International Immunopharmacology (2008) **8**, 1423–1432

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Immunomodulatory properties of two wheat bran fractions — aleurone-enriched and crude fractions — in obese mice fed a high fat diet

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Received 4 April 2008; received in revised form 15 May 2008; accepted 27 May 2008

KEYWORDS
Wheat bran;
Aleurone;
High fat diet;
GLP-1;
Obesity;
Cytokines;
Immunomodulation

Abstract

Several data suggest that fermentable dietary fibers could play a role in the control of obesity and associated metabolic disorders. In mice, dietary fructans, which are extensively fermented in caeco-colon by *bifidobacteria*, decrease fat mass development and modulate gastrointestinal peptides involved in the control of food intake (namely glucagon-like peptide (GLP)-1). The aim of this study was to compare the effect of two cereal bran fractions isolated from wheat – aleurone-enriched and crude fractions – in a nutritional model of obesity. In a first experiment, we confirmed that 2 weeks of treatment with a high fat (HF) diet is sufficient to exhibit glucose intolerance and to increase adiposity in mice. In the second experiment, mice were fed a HF or a HF diet enriched with 10% wheat bran fractions during 3 weeks. None of the wheat bran fractions modified body weight, adipose tissue mass, glucose or lipid homeostasis. Wheat bran fractions increased *bifidobacteria* and *lactobacilli* in the caecal content without any effect on caecal enlargement and on GLP-1 precursor expression in the colon. Furthermore, wheat bran fractions decreased circulating interleukin 6 (IL-6) and CD68 mRNA in the visceral adipose tissue, suggesting a decrease in recruited-tissue macrophages. We propose that specific and early immunomodulatory properties of cereal products with prebiotic properties, may occur in obese mice independently of extensive gut fermentation.

Abbreviations: AX, arabinoxylan; GLP-1, glucagon-like peptide-1 7-36 amide; HF, high fat; IL, interleukin; LPS, lipopolysaccharides; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1; OFS, oligofructose; OGTT, oral glucose tolerance test; NDC, non digestible carbohydrates; SCFA, short chain fatty acids; TNF-α, tumor necrosis factor-alpha.

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doi:10.1016/j.intimp.2008.05.015

1. Introduction

In most Western countries, cereals represent a major source of dietary fibers but their consumption has decreased dramatically over the last century [1]. In addition, cereals are consumed as more refined products than in the past. This phenomenon participates to the drop in daily fiber intake. In parallel, diabetes, obesity and cardiovascular diseases, clustered in the metabolic syndrome, have emerged as major health problems. It has been suggested that the decrease in fibers intake contributes to the development of these health disorders [2–4]. Although the pathogenesis of metabolic syndrome is complex and not fully understood, obesity and insulin resistance are accompanied by an altered profile of a number of hormones and cytokines. Gut peptides controlling food intake and insulin response/secretion – such as glucagon-like peptide-1 7-36 amide (GLP-1) – might play a role in the regulation of satiety and insulin resistance [5,6]; on the other hand, obesity and low tone inflammation are now clearly linked, since the discovery of the role of proinflammatory cytokines – mostly IL-6, TNFα – in metabolic alterations [7,8]. An efficient approach in the prevention and treatment of this syndrome should involve lifestyle changes, including appropriate nutrition. Among dietary advices, restoring a higher fiber intake could be essential. The interest of fibers in the management of metabolic syndrome relates to its food origin, to its specific chemical structure and physical properties, or to its fermentation in the gut [9]. On the basis of studies in animals and humans, it has been proposed that the intake of highly fermentable non digestible carbohydrates (NDC), could be interesting nutrients prone to increase satiety, to improve glucose tolerance, to lower hepatic and serum lipids, and even to control hypertension [3,10–15]. Interestingly, in view of the results obtained in animals fed a diet containing oligofructose (OFS), a short chain/highly fermentable fructans, these effects seem to be linked to both the higher expression of proglucagon and level of GLP-1 in the proximal colon, that drives an increase in the gut peptide in the portal vein [11,12]. This peptide is considered as a key hormone in the control of food intake, glucose tolerance, and insulin secretion (therefore called "incretin") by pancreatic β-cells [5,16]. Fermentable dietary fibers, such as fructans, decrease glycemia, triglyceridemia, and modulate fermentation-related events (increase in caecal tissue and content; GLP-1 production), within 3 weeks of treatment [11,17–20]. Of particular interest, we have recently demonstrated that OFS treatment reduced the development of glucose intolerance following a HF, carbohydrate-free diet, a classical nutritional model leading to disorders associated with obesity; the antidiabetic effect of OFS being largely dependent on GLP-1 action [12].

Wheat bran is a by-product of conventional milling and is commercially available in large quantities. Different approaches have been recently proposed to incorporate wheat bran in food products, in order to optimize the nutritional composition or to produce interesting physiological effects. In fact, cereal products have been shown to regulate carbohydrate and lipid homeostasis [2,15]. However, the quantity and the quality of dietary non starch NDC present in cereal products, such as fructans, is too low to allow those fermentable fibers to have a substantial influence on the nutritional properties of those cereal products [2,21].

Bran, which is composed of 50% pericarp/testa and 50% aleurone layer, is particularly rich in dietary fibers (50% of the bran weight) [2,22]. The pericarp testa is constituted of lignified cell walls, where cellulose microfibrils are dispersed in acidic arabinoxylans (AX) [2]. AX represent about half the dietary fibers component of wheat bran or wheat aleurone whereas cellulose and β-glucans contributed only smaller parts [22]. In fact, isolation of aleurone from wheat bran resulted in considerable differences in the degrees of branching in AX [22–24]. The physiological effect of AX is largely unknown but several studies indicate that they behave like a rapidly fermentable, soluble fiber in the colon with impact on lipid and glucose metabolism in human and/or in rats [25–30].

