FOOD & FUNCTION

Influence of dietary supplementation with dextrin or oligofructose on the hepatic redox balance in rats

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We assessed the impact of oligofructose (OFS) and dextrin (DEX) as diet supplements on hepatic redox state. Rats were fed either a 10% OFS or a 10% DEX supplemented diet for 9 wk. In the DEX diet group, the levels of hepatic protein carbonylation were decreased by 63%. Total glutathione and reduced glutathione (GSH) contents were reduced in the OFS and DEX diet groups by around 20%. DEX supplementation significantly reduced oxidized glutathione (GSSG) levels resulting in a 33% increase in the GSH/GSSG ratio. The activity of the hepatic antioxidant enzymes was not changed by either OFS or DEX supplementation. OFS supplementation caused a decrease in serum levels of triglycerides (36%), cholesterol (24%), HDL (16%) and LDL (17%). DEX supplementation only reduced triglycerides (32%) and urea (22%). Both diets increased serum levels of acetate by fivefold and propionate by twofold, but DEX diet decreased butyrate levels by 75%. Due to their different composition/structure these two dietary fibers affected metabolism in different ways. Diet supplementation with 10% DEX can potentially improve host health, by protecting the liver from protein carbonylation and by improving GSH/GSSG ratio and diet supplementation with 10% OFS can improve the lipid profile.

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After Gibson and Roberfroid, prebiotics (typically non-digestible carbohydrates, commonly nutritionally classed as dietary fibers) have been redefined as selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora. Western diet (mostly fructose-rich, lipid-rich and fiber-poor diet) modifies gastrointestinal microflora quality, which is associated with increased endotoxemia, inflammation, steatosis, body weight gain, fat mass development and type 2 diabetes. Prebiotics aid overcoming major consequences of these eating habits since changing the gut microbiota with prebiotics may participate in the control of the development of metabolic diseases associated with obesity [1, 2].

Few studies have been done on the ability of dietary fibers to improve host health by modulation of the redox balance. However, it has been reported a reduced susceptibility of cardiac tissue to lipid peroxidation of inulin-type

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Abbreviations: OFS, oligofructose; **CT,** control group; **DEX,** dextrin; **GSH,** reduced glutathione; **GSSG,** oxidized glutathione; **TG,** plasma triglycerides

fructan supplementation in fructose-fed rats [3] and protection against the pro-oxidant effect of fructose [4].

With this in mind, we investigated the effects of Nutriose® FB 06, a source of dextrin (DEX) that has been suggested to have prebiotic characteristics, and Raftilose® P95, a commercially available oligofructose (OFS) classified as a prebiotic food supplement (both dietary fibers), on the hepatic redox status. Its alteration (towards a pro-oxidant state) is being described as one of the major players in western diet-induced hepatic lesions [5].

Nutriose® FB 06 is produced from wheat starch, with up to 85% of fiber content (dry substance), and a high number of α-1,6 linkages and non-digestible glucoside linkages, such as α-1,2 and α-1,3 [6]. The final product is a mixture of glucose polymers with a narrow range of molecular weight and a degree of polymerization (DP) range from 12 to 15. Nutriose® FB 06 was made commercially available in 2004 and research has mainly focused on its gastrointestinal effects in humans. It has been shown to be mostly resistant to digestion in the small intestine (15% is enzymatically digested, 75% is slowly and progressively fermented in the colon, producing SCFA, and 10% is excreted) [7]. Raftilose® P95 is obtained from enzyme hydrolysis of chicory inulin, composed of a mixture of glucosyl-(fructosyl)$_n$-fructose (64%) and (fructosyl)$_n$-fructose (36%) and has a DP range from 2 to 8. Unlike Nutriose® FB 06, Raftilose® P95 reaches the colon practically intact, where it is fermented, leading to the production of SCFA [8].

Animals were randomly divided into three groups: (i) control group (CT; $n = 5$), fed with a standard rat diet (Diet A04, Panlab, Spain); (ii) OFS diet group ($n = 5$), fed with a standard diet supplemented with 10% Raftilose® P95 and (iii) DEX diet group ($n = 5$), fed with a standard diet supplemented with 10% Nutriose® FB 06. Briefly, animals were treated during 9 wk, placed in metabolic cages for 96 h during the 1st, 5th and 9th weeks.

