Rice Bran Oil Improves Insulin Resistance by Affecting the Expression of Antioxidants and Lipid-Regulatory Genes

Mervat A. Ahmed1 · Mona A. Mohamed2 · Laila A. Rashed3 · Sohaier A. Abd Elbast1 · Elham A. Ahmed1

Abstract The present study investigated the molecular effects of rice bran oil (RBO) on lipid-regulatory genes (sterol regulatory element binding protein-1 [Srebf1] and peroxisome proliferator-activated receptors-α [Ppara]) and the expression of catalase (CAT) and superoxide dismutase (SOD1) genes in insulin-resistant rats. Rats were divided into five groups: animals that received standard diet (control); rats fed standard diet containing RBO as the sole source of fat (RBO); a high-fructose diet (HFD) group, which was further divided into two subgroups: rats fed HFD either for only 1 month (HFD1) or for 2 months (HFD2) and rats fed HFD containing RBO for 1 month; while rats in the last group fed HFD for 30 days then treated with RBO for another 30 days. The HFD induced a state of insulin resistance (IR) as indicated by the hyperinsulinemia and elevated homeostasis model assessment insulin resistance index. Hepatic lipid levels and radical scavenging enzymes were altered by the HFD. Lipid-regulatory genes, Srebf1 and Ppara, were upregulated while Sod1 and Cat were downregulated in insulin-resistant rats. Addition of RBO to the two diet regimens alleviated the disorders of IR to some extent. RBO reduced the hepatic levels of triacylglycerol, malondialdehyde, SREBP, and PPAR-α mRNA. Hepatic SOD and CAT were elevated at gene and protein levels. The HFD induces de novo lipogenesis by upregulating the lipid-regulatory genes resulting in increased serum and hepatic triacylglycerol. Moreover, IR induced by the HFD caused a state of oxidative stress. Supplementation of RBO to fructose-fed rats not only improves insulin resistance but also downregulates lipogenic genes and improves the unbalanced oxidative status.

Keywords Antioxidant enzymes · Insulin resistance · PPAR-α · Rice bran oil · SREBP-1


Abbreviations

- ALT: alanine aminotransferase
- AMV: avian myeloblastosis virus
- AST: aspartate aminotransferase
- CAT: catalase
- dNTP: deoxy nucleotide triphosphate
- GPx: glutathione peroxidase
- HFD: high-fructose diet
- HMG-CoA: 3-hydroxy-3-methyl glutaryl coenzyme A
- HOMA-IR: homeostasis model assessment insulin resistance
- IR: insulin resistance
- MDA: malondialdehyde
- NADPH: nicotinamide adenine dinucleotide phosphate
- (reduced form)
- PPAR-α: peroxisome proliferator-activated receptors-α
- RBO: rice bran oil
- SOD: superoxide dismutase
- SREBP-1: sterol regulatory element binding protein-1
- TAG: triacylglycerol
Introduction

Insulin resistance (IR) is one of the main causes of metabolic syndrome, which is increasing at a terrifying level, due to the increasing consumption of fructose (Abdel-Kawi, Hassanin, & Hashem, 2016; Guo, 2014; Tappy & Lé, 2010). A high-fructose diet (HFD) has been used to induce insulin resistance characterized by hyperglycemia and hyperinsulinemia in rodents (Kannapan, Jayaraman, Rajasekar, Ravichandran, & Anuradha, 2006; Mahfouz, Ghanem, & Mohamed, 2010; Rajasekar, Kaviarasen, & Anuradha, 2005).

Rice bran oil (RBO) is characterized by its mild flavor, high smoke point, and long shelf stability, in addition to its high content of linoleic and oleic fatty acids and natural antioxidants such as y-oryzanol, phytosterols, tocopherols, and tocotrienols (Friedman, 2013; Liang et al., 2014). RBO was reported as a lipid-lowering agent (Abd Elbast, Rashed, Mohamed, Ahmed, & Ahmed, 2016; Berger et al., 2005), which protects against diabetes mellitus (Posuwan et al., 2013), cancers (Shih et al., 2011), brain aging, and neurodegenerative diseases (Stephanie et al., 2013) due to its antioxidant content.

The current study was designed to investigate the molecular effects of RBO on lipid metabolism through studying the expressions of the hepatic lipid-regulatory genes, sterol regulatory element binding protein-1 (Srebf1), peroxisome proliferator-activated receptors-α (Ppara), and the antioxidant enzymes superoxide dismutase (Sod1) and catalase (Cat) in insulin-resistant rats.

