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Effects of whole-grain cereal foods on plasma short chain fatty acid concentrations in individuals with the metabolic syndrome

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ABSTRACT

Objective: Short chain fatty acids (SCFAs) derived from dietary fiber fermentation by gut microbiota have been identified as one of the mechanisms behind the association between habitual whole-grain intake and a lower risk of cardiometabolic diseases. The aims of the present work are: (1) to evaluate whether a whole-grain wheat-based diet may increase SCFAs concentration, and (2) to identify possible associations between SCFAs and metabolic changes observed after the nutritional intervention.

Methods: Fifty-four subjects participated in the trial. They underwent a 12-wk dietary intervention based on whole-grain or refined cereal products. At baseline and after the intervention, glucose, insulin, triacylglycerol, inflammatory markers (hs-CRP, IL-1 ra, IL-6, and TNF-α), and SCFAs plasma concentrations were evaluated.

Results: After the intervention, in the whole-grain group fasting plasma propionate concentrations were higher than at baseline, whereas a reduction was detected in the control group. The absolute changes (end of trial minus baseline) in fasting plasma propionate concentrations were significantly different between the two groups ($P = 0.048$). The absolute changes of fasting propionate correlated with cereal fiber intake ($r = 0.358$, $P = 0.023$), but no significant correlations with clinical outcomes were found. However, postprandial insulin was significantly decreased in the group having the absolute changes of fasting propionate concentration above the median value ($P = 0.022$ versus subjects with fasting propionate changes below the median value).

Conclusions: A 12-wk whole-grain wheat-based diet increases fasting plasma propionate. This increase correlates with the cereal fiber intake and is associated with lower postprandial insulin concentrations.

Introduction

Whole-grain consumption has been associated with a reduced risk of cardiovascular disease, overweight/obesity and type 2 diabetes [1,2].

One of the proposed mechanisms behind these associations is the colonic fermentation of cereal fiber, leading to the production of short chain fatty acids (SCFAs): acetate, propionate, and butyrate. In fact, many studies have speculated on the role of SCFAs in the modulation of glucose and lipid metabolism [3,4]. Nevertheless, evidence from clinical trials is still scant and

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controversial [5–8]. This may be due, at least in part, to the high variability of fiber fermentation by colonic microbiota. In vitro [9, 10] and in vivo [4] studies have demonstrated that the rate of fiber fermentation depends strictly on the type of cereal; in particular, rye and barley seem to be more fermentable than wheat. Moreover, study duration may also play a role. In fact, Freeland et al. [11] have shown that wheat also increases colonic SCFAs production, even though it takes time to be able to appreciate a significant increase of fiber fermentation products in plasma (9 mo).

Thus, the aim of this study was to evaluate whether a 12-wk consumption of a diet rich in whole-grain products (mainly whole wheat) may influence the production of SCFAs in subjects with the metabolic syndrome. In addition, we hypothesized a role of SCFAs in the modulation of postprandial glucose, insulin, and triacylglycerol concentrations and subclinical inflammation.

Material and methods

Subjects and study design

Fifty-four overweight/obese subjects (23 men and 31 women) with the metabolic syndrome were randomly assigned to an isoenergetic diet based on either whole-grain cereal products (whole-grain group, n = 26) or refined cereal goods (control group, n = 28) for 12-wk.

Details on the study design have been published elsewhere [12]. In short, participants were encouraged not to change their habitual meat, dairy products, eggs, fish, fruit, vegetable, and fat intake during the study. The only difference between the whole-grain and the control diet was the inclusion of a fixed amount of whole-grain or refined cereal products as the main carbohydrate source. Therefore, the two diets were designed to have the same energy intake and nutrient composition (18% protein, 30% fat, 52% carbohydrates); they were different only for cereal-foods consumed and for the cereal fiber intake.

At baseline and at the end of the intervention, fasting blood samples were taken in K2-EDTA tubes for SCFAs measurements and fasting and postprandial blood samples (over 3 h) for the evaluation of glucose, insulin, triacylglycerol, and inflammatory markers (hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; IL-1 ra, interleukin-1 receptor antagonists; and TNF-α: tumor necrosis factor-α). During the study, all subjects were asked to fill in a 7 d food record every mo to assess dietary compliance.

