Whole-grain wheat consumption reduces inflammation in a randomized controlled trial on overweight and obese subjects with unhealthy dietary and lifestyle behaviors: role of polyphenols bound to cereal dietary fiber[1–5]

Paola Vitaglione, Ilario Mennella, Rosalia Ferracane, Angela A Rivellese, Rosalba Giacco, Danilo Ercolini, Sean M Gibbons, Antonietta La Storia, Jack A Gilbert, Satya Jonnalagadda, Frank Thielecke, Maria A Gallo, Luca Scalfi, and Vincenzo Fogliano

ABSTRACT

Background: Epidemiology associates whole-grain (WG) consumption with several health benefits. Mounting evidence suggests that WG wheat polyphenols play a role in mechanisms underlying health benefits.

Objective: The objective was to assess circulating concentration, excretion, and the physiologic role of WG wheat polyphenols in subjects with suboptimal dietary and lifestyle behaviors.

Design: A placebo-controlled, parallel-group randomized trial with 80 healthy overweight/obese subjects with low intake of fruit and vegetables and sedentary lifestyle was performed. Participants replaced precise portions of refined wheat (RW) with a fixed amount of selected WG wheat or RW products for 8 wk. At baseline and every 4 wk, blood, urine, feces, and anthropometric and body composition measures were collected. Profiles of phenolic acids in biological samples, plasma markers of metabolic disease and inflammation, and fecal microbiota composition were assessed.

Results: WG consumption for 4–8 wk determined a 4-fold increase in serum dihydroferulic acid (DHFA) and a 2-fold increase in fecal ferulic acid (FA) compared with RW consumption (no changes). Similarly, urinary FA at 8 wk doubled the baseline concentration only in WG subjects. Concomitant reduction in plasma tumor necrosis factor-α (TNF-α) after 8 wk and increased interleukin (IL)-10 only after 4 wk with WG compared with RW ($P = 0.04$) were observed. No significant change in plasma metabolic disease markers over the study period was observed, but a trend toward lower plasma plasminogen activator inhibitor 1 with higher excretion of FA and DHFA in the WG group was found. Fecal FA was associated with baseline low Bifidobacteriales and Bacteroidetes abundances, whereas after WG consumption, it correlated with increased Bacteroidetes and Firmicutes but reduced *Clostridium*. TNF-α reduction correlated with increased *Bacteroides* and *Lactobacillus*. No effect of dietary interventions on anthropometric measurements and body composition was found.

Conclusions: WG wheat consumption significantly increased excreted FA and circulating DHFA. Bacterial communities influenced fecal FA and were modified by WG wheat consumption. This trial was registered at clinicaltrials.gov as NCT01293175. *Am J Clin Nutr* 2015;101:251–61.

Keywords bioavailability, ferulic acid, inflammation, obesity, wholegrain wheat

INTRODUCTION

Epidemiologic evidence indicates that whole-grain (WG)[6] consumption substantially lowers the risk of chronic diseases, such as cardiovascular disease, diabetes, and cancer, and plays a role in body weight management and digestive health. Dietary guidelines worldwide recommended increasing WG consumption by replacing refined grains (1, 2).

Recently, some doubts on the epidemiologic links between WG consumption and disease prevention arose (3), and intervention studies about subclinical inflammation and body weight showed discrepant findings (4, 5). However, convincing evidence to support beneficial effects of WG intake on vascular disease prevention was provided (6).

There is still a knowledge gap on the mechanisms underpinning WG health benefits. It is known that WG physical structures help in reducing glucose and lipid absorption,

[1] From the Department of Agricultural and Food Science, University of Naples "Federico II," Portici (NA), Italy (PV, IM, RF, DE, ALS, and VF); the Departments of Clinical Medicine and Surgery (AAR) and Public Health (LS), University of Naples "Federico II," Napoli, Italy; Institute of Food Science, National Research Council, Avellino, Italy (RG); Graduate Program in Biophysical Sciences (SMG) and Department of Ecology and Evolution (JAG), University of Chicago, Chicago, IL; Institute for Genomics and Systems Biology, Argonne National Laboratory, Lemont, IL (SMG and JAG); General Mills Bell Institute of Health and Nutrition, Minneapolis, MN (SJ); Cereal Partners Worldwide S.A., Lausanne, Switzerland (FT); and Centro Diagnostico San Ciro, Portici, Italy (MAG).

[2] Supported by General Mills Bell Institute of Health and Nutrition with an unconditional grant.

[3] Supplemental Tables 1–4 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

[4] Present address for SJ: Kerry, Beloit, WI.

[5] Address correspondence to P Vitaglione, Dipartimento di Agraria, via Università, 100, Portici (NA), Italy. E-mail: paola.vitaglione@unina.it.

[6] Abbreviations used: DHFA, dihydroferulic acid; FA, ferulic acid; MS, mass spectrometry; MS/MS, tandem mass spectrometry; OTU, operational taxonomic unit; PAI-1, plasminogen activator inhibitor 1; QIIME, Quantitative Insights into Microbial Ecology; WG, whole grain

Received March 18, 2014. Accepted for publication November 7, 2014.

First published online December 3, 2014; doi: 10.3945/ajcn.114.088120.

dietary fiber can contribute to improve several gut functions, and many bran and germ phytochemicals may exert antioxidant and anti-inflammatory properties (7, 8). The bifidogenic effect of WG found in some studies suggested a role of the microbiota in triggering amelioration of gut and systemic inflammation, explaining some of the metabolic benefits attributed to WG consumption (9–15). The interplay between microbiota and the polyphenols bound to WG fiber might explain some of WG health benefits (16). WGs are a rich source of phenolic compounds, mainly hydroxycinnamic acids (17–20), with ferulic acid (FA) being the most abundant. The FA concentration varies depending on cereal variety and milling procedure (21), and in WG wheat, it ranges from 4.5 to 1270 mg/kg (22). From the chemical point of view, FA and 95% of the grain phenolic compounds are covalently bound to arabinoxylan chains of cell wall polysaccharides through ester bonds (23). WG fiber can deliver phenolic compounds into the lower gut, and the slow and continuous release of FA by the action of gut microbiota metabolism may increase circulating FA and its metabolites, thus providing an amelioration of subclinical inflammation and the long-term benefits associated with WG consumption (16).

However, to our knowledge, no intervention study has been performed to determine the bioavailability of WG polyphenols and to ascertain their role in preventing chronic disease over long-term consumption.

