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Xanthohumol and 8-prenylnaringenin ameliorate diabetic-related metabolic dysfunctions in mice☆,☆☆

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Abstract

Type 2 diabetes mellitus (T2DM) is a chronic disease characterized by metabolic disturbances in specific tissues. The present work aimed to analyze the effects of xanthohumol (XN) and 8-prenylnaringenin (8PN), two beer-derived polyphenols, in liver and skeletal muscle lipid and glycolytic metabolism in T2DM mice model. Thirty C57Bl/6 mice were randomly divided into five groups: standard diet (control), high-fat diet (DM), high-fat diet plus ethanol (DM-Ethanol), high-fat diet plus 10 mg/L XN (DM-XN) and high-fat diet plus 10 mg/L 8PN (DM-8PN) during 20 weeks. Fasting blood glucose and insulin tolerance tests were performed 1 week before sacrifice. At the end of the study, blood, liver and skeletal muscle were collected. Both XN and 8PN treatments prevented body weight gain; decreased glycemia, triglyceride, cholesterol and alkaline phosphatase levels; and improved insulin sensitivity. Polyphenols promoted hepatic and skeletal muscle AMP-activated protein kinase (AMPK) activation, diminishing the expression of target lipogenic enzymes (sterol regulatory element binding protein-1c and fatty acid synthase) and acetyl-CoA carboxylase activity. Moreover, both XN and 8PN treatments decreased VEGFR-1/VEGFB pathway, involved in fatty acid uptake, and increased AS160 expression, involved in GLUT4 membrane translocation. Presented data demonstrated that both XN and 8PN treatment resulted in AMPK signaling pathway activation, thus suppressing lipogenesis. Their consumption prevented body weight gain and improved plasma lipid profile, with significant improvement of insulin resistance and glucose tolerance. XN- or 8PN-enriched diet could ameliorate diabetic-associated metabolic disturbances by regulating glucose and lipid pathways.

Keywords: Type 2 diabetes mellitus; Metabolism; Liver; Skeletal muscle; Polyphenols

1. Introduction

The prevalence of obesity and type 2 diabetes mellitus (T2DM) has dramatically increased over the past decades, and their associated metabolic complications are considered a major health problem affecting more than 400 million adults worldwide [1]. As a hallmark sign of T2DM, hyperglycemia is one of the main causes of glucose control and lipid metabolism impairments, early outcomes of insulin resistance. The available treatment strategies for diabetes management are not completely efficient, as highlighted by the markedly increased morbidity and mortality rates in diabetic patients. According to this, lifestyle modification and improved pharmacological preventive and therapeutic approaches for T2DM are needed. In recent years, studies using novel pharmacological treatments and functional foods

Abbreviations: 8PN, 8-prenylnaringenin; ACC, acetyl-CoA carboxylase; AUC, area under the curve; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AMPK, AMP-activated protein kinase; CD36, cluster of differentiation 36; FAS, fatty acid synthase; FAT, fatty acid translocase; HFD, high-fat diet; HOMA, homeostasis model assessment of insulin resistance; IPITT, intraperitoneal insulin tolerance test; OGTT, oral glucose tolerance test; PFKFB3, 6-phosphofructo-2-kinase fructose-2,6-bisphosphatase 3; SREBP-1c, sterol regulatory element-binding protein 1c; VEGF-B, vascular endothelial growth factor B; VEGFR-1, vascular endothelial growth factor receptor 1; T2DM, type 2 diabetes mellitus; XN, xanthohumol.

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to regulate energy metabolism have been conducted in order to control T2DM [2]. Growing evidence indicates that polyphenols, apart from antioxidant activity, possess health-promoting properties associated with the prevention and therapeutic approaches being associated with low risk for the development and progression of chronic diseases, namely, diabetes and cardiovascular disease [3,4]. Several epidemiologic studies suggest that a polyphenol-enriched diet is an important strategy to prevent obesity and related chronic diseases, namely, T2DM. Both xanthohumol (XN) and 8-prenylnaringenin (8PN) beer-derived polyphenols have been shown to possess interesting biological effects, namely, antidiabetic, anti-inflammatory and anticarcinogenic [5–8]. However, the underlying mechanisms of metabolic action of XN and 8PN in lipid and glucose pathways have not been thoroughly investigated.

