Insulin Resistance Induced by a High Fructose Diet in Rats Due to Hepatic Disturbance

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ABSTRACT

High consumption of dietary fructose is accused of being responsible for the development of the insulin resistance (IR) syndrome. Concern has arisen because of the realization that fructose, at elevated concentrations, can promote metabolic changes that are potentially deleterious. Among these changes is IR which manifests as a decreased biological response to normal levels of plasma insulin. Therefore, this experiment was designed to evaluate the role of high fructose diet on metabolic syndrome in rats.

The experimental animals were divided into two batches. The control batch received a control diet; the second batch was given a high-fructose diet as the sole source of carbohydrate. The rats were continued on the dietary regimen for 1, 2 & 3 months. After the experimental periods, fructose-fed rats groups showed significant elevations in the levels of glucose, insulin sensitivity, liver function tests, nitric oxide and tumor necrosis factor-α when compared to their corresponding values in the rats fed normal diet. Moreover, liver lipid peroxidation [thiobarbituric acid-reactive substance (TBARS) and lipid hydroperoxide concentrations were remarkably increased in high-fructose-fed rats according to the time of administration (1, 2 & 3 months). On the other hand, the activities of enzymatic antioxidants (glutathione reductase and glutathione peroxidase) and glyoxalase I & II were significantly declined in this group. In conclusion, high fructose feeding raises liver dysfunction and causes the features of metabolic syndrome (insulin resistance) in rats dependent on the time of administration due to different mechanisms which were discussed in this work according to available recent researches.

Key Words: Insulin Resistance / Fructose / Hepatic Disturbance / Rats.

INTRODUCTION

Insulin resistance syndrome (IR) is a cluster of related variables that included resistance to insulin stimulated glucose uptake, glucose intolerance, hyperinsulinemia, hypertension and dyslipidemia ¹. Insulin resistance and many facets of the metabolic syndrome are often linked to the macronutrient content of the diet and there is evidence that excessive consumption of macronutrients such as carbohydrates, fats and even protein may eventually lead to the development of insulin resistance ². Insulin resistance can also be induced by consumption of refined carbohydrates and excessive intake of these may be particularly deleterious with respect to increase the risks of insulin resistance ³. Specifically, diets high in fructose have been shown to contribute to weight gain, hyperlipidemia and metabolic disturbances ⁴.

Fructose is widely used as sweetener in food processing ⁵. It is sweeter than glucose (over than 2-folds) and it is transformed into lipids in the fastest pathway among all carbohydrates and therefore...
blamed for serious atherogenic effect. Fructose does not have insulin releasing effect and does not need insulin for its metabolism (6).

The consumption of fructose has increased worldwide in the past two decades. This increase is largely because of an augmentation in the consumption of soft drinks and many other beverages high in fructose and the consumption of foods such as breakfast cereals, baked goods, condiments and desserts sweetened with sucrose and high fructose corn syrup (7). Furthermore, consumption of a high-fructose diet promotes development of three of the pathological characteristics associated with metabolic syndrome: visceral adiposity, dyslipidemia and insulin resistance (7).

In human, insulin resistance may result from inherited factors or it may develop through lifestyle and environmental effectors (8). Non-genetic factors such as increased consumption of dietary fructose might be one of the environmental factors contributing to the development of obesity and the accompanying abnormalities in insulin resistance syndrome (7). The glucose metabolism and glucose uptake pathways are disturbed through an overload of fructose. These perturbed metabolisms caused enhanced rate of lipogenesis and triacylglycerol synthesis through high concentration of glycerol and acyl molecules from fructose catabolism which leads to insulin resistance observed in both human and animal models (9).

Also, Animal studies have suggested that a diet high in fructose (>60/100g) induces insulin resistance in animals (10). Fructose consumption has been shown to induce dyslipidaemia, low grade hepatic inflammation and the activation of stress-sensitive pathways in the liver. There is evidence for lipotoxicity in the livers of fructose-fed animals. Furthermore, high-fructose diet-fed rats display hepatic oxidative damage and altered lipid metabolism due to hepatic stress as a result of the burden of fructose metabolism (11).

