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Hormetic modulation of hepatic insulin sensitivity by advanced glycation end products

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ABSTRACT

Because of the paucity of information regarding metabolic effects of advanced glycation end products (AGEs) on liver, we evaluated effects of AGEs chronic administration in (1) insulin sensitivity; (2) hepatic expression of genes involved in AGEs, glucose and fat metabolism, oxidative stress and inflammation and; (3) hepatic morphology and glycogen content. Rats received intraperitoneally albumin modified (AlbAGE) or not by advanced glycation for 12 weeks. AlbAGE induced whole-body insulin resistance concomitantly with increased hepatic insulin sensitivity, evidenced by activation of AKT, inactivation of GSK3, increased hepatic glycogen content, and decreased expression of gluconeogenesis genes. Additionally there was reduction in hepatic fat content, in expression of lipogenic, pro-inflamatory and pro-oxidative genes and increase in reactive oxygen species and in nuclear expression of NRF2, a transcription factor essential to cytoprotective response. Although considered toxic, AGEs become protective when administered chronically, stimulating AKT signaling, which is involved in cellular defense and insulin sensitivity.

1. Introduction

Advanced glycation end products (AGEs) are a group of compounds formed as a result of nonenzymatic covalent interaction between reducing sugars and amino groups of proteins, lipids and nucleic acids (Vlassara and Palace, 2002,Sadowska-Bartosz and

Abbreviations: AGEs, Advanced glycation end products; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; Nfkb1, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; PI3K, phosphoinositide 3-kinase; AKT, serine/threonine-specific protein kinase; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; GS, glycogen synthase; TC, total cholesterol; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NEFA, non-esterified fatty acids; ITT, Insulin tolerance test; kITT, constant rate of blood glucose disappearance; SREBP, sterol regulatory element-binding protein 1; $V_{v[f]}$, volumetric density of fat globules; PAS, periodic acid-Schiff; NAFLD, non-alcoholic fatty liver disease; αSMA, α-smooth muscle actin; NRF2, nuclear factor-erythroid 2-related factor; KEAP1, Kelch-like ECH-associated protein; mTOR, mammalian target of rapamycin; *Ager*, Receptor for advanced glycation end-products; Lgals3, Receptor for advanced glycation end-products 3; *Ddost*, Advanced glycation end product receptor 1/OST48; *Pck1*, Phosphoenolpyruvate carboxykinase; *G6pc*, Glucose-6-phosphatase; *Slc2a2*, Solute carrier 2a2; *Foxa2*, Forkhead box A2; *Srebf1*, Sterol regulatory element binding transcription factor 1; *Srebf2*, Sterol regulatory element binding transcription factor 2; *Acaca*, Acetyl-CoA carboxylase; *Fasn*, Fatty acid synthase; *Tnf*, Tumor necrosis factor; *Il6*, Interleukin 6; *Hmgb1*, High-mobility group box 1; *Sod1*, Superoxide dismutase 1; *Cat*, Catalase; *Txnip*, Thioredoxin interacting protein; IRS2, Insulin receptor substrate 2; DHE, Dihydroethidium.

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Bartosz, 2015,Ottum and Mistry, 2015). AGEs formation is part of the normal aging process, however, it is accelerated in conditions of chronic hyperglycemia and other homeostatic imbalances. AGEs can also be obtained from exogenous sources, such as high fat/sugar heat-processed foods, abundant in modern diets (Uribarri et al., 2010).

AGEs bind to cell surface receptors such as the receptor for advanced glycation end products (RAGE) and AGE-specific complex receptors AGER1/OST48, and AGER3/galectin-3 (Ott et al., 2014,Sato et al., 2006). RAGE activation stimulates formation of reactive oxygen species (ROS) and expression of NFkB and pro-inflammatory cytokines, creating a vicious cycle that exacerbates AGEs formation (Xie et al., 2013,Tóbon-Velasco et al., 2014,Kislinger et al., 1999,Yamagishi and Matsui, 2015). In contrast, AGER1 exerts antioxidant effects, suppresses RAGE signaling and removes circulating AGEs (Li et al., 1996,Vlassara and Striker, 2011,Vlassara et al., 2009,Cai et al., 2012).

There is evidence suggesting that AGEs contribute to chronic liver disease (Basta et al., 2011,Lorenzi et al., 2011,Guimarães et al., 2010,Iwamoto et al., 2008,Wu et al., 2010,Goodwin et al., 2013,Gaens et al., 2012). RAGE is expressed in various liver cell types and chronic AGEs administration exacerbated liver injury in rats submitted to a bile duct ligation (Goodwin et al., 2013). Although numerous studies had evaluated AGEs as a potential cause of hepatic injury, we could not find descriptions of the effects of these compounds on the hepatic metabolism. The liver is a insulin-target tissue critical for glucose homeostasis; in this organ, insulin-receptor-mediated PI3K and AKT stimulation inhibits gluconeogenesis and stimulates glycogen synthesis (Barthel and Schmoll, 2003,Kubota et al., 2000,Michael et al., 2000).

In view of the key role of the liver in glucose homeostasis and of the paucity of information regarding the metabolic effects of AGEs on the liver and given the abundance of AGEs in the Western-diet, we evaluated the effects of chronic AGEs administration in (1) variables associated with glucose homeostasis; (2) hepatic expression of genes involved in AGEs, glucose and fat metabolism, oxidative stress and inflammation; (3) hepatic status of proteins involved in insulin signaling and antioxidant defense; and (4) hepatic morphology and glycogen content.

