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Protein carbonylation associated to high-fat, high-sucrose diet and its metabolic effects

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Abstract

The present research draws a map of the characteristic carbonylation of proteins in rats fed high-caloric diets with the aim of providing a new insight of the pathogenesis of metabolic diseases derived from the high consumption of fat and refined carbohydrates. Protein carbonylation was analyzed in plasma, liver and skeletal muscle of Sprague–Dawley rats fed a high-fat, high-sucrose (HFHS) diet by a proteomics approach based on carbonyl-specific fluorescence-labeling, gel electrophoresis and mass spectrometry. Oxidized proteins along with specific sites of oxidative damage were identified and discussed to illustrate the consequences of protein oxidation. The results indicated that long-term HFHS consumption increased protein oxidation in plasma and liver; meanwhile, protein carbonyls from skeletal muscle did not change. The increment of carbonylation by HFHS diet was singularly selective on specific target proteins: albumin from plasma and liver, and hepatic proteins such as mitochondrial carbamoyl-phosphate synthase (ammonia), mitochondrial aldehyde dehydrogenase, argininosuccinate synthetase, regucalcin, mitochondrial adenosine triphosphate synthase subunit beta, actin cytoplasmic 1 and mitochondrial glutamate dehydrogenase 1. The possible consequences that these specific protein carbonylations have on the excessive weight gain, insulin resistance and nonalcoholic fatty liver disease resulting from HFHS diet consumption are discussed.

Keywords: High-fat, high-sucrose diet; Obesity; Insulin resistance; NAFLD; Protein carbonylation; Sprague–Dawley rat

1. Introduction

Protein carbonylation constitutes the most common biomarker of oxidative protein damage and can be induced by the attack of reactive oxygen species (ROS) [1]. The consumption of high-caloric diets associated to a westernized lifestyle has been linked to the generation of an excess of ROS, particularly superoxide anion through the mitochondrial electron-transport chain, and to the development of multiple metabolic disorders such as obesity, hypertension, hyperglycemia, hyperinsulinemia or dyslipidemia [2–4]. As a consequence of this ROS overproduction, increased protein carbonylation levels have been described occurring together with these diet-induced disorders [5,6].

Protein carbonylation is an irreversible oxidative posttranslational modification, although cells exhibit native enzymatic systems that eliminate altered proteins and maintain cellular homeostasis and survival. These damage response systems are typically proteasome in the cytosol and adenosine triphosphate (ATP)-dependent proteases in mitochondria [7]. When these enzymatic systems come to fail, carbonylated proteins accumulate in the cell, and cellular functions are disrupted because carbonylated proteins either lose their catalytic and structural integrity, or interrupt regulatory pathways [8].

Protein oxidative damage has been traditionally accepted to be a random process since ROS generally attack protein nonenzymatically and radical species such as hydroxyl radicals are so reactive that are they able to oxidize most amino acids [9]. However, several studies have revealed that protein carbonylation is highly selective in diseases such as Alzheimer [10] or uremia [11], or even during the natural aging process. A dietary intervention study of Wistar rats supplemented with fish oils also showed such specificity by down-regulating carbonylation of several plasma and liver proteins [12]. However, to date, few studies have addressed protein oxidation linked to metabolic disorders induced by diet high in fat and/or sugars, and those that exist performed essentially total protein carbonylation measurements [13–17].

The aim of the present study is to evaluate comprehensively the impact of oxidative stress triggered by high-fat, high-sucrose (HFHS) diets on irreversible oxidative protein damage and, particularly, on protein carbonylation. Recent investigations indicated that obesity and insulin resistance are accompanied by an increased carbonylation of certain adipose-regulatory proteins [6,18]. The present work is

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focused on the explicit carbonylation of plasma, liver and skeletal muscle proteins since these tissues play a key role in the development of metabolic disorders [19], and they have not been addressed before. To this purpose, we analyzed total and individual protein carbonylation in plasma, liver and skeletal muscle from rats fed a HFHS diet for 22 weeks compared with control rats fed a low-fat diet. Proteins susceptible to oxidation and carbonylation sites were identified by using a proteomics approach based on gel electrophoresis and protein identification by tandem mass spectrometry (MS/MS). Protein carbonylation was correlated with morphological measurements, and biochemical parameters of lipid and carbohydrate metabolism, lipid peroxidation products and endogenous antioxidant systems, and discussed in terms of its potential implication on the metabolic disorders observed.