Materials and Methods

Animals

A total of 50 adult female Sprague Dawley rats weighing 140–220 g were used throughout this study. These animals were purchased from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Helwan, Egypt). Rats were divided into five groups (10 rats each) and housed in steel cages (five per cage) at constant environmental temperature (25 ± 5°C) and humidity (50 ± 10%) with a dark and light cycle (12 h). Animals were maintained for a week on a standard diet as an acclimatization period. Food and water were provided ad libitum. The use of animals in these experimental procedures were reviewed and approved by the Research Ethics Committee (Faculty of Science, Al-Azhar University, Egypt).

Preparation of Diets

The standard and high-fructose (60 g/100 g) diets were prepared as described previously by Rajasekar et al. (2005), while the diet containing RBO (10%) was prepared according to Wang et al. (2007).

Study Design

Animals were divided into five groups: group I: normal control (NC): animals fed standard diet and left intact without any treatment. Group II: RBO: rats in this group fed normal diet contain RBO as the sole source of fat. Group III: HFD: this group was further divided into two subgroups: rats in each subgroup fed HFD either for only 1 month (HFD1) or 2 months (HFD2) serving as reference groups for the corresponding treated groups. Group IV: (HFD1 + RBO): rats in this group fed HFD containing RBO for 1 month. Group V: (HFD2 + RBO): rats in this group fed HFD for 30 days, and then received HFD with RBO for another 30 days. The animals were maintained in their respective groups for 4 weeks except group V. Body weights of rats in all groups were recorded weekly throughout the experimental period and body weight gain was calculated at the end of the feeding period.

Blood Collection and Tissue Sampling

At the end of the experimental period, rats were weighed then anesthetized with urethane (99%, Sigma-Aldrich, St. Louis, MO, USA) at a dose of 1 g/kg body weight intraperitoneally. Blood samples were taken from the retro-orbital venous plexus after overnight fasting. Each blood sample was divided into two tubes: a fluoride-containing tube for plasma glucose determination and a plain tube for the biochemical analysis. Blood was centrifuged at 4000 rpm for 5 min, then serum obtained was kept at −20°C.

Liver was quickly removed and perfused with phosphate buffer saline (pH 7.4) to remove any red blood cells and clots, dried by filter paper, and weighed.

Preparation of Liver Homogenate for Lipid Determination

Hepatic cholesterol and triacylglycerol (TAG) were extracted according to the method of Bligh and Dyer (1959) with some modifications. Briefly, 0.5 g of liver was homogenized with a mixture solvent of chloroform and methanol (2:1) of 5 mL. The tissue homogenate was centrifuged at 4000 rpm for 15 min where two layers were separated. The upper methanol-water layer was removed by suction and the lower chloroform layer containing the total lipids was evaporated in a water bath at 40°C till dryness. The dried lipid extract was redissolved in 1 mL methanol, and then applied for determination of total cholesterol and triacylglycerol.
Preparation of Liver Homogenate for the Determination of Malondialdehyde, CAT, and SOD1

The tissue homogenate was prepared according to the method of Nishikimi, Roa, and Yogi (1972). Exactly 0.5 g of hepatic tissue was weighed and homogenized using an automatic homogenizer (RAT-MICCRA D-S, MICCRA GmbH, Heitersheim, Germany) in 10 mL of ice-cold 0.05 mM potassium phosphate buffer solution (pH 7.4) to yield ultimately 5% (w/v) whole liver homogenate. The homogenates were centrifuged at 5000 rpm for 15 min at 4°C, and then the supernatant was used for the determination of malondialdehyde (MDA), SOD, and CAT.

**Biochemical Assay**

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using commercial assay kits (Diamond Diagnostics, Cairo, Egypt). The fasting plasma glucose level was determined using the enzymatic colorimetric method (Sharp, 1972), while serum insulin was measured using the enzyme-linked immunoassay (Rat Insulin ELISA kit, Glory Science Co., Del Rio, TX, USA) (Dahir, Cook, & Self, 1992). The homeostasis model assessment insulin resistance (HOMA-IR) index was calculated according to the formula (Pickavance, Tadayyon, Widdowson, Buckingham, & Wilding, 1999):

\[
\text{HOMA-IR} = \left(\frac{\text{fasting insulin (μIU/mL)} \times \text{fasting glucose (mmol/L)}}{22.5}\right)
\]

Hepatic total cholesterol and triacylglycerol were extracted as mentioned and were determined using commercial assay kits (Diamond Diagnostics). Hepatic MDA was assayed according to the method of Ohkawa, Ohishi, and Yagi (1979), while hepatic SOD1 and CAT were determined according to the methods of Nishikimi et al. (1972) and Aebi (1984), respectively.