Here, an 8-wk double-arm randomized controlled trial in 80 healthy overweight/obese subjects sharing suboptimal dietary and lifestyle behaviors was performed by daily replacing exact amounts of specific refined wheat products with a WG wheat product (WG group) or selected refined wheat products (control group). The metabolic profiles of phenolic acids in blood, urine, and feces were obtained, and the concomitant change in fecal microbiota composition and obesity-related inflammation and chronic disease risk were determined.

SUBJECTS AND METHODS

Food products

A 100% WG wheat product was used in this study (Shredded Wheat; Cereal Partners Worldwide). It was selected among several commercial products for its WG wheat content (100% WG) and for the amount of polyphenols bound to dietary fiber. Two refined wheat products were selected from the market to guarantee a nutritionally well-balanced placebo for the WG product ["Magretti" crackers (Galbusera) and "Mulino Bianco" toasted sliced breads (Barilla)].

Subjects

Recruitment was performed at the Department of Agricultural and Food Science of the University of Naples, Naples, Italy. Subjects were recruited into the study by public announcements in a local newspaper, through social networks, and among students and staff of the department. The selection was carried out by interview on health status and dietary and behavioral lifestyle factors, collection of anthropometric data, and a 7-d food diary recall. The following were eligible to participate: men and women aged >18 y with a BMI (in kg/m^2) 25–35; a habitual diet characterized by the absence of WG cereals and cereal bran–containing products, probiotics, vitamins/minerals supplements, or complementary and alternative medicines; intake of fruit and vegetables ≤3 servings/d (300 g/d); and a low level of physical activity (total physical activity <500 metabolic equivalent min/wk).

Subjects having any type of disease (functional or metabolic disease, including hyperlipidemia, diabetes, and metabolic syndrome) or food allergy, dieting or under a controlled dietary regimen over the previous 3 months, receiving any type of drug therapy or using drugs over the previous 3 months, participating in other trials, or who were pregnant or lactating were excluded.

Eligible subjects who agreed to participate entered into the study by signing a written informed consent.

Study design

This 8-wk placebo-controlled randomized controlled trial had a double-arm parallel design. Once enrolled by the study nutritionist and physician, subjects were randomly assigned by the dietitian to the WG or the control group on the basis of a randomization sequence that was previously generated by the statistician with the use of a computer-generated permuted blocks (n = 5) randomization scheme.

The dietary intervention was tailored for each subject and consisted of isocaloric replacement of a specific amount of some refined wheat products (mainly bread, pasta, or sliced toasted bread) habitually consumed by subjects with the selected food products. For 8 wk, WG subjects included in their diet 70 g/d (3 biscuits/d) of WG product, whereas control subjects included 1 package (33 g) of crackers and 3 slices of toasted bread (~27 g). In addition, all subjects were instructed to consume the same amount of seasonings they usually ate (if any) with the replaced foods to maintain an unchanged overall nutritional composition of their diets, as well as consume the same amount of fruit and vegetables and maintain the same level of physical activity.

The nutritional composition and the phenolic acid content (including total, free, and bound to dietary fiber amount of each compound) of WG and refined wheat portions consumed daily by volunteers are reported in **Table 1**.

Study protocol

The study protocol, approved by the Ethics Committee of the University of Naples, is illustrated in **Figure 1**. Food products were supplied at baseline and after 4 wk at the Department of Agricultural and Food Science of the University of Naples. Compliance with the dietary treatments was assessed every 2 wk by self-recorded 4-d (3 working days and 1 weekend day) food diaries and also every 4 wk by weighing the uneaten foods returned by subjects; moreover, phone call interviews at 2 and 6 wk were done by an expert dietitian to monitor compliance with the protocol, and physical activity level was assessed by the International Physical Activity Questionnaire (24). At baseline and every 4 wk of treatment, fasting participants returned to the laboratory to provide blood and urine samples, as well as anthropometric and body composition data. During those occasions, they also delivered a fecal sample (collected the day before and stored at −20°C until arrival) and food diaries filled at weeks 2–4 or 6–8. Biological samples were

TABLE 1
Nutritional composition and phenolic acid profile of a daily portion of WG wheat (70 g) and refined wheat products (60 g, cumulative of 2 products) consumed in this study by WG and control subjects, respectively[1]

Characteristic	WG wheat product	Refined wheat products
Proteins, g	7.8	6.5
Carbohydrates, g	45.8	45.7
Sugars	0.6	1.2
Fats, g	1.7	2.2
Saturated	0.3	0.3
Dietary fiber, g	8.0	2.2
Energy, kcal, kJ	229.5, 960.2	222.4, 930.5
Phenolic compounds, mg		
Ferulic acid (total)	96.7	2.6
Free	0.3	2.6
Bound	96.4	—
Sinapic acid (total)	26.5	—
Free	0.2	—
Bound	26.3	—
Coumaric acid (total)	9.4	—
Free	Traces	—
Bound	9.4	—
Gallic acid (total)	1.9	—
Free	0.1	—
Bound	1.8	—
Syringic acid (total)	1.8	Traces
Free	0.3	Traces
Bound	1.5	—
Vanillic acid (total)	1.6	Traces
Free	0.2	Traces
Bound	1.4	—
Salycilic acid (total)	0.5	—
Free	0.1	—
Bound	0.4	—
Caffeic acid (total)	0.3	—
Free	Traces	—
Bound	0.3	—
Total phenolics	138.7	2.6
Free	1.2	2.6
Bound	137.5	—

[1]WG, whole grain.

collected, treated, and analyzed as required for the specific procedures by personnel, who were blinded to the assignment of interventions.

Determination of phenolic compounds in serum, urine, and feces

Blood samples were collected in serum tubes for gel separation and immediately centrifuged at 2600 × *g* for 10 min at 4°C. Urine samples were immediately treated with 0.005% of butylated hydroxytoluene. Feces were diluted in a 1:10 (wt:vol) ratio in phosphate-buffered saline (10 mmol/L) containing 0.005% of butylated hydroxytoluene, vortexed, and centrifuged at 2600 × *g* for 15 min at 4°C. Serum, urine, and fecal supernatants were stored at −40°C before analysis.

Phenolic compounds were extracted and analyzed by HPLC–tandem mass spectrometry (MS/MS) as described recently (25). Briefly, 500 μL serum and 1.5 mL urine and fecal suspensions were extracted by ethyl acetate (1.5 mL × 2 or × 3 times, respectively); supernatants were dried under nitrogen flow and dissolved in 50 μL methanol/water (70:30); 30 μL was injected into the HPLC–MS/MS system.

