EXPERIMENTAL STUDY OF THE POSSIBLE EFFECT OF VALSARTAN ON INSULIN RESISTANCE IN FRUCTOSE- FED ALBINO RATS

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ABSTRACT
Insulin resistance (IR) is a consequence of high fructose fed diet in rats. The current study was carried out to declare if tumor necrosis factor-alpha (TNF-α) exerts a partial role in the development of IR in non-obese rat model; fructose fed rats (FFR) like that happens in obese rat models. We evaluate the influence of valsartan (a selective blocker of angiotensin receptor type-1) in comparison to metformin (a known insulin sensititizer) on enhancement of insulin sensitivity in FFR. Rats were divided into 2 equal groups (36 rats /group), one group received high fructose diet to induce insulin resistance and the other included standard diet fed rats. Each group is further divided into 3 equal subgroups, (standard diet + saline), (FFR+ saline), (Standard diet + metformin), (FFR+ metformin), (standard diet+ valsartan) and (FFR+ valsartan). In all rats, body weight, fasting serum glucose, fasting serum insulin, insulin sensitivity test, fasting glucose insulin ratio (FGIR), serum TNF-α and serum malondialdehyde (MDA) were measured. Results revealed that administration of valsartan to FFR produced a comparable improvement of insulin resistance. In addition valsartan treatment in FFR produced significant decrease in serum TNF-α and MDA. It could be concluded that TNF-α and angiotensin II might regulate insulin sensitivity in non-obese FFR.

INTRODUCTION
Insulin resistance occurs in a wide
(Standard and high fructose were given throughout the whole study (9 weeks). The rats of each group further divided into 3 equal sub-groups (12 rats each) as the following:

-Sub-group (1): served as a control, received standard diet for 9 weeks and treated in the last 3 weeks of the experiment with intragastric 0.5ml of normal saline (the vehicle used to dissolve drugs).

-Sub-group (2): Received high fructose diet and treated with saline (insulin resistant control).

-Sub-group (3): Comprised rats that fed standard diet for 9 weeks and treated intragastrically with metformin in a dose of 200 mg/kg/day in the last 3 weeks of the experiment (10).

-Sub-group (4): Consisted of rats fed high fructose diet for 9 weeks and after confirmation of being insulin resistant as determined from the results of fasting serum insulin (11), they treated intragastrically in the last 3 weeks with metformin as previously mentioned regimen.

-Sub-group (5): Comprised rats fed standard diet and treated with valsartan in a dose of 30 mg/kg/day, intragastrically in the last 3 weeks of the experiment (12).

-Sub-group (6): FFR treated with valsartan as previously mentioned.

Diet composition according to Fauré et al. (10) is shown in the following table; (table 1):

<table>
<thead>
<tr>
<th>Component ingredient</th>
<th>Standard diet</th>
<th>High fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>38</td>
<td>15.96</td>
</tr>
<tr>
<td>Fructose</td>
<td>-</td>
<td>33.64</td>
</tr>
<tr>
<td>Wheat starch</td>
<td>20</td>
<td>8.40</td>
</tr>
<tr>
<td>Casein</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Cellulose</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Vitamins</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

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RESULTS

Animals fed high fructose diet showed non-significant change in body weight (BW) as compared to rats fed standard diet. Either valsartan or metformin treatment induced no significant change in BW (tab 2).

FFR developed significant insulin resistance as evidenced by impaired response (reduction in plasma glucose) to IP insulin administration and also from fasting serum insulin and FGIR (tab3, 4 &fig1, 2). Furthermore FFR showed significant increase in serum MDA and serum TNF-α levels but they showed no change in fasting serum glucose level. These findings are in comparison to rats fed standard diet (tab 5 & fig 3). Daily intragastric administration of either metfor-

min (200 mg/kg/day) or valsartan (30 mg/kg/day) in the last 3 weeks of the experiment to FFR induced significant enhancement of insulin sensitivity and improvement of FGIR. In addition, these rats showed significant decrease in fasting serum insulin, serum MDA, serum TNF-α levels and no significant change in fasting serum glucose level. These results are in comparison to FFR untreated rats and rats fed standard diet. Furthermore administration of either metformin or valsartan as in the previous regimen to rats fed standard diet produced non-significant changes in all parameters as compared to that fed standard diet untreated group (tab3, 4,5 fig1, 2,3).
Table (4): Fasting glucose insulin ratio (FGIR) in rats fed either standard diet or high fructose diet (drug treated and untreated), means ± SEM

