Biochemical investigation of an experimentally induced metabolic syndrome in rats

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ABSTRACT

Metabolic syndrome (MS) is a complex condition characterized by insulin resistance, hyperglycemia, dyslipidemia, and obesity. This project aims to induce MS in rats and then a demonstration of the main biochemical parameters. In male, Sprague-Dawley rats, MS has been prompted suitably and relatively by fast (six weeks) approach through a high fructose in drinking water (40%). It has been found that serum urea, creatinine, and total bilirubin raise in MS significantly. Moreover, dyslipidemia has arbitrated via some considerable lipid profile deviations. In addition, BMI, blood glucose, and insulin monitoring evidently ensure achievement of MS. It is concluded that a well-established rat model of MS could be employed by a 40% fructose in drinking water.

Key words: Dyslipidemia, Fructose, Insulin resistance, Metabolic syndrome.

INTRODUCTION

Metabolic syndrome (MS) or insulin resistance syndrome, proposed by Gerald Reaven, is the major worldwide metabolic disorder contributed to the challenge of human health. (Reaven, 1988; Farag et al., 2018). Actually, about 20-30% of the global population may suffer from MS (Kennedy et al., 2010), as well as many animal species like dogs (Tvarijonaviciute et al., 2012), cattle (Pampori et al., 2012), laboratory animals (Zhou et al., 2014), and horses it may be donated to laminitis (Morgan et al., 2015). Obesity, insulin resistance, hyperglycemia, dyslipidemia, and hypertension are the main features of MS (Zhou et al., 2014).

The pathophysiology of MS was not absolutely recognized, therefore a number of suppositions were submitted. The crucial one suggests that MS was the consequence of the genetics, standard of life, and diet interactions (Martinez et al., 2018), but insulin resistance remains the leader of this syndrome (Sudarsanam et al., 2010; McCracken et al., 2018). On the account of the high mass of adipose tissue in MS individual that results from negative energy balance, a great amount of lipids, particularly free fatty acids (FFAs) were liberated into the bloodstream leading to the negative triggering response of tissues (muscles) to insulin through inhibiting of insulin-mediated glucose uptake. Consequently, hyperglycemia evokes the beta-cells of the pancreas to more insulin secretion which results in hyperinsulinemia (Sears and Perry, 2015). On the other hand, insulin resistance and elevated FFAs in blood inhibit glycogenesis develop more hyperglycemia and hypetriacylglycerolemia due to the principal role of insulin in the depression of lipolysis. Again, insulin resistance results in great lipolysis making more FFAs, progressing the deleterious cycle (McCracken et al., 2018).

Kennedy et al., (2010) has proposed that leptin-deficient mouse (Lep<sup>ob/ob</sup>) is a vital genetic model for MS aroused by spontaneous mutation. Nowadays, it can be experimentally induced in mice. Whereas, Bezerra and Oliveira (2013) have documented that in the case of obesity, mature adipocytes secrete more adipokines (a cytokine delivered from adipose tissues, contributed to insulin resistance), leptin, adiponectin, and proinflammatory cytokines (interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha)). IL-6 and TNF-alpha promote lipolysis and hepatic fatty acid synthesis. They are also expanding insulin resistance through negatively direct interaction with insulin receptors. Recently, a proteomic study of certain liver proteins, like fructose 1,6-bisphosphatase, 1-pyruvate dehydrogenase, fatty acid synthase, Acyl-CoA synthetase 1, has indicated variable changes in the metabolism of carbohydrates and lipid, and related pathways (Hsieh et al., 2016).

Actually, MS is not entirely understood and it is in need of much more exploration (McCracken et al., 2018), i.e. little attention has been paid to the assessment of the biochemical changes correlated to experimentally induced MS in the rat as a model for this syndrome. In fact, this project aims to the induction of MS in the rat by using a simplified way and demonstration of the related biochemical profiles.

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MATERIALS AND METHODS

Experimental animals: Three months old 30 male Sprague-Dawley rats weight 180-200g were housed in the standard cages (dimension of 43×29×15 cm), under suitable conditions of humidity, temperature (22±2 °C), and 12-hour light-dark cycle, in the animal house unit, college of veterinary medicine, University of Mosul, during March-April 2018. At the end of experiments, all animals were sacrificed using diethyl ether (99.5%) (Scharlau, Spain) anesthesia.