An HPLC system consisting of 2 micropumps (PerkinElmer Series 200), coupled with an API 3000 Triple Quadrupole mass spectrometer (Applied Biosystem Sciex), was used, and elution was achieved with a Phenomenex Luna 3μ C18 (2) 100-A (50 × 2.00 mm) column by using water/acetonitrile/formic acid [94.9:5:0.1 (by volume)] and acetonitrile/formic acid [99.9:0.1 (vol:vol)] as mobile phases, a flow rate of 200 μL/min, and a linear gradient. Phenolic acids were detected and quantified through electrospray ionization MS/MS analysis (negative mode ionization, multiple-reaction monitoring mode tracking) by using the MS parameters and specific calibration curves [for method details, see Vitaglione et al. (25)].

To normalize the excretion rate of urinary phenolic compounds, we stored 1-mL aliquots of urine at −40°C and measured urinary creatinine concentration by an automated system based on the buffered Jaffe reaction and analyzed by the COBAS Integra (Roche Diagnostic Ltd.).

Determination of markers of metabolic and inflammatory disease in plasma

Metabolic disease intermediate markers were determined in duplicate in 12.5 μL plasma by using the Bio-Plex Pro human diabetes immunoassays multiplex kit (Bio-Rad) and Luminex Technology (Bio-Plex; Bio-Rad), according to the

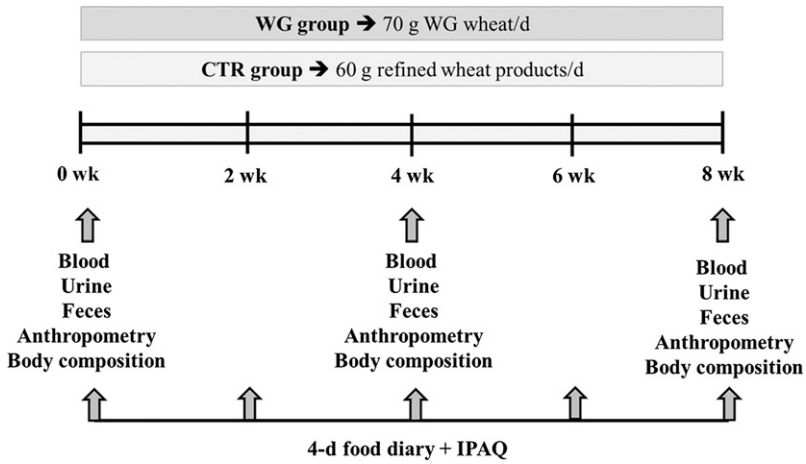

FIGURE 1 Schematic outline of the study protocol. CTR, control; IPAQ, International Physical Activity Questionnaire; WG, whole grain.

manufacturer's instructions. Blood samples were collected into EDTA-coated tubes and were immediately added with protease inhibitors, such as dipeptidylpeptidase IV inhibitor (Millipore) and phenylmethanesulfonyl fluoride (Sigma). They were centrifuged at 2400 × *g* per 10 min at 4°C, and the supernatants were stored at −40°C before analysis.

The Bio-Plex Pro immunoassay kits allowed the simultaneous quantification of the following biomarkers: C-peptide, ghrelin, glucose-dependent insulinotropic peptide, glucagon-like peptide 1, glucagon, insulin, leptin, plasminogen activator inhibitor 1 (PAI-1), resistin, visfatin, adiponectin and adipsin, IL-6, IL-10, and TNF-α. The sensitivity levels of the assay (in pg/mL) correspond to the following: C-peptide, 14.3; ghrelin, 1.2; glucose-dependent insulinotropic peptide (total), 0.8; glucagon-like peptide 1 (active), 5.3; glucagon, 4.8; insulin, 1; leptin, 3.1; PAI-1, 2.2; resistin, 1.3; and visfatin, 37.1.

The interassay variation (% CV) was 4%, and the intra-assay variation (% CV) was 5%.

Glycemia

Glycemia was measured immediately before the blood draw by finger pricking and using a bedside glucometer (OneTouch Sure Step; Life Scan Inc.). Accuracy of the glucometer was evaluated by the manufacturer by using least squares linear regression analysis and found to be 97% "clinically accurate" compared with reference (YSI2700) results.

Determination of plasma lipids

Cholesterol and triglycerides were assayed on plasma and HDL by enzymatic colorimetric methods (ABX Diagnostics, Roche Molecular Biochemicals, and Wako Chemicals GmbH) on a Cobas Mira autoanalyzer (ABX Diagnostics). HDL was isolated from plasma by a precipitation method with a sodium phosphotungstate and magnesium chloride solution.

Determination of the fecal microbiota by 16S rRNA gene sequencing and data analysis

Microbial DNA extraction was carried out by using the PowerSoil DNA isolation kit (MoBIO Laboratories Inc.) with 250 mg fecal samples collected at baseline and at the end of intervention (8 wk). The V4 region of the 16S rRNA gene (515F-806R) was amplified by using the Earth Microbiome Project barcoded primer set. PCR conditions and library preparation were as described previously (26, 27). Sequencing was carried out by using the Illumina MiSeq platform (Argonne Core Sequencing Facility).

Sequence data processing and analyses were performed with scripts from the Quantitative Insights into Microbial Ecology (QIIME) software package, version 1.5.0 (28), by using default parameters. Raw sequence files were quality filtered and demultiplexed with the split_libraries_fastq.py script in QIIME, with default settings (28). After demultiplexing, 1,615,683 sequences remained. The pick subsampled reference otus, through the otu table.py script, was used to generate 97% operational taxonomic unit (OTU) clusters (open reference OTU picking), an OTU table (singletons removed), a representative sequence file (based on cluster centroids), an alignment of the representative sequences, and a phylogenetic tree based on the alignment. Sequence alignments were carried out by using PyNAST (28). The above OTU picking workflow has been renamed pick_open_reference_otus.py in the latest version of QIIME (version 1.8.0). The February 4, 2011, release of Greengenes was used as the reference database for OTU picking (29). In the final OTU table, there were 22,019 nonsingleton OTUs, and the number of sequences per sample varied from 3743 to 55,750 (median of 16,018, excluding 3 samples that failed to sequence properly). Therefore, all samples were rarified to 3740 sequences per sample before downstream analyses. Statistical tests were run by using the otu_category_significance.py (ANOVA) and compare_categories.py (ADONIS, ANOSIM, and MRPP) scripts in QIIME, as previously reported (27). Weighted and unweighted UniFrac (30) distance matrices were used for constructing principal coordinate analysis plots.