**Real-Time Polymerase Chain Reaction**

**RNA Isolation**

Hepatic mRNA was extracted from liver using a single-step method using TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was incubated at 70°C for 10 min to prevent secondary structures. The RNA was supplemented with MgCl₂ (25 mM), RTase buffer (10×), deoxy nucleotide triphosphate (dNTP) mixture (10 mM), oligo d(t) primers, RNase inhibitor (20 U), and avian myeloblastosis virus (AMV) reverse transcriptase (20 U/μL). This mixture was incubated at 42°C for 1 h. Primer sequences are shown in Table 1.

**Reverse Transcription**

Exactly 1 μg of RNA was used in the subsequent cDNA synthesis reaction, which was performed using the Reverse Transcription System (Promega, Madison, WI, USA). Total RNA was incubated at 70°C for 10 min to prevent secondary structures. The RNA was supplemented with MgCl₂ (25 mM), RTase buffer (10×), deoxy nucleotide triphosphate (dNTP) mixture (10 mM), oligo d(t) primers, RNase inhibitor (20 U), and avian myeloblastosis virus (AMV) reverse transcriptase (20 U/μL). This mixture was incubated at 42°C for 1 h. Primer sequences are shown in Table 1.

**Quantitative Real-Time Polymerase Chain Reaction**

Quantitative real-time polymerase chain reaction was performed in an optical 96-well plate with an ABI PRISM 7500 fast sequence detection system (Applied Biosystems, Carlsbad, CA, USA) under universal cycling conditions (10 min at 95°C followed by 40 cycles of 15 s at 95°C and 60 s at 60°C). Reaction contained SYBR Green Master Mix (Applied Biosystems), gene-specific forward and reverse

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequence Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ppara</td>
<td>Forward: 5’ACTGTTGCAAGAGATCTACAGAG3’</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’TTGTCTGTCACGTTCGAAATC3’</td>
</tr>
<tr>
<td>Srebf1</td>
<td>Forward: 5’AGCCCAAGGAAAGAGAT3’</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’GCCATAGTGCAAGGT3’</td>
</tr>
<tr>
<td>Sod1</td>
<td>Forward: 5’GCAGAAGGCAAAGGTGA3’</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’TAGCAGGACAGCATGAG3’</td>
</tr>
<tr>
<td>Cat</td>
<td>Forward: 5’TATTGCCTTCGGATTCT3’</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’ATGCCCTGTCAGTTGCT3’</td>
</tr>
<tr>
<td>Actb</td>
<td>Forward: 5’TCTGGCAACACACCTTACATG3’</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’AGC ACAGCCTGGATAGCAG3’</td>
</tr>
</tbody>
</table>

CAT, catalase; PPAR-α, peroxisome proliferator-activated receptors-α; RT-PCR, real-time polymerase chain reaction; SOD, superoxide dismutase; SREBP-1, sterol regulatory element binding protein-1
primers (10 μM), and cDNA and nuclease-free water. Data were analyzed with the ABI Prism sequence detection system software and quantified using the v17 Sequence Detection Software from PE Biosystems (Foster City, CA, USA). Relative expression of studied genes was calculated using the comparative threshold cycle method. All values were normalized to the beta actin, which was used as the control housekeeping gene (Livak & Schmittgen, 2001).

Statistical Analysis

The data are presented as mean ± SE. One-way ANOVA followed by post hoc LSD analysis was performed using the statistical package for social science version 16 to compare all the treated groups. The value of \( p \leq 0.05 \) was considered statistically significant.

Results

Table 2 revealed significant elevation \(( p < 0.001)\) in serum ALT in both HFD1 and HFD2 groups (with % changes 53.27 and 35.92, respectively), compared to the NC group. Moreover, the HFD1 group exhibits significant elevation \(( p < 0.02)\) in the serum level of ALT, compared to the RBO group. In addition, significant reductions \(( p < 0.01)\) were observed in the serum ALT level in both the prophylactic (HFD1 + RBO) and therapeutic (HFD2 + RBO) groups, compared to the HFD1 and HFD2 groups. On the other hand, the level of serum AST was significantly reduced \(( p < 0.001)\) in HFD1 and HFD1 + RBO, compared to the NC group. Serum AST showed significant elevation \(( p < 0.001)\) in the HFD2 group, compared to the HFD1 group.