After one week of acclimatization, rats were divided randomly into three groups (10 rats per group) according to drinking water; control group on tap water, F20% group, and F40% group. All three groups diet and water ad libitum, and the chow were standard for rats (NRC, 1995). The experimental procedures were approved ethically by the college of veterinary medicine, University of Mosul.

Fructose drinking water: Freshly fructose drinking water was prepared every alternative day depending on weight to volume formula (Wong et al., 2016) by using D (-)-fructose 98.5-102% (Scharlau, Spain), 20g of fructose dissolved in 100ml of tap water to prepare 20% fructose drinking water (F20%), and 40g of fructose dissolved in 100ml of tap water to prepare 40% fructose drinking water (F40%). Bottles of drinking water were covered using aluminum foil to avoid fermentation.

Physiological parameters: As far as body weight, an electronic weighing scale with an accuracy of 0.01 g, has been used whereas body length, and abdominal circumference has been measured by scale tape at the beginning and end (six weeks later) of the experiment. Body length measurement was from nose to the origin of the tail (Poudyal et al., 2010). Abdominal circumference was determined about the anterior region of the abdomen. Anyhow, body weight gain calculated from values of initial and final weight, while body mass index (BMI) was found as BMI = body weight (g)/ body length² (cm²) (Novelli et al., 2007; Nutan and Kochar, 2014). All measurements were done in anesthetized rats.

Biochemical analysis: The rats were starved overnight, then blood was collected from the retro-ocular vein by a heparinized capillary tube. The blood was immediately centrifuged at 3000xg for 15 minutes. The achieved serum was kept at -20 °C until biochemical analysis. Fasting serum glucose (FSG), total protein, albumin, globulin, urea, creatinine, alanine transaminase (ALT), aspartate transaminase (AST), and total bilirubin, and lipid profiles; cholesterol, TG VLDL, LDL, and HDL were estimated using automated enzymatic method (Genri-GS200-China). While atherogenic index (AI) was calculated as:

\[ AI = \frac{\text{total cholesterol} - \text{HDL}}{\text{HDL}} \]

Alfa 2


Serum insulin was measured using RayBio® rat insulin ELISA kit (3607 Parkway Lane, GA 30092, USA), whereas HOMA-IR (homeostatic model assessment of insulin resistance), and HOMA-â index (homeostatic model assessment of β-cell function) were calculated according to Zhou et al., (2014) just as follows:

\[ \text{HOMA-IR} = \frac{\text{FSG} \times \text{FSI}}{22.5} \]

\[ \text{HOMA-β index} = 20 \times \frac{\text{FSI}}{\text{FSG}} - 3.5 \]

Statistical analysis: By using Statistical Package for Social Sciences (SPSS, version 22), all experimental data were examined and characterized as mean values with their standard deviation (Mean ± SD) and subjected to one-way analysis of variance (ANOVA), a probability (p) of 0.05 and 0.001 were considered as the significant (p≤0.05) and highly significant (p≤ 0.001), respectively (Sweet and Grace-Martin, 2011).

RESULTS AND DISCUSSION

Laboratory animals, mainly mice and rats, were employed in MS projects, either transgenic or diet- or drinking water-induced, and because the syndrome in the human and most animals usually develop from dietary source, therefore MS was induced in mice and rats by high fructose diet (Wong et al., 2016) or high fructose drinking water (Al-Agele and Khudiar, 2016). Biochemical parameters fluctuations associated with MS induced in rats by fructose in drinking water have not been thoroughly investigated. Therefore, it is believed that more investigations are required in this area.

In this project, MS was established in adult male normal Sprague-Dawley rats (without genetic modification) through 40% (but not 20%) fructose in drinking water. MS was confirmed by observed obesity, hyperglycemia, insulin resistance, and dyslipidemia (Wong et al., 2016).