2. Materials and methods

2.1. Materials and reagents

Fluorescein-5-thiosemicarbazide (FTSC) was purchased from Invitrogen (Carlsbad, CA), and porcine sequencing-grade modified trypsin was from Promega (Madison, WI). Ketamine chlorhydrate (Imalgene 1000) was purchased from Merial Laboratorios S.A. (Barcelona, Spain), and xylacine (Rompun 2%) was from Quimica Farmaceutica S.A. (Barcelona, Spain). ProteoBlock protease inhibitor cocktail was purchased from Thermo Fisher Scientific Inc. (Rockford, IL). Phenylmethylsulfonyl fluoride (PMSF); dithiothreitol (DTT); iodoacetamide; ethylenediaminetetraacetic acid (EDTA); trichloroacetic acid (TCA); Tris–HCl; 3,3-cholaminopropyl-dimethylammonio-1-propanesulfonate (CHAPS); sodium phosphate; magnesium chloride anhydrous; 2,2 -azobis(2-amidinopropane) dihydrochloride (AAPH); 3 ,6 -dihydroxyspiro[isobenzofuran-1[3H],9 [9H]-xanthen]-3-one (fluorescein); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox); 2-thiobarbituric acid; 1,1,3,3,-tetraethoxypropane; propyl gallate and bicinchoninic acid (BCA) were purchased from Sigma-Aldrich (St. Louis, MO). Urea, thiourea, sodium dodecyl sulfate (SDS), glycine, glycerol and Serdolit MB-1 were obtained from USB (Cleveland, OH). IPG buffer; Pharmalyte 3–10; ammonium persulfate; bromophenol blue; and 1,2-bis(dimethylamino)ethane were purchased from GE Healthcare Science (Uppsala, Sweden). Acrylamide; bis-*N,N* -methylene-bis-acrylamide; and Bio-Rad protein assay were obtained from Bio-Rad (Hercules, CA). All other chemicals and reagents used were of analytical reagent grade, and water was purified using a Milli-Q system (Millipore, Billerica, MA).

2.2. Animals and experimental design

Male 22-week-old Sprague–Dawley rats weighing between 500 and 600 g (Janvier, Le Genest-St-Isle, France) were housed in animal cages (n=2–3/cage) with constantly regulated temperature (22°C±2°C), humidity (50%±10%) and light-controlled room (lights on 06:30–18:30). The animals were randomly assigned to one of two dietary groups: a control group (n=5) fed the reference diet Teklad Global 2014 (Harlan Teklad Inc., Indianapolis, IN) and a group (n=5) fed a HFHS based on TD. 08811 diet (Harlan Teklad Inc.). Rats had *ad libitum* access to water and food. Food intake and water consumption were registered daily throughout the study (means are shown in Table 1). After 22 weeks of experiment, rats were fasted overnight, anesthetized intraperitoneally with ketamine and xylacine (80 mg/kg and 10 mg/kg body weight, respectively) and then killed by exsanguination. The control diet composition was (% by weight) 4.3% fat (3.9% soybean oil), 46.7% available carbohydrate (41% corn and wheat starches), 16.3% protein, 21.5% total fiber, 4.6% minerals and 1.2% vitamins. Fats provided 13.3% of calories, carbohydrates provided 64.3%, and proteins provided 22.5%. Total energy density was 2.9 kcal/g. HFHS diet composition was (% by weight) 24.3% fat (21% anhydrous milk fat, 2% soybean oil), 43.5% available carbohydrate (32.4% sucrose), 20.9% protein, 4.7% total fiber (from cellulose), 4.1% minerals, 1.8% vitamins (from American Institute of Nutrition 93-VX (94047)) and 0.004% antioxidants. Fats provided 45.9% of calories, carbohydrates provided 36.5% (27% from sucrose), and proteins provided 17.6%. Total energy density was 4.7 kcal/g. The approximate fatty acid profile of control diet (% of total fat) was 18% saturated fatty acids (SFA), 20% monounsaturated fatty acids (MFA) and 62% polyunsaturated fatty acids (PUFA). In the HFHS diet, the fatty acid profile was 61% SFA, 31% MUFA and 8% PUFA. The HFHS diet contained about 0.05% of cholesterol, mainly from the anhydrous milk fat. Control diet can contain small amounts of cholesterol, likely less than 0.001%. All the procedures followed the European Union guidelines for the care and management of laboratory animals, and all efforts were made to minimize suffering. The pertinent permission for this specific study was obtained from the CSIC (Spanish Research Council) Subcommittee of Bioethical Issues (ref. AGL2009-12 374-C03-03).

2.3. Sample collection

Total abdominal fat, corresponding to the sum of epididymal, perirenal and mesenteric depots, liver and skeletal muscle from hind leg were excised, washed with 0.9% NaCl solution, weighed and immediately frozen in liquid nitrogen upon sacrifice.