The aim of this study was to compare the physiological effects of dietary supplementation with wheat bran crude fraction or the aleurone-enriched fraction on 1) key parameters known to be modulated by OFS (fermentation and GLP-1 production) and 2) the capacity of these both cereal products to counteract the development of metabolic disorders induced by a HF diet, a nutritional model leading to obesity, insulin resistance and glucose intolerance associated with low grade chronic systemic inflammation [12,31].

2. Materials and methods

2.1. Animals and diet

Male C57bl6/J mice (8 weeks old at the beginning of the experiment, Charles River, Brussels, Belgium) were housed in a controlled environment (12 h daylight cycle, lights off at 6 pm) with free access to food and water in groups of 4 per cage.

2.1.1. Experiment 1

Control (CT) mice ($n=8$) were fed a A04 standard diet (A04, UAR, Villemoisson-sur-Orge, France), whereas HF-treated mice ($n=8$) received a diet containing 49.5% fat – corn oil and lard – (g/100 g of total dry diet), 37% protein, 10% cellulose, 3.5% mineral/vitamins mixture for 2 weeks. The energy content of the HF diet was 72% of fat, 28% protein, and <1% carbohydrates. One set of mice was 6 h fasted in order to perform an oral glucose tolerance test (OGTT, see below) whereas another set of mice was killed by cervical dislocation after a 5 hour period of fasting in order to dissect and to weigh adipose tissues (subcutaneous and visceral).

2.1.2. Experiment 2

Mice ($n=8$ per group) were divided into 3 groups and were fed a HF diet or a mix of 90% HF diet and 10% wheat bran (aleurone-enriched or crude fractions) for 3 weeks. Crude fraction was prepared by Dr G. Sinnaeve (CRA-W, Département Qualité des productions agricoles, Gembloux, Belgium) and aleurone was commercially available as Leuron™ (Healthbalance, Uzwil, Switzerland). The composition of the 2 wheat bran fractions in term of soluble and insoluble fibers as well as fermentable NDC – fructan and resistant starch – is presented in Table 1 [21]. One week prior to sacrifice, OGTT was performed on 6 hour-fasted mice (see below). At the end of the experiment 2, mice were anaesthetized after a 5 hour period of fasting by intra-peritoneal injection of sodium pentobarbital solution (using 60 mg/kg of body weight, Nembutal®, Sanofi Santé Animale, Brussels, Belgium). Portal vein blood samples were collected in EDTA tubes (Sarstedt, Nümbrecht, Germany) containing dipeptidyl peptidase IV inhibitor (DPPIV inhibitor, Linco Research, St Charles, USA); after centrifugation, plasma was stored at −80 °C for GLP-1 assessment. Blood from vena cava was collected in EDTA tubes and plasma was stored at −80 °C for lipid and cytokine assessments.

Table 1 Proportion of dietary fibers in wheat bran fractions

g/100 g	Insoluble fiber	Soluble fiber	Fructan	Resistant starch
Crude wheat bran fraction	10.0	6.6	3.2	0.2
Aleurone-enriched wheat bran fraction	50.8	3.3	3.4	0.1

Caecum (full and empty), proximal colon, liver and adipose tissues (epididymal, subcutaneous and visceral) were precisely dissected weighed, immersed in liquid nitrogen, and stored at −80 °C, for further analysis.

All experiments were approved by the local ethics committee and housing conditions were as specified by the Belgian Law of November 14, 1993 on the protection of laboratory animals (agreement no. LA 1230314).

2.2. Oral glucose tolerance test (OGTT)

Oral glucose tolerance test was performed after 2 weeks of treatment in mice previously fasted for 6 h. Glucose was orally administered (3 g/kg body weight of 66% (wt/vol) glucose solution) and blood glucose was determined through a glucose meter (Roche Diagnostics) on 3.5 μl of blood collected from the tip of the tail vein before glucose load (−30 min and 0 min) and after glucose load (15, 30, 60, 90, 120 min). 20 μl of blood was sampled 30 min before and 15 min following the glucose load to assess plasma insulin concentration.

2.3. Blood parameters

Plasma insulin concentration was determined in 5 μl of plasma collected from tail blood using an ELISA kit (Mercodia, Uppsala, Sweden).

Concentrations of GLP-1 (7-36) amide were measured in the portal plasma using an Elisa Kit, specific for GLP-1 (7-36) amide without cross reactivity towards GLP-1 (9-36) amide, GLP-2 and glucagon (GLP-1 active Elisa kit, Linco Research, St Charles, USA) [11].

Plasma triglycerides, cholesterol and non-esterified fatty acids concentrations were measured in the plasma obtained from the vena cava using kits coupling enzymatic reaction and spectrophotometric detection of reaction end-products (Elitech diagnostics, Brussels, Belgium and Wako, Brussels, Belgium). Plasma tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-1α, IL-1β, monocyte chemoattractant protein (MCP)-1, and macrophage inflammatory protein (MIP)-1α were determined in 25 μl of plasma obtained from the vena cava using a Multiplex kit (Bio-Rad, Nazareth, Belgium) and measured by using Luminex technology (Bio-Plex System™, Bio-Rad).