Determination of anthropometric measurements and body composition

All measurements were performed by the same operator following standard procedures.

Height of subjects was measured during the selection phase to the nearest 0.5 cm with a stadiometer (Model 213; Seca). Weight was measured, after voiding, with subjects wearing light clothing to the nearest 0.1 kg on a digital scale (Model 703; Seca).

Waist circumference was measured on undressed subjects at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor.

Body composition was determined by conventional bioelectrical impedance analysis with a single-frequency 50-kHz bioelectrical impedance analyzer (BIA 101 RJL; Akern Bioresearch) in the postabsorptive state, at an ambient temperature of 22–24°C, after voiding and after being in the supine position for 20 min.

Body composition was calculated from bioelectrical measurements and anthropometric data by applying the software provided by the manufacturer by using validated predictive equations for total body water, fat mass, and fat-free mass.

Statistical analysis

The sample size needed to detect an effect of WG treatment on primary outcome (FA bioavailability) and secondary outcome (metabolic and inflammatory markers) was defined on the basis of previous studies. From post hoc analysis of data collected by Costabile et al. (9), we calculated that 25 participants in each treatment group would give sufficient power (α error of 0.05, 80% power, and 2-sided testing) to detect a 50% change in plasma FA. In addition, considering an α error of 0.05, a power of 0.80, and 2-sided testing, we estimated that a sample size of 28 participants would be adequate to detect a 10% change in fasting total cholesterol by using variation in accordance with other studies (31–33) and to detect a 30% change of circulating IL-6 by using variation in accordance with Martínez et al. (12). We calculated that 30 participants would be adequate to detect a 15% change in fasting TNF-α by using variation in accordance with Katcher et al. (32) and Price et al. (34). The participant number was increased to 40 per group because of possible dropouts.

All values are reported as means ± SEMs. Kolmogorov-Smirnov and Shapiro tests were used to evaluate the normality of distribution of all monitored variables, and logarithmic transformation was applied to nonnormally distributed data. Differences of variables between baseline and over time within and between interventions were tested by 2-factor ANOVA with repeated measures on one factor in combination with Tukey post hoc tests; $P < 0.05$ was considered statistically significant. Pearson correlation coefficients were calculated to assess bivariate associations between data sets ($P < 0.05$ was considered significant).

Statistical analyses were performed by using Statistical Package for Social Sciences (version 16.0; SPSS Inc.). The microbiota composition and the relative statistical associations were determined by using specific scripts from the QIIME software (28), as described above.

RESULTS

Compliance with the treatment

The study recruitment and follow-up started in January 2011 and March 2011, respectively, and the study was completed in May 2013. No adverse events were identified in WG and control groups over the study period. Twelve subjects (4 from the WG and 8 from the control groups) dropped out of the study during the second and third weeks for personal reasons unrelated to the intervention. The reasons included the need for taking antibiotics for 3 subjects and particular personal and familial events that voluntarily and involuntarily constricted volunteers to change their dietary habits and behavior, such as change/loss of job for 4 subjects, mourning for 2 subjects, and health conditions of parents/son for 3 subjects. Sixty-eight subjects (36 in the WG group and 32 in the control group) completed the study and were included in the analyses (**Figure 2**). Their general characteristics are reported in **Table 2**.

The analysis of food diaries and the weight of foods returned by subjects over the study period showed a good compliance of subjects to the treatments (**Supplemental Table 1**). No significant difference between groups in energy intake and macronutrient composition of diets over time was found (**Table 3**). WG wheat consumption resulted in a significant increase in total dietary fiber in WG subjects at 4 and 8 wk compared with baseline and with control subjects. Over the study period, WG subjects consumed 61 ± 1.5 g/d (~2.5 biscuits) of WG product (out of the assigned 70 g, 3 biscuits). This WG wheat provided ~7.1 g/d of cereal dietary fiber, which well matched the increased intake of total dietary fiber in this group. No differences in dietary fiber from any other source except WG or refined wheat products were found over the study period (**Figure 3**).

Anthropometric data, body composition, glycemia, and plasma lipids

No significant variations in anthropometric data, body composition, plasma lipids, and glycemia were found over the study period within and between groups (**Supplemental Table 2**).

Phenolic acids in serum, urine, and feces

Supplemental Table 3 reports concentrations of phenolic acids retrieved in biological samples monitored over the study period. As expected, a greater number of phenolic acid compounds were detected in urine and feces than in serum samples (13 and 14 compared with 6, respectively). No significant difference was found among baseline concentrations of single and total phenolic acids in biological samples from WG and control subjects. As expected, no significant variation over the study period for any of the monitored compounds was found in control subjects. On the contrary, WG consumption resulted in a significant 4.2- and 5-fold increase in serum dihydroferulic acid (DHFA) concentration, as well as a 1.3- and 0.8-fold increase in

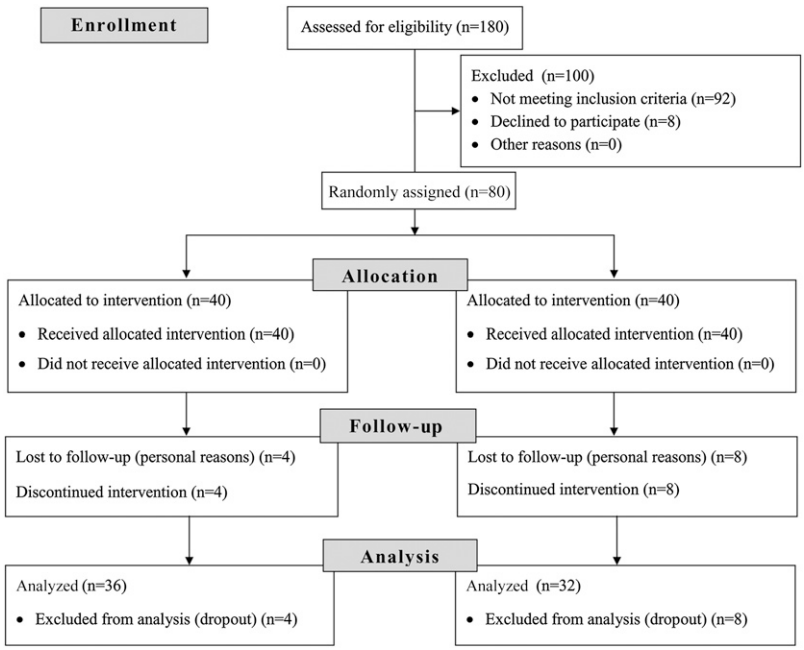

FIGURE 2 Participant flow over the study period.