The design of the trial was approved by the "Federico II" University Ethics Committee and followed the Helsinki Declaration guidelines. All participants provided written informed consent.

The study was registered with ClinicalTrials.gov (identifier NCT00945854).

Experimental procedures

Plasma glucose and triacylglycerol concentrations were assayed by enzymatic colorimetric methods (ABX Diagnostics, Montpellier, France; Roche Molecular Biochemicals, Mannheim, Germany; Wako Chemicals GmbH, Neuss, Germany, respectively) on a Cobas Mira autoanalyzer (ABX Diagnostics, Montpellier, France). Plasma insulin concentrations were measured by an enzyme-linked immunosorbent assay for the specific determination of biologically active insulin (DAKO Insulin, DAKO Diagnostics, Ely, UK). The inflammatory markers (hs-CRP, IL-1 ra, IL-6, and TNF-α) were determined in Germany at the University of Ulm, in the laboratory of the Department of Internal Medicine II-Cardiology, as described by de Mello et al. [13].

Acetate, propionate, and butyrate acids were evaluated in plasma at baseline and at the end of the dietary intervention. Before the analyses, 400 mL-samples were deproteinated using 50 μL metaphosphoric acid (16% W/V) at 60° C for 30 min and 50 μl of internal standard (isovaleric acid 80 μM) was added to each sample. After centrifugation (8000 rpm for 30 min at room temperature) all samples were filtered. Then, SCFAs concentrations were analyzed by gas-chromatography (Hewlett Packard 5890 Series II), according to Remesey and Demigne [14].

Statistical analyses and calculations

Results were expressed as mean ± SEM. Variables not normally distributed were analyzed after logarithmic transformation or as medians.

To test the effects in each intervention group, 12-wk concentrations were compared to the baseline concentration by a paired sample *t* test. Differences between the two experimental diets, expressed as absolute changes (12-wk value minus baseline value) were evaluated by one-way analysis of variance (ANOVA). Bivariate associations were assessed by Pearson's correlation. A P value <0.05 was considered significant. Statistical analysis was performed according to standard methods using the Statistical Package for Social Sciences software version 21.0 (SPSS, Chicago, IL, USA).

Results

Subjects characteristics and dietary compliance

SCFAs measurement was performed in 40 subjects who were, therefore, included in this analysis: 19 subjects (7 M/12 F) in the control group and 21 subjects (9 M/12 F) in the whole grain group. The two experimental groups were similar at baseline for anthropometric parameters as well as for all clinical and metabolic parameters and inflammatory markers (Table 1). A significant reduction of postprandial insulin and triacylglycerol responses were observed at the end of the intervention in the whole-grain group compared to the control group (postprandial insulin: −17.2 ± 10 versus 13.6 ± 6.7 μU/mL, whole grain and control group respectively, $P < 0.05$; postprandial triacylglycerols: −22.6 ± 13 versus 11.6 ± 8.9 mg/dL, whole-grain and control group respectively, $P < 0.05$) [12].

Table 1
Clinical data, fasting plasma metabolic parameters and inflammatory markers at baseline and after the intervention in the two experimental groups