2.4. Quantification of *Bifidobacterium* spp. and *Lactobacillus* spp. in caecal contents

The caecal contents collected post mortem from mice were stored at −80 °C. The QIAamp DNA Stool Minikit (Qiagen) was used to extract DNA from stool sample according to the manufacturer's instructions. The primers and probes used to detect *Bifidobacterium*

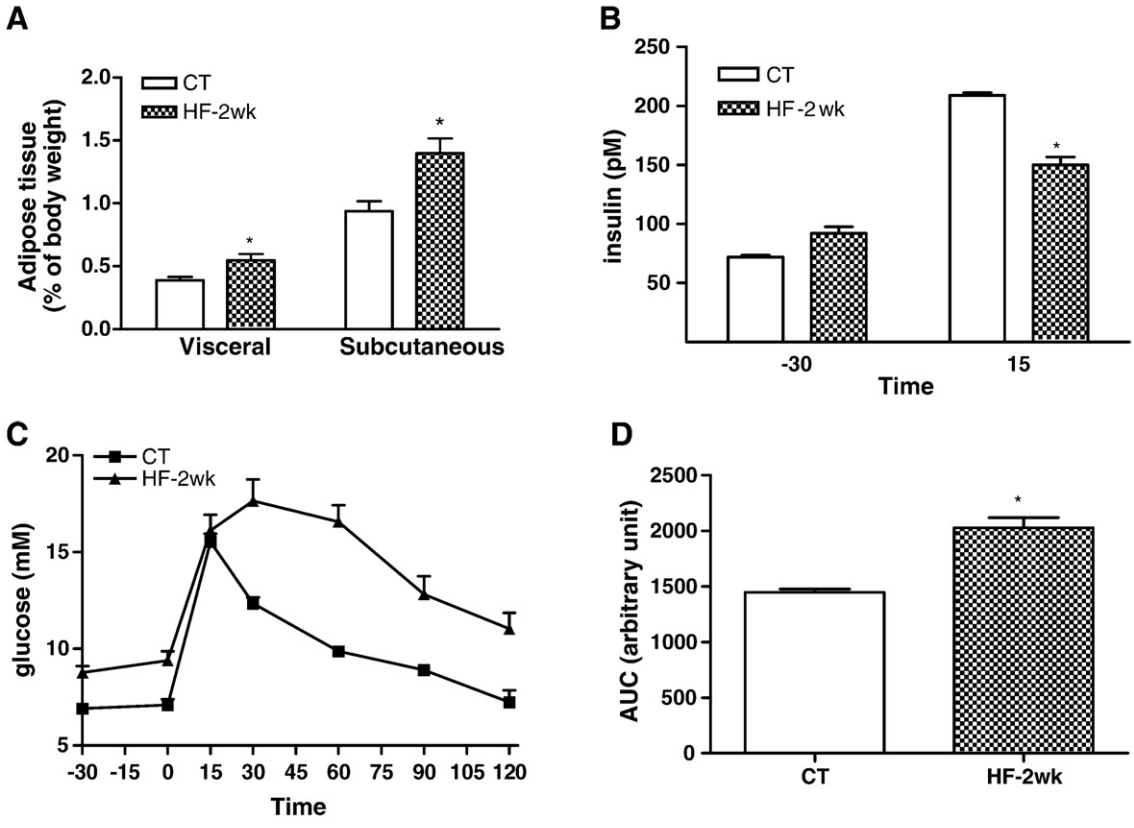

Figure 1 Adiposity (A) and oral glucose tolerance test (B,C,D) of mice fed a high fat (HF) diet or a standard diet (CT) for 2 weeks. A: visceral and subcutaneous adipose tissue weight; B: plasma insulin concentrations 30 min before and 15 min after the oral glucose load. C: Plasma glucose after the oral glucose load; D: area under curve (AUC) of the glucose excursion after the oral glucose load. *$p<0.05$, student *t* test.

Table 2 Body weight and organ weights of mice fed a high fat (HF) diet or a HF diet supplemented with 10% bran fractions for 3 weeks

	HF	HF-crude	HF-aleurone
Body weight (g)	25.21 ± 0.66	25.53 ± 0.37	25.75 ± 0.52
Liver (g/100 g body weight)	3.86 ± 0.15	3.54 ± 0.10	3.70 ± 0.09
Spleen (g/100 g body weight)	0.39 ± 0.06	0.27 ± 0.01	0.28 ± 0.01
Empty caecum (g/100 g body weight)	0.27 ± 0.01	0.27 ± 0.01	0.30 ± 0.01
Adipose tissues (g/100 g body weight)			
Visceral	0.51 ± 0.07	0.66 ± 0.11	0.67 ± 0.07
Epididymal	1.62 ± 0.29	1.72 ± 0.19	1.76 ± 0.20
Subcutaneous	1.37 ± 0.25	1.58 ± 0.15	1.34 ± 0.16

Values are mean ± SEM; $p > 0.05$, ANOVA.

spp. and *Lactobacillus* spp. were based on 16S rRNA gene sequences. The PCR amplification reactions were carried out as follows, 2 min at 50 °C, 10 min at 95 °C, followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C, and detection was carried out on an ABI Prism 7900 sequence detection system (Applied Biosystems, Foster City, CA, USA). Each assay was performed in duplicate in the same run. The cycle threshold of each sample was then compared to a standard curve made by diluting genomic DNA (10-fold serial dilution) from cultures. Cell counts before DNA extraction were determined with the Neubauer hemocytometer. To determine the sensitivity and specificity of the assays, the PCR assays were confirmed by using a set of intestinal bacterial species as controls. Group-specific primers based on 16S rDNA sequences PCR assay are F-Bifidobacterium CGCGTCYGGTGTGAAAG, R-Bifidobacterium CCCCACATCCAGCATCCA, BHQ-1-bifido AACAGGATTAGATACCC; F-Lactobacillus GAGGCAGCAG-TAGGGAATCTTC, R-Lactobacillus GGCCAGTTACTACCTCTATCCTTCTTC, BHQ-1-lacto ATGGAGCAACGCCGC.

