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# Curcumin prevents inflammatory response, oxidative stress and insulin resistance in high fructose fed male Wistar rats: Potential role of serine kinases

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ABSTRACT

Emerging evidence suggests that high fructose consumption may be a potentially important factor responsible for the rising incidence of insulin resistance and diabetes worldwide. The present study investigated the preventive effect of curcumin on inflammation, oxidative stress and insulin resistance in high fructose fed male Wistar rats at the molecular level. Fructose feeding for 10 weeks caused oxidative stress, inflammation and insulin resistance. Curcumin treatment attenuated the insulin resistance by decreasing IRS-1 serine phosphorylation and increasing IRS-1 tyrosine phosphorylation in the skeletal muscle of high fructose fed rats. It also attenuated hyperinsulinemia, glucose intolerance and HOMA-IR level. Curcumin administration lowered tumor necrosis factor alpha (TNF-α), C reactive protein (CRP) levels and downregulated the protein expression of cyclo-oxygenase 2 (COX-2), protein kinase theta (PKCθ). In addition, inhibitor κB alpha (IκBα) degradation was prevented by curcumin supplementation. Treatment with curcumin inhibited the rise of malondialdehyde (MDA), total oxidant status (TOS) and suppressed the protein expression of extracellular kinase ½ (ERK ½), p38 in the skeletal muscle of fructose fed rats. Further, it enhanced Glutathione Peroxidase (GPx) activity in the muscle of fructose fed rats. At the molecular level, curcumin inhibited the activation of stress sensitive kinases and inflammatory cascades. Our findings conclude that curcumin attenuated glucose intolerance and insulin resistance through its antioxidant and anti-inflammatory effects. Thus, we suggest the use of curcumin as a therapeutic adjuvant in the management of diabetes, obesity and their associated complications.


## 1. Introduction

The prevalence of diabetes worldwide is a global health concern as it is projected to rise 592 million by 2035 [1]. Insulin resistance plays a pivotal role in the development of type 2 diabetes. Inadequate response of peripheral tissue to normal circulating levels of insulin to regulate whole body glucose homeostasis is known as insulin resistance and this is an early metabolic defect associated with overt type 2 diabetes mellitus (T2DM). Under normal physiological circumstances, skeletal muscle contributes to 70–85% of whole body insulin stimulated glucose uptake, utilization and storage thereby maintains systemic glucose metabolism [2]. Since skeletal muscle is considered as a primary tissue for whole body glucose homeostasis, investigating the pathogenesis of insulin resistance in the skeletal muscle may be beneficial in the management of diabetes and its associated complications.

The primary cause for the current epidemic of diabetes and obesity worldwide is due to a modern lifestyle and an increase in the consumption of high-sugar diets especially fructose [3]. Recent findings have reported that increased consumption of fructose contributes to the constellation of abnormalities such as hyperinsulinemia, glucose intolerance, insulin resistance, hypertension, and dyslipidemia [4]. Animal studies have shown that high fructose feeding causes oxidative stress, low grade inflammation and altered lipid metabolism and plays a major role in the genesis of insulin resistance. The development of insulin resistance leads to impaired insulin signalling and reduced glucose uptake.

Several lines of evidence from in-vitro and in-vivo models have

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demonstrated that hyperglycemia and elevated plasma free fatty acids cause enhanced production of reactive oxygen species (ROS) which further triggers the production of pro-inflammatory cytokines such as TNF-α, IL6, IL1β, etc. [5]. The increased production of ROS leads to oxidative stress which further activates multiple stress sensitive kinases (NF-κB, p38 MAPK, JNK and ERK ½) [6]. The activation of stress sensitive kinases and inflammatory cascades contribute to impaired insulin signalling. As a consequence, inactivation/inhibition of other substrates involved in the insulin signalling pathway like protein kinase B (PKB), IP3, Grb2 leads to reduced glucose uptake ultimately insulin resistance [7]. Though several experimental evidence suggests that fructose consumption is associated with insulin resistance the possible mechanisms responsible for the same remain unclear. Hence, we investigated the mechanisms involved in the development of insulin resistance in the high fructose fed male Wistar rats.

Though pharmacological strategies are available for the management of diabetes, yet their associated complications are difficult to manage. The lack of effective pharmacological management for diabetic complications has led to a search for effective natural products as alternative remedy. Curcumin, a natural phytochemical present in *Curcuma longa* (turmeric) is used in food preparations as spice, colouring and flavouring agent. Curcumin has been shown to decrease blood glucose levels and inflammatory response in diabetic animal models [8]. Furthermore, numerous studies suggest that curcumin has a beneficial role in diabetes by lowering blood glucose levels in STZ-induced type 1 diabetic rats, KK-Ay type 2 diabetic mice [9] and db/db mice [10]. A study reported that curcumin improved insulin and leptin resistance in fructose fed rats through inhibition of hepatic protein-tyrosine phosphatase 1B [11]. Although studies have shown that curcumin can improve insulin resistance in diabetic mouse models, the beneficial effects of curcumin against high fructose induced metabolic derangement has not been elucidated at the molecular level. Hence, the present study investigated the preventive effects of curcumin against inflammation, oxidative stress and insulin resistance in fructose fed rats at the molecular level.

