Journal of Functional Foods 26 (2016) 350–362

Available online at www.sciencedirect.com

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

Anti-obesity and insulin-sensitising effects of a glycosaminoglycan mix

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ARTICLE INFO

Article history:
Received 10 April 2016
Received in revised form 18 July 2016
Accepted 22 July 2016
Available online 17 August 2016

Keywords:
Glycosaminoglycans
Weight loss
Fat loss
White adipose tissue
Oxidative metabolism

ABSTRACT

The potential of a mixture of glycosaminoglycans (GAGs) contained in an oral supplement for joint discomfort (Oralvisc™) to prevent diet-induced obesity (DIO) and to serve as co-adjuvant in weight loss strategies was studied. Oral treatment with the GAG mix did not counteract the development of DIO in obesity-prone mice but reduced food intake under normal fat diet and led to increased insulin sensitivity under both normal and high fat diets. During reversal of DIO, GAG-treated obese mice showed higher and faster loss of body fat and displayed decreased endpoint adiposity, leptinaemia and hepatic steatosis and signs of increased insulin sensitivity compared with vehicle-treated controls. Expression of oxidative metabolism-related genes and mitochondrial DNA content were increased in visceral white fat depots of GAG-treated animals. The results sustain this GAG mix in functional foods/supplements for obesity and particularly for the (frequent) combination of obesity and joint dysfunction symptoms.

1. Introduction

The prevalence of obesity has increased alarmingly in the last decades both in developing and developed countries and both among adults and children (Ng et al., 2014), reaching pandemic levels according to the World Health Organization (World Health Organization, 2015). Obesity is associated with comorbidities such as liver steatosis, hypertension, dyslipidaemia, insulin resistance, impaired glucose tolerance

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Abbreviations: *B2m*, beta-2 microglobulin; BAT, brown adipose tissue; CCK, cholecystokinin; *Cox5a*, cytochrome c oxidase subunit Va; *Cpt1a*, carnitine palmitoyltransferase 1a (liver isoform); *Cpt1b*, carnitine palmitoyltransferase 1b (muscle isoform); DIO, diet-induced obesity; eWAT, epididymal white adipose tissue; *Fasn*, fatty acid synthase; GAGs, glycosaminoglycans; *Gdi1*, guanosine diphosphate (GDP) dissociation inhibitor 1; *Gpam*, glycerol-3-phosphate acyltransferase; HF, high fat; HOMA-IR, homeostatic model assessment for insulin resistance; ipGTT, intraperitoneal glucose tolerance test; iWAT, inguinal white adipose tissue; *Lep*, leptin; *Lipe*, hormone sensitive lipase; *Lrp10*, low-density lipoprotein receptor-related protein 10; *Mfn2*, mitofusin 2; *Mlxipl*, carbohydrate response element binding protein; mtDNA, mitochondrial DNA; NEFA, non-esterified fatty acids; NF, normal fat; *Pnpla2*, adipose triglyceride lipase; *Ppara*, PPARα, peroxisome proliferator-activated receptor alpha; *Ppargc1a*, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; RHAMM, hyaluronan mediated motility receptor; R-QUICKI, revised quantitative insulin sensitivity check index; rWAT, retroperitoneal WAT; *Srebf1*, sterol regulatory element binding protein 1; *Ucp1*, uncoupling protein 1; WAT, white adipose tissue

http://dx.doi.org/10.1016/j.jff.2016.07.022
1756-4646/

and, very often, joint problems (osteoarthritis), which are all common components of the metabolic syndrome (Moller & Kaufman, 2005; Zhuo, Yang, Chen, & Wang, 2012). Obesity poses therefore a major health and cost burden on affected individuals and societies.

Conventional weight loss strategies relying exclusively on food restriction and increases in physical activity are difficult to follow and have proven ineffective against the current obesity pandemic. Co-adjuvants effectively complementing a negative energy balance are sought. In this context, food ingredients have been identified that may favour leanness by affecting specific biochemical processes related to the control of energy balance and body adiposity (Kim & Park, 2011; Kovacs & Mela, 2006). Among these processes, activation of thermogenesis in brown adipose tissue (BAT) and of substrate oxidation in white adipose tissue (WAT) is receiving much attention nowadays (Bonet, Oliver, & Palou, 2013; Dulloo, 2011; Flachs, Rossmeisl, Kuda, & Kopecky, 2013; Palou, Pico, & Bonet, 2013). Contrary to previous assumptions, it is now well accepted that adult humans do possess functional BAT (Lee & Greenfield, 2015; Nedergaard, Bengtsson, & Cannon, 2007), which is a tissue endowed with a high oxidative capacity, specialised in the regulated dissipation of energy as heat through the uncoupling of substrate oxidation from ATP synthesis via the activity of mitochondrial uncoupling protein 1 (UCP1) (Cannon & Nedergaard, 2004). Moreover, it is now clear that a variety of stimuli (of which most also activate BAT) can enhance mitochondrial oxidative metabolism in WAT depots, in some cases linked to the emergence of brown-adipocyte like cells, a process known as WAT browning (Bonet et al., 2013; Lo & Sun, 2013). Activated BAT and *browned* WAT have high capacity for energy expenditure and for lipid and glucose uptake and oxidation (Bartelt et al., 2011; Cannon & Nedergaard, 2004; Mossenbock et al., 2014) and are therefore seen as promising targets in obesity and related metabolic disorders such as hyperlipidaemia and diabetes (Bonet et al., 2013; Harms & Seale, 2013).