2.5. Liver lipid analysis

Triglycerides and cholesterol were measured in the liver tissue as previously described for blood samples, after an extraction with chloroform-methanol according to Folch et al. [32]. Protein concentration was measured by the method of Bradford using bovine serum albumin as standard [33].

2.6. Real-time quantitative PCR

Total RNAs from liver, proximal colon and visceral adipose tissue were prepared using TriPure reagent (Roche, Basel, Switzerland) as described before [34]. PCRs were performed using an AbiPrism 5700 Sequence Detection System instrument and software (Applied Biosystems, Foster City, CA, USA) as described [34]. Primer sequences for the targeted mouse genes are available upon request (audrey.neyrinck@uclouvain.be).

2.7. Statistical analysis

Results are presented as mean ± SEM. For the experiment 1, statistical analysis was performed by student *t* test using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA). For the experiment 2, statistical analysis was performed by one-way ANOVA followed by post hoc test (Tukey HSD (GraphPad Software, San Diego, CA, USA). When variances are heterogeneous, logarithmic transformations of individual values are used before statistical analysis. $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Experiment 1: Effect of a HF diet after 2 weeks of treatment

Fig. 1 shows that HF diet significantly increased adiposity in the visceral and subcutaneous adipose tissues (Fig. 1A). Furthermore, HF fed mice exhibit glucose intolerance (Fig. 1C and D) together with a lower capacity to secrete insulin after a glucose load (OGTT) (Fig. 1B).

3.2. Experiment 2: Effect of bran fractions added in the HF diet

3.2.1. Body and organ weights

Body weight was not modified through the addition of wheat bran fractions in the HF diet (Table 2). Food intake, taking into account spillage, was recorded twice a week; total food intake was 230 ± 10, 263 ± 45 and 294 ± 26 g for HF, HF-crude and HF-aleurone groups, respectively ($p > 0.05$, ANOVA). Furthermore, no modification was observed in liver or adipose tissue weights. Interestingly, we observed a lower spleen weight (by about 30%) in both groups treated with bran fractions ($p = 0{,}06$; ANOVA). Caecal tissue as well as caecal content was slightly increased by the addition of wheat aleurone in the HF diet, but this effect was not significant (Table 2 and Fig. 2, respectively).

3.2.2. Analysis of prebiotic effect

We performed enumeration of *lactobacilli* and *bifidobacteria* in the caecal content of mice (Fig. 3). Interestingly, wheat bran fractions increased the number of those both lactic acid-producing bacteria; the increase of *bifidobacteria* was highly pronounced for the aleurone-enriched fraction.

3.2.3. Analysis of carbohydrate homeostasis: glucose tolerance and glucose-induced insulin secretion (OGTT)

Fig. 4A and B showed glucose excursion and area under the curve (AUC) data after an oral load in glucose to mice 2 weeks after the beginning of the dietary treatments. Neither wheat bran nor aleurone-enriched fraction improved glucose tolerance compared to HF mice. In the same way, fasting insulinemia and

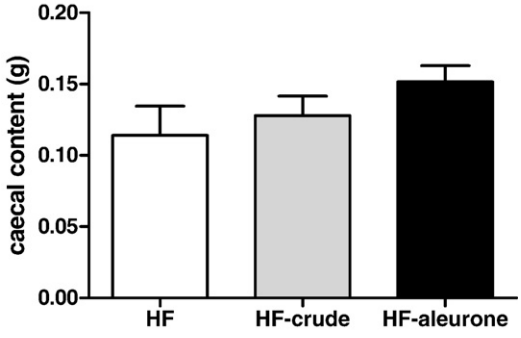

Figure 2 Caecal content of mice fed a high fat (HF) diet or a HF diet supplemented with 10% bran fractions for 3 weeks. $p > 0.05$, ANOVA.

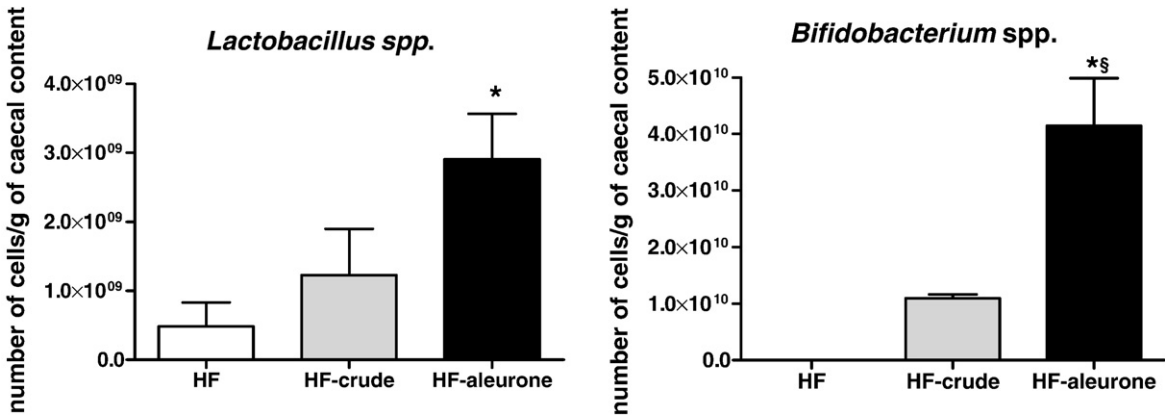

Figure 3 Quantification of *Bifidobacterium* spp. and *Lactobacillus* spp. in the caecal content of mice fed a high fat (HF) diet or a HF diet supplemented with 10% bran fractions for 3 weeks. *$p<0.05$ versus HF mice; §versus HF-crude, ANOVA.

insulin secretion following glucose load were not significantly modified by the addition of wheat bran fractions (Fig. 4C and D).