## 2. Materials and methods

### 2.1. Materials

All the reagents and chemicals were of molecular grade and obtained from Sigma Aldrich (USA), Merck (India) or SRL (India). Horseradish Peroxidase-conjugate goat anti-rabbit IgG and goat anti-mouse IgG and primary antibody IκBα were procured from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and β-actin were from Biolegend (San Diego, CA, USA). The primary antibodies, Insulin receptor substrate ($IRS1^{tyr1222}$, $IRS1^{ser307}$), ERK ½, phospho ERK ½, p38 and COX-2 were procured from Cell Signaling Technology (CST, MA, USA). Glutathione peroxidase (GPx) was obtained from Abcam (USA). The primary antibody PKC theta total and phospho PKC theta and enhanced chemiluminescence substrate (ECL) were procured from Pierce (West Pico Super Signal, Pierce, Thermo Fisher Scientific and Marietta, USA).

### 2.2. Animals

The study protocol was approved by Institute Animal Ethics Committee. Five month old male Wistar rats weighing 250–300 g were used for this study. They were maintained in polycarbonate cages under a 12-h light/12-h dark cycle with food and water ad libitum. According to the experimental design, the rats were randomized into four different groups (10 rats in each group). The experiment was carried out for the period of ten weeks.

### 2.3. Experimental groups

- Control group – Received standard rodent pellet
- Control + Curcumin group – Received standard rodent pellet and curcumin
- Fructose group – Received chow supplemented with 60% fructose
- Fructose + Curcumin group – Received chow supplemented with 60% fructose and curcumin

### 2.4. High fructose diet and curcumin supplementation

The high fructose diet (60% (w/w) was prepared as the method described by Hwang Ho et al. [12]. Curcumin was procured from Sigma–Aldrich, St. Louis, MO. Curcumin in the dose of 200 mg/kg body weight was dissolved in 0.1% carboxy methyl cellulose and given orally to the rats [13]. Fasting blood samples were collected at the end of the study. The plasma was separated from the blood samples and used for the estimation of biochemical parameters and inflammatory markers. At the end of the experiment, all the animals were sacrificed and skeletal muscle was collected and stored at −70 °C for immunoblotting analysis of insulin signalling and inflammatory pathways.

### 2.5. Intra-peritoneal glucose tolerance test (IPGTT)

After an overnight fasting for 12–15 h, intraperitoneal glucose tolerance test (IPGTT) was performed for all experimental rats. Blood samples were drawn initially and then the animals were injected with 2 g/kg body weight of glucose intraperitoneally. At 30, 60 and 120 min blood samples were obtained after the glucose injection [14,15]. The samples were used for the estimation of plasma glucose and insulin. Area under curve (AUC) was calculated based on the plasma glucose values obtained during IPGTT. The graph pad prism software 6 was used for the analysis of area under curve.

### 2.6. Biochemical assay

#### 2.6.1. Estimation of biochemical parameters

The fasting plasma glucose was estimated using reagent kits adapted for fully automated Clinical Chemistry Analyser (AU-400, Olympus, Japan) as per the manufacturer's instructions. Plasma ultra-sensitive rat insulin was estimated by ELISA kit (Crystal Chem, Downers Grove, IL, USA). The homeostasis model assessment index (HOMA-IR) was calculated using the formula [fasting glucose (mmol/L) X fasting insulin (μIU/mL)]/22.5] [16].

#### 2.6.2. Measurement of plasma inflammatory markers and adipokines

The plasma inflammatory proteins tumor necrosis factor alpha (TNFα, Gen-Probe, Diaclone SAS, France) and C-reactive protein (CRP, immunology Consultants Laboratory, Portland) were estimated by enzyme linked immuno-sorbet assay (ELISA) according to the manufacturer's instructions. The plasma levels of adipokines such as adiponectin and resistin were estimated using ELISA kits from Ray Biotech Inc (Norcross, USA).

#### 2.6.3. Estimation of skeletal muscle triacylglycerol

The muscle tissues were homogenized in chloroform and methanol (2:1, v/v) mixture, total lipids was extracted according to the method of Folch et al. [17]. Triacylglycerol (TAG) content was estimated using commercial enzyme kits (Genuine Biosystems, Chennai, India). TAG measurements were normalized to the weight