The current study focused on insulin resistance, hyperglycemia, hyperinsulinemia and increase in lipid peroxidation associated with an exaggerated response to glucose challenge due to the increment dietary fructose consumption in rats which might be one of the environmental factors contributing to the development of oxidative stress due to hepatic disturbance.

**MATERIAL AND METHODS**

Forty male albino rats (*Rattus rattus*) whose body weight ranged from 100±10g were reared in the Animal House of Nuclear Research Center, Inshas, Egypt and cared for in accordance with the institution’s guidelines. The animals were housed in an environmentally controlled room that was maintained at a temperature of 22±2°C and humidity 55±5%, with a 12-hour light/dark cycle. The animals received a standard pellet diet (12) and tap water *ad libitum*.

After acclimatization for one week, the animals were randomly divided into four groups (n=10 in each group). The first group was fed on a control diet, containing 50% corn starch, 20% soya bean meal, 17% sugar, 5% corn oil, 5% cellulose and 3% vitamins & mineral mixture. The 2nd, 3rd and 4th groups were fed on the same composition as the control diet, except that corn starch was replaced with an equal amount of fructose for one, two and three months respectively. At the end of each experimental period, animals were scarified and blood samples were collected from rats on each time interval. Sera were separated and divided into considerable aliquots to avoid the effects of repeated thawing and freezing. All specimens were stored at -40°C until use. Liver samples were carefully excised from each rat, immediately immersed and washed carefully with cold physiological saline solution (0.9% NaCl), quickly frozen in liquid nitrogen to avoid any distraction in the tissue and finally stored at -40°C till determination.
Determination of carbohydrate related parameters:

Serum glucose concentration was estimated by enzymatic method of Trinder (13). The kit was purchased from Diagnosticum Zrt. Co., Budapest, Hungary.

Serum rat insulin was estimated by radioimmunoassay (RIA) using solid phase component system according to the methods of Marschner et al. (14). The kits were purchased from Diagnostic Millipore Co, Missouri, USA.

The homeostatic model assessment (HOMA) value as a measure of IR was calculated using the following formula: fasting insulin (µU/L) × fasting glucose (mmol/L)/22.5 as described by Matthews et al. (15).

Determination of serum liver profile:

The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT) and lactate dehydrogenase (LDH) were determined kinetically according to Bergmeyer et al. (16), Rosalki & Tarlow (17) and Buhl & Jackson (18) respectively. The kits were purchased from Diagnosticum Zrt. Co., Budapest, Hungary. The concentrations of serum total bilirubin, total protein and albumin were estimated colourimetrically using commercial kits (Bio-Tec, U.K) according to Jendrassik & Grof (19), Weichselbaum (20) and Doumas et al. (21) respectively.

Determination of enzymes antioxidants:

Glutathione reductase (GR) and glutathione peroxidase (Gpx) activities were assayed using enzyme linked immunosorbent assay (ELISA) according to Goldberg and Spooner (22) and Zhang et al. (23) respectively. The kits were purchased from Bio Vision’s Co. USA.

Determination of lipid peroxidation:

Lipid hydroperoxide (LHP) in hepatic tissue was determined using ELISA technique according to Nakamura & Maeda (24). The kit was purchased from amsbio, Life Science, Int. Co. USA. However, hepatic thiobarbituric acid reactive substance (TBARS) was measured using a commercial enzyme immunoassay (EIA) technique according to Pedeson et al. (25). The analysis was performed with a colorimetric commercial kit (OXIS Co., USA).