Parameters	Control group (n = 19)			Whole-grain group (n = 21)			ANOVA for Δ
	Baseline	12 week	Δ	Baseline	12 week	Δ	
Age (years)	58.4 ± 1.6			57.2 ± 1.9			
Sex (M/F)	7/12			9/12			
BMI (kg/m^2)	31.5 ± 1.3	31.3 ± 1.3	−0.20 ± 0.2	32.1 ± 1.4	31.9 ± 1.4	−0.20 ± 0.2	0.992
Fasting glucose (mg/dL)	105 ± 2.8	105 ± 0.2	−0.72 ± 1.5	103 ± 2.2	103 ± 2.7	−0.38 ± 1.7	0.882
Fasting insulin (μU/mL)	11.8 ± 1.3	11.7 ± 1.6	−0.11 ± 1.5	14.1 ± 1.9	13.3 ± 1.1	−0.47 ± 0.9	0.726
HOMA	3.08 ± 0.3	3.09 ± 0.5	0.01 ± 0.4	3.61 ± 0.5	3.36 ± 0.3	−0.25 ± 0.3	0.615
Triacylglycerols (mg/dL)	133 ± 9.5	136 ± 13	2.95 ± 12	153 ± 36	137 ± 15	−16.9 ± 28	0.538
Total cholesterol (mg/dL)	201 ± 8.8	208 ± 7.5	6.47 ± 5.0	200 ± 10	201 ± 10	1.10 ± 5.1	0.457
HDL-cholesterol (mg/dL)	38.7 ± 1.5	39.8 ± 1.5	1.11 ± 1.2	42.9 ± 3.2	42.3 ± 3	−0.62 ± 1.2	0.324
hs-CRP (mg/dL)	2.27 ± 0.4	2.39 ± 0.4	0.12 ± 0.2	2.52 ± 0.5	2.44 ± 0.5	−0.08 ± 0.4	0.693
IL-6 (pg/mL)	1.69 ± 0.3	1.70 ± 0.3	0.01 ± 0.2	1.84 ± 0.2	2.23 ± 0.3	0.39 ± 0.2	0.161
IL-1 ra (pg/mL)	316 ± 73	311 ± 63	−5.17 ± 14	380 ± 61	378 ± 61	−1.40 ± 24	0.897
TNF-α (pg/mL)	1.07 ± 0.4	1.31 ± 0.5	0.24 ± 0.1	1.71 ± 0.6	1.50 ± 0.6	−0.21 ± 0.3	0.232

BMI, body mass index; HDL, high-density lipoproteins; hs-CRP, high-sensitivity C-reactive protein; HOMA, homeostasis model assessment; IL-1 ra, interleukin-1 receptor antagonists; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; ANOVA, analysis of variance
Data are expressed as mean ± SEM

Table 2
Fasting concentrations of plasma acetate, propionate and butyrate measured in the two experimental groups

SCFA (μmol/L)	Control group (n = 19)			Whole-grain group (n = 21)			ANOVA for Δ
	Baseline	12 week	Δ	Baseline	12 week	Δ	
Acetate	204 ± 20	178 ± 20	−26.1 ± 18	179 ± 14	154 ± 18	−25.2 ± 20	0.974
Propionate	7.58 ± 1.1	6.29 ± 0.9	**−1.30 ± 0.9**	5.61 ± 0.6	7.11 ± 0.9	**1.50 ± 1.0**	**0.048**
Butyrrate	5.58 ± 0.9	5.69 ± 0.9	0.11 ± 0.7	4.37 ± 0.6	4.74 ± 0.6	0.37 ± 0.7	0.623

Data are expressed as mean ± SEM
Bold values are used to highlight statistically significant results

No changes were observed in the other clinical outcomes (Table 1).

As reported earlier [12], no differences were observed in diet composition at baseline between the two experimental groups. During the intervention, dietary compliance was adequate according to the 7-d food records in both groups. Total and cereal fiber intake were significantly different between the two experimental groups at the end of the trial, as expected from the controlled study design (total fiber: 40.2 ± 1.2 versus 22.1 ± 0.9 g/d whole-grain and control group respectively, $P < 0.001$; cereal fiber: 28.9 ± 1.1 versus 11.8 ± 0.4 g/d, whole-grain and control group respectively, $P < 0.001$). Moreover, we found a significant difference in the absolute change of total plasma alkylresorcinol concentrations, a well-known marker of whole-wheat intake [15], between the two experimental groups (88.3 ± 16 versus −19.7 ± 7.0 nmol/L, whole-grain and control group, respectively $P < 0.001$). No other significant differences were observed between the two study groups in relation to the energy content and nutrient composition of the diet during the trial [12].

Short chain fatty acids concentrations

The concentrations of SCFAs detected at baseline and after the experimental period are shown in Table 2.