3.2.4. Analysis of lipid homeostasis

Table 3 shows that wheat bran fractions-enriched diets did not affect cholesterol and triglyceride concentrations in the serum. However, in the liver, we observed a decrease in cholesterol content of about 15% for both fractions compared to HF diet alone and a lower triglyceride content of about 18% for crude fraction (not significant, $p>0.05$, ANOVA). Non-esterified fatty acids were not significantly modified by any treatment although their concentrations were increased by the wheat bran fractions.

3.2.5. GLP-1 and proglucagon mRNA concentrations

The concentration of portal GLP-1 and its colonic precursor (proglucagon mRNA) was unchanged by the addition of wheat bran fractions in the diet (Fig. 5).

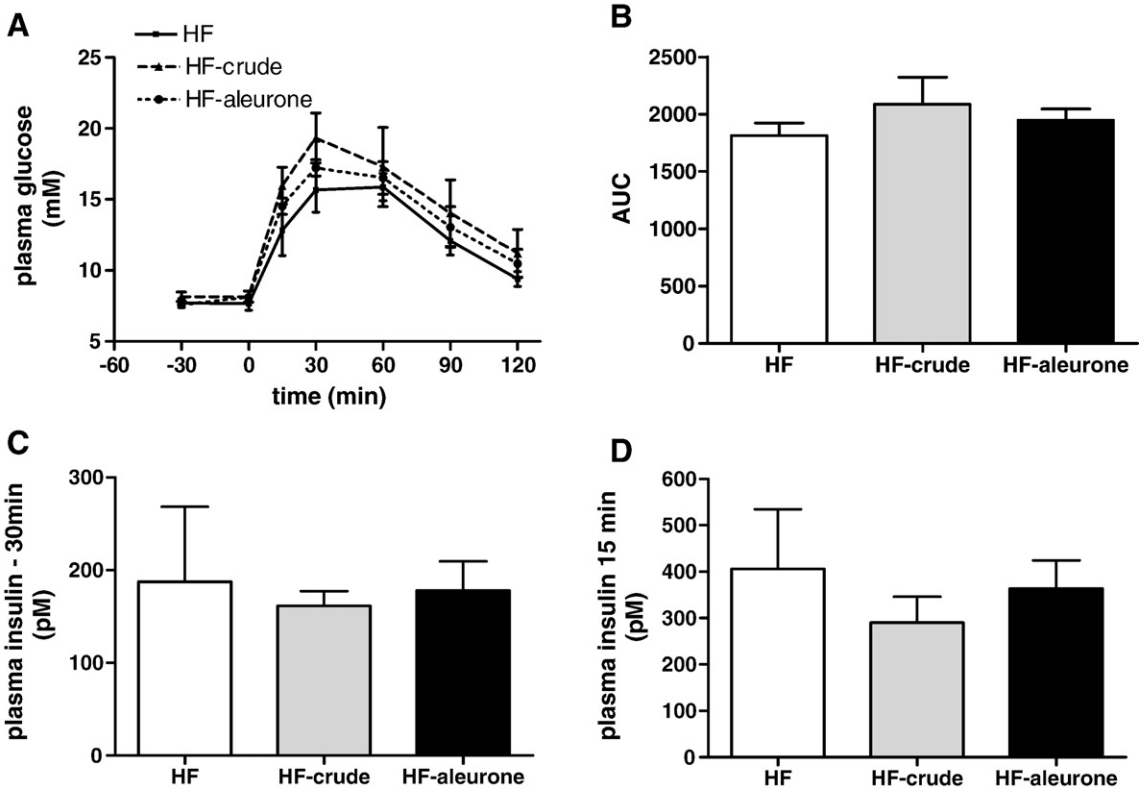

Figure 4 Glucose tolerance and glucose-induced insulin secretion of mice fed a high fat (HF) diet or a HF diet supplemented with 10% bran fractions for 2 weeks (1 week prior to sacrifice) following an oral glucose load (3 g/kg). Plasma glucose (A); area under curve (AUC) of the glucose excursion after the oral glucose load (B); plasma insulin concentrations 30 min (C) before and 15 min (D) after the oral glucose load. $p>0.05$, ANOVA.

Table 3 Serum lipids and liver lipids of mice fed a high fat (HF) diet or a HF diet supplemented with 10% bran fractions for 3 week

	HF	HF-crude	HF-aleurone
Serum lipids (mM)			
Triglycerides	0.438 ± 0.029	0.436 ± 0.034	0.419 ± 0.033
Cholesterol	2.561 ± 0.170	2.570 ± 0.169	2.686 ± 0.114
Non-esterified fatty acids	3.913 ± 0.608	5.323 ± 0.787	5.423 ± 0.493
Liver lipids (nmol/mg protein)			
Triglycerides	56.74 ± 13.04	46.60 ± 9.61	56.33 ± 8.07
Cholesterol	28.61 ± 1.50	24.89 ± 1.36	24.67 ± 1.55

Values are mean ± SEM; $p > 0.05$, ANOVA.

