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Diet-induced disorders in rats are more efficiently attenuated by initial rather than delayed supplementation with polyphenol-rich berry fibres

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ABSTRACT

The effects of blackcurrant and strawberry fibre on high-fat diet-induced metabolic disorders in rats were compared. The blackcurrant and strawberry preparations were obtained from fruit pomace and contained 66.5 and 51.3% dietary fibre and 4.9 and 5.5% polyphenols, respectively. A high-fat diet was supplemented with the blackcurrant or strawberry fibre for the entire 8 weeks of experimental feeding or for 4 weeks, starting at week 5. Obesity, dyslipidaemia, hyperinsulinaemia and altered microbial metabolism in the distal intestine were noted in rats fed a high-fat diet. The 8-week supplementation with blackcurrant fibre decreased body weight, whereas both fibres decreased epididymal fat mass, increased the caecal concentration of short-chain fatty acids, prevented hyperinsulinaemia and decreased cholesterolaemia. Thus, pomace-derived blackcurrant and strawberry fibre attenuate disorders induced by a high-fat diet; however, initial dietary supplementation is more efficient than the same, but delayed, intervention.

1. Introduction

Edible berries are popularly consumed fruits both in fresh form and as ingredients of food products, such as yogurts, beverages, jams and jellies (Nile & Park, 2014). Some of the most popular berries that are processed by the food industry are blackcurrants and strawberries. These fruits are a rich source of nutrients and bioactive compounds, especially polyphenols, which may prevent the development of diet-related disorders, such as obesity, cardiovascular disease and diabetes, or attenuate their progression (Giampieri et al., 2012; Nile & Park, 2014). The two main classes of polyphenols in blackcurrants and strawberries are anthocyanins and proanthocyanidins; strawberries also contain high amounts of ellagitannins (Buendía et al., 2010; Maatta, Kamal-Eldin, & Törrönen, 2001). However, it is thought that the beneficial effects of polyphenols are synergistic rather than being a

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consequence of precise constituents (Giampieri et al., 2012). So far, a wide range of biological activities has been ascribed to strawberry polyphenols, including lipid-lowering, anti-obesity and improved insulin sensitivity (Giampieri et al., 2012; Prior et al., 2008). Recent studies have also shown that blackcurrant polyphenols can attenuate weight gain and hyperlipidaemia and improve glucose metabolism in mice with diet-induced metabolic disorders (Benn et al., 2015; Esposito et al., 2015). Importantly, some of these favourable effects were not seen if the gut microbiota was disrupted by antibiotic treatment (Esposito et al., 2015), which is in line with findings that polyphenols are mainly metabolised and absorbed in the distal intestine (Selma, Espin, & Tomas-Barberan, 2009).

A well-known group of indigestible food components that can beneficially affect the distal intestine and its microbiota is dietary fibre. In addition, dietary fibre can increase satiety and is partly responsible for the regulation of glucose and lipid metabolism in the organism; it can also reduce postprandial glycaemia and blood cholesterol levels (Babio, Balanza, Basulto, Bulló, & Salas-Salvadó, 2010). An alternative and relatively unstudied source of dietary fibre is fruit pomace, which is a by-product of juice manufacture. Fruit pomace is usually dumped or composted, but some attempts to use blackcurrant press residues as an ingredient in purees and extruded snacks have already been made (Keenan, Brunton, Gormley, & Butler, 2012; Makila et al., 2014). Blackcurrant and strawberry pomace contain high amounts of dietary fibre, exceeding 50% dry weight (Pieszka, Gogol, Pietras, & Pieszka, 2015; Sójka, Klimczak, Macierzyński, & Kołodziejczyk, 2013; Sójka & Król, 2009). Additionally, they are also rich sources of polyphenols, especially tannins and to a lesser extent anthocyanins (Sójka et al., 2013; Sójka & Król, 2009), whose biological activity may still be significant and may contribute to the beneficial effects of dietary fibre (Jaroslawska et al., 2011; Jurgoński, Milala, Juśkiewicz, Zduńczyk, & Król, 2011). Indeed, our previous study showed that a polyphenol-rich pomace from strawberry more efficiently attenuated metabolic disorders induced in rats by a high-fructose diet than a strawberry pomace without most of polyphenols, which were removed by extraction (Jaroslawska et al., 2011).