Fasting plasma concentrations of acetate, propionate, and butyrate were similar in both groups at baseline. After the intervention, fasting plasma propionate concentration increased in respect to baseline in the whole grain group, whereas a reduction was detected in the control group. Consequently, the absolute changes in fasting plasma propionate concentration between 12 wk and the baseline values were significantly different between the whole grain and the control group (+1.50 ± 1.0 versus −1.30 ± 0.9 μmol/L, respectively; $P = 0.048$; Table 2).

Short chain fatty acids and clinical outcomes

At the end of the intervention, the absolute change in fasting plasma propionate concentration was positively and significantly correlated with the cereal fiber intake (r = 0.358, $P = 0.023$) (Fig. 1).

No significant correlations were found between fasting SCFAs concentrations and clinical outcomes. Nevertheless, when the groups were stratified according to the absolute changes between the end of the trial and baseline of fasting plasma propionate (median value: 0.0500 μmol/L), we found a lower average postprandial plasma insulin concentration in the group above the median than in the group below the median value (−17.4 versus 12.4 μU/mL, respectively; $P = 0.022$, Fig. 2).

Discussion

In this study, we detected an increase in fasting propionate plasma levels after 12-wk consumption of a diet rich in whole-grain foods. In addition, this increase was positively correlated with the cereal fiber intake. This finding is strengthened by the optimal compliance to the recommended diets assessed by plasma alkylresorcinol concentrations, a marker of whole-wheat intake as reported above.

These results suggest that also fiber present in whole-grain wheat foods, like that present in other cereal foods, can be fermented and induce a modification of colonic microbiota in the time frame of 12 wk.

In the study of Freeland et al. [11], SCFAs production was detectable only after 9 mo of wheat consumption, even if the amount of dietary fiber was equal to that provided by our experimental diet (40.0 versus 40.2 g/d, respectively).

In our study, correlation analyses did not show any relation between fasting propionate and metabolic parameters or inflammatory makers. However, if the study population was stratified according to the median value of the absolute change (end of the trial minus baseline) of plasma propionate

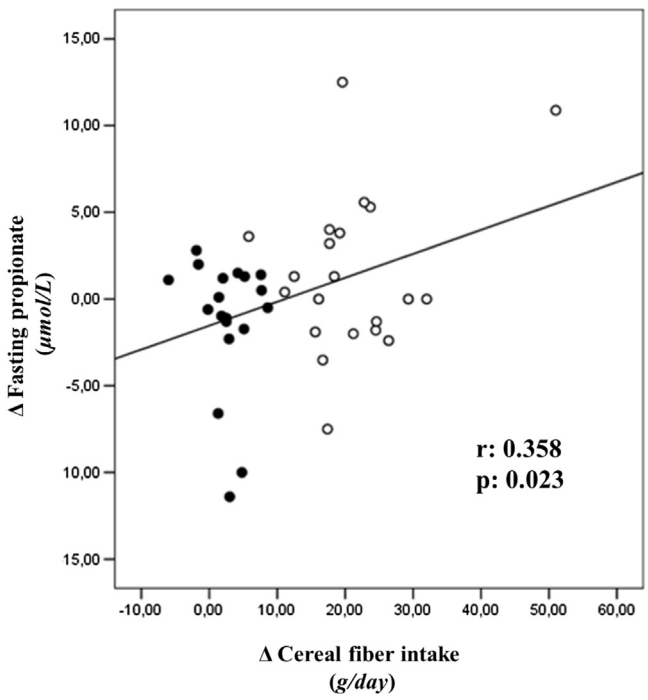

Fig. 1. Pearson correlation between the absolute changes (end of the trial *minus* baseline) of cereal fiber intake and fasting propionate. Black circle, control group; white circle, whole-grain group.