Plasma glucose levels were significantly elevated \(( p < 0.001)\) in rats fed HFD for 1 month with % changes reaching 50.33 and 53.4, compared to the NC and RBO groups, respectively (Table 2). Addition of RBO to the HFD diet improves the plasma glucose level \(( p < 0.001, \text{ with % change reached } 20.9)\), as compared to HFD1. Rats fed HFD diet for 2 months revealed a significant reduction in plasma glucose \(( p < 0.01)\), compared to rats fed HFD for only 1 month.

With regard to serum insulin, the HFD induced hyperinsulinemia in rats (HFD1 and HFD2) \(( p < 0.001, \text{ with % changes reached } 96.25 \text{ and } 188.73, \text{ respectively})\), compared to normal rats. Moreover, rats fed HFD for 2 months showed highly significant elevation in serum insulin \(( p < 0.001)\), compared to rats fed HFD for 1 month. Although, prophylactic (HFD1 + RBO) and therapeutic (HFD2 + RBO) groups showed an improvement in their hyperinsulinemia \(( p < 0.01 \text{ and } p < 0.001, \text{ respectively})\) compared to their respective control groups (HFD1 and HFD2), their insulin level is still highly elevated compared to the control group. Data in Table 2 revealed significant elevation in HOMA-IR \(( p < 0.001)\) in both HFD1 and HFD2 with % change reaching 139.73 and 220.1 as compared to the control group. Rats fed HFD for 2 months showed pronounced elevation in

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NC</th>
<th>RBO</th>
<th>HFD1</th>
<th>HFD1 + RBO</th>
<th>HFD2</th>
<th>HFD2 + RBO</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT Mean ± SE</td>
<td>26.45 ± 1.1</td>
<td>32.61 ± 0.94</td>
<td>40.54 ± 3.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>32.14 ± 1.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35.95 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.86 ± 1.53&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Range (21.67–29.99)</td>
<td>(29.00–37.6)</td>
<td>(29.00–52.00)</td>
<td>(27.01–43.34)</td>
<td>(25.00–56.47)</td>
<td>(22.00–36.92)</td>
<td></td>
</tr>
<tr>
<td>AST Mean ± SE</td>
<td>13.47 ± 0.69</td>
<td>17.93 ± 1.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.42 ± 0.28&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.70 ± 0.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>12.12 ± 1.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.89 ± 0.66&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Glucose Mean ± SE</td>
<td>120 ± 3.95</td>
<td>117.6 ± 4.37</td>
<td>180.4 ± 10.13&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>142.7 ± 2.48&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>134.2 ± 4.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>125.5 ± 7.23</td>
</tr>
<tr>
<td>Insulin Mean ± SE</td>
<td>7.46 ± 0.36</td>
<td>8.85 ± 0.36</td>
<td>14.64 ± 0.83&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>11.79 ± 0.75&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>21.54 ± 1.16&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>11.55 ± 0.73&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Range (6.50–10.1)</td>
<td>(7.50–10.5)</td>
<td>(10.6–16.7)</td>
<td>(9.03–14.9)</td>
<td>(17.6–25.5)</td>
<td>(8.90–15.7)</td>
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</tr>
<tr>
<td>HOMA-IR Mean ± SE</td>
<td>2.24 ± 0.11</td>
<td>2.56 ± 0.13</td>
<td>5.37 ± 0.56&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.14 ± 0.26&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.17 ± 0.55&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>3.53 ± 0.26&lt;sup&gt;ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>Range (1.81–275)</td>
<td>(1.67–3.00)</td>
<td>(2.24–7.33)</td>
<td>(3.05–5.33)</td>
<td>(5.51–10.13)</td>
<td>(2.64–5.58)</td>
<td></td>
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</tbody>
</table>

The mean difference is significant at \( p < 0.05 \). Each group contains 10 rats. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HFD, high-fructose diet; HOMA-IR, homeostasis model assessment insulin resistance; NC, normal control; RBO, rice bran oil.