Not much research has been done on the effects of using DEX as opposed to using OFS (see [9] for review), as a food supplement. The metabolic parameters of OFS and DEX diets are presented in Supporting information Table S1. A diet supplemented with OFS or DEX did not result in change in liquid intake or urine volume. Rats fed with OFS diet displayed a period of adjustment as evidenced by a significantly lower solid intake compared with rats fed with CT or DEX diets during the 1st and 5th weeks of treatment. During the 9th week, solid intake was similar between rats fed with CT, OFS or DEX diets and there was a significantly increased production of feces by rats fed with OFS or DEX diets compared with rats fed with CT diet, in accordance with literature, as a result of fermentation of carbohydrates that escape digestion, improving the intestinal environment, increasing stool volume and producing SCFA [10, 11]. Liquid intake was significantly higher in the OFS group and lower in the DEX group only in the 1st week of treatment. OFS or DEX supplementation did not result in change in urine volume.

Although the net weight gain of rats in both fiber diets was not significantly different from those in the CT diet, the net weight gain of rats in the DEX diet was found to be significantly higher than those in the OFS diet.

In order to study the antioxidant properties of Nutriose® FB 06 and Raftilose® P95, we assessed in rats' liver the levels of oxidative damage to lipids and proteins (Fig. 1), of endogenous antioxidant enzymes (Supporting information Table S2) and of glutathione (Fig. 2).

A diet supplemented with DEX did not produce a significant change in the level of hepatic lipid peroxidation, but significantly decreased the amount of protein carbonylation (64%) and significantly increased (46%) the reduced/oxidized glutathione (GSH/GSSG) ratio. An increase of the GSH/GSSG ratio translates into an increased antioxidant capacity as a higher proportion of available glutathione is in its reduced form [12] and may reflect a decreased production and/or an increased scavenging of reactive species. Taken together, these data suggest a protective outcome of DEX ingestion.

Contrary to the effects observed in the DEX diet group, supplementing a diet with OFS did not significantly change the hepatic oxidative stress parameters evaluated, except for a significant reduction in the amount of total glutathione resulting from a significant reduction in the GSH content

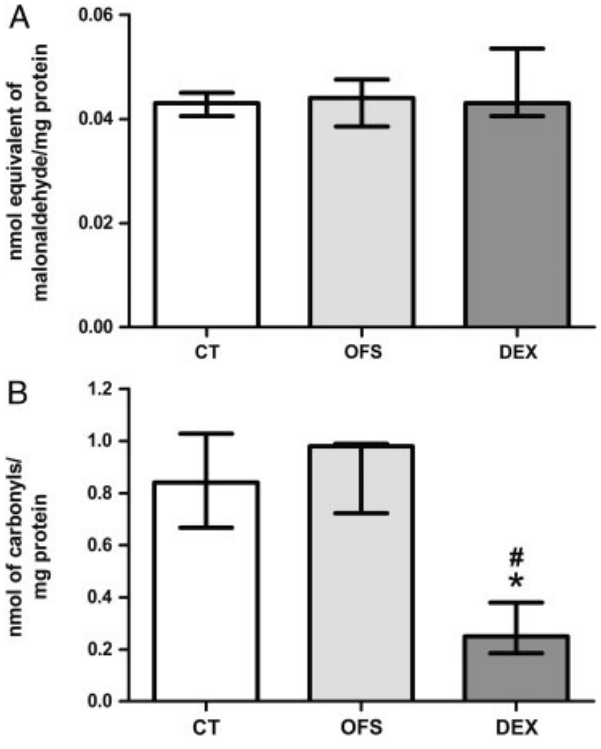

Figure 1. Oxidative stress biomarkers levels measured in liver homogenates of rats fed with standard diet (CT), CT supplemented with 10% Raftilose® P95 (OFS) or with 10% Nutriose® FB 06 (DEX), for 9 wk: (A) lipid peroxidation and (B) protein carbonylation. Values are presented as medians ± IQR ($n = 5$), $^{*}p < 0.05$, compared with CT and $^{\#}p < 0.05$, compared with OFS. (Kruskal–Wallis followed by Newman–Keuls test).

