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Betaine supplementation protects against high-fructose-induced renal injury in rats☆

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Abstract

High fructose intake causes metabolic syndrome, being an increased risk of chronic kidney disease development in humans and animals. In this study, we examined the influence of betaine on high-fructose-induced renal damage involving renal inflammation, insulin resistance and lipid accumulation in rats and explored its possible mechanisms. Betaine was found to improve high-fructose-induced metabolic syndrome including hyperuricemia, dyslipidemia and insulin resistance in rats with systemic inflammation. Betaine also showed a protection against renal dysfunction and tubular injury with its restoration of the increased glucose transporter 9 and renal-specific transporter in renal brush bolder membrane and the decreased organic anion transporter 1 and adenosine-triphosphate-binding cassette transporter 2 in the renal cortex in this model. These protective effects were relevant to the anti-inflammatory action by inhibiting the production of inflammatory cytokines including interleukin (IL)-1β, IL-18, IL-6 and tumor necrosis factor-α in renal tissue of high-fructose-fed rat, being more likely to suppress renal NOD-like receptor superfamily, pyrin domain containing 3 inflammasome activation than nuclear factor κB activation. Subsequently, betaine with anti-inflammation ameliorated insulin signaling impairment by reducing the up-regulation of suppressor of cytokine signaling 3 and lipid accumulation partly by regulating peroxisome proliferator-activated receptor α/palmityltransferase 1/carnitine/organic cation transporter 2 pathway in kidney of high-fructose-fed rats. These results indicate that the inflammatory inhibition plays a pivotal role in betaine's improvement of high-fructose-induced renal injury with insulin resistance and lipid accumulation in rats.

Keywords: Betaine; Fructose; Renal injury; Renal inflammation; Insulin resistance

1. Introduction

Fructose is widely used as an industrial sweetener in soft drinks, fruit juices, baked goods, jams, syrups and candies in the world. Intake of fructose has increased dramatically over the last century, especially in Western countries, which likely contributes to secular trends in the prevalence of metabolic diseases, such as hepatic steatosis, hypertension, cardiovascular disease and diabetes [1–5]. Animal studies also have proved that high fructose intake causes the development of metabolic syndrome including hyperuricemia, hyperinsulinemia, hyperleptinemia, dyslipidemia, insulin resistance and chronic inflammation [6–8]. It is also awaked that high fructose intake contributes to an increased incidence of chronic kidney diseases with renal tubulointerstitial injury [9–11]. High-fructose-induced hyperuricemia is associated with a decreased renal uric acid excretion [12]. In our previous study, renal tubular injury in high-fructose-fed rats was epitomized by the deregulation of renal urate transport-related proteins, resulting in the increased reabsorption and the decreased excretion of renal uric acid [13]. Insulin resistance has been suggested to enhance urate reabsorption by up-regulating urate anion exchanger 1 expression and reduce urate excretion by down-regulating urate transporter expression in diabetic rats [14,15]. These abnormalities may aggravate hyperuricemia and renal injury, playing a causal role in endothelial dysfunction in high-fructose-induced metabolic diseases [16,17].

Uric acid is identified as an endogenous adjuvant that drives the activation of NOD-like receptor superfamily, pyrin domain containing 3 (NLRP3) inflammasome [composed of NLRP3, apoptosis-associated speck-like protein (ASC) and caspase-1] [18,19]. Caspase-1 activation stimulates the maturation of interleukin (IL)-1β and IL-18 and in turn increases tumor necrosis factor (TNF)-α production [20]. These inflammatory cytokines can cause renal injury and contribute to the progression of chronic kidney disease [21,22]. Additionally, nuclear factor κB (NF-κB) pathway activation increases IL-1β and TNF-α expression and stimulates inflammatory cascade in renal tissue of fructose-fed rats [23]. In fact, inflammatory cytokines such as IL-6 and

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TNF-α can stimulate the up-regulation of suppressor of cytokine signaling 3 (SOCS3), which impairs insulin receptor substrate 1 (IRS1)/protein kinase B (Akt) signaling pathway [24]. Furthermore, inflammation stress disrupts cellular cholesterol export and exacerbates lipid accumulation associated with down-regulation of renal peroxisome proliferator-activated receptor α (PPARα) [25]. In our previous study, high-fructose feeding induced renal NLRP3 inflammasome activation and inflammatory cytokine production, with reduction of expression of renal PPARα and its control genes palmityltransferase 1 (CPT1) and carnitine/organic cation transporter 2 (OCTN2) in rats [13]. These observations suggest that renal inflammatory response mediated by the NLRP3 inflammasome and NF-κB activation may play a pivotal role in high-fructose-induced insulin resistance, lipid accumulation and renal injury. Thus, improvement of insulin resistance and lipid accumulation by inhibiting inflammation can help to protect against renal injury [26,27].