3.2.6. Analysis of inflammatory tone

TNF-α, IL-6, IL-1α, IL-1β, MCP-1, and MIP1α were measured in the plasma of mice (Fig. 6). TNF-α and IL-6 levels were decreased in the group receiving the crude fraction with a significant *p* value for IL-6 compared to HF group. Aleurone-enriched fraction decreased all inflammatory mediators, except IL-1α, although statistical analysis does not reveal any significant effect. Furthermore, we measured mRNA concentrations of IL-1 β, IL-6, TNF-α and CD68 in the visceral adipose tissue (Fig. 7). Although mRNA content of IL-1 β, IL-6 and TNF-α decreased in mice receiving bran fractions, there is no significant *p* value. Interestingly, the adipose levels of CD68 mRNA were significantly lower in mice treated with both bran fractions as compared to HF mice.

4. Discussion

Several data suggest that dietary fibers could play a role in the control of obesity and associated metabolic disorders [3,9–15]. The interesting effect could be linked to their influence on food intake, glycemic index, and lipid homeostasis [35]; recent data suggest that the gut microflora can be related to obesity and metabolic response towards a HF diet [36–38]. We have recently shown that fermentable carbohydrates such as fructans, are able to improve diabetes and obesity due to a HF diet in mice, this effect being clearly linked to their characteristics of fermentation in the gut [34]. The aleurone fraction found in cereal grains is particularly rich in AX, that could be fermentable compounds interesting to take into account to explain some beneficial effects of cereal products [25,26]. The aim of this study was to investigate the putative metabolic effect of two wheat bran fractions (crude and aleurone-enriched fractions), added in a diet promoting obesity in mice for 3 weeks. Metabolic alterations induced by HF feeding are early events since 1) in the first experiment, we show that fat mass development, glucose intolerance and a lower glucose-induced insulin secretion appeared as early as 2 weeks after the start of the dietary treatment; 2) inflammatory markers increased in the liver and in the adipose tissues after 2 weeks [31]. The concentration of 10% bran fractions was chosen since it is a dose which fermentable fructans are able to significantly improve diabetes, fat mass development, and inflammation [12]. Except a slight decrease in hepatic cholesterol content, 10% of wheat bran fractions did not induce any significant modification in lipid homeostasis. The lower cholesterol absorption observed in rats fed with soluble corn bran AX could be one mechanism contributing to the lower hepatic cholesterol level [25]. Gut fermentation has to be taken into account in the interpretation of the metabolic effects of dietary NDC and fibers. The fermentation of NDC in the caeco-colon leads to the production of short chain fatty acids (SCFA), propionate being an inhibitor of hepatic lipid synthesis [18,39–41]. Propionate, which is largely produced through the fermentation of fructans, has been shown to decrease cholesterol synthesis in different models [41]. Furthermore, fiber fermentation, through to the production of SCFA, might also be implicated in the modulation of expression of the gut-derived proglucagon gene and subsequently, secretion of proglucagon-derived peptides, in particular GLP-1 [42–44]. This peptide acts as incretin hormone and is known as an antidiabetic agent that combines insulinotropic and anorexic effects [45]. Previous data suggest that there is a strong association between the increase in proglucagon expression in the proximal colon and the beneficial effects of fermentable fructans on glycemia, fat mass development, and/or body weight gain [19]. The production and the response towards GLP-1 seem a prerequisite for fermentable NDC to provoke a decrease in body weight, fat mass development, and to improve glucose response [12]. In the present study, there is no evidence of extensive gut fermentation due to wheat bran fraction

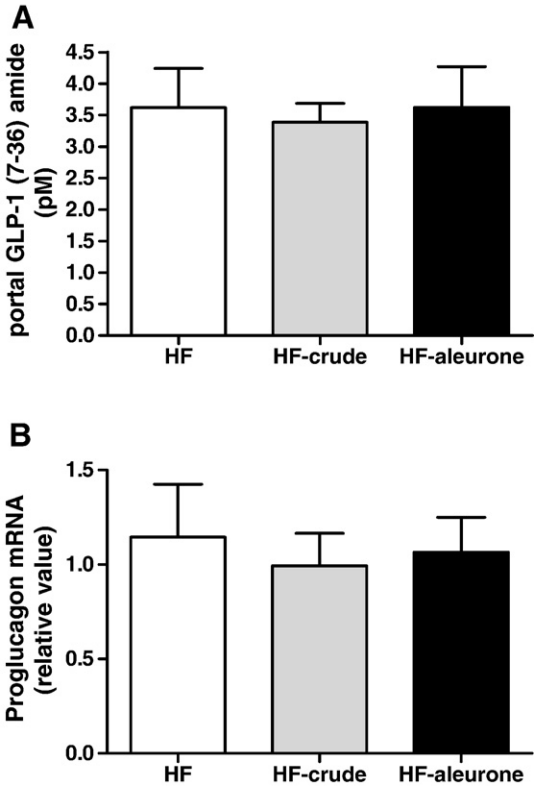

Figure 5 Glucagon-like peptide-1 (GLP-1) concentration in the portal vein (A) and proglucagon mRNA content in the proximal colon (B) of mice fed a high fat (HF) diet or a HF diet supplemented with 10% bran fractions for 3 weeks. $p > 0.05$, ANOVA.