Physiological parameters: As can be seen in the Table 1, body weight, body weight gain, and BMI were increased significantly (p≤ 0.05) in treated groups when compared with the initial and with the control group. Remarkable high body weight gain and BMI must have been referred to obesity which is undoubtedly one of the cardinal dramatic phenomena of the MS (Hsieh et al., 2016). Fructose in drinking water directly contributes to hyperglycemia resulting in positive energy balance which, by the time, encourage adipocyte hypertrophy and hypertrophy, as an attempt to storage “excess fuel”, giving rise to overweight.

Biochemical parameters: Total protein, albumin, globulin, and ALT have non-significant changes in treated groups as illustrated by Table 2. Whereas urea and creatinine were increased (p≤ 0.001) in F40% group at the end of experiments. There is depression (p≤ 0.05) in AST and a total bilirubin level of both treated group as compared with their initials and with the control group. Raised blood urea...
could result from high production or impaired excretion, that may be due to various conditions (Rodwell et al., 2015). Whereas Meyer and Hostetter, (2007) supposed the strong relationship between high levels of serum urea and creatinine with insulin resistance, oxidative stress and free radicals production, and systemic inflammation characterized by elevated level of TNF-alpha, IL-6. It could be suggested that hyperglycemia may have deleteriously effected on the renal tissue impairing the glomerular function in the clearance of urea. On the other hand, Al-Agele and Kuduair (2016)

### Table 1: Effects of fructose drinking water (F20% and F40%) on physiological parameters.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>F20%</th>
<th>F40%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>6 weeks</td>
<td>Initial</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>189 ± 5.3</td>
<td>254 ± 6.7</td>
<td>193 ± 9.2</td>
</tr>
<tr>
<td>Body weight gain (g) (%)</td>
<td>-</td>
<td>65 (34.39) ± 4.3</td>
<td>-</td>
</tr>
<tr>
<td>BMI</td>
<td>0.39 ± 0.02</td>
<td>0.40 ± 0.04</td>
<td>0.36 ± 0.05</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>22 ± 1.2</td>
<td>25 ± 1.4</td>
<td>23 ± 1.1</td>
</tr>
<tr>
<td>Abdomen circumference (cm)</td>
<td>17.9 ± 1.5</td>
<td>20.2 ± 1.8</td>
<td>17.3 ± 2.0</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 10 rats for each group.
A-D represent a significant difference (p ≤ 0.05).
a-d represent a highly significant difference (p ≤ 0.001).
A, a indicate a significant difference of 6 weeks group as compared to initial group within F20%.
B, b indicate a significant difference of 6 weeks group as compared to initial group within F40%.
D, d indicate a significant difference of 6 weeks of F40% as compared to 6 weeks of control.
Absence of letters means non-significant differences

### Table 2: Effects of fructose drinking water (F20% and F40%) on biochemical parameters.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>F20%</th>
<th>F40%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>6 weeks</td>
<td>Initial</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.4 ± 0.5</td>
<td>6.2 ± 0.4</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.8 ± 0.3</td>
<td>3.5 ± 0.4</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>2.7 ± 0.1</td>
<td>2.8 ± 0.3</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>17 ± 2.2</td>
<td>16.8 ± 2.9</td>
<td>16.6 ± 3</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.59 ± 0.2</td>
<td>0.55 ± 0.1</td>
<td>0.67 ± 0.1</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>39 ± 5</td>
<td>37 ± 6.7</td>
<td>41 ± 3.8</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>132 ± 13</td>
<td>115 ± 9</td>
<td>137 ± 11</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.06 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.03</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 10 rats for each group.
A-D represent a significant difference (p ≤ 0.05).
a-d represent a highly significant difference (p ≤ 0.001).
A, a indicate a significant difference of 6 weeks group as compared to initial group within F20%.
B, b indicate a significant difference of 6 weeks group as compared to initial group within F40%.
C, c indicate a significant difference of 6 weeks of F20% as compared to 6 weeks of control.
D, d indicate a significant difference of 6 weeks of F40% as compared to 6 weeks of control.
Absence of letters means non-significant differences