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Standard diet for 9 weeks and treated with saline in the last 3 weeks</th>
<th>FFR for 9 weeks and treated with intragastric saline in the last 3 weeks</th>
<th>Standard diet + metformin (200 mg/kg/day, intragastric in the last 3 weeks)</th>
<th>FFR+ Metformin 200mg/kg/day, intragastric in the last 3 weeks</th>
<th>Standard diet + valsartan 30 mg/kg/day, intragastric in the last 3 weeks</th>
<th>FFR + valsartan 30mg/kg/day intragastric, in the last 3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting serum glucose (mg/dl)</td>
<td>98.3±1.1</td>
<td>100.2±1.7</td>
<td>96.5±1.4</td>
<td>99.1±1.3</td>
<td>99.2±2.4</td>
<td>101.1±0.7</td>
</tr>
<tr>
<td>Fasting serum insulin (µU/ml)</td>
<td>13.1±0.05</td>
<td>35.3±3.2*</td>
<td>13.3±0.1</td>
<td>14.1±0.3</td>
<td>13.8±0.06</td>
<td>14.1±0.01</td>
</tr>
<tr>
<td>FGIR</td>
<td>7.5</td>
<td>2.8</td>
<td>7.3</td>
<td>7.03</td>
<td>7.1</td>
<td>7.17</td>
</tr>
</tbody>
</table>

* P value is significant (< .05) between high fructose fed group treated with saline and standard diet fed group treated with Saline.

FFR = Fructose fed rats.
IS = Insulin sensitive (FGIR ≥ 7).
IR = insulin resistance (FGIR < 7).
SEM = Standard error of mean.

Table (5): Effect of administration of either metformin (200mg/kg/day) or valsartan (30mg/kg/day) in the last 3 weeks, intragastrically on fasting serum glucose, fasting serum insulin, serum MDA and serum TNF-α in FFR and in rats fed standard diet. Mean ± SEM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Standard diet fed rats + saline treatment</th>
<th>FFR + saline treatment</th>
<th>Standard diet + Metformin</th>
<th>FFR+ Metformin</th>
<th>Standard diet + valsartan</th>
<th>FFR + valsartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting serum glucose (mg/dl)</td>
<td>98.3±1.1</td>
<td>100.2±1.7</td>
<td>96.5±1.4</td>
<td>99.1±1.3</td>
<td>99.2±2.4</td>
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<td>14.1±0.3</td>
<td>13.8±0.06</td>
<td>14.1±0.01</td>
</tr>
<tr>
<td>Serum MDA (µmol/ml)</td>
<td>4.7±0.03</td>
<td>18.3±1.1</td>
<td>4.9±0.02</td>
<td>9.3±0.3</td>
<td>5.1±0.1</td>
<td>10.2±0.1</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>0.93±0.001</td>
<td>4.1±0.05</td>
<td>0.96±0.003</td>
<td>0.98±0.03*</td>
<td>0.97±0.02</td>
<td>1.4±0.001*</td>
</tr>
</tbody>
</table>

SEM = Standard error of mean.

FFR = fructose fed rats.
* P value is significant (< .05) between (FFR treated with saline) & (standard diet fed rats treated with saline).
* P value significant (<.05) between (FFR + metformin) and (FFR+ saline).
* Significant difference between (FFR + valsartan) and (FFR + saline).