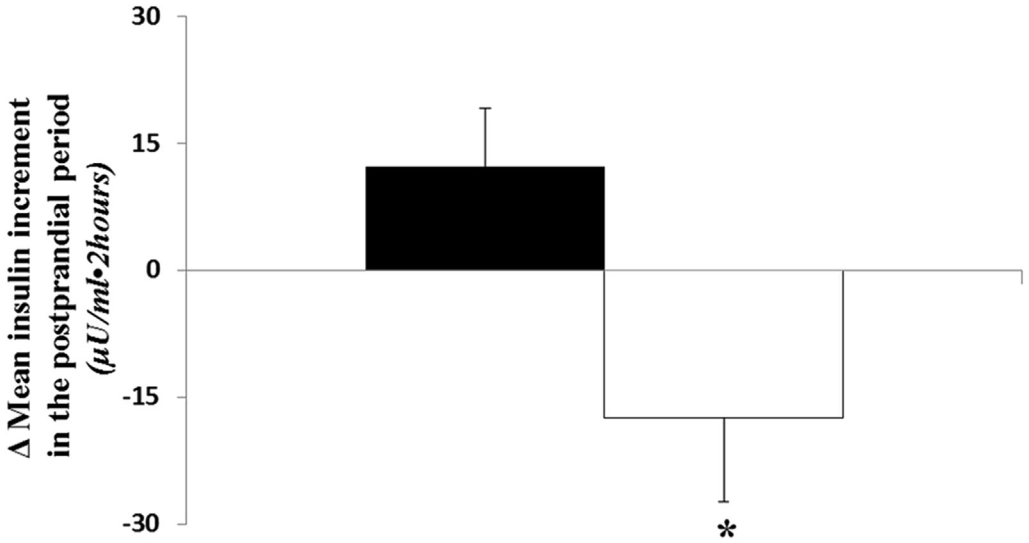

Fig. 2. Mean postprandial insulin increment according to the absolute changes (end of the trial minus baseline) of plasma fasting propionate (below and above the median). Data are expressed as mean ± SEM. Black, values below the median; white, values above the median. $^{*}P = 0.020$ (versus below the median; one-way ANOVA).

concentrations, a reduction of postprandial insulin was observed in subjects above the median value when compared to subjects below the median. This result is in line with other studies evaluating the effect of propionate-enriched cereal products on postprandial insulin response [16–19].

In our trial we did not observe any improvement of peripheral insulin sensitivity (evaluated by IVGTT-derived indices), therefore, we assumed that the reduction of postprandial insulin was a marker of improved insulin sensitivity at the splanchnic level. Propionate is produced by colonic fermentation and, after intestinal absorption, it reaches the liver where it is metabolized. Therefore, we can hypothesize that a higher propionate production in the colon during the whole-grain diet may have improved insulin action specifically at the level of the liver.

Unfortunately, we did not perform any measurement of hepatic insulin sensitivity. However, Luo et al. [20] demonstrated that a 4-wk consumption of short-chain fructooligasaccharides, a high-fermentable fiber, reduces hepatic glucose production in healthy subjects, which is in line with an improvement of hepatic insulin sensitivity.

Undoubtedly, dietary strategies to modulate the postprandial insulin response are necessary tools in the management and prevention of cardiometabolic diseases. In this light, the results of our trial, in agreement with the evidence available in the literature, suggest that habitual whole wheat consumption may help to achieve this goal.

One limitation of our study is the assessment of SCFAs in peripheral blood, which did not allow us to evaluate the amount of SCFAs reaching the liver (this represents the major site of their effects).

The assessment of fecal SCFAs would have been helpful to evaluate the extent of colonic fermentation in the two experimental groups; unfortunately, fecal samples were not collected. Moreover, we have no information on colonic microbiota composition.

In addition, we cannot exclude that the sample size and the study duration were not sufficient to detect significant correlations between SCFAs and clinical outcomes.

The strengths of our study are the well-controlled study design, the optimal compliance to the experimental diets of the participants and the prolonged exposure to a rather high consumption of whole grain wheat products.

Conclusion

The habitual consumption of whole grain wheat foods may promote colonic fiber fermentation, as suggested by the increase of fasting propionate concentrations observed in the whole-grain group at the end of the dietary intervention. In addition, propionate may be involved in the reduction of postprandial insulin concentrations observed after the whole-grain diet. Further studies are needed to clarify this effect, meanwhile, this study adds new information on the potential benefits of whole-grain consumption in line with present dietary recommendations.

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