Journal of Functional Foods 67 (2020) 103856

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

A polyphenol-rich Calafate (*Berberis microphylla*) extract rescues glucose tolerance in mice fed with cafeteria diet

J. Soto-Covasich[a], M. Reyes-Farias[b], R.F. Torres[c,d], K. Vasquez[b], L. Duarte[b], J. Quezada[b], P. Jimenez[b], M.T. Pino[e], L. Garcia-Nannig[f], L. Mercado[g], D.F. Garcia-Diaz[b,*]

[a] *Programa de Doctorado en Biotecnologia, Pontificia Universidad Catolica de Valparaiso-Universidad Tecnica Federico Santa Maria, Chile*
[b] *Departamento de Nutricion, Facultad de Medicina, Universidad de Chile, Santiago, Chile*
[c] *Laboratorio de Biologia, Fundacion Cultura Cientifica, Valdivia, Chile*
[d] *Departamento de Ciencias Basicas, Facultad de Medicina, Universidad Catolica de la Santisima Concepcion (UCSC), Concepcion, Chile*
[e] *Instituto de Investigaciones Agropecuarias, Santiago, Chile*
[f] *Advanced Center for Chronic Diseases (ACCDiS), Universidad de Chile, Santiago, Chile*
[g] *Laboratorio de Inmunologia, Instituto de Biologia, Pontificia Universidad Catolica de Valparaiso, Chile*

ARTICLE INFO

Keywords:
Obesity
Adipose tissue
Inflammation
Glucose tolerance
Natural product

ABSTRACT

The establishment of a chronic inflammatory state in the adipose tissue contributes to obesity-associated insulin resistance. Hence, disrupting the inflammatory response elicited by obesity remains a relevant target to tackle the modern-world pandemic. We evaluated the anti-inflammatory and insulin-sensitizing effect of Calafate (*Berberis microphylla*) by producing and characterizing a polyphenol-pure Calafate extract (PPCE). C57BL/6 mice fed with cafeteria diet for 14 weeks were administered PPCE (50 mg/Kg/day) for 4 weeks. PPCE administration rescued glucose tolerance and insulin-elicited AKT phosphorylation in white adipose tissue of diet-induced insulin-resistant mice. Furthermore, the cafeteria diet-induced expression of TNF-alpha and F4/80 was attenuated by PPCE administration, suggesting that PPCE rescues insulin sensibility by ameliorating the obesity-associated inflammatory state. Altogether, our data shows that Calafate represents a natural source of polyphenols with glucose tolerance-improving properties *in vivo*, suggesting a potential use of PPCE as a complementary tool against insulin resistance.

1. Introduction

Processed food are the major source of energy intake in high-income countries and processed food consumption is associated to the development of obesity (Hall et al., 2019; Poti, Braga, & Qin, 2017). In rodents, diets consisting of processed foods are known as Cafeteria Diet (CD). CD have been used to study diet-induced obesity and associated disorders, including insulin resistance, cardiovascular disease and diabetes. Representing a relevant model to study obesity-associated comorbidities (Gomez-Smith et al., 2016).

Obesity is a worldwide health burden that caused around 3.4 million deaths in 2010 (Lim et al., 2012). Obesity is characterized by an excessive accumulation of fat in adipose tissue contributing to the generation of a low-grade chronic inflammatory state with concomitant macrophage infiltration (Gustafson, Hedjazifar, Gogg, Hammarstedt, & Smith, 2015). CD consumption induces an inflammatory state in rodents (Toledo et al., 2019; Zeeni, Dagher-Hamalian, Dimassi, & Faour, 2015), that is associated to robust macrophage infiltration to the adipose tissue (Sampey et al., 2011). The interaction between macrophages and adipocytes causes an increase in pro-inflammatory cytokines such as Tumor Necrosis Factor alpha (TNF-alpha), Monocyte chemoattractant protein-1 (MCP-1) and nitric oxide (NO). The increase in adipokines positively correlates with body adiposity (Ferrante, 2007), contributing to the generation and maintenance of a chronic inflammatory state associated to obesity (Shoelson, Lee, & Goldfine, 2006).