Determination of serum nitric oxide:

Total nitrite concentration, as an index of tissue NO level was determined by the method of Rock et al. (26) using Griess reagent. Nitrate was reduced to nitrite by the addition of nitrate reductase. The samples were treated with 70% sulphosalicylic acid. The sulphanilamide–diazonium salt was then reacted with n- (1-naphthyl) ethylenediamine (0.3%) to produce a chromophore, the color of which was read at 540nm. So, total nitric oxide (TNO) was estimated in serum using a commercial enzyme immunoassay (EIA) technique (Cayman Chemical Company, USA).

Determination of serum rat tumor necrosis factor-α (rat TNF-α):

Serum rat tumor necrosis factor-α (rat TNF–α) was determined using ELISA technique (R&D Systems, Inc.; Minneapolis, USA).
Determination of glyoxalase I & II :

Glyoxalase I was assayed by measuring the rate of formation of S-D-lactoylglutathione. The assay mixture contained 7.9mM MG, 1mM GSH, and 14.6mM magnesium sulfate, and 182mM imidazole HCl, pH 7.0. After 5 minutes, 0.1 ml of sample (50 µg protein) was added and increase in absorption at 240 nm was measured and the activity was calculated using the coefficient 2.86/mM/cm\(^{(27)}\). The enzyme activity is calculated as µmole/min/g of the product formed. One unit of the enzyme is defined as the amount of enzyme catalyzing the formation of 1µmol of S-D-lactoylglutathione/min/mg protein under the assay conditions.

Glyoxalase II was assayed by measuring formation of GSH from S-D-lactoylglutathione. The reaction was started by the addition 0.5ml of 1.5mM S-D-lactoylglutathione to 0.1ml of sample and GSH formation was measured after 15 min by reaction with 0.75 mM DTNB. The activity was expressed as mmol GSH formed/min/mg protein \(^{(28)}\). The kits of glyoxalase I & II were purchased from amsbio, Life Science Int. Co. USA.

Statistical analysis :

Statistical deference between the means were assayed by using one way analysis of variance (ANOVA) followed by Duncan’s multiple range tests according to Snedecor & Cochran \(^{(29)}\).

RESULTS AND DISCUSSION

In this work, the fructose fed rats represents a model for the metabolic syndrome including insulin resistance and growing evidence suggests a role for inflammation and oxidative stress in this model. Dietary fructose is a monosaccharide which can induce metabolic disorders including insulin resistance, hyperinsulinemia, hypertension and dyslipidemia which is of pathophysiologic importance for the development of diabetes and atherosclerosis \(^{(30)}\). There are many reports in the literature describing an increase in body weight, glycemia and insulinemia with the utilization of high-fructose diets in both animal \(^{(31)}\) and human \(^{(32)}\).

In the current study, consumption of high fructose diet to rats led to a considerable increment in both levels of serum glucose and insulin which produce a remarkable reduction in the insulin sensitivity which is indicated by higher values of insulin resistance (HOMA) as compared to their corresponding normal group for three time intervals (Table 1). These results may be due to a defect in insulin binding caused by decreasing receptor number or their affinity, or/and defects at the level of effect molecules such as glucose transporters and activities of their enzymes involved in glucose metabolism \(^{(33)}\). Moreover, these data may be attributed to the alterations in insulin signaling which led to a remarkable disturbance in de novo lipogenesis and leptin regulation as a result of hepatic metabolism of fructose \(^{(34)}\).

Glucose uptake pathway and glucose metabolism are disturbed through an overload of fructose. These perturbed metabolisms caused enhancement in the rate of lipogenesis and triacylglycerol synthesis through high concentration of glycerol and acyl molecules from fructose catabolism which leads to insulin resistance observed in both human and animal models \(^{(9)}\).

In short-term studies in human, fructose ingestion did not have a deleterious effect on glucose metabolism except when it was fed in very large amounts. On the contrary, it generally improved glycemic control, presumably because only a small proportion of ingested fructose is converted to glucose \(^{(35)}\). In one study, nine healthy individuals, 10 with impaired glucose tolerance, and 17 with type II diabetes were given a 50-g load of glucose, sucrose or fructose. In all three groups, ingestion of
fructose, compared with glucose or sucrose, when given either alone or with a meal, resulted in significantly lower insulin responses, serum glucose levels and glycosuria (36).