TABLE 2
General characteristics of participants at baseline[1]

	WG (n = 36)		CTR (n = 32)	
Characteristic	Mean ± SEM	Range	Mean ± SEM	Range
Subjects, n	36		32	
Sex, M/F, n	11/25		12/20	
Age, y	40 ± 2	19–67	37 ± 2	21–62
BMI, kg/m^2	30.0 ± 0.5	25.0–34.9	29.5 ± 0.4	25.6–34.9
Total cholesterol, mg/dL	176.8 ± 5.6	116–195	179.7 ± 4.8	112–190
HDL cholesterol, mg/dL	49.5 ± 2.4	24–79	48.9 ± 1.9	32–74
Triglycerides, mg/dL	95.2 ± 8.2	43–145	87.6 ± 5.9	51–144
Glycemia, mg/dL	93.9 ± 2.1	56–121	95.9 ± 1.7	72–114
Waist circumference, cm	100.0 ± 1.9	76–119	98.6 ± 2.2	75–125
Hip circumference, cm	110.5 ± 1.0	99–125	108.5 ± 1.1	96–126
Fat-free mass, %	63.2 ± 1.2	54–75	61.3 ± 2.8	28.3–73
Fat mass, %	36.8 ± 1.2	25–46	33.3 ± 1.6	18.8–43
Total PA, MET min/wk	287.5 ± 17.3	220–357	317.5 ± 15.0	260–375

[1]There were no statistical differences between the groups at baseline. CTR, control group; MET, metabolic equivalent; PA, physical activity; WG, whole-grain group.

fecal FA concentration, after 4 and 8 wk, respectively, and a 0.8-fold increase in FA urinary excretion after 8 wk compared with baseline within and between groups (**Figure 4**). In the WG group, a trend of increased urinary DHFA after 4 wk (P = 0.08) and 8 wk (P = 0.09) compared with baseline and also with the control group (P = 0.06) after 8 wk was observed.

In WG subjects (but not in control subjects), FA serum concentrations at 4 and 8 wk significantly correlated with serum DHFA (Pearson; r = 0.734, $P < 0.001$, n = 36 and r = 0.684, $P < 0.001$, n = 36), whereas urinary FA correlated with fecal (r = 0.331, P = 0.004, n = 36 and r = 0.431, P = 0.002, n = 36) and urinary (r = 0.231, P = 0.002, n = 36 and r = 0.411, P = 0.001, n = 36) DHFA; interestingly, in subjects experiencing increased urinary FA, a positive variation of fecal FA also was found (r = 0.618, P = 0.032, n = 29) after an 8-wk intervention.

Metabolic disease and inflammatory markers in plasma

No difference at baseline and no variation over the study period were found for diabetes and obesity markers within and between groups (**Supplemental Table 4**). Plasma concentrations of inflammatory status markers (**Table 4**) were similar at baseline for both groups. However, significant modifications over the study period were found between groups and within the WG group. In

TABLE 3
Energy intake and macronutrient composition of individual diets over the study period[1]

	WG (n = 36)			CTR (n = 32)		
Characteristic	0 wk	4 wk	8 wk	0 wk	4 wk	8 wk
Energy, kcal	1600.4 ± 95.4	1622.3 ± 87.8	1553.7 ± 78.7	1615.6 ± 87.5	1570.7 ± 69.4	1561.5 ± 85.6
Carbohydrates						
Total, g	198.0 ± 13.1	195.2 ± 10.1	189.4 ± 10.0	186.9 ± 11.3	183.2 ± 10.2	181.0 ± 11.0
Dietary fiber, g	15.6 ± 1.2	19.2[2] ± 1.0	19.5[2] ± 0.9	14.2 ± 1.0	13.9 ± 0.9	12.4 ± 0.9
% Energy	47.5 ± 1.0	46.0 ± 1.3	46.2 ± 1.1	45.0 ± 1.4	44.7 ± 1.4	44.9 ± 1.2
Proteins						
g	66.3 ± 3.9	71.0 ± 3.7	70.2 ± 3.2	69.5 ± 5.0	69.4 ± 3.6	69.1 ± 4.2
% Energy	16.8 ± 0.4	17.8 ± 0.5	18.5 ± 0.5	17.0 ± 0.6	17.9 ± 0.7	17.7 ± 0.5
Fats						
Total, g	60.0 ± 3.7	62.7 ± 4.4	58.4 ± 3.7	65.3 ± 4.1	61.1 ± 3.4	61.9 ± 4.1
Saturated, g	22.8 ± 2.5	25.7 ± 3.3	24.2 ± 3.0	23.1 ± 3.2	21.6 ± 2.3	22.5 ± 2.4
Monounsaturated, g	25.7 ± 1.7	25.9 ± 1.8	23.7 ± 1.7	28.9 ± 1.9	26.2 ± 1.5	26.3 ± 1.9
Polyunsaturated, g	12.3 ± 1.6	10.2 ± 1.1	9.7 ± 1.2	12.5 ± 1.8	10.7 ± 1.1	9.8 ± 1.1
Total, % energy	34.0 ± 0.9	34.4 ± 0.9	33.5 ± 0.8	36.2 ± 1.2	35.1 ± 1.1	35.8 ± 1.2
Alcohol						
g	4.7 ± 1.8	5.0 ± 1.4	4.7 ± 1.7	4.1 ± 1.2	5.2 ± 1.8	3.7 ± 1.5
% Energy	1.7 ± 0.6	1.8 ± 0.5	1.8 ± 0.6	1.8 ± 0.6	2.3 ± 0.9	1.6 ± 0.7

[1]All values are means ± SEMs. No significant differences were present between the groups at baseline. CTR, control group; WG, whole-grain group.

[2]From ANOVA and Tukey post hoc test: $P < 0.05$ for the difference between a given week and baseline values within treatments and for the difference between treatments at a given week.