Betaine (trimethylycine) is distributed widely in animals, plants and microorganisms [28]. Its rich dietary sources include wheat, shellfish, spinach and sugar beets. Betaine insufficiency is associated with lipid metabolism disorder, diabetes, metabolic syndrome and vascular diseases in patients [29]. On the other hand, dietary betaine suppresses NF-κB activation during inflammation response and improves the deleterious conditions related to lipid metabolism disorders [30,31]. Betaine reduces the expression of IL-6 and TNF-α in human adipocytes caused by hypoxia, showing its potential to reduce the risk for inflammation-related diseases [30,32]. Additionally, betaine supplementation prevents hepatic steatosis and insulin resistance in a high-fat-diet-fed animals [33,34] and up-regulates hepatic PPARα and CPT1 expression to alleviate hepatic triglyceride (TG) accumulation in apolipoprotein-E-deficient mice [35]. Therefore, we hypothesized that betaine would be an important nutrient for the prevention of chronic renal disease, which is associated with renal inflammation, insulin resistance and lipid accumulation.

In the present study, we found that betaine improved high-fructose-induced hyperuricemia, insulin resistance, dyslipidemia and systemic inflammation in rats. Betaine also showed a renal protection against renal dysfunction and tubular injury in this model. Its renal protection was due to the inhibition of renal inflammation through suppressing renal NLRP3 inflammasome activation more than NF-κB activation, which contributed to the amelioration of insulin resistance and lipid accumulation in renal tissue of high-fructose-fed rats. The suppression of renal NLRP3 inflammasome activation by betaine was firstly proved.

2. Materials and methods

2.1. Chemicals and antibodies

Betaine was purchased from Sigma (St. Louis, MO, USA). Fructose was bought from Jiakangyuan Technology (Peking, P.R. China). Assay kits of urate, creatinine, blood urea nitrogen (BUN), glucose, TG, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and total cholesterol (TC) were purchased from Jiancheng Biotech (Nanjing, P.R. China). Hematoxylin–eosin (H&E) and oil red O reagents were also provided by Jiancheng Biotech (Nanjing, P.R. China). Enzyme-linked immunosorbent assay (ELISA) kits for IL-1β, IL-18 and IL-6 assays were purchased from IBL (Minneapolis, MN, USA). ELISA kit for TNF-α assays was obtained from R&D (Minneapolis, MN, USA). The antibodies of glucose transporter 9 (GLUT9), renal-specific transporter (RST) and organic anion transporter 1 (OAT1) for rat were supported by Sai-Chi Biotech (Beijing, P.R. China). The antibody of NLRP3 for rat was purchased from Novus Biologicals (Littleton, CO, USA). The antibodies of ASC, caspase-1, thioredoxin-interacting protein (TXNIP) and OCTN2 for rat were obtained from Abcam (Cambridge, MA, USA). The antibodies of adenosine-triphosphate-binding cassette transporter 2 (ABCG2), IRS1, p-IRS1 (Ser), p-IRS1 (Tyr), AKT, p-AKT, SOCS3, NF-κB, p-NF-κB and PPARα for rat were supported by Cell Signaling Technology (Danvers, MA, USA). The antibody of CPT1 for rat was purchased from Bioss (Beijing, P.R. China). Rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) monoclonal antibody (KC-5G5) was from Kangcheng Biotech (Shanghai, P.R. China). Goat anti-rabbit IgG-HRP (SB-200) was obtained from Jingmei Biotech (Shanghai, P.R. China). Glucose, insulin, chloroform, methanol and other chemicals used in this study were obtained commercially.

