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Original Research

Glucocorticoid Antagonism Reduces Insulin Resistance and Associated Lipid Abnormalities in High-Fructose-Fed Mice

Emayavaramban Priyadarshini MSc, Carani Venkatraman Anuradha MSc, MPhil, PhD *

Department of Biochemistry and Biotechnology, Annamalai University, Tamil Nadu, India

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ABSTRACT

Objectives: High intake of dietary fructose causes perturbation in lipid metabolism and provokes lipid-induced insulin resistance. A rise in glucocorticoids (GCs) has recently been suggested to be involved in fructose-induced insulin resistance. The objective of the study was to investigate the effect of GC blockade on lipid abnormalities in insulin-resistant mice.
Methods: Insulin resistance was induced in mice by administering a high-fructose diet (HFrD) for 60 days. Mifepristone (RU486), a GC antagonist, was administered to HFrD-fed mice for the last 18 days, and the intracellular and extracellular GC levels, the glucocorticoid receptor (GR) activation and the expression of GC-regulated genes involved in lipid metabolism were examined.
Results: HFrD elevated the intracellular GC content in both liver and adipose tissue and enhanced the GR nuclear translocation. The plasma GC level remained unchanged. The levels of free fatty acids and triglycerides in plasma were elevated, accompanied by increased plasma insulin and glucose levels and decreased hepatic glycogen content. Treatment with RU486 reduced plasma lipid levels, tissue GC levels and the expression of GC-targeted genes involved in lipid accumulation, and it improved insulin sensitivity.
Conclusions: This study demonstrated that HFrD-induced lipid accumulation and insulin resistance are mediated by enhanced GC in liver and adipose tissue and that GC antagonism might reduce fructose-induced lipid abnormalities and insulin resistance.

Mots clés :
11β-hydroxystéroïde déshydrogénase de type 1
glucocorticoïdes
récepteur des glucocorticoïdes
insulinorésistance
accumulation des lipides
RU 486

RÉSUMÉ

Objectifs : L'apport élevé de fructose en provenance des aliments cause la perturbation du métabolisme des lipides et provoque l'insulinorésistance induite par les lipides. Il a récemment été suggéré qu'une augmentation des glucocorticoïdes (GC) était impliquée dans l'insulinorésistance induite par le fructose. L'objectif de la présente étude était d'examiner l'effet du blocage des GC sur les anomalies lipidiques chez les souris insulinorésistantes.
Méthodes : L'insulinorésistance était induite chez les souris par l'administration d'un régime à teneur élevée en fructose (RTÉFr) durant 60 jours. La mifépristone (RU 486), un antagoniste des GC, était administrée chez les souris nourries selon un RTÉFr durant les 18 derniers jours, puis les concentrations intracellulaires et extracellulaires des GC, l'activation du récepteur des glucocorticoïdes (RG) et l'expression des gènes régulés par les GC qui interviennent dans le métabolisme des lipides étaient examinées.
Résultats : Le RTÉFr augmentait le contenu intracellulaire en GC dans le foie et le tissu adipeux et améliorait la translocation nucléaire du RG. La concentration plasmatique des GC demeurait inchangée. Les concentrations plasmatiques d'acides gras libres et de triglycérides étaient élevées, et accompagnées par l'augmentation des concentrations plasmatiques de l'insuline et du glucose, et de la diminution du contenu hépatique en glycogène. Le traitement par RU 486 réduisait les concentrations plasmatiques des lipides, les concentrations tissulaires des GC et l'expression des gènes ciblés par les GC impliqués dans l'accumulation des lipides, et améliorait l'insulinosensibilité.
Conclusions : Cette étude démontrait que l'accumulation des lipides et l'insulinorésistance induites par le RTÉFr sont médiées par l'augmentation des GC dans le foie et les tissus adipeux, et que l'antagoniste des GC réduirait les anomalies lipidiques et l'insulinorésistance induites par le fructose.

* Address for correspondence: Carani Venkatraman Anuradha MSc, MPhil, PhD, Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar 608 002, Tamil Nadu, India.
E-mail address: cvaradha@yahoo.com, cvaradha975@gmail.com

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Introduction

Glucocorticoid hormones (GCs) orchestrate carbohydrate, lipid and protein metabolism and regulate energy homeostasis through genomic and nongenomic actions in cells. The genomic effects of GCs are mediated through ligand-dependent activation of the glucocorticoid receptor (GR), which is expressed in almost every cell type (1). Activated GR acts as a transcription factor and targets the genes related to glucose and triglyceride (TG) metabolism (2). Chronic excess of GCs is associated with insulin resistance, while reduction of GCs improves insulin sensitivity (3). For example, the levels of circulating GCs are high in patients with insulin resistance, and increased GC production induces hypercortisolism-mediated insulin resistance through activation of the GR (4). Overexpression of 11-β-hydroxysteroid dehydrogenase type 1 (11βHSD1), a ketoreductase that enhances tissue availability of GC results in a phenotype that resembles the characteristic features of the metabolic syndrome (MS) such as obesity, glucose intolerance and hypertriglyceridemia, whereas 11βHSD1 deficiency increases insulin action and is associated with decreased TG levels and enhanced fatty acid oxidation (5).

Chronic administration of fructose as the only source of carbohydrate in animals produces metabolic changes, insulin resistance and other components of the MS (6). Studies in C57BL/6 mice have observed the development of fatty liver and insulin resistance by administering 60% fructose in the diet (7) or 30% fructose solution (8). C57BL/6 mice consuming a 20% fructose diet developed higher visceral fat weights, increased numbers of liver fat depots and increased hepatic TG accumulation without changes in body weight gain, glucose tolerance or glycogen storage when compared to control mice (9,10). Furthermore, studies from our laboratory have shown that chronic administration of fructose (60% in diet for 60 days) produces metabolic changes, insulin resistance and other components of the MS in Swiss albino mice (11). Other investigators have also noticed that Swiss albino mice develop insulin resistance after consuming a 10% fructose solution for 10 weeks (12). The sequence of events occurring in the development of diabetes in mice is shown to be similar to that observed in human type 2 diabetes (13), and the phenotypic changes in this diet model mimic the characteristic features of the MS in humans. Therefore, mice models are considered to be valid for clinical studies.