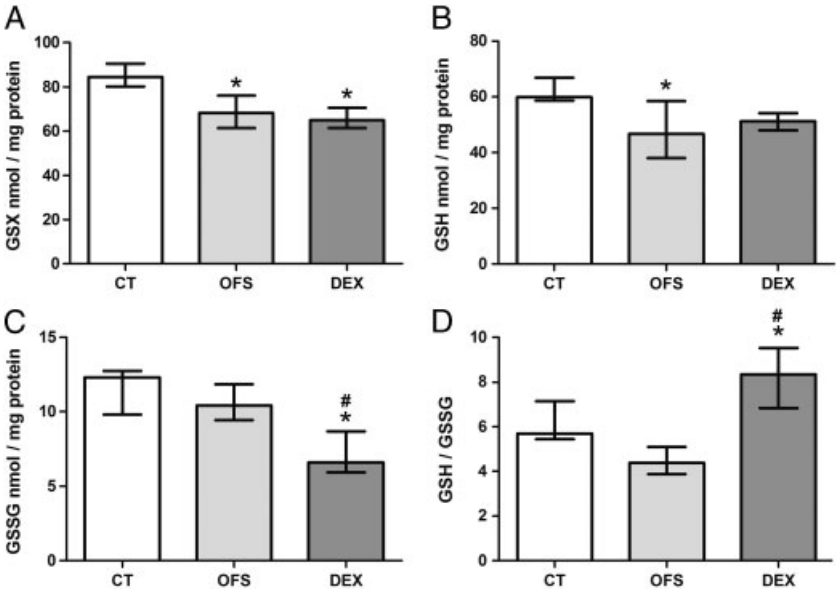

Figure 2. Glutathione levels (nmol/mg of protein) in liver homogenates of rats fed with standard diet (CT), CT supplemented with 10% Raftilose® P95 (OFS) or with 10% Nutriose® FB 06 (DEX), for 9 wk: (A) total glutathione, (B) oxidized glutathione, (C) reduced glutathione and (D) GSH/GSSG ratio. Values are presented as medians ± IQR ($n = 5$), $^{*}p < 0.05$, compared with CT and $^{\#}p < 0.05$, compared with OFS. (Kruskal–Wallis followed by the Newman–Keuls test).

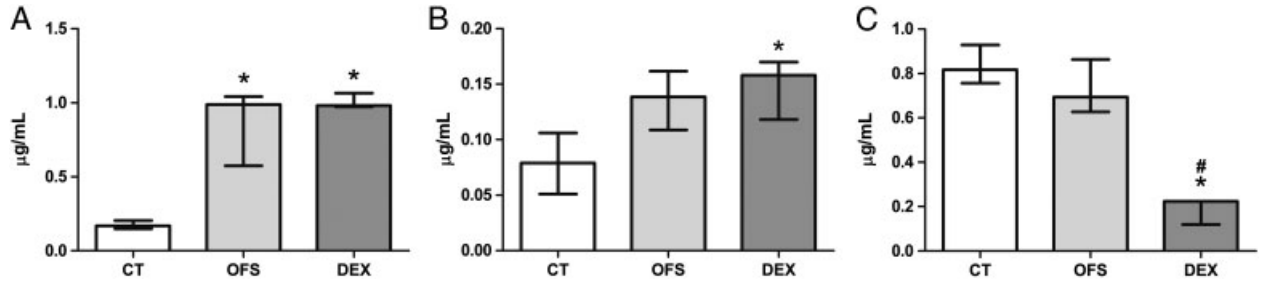

Figure 3. Serum levels of SCFA levels of rats treated with standard diet (CT), CT supplemented with 10% Raftilose® P95 (OFS) or with 10% Nutriose® FB 06 (DEX) for 9 wk: (A) acetate, (B) propionate and (C) butyrate. Values are presented as medians ± IQR ($n = 5$), $^{*}p < 0.05$, compared with CT and $^{\#}p < 0.05$, compared with OFS. (Kruskal–Wallis followed by the Newman–Keuls test).

(similar to what was observed with DEX). However, since there was no significant change in the GSSG content in the liver of these rats the calculated GSH/GSSG ratio remained similar to the values found for the CT diet group. Therefore, no protective effect was observed by feeding rats with the OFS diet.