Adipokines interferes with the pivotal PI3K/AKT pathway, rendering adipose tissue inflammation as a link between obesity and insulin resistance (Huang, Liu, Guo, & Su, 2018). Ultimately, obesity-induced alterations in insulin signaling and impaired glucose homeostasis

Abbreviations: BAT, Brown adipose tissue; CD, Cafeteria diet; C-3-GE, Cyanidin-3-glucoside equivalents; Chow, Chow diet; FRAP, Ferric reducing antioxidant power; GAE, Gallic acid equivalents; LC-MS, Liquid chromatography coupled to mass spectrometry; MCP-1, Monocyte chemoattractant protein-1; NO, Nitric oxide; PPCE, Polyphenol-pure Calafate extract; T2D, Type 2 diabetes; TNF-alpha, Tumor Necrosis Factor alpha; WAT, White adipose tissue

* Corresponding author at: Departamento de Nutricion, Facultad de Medicina, Universidad de Chile, Independencia 1027, Santiago, Chile.
E-mail address: dgarcia@med.uchile.cl (D.F. Garcia-Diaz).

https://doi.org/10.1016/j.jff.2020.103856
Received 26 June 2019; Received in revised form 11 February 2020; Accepted 13 February 2020
Available online 28 February 2020

are the major contributors to the generation of type 2 diabetes (T2D) (Dandona, Aljada, & Bandyopadhyay, 2004). Evidence suggest that disrupting the inflammatory response elicited by obesity allows a recovery of insulin sensitivity (Chen, Lee, Leu, & Wang, 2019; Ruiz & Haller, 2006).

The Calafate (*Berberis microphylla*) is a native shrub that grows in Chilean and Argentinian Patagonia (Ruiz et al., 2010). Calafate fruits are dark purple, black, or bluish berries that show a high polyphenol content and a high antioxidant capacity (Ramirez, Zambrano, Sepulveda, Kennelly, & Simirgiotis, 2015). Polyphenols have been shown to ameliorate the inflammatory state associated to obesity and to improve insulin sensitivity (Anhe et al., 2017). Interestingly, we previously showed that a Calafate extract prevented the inflammation-elicited alteration of insulin-induced glucose uptake using an *in vitro* model of inflammation, suggesting an insulin-sensitizing role for Calafate (Reyes-Farias et al., 2015, 2016). However, this has not been evaluated *in vivo*. In this work, we aim to evaluate the effects of Calafate extract treatment on body weight, inflammation, glucose tolerance and insulin signaling pathway in a preclinical model of insulin-resistance.

2. Material and methods

2.1. Mice

Male C57BL/6 mice of 6–7 weeks of age and weighing 22 ± 1 g were used throughout the study. Mice were kept in an animal facility in a 12/12 h light/dark cycle, room temperature was kept constant at 20 ± 2 °C. 3–4 mice were co-housed with free access to food and water. All the experiments were approved by the Animal Ethics Committee of the University of Chile (FMUCH, Chile, protocol N° CBA #0787) (Santiago, Chile).

2.2. Experimental design

Mice were randomly assigned to one of the following experimental groups: i) A control group was always kept on a chow diet (Chow) ii) A group was kept on cafeteria diet throughout the study (CD) iii) A third group was obtained by randomly dividing the CD group after 10 weeks of cafeteria diet feeding, this group of mice received PPCE in the drinking water during 4 weeks (CD + PPCE). This group continued the cafeteria diet regime during the time PPCE was administered. Mice body weight was registered weekly. For collecting tissues, mice were intraperitoneally injected with a ketamine-xylazine mixture (91 and 9 mg/kg respectively) and euthanized by cervical dislocation. Cardiac puncture was used to collect the blood used for lipid profiling. Tissues were kept at −80° until used.

The cafeteria diet (62.9% kcal fat, 20.2% kcal carbohydrates and 16.9% kcal protein) was prepared as previously described (Garcia-Diaz et al., 2007; Paternain et al., 2011). The chow used for control feeding was Champion® Animal nutrition, Santiago, Chile (25.3% kcal fat, 54.9% kcal carbohydrates and 19.8% kcal proteins).

