diabetes, serum GPC4 level decreased with the presence of the high blood glucose level and the hyperinsulinemia, but it increased in HFD-fed mice that can maintain normal glycaemia and normal insulinemia when compared to controls, but in humans, Ussar et al., [3] found that serum GPC4 level was elevated in overweight and obese subjects when compared to controls, and was positively correlated with BMI, WHR, insulin level, and HOMA-IR. But, they did not record any relation of GPC4 level with fasting plasma glucose, TC, LDL, or HDL.

On the other hand, Yoo et al., [8] disagreed with us in that plasma GPC4 level had no significant correlation with BMI, and had significant positive correlations with each of glucose, and the ratio of visceral to subcutaneous fat in Korean women with NAFLD disease. However, the same study of Yoo et al., [8] coincided with the present work, in that, plasma GPC4 level showed significant positive correlations with HOMA-IR, TG, ALT, and AST levels.

In line with the findings of this study, Li et al., [46] showed that circulating plasma GPC4 levels were elevated, and positively correlated with serum insulin level and HOMA-IR value in overweight and obese subjects with IGT when compared to non-obese subjects with NGT. And, at the same time, this study revealed significant reduction in plasma GPC4 level in newly diagnosed type II diabetes even less than its value in normal healthy controls with NGT, and found no correlation of GPC4 with serum insulin or HOMA-IR value in the newly diagnosed diabetic group. But, they recorded negative correlations between GPC4 and each of fasting plasma glucose and glycosylated haemoglobin (HbA1c) in IGT and diabetic groups [46].

Li et al., [46] interpreted these changes in serum GPC4 level from prediabetic to diabetic state as the increase of insulin level in the early prediabetic state with IGT leads to the activation of Glycosylphosphatidylinositol-specific Phospholipase D1 (GPLD1) [47,48] which in turn is responsible for cleavage of GPC4 [49,50], and this will lead to increased circulating GPC4 level. With the development of prediabetic state to type II diabetes, the progression of insulin resistance leads to a decrease in the activity of GPLD 1 and a reduction in the circulating GPC4 levels [46].

Moreover, discrepancies in the relationship of GPC4 level to insulin resistance might be described as a result of the bell-shaped profile of GPC4 level from normal to a prediabetic (IGT), and to a diabetic state. So, it is not easy to record its relation to insulin resistance in recently discovered type II diabetes [46].

In contrast with our findings, Lee et al., [32] found that, in type II diabetes, plasma GPC4 level did not show any significant changes among quartile groups based on BMI and HOMA-IR. And, they reported no correlations between GPC4 level and BMI or HOMA-IR in these quartile groups. At the same time, they observed that GPC4 level was negatively associated with HOMA-IR among quartile group based on HOMA-IR.

Also, Lee et al., [32] disagreed with us, in that they recorded no significant correlations between GPC4 and any of WHR, TC, TG, LDL, and ALT. And they reported a negative correlation of GPC4 with AST. But, they agreed with us, in that they did not find any correlation between GPC4 and either plasma glucose or HDL.

Concerning CRP, it was observed that its level increased progressively in all HFD-fed rat groups with different stages of NAFLD indicating different degrees of the inflammatory process of NAFLD, and these results are in agreement with other studies [42,43].

In addition, a significant positive correlation of serum GPC4 level with CRP was reported in all HFD-groups that could be supported by the study
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of Linder and Fisher [51] who found that plasma GPC4 is elevated in patients with severe sepsis/septic shock and positively correlated with CRP and other markers of systemic inflammation.

Our findings could be interpreted as the significant increase of serum GPC4 level observed in the HFD-fed rats for 4 and 12 weeks (groups IIA, IIB respectively), may be considered as a compensatory mechanism by which fat tries to overcome IR [Sanal, 2015]. As, the increase in serum insulin level will increase circulating GPC4 level by increasing its cleavage. While, the significant reduction in serum GPC4 level in HFD-fed rats for 24 weeks (group IIC) may be due to decompensation occurred with the progression of hyperinsulinemia and IR, as the unreasonable elevation of insulin suppress the cleavage of GPC4 and decreasing its circulating level [48,50].

In addition, the explanation for the different correlations reported between serum GPC4 level and HOMA-IR in this study among different HFD-groups may be related to the bell-shaped profile of GPC4 level through different conditions of IR [46].

Discrepancies, between the results of this study, and those of others might be related to using different kits or animal models, various duration and study design.

Conclusions:

Serum GPC4 level increased and positively correlated with insulin and HOMA-IR in this study among different HFD-groups may be related to the bell-shaped profile of GPC4 level through different conditions of IR [46].

Further studies are required to clarify the physiological role of GPC4 in the pathogenesis of NAFLD.