2. Materials and methods

2.1. Animals and ethics statement

Experimental protocols were conducted in accordance with guidelines of Brazilian College for Animal Experimentation and were approved by the Institutional Animal Care and Research Advisory Committee. Rats were obtained in the central animal facility of University of São Paulo Medical School. Male Wistar rats (207.6 ± 13.83 g) were housed in normal cages in a climate-controlled environment with free access to water and standard rat chow. Rat serum albumin (RSA) was purchased from Sigma-Aldrich (Taufkirche) and modified by advanced glycation (AlbAGE) as previously described (Machado et al., 2006).

2.2. Experimental protocol

Rats were randomly assigned to two experimental groups and treated for 12 weeks: Control group (AlbCTRL, $n = 11$) receiving unmodified RSA (20 mg/kg/day) and AGE group (AlbAGE, $n = 12$) receiving AGE-modified RSA (20 mg/kg/day), intraperitoneally (i.p.), as previously described (Coughlan et al., 2011). After treatment, rats were anesthetized and perfused with phosphate-buffered saline through abdominal aorta. The liver was quickly excised and a fragment of tissue was snap frozen and stored at −80 °C until molecular biology analyzes; other fragment was fixed in 10% formaldehyde for 24 h at room temperature, then dehydrated and processed for paraffin-embedding for histological analyzes.

2.3. Blood analyses

Before the procedure, the animals were food deprived for 12 h. Blood samples were collected from the caudal vein. Plasma was used for measurements of TC, TG, AST and ALT by enzymatic/colorimetric method. Blood glucose was measured by a glucometer (ACCU-CHEK®; Roche, Basel) and insulinemia was measured by enzymatic immunoassay using specific rat/mouse insulin ELISA kit (Millipore, Bedford).

Serum was used for NEFA measurement (Randox Laboratories LTD, Crumlin, UK). Whole-body insulin sensitivity was assessed by the insulin tolerance test (ITT), as previously described (Okamoto et al., 2011).

2.4. Gene expression analyses by quantitative PCR

Approximately 200 mg of liver were pulverized in liquid nitrogen, followed by RNA isolation using TRIzol LS Reagent (Life Technologies, Carlsbad) and a RNA extraction kit (Qiagen Sciences, Germantown). Two μg of total RNA were reverse-transcribed using random hexamers (High Capacity cDNA Reverse Transcription Kit; Life Technologies) following the manufacturer's guidelines. Quantitative real-time PCR was performed in duplicate using the StepOnePlus System (Life Technologies) with 100 ng of cDNA and the TaqMan assay system (Life Technologies) All employed probes spanned an exon junction: *Ager* (Rn01525753_g1), *Lgals3* (Rn00582910_m1), *Ddost* (Rn01518759_m1), *G6pc* (Rn00689876_m1), *Pck1* (Rn01529014_m1), *Slc2a2* (Rn00563565_m1), *Foxa2* (Rn01415600_m1), *Srbef1* (Rn01495769_m1), *Srbef2* (Rn01502638_m1), *Acaca* (Rn00573474_m1), *Fasn* (Rn00569117_m1), *Nfkb1* (Rn01399583_m1), *Tnf* (Rn01525859_m1), *Il6* (Rn0141030_m1), *Hmgb1* (Rn02377062_g1), *Sod1* (Rn0566938_m1), *Cat* (Rn00560930_m1), *Txnip* (Rn01533891_g1). Data were normalized by the mean expression of two reference genes (*Actb*; Rn00667869_m1 and *Ppib;* Rn03302274_m1). Relative levels of mRNA expression were calculated using comparative cycle threshold (Ct) ($2^{-\Delta\Delta Ct}$) method (Livak and Schmittgen, 2001).

2.5. Western blotting

For AKT, GSK3 and HMGB1 protein analyses, liver samples were homogenized in ice-cold solubilization buffer (100 mM Tris [pH 7.5]; 10 mM EDTA; 10% SDS; 100 mM NaF; 10 mM Na^+ pyrophosphate; 10 mM Na^+orthovanadate). The protein extracts were incubated at 95 °C for 10 min and then centrifuged at 16,000 g at 4 °C for 40 min. For SREBP1 analysis, liver was homogenized in ice-cold RIPA buffer supplemented with a protease inhibitor mixture. For NRF2 protein analysis, cytosolic and nuclear protein fractions samples were obtained based on previously described (Andrews and Faller, 1991). Briefly, samples were pulverized in liquid nitrogen, suspended in ice-cold PBS containing dithiothreitol (DTT; 200 mM) and phenylmethylsulphonyl fluoride (PMSF; 200 mM), and centrifuged at 1000g for 10 min. The supernatant was recovered as the cytosolic fraction. The pellet, containing the nucleus, was suspended in hypotonic buffer (10 mM HEPES pH 7.9, 1.5 mM MgCl, 10 mM KCl, 0.2 mM DTT, 0.2 mM PMSF) and incubated for 10 min. NP-40 was added to a final concentration of 0.4%, and samples were centrifuged at 15,000 g for 30 s. The resulting pellet was then incubated for 20 min in a high salt extraction buffer (20 mM HEPES pH 7.9, 1.5 mM MgCl, 0.5 mM EDTA, 0.2 mM DTT,