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Original Contribution

SREBP-1c in nonalcoholic fatty liver disease induced by Western-type high-fat diet plus fructose in rats

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ABSTRACT

This study concentrated on the initial events triggering the development of nonalcoholic fatty liver disease induced by a high-fat plus fructose (HF-F) diet and on the possibility of delaying nonalcoholic fatty liver disease progression by adding dehydroepiandrosterone (DHEA) to the diet. Sterol regulatory element binding protein-1c (SREBP-1c) activation plays a crucial role in the progression of nonalcoholic fatty liver disease induced by an HF-F diet. This study investigated the protective effects of DHEA, a compound of physiological origin with multitargeted antioxidant properties, against the induction of SREBP-1c and on liver insulin resistance in rats fed an HF-F diet, which mimics a typical unhealthy Western diet. An HF-F diet, fortified or not with DHEA (0.01%, w/w), was administered for 15 weeks to male Wistar rats. After HF-F the liver showed unbalanced oxidative status, fatty infiltration, hepatic insulin resistance, and inflammation. The addition of DHEA to the diet reduced both activation of oxidative-stress-dependent pathways and expression of SREBP-1c and partially restored the expression of liver X-activated receptor-α and insulin receptor substrate-2 genes. DHEA supplementation of the HF-F diet reduced de novo lipogenesis and delayed progression of nonalcoholic fatty liver disease, demonstrating a relationship between oxidative stress and nonalcoholic fatty liver disease via SREBP-1c.

Nonalcoholic fatty liver disease (NAFLD)[1] is the most common liver disease in Western countries [1]; its clinical relevance is connected to its possible evolution toward nonalcoholic steatohepatitis (NASH) and, finally, to end-stage liver disease, including cirrhosis and hepatocellular carcinoma [2]. An increased prevalence of NAFLD has been observed in persons with obesity, metabolic syndrome, and type 2 diabetes, a subset of whom develop NASH [3]. The "two-hit" hypothesis has become an important theoretical framework in understanding the pathogenesis of liver damage in these patients [4]. Although initial and subsequent mechanisms are not entirely distinct, the first hit mainly consists of triglyceride and fatty acid accumulation in the liver. The second hit involves oxidative stress and inflammation of the liver. Both conditions can be induced by high-energy diets, which are believed to play a key role in insulin resistance and dyslipidemia, resulting in increased oxidative stress and a proinflammatory environment [5].

Sterol regulatory element binding protein-1c (SREBP-1c) plays a unique role in the expression of genes involved in hepatic triglyceride synthesis and may also play a major role in the pathogenesis of NAFLD [6]. The regulation of SREBP-1c is dependent on nutritional status and many other factors, primarily insulin levels, liver X-activated receptors (LXR), and Sp1 and nuclear factor Y elements [7]. Although precise mechanisms leading to the activation of the SREBP-1c promoter have yet to be clarified, it is now certain that SREBP-c1 is strongly induced by saturated fatty acids, possibly through their ability to promote inflammation [8]. In the liver, exposure to large quantities of fructose and fats leads to rapid stimulation of lipogenesis with accumulation of triglycerides and contributes to hepatic insulin resistance [9].

This study investigated the role of oxidative-related pathways in the induction of SREBP-1c in rats fed a high-fat diet plus fructose (HF-F) that mimics a typical unhealthy Western diet [10]. Attention was focused on the initial events triggering the development of NAFDL induced by HF-F and on the possibility of delaying NAFDL and the progression to NASH by adding dehydroepiandrosterone (DHEA) to the diet.

Abbreviations: NAFDL, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SREBP-1c, sterol regulatory binding protein-1c; LXR, liver X-activated receptors; HF-F, high-fat diet plus fructose; DHEA, dehydroepiandrosterone; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEFA, nonesterified fatty acid; HOMA, homeostasis model assessment; GSH, reduced glutathione; GSSG, oxidized glutathione; HNE, 4-hydroxynonenal; SOD, total superoxide dismutase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NF-κB, nuclear factor κB; IRS2, insulin receptor substrate; GLUT2, glucose transporter.

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DHEA is a steroid of physiological origin that possesses multi-targeted antioxidant properties: it has been shown to prevent hyperglycemia-induced tissue damage in several in vivo and in vitro models [11–14]. Moreover, a recent report provides novel evidence that more histologically advanced NAFLD is associated with low circulating levels of DHEA and suggests a mechanistic role of DHEA in the histological progression of NAFLD to NASH [15]. DHEA is also reported to influence insulin sensitivity [16] and to display antiobesity properties in rodents, reducing lipogenesis [17].

In this study, rats fed an HF-F diet for 15 weeks showed hyperlipidemia, insulin resistance, liver steatosis, oxidative stress, and an increased expression of liver SCREBP-c1. Low-dose DHEA supplementation of the HF-F diet prevented signs of NAFLD.

Materials and methods

Materials

Unless otherwise indicated all compounds were purchased from Sigma Chemical Co. (St. Louis, MO, USA), antibodies were from Chemicon (Temecula, CA, USA) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), and materials for real-time PCR were from Bio-Rad Laboratories (Hercules, CA, USA).