Table (1): Disturbance in carbohydrate related parameters in fructose fed rats at different time intervals (Mean ± SE).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>High fructose diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>One month</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.263 ± 0.74 A</td>
<td>10.951 ± 0.87 B</td>
</tr>
<tr>
<td>Insulin (µU/L)</td>
<td>26.941 ± 1.06 A</td>
<td>34.817 ± 1.26 B</td>
</tr>
<tr>
<td>Insulin Resistance (HOMA)</td>
<td>7.499 ± 0.81 A</td>
<td>16.946 ± 1.17 B</td>
</tr>
</tbody>
</table>

- Means bearing different superscripts (A,B,C&D) within the same row are differ significantly (P<0.05).

Houstis et al. (37) recorded a significant increase in the glucose and insulin concentration in rats fed high fructose diets, indicating that the ability of insulin to stimulate glucose disposal is markedly impaired in peripheral tissues associated with insulin resistance by fructose feeding. Also, Suga et al. (38) suggested that fructose feeding decreases the efficacy of insulin extraction by the liver, which retards insulin clearance from the circulation.

Hepatic metabolism of fructose leads to alterations in the activities of key enzymes of glucose metabolism and activation of stress sensitive pathway that may desensitize insulin signaling (11). Fructose is phosphorylated in the liver by adenosine triphosphate to form fructose-1-phosphate. The reaction is catalyzed by the enzyme fructokinase. Fructose-1-phosphate is split by aldolase-B into glyceraldehyde and dihydroxyacetone phosphate. Both of which can be further metabolized in the glycolytic pathway. In contrast, hepatic glucose metabolism is limited by the capacity to store glucose as glycogen and, more importantly, by the inhibition of glycolysis and further glucose uptake resulting from the effects of citrate and ATP to inhibit phosphofructokinase. The products of fructose metabolism in the glycolytic pathway of the liver are glucose, glycogen, lactate and pyruvate. Because fructose uptake by the liver is not inhibited at the level of phosphofructokinase, fructose consumption results in larger increases of circulating lactate than does consumption of a comparable amount of glucose (38).

There is now much emerging evidence that chronic consumption of high-fructose diets contributes to excessive formation of reactive oxygen species (ROS). This leads to induced oxidative stress and mediated insulin resistance (37). Moreover, an increase in cellular ROS accumulation directly triggers the activation of serine/threonine kinase cascades such as c-Jun N-terminal kinase and nuclear factor-kappa B that, in turn, phosphorylate multiple targets, including the insulin receptor and the insulin receptor substrate (IRS) proteins (39). Increased serine phosphorylation of IRS directly decreases its ability to undergo tyrosine phosphorylation and accelerate the degradation of IRS-1, causing impaired glucose uptake in muscle, liver and adipose tissues (39).

In the current study, hepatic damage in fructose-fed rats was evident from the elevation in the activities of AST, ALT, GGT, LDH associated with a significant increment in the level of bilirubin levels as well as the decline in total protein and albumin levels compared to their values in the corresponding normal fed rats groups throughout three time intervals (1, 2 & 3 months) (Table 2). These biochemical changes reflected the hepatocellular damage including a reduction in the synthetic capacity of the liver in fructose-fed rats. Liver damage was further confirmed by decreased activity of glutathione peroxidase and glutathione reductase as well as significant increases in the levels of
TBARS and lipid hydroperoxide (Table 3). These data are in agreement with other investigations that reported a significant increase in lipid peroxidation and reduction of hepatic antioxidant enzyme activities in rats fed high fructose diet \(^{40,41}\).