The present study aims to identify the pathways involved in the metabolic disarrangements and to address the putative role of polyphenols in counteracting the development of T2DM. Our attention has focused in both liver and skeletal muscle, important metabolic tissues in the regulation of energy metabolism and body homeostasis. AMP-activated protein kinase (AMPK) is a key metabolic regulator present in both tissues and plays an important role in the control of glucose and lipid metabolism [9,10]. Evidence suggests that a decrease in AMPK activity may cause metabolic disorders. Thus, modulation of this regulatory enzyme could be a promising target for treating metabolic disturbances. Once activated, AMPK suppresses the expression of sterol regulatory element-binding protein (SREBP)-1c, an important transcription factor involved in the fatty acid biosynthesis and lipid uptake [11–15]. SREBP-1c primarily regulates lipogenic enzymes such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), which in turn inhibit glucose production and lipid biosynthesis and stimulate fatty acid β-oxidation [9,16,17]. Hence, AMPK-dependent phosphorylation of SREBP-1c might be a potential approach to treat lipid disarrangements as in T2DM and other metabolic diseases [15,18,19].

Free fatty acids are taken up through specific transporter proteins, with the scavenger receptor cluster of differentiation 36 (CD36) being the best characterized fatty acid translocase [20]. CD36 was linked to lipid metabolism accounting for the increased rate of fatty acid transport into several tissues of high-fat diet (HFD)-fed animals [20,21]. Increased expression of hepatic and muscle CD36 protein in response to an HFD is sufficient to exacerbate triglyceride storage and secretion, contributing to the dyslipidemia associated with T2DM development [20–22]. Some studies have demonstrated that polyphenols down-regulate CD36 gene expression displaying lipid-lowering effect [23]. However, the potential of XN and 8PN in this transporter expression has never been addressed. Hagberg and colleagues have recently proposed the involvement of vascular endothelial growth factor receptor-1 (VEGFR-1) and its ligand, vascular endothelial growth factor B (VEGF-B), in the lipid transport and uptake from endothelial cells to tissues [24,25].

Moreover, it is well documented that the deregulation on glucose uptake by skeletal muscle in T2DM is primarily modulated by insulin-sensitive GLUT4 through PI3K/Akt pathway [26]. In order to understand the involvement of polyphenol consumption in glucose uptake, AS160, an Akt substrate, was assessed in the present work as a mediator of the GLUT4 translocation. Furthermore, the expression of an important glycolytic regulator also involved in insulin signaling, 6-phosphofructo-2-kinase fructose-2,6-bisphosphatase 3 (PFKFB3), was also assessed in our study. PFKFB3 regulates glycolysis through the production of fructose 2,6-bisphosphate, which is a potent allosteric activator of 6-phosphofructo-1-kinase, the glycolysis rate-limiting step [27,28]. A study conducted by Trefely and coworkers demonstrated that the *in vitro* inhibition of PFKFB3 suppressed insulin-stimulated glucose uptake and GLUT4 translocation, impairing insulin signaling and exacerbating insulin resistance [28].

Herein, we evaluated the diabetic-preventive effect of XN and 8PN using an HFD-induced diabetic mice model by exploring the molecular mechanisms associated with diabetic metabolic dysfunction.