2.3. Calafate extract

Calafate fresh fruits were donated by the Institute of Agricultural Research of Chile (INIA). The raw extract was prepared as follows: 535 g of fresh Calafate fruit were dried for 72 h at 40 °C and pulverized in a mortar. A 24 h extraction was performed using methanol:water (1:1 v/v) and followed by methanol evaporation at 40 °C using a rotating evaporator. The raw extract was resuspended in 450 mL ultrapure water and loaded onto an Amberlite (R) XADH7 column (Sigma-Aldrich, San Luis, MO, USA). The PPCE was obtained by eluting the phenolic compounds with methanol acidified with 0.1% HCl. Methanol was evaporated as described above. Finally, the PPCE was resuspended in 250 mL ultrapure water and maintained at −20 °C until required. The PPCE was administered in the drinking water at a dose of 50 mg polyphenol/Kg daily. Polyphenol stability was evaluated in PPCE frozen samples and in the PPCE beverage using Folin ciocalteu phenol method. The PPCE beverage was replaced each second day to avoid polyphenol degradation.

2.4. Extract characterization

Total polyphenolic content was determined by the Folin ciocalteu phenol method measured at 765 nm (Singleton, 1985). Results were expressed as gallic acid equivalents (GAE). Total anthocyanins were measured by the differential pH method (Wrolstad, 1993). The absorbances were measured at 515 and 700 nm using buffers pH 1.0 and 4.5, respectively. Results were expressed as mg cyanidin-3-glucoside equivalents (C-3-GE)/100 g dry weight (DW). The antioxidant activity was measured by the ferric reducing antioxidant power (FRAP) method measured at 593 nm (Benzie & Strain, 1996). Results were expressed as mmol Fe^{+2}/100 g DW. The anthrone method was used to determine the total carbohydrate content. Measures were taken at 630 nm (Somani, Khanade, & Sinha, 1987).

2.4.1. Liquid chromatography coupled to mass spectrometry (LC-MS)

The analyses were conducted using an Agilent 1100 HPLC (Agilent Technologies Inc., CA, USA) system coupled with a Esquire 4000 ion trap LC/MS system (Bruker Daltoniks, Germany), using a C18 column (5 μm, 4.6 mm i.d × 150 cm, Luna, Phenomenex Inc, CA,USA) as previously described (Ruiz et al., 2010). Briefly, The mobile phase was water: acetonitrile: formic acid (87:3:10 v/v/v, solvent A) and water: acetonitrile: formic acid (40:50:10 v/v/v, solvent B) at a flow rate of 0.8 mL/min, using the following elution gradient: 0–15 min, 6% B; 15–30 min, 30% B; 30–35 min, 60% B; 35–41 min, 6% B; 41–50 min, 6% B. The mass spectral data were acquired in positive mode; ionization (nebulization) was performed with nitrogen as drying gas at 50 psi, 365 °C and at a flow rate of 10 L/min and capillary voltage 3000 V. All scans were performed in the range 50–1400 *m/z*. The trap parameters were set in ion charge control using manufacturer default parameters. Collision induced dissociation was performed by collisions with the helium background gas present in the trap. Fragmentation was set with Smart Frag.

2.5. Glucose tolerance test

Mice from all experimental conditions, including controls, were subjected to the glucose tolerance test. Mice were fasted for 12 h before evaluation took place. The glucose load (1.0 g/kg body weight) was injected intraperitoneally and tail blood samples were collected 0, 15, 30, 45, 60, 120 and 150 min after glucose load. Glucose concentration was determined using a glucometer (FreeStyle Optium, Abbott, Chicago, USA) and area under the curve was estimated using the baseline. Tests were performed after 9 and 13 weeks of cafeteria diet feeding.

2.6. Histological analysis

Epididymal adipose tissue samples were fixed in 4% para-formaldehyde for 14 h at 4 °C and processed for paraffin sections. Adipose tissue sections (7 μm) were stained with hematoxylin and eosin (H&E) and images were taken using a Canon EOS Rebel T3 camera mounted on a microscope (Zeiss Axioscope microscope, Zeiss, Oberkochen, Germany). The images were processed as previously described and imageJ software was used to measure adipocyte size (Parlee, Lentz, Mori, & MacDougald, 2014).

2.7. Quantitative real-time PCR

Total RNA from epididymal tissue was extracted using Trizol (Invitrogen, Carlsbad, CA, USA). Purified RNA was treated with DNase