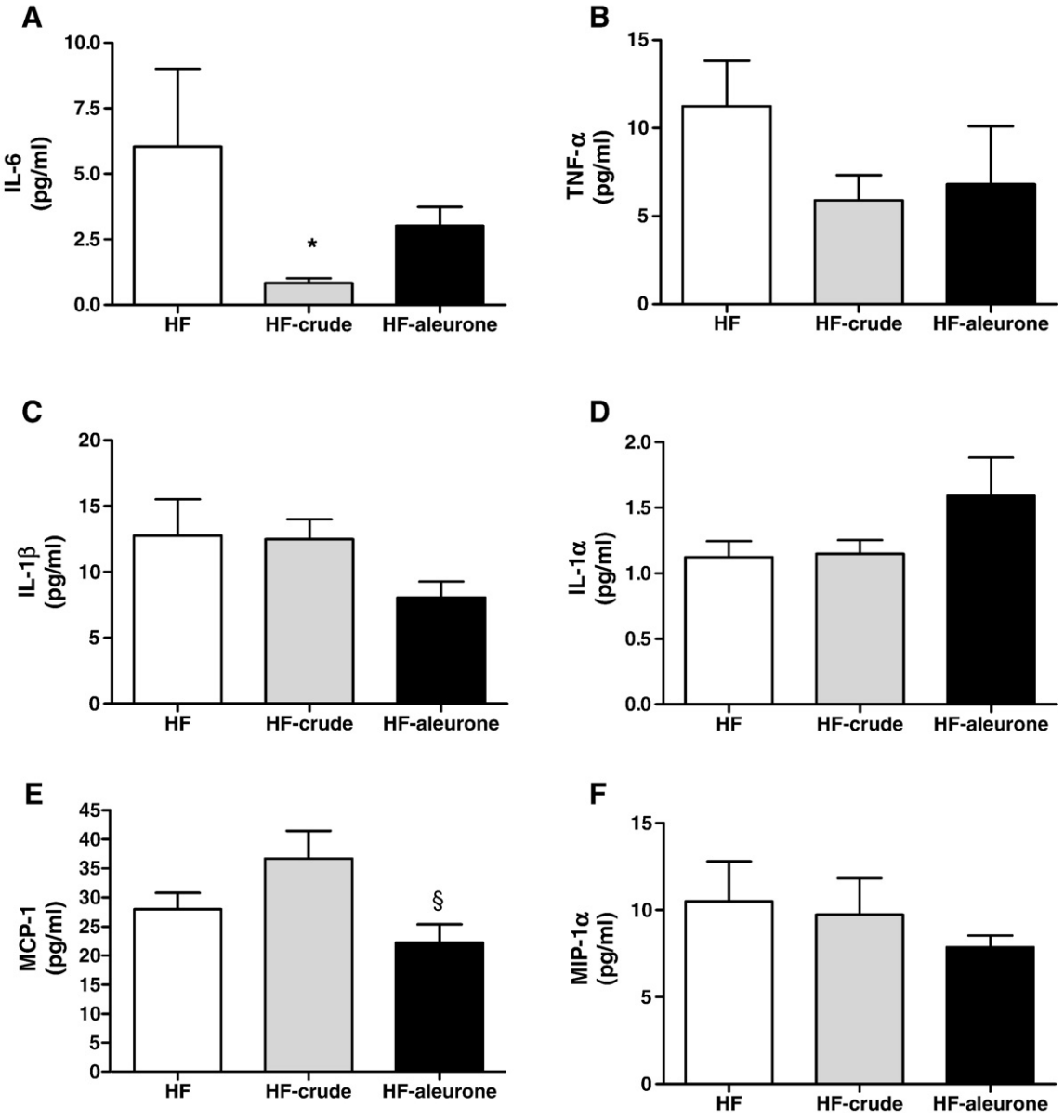

Figure 6 Plasma tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-1α, IL-1β, monocyte chemoattractant protein (MCP)-1, and macrophage inflammatory protein (MIP)-1α concentrations in mice fed a high fat (HF) diet or a HF diet supplemented with 10% bran fractions for 3 weeks. *$p<0.05$ versus HF mice; §versus HF-crude, ANOVA.

addition in the HF diet since there is no increase in caecal content or caecal tissue weight 3 weeks after the beginning of the dietary treatment. Addition of wheat bran fractions at a concentration of 10%, representing only few percentages of fermentable fiber, is probably not sufficient to induce important gut fermentation. Interestingly, we demonstrated an important bifidogenic effect of aleurone-enriched bran fractions in the caecal content, independently of caecal enlargement. However, the concentration of portal GLP-1 was unchanged whatever was the dietary treatment, a phenomenon correlated with the identical level of its precursor (proglucagon mRNA) in the colon. Furthermore, wheat bran or aleurone-enriched fraction did not affect fat mass development, body weight, glucose tolerance or insulin response following a load of glucose, probably due to the lack of effect of wheat bran fractions on GLP-1 secretion related to caecal enlargement. We have focused our attention on the modulation of peptide expression in the proximal colon, because we have previously shown that the content of GLP-1 in the proximal colon is three fold higher than in the distal colon [11]. However, some data suggest that wheat bran or arabinoxylan-rich fiber are extensively fermented in the distal part of the colon [26,46]. Therefore, it would be interesting, before excluding any effect of wheat bran fractions, to have a study fully devoted to analyse the fermentation pattern – and its related GLP-1 production – in the different segments of the intestine.

The major positive result obtained upon this study is the impact of wheat bran fractions on inflammation. Recently, it was suggested that obesity and insulin resistance are features