a, significance versus NC; b, significance versus RBO; c, significance versus HFD1; d, significance versus HFD2.
Table 3 Hepatic total cholesterol (mg/g tissue), triacylglycerol (mg/g tissue), and MDA (nmol/g tissue) as well as SOD and CAT (U/g tissue) enzyme activity in different experimental groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NC</th>
<th>RBO</th>
<th>HFD1</th>
<th>HFD1 + RBO</th>
<th>HFD2</th>
<th>HFD2 + RBO</th>
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</thead>
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<td>Cholesterol</td>
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<tr>
<td>Mean ± SE</td>
<td>11.56 ± 1.25</td>
<td>9.17 ± 0.88</td>
<td>13.1 ± 1.09b</td>
<td>9.64 ± 0.35c</td>
<td>11.51 ± 0.48</td>
<td>11.24 ± 1.26</td>
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<tr>
<td>Triacylglycerol</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>18.96 ± 1.90</td>
<td>20.99 ± 2.22</td>
<td>57.96 ± 5.10ab</td>
<td>37.37 ± 3.74abc</td>
<td>42.52 ± 5.98abc</td>
<td>44.78 ± 3.51ab</td>
</tr>
<tr>
<td>Range (10.81–29.19)</td>
<td>(11.21–28.18)</td>
<td>(44.44–87.07)</td>
<td>(22.53–52.93)</td>
<td>(29.29–76.46)</td>
<td>(35.86–70.10)</td>
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<tr>
<td>MDA</td>
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<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>53.54 ± 2.88</td>
<td>47.96 ± 4.61</td>
<td>78.48 ± 1.99ab</td>
<td>40.34 ± 3.57c</td>
<td>60.92 ± 3.05c</td>
<td>40.94 ± 3.30d</td>
</tr>
<tr>
<td>Range (38.04–63.01)</td>
<td>(30.46–62.75)</td>
<td>(70.85–84.97)</td>
<td>(24.44–53.20)</td>
<td>(46.55–70.85)</td>
<td>(25.49–52.81)</td>
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</tr>
<tr>
<td>SOD1</td>
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</tr>
<tr>
<td>Mean ± SE</td>
<td>2.27 ± 0.14</td>
<td>2.68 ± 0.26ab</td>
<td>0.66 ± 0.10ab</td>
<td>1.24 ± 0.09abc</td>
<td>0.42 ± 0.08a</td>
<td>0.90 ± 0.08abcd</td>
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<tr>
<td>Range (1.70–2.80)</td>
<td>(1.90–4.10)</td>
<td>(0.23–1.06)</td>
<td>(0.82–1.6)</td>
<td>(0.19–0.81)</td>
<td>(0.54–1.30)</td>
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<tr>
<td>CAT</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>3.86 ± 0.24</td>
<td>3.28 ± 0.42</td>
<td>0.64 ± 0.07ab</td>
<td>1.66 ± 0.11abc</td>
<td>0.33 ± 0.04a</td>
<td>1 ± 0.05abcd</td>
</tr>
<tr>
<td>Range (2.60–4.80)</td>
<td>(2.09–4.80)</td>
<td>(0.27–0.84)</td>
<td>(1.06–2.04)</td>
<td>(0.17–0.47)</td>
<td>(0.43–1.20)</td>
<td></td>
</tr>
</tbody>
</table>

The mean difference is significant at p < 0.05. Each group contains 10 rats. CAT, catalase; HFD, high-fructose diet; MDA, malondialdehyde; NC, normal control; RBO, rice bran oil; SOD, superoxide dismutase.

a, significance versus NC; b, significance versus RBO; c, significance versus HFD1; d, significance versus HFD2.

HOMA-IR (p < 0.001) compared to rats fed HFD for only 1 month. Addition of RBO to the HFD either for the first day or after 1 month on the HFD, HOMA-IR was decreased significantly (p < 0.02, 0.001) as compared to its respective control group. Rats fed HFD for 2 months showed pronounced elevation in HOMA-IR (p < 0.001), compared to rats fed HFD for only 1 month.

Significant elevation in the hepatic cholesterol concentration (p < 0.05) was recorded in the HFD1 group, compared to the NC group (Table 3). However, the (HFD1 + RBO) (prophylactic) group showed a significant reduction (p < 0.01) in the hepatic cholesterol concentration, compared to the HFD1 group. For hepatic triacylglycerol, significant elevations (205.7%, 97.1%, 124.26%, and 136.18%) were observed in all experimental groups except RBO, compared to NC. Administration of RBO in a diet with high fructose for 1 month reduced hepatic triacylglycerol significantly (p < 0.001), compared to its corresponding control (HFD1). Surprisingly, feeding HFD for 2 months reduced the hepatic triacylglycerol (p < 0.001), compared to (HFD1) as illustrated in Table 3.