Recently, it has been suggested that amplified GC action promotes fructose-induced insulin resistance. Ad libitum access to water containing fructose (16%) increased 11βHSD1 activity in rat liver and mesenteric adipose tissue within 24 hours of exposure (14). Kovačević et al (15) demonstrated that rats drinking 10% fructose solution for 9 weeks had elevated 11βHSD1 and GC levels and lipid accumulation in adipose tissue. Indeed, Bursac et al (16) noted that feeding the 10% fructose solution for 9 weeks enhanced the pre-receptor metabolism of GC and the nuclear translocation of GR.

A high-fructose diet has been documented to induce insulin resistance primarily through upregulation of the lipogenesis pathway (17). We hypothesized that fructose-induced changes could involve dysregulation of GC action and that inhibition of intracellular GC action could alleviate the detrimental effects of fructose. In order to clarify this, the present study analyzed the expression of proteins and genes that regulate GC action and those involved in TG metabolism in liver and adipose tissue of fructose-fed mice, treated or untreated with mifepristone (RU486), a GR antagonist.

Methods

Chemicals, assay kits, antibodies and primers

RU486 was procured from Sigma Chemical (St. Louis, Missouri, United States [US] [product #M 8046]), and the high-density lipoprotein-cholesterol (HDL-c) assay kit was purchased from Agappe Diagnostics (Kerala, India). Fine chemicals and solvents of analytic grade were obtained from HIMEDIA Laboratories (Mumbai, India) or Sisco Research (Mumbai, India).

Antibodies against 11β-HSD1 (Cayman Chemical, Ann Arbor, Michigan, US; 1:50); GR (cell signalling, 1:1000); heat shock protein 90 (Hsp90) (cell signalling, 1:1000); glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Abcam, Cambridge, UK) (cell signalling, 1:1000); histone H3 (cell signalling, 1:1000) and calnexin (Abcam) (cell signalling, 1:1000) were purchased as indicated. Anti-rabbit monoclonal secondary antibody was procured from Cell Signaling Technology (Danvers, Massachusetts, US). Immobilon polyvinylidene fluoride (PVDF) (Millipore, Billerica, Massachusetts, US) membrane and an enhanced chemiluminescence kit (Super Signal West Pico Chemiluminescent Substrate; Thermo Scientific, Rockford, Pennsylvania, US) were used for immunoblotting.

TRIzol reagent (Genei, Bangalore, India), M-MuLV-reverse transcriptase (Thermo Scientific, Pittsburgh, Pennsylvania, US) and Oligo dT primers (Genei, Bangalore, India) were obtained from standard manufacturing companies. Forward and reverse primers were purchased from Sigma-Aldrich (St. Louis, Missouri, US), and SYBR green master mix was purchased from Kapabiosystems (Wilmington, Massachusetts, US).

Animal maintenance

Male Mus musculus albino mice of Swiss strain 8 weeks of age, weighing 25 to 30 grams were obtained from the Central Animal House, Rajah Muthiah Medical College and Hospital (RMMC and H). The animals were housed individually in polypropylene cages for an experimental period of 60 days. The animal room was maintained under hygienic conditions at a temperature ranging from 22°C to 24°C with a 12-hour light (8 AM to 8 PM)/12-hour dark (8 PM to 8 AM) cycle and constant humidity. The study was approved by the Institutional Animal Ethical Committee (IAEC), RMMC and H, Annamalai Nagar (#160/1999/CPCSEA/977). Animal handling was done according to the guidelines of the IAEC.

Diet

The diet was freshly prepared in the laboratory. The composition of fructose diet was identical to the starch diet except that the cornstarch was replaced with an equal quantity of fructose. The diet provided a metabolizable energy of 3.650 kcal/g, of which 65.75% was obtained from starch in the control diet and from fructose in the fructose diet. Table 1 gives the composition of diet, and Table 2 shows the percentages of distribution of calories. The diet composition and the duration of the study are based on our earlier study

Table 1
Composition of diet

Ingredients	Control diet (g/kg)	High-fructose diet (g/kg)
Cornstarch	600.0	-
Fructose	-	600.0
Casein (fat-free)	200.0	200.0
Methionine	7.0	7.0
Groundnut oil	50.0	50.0
Wheat bran	106.0	106.0
Salt mixture*	35.0	35.0
Vitamin mixture†	2.0	2.0

* The composition of mineral mix (g/kg): $MgSO_4.7H_2O$-30.5; NaCl-65.2; KCl-105.7; KH_2PO_4-200.2; $3MgCO_3$-3.65, $Mg(OH)_2.3H_2O$-38.8; $FeC_6H_5O_7.5H_2O$-40.0; $CaCO_3$-512.4; KI-0.8; NaF-0.9; $CuSO_4.5H_2O$-1.4; $MnSO_4$-0.4 and $CONH_3$ -0.05.

† 1 kg of vitamin mix contained thiamine mono nitrate, 3 g; riboflavin, 3 g; pyridoxine HCl, 3.5 g; nicotinamide, 15 g; d-calcium pantothenate, 8 g; folic acid, 1 g; d-biotin, 0.1 g; cyanocobalamin, 5 mg; vitamin A acetate, 0.6 g; α-tocopherol acetate, 25 g and choline chloride, 10 g.