Table 1
Body measurements and metabolic variables in rats

		Control (n=5)	HFHS (n=5)
Final body weight (g)		722.4 (95.6)	918.2 (93.3) *
Food intake (g/day)		26.7 (1.2)	21.9 (0.5) *
Energy intake (kcal/day)		77.4 (8.8)	101.0 (8.4) *
Total abdominal fat weight (g)		32.4 (13.8)	60.7 (9.3) **
Adiposity index (%) [a]		4.4 (1.2)	6.7 (1.5) *
Liver weight (g)		15.6 (3.6)	24.8 (6.8) *
Total liver lipids (mg/g)		2.3 (0.2)	8.8 (1.9) **
Hepatosomatic index (%) [b]		2.1 (0.3)	2.7 (0.8)
Total cholesterol (mmol/L)		2.87 (0.40)	2.26 (0.20)
LDL-C (mmol/L)		0.42 (0.11)	0.29 (0.04)
HDL-C (mmol/L)		0.73 (0.06)	0.60 (0.06)
Triglycerides (mmol/L)		2.13 (0.34)	1.71 (0.33)
HbA1c (%)		5.7 (0.4)	5.0 (0.1)
Plasma glucose (mmol/L)		4.1 (0.4)	4.5 (0.5)
Plasma insulin (pmol/L)	Week 5	0.25 (0.10)	1.16 (0.11) **
	Week 9	0.27 (0.12)	0.80 (0.03) **
	Week 13	0.29 (0.14)	0.88 (0.05) **

Results are means (standard deviation); n, number of rats.
[a] Adiposity index: (total abdominal fat×100)/body weight.
[b] Hepatosomatic index: (liver weight×100)/body weight.
* P<.05 vs. control group.
** P<.01 vs. control group.

The adiposity index [(total abdominal fat×100)/body weight] and hepatosomatic index [(liver weight×100)/body weight] were determined. One part of dissected livers was used for histological analysis. Blood samples were collected on a tube with EDTA by cardiac puncture, and plasma and erythrocyte samples were separated by centrifugation (850*g*, 4°C, 15min). Plasma samples for protein oxidation measurement were supplemented with 5 mM PMSF. All samples were stored at −80°C until analysis.

2.4. Biochemical measurements

Triglycerides, cholesterol, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol were measured by spectrophotometric methods (SpinReact Kits, Girona, Spain) [20,21]. The percentage of glycated hemoglobin, or HbA1c, in the blood was determined according to Sharp et al. [22]. Blood glucose levels were measured by an enzyme electrode method using the Ascensia ELITE XL blood glucose meter (Bayer Consumer Care AG, Basel, Switzerland). Plasma insulin concentrations were measured using a Rat/Mouse Insulin ELISA kit according to the manufacturer's instructions (Millipore Corporation, Billerica, MA).

2.5. Determination of lipid peroxidation levels and antioxidant systems in blood and liver samples

For estimating lipid peroxidation levels, we measured three different products of lipid peroxidation: lipid hydroperoxides (primary lipid oxidation product), conjugated dienes (intermediate oxidation product) and malondialdehyde (MDA) (end oxidation product). The first step for the estimation of lipid hydroperoxides and conjugated dienes was to extract liver lipids with dichloromethane [23] and to determine total lipid content [24]. Then, conjugated dienes were measured following the American Oil Chemists' Society method [25], and lipid hydroperoxides were measured following the method of Chapman and Mackay [26]. We also measured total MDA concentrations in plasma and liver. Total MDA was derivatized with thiobarbituric acid (TBA) after protein hydrolysis [27] and precipitation [28], and determined by high-performance liquid chromatography fluorescence according to Fukunaga et al. [29]. Total superoxide dismutase (SOD) activity was measured in plasma and liver following the method of Misra and Fridovich [30]. Catalase (CAT) activity was measured in plasma and liver according to the procedure of Cohen et al. [31]. Glutathione peroxidase (Gpx) and glutathione reductase (GR) activities were determined according to Wheeler et al. [32]. The oxidized and reduced glutathione balance (GSSG/GSH) was measured by the Hissin and Hilf fluorometric method [33]. The oxygen radical absorbance capacity (ORAC) from plasma and liver was determined according to Ou et al. [34]. ORAC measures antioxidant scavenging activity against peroxyl radicals induced by AAPH using fluorescein as fluorescent probe and Trolox as standard and quality control.

2.6. Liver histological studies

Livers fixed in formalin were dehydrated in alcohol and embedded in paraffin. Serial liver sections (3 μm) were stained with haematoxylin/eosin (Harris Hematoxylin, Química Clínica Aplicada, S.A., Tarragona, Spain). Liver injury such as steatosis, lobular inflammation and fibrosis was evaluated by histological examination under a light microscope, as previously described [35]. Briefly, ten 200× light microscopic fields were viewed on each section and scored for the severity of hepatic steatosis, inflammation and fibrosis according to the following criteria: for hepatic steatosis (expressed as percent of