### Table 3: Effects of fructose drinking water (F20% and F40%) on glucose and insulin.

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>F20%</th>
<th>F40%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>6 weeks</td>
<td>Initial</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>6.44 ± 0.40</td>
<td>6.65 ± 0.51</td>
<td>6.31 ± 0.39</td>
</tr>
<tr>
<td>Insulin (µIU/l)</td>
<td>17.8 ± 1.1</td>
<td>18.3 ± 0.8</td>
<td>17.5 ± 1.3</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>5.09</td>
<td>5.40</td>
<td>4.90</td>
</tr>
<tr>
<td>HOMA-β index</td>
<td>121.08</td>
<td>116.19</td>
<td>124.55</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 10 rats for each group.
A-D represent a significant difference (p ≤ 0.05).
a-d represent a highly significant difference (p ≤ 0.001).
B, b indicate a significant difference of 6 weeks group as compared to initial group within F40%.
D, d indicate a significant difference of 6 weeks of F40% as compared to 6 weeks of control.
Absence of letters means non-significant differences
believed that renal damage by fructose may be due to enhancing proteinuria and renal incompetence to excrete fructose.

The serum creatinine level estimation was utilized to monitoring of renal failure progressing. High creatinine may be the byproduct of muscular energy metabolism that shifts to depend on non-glucose sources to attain energy due to insulin resistance to which a little amount of glucose can be utilized. Likewise, the raised level of both serum total bilirubin may indicate liver injury, while serum AST due to various tissues damage (Rodwell et al., 2015).

Serum glucose, insulin, and HOMA-IR were elevated (p ≤ 0.001) in the group of F40%. Table 3. In the same group, HOMA-β index was depressed (p ≤ 0.05). Hyperglycemia that concomitant MS agrees with almost all results of other papers dealt with MS. Actually, it has been believed that insulin resistance evident by higher HOMA-IR is the leading contributor to the pathogenesis of MS (Mehta et al., 2010). In insulin resistance, the normal concentration of insulin cannot give rise to an adequate response or predicted effect, particularly in adipose tissue, muscles, and liver. As a compensatory mechanism pancreatic, beta cells produce more insulin that ensured by depressed HOMA-β index in our findings, to overcome hyperglycemia (Nolan et al., 2015). When insulins combined with their receptors, tyrosine phosphorylation of substrates lead to the activation of two parallel cell signaling pathways; phosphoinositol 3-kinase (PI3K) and mitogen activated protein kinase (MAPK). In the case of insulin resistance, MAPK works ordinaril, in contrast to PI3K which lacks their usual path triggering trouble in related signaling pathways, ultimately reduced endothelial NO production that contributing to atherosclerosis (Lawan et al., 2018).

Lipid profiles were presented in Table 4, although, there were age-related changes, it is clear that the total cholesterol, TG, VLDL, LDL were elevated significantly and HDL decreased as compared with their initial groups and controlled groups. Anyway, disorders related to adipose tissue resulting in a defect in the metabolism of FFAs that worse insulin resistance. However, Rodwell et al., (2015) suggest that large amount of FFAs encourages more production of lipoproteins and promote the process of gluconeogenesis which subsequent in dyslipidemia. Again, insulin resistance may be the creator of dyslipidemia by several means. It is familiar that in normal conditions, insulin suppresses lipolysis in adipocytes. Therefore, when insulin action is unsuitable, lipolysis will grow to produce more FFAs in the liver and subsequently the excess amount of TG will form (Yokozawa et al., 2006). Further, FFAs support formation of apoB is the chief lipoprotein of VLDL. Eventually, serum VLDL will be elevated. Insulin contributes to apoB degradation via the PI3K pathway, but when insulin resistance exists, more VLDL will be produced. Finally, it is believed that insulin regulates the activity of lipoprotein lipase that adjusts VLDL metabolism and clearance so insulin resistance causes dyslipidemia through excessive VLDL production. However, VLDL uptake to remnant lipoprotein and LDL (both accused to the development of atherosclerosis), while TG in VLDL has a preference to uptake to HDL, TG enriched HDL consider favored substrate to the hepatic lipase, therefore HDL clear immediately leading to low serum HDL level (Chiang et al., 2011). Future work should focus on the detailed molecular pathophysiology of induced MS.

CONCLUSION

A well-established rat model of MS could be employed simply by 40% fructose in drinking water, that is ready for the further future work, and it seems that insulin resistance is essential to MS and related biochemical changes. To the knowledge of the authors, certain parameters as urea, creatinine, and AST were examined in MS for the first time.
REFERENCES