2. Material and methods

2.1. Animals and experimental mice treatment

Thirty-six-week-old male C57Bl/6 mice (Charles-River, Spain) were randomly divided into five experimental groups ($n=6$) and fed *ad libitum* with the following: standard diet (C); HFD (obtained from Research Diets, #D12451, New Jersey, USA) (DM); HFD plus 0.1% ethanol in drink water (DM-Ethanol); HFD and 10 mg/L XN (Hopsteiner, Germany) in 0.1% ethanol (DM-XN) and HFD plus 10 mg/L 8PN in 0.1% ethanol (DM-8PN) during 20 weeks. The 8PN was synthetized according to previously described procedures [29]. This mouse strain is prone to develop T2DM under HFD ingestion, displaying significant dyslipidemia, insulin resistance and glucose intolerance, as demonstrated by others [30]. During the treatment period, body weight and glycemia were monitored weekly; food and beverage intake were controlled every 2 days. Beverages were renewed every 2 days and were kept in dark bottles to avoid compound degradation. After 20 weeks of treatment, animals were then sacrificed, and skeletal muscle and liver were frozen at −80°C for molecular analyses or fixed in 10% neutral-buffered formalin, dehydrated and paraffin-embedded for histological assays. Three-micrometer-thick tissue sections were used for hematoxylin–eosin histological staining. Blood was also collected for biochemical analyses.

Animals were maintained under controlled conditions of temperature (23°C±5°C), humidity (35%±5%), and 12-h light/dark cycles. All animal experiments were conducted at the animal house located at the Faculty of Medicine, University of Porto, and were carried out by trained technicians in accordance with the European Community policy for Experimental Animal Studies [European Community law dated November 24, 1986 (86/609/CEE), with addendum from June 18, 2007 (2007/526/CE)].

2.2. Oral glucose tolerance test (OGTT) and intraperitoneal insulin tolerance test (IPITT)

All animals were fasted overnight after 19 weeks of treatment. To perform the OGTT, all mice received a glucose solution of 1 g/kg body weight by oral gavage. To analyze IPITT, animals were injected intraperitoneal with 0.75 U/kg body weight of insulin (Sigma, Portugal). Blood glucose concentrations were measured 30 min before; at baseline; and thereafter at 15, 30, 60, 90 and 120 min after the glucose or insulin administration with Precision Xtra Plus test strips and an Optium Xceed device (Abbott Diabetes Care, Ltd., Maidenhead, UK) according to the manufacturer's instructions. Results are expressed as mean ± S.D. of the total area under the curve (AUC) calculated for each measurement.

2.3. Systemic biochemical measurements

Plasma biochemical markers were assessed at the Department of Clinical Pathology, São João Hospital Center, using Olympus AU5400 automated clinical chemistry analyzer (Beckman-Coulter, Izasa, Porto, Portugal). Hepatic function markers were determined such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels, and metabolic status was evaluated through glucose, uric acid, triglycerides (TG), total cholesterol, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL, calculated according to Friedewald's equation) and high-density lipoproteins (HDL) levels. Plasma insulin levels were measured using a rat/mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (EZRMI-13K; Merck Millipore, Madrid, Spain).

2.4. Insulin sensitivity and resistance indexes

Insulin sensitivity and resistance indexes were calculated as follows: quantitative insulin sensitivity check index (QUICKI)=1/[log(I0) log(G0)], where I0 is fasting insulin (U/ml) and G0 is fasting glucose levels (mg/dl), and homeostasis model assessment (HOMA)=(G0 I0)/22.5, where glucose was expressed as mg/dl and insulin was expressed as μU/ml [31].

2.5. Extraction and quantification of liver TG and cholesterol levels

TG and cholesterol were extracted from frozen liver samples and were determined by colorimetric quantification kits for triglyceride (ab65336; Abcam, UK) and cholesterol (ab65359; Abcam, UK), according to the manufacturer's instructions.

2.6. Tissue VEGF-B quantification by ELISA

Liver and skeletal muscle VEGF-B concentration was quantified using Quantikine mouse VEGF-B (ABIN869657; Antibodies-online, USA) ELISA kit, using a microplate reader (Thermo Fisher Scientific, USA) in accordance with the manufacturer's instructions.