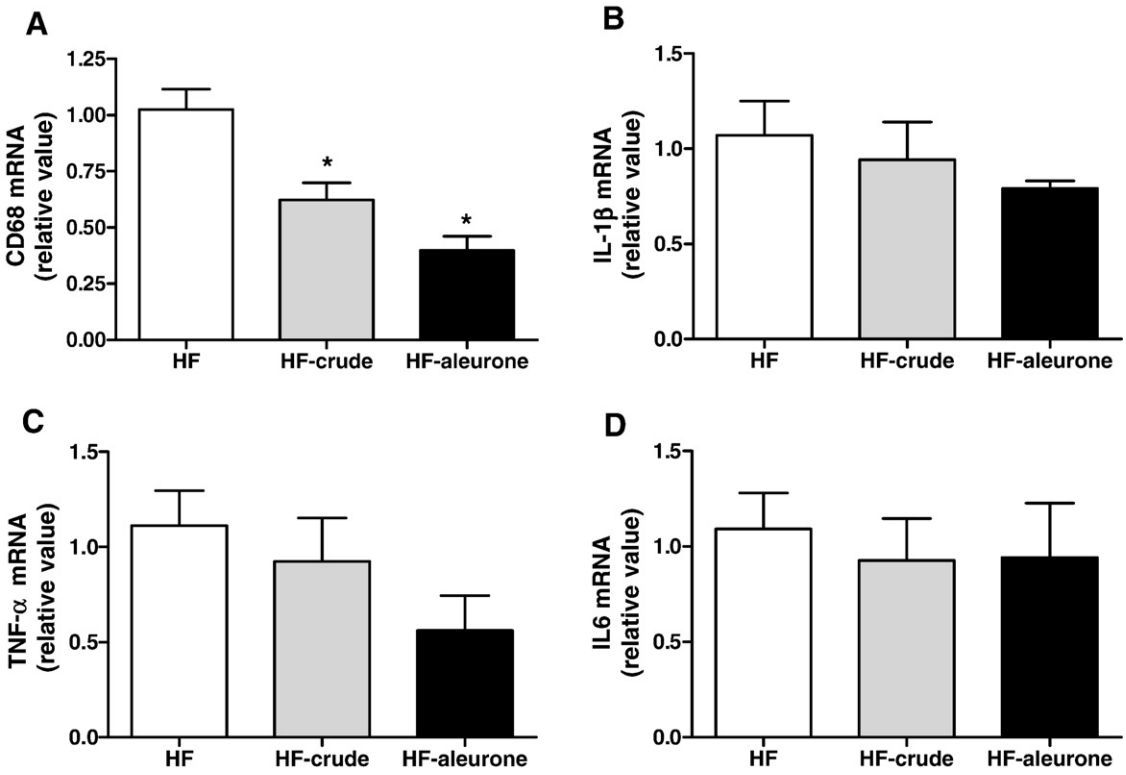

Figure 7 Plasma tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1 β, IL-6, and CD68 mRNA concentrations in visceral adipose tissue of mice fed a high fat (HF) diet or a HF diet supplemented with 10% bran fractions for 3 weeks. *$p<0.05$ versus HF mice, ANOVA.

associated with a proinflammatory status [8]. Results obtained by Cani et al. [12,31,34,38] demonstrated that HF diet-fed mice developed insulin resistance and inflammation by a mechanism directly dependent on a gut bacterial Gram negative derived compound, namely lipopolysaccharide (LPS) and also that OFS may exert indirect anti-inflammatory actions when mice were fed a HF diet, independently of GLP-1 action [12,31,34,38]. This is the first study showing that wheat bran has an impact on proinflammatory mediators in a nutritional model inducing obesity and diabetes. Indeed, crude fraction of wheat bran significantly decreased a major proinflammatory cytokine – IL-6 – accompanied by a lower TNF-α plasma concentration. Furthermore the pattern of proinflammatory mediators was lower when aleurone-enriched fraction was added in the HF diet. Several studies demonstrated that many inflammation and macrophages-specific genes, such as CD68, IL-1 and IL-6 are dramatically upregulated in white adipose tissue in mouse model of HF diet-induced obesity [31,47]. Here, we have shown that CD68 mRNA, a macrophage-specific gene, decreased in the visceral adipose tissue when bran fractions were added in the HF diet. The mechanisms involved in the development of metabolic endotoxemia-induced inflammation and the corresponding metabolic disorders in response to HF feeding are associated with an increased intestinal permeability [34,38]. The modulation of gut bacteria following high fat diet strongly increased intestinal permeability. In fact, specific strategy for modifying gut microbiota in favour of bifidobacteria, through the use of prebiotics (OFS), prevents the deleterious effect of HF diet-induced metabolic diseases, in particular, inflammation [34]. Therefore, we postulated that the lower inflammation and the lower macrophages infiltration in visceral adipose tissue due to supplementation with wheat bran fractions may be linked to their bifidogenic effect. However, although increasing evidence has established correlative and causative links between chronic inflammation – present in white adipose tissue – and the development of insulin resistance and type 2 diabetes mellitus [7,8,47–49], we were unable to highlight any effect on glucose homeostasis. Nevertheless, modulation of immune function by nutrients is an emerging research area in the field of nutrition.

The present study indicates that the modulation of inflammation by dietary fibers occurs as early as 3 weeks after the beginning of the dietary treatment and does not require an extensive fermentation. However, the changes of inflammatory cytokines pattern upon dietary fibers feeding does not necessarily lead to an improvement of metabolic disorders associated to obesity. In recent studies, we have shown that both OFS and laminarin (a marine β-glucan), added in the diet (10%), protect the liver from injury induced by a single injection of LPS in rats, by modulating hepatic immune cell activities and LPS-induced cytokines [50,51]. Therefore, it should be interesting to investigate nutritional approach of wheat bran fraction effects in a model of systemic inflammatory diseases. Moreover, in view of the observed modulation of cytokine profile upon wheat bran fractions (decrease in circulating IL-6), their influence on colon cancer in an adequate model would also be an interesting perspective [52–55].

Acknowledgements

Financial support has been provided by the Ministry of the Walloon Region (General Directory of Agriculture). We thank Dr Sinnaeve (CRA-W, Département Qualité des productions agricoles, Gembloux, Belgium) for providing crude fraction of wheat. P.D. Cani is a postdoctoral researcher from the Fonds de la Recherche Scientifique (Belgium).

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