References


العلاقة بين مستوي مصل الدم لمادة الجليبيكين-4 والمؤشرات الأيضية في مرض الكبد الدهني غير الكحولي الناجم عن التجربة في ذكور الجرذان البيضاء البالغة

خلفية البحث: مادة الجليبيكين-4 هي مادة دهنية جديدة تُثير على إشارة الانسيولين وتتأثر الخلايا الدهنية، ووجد أن مستواها في مصل الدم مرتبطة بالمؤشرات التي لها علاقة بالسمة مثل فرق سكر الدم ومقاومة الانسيولين، وإنزيماً الكبد المرتبطة مثل إنتروليتين الأمينية والأليينين الأميني تتراسفورمات.

وبعد مرور الكبد الدهني غير الكحولي واحداً من مظاهر متلازمة التمثيل الغذائي، وعادة ما يرتبط مستوي مقاومة الانسيولين وعمر دهنيات الدم، ومستويات إنزيماً الكبد المرتبطة. أجريت دراسات قليلة لتوضيح دور هذه المادة في تطور مرض الكبد الدهني غير الكحولي في الإنسان، وعلاقته بالمؤشرات الأيضية الأخرى ولكن لم يتم توضيح دورها الدقيق بعد.

الهدف من البحث: هدفنا الدائرة إلى تحديد مستوى مادة الجليبيكين-4 في مصل الدم في مرض الكبد الدهني غير الكحولي الناجم عن إتباع نظام غذائي عالي الدهون في ذكور الجرذان البيضاء البالغين، ودراسة علاقتها بمادة الجليبيكين الآخرى.

مواد وطرق البحث: وشملت الدراسة إجمالي ستون من ذكور الجرذان البيضاء الأصحاء البالغين، وتم تقسيمهم إلى مجموعتين رئيسين: المجموعة الضابطة الأولى وعدها ثلاثين يتغذى فيها الفئران على الطعام المتتابع، وتم تقسيمهم وفقاً لفترة التغذية إلى ثلاث مجموعات فرعية متساوية، مجموعة فرعية (أ): لمدة 4 أسابيع، ومجموعة فرعية (ب)، لمدة 12 إسبوعاً، ومجموعة فرعية (ج)، لمدة 24 إسبوعاً. المجموعة الثانية وعدها ثلاثين يتغذى فيها الفئران نظام غذائي عالي الدهون، وتم تقسيمهم أيضاً وفقاً لفترة التغذية إلى ثلاث مجموعات فرعية متساوية: المجموعة الفرعية (أ): لمدة 4 أسابيع، المجموعة الفرعية (ب)، لمدة 12 إسبوعاً، والمجموعة الفرعية (ج)، لمدة 24 إسبوعاً.

وقد تم قياس مؤشر كتلة الجسم، ومحيط البطن، ومستوي الجليبيكين في مصل الدم، والجلوكوز، والبروتينات. كما تم تقييم نوع جودة الذئب، والإنسولين، والكولسترول الكلي، والدهون الثلاثية، والبروتينات الدهنية عالية الكثافة، والبروتينات الدهنية منخفض الكثافة، وإنزيمات الكبد والبروتينات النشط (ج) وكذلك تم ترشيح كبد الجرذان لعمل تحليل النتائج.

النتائج: أظهرت النتائج أن مستوى الجليبيكين-4 في مصل الدم يرتبط بشكل إيجابي مع مؤشر كتلة الجسم، ومحيط البطن، والجلوكوز، والإنسولين، والكولسترول الكلي، والدهون الثلاثية، والبروتينات الدهنية عالية الكثافة، والبروتينات الدهنية منخفض الكثافة، وإنزيمات الكبد والبروتينات النشط (ج) في جميع مجموعات الجرذان التي تم تقسيمها بنظام غذائي عالي الدهون.

وفيما يتعلق بمقياس مقاومة الأنسولين، فقد وجد أن مستوى الجليبيكين-4 في مصل الدم كان مرتبطاً به بشكل إيجابي فقط في الجرذان التي تم تقسيمها بنظام غذائي عالي الدهون لمدة 24 إسبوع ولم يتم تسجيل أي ارتباطات مع المجموعة التي تم تقسيمها لمدة 24 إسبوع.

الاستنتاج: هنا إرتفاعاً في مستوى الجليبيكين-4 في مصل الدم في الجرذان التي تم تقسيمها بنظام غذائي عالي الدهون لمدة 4 و12 إسبوع كائنة تعويضية للتغلب على مقاومة الأنسولين، وتم ملاحظة إنخفاض في مستوى في المجموعة ذات 24 إسبوع بسبب فشل التعويض نتيجة للتقدم المبكر في مقاومة الأنسولين.