Additional evidence that fructose can cause liver damage is that intravenous administration of fructose (250 mg/kg of body weight over five minutes) to healthy volunteers resulted in a 75% reduction in the hepatic concentration of adenosine triphosphate \(^{42}\). Patients with obesity-related non-alcoholic steatohepatitis recovered significantly more slowly from fructose-induced hepatic adenosine triphosphate (ATP) depletion than did healthy age and sex-matched controls \(^{43}\). The effect of oral fructose on hepatic ATP levels has not been examined. However, the findings from the intravenous studies are consistent with the hypothesis that ingestion of large amounts of fructose could overwhelm the capacity of the liver to metabolize it, resulting in transient hepatic dysfunction. Repeated episodes of fructose-induced hepatic stress could lead to progressive hepatic injury. Moreover, fructose appears to be more toxic to a disease liver than to a healthy one \(^{44}\).

Systemic oxidative stress is associated with insulin resistance, which manifests as decreased glutathione peroxidase (GPx) and glutathione reductase (GH-R). This is possibly due to increase the oxidative stress on one hand and decrease the activities of different antioxidative enzymes on the other. The significant reduction of glutathione peroxidase and glutathione reductase in rats fed high fructose diet for three time intervals (1, 2 & 3 months) could be attributed to the fact that the high fructose delivery to the liver may generate stress activating molecules, such as methylglyoxal and/or d-glyceraldehyde. These molecules can serve as substrates for advanced glycation end products (AGEs) which could activate NADPH oxidase in endothelial cells \(^{11}\). Activation of NADPH oxidase could also cause endothelial cells lack cystathionine β synthase (CβS).

### Table (2): Disturbance in liver profile in fructose fed rats at different time intervals (Mean ± SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>High fructose diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>One month</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>123.71 ± 2.46 (^A)</td>
<td>160.75 ± 2.86 (^B)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>29.63 ± 1.27 (^A)</td>
<td>44.89 ± 1.52 (^B)</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>14.52 ± 0.91 (^A)</td>
<td>32.71 ± 1.34 (^B)</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>271.35 ± 3.47 (^A)</td>
<td>311.89 ± 3.87 (^B)</td>
</tr>
<tr>
<td>T. Bilirubin (mg/dL)</td>
<td>0.29 ± 0.03 (^A)</td>
<td>0.58 ± 0.07 (^B)</td>
</tr>
<tr>
<td>T. Protein (g/dL)</td>
<td>6.28 ± 0.49 (^A)</td>
<td>5.71 ± 0.44 (^B)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.11 ± 0.31 (^A)</td>
<td>3.76 ± 0.27 (^B)</td>
</tr>
</tbody>
</table>

- Means bearing different superscripts (A,B,C&D) within the same row are differ significantly (P<0.05).

Reactive oxygen species (ROS) can themselves reduce the activity of antioxidant enzymes. The abnormal increase of H\(_2\)O\(_2\), which has high membrane permeability, induces toxic injury by conversion to HO \(^-\) via the Fenton reactions in liver, which is rich in Fe\(^{2+}\) and Cu\(^{1+}\) \(^{45}\). Glutathione peroxidase is a selenium-containing enzyme, which catalyzes the conversion of hydrogen peroxide and various hydroperoxides using GSH as a reducing agent to form water and corresponding alcohols respectively \(^{46}\). The observed depletion in GP\(_X\) activity could be a consequence of the deficiency of
selenium than other selenoproteins \(^{(47)}\) and/or elevation in plasma nitric oxide level that may inactivate or modify several enzymes that catalyze reactions essential metabolism including GPx \(^{(48)}\). Furthermore, Datta \textit{et al.} \(^{(49)}\) reported that the depression in SOD activity in fructose fed rats may be due to the elevation in formation of protein glycation by fructose which is more reactive reducing sugar compared to others (glucose and lactose). Also, GSH level was decreased in fructose group could be due to increase in the utilization of trap free radicals and/or decrease the regeneration as evident with the lower activity of glutathione reductase enzyme.