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DISCUSSION

In the present study, the comparable effect of either administration of metformin or valsartan on insulin resistance induced by high fructose diet was examined. Insulin resistance was evaluated on the basis of FGIR and insulin sensitivity test (13,15). This is in addition to fasting serum insulin where hyper-insulinemia has been used as an index of insulin resistance (15). In the current study rats fed high fructose diet showed a significant insulin resistance as evidenced by impaired response to IP insulin injection in comparison to rats fed standard diet (table 3,4 & fig1, 2). These results are in consistent with previous studies (18,19,20,21); as they concluded that high fructose diet induced insulin resistance in rats within 6 weeks due to post-receptor defects. These defects inhibited transport of insulin, which decreased the ability of insulin to stimulate glucose uptake to muscle and fat cells. In addition they reported that this insulin resistance was accompanied by increased fasting serum insulin but with production of non-significant change in fasting serum glucose, which is also in agreement with the results of the current work.
et al. (29) found that AT1 receptors blockade increased insulin sensitivity in KK-Ay mice via stimulating insulin signaling cascade and consequent enhancement of GLUT4 translocation to plasma membrane. In consistent with the present study, Lau et al., Zhao et al., and Juan et al (30,31,32) demonstrated that oxidative stress plays an important role in AngII-mediated insulin resistance. It has been indicated that reactive oxygen species (ROS) play a pivotal role in the development of insulin resistance and that AngII is involved in the regulation of ROS production.

On the light of the present study, it could be concluded that, TNF-α and Ang II might regulate insulin sensitivity in non-obese normoglycemic rats as proved to exert such effect in obese hyperglycemic rats. In addition our results provide some information to understand the clinical relevance of the effect of angiotensin receptor blocker (ARB); valsartan on insulin sensitivity and the onset of diabetes, thereby preventing cardio-vascular events associated with insulin resistance in normoglycemic non-obese. We are in need to further human studies to support this observation.

REFERENCES


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الخامسية: لاتعاني من مقاومة لعوامل الأنسولين (غذاء قياسي) وعولجت يومياً عن طريق الفم بدواء الفالسرتان بجرعة 20 مجم/كم في مدى الثلاثة أسابيع الأخيرة من الدراسة.

السادسة: تعاني من مقاومة لعوامل الأنسولين وعولجت مثل الخمسية.

وتم قياس مدى الحساسية لعوامل الأنسولين عن طريق اختبار تأثير حقن وحدة واحدة من الأنسولين المائي لكل كجم من وزن الجسم في الفضاء البريتوتي متبوعاً بقياس مستوى الجلوكوز في البلازما مباشرة قبل حقن الأنسولين وبعد 15 دقيقة، 30 دقيقة، 45 دقيقة من الحقن على التوالي. وكذلك تم تقسيم نسبة الجلوكوز والأنسولين الصائم في المصل وكذلك مستوى معمول تحلل الأورام - ألفا ومستوى المالونيدايد (كعاملة للأكسدة).

ويمكن تلخيص نتائج هذا البحث في النقاط الآتية:

- تغذية الفئران بغذاء عالى المحتوى من سكر (الفركتوز) أدى إلى حدوث مقاومة لعوامل الأنسولين التي ظهرت في صورة خلل ذو دلالة إحصائية في الاستجابة لحقن الأنسولين في الفضاء البريتوتي وكذلك في مستوى الأنسولين الصائم في المصل وكذلك على نسبة كل من الجلوكوز والأنسولين الصائم مع عدم التأثير على مستوى الجلوكوز الصائم في المصل. كما صحب ذلك ارتفاع ذو دلالة إحصائية في مستوى معمول تحلل الأورام - ألفا ومستوى المالونيدايد في المصل.

- عند أعطاء أي من دواء الميتفورمين أو الفالسرتان للذئاب التي تعاني من مقاومة لعوامل الأنسولين حدث تأثير إيجابي ومخيد على المقاومة لعوامل الأنسولين وكذلك حدث نقص ذو دلالة إحصائية في مستوى الأنسولين الصائم في المصل، مع معمول تحلل الأورام - ألفا وكذلك المالونيدايد مع عدم حدوث تغيير في مستوى الجلوكوز الصائم وذلك بالمقارنة مع الفئران التي تعاني من المقاومة ولم تعط هذه الأدوية.

وعلى ضوء هذه يمكن استنتاج أن لدواء الفالسرتان تأثير مقارب لدواء الميتفورمين على التحكم في التأثير المقاوم لعوامل الأنسولين في الفئران غير السمينة وذلك عن طريق اقتراح مستوي معمول تحلل الأورام - ألفا وكذلك الشفوق الحرة.