Berry pomaces are concentrated sources of fibre–polyphenol complexes and, after processing, they could be considered as important ingredients of functional food designed for reducing the development of obesity and obesity-related disorders. Thus, the aim of this study was to compare the effects of polyphenol-rich blackcurrant and strawberry fibre preparations obtained from fruit pomace on high-fat (HF) diet-induced metabolic disorders in rats. Moreover, we hypothesised that the initial addition of blackcurrant or strawberry fibre-polyphenol complexes to a HF diet would be more efficient in the attenuation of metabolic disorders than the same nutritional intervention delayed in time.

2. Materials and methods

2.1. *Fibre preparations and their chemical analysis*

Blackcurrant and strawberry fruit pomaces, by-products of the manufacture of concentrated juices (Alpex Co., Łęczeszyce, Poland), were used to prepare fibre preparations. The fresh pomaces from a Bücher type press were dried in a convection oven at a temperature ≤70 °C until the moisture content fell below 5%; this material was then passed through sieves. The seedless fractions were granulated to 0.5–2.0 mm pellets (Lab-mill 1 QC-114, Labor-MIM, Budapest, Hungary) and comprised the blackcurrant and strawberry fibre preparations. The preparations were then analysed for dry matter, crude protein, fat and ash and total and insoluble dietary fibre using AOAC/2005 methods 934.01, 920.152, 930.09, 940.26, 985.29, and 993.19, respectively (Horwitz & Latimer, 2007). Soluble dietary fibre was calculated as the difference between total dietary fibre and insoluble dietary fibre. The nitrogen-free extract was calculated by adding water, fibre, crude protein, fat and ash and subtracting that sum from 100.

High-performance liquid chromatography (HPLC) analysis of phenolic compounds in the blackcurrant and strawberry fibre preparation was performed in accordance with previously described procedures (Sójka, Guyot, Kołodziejczyk, Król, & Baron, 2009; Sójka et al., 2013). Polyphenol compounds in the blackcurrant fibre were extracted by a five-step method using a mixture of methanol/water/formic acid at a ratio of 50:48:2 (v/v/v), as previously described (Sójka & Król, 2009). Anthocyanins and flavonols in the obtained extracts were determined using HPLC (Knauer Smartline system with a photodiode array detector, Berlin, Germany) coupled to a Gemini C18 column (110 Å, 150 × 4.60 mm; 5 µm, Phenomenex, Torrance, CA, USA). Phase A was 10% formic acid in water, and phase B was a solution composed of acetonitrile/water/formic acid at a ratio of 50:40:10 (v/v/v); the flow rate was 1 mL/min, the sample volume was 20 µL and the column temperature was 40 °C. The gradient was as follows: 12% phase B for 0–0.6 min, 12–30% B for 0.6–16 min, 30–100% B for 16–20.5 min, 100% B for 20.5–22 min, 100–12% B for 22–25 min, 12% B for 25–35 min. The strawberry fibre was extracted with a three-step method using 70% acetone, according to Sójka et al. (2013), the acetone was then evaporated using vacuum rotary evaporator and the residue was dissolved in 70% glycerol. A part of the obtained glycerol solution was directly used for the determination of unconjugated ellagic acid, anthocyanins and flavonols, whereas the rest was first hydrolysed in an acidic environment for ellagitannin breakdown. The total ellagitannin content was determined based on the amount of ellagic acid released during hydrolysis (Sójka et al., 2013). Free and released ellagic acid, anthocyanins and flavonols were determined in both types of sample after a 10-fold dilution in methanol using the aforementioned HPLC system and a longer Gemini C18 column (110 Å, 250 × 4.60 mm; 5 µm, Phenomenex). Phase A was 0.05% phosphoric acid in water, and phase B was 0.05% phosphoric acid in 80% acetonitrile; the flow rate was 1.25 mL/min, the sample volume was 20 µL and the temperature was 35 °C. The gradient programme was as follows: 5% B for 0–5 min, 5–15% B for 5–10 min, 15–40% B for 10–35 min, 40–73% B for 35–40 min, 73% B for 40–44 min, 73–5% B for 44–46 min and 5% B for 46–54 min. Identification and quantification of the polyphenols were performed according to the procedures described elsewhere (Sójka et al., 2009, 2013; Sójka & Król, 2009). Ellagic acid and flavonols were detected at a wavelength of 360 nm, whereas anthocyanins at 520 nm. Standards were purchased from Extrasynthese (Genay, France).