Significant elevation (p < 0.001) in the hepatic MDA concentration was observed in HFD1 (change % 46.58), compared to the NC group. Addition of RBO to the HFD reduced the concentration of MDA (p < 0.001), compared to HFD1. Rats fed HFD for 2 months reduced the hepatic MDA concentration (p < 0.002), compared to rats fed HFD for 1 month. Moreover, addition of RBO after feeding HFD for 1 month reduced the hepatic MDA significantly (p < 0.02), compared to rats fed HFD for 2 months. Hepatic enzyme activity for SOD and CAT were decreased in all experimental groups except RBO, compared to the NC group. Addition of RBO to the two diet regimens improved the concentration of these antioxidant enzymes (p < 0.005, p < 0.001, respectively), compared to HFD1. While only hepatic SOD activity was significantly elevated in the therapeutic (HFD2 + RBO) group (p < 0.02), compared to HFD2 (Table 3).

Fig. 1 Effect of rice bran oil (RBO) on sterol regulatory element binding protein-1 (Srebf1) expression in hepatocytes as determined by the real-time polymerase chain reaction (RT-PCR). Data are presented as the mean value. The effects of RBO were analyzed using ANOVA followed by LSD analysis for multiple comparisons. The mean difference is significant at p < 0.05. Each group contains 10 rats.
A highly significant reduction was observed in the Srebf1 mRNA level (p < 0.001) in the HFD1 and HFD2 groups, compared to the NC and RBO groups. On the other hand, both the prophylactic (HFD1 + RBO) and therapeutic (HFD2 + RBO) groups showed significant reductions in the Srebf1 mRNA level (p < 0.001) compared to their respective control groups (HFD1 and HFD2, respectively), their Srebf1 mRNA level is still significantly elevated compared to the NC and RBO groups (p < 0.001).

Discussion

The modern lifestyle and the increased consumption of fructose induce insulin resistance (IR), which leads to hyperglycemia due to the impaired glucose uptake, alterations in lipid metabolism, and oxidative stress the main cause of the pathological changes in insulin resistance (Hussein, Abd El-Hamid, & Hemdan, 2013; Misra & Khurana, 2009; Ye, 2013). Enzymes like ALT and AST are present mainly in the liver, but also can be found in other organs (Jacobs, 1996). ALT is produced in hepatocytes, a very specific marker of hepatocellular injury and usually rises in conjunction with AST (Hall & Cash, 2012). High levels of serum ALT and AST were observed by de Castro...
Many studies proved the induction of insulin resistance by feeding rats with HFD (Abd El-Wahab, Mohamed, El Sayed, & Bawouny, 2017; Mahfouz, Ghanem, & Mohamed, 2009; Mohamed, 2010; Shawky, Shehatou, Abdel Rahim, Suddek, & Gameil, 2014) and was established in the current study. Feeding rats with HFD for 1 and 2 months (HFD1 and HFD2, respectively) increased the hepatic insulin insensitivity as indicated by the significant elevation of serum insulin and HOMA-IR. HOMA-IR was (5.37) for HFD1 and (7.17) for HFD2, which exceeded the previously reported cutoff value (2.29), indicating severe insulin resistance (Radikova et al., 2006).

Surprisingly, plasma glucose in rats fed HFD for 8 weeks was significantly lower than rats fed HFD for 4 weeks and was insignificant, compared to NC rats. In addition, rats fed HFD for 8 weeks showed pronounced elevation in the insulin level and HOMA-IR, compared to rats fed fructose for only 4 weeks. The development of hyperglycemia may be due to the impaired glucose uptake and conversion of fructose to glucose (Konopelnuk, Yurchenko, Karpovets, & Ostapchenko, 2015). Moreover, enzymes that regulate the hepatic carbohydrate metabolism may be altered by the chronic fructose feeding. It was reported that the activity of glucokinase was decreased, while the glucose-6-phosphatase activity was increased (Faure et al., 1997). In response to reduced insulin sensitivity, the synthesis of insulin is increased by a compensatory mechanism as an adaptive increase of circulating hormone concentration (Rafacho, Quallio, Ribeiro, Taboga, & Paula, 2010) as observed in the present results.

The liver removes glucose from the portal vein after a meal converting it into glycogen (Feinman & Fine, 2013). Koo et al. (2008) observed higher levels of hepatic glycogen in fructose-fed rats due to its conversion to glucose by gluconeogenesis as a protective mechanism against hepatic fat accumulation (Lopez-Soldado et al., 2015), and hyperglycaemia (Ros, Garcia-Rocha, Calbo, & Guinovart, 2011). These reports may explain the reduced levels of serum glucose, hepatic TAG, and cholesterol observed in the HFD2 group.