This study examined two indices of lipid peroxidation; the TBARS and the lipid hydroperoxides (LHP) in liver tissue of control and experimental animals. Although, the thiobarbituric acid (TBA) test is a very non-specific technique, it can offer an empirical window on the complex process of lipid peroxidation and is used widely as a marker. The TBA test analyses the end products derived from hydroperoxide transformation, metabolism or decomposition \(^{(50)}\). In the current study, the levels of TBARS and hydroperoxides were increased in the liver of fructose-fed rats. Fructose feeding can induce free radical formation by a number of mechanisms. It causes downregulation of the key enzymes of the hexose monophosphate pathway, namely glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase that generate a reduced environment in the form of NADPH and NADH \(^{(51)}\). Impaired regeneration of NADPH could result in an increase in the oxidative state of the cell. Further, heightened catabolism of fructose would result in energy depletion in cells, making them more susceptible to peroxidation. In addition to this, hyperglycaemia, hypertriglyceridaemia and hyperinsulinaemia produced by fructose feeding can be related to increased lipid peroxide levels found in these rats \(^{(52)}\).

**Table (3) : Disturbance in enzymes antioxidants, lipid peroxidation and glyoxalase I & II in fructose fed rats at different time intervals (Mean ± SE).**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>High fructose diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>One month</td>
</tr>
<tr>
<td>Glutathione reductase (µmol of NADPH oxidized/hr/mg protein)</td>
<td>34.823 ± 1.07 (^{A})</td>
<td>30.569 ± 0.92 (^{B})</td>
</tr>
<tr>
<td>Glutathione peroxidase (µmol of GSH utilized/hr/mg protein)</td>
<td>5.421 ± 0.37 (^{A})</td>
<td>5.047 ± 0.332 (^{B})</td>
</tr>
<tr>
<td>Lipid hydroperoxide (nmol/mg tissue)</td>
<td>1.27 ± 0.069 (^{A})</td>
<td>1.653 ± 0.076 (^{B})</td>
</tr>
<tr>
<td>TBARS (nmol/mg tissue)</td>
<td>1.623 ± 0.079 (^{A})</td>
<td>2.197 ± 0.092 (^{B})</td>
</tr>
<tr>
<td>Glyoxalase-I (µmol/min/mg protein)</td>
<td>13.39 ± 0.732 (^{A})</td>
<td>10.126 ± 0.659 (^{B})</td>
</tr>
<tr>
<td>Glyoxalase-II (µg of GSH consumed/min/mg protein)</td>
<td>5.636 ± 0.392 (^{A})</td>
<td>4.972 ± 0.365 (^{B})</td>
</tr>
</tbody>
</table>

- Means bearing different superscripts (A,B,C&D) within the same row are differ significantly (P<0.05).

So, increased TBARS and LHP levels in liver tissues suggest the elevation in the ROS formation and oxidative deterioration of lipids. The TBARS test analyzes the end-products derived from hydroperoxide transformation, metabolism or decomposition \(^{(53)}\) while LHP measures the rate of initiation of lipid peroxidation and their decomposition to other products. ROS could be enhanced during fructose feeding by well described mechanisms like autooxidation and glycation due to hyperglycemia \(^{(54)}\). These data are recorded in the current work. In addition, hyperinsulinemia,
depletion of ATP due to increased catabolism of fructose, increased aldehyde formation and reduced generation of reducing equivalents could be the other contributing mechanisms (51).

Modulation of the glyoxalase system (glyoxalase I & II) has been observed in fructose-fed rats and during the onset of diabetes as well as in the development of clinical metabolic complications (55). Patients with diabetic complications had a propensity to maintain relatively high levels of plasma glyoxal and S-D-lactoylglutathione as a result of a significant decrease in the activities of glyoxalase I and II (56).