Animals and treatments

Male Wistar rats (Harlan Laboratories, Udine, Italy) weighing 200–220 g were cared for in compliance with the Italian Ministry of Health Guidelines (No. 86/609/EEC) and with the *Principles of Laboratory Animal Care* (NIH No. 85–23, revised 1985). The scientific project, including animal care, was supervised and approved by the local ethical committee. The animals were divided into four groups: (group 1) rats maintained on standard lab chow, consisting of 24% protein, 11% fat, and 65% carbohydrate (percentages by weight) and water ad libitum (control group); (group 2) rats maintained on standard lab chow fortified with 0.01% (w/w) DHEA (DHEA group); (group 3) rats maintained on a high-fat diet plus 4% cholesterol plus 10% (w/v) fructose dissolved in the drinking water (HF-F group); and (group 4) rats maintained on the HF-F diet fortified with 0.01% (w/w) DHEA (HF-F-DHEA group). The high-fat diet consisted of 17% protein, 26% fat plus 4% cholesterol and 53% carbohydrate. Rats were fed the standard or HF-F diet with or without DHEA for 15 weeks. Body weight and water and food intake were recorded weekly. Rats were then anesthetized with 20 mg/kg bw of Zoletil 100 (Virbac S.r.l., Carros, France) and killed by aortic exsanguination. Blood was collected and plasma isolated. Glycemia was measured with the Accu-Check Compact kit (Roche Diagnostics, Mannheim, Germany). The liver was isolated, weighed, cut into a number of portions, and stored at −80°C.

Oral glucose tolerance test

After a fasting period of 12 h, 1 day before the rats were due to be killed, a 50% (w/v) glucose solution was administered orally at 3 g/kg. Blood samples were collected from the caudal vein after glucose loading.

Biochemical parameters

The plasma lipid profile was determined by measuring the contents of triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) by standard enzymatic procedures using reagent kits (Hospitex Diagnostics, Florence, Italy). Nonesterified fatty acid (NEFA) levels were determined with a commercially available kit (WAKO Pure Chemical, Osaka, Japan).

The plasma insulin level was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala, Sweden). Insulin sensitivity was calculated using the homeostasis model assessment (HOMA): fasting glucose (mmol/L)×fasting insulin (μg/L)/22.5. Leptin and adiponectin levels were measured using an ELISA kit (Leptin Enzyme Immunoassay Kit, SPI-BIO, Montigny le Bretonneux, France; Adiponectin ELISA kit, Otsuka Pharmaceuticals, Japan). Plasma DHEA was determined by specific radioimmunoassay DSL-9000 (Diagnostic System Laboratories, Oxford, UK).

Preparation of tissue extracts

Cytosolic fractions from homogenized liver were prepared by the method of Meldrum et al. [18] with modification. Total extracts were obtained from liver homogenized at 10% (w/v) directly in extraction buffer and centrifuged at 1000 *g* for 5 min at 4 °C. Supernatants (total extract) were stored at −80°C until use. Protein content was determined using the Bradford assay [19]. Total liver homogenates were prepared at 20% (w/v) in PBS by Polytron and stored at −80°C.

Pro-oxidant–antioxidant balance

Hydrogen peroxide (H_2O_2) was determined in the plasma and in the cytosolic fraction as described by Zoccarato et al. [20].

Reduced (GSH) and oxidized glutathione (GSSG) contents were evaluated in plasma and cytosolic fractions of the liver by the method of Owens and Belcher [21]. The GSSG content (expressed as μg/mg protein) is the total glutathione content minus the GSH content.

The 4-hydroxynonenal (HNE) concentration was detected in the cytosolic fraction and plasma by an HPLC procedure using an RP-18 column from Merck (Rahway, NJ, USA). The mobile phase used was 42% acetonitrile/bidistilled water (v/v). Serial concentrations of HNE (0.5–10 μmol/L) were used to prepare a standard curve [22].

Total superoxide dismutase (SOD) activity was assayed in the plasma and in the cytosolic fraction by the method of Flohè and Otting [23].

Liver triglyceride level

Hepatic TG was extracted from total tissue homogenate and detected using reagent kits (Hospitex Diagnostics, Florence, Italy).

Liver necrosis

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined in plasma by standard enzymatic (UV) procedures using reagent kits (Hospitex Diagnostics).

Proinflammatory parameter

The content of tumor necrosis factor-α (TNFα) was determined in plasma and in total liver extract. Protein content was determined and normalized before the samples were analyzed with a rat TNFα ELISA kit (Diaclone, Cedex, France).

Western blot

NF-κB p65 in the cytosol and NF-κB p65 and SREBP-1c in the liver nuclear extract were detected by the Laemmli method [24]. Protein aliquots (80 μg) were separated on 10% SDS–polyacrylamide gels and then blotted onto nitrocellulose membranes. The membranes were incubated with mouse monoclonal antibody against NF-κB p65 and a rabbit polyclonal antibody against SREBP-1c. Anti-β-actin antibody served as loading control for cytosolic proteins, and α-laminin for nuclear proteins. Specific bands were quantified by densitometry using analytic software (Multi-Analyst; Bio-Rad) and the net intensity