The activity levels of glutathione dependent antioxidant enzymes and of the scavenger enzymes superoxide dismutase and catalase were not significantly changed by either OFS or DEX diets (Supporting information Table S2). Therefore, a protective effect on the liver of a diet supplemented with DEX cannot be explained by the activity levels of these enzymes.

Serum biochemistry analysis and determination of serum levels of a selection of SCFA were performed in search of clues to the possible source of the protective effect observed from supplementing a diet with DEX.

The serum analysis showed (Supporting information Table S3) that supplementing a diet with OFS reduced mainly plasma triglycerides (TG) (25%), but also cholesterol (17%), HDL (15%) and LDL (14%). Comparatively, supplementing a diet with DEX resulted only in a significant reduction of TG (27%). Serum biochemical analysis also showed that the OFS diet increased glucose levels (8%), while DEX diet decreased urea levels (21%).

Urea production occurs almost exclusively in the liver and is the fate of most ammonia generated by amino acid catabolism. Amino acids undergo oxidative degradation under several metabolic circumstances [13]. Therefore, the reduction in serum urea observed in the DEX diet group could be, partially, explained by a decreased catabolism of amino acids. Another contributing factor could be that absorption of ammonia from the colon is reduced due to stimulation of colonic bacteria by DEX reducing bacterial ammonia production, increasing bacterial ammonia uptake for their own metabolism and/or increasing fecal urea/nitrogen excretion [14].

Several studies have shown that high urea levels cause oxidative stress. In renal medullary cells, high urea levels increased gene and protein expression of growth arrest and DNA damage-inducible protein 153, which was prevented by pre-treatment with the antioxidant *N*-acetylcysteine, and

decreased the level of GSH content [15]. It was later shown that low levels of urea (5 mM), a normal plasma level, also produce protein carbonylation and DNA double-strand breaks in renal cells in vitro and in vivo [16]. Therefore, low urea production reflected by low urea serum levels as a result of treatment with DEX supplemented diet might have contributed to the extensive decrease in protein carbonylation. However, this may not be the sole contributing factor since the decrease in serum urea was about three times less than the decrease in protein carbonylation.

SCFA serum levels showed a similar increase in acetate (~five-fold) and propionate (~two-fold) in the OFS and DEX diet groups, but the DEX diet produced a decrease in butyrate levels (78%) (Fig. 3). This outcome was probably due to the different compositions/structures of the dietary fibers used that resulted in different fermentation end products in the colon. SCFA arise from bacterial fermentation of polysaccharide, oligosaccharide, protein, peptide and glycoprotein precursor in the colon [17]. The production of SCFA is determined by several factors, including the numbers and types of bacteria present in the colon, substrate source and gut transit time [18]. The carbohydrates present in the diet influence not only the amount but also the proportion of the SCFA produced during fermentation.

It has been shown that butyrate impairs energy metabolism in isolated perfused liver of rats as a result of an increase in the flavin adenine dinucleotide ($FADH_2$)/nicotinamide adenine dinucleotide (NADH) ratio due to β-oxidation [19]. The initial rate of adenosine 5′-triphosphate (ATP) net consumption, in hepatocytes, has been shown to be higher in the presence of butyrate than acetate associated with intracellular pH regulation [20]. Therefore, the decrease in serum butyrate observed could result in lower ATP consumption reducing the need to generate metabolic energy from protein. In this regard, we have observed a concordant decrease in serum urea from rats fed with DEX supplemented diet.

Taken together with what is known about Nutriose® FB 06 and Raftilose® P95, our results suggest that due to the different compositions/structures, these two dietary fibers produce different fermentation end products that once absorbed into the blood and transported to the liver distinctly affect the metabolism.

Our data show that feeding rats with a diet supplemented with 10% DEX can potentially improve host health by protecting the liver from protein carbonylation and by improving the GSH/GSSG ratio. Considering that the western diet is associated with increased liver inflammation, a decrease in the carbonyls levels could be beneficial, since macrophages activated under conditions of oxidative or carbonyl stress can lead to a more enhanced inflammatory response [21]. Feeding rats with a 10% OFS supplemented diet did not produce major changes on the rat hepatic redox state but produced beneficial effects on the lipid profile.

The authors have declared no conflict of interest.

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