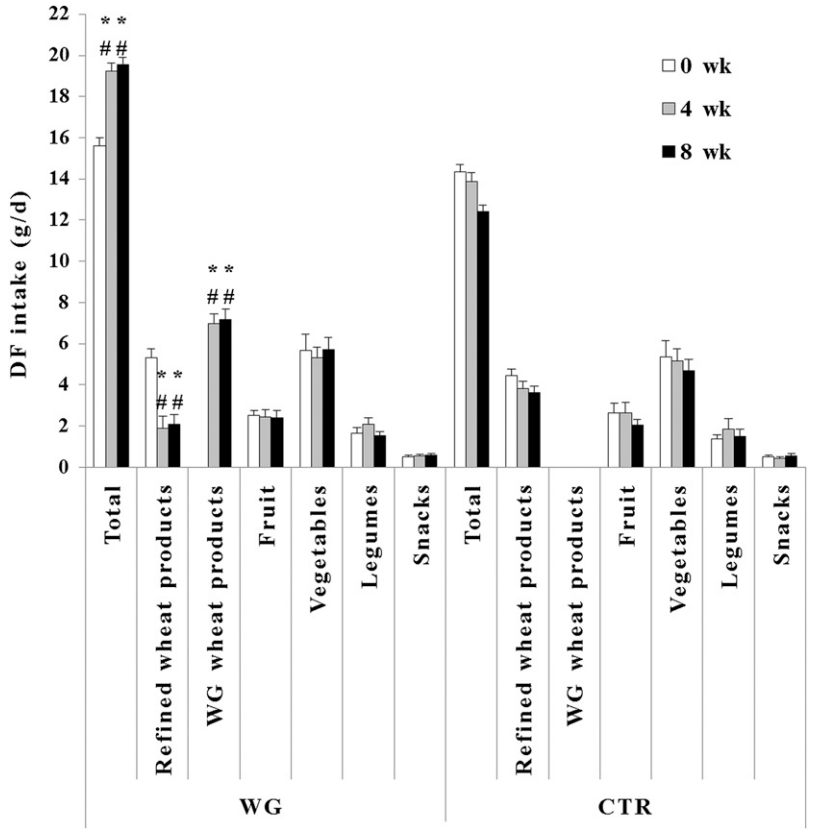

FIGURE 3 Mean (±SEM) daily intakes of DF, total and from each dietary source, over the study period in WG ($n = 36$) and CTR ($n = 32$) groups. From ANOVA and Tukey post hoc test: $^{*}P < 0.05$ for the difference between a given week and baseline values within treatments; $^{\#}P < 0.05$ for the difference between treatments at a given week. CTR, control; DF, dietary fiber; WG, whole grain.

the WG group, there was a significant reduction in inflammatory TNF-α after 8 wk compared with baseline and the control group, as well as a significant increase in anti-inflammatory IL-10 after 4 wk compared with baseline and the control group but not compared with 8-wk data in either group. Moreover, a trend of reduction in IL-6 at 8 wk compared with 4 wk in the WG group compared with the control group was found ($P = 0.06$).

The urinary excretion of FA and DHFA over WG treatment (after 4 and 8 wk of intervention) tended to be negatively correlated with plasma PAI-1 concentrations (Pearson; $r = -0.264$, $P = 0.075$, $n = 36$ and $r = -0.341$, $P = 0.061$, $n = 36$ for FA; $r = -0.271$, $P = 0.071$, $n = 36$ and $r = -0.302$, $P = 0.059$, $n = 36$ for DHFA).

Microbiota composition: effect of dietary treatments and impact on circulating and excreted FA and inflammatory/metabolic markers

Microbial community data were analyzed by comparing OTU composition between subjects, with treatment group, age, and sex as independent variables. Data showed that fecal microbial community structure was significantly different between men and women ($P < 0.05$), whereas no significant variation was found in relation to dietary treatments or age. Weighted and unweighted UniFrac phylogenetic metrics (measures of overall community composition) clearly showed that the microbial community structure of WG and control subjects was not significantly different; in fact, the different categories of individuals did not form discrete clusters in the principal coordinate analysis plot, suggesting the overall microbiota to be similar (**Figure 5**). In addition, no difference was observed between WG subjects at time zero and after the treatment (Figure 5). However, individual bacterial taxa showed significant variation in relative abundance in relation to diet and sex. Specifically, *Prevotella* significantly increased from 1.8% to 3.5%, whereas other taxa were significantly reduced in WG subjects ($P < 0.05$), including *Dialister* (from 2.5% to 0.6%), *Bifidobacterium* (from 6.6% to 5.3%), *Blautia* (from 9.7% to 6.7%), and *Collinsella* (from 1.8% to 0.9%).

Pearson correlations were used to evaluate the potential interplay between baseline microbiota composition and circulating FA, as well as that between fecal FA and microbiota over WG treatment. Results showed that in WG subjects, a lower baseline relative abundance of Bifidobacteriales (Actinobacteria) of 5.0% ($r = -0.74$, $P = 0.014$, $n = 34$) and Bacteroidetes of 9.6% ($r = -0.66$, $P = 0.02$, $n = 29$) was associated with an increased release of FA in the gut and urinary excretion, respectively. After the WG treatment, fecal FA was associated with an increase in the relative abundances of Bacteroidetes from 9.6% to 14.5% ($r = 0.76$, $P = 0.01$, $n = 34$) and Firmicutes from 75.3% to 79.7% ($r = 0.64$, $P = 0.04$, $n = 34$), whereas a reduction of *Clostridium* from 3.1% to 1.6% ($r = -0.72$, $P = 0.02$, $n = 34$) was registered.

No significant correlation was found between any OTU at baseline and specific variation of any metabolic or inflammatory marker in both treatment groups. Interestingly, the reduction of TNF-α after 8 wk of WG consumption correlated with an