Judging from the data in table (3), rats supplemented with high fructose diet showed a significant (p<0.05) decline in activities of the glyoxalases I and II than those of their corresponding control group depending on the time of administration (1, 2 & 3 months). Reactive aldehydes, particularly methyl glyoxal, that are formed during the normal course of fructose metabolism, are scavenged by an efficient detoxification system that comprises two enzymes, glyoxalases I and II (53). Glyoxalase I catalyse the condensation of GSH with the reactive aldehyde to form hydroxyacylglutathione, while glyoxalase II hydrolyses the product of glyoxalase I to GSH and aldonates (57). Excess methylglyoxal and aldehydes could be formed by the hepatic metabolism of fructose through the glycolytic intermediate. These products can in turn activate the stress response pathways. Modulation of the glyoxalase system occurs during the onset of diabetic complications (40). Decreased glyoxalase I and II activity represents a harmful situation, as the disposal of methylglyoxal as well as products of lipid peroxidation is less efficient (41).

Nitric oxide (NO) is a messenger molecule that plays an important role in a wide variety of physiological functions, including hemodynamics, glucose uptake and anti-inflammatory action (58). In the current study, table (4) showed that total nitric oxide levels were increased in fructose fed rats during the whole experimental periods (1, 2 & 3 months). This change is directly proportional with the time of administration. This result may be due to the alteration in the expression gene of nitric oxide synthase enzyme (eNOS). Several studies have shown that NO is associated with insulin resistance (59,60). These results may be due to reactive oxygen species which reduce NO bioavailability by inactivating it to ONOO’. In this respect, the elevation of serum NOx might indeed and reflect the impairment of NO bioavailability since ONOO’, as well as, NO are metabolized into nitrites and nitrates (61).

Table (4): Disturbance in nitric oxide and tumor necrosis factor-α in fructose fed rats at different time intervals (Mean ± SE).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Insulin Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One month</td>
<td>Two months</td>
</tr>
<tr>
<td>TNO (µmol/L)</td>
<td>20.37 ± 0.97 A</td>
<td>31.492 ± 1.21 B</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>5.182 ± 0.216 A</td>
<td>7.368 ± 0.281 B</td>
</tr>
</tbody>
</table>

- Means bearing different superscripts (A,B,C&D) within the same row are differ significantly (P<0.05).

One factor that plays a role in the development of insulin resistance is the tumor necrosis factor alpha (TNF-α). TNF-α is a pleiotropic cytokine which occurs in many pathological processes such as inflammation, allergy, congestive heart failure, etc. (62). It has been discovered that TNF-α inhibits insulin signal transduction, influence lipid metabolism and modulates many other factors involved in the pathogenesis of insulin resistance [e.g. the peroxisome proliferators activated receptor-γ (PPAR-γ), adiponectin and resistin] (62).
Therefore, TNF-α found in pathophysiological states significantly stimulated LDL receptor function and gene expression. Much attention has been paid to the roles of TNF-α in insulin resistance and lipid metabolism in liver, adipose and muscle tissues. Exogenous TNF-α and overexpression of TNF-α in adipose tissue and muscle are known to induce an insulin-resistant state in both animal models and humans.

In the present study, the levels of tumor necrosis factor-α were significantly (p<0.05) increased in fructose fed rats when compared to control ones throughout the all time intervals (Table 4). The tumor necrosis factor (TNF-α), is thought to represent the first step towards the subsequent development of liver fibrosis. Damaged liver cells generate ROS which stimulate the release of proinflammatory cytokines like TNF-α from hepatocytes and Kupffer cells thereby increasing the severity of inflammatory stress in the liver. Also, because fat accumulates in the liver, there is sustained hepatic generation of proinflammatory cytokines from the Kupffer cells, leading to a vicious cycle of worsening insulin resistance and severity of steatohepatitis. Increased TNF-α production led to suppress insulin receptor signal transduction in fructose-fed rats. Miller & Adeli, suggested a link between inflammation and insulin resistance. A recent study also showed that plasma concentrations of IL-1 and IL-6 were higher in high fructose-fed.

In conclusion, this work suggests that the consumption of excessive amounts of fructose can lead to a wide range of health problems including insulin resistance and liver dysfunction dependent on the duration of fructose intake.

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