The present study demonstrated that administration of RBO reduced the level of insulin and HOMA-IR, compared to NC and fructose-fed rats (HFD1 and HFD2). These results agree with those of Abd El-Wahab et al. (2017), who reported that HFD containing RBO improves insulin sensitivity and reduces lipo- and glucotoxicity. Shakib, Gabriel, and Gabriel (2014) showed that the glucose lowering effect of RBO may be due to its content of oleic acid. While Vafa, Haghhighat, Moslehi, Eghtesadi, and Heydari (2015) suggested that tocotrienols of RBO can improve insulin sensitivity through activating peroxisome proliferator-activated receptors (PPAR). Binding of toco-trienols to PPAR promotes insulin-mediated glucose uptake through increasing the expression of glucose transporter 4 (Fang, Kang, & Wong, 2010).

The hepatic cholesterol concentration was elevated in fructose-fed rats for 4 weeks; however, the addition of RBO reduced the elevated cholesterol level. Many studies reported significant elevation in hepatic cholesterol of HFD-fed rats (Al-Okbi et al., 2014; Mahfouz et al., 2010; Mohamed, 2010). 3-Hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase activity, a rate-limiting enzyme in the cholesterol biosynthetic pathway, is mediated by the changes in the phosphorylation state of the enzyme that is affected by insulin (Ness, Zhao, & Wiggins, 1994). Therefore, the absence of the insulin signal due to the insulin resistance state inhibits the hepatic HMG-CoA reductase activity.

On the other hand, present data showed significant elevation in the hepatic triacylglycerol concentration in all studied groups except the RBO group, compared to the control group. Schaalan, El-Abhar, Barakat, and El-Denshary (2009) reported that consumption of fructose and fats causes stimulation of hepatic lipogenesis with accumulation of triacylglycerol, which contributes to hepatic insulin resistance. The present results are in line with those of Chou, Ma, Cheng, and Lai (2009) who reported that rats fed with the RBO diet had a suppressed hepatic triacylglycerol concentration that is associated with improved insulin sensitivity.

Both hyperinsulinemia and hyperglycemia, in addition to fructose itself, create a state of oxidative stress as a result of free-radical production. Free radicals can be produced by autoxidation of glucose and enhanced glycation (Rajasekar & Anuradha, 2007; Reddy, Ramatholisamma, Karuna, & Saralakumari, 2009). HFD-fed rats display hepatic oxidative damage due to generation of reactive oxygen species, which attack the polyunsaturated fatty acids causing lipid peroxidation and resulting in the formation of free radicals that cause damage to certain cell structures (Araghi, Seiyi, Sayrafi, & Sadighara, 2016; Ghatak & Panchal, 2012; Kelley, Allan, & Azhar, 2004). In the present study, insulin-resistant rats (HFD1 and HFD2) displayed impairment in the antioxidant defense system indicated by a highly significant reduction in hepatic SOD and CAT activities accompanied by significant elevation in hepatic MDA, compared to the control group. The current results...
agree with those of many previous studies (Abd El-Wahab et al., 2017; Abdel-Kawi et al., 2016; Mahfouz et al., 2010; Rajasekar, Viswanathan, & Anuradha, 2008).

The improvement in antioxidant capacity, which was characterized by increasing the activity of SOD and CAT, in addition to the reduction of the hepatic MDA content, was noticeable and pronounced in rats fed RBO-containing diets (HFD1 + RBO and HFD2 + RBO) as compared to their respective control. These results are in harmony with those reported by Al-Okbi et al. (2014), Araghi et al. (2016), and Abd El-Wahab et al. (2017). The most important antioxidants in RBO are γ-oryzanol and tocotrienols (Chotimarkorn, Benjakul, & Silalai, 2008; Posuwan et al., 2013). These phytochemicals elicit antioxidant properties either by donating a hydrogen atom to lipid radicals (Siddiqui, Khan, & Siddiqui, 2010) or by increasing the activities of some antioxidant enzymes such as SOD, CAT, glutathione peroxidase (GPx), nicotinamide adenine dinucleotide phosphate reduced form (NADPH): quinone oxidoreductase, and GPx (Hsieh & Wu, 2008; Kuhad & Chopra, 2009; Lee & Kim, 2010; Son, Rico, Nam, & Kang, 2010; Weng-Yew, Selvaduray, Ming, & Nesaretnam, 2009).