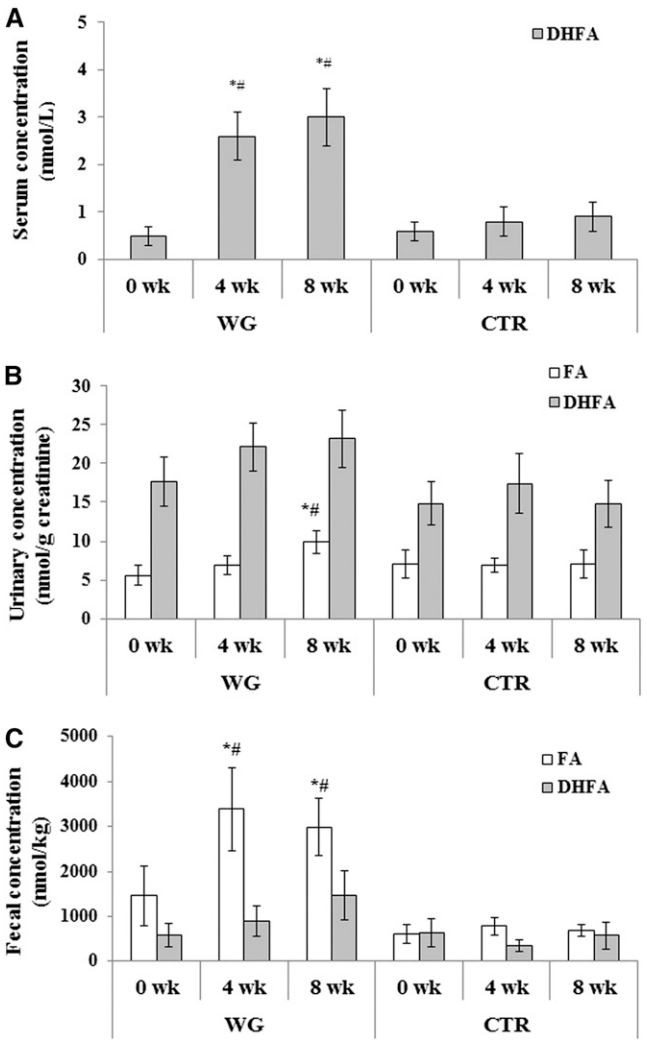

FIGURE 4 Mean (±SEM) concentrations of FA and DHFA in serum (A), urine (B), and feces (C) over the study period in the WG (n = 36) and CTR (n = 32) groups. From ANOVA showing a significant ($P < 0.05$) treatment × time interaction and Tukey post hoc test on serum DHFA and urinary and fecal FA concentrations: $^{*}P < 0.05$ for the difference between a given week and baseline values within treatments; $^{\#}P < 0.05$ for the difference between treatments at a given week. CTR, control; DHFA, dihydroferulic acid; FA, ferulic acid; WG, whole grain.

increased abundance of fecal *Bacteroides* from 9.9% to 14.7% ($r = -0.637$, $P = 0.002$, $n = 31$) and *Lactobacillus* from 0.03% to 0.12% ($r = -0.572$, $P = 0.021$, $n = 31$).

DISCUSSION

In this study, the phenolic profile of serum, urine, and feces on WG wheat consumption; their effect on metabolic and inflammatory parameters; and the correlations with changes in the fecal microbiota were assessed. Overweight/obese subjects with suboptimal lifestyle factors (such as limited fruit and vegetable intake and low physical activity) were considered in this study because they were suitable subjects to verify *1*) the distribution of WG polyphenols among the main biological fluids by reducing the interference of other major dietary sources of polyphenols (35), *2*) the hypothesis that WG polyphenols might prevent the development of some pathophysiologic pathways that are possibly unbalanced in these subjects (although they were still healthy) (16), and *3*) the interplay among circulating and excreted WG polyphenols, gut microbial community composition, and health benefits possibly induced by WG wheat consumption in a population at risk for developing chronic diseases (14, 36–40).

Biochemical data showed that among 15 phenolic acids monitored in serum, urine, and feces, an 8-wk consumption of WG resulted in a significant increase in urinary and fecal FA and serum DHFA concentrations. The observation that FA concentration can increase in the blood on WG wheat consumption was conceptually in agreement with a previous study conducted in healthy normal-weight subjects (9), whereas in a recent study in overweight healthy subjects, a 4 wk-consumption of bread and cereals enriched with an aleurone fraction failed to increase serum FA (34). Interestingly, in the present study, WG consumption also significantly increased serum DHFA concentration, which is positively correlated with serum FA, whereas excreted DHFA correlated with urinary FA. DHFA is a well-known microbial metabolite derived from FA and chlorogenic acid, absorbable through the colon and retrievable in serum and urine (14, 41–46). In this study, WG wheat represented the unique dietary source of FA (~97 mg/d), differentiating the WG from the control group, and therefore these findings indicated that FA can be absorbed from WG wheat, is released in the gut, and is mainly converted to DHFA by microbiota.

Moreover, the study of gut microbial communities showed that FA was mostly retrieved in the blood and excreted in urine in subjects harboring a low relative abundance of Bacteroidetes (phylum) and Bifidobacteriales (order) at baseline. After 8 wk, these subjects experienced an increase of Bacteroidetes and total Firmicutes, although within Firmicutes, a reduction of *Clostridium* relative abundance took place.

Previous in vitro studies showed that the release of FA in the colon might be associated with wheat bran polysaccharide fermentation and sustained by the action of bacterial extracellular xylanase and FA esterase (47, 48). These enzymes are mainly synthetized by bacterial species belonging to the genera *Lactobacillus* and *Roseburia* (Firmicutes), *Bifidobacterium* (Actinobacteria), and *Bacteroides* and *Prevotella* (Bacteroidetes) in the presence of arabinoxylans with esterified FA (49–53). Thus, it can be hypothesized that in overweight/obese subjects who showed a low abundance of Bacteroidetes and Bifidobacteriales, Firmicutes were mainly responsible for the fermentation of WG polysaccharides and the released FA once WG wheat was introduced in the diets.

The contemporary observation of an increase in the relative abundance of *Prevotella* and a significant positive correlation between fecal FA and the abundance of the whole Bacteroidetes (although not *Prevotella* alone) in WG subjects suggests that *Bacteroides* may also have a role in intestinal release of WG FA. These findings are in agreement with Lappi and coworkers (13), who found a trend toward reduced *Bacteroides* and *Prevotella* and increased *Clostridium* in Finnish subjects with metabolic syndrome who replaced rye bread with white wheat bread for 12 wk (13). However, those authors concluded that dietary fats explained *Bacteroides* changes better than did WG, whereas in the present study, fats did not affect the WG-microbiota interplay.

Altogether, these findings suggest that in this study, the intestinal release of WG FA might be activated by Firmicutes and sustained over time with the contribution of Bacteroidetes.