Glycosaminoglycans (GAGs) are a special group of carbohydrates found in food that exert biological effects beyond basic nutrition. Chemically, GAGs are high molecular weight linear polymers formed by a repeating disaccharide unit composed of an amino sugar and a uronic acid. GAGs are used in different therapeutic applications, notably for joint health. Hyaluronic acid – a polymer of N-acetyl-glucosamine and glucuronic acid disaccharide units – is a main and functionally very relevant component of cartilage and synovial fluid. Amelioration of joint dysfunction symptoms following not only intra-articular injection but also oral intake of hyaluronic acid or hyaluronic acid-rich mixtures/extracts has been described in affected humans (Martinez-Puig, Möller, Fernández, & Chetrit, 2013; Nelson et al., 2013, 2015; Sanchez et al., 2014; Sola et al., 2015; Tashiro et al., 2012).

In a previous in vitro study we reported that a mix of GAGs consisting of hyaluronic acid and dermatan sulphate (1:0.25, w/w) that is contained in an oral supplement for joint discomfort (Oralvisc™) can suppress spontaneous and hormonally-induced adipogenesis and induce the expression of cartilage extracellular matrix proteins in primary mouse embryo fibroblasts (Petrov et al., 2015). Moreover, the GAG mix and especially its minor component, dermatan sulphate – a polymer of sulphated N-acetyl-galactosamine and iduronic acid disaccharide units – enhanced the expression of a collection of genes related to mitochondrial oxidative metabolism in the cells in the adipose differentiated state (Petrov et al., 2015). In the present work we aimed to test through animal intervention studies the potential applicability of this GAG mix composition in the prevention of diet-induced obesity and as a co-adjuvant in weight loss strategies.

2. Materials and methods

2.1. Study design

Animal protocols followed in this study were reviewed and approved by the Bioethical Committee of the University of the Balearic Islands. Guidelines for the use and care of laboratory animals of this University were followed.

2.1.1. Experiment 1

Six-week-old C57BL6/J male mice (Charles River Laboratories, Barcelona, Spain) housed at 22 °C with a 12-hour light/dark period were used. At the beginning of the experiment, the animals were distributed into six experimental groups: mice fed *ad libitum* with a normal fat (NF) or a high fat (HF) diet and treated daily with vehicle (water, controls, C) or the Oralvisc™ GAG mix composition (Bioiberica S.A., Palafolls, Barcelona, Spain; referred from hereafter as GAG mix) at a dose of 0.45 mg/mouse/day or 1.8 mg/mouse/day (D1 and D2, respectively) (n = 8 animals/group, distributed in two cages). The GAG mix contained hyaluronic acid (CAS number 9067-32-7; molecular weight: 800,000 Da) and dermatan sulphate (CAS Number: 54328-33-5; average molecular weight: 30,000 Da) at a ratio (1:0.25, w/w) obtained by chemical extraction from rooster combs. Defined diets (from Research Diets, Inc., New Brunswick, NJ, USA) were used; the NF diet (D12450B) provided 15.9 kJ/g and 10% energy from fat and the HF diet (D12451) 19.5 kJ/g and 45% energy from fat. Treatments were administered orally with the aid of a pipette. The experimental period was 19 weeks, during which body weight, body composition and energy intake were tracked. Body composition was analysed using an Echo MRI body composition analyser (EchoMRI, LLC, Houston, TX, USA). Energy intake was estimated on a per-cage basis from the actual amount of food consumed by the animals and its caloric equivalence.

2.1.2. Experiment 2

Twenty six-week-old C57BL6/J diet-induced obese (DIO) male mice displaying 24% excess body weight as a result of HF diet feeding as in experiment 1 were used. At the start of the experiment (day 0), the DIO mice were switched to the NF diet and distributed into two groups that received daily by oral route the vehicle (water, control group, initial body weight: 35.4 ± 1.8 g) or the GAG mix at a dose of 3 mg/mouse/day (treatment group, initial body weight: 35.6 ± 1.5 g) (n = 7–8 animals/group). Animals were housed in pairs at 22 °C with a 12-hour light/dark period, under *ad libitum* feeding conditions. Body weight, body composition and food intake were regularly monitored during 32 days, after which the animals were euthanised by decapitation, within the first 2 h of the light cycle. Tissues including