Moreover, HFD downregulated the hepatic levels of SOD and Cat mRNA after 4 and 8 weeks of feeding. These results agree with Cavarape et al. (2001) who recorded downregulation in hepatic Cat and Sod1 expression in HFD-fed rats. While disagree with the results of Jarukamjorn, Jearapong, Tresini, 2000). This hypothesis was confirmed by the improvement observed in groups fed HFD contained RBO, where addition of RBO to HFD enhanced the oxidative stability and improved the antioxidant system through upregulating SOD and CAT expressions.

The activity of hepatic SREBP is reported to be increased in fructose-fed mice (Miyazaki et al., 2004), and this explains the upregulation of Srebf1 gene in rats fed HFD for 4 and 8 weeks. Unlike glucose, fructose directly provides substrates for triacylglycerol biosynthesis without negative feedback, in addition to upregulating transcription factors and enzymes involved in lipogenesis, including SREBP1. Thus, induction of hepatic triacylglycerol synthesis observed in this study is mediated by SREBP that binds to the sterol regulatory elements of the lipogenic genes activating their transcription (Horton, Goldstein, & Brown, 2002). The current results are in harmony with those of Nagai et al. (2002); Kanuri, Spruss, Wagnerberger, Bischoff, and Bergheim (2011); Spruss, Kanuri, Uebel, Bischoff, and Bergheim (2011); Nomura and Yamanouchi (2012); and Clayton, Vickers, Bernal, Yap, and Sloboda (2015). Peroxisome proliferator-activated receptor-alpha (PPAR-α) directly controls the transcription of genes involved in fatty-acid uptake, peroxisomal and mitochondrial β-oxidation pathways, and triacylglycerol catabolism, and so influences the intracellular lipid and carbohydrate metabolism (Evans, Barish, & Wang, 2004; Lefebvre, Chinetti, Fruchart, & Staels, 2006). The relation between fructose feeding and hepatic expression of PPAR-α or its sensitive genes is controversial. Some studies have recorded downregulation (Huang et al., 2009; Nagai et al., 2002; Roglans et al., 2007), whereas others reported no change in the expression of hepatic Ppara (Kim, Okubo, Juneja, & Yokozawa, 2010; Mori, Kondo, Hase, & Murase, 2011).

In the present study, HFD induced hepatic de novo lipogenesis through upregulating the expression level of lipid-regulatory genes, Srebf1 and Ppara, in the HFD1 and HFD2 groups. The present data agree with the results of Clayton et al. (2015) who reported that fructose-fed mother rats at postnatal day 10 demonstrated an increase in PPAR-α. In contrast, Nagai et al. (2002) concluded that fructose or its metabolites downregulate the expression of Ppara in primary cultured hepatocytes.

Fasting increases the hepatic expression of Ppara and its target genes, resulting in enhanced β-oxidation (Palou et al., 2008). Although HFD induced hyperinsulinemia, the induction of PPAR-α in the present study might be due to the insulin resistance state, which resembles the fasting state in poor insulin signaling. The presence of increased concentration of hepatic triacylglycerol confirms that hypothesis.

Treatment with RBO reversed the upregulated hepatic mRNA levels of Srebf1 and Ppara caused by the HFD, compared to their respective control groups (HFD1and HFD2). These results agree with Nagai et al. (2002) who reported that RBO treatment suppressed the increased hepatic de novo lipogenesis. Consequently, the inhibitory effect of RBO may be due to the unsaponifiable matter, which downregulates SREBP-1c (Ham et al., 2016).

Fukuoka et al. (2014) showed that treatment of HepG2 cells with triterpene alcohols, the characteristic components of RBO, decreased the gene expression of Srebf1_v3. Moreover, the expression of Srebf1_v3 was previously reported to be directly regulated by insulin (O’Brien, Streeper, Ayala, Stademaiter, & Hornbuckle, 2001). Consequently, the observed improvement of insulin sensitivity due to RBO affects the gene expression of Srebf1 as illustrated in the current results. In contrast, insulin was reported to negatively regulate Ppara expression (Nagai et al., 2002). Therefore, the downregulation of PPAR-α expression by the addition of RBO may be due to the improvement of the insulin resistance state of fructose-fed rats that was proved by decreased HOMA-IR.
HFD is one of the most important causes of IR as proved in this study. RBO alleviates the IR to some extent by improving the insulin sensitivity through its tocotrienol content. RBO also downregulates the lipid-regulatory genes and improves the unbalanced oxidative status by upregulating the antioxidant enzymes. These effects may be due to the improvement in insulin sensitivity.

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Conflict of Interest The authors declare that they have no conflicts of interest.

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