2.2. Animals and experiment protocol

Male Sprague–Dawley rats, weighing 180–200 g, were purchased from the Experimental Animal Center of Nanjing Medical University (Nanjing, P.R. China) and housed in a temperature- and humidity-controlled environment with a 12-h light–dark cycle. The animals had access to diet and water *ad libitum*. All animal use procedures were conducted in accordance with Chinese legislation on the use and care of laboratory animals and were approved by the Institute for Experimental Animals of Nanjing University.

Fifty rats were divided into two experimental groups: the control group (n=10) had standard diet (commercial food, Keaoxieli Co. Ltd., Peking, P.R. China) and drinking water (available *ad libitum*) and the high-fructose-fed group (n=40) had standard diet and 10% fructose solution in drinking water (available *ad libitum*) during 8 weeks. The fructose level used in this study was decided according to the previous animal studies [6–8]. After 4 weeks, high-fructose-fed rats were divided into four subgroups (10 rats per group). One group (vehicle group) still received 10% fructose in drinking water. The other three groups were fed with betaine at 62.5, 125 and 250 mg/kg together with 10% fructose in drinking water for an additional 4 weeks, respectively. The dosages of betaine were selected according to our preliminary experiment and other reports [30,36]. The precise dosage of betaine was calculated for each rat per day according to body weight, which was dissolved in a fixed amount of 10% fructose solution. Daily food and fluid intake was measured during the treatment. Caloric intake for high-fructose-fed rats was calculated as the sum of calories ingested as food and fructose solution.

2.3. Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)

During the last week of the feeding period, OGTT and ITT were performed, respectively. Rats were orally administered with glucose (1.5 g/kg body weight) or intraperitoneally injected with insulin (0.8 U/kg body weight). Tail-vein blood samples were collected at 0, 30, 60, 90 and 120 min after glucose or insulin treatment and then centrifuged (4000×g) at 4°C for 10 min to get serum for glucose assay. The integrated area under the curve (AUC) analysis for OGTT and ITT was calculated by the following formula: $AUC=(C_0+C_{30}+C_{60}+C_{90}+C_{120})\times 120/2$. C was the concentration of serum glucose at the time point.

2.4. Urine sample collection

Rats were removed to a metabolic cage and had free access to standard diet and water. Urine sample was collected for 24 h, and the volume was recorded for each group. Urine samples were centrifuged at 2000×g for 10 min to remove the particulate contaminants, and the supernatant was used for uric acid and creatinine analysis.

2.5. Blood and tissue samples collection

After the OGTT and ITT, animals were allowed 3 days to recover from wounds. To avoid the fluctuation of hormone levels due to circadian rhythms, rats were killed by decapitation at 9:30–10:30 a.m. after a 16-h fast. Kidney tissues were dissected quickly on ice, and parts of them were immediately fixed for H&E and oil red O staining analysis, while others were stored in liquid nitrogen for Western blot analysis. Blood samples were collected and centrifuged (3000×g) at 4°C for 10 min to get serum frozen at −80°C for biochemical assays.

2.6. Urate handling investigation

Serum and urinary urate were determined by the phosphotungstic acid method. Creatinine and BUN levels in serum and urine were measured using standard diagnostic kits, respectively. Urate concentrations in urine (Uur) and serum (Sur) and creatinine concentrations in urine (Ucr) and serum (Scr) were used to evaluate the fractional excretion of uric acid (FE_{UA}) by the following formula: $FE_{UA}=(Uur\times Scr)/(Sur\times Ucr)\times 100$, expressed as a percentage.

2.7. Determination of lipid contents in serum and renal tissue

Lipid contents in renal tissues were extracted according to the method reported by Folch et al. (1957) [37]. Renal tissues were homogenized with chloroform/methanol (2/1) to a final volume 20 times of wet tissue weight. After dispersion, whole mixture was agitated during 15–20 min in an orbital shaker at room temperature. The homogenate was centrifuged (12,000×g) at 4°C for 10 min to get lipid phase for assays. TG, TC, LDL and HDL levels in serum and TG and TC contents in renal homogenate were determined by standard diagnostic kits, respectively.

2.8. Determination of proinflammatory cytokine levels in serum and kidney

Renal tissues were homogenized in 10 weight/volume of sodium chloride on ice and then centrifuged at 10,000×g for 15 min at 4°C. The supernatants were collected for assays. Serum and kidney IL-1β, IL-18, IL-6 and TNF-α levels were determined by ELISA kits.