TABLE 4
Plasma concentrations of inflammatory status markers over the study period[1]

	WG (pg/mL) (n = 36)			CTR (pg/mL) (n = 32)			WG compared with CTR, P[2]		
	0 wk	4 wk	8 wk	0 wk	4 wk	8 wk	Δ_{4-0}	Δ_{8-0}	Δ_{8-4}
IL-6	57.5 ± 7.5	69.5 ± 11.2	46.9 ± 4.0	65.5 ± 11.4	56.3 ± 7.5	60.2 ± 7.2	—	—	—
IL-10	26.9 ± 3.0	41.7 ± 2.8[3]	26.8 ± 3.2[4]	28.8 ± 5.1	27.5 ± 4.3	27.9 ± 3.89	0.04	0.29	0.03
TNF-α	341.9 ± 25.5	370.1 ± 30.5	243.0 ± 26.0[3,4]	321.9 ± 52.1	314.9 ± 50.3	329.8 ± 50.6	0.15	0.04	0.20

[1]All values are means ± SEMs. Data were log transformed before analysis. CTR, control group; WG, whole-grain group.
[2]P values for the difference between WG and CTR with respect to the pairwise time point differences (Δ) were calculated when a significant treatment × time interaction was found; no significant differences were present between the groups at baseline.
[3]$P < 0.05$ compared with baseline (ANOVA and Tukey post hoc test).
[4]$P < 0.05$ compared with 4 wk (ANOVA and Tukey post hoc test).

Moreover, the reduction of *Clostridium* in subjects experiencing higher FA release might be attributable to competition with other species or to a direct antimicrobial effect of FA toward clostridia (54).

Data on inflammatory markers have shown a significant reduction of inflammatory TNF-α and a trend toward reduced IL-6 after 8 wk, as well as an increase of the anti-inflammatory IL-10 after 4 wk of WG consumption. Two previous intervention trials demonstrated the ability of WG consumption to ameliorate subclinical inflammation (12, 32), whereas many others studies failed to find such a positive association (31, 33, 55, 56). In the study by Katcher and coworkers (32), the reduction of inflammation followed the inclusion of WG in hypocaloric and healthy diets, and in the study by Martínez and coworkers (12), the nutritional composition of diets was not controlled.

The strong point of the present study is that the amelioration of individual inflammatory status was found in the context of a controlled energy and nutritionally balanced replacement of refined wheat with WG wheat, and it was not accompanied by any modification of body weight.

Moreover, the correlation between a reduced TNF-α and an increased abundance of *Bacteroides* (as observed in subjects with a higher bioaccessibility of FA) and *Lactobacillus* (known for releasing feruloyl-esterase activity in the gut as discussed above) provided a further potential link between the increase of serum FA and the amelioration of inflammation in our subjects. In addition, the trend toward an inverse correlation found between urinary FA and DHFA with PAI-1 concentration suggested a role for WG FA and its gut metabolite in triggering mechanisms that may result in a reduced risk of cardiovascular disease, diabetes, and others pathologies associated with obesity and low-grade inflammatory status (57, 58).

PAI-1 is a well-known biomarker of cardiovascular disease risk, metabolic syndrome (59), nonalcoholic fatty liver disease (60), and cancers (61). An inverse association between WG consumption and PAI-1 was found in a recent observational study (62), but previous intervention studies failed to find a significant effect of WG consumption on this marker (31, 63). The trend found in this study might suggest that WG's effects on metabolic diseases may be better observed in subjects who have the ability of releasing and metabolizing the FA bound to dietary fiber; this may explain the conflicting evidence found thus far. According to this hypothesis, the benefits of WG wheat polyphenols are mediated by the metabolic activity of the gut microbiota, as already observed for soybean- or ellagitannin-rich foods, whose health benefits are linked to the ability of individual bacterial taxa to convert genistein into equol and ellagitannins/ellagic acid into urolithins, respectively (64, 65). On the other hand, it cannot be excluded that other bioactive components in WG such as resistant starch, betaine, and some minerals might have contributed together with FA (directly or through their microbiota metabolites but in the absence of a prebiotic effect) to ameliorate inflammation (7).

In conclusion, in this study, it was demonstrated for the first time that WG wheat FA is released and absorbed in the gut and is likely metabolized by gut microbiota, and DHFA is the most

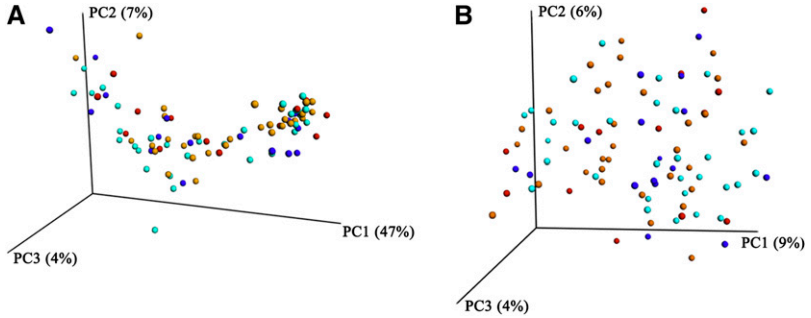

FIGURE 5 PC analysis of weighted (A) and unweighted (B) UniFrac distances for 16S ribosomal RNA gene sequence data from whole-grain subjects before (orange dots) and after (green dots) 8 wk of intervention, as well as control subjects before (red dots) and after (blue dots) 8 wk of intervention. PC, principal coordinates.

abundant circulating metabolite in overweight/obese subjects. Even though WG wheat did not cause significant modification of microbial community composition or structure, there were significant relationships between FA release in the gut and relative abundance of Firmicutes at baseline and Bacteroidetes following WG consumption. The increased abundance of these bacteria together with *Lactobacillus* was associated with the ameliorated inflammatory status of subjects receiving WG treatment, which may suggest that WG FA may play a role in reducing the risk of pathologies associated with subclinical inflammation. This was also supported by evidence that a greater excretion of FA and DHFA in urine, reflecting a better release, metabolism, and absorption of the compounds, was associated with a trend toward lower PAI-1 plasma concentrations.

Because no specific correction was made for multiple comparisons, possibly leading to some false-positive findings, some results of the study should be cautiously taken into account, and a more detailed study may be warranted. The application of a completer analysis instead of an intention-to-treat analysis of data might also be seen as a study limitation. However, it was preferred because dropouts in both groups left the study for personal reasons within the first 3 wk, no data were available after baseline, and the power of the study was unaffected by the exclusion from analysis of those few dropouts (66).

In addition, in this study, unblinded participants might have led to possible biases in psychological response and compliance to the dietary interventions, whereas the blinded outcome assessors guaranteed unbiased interaction with participants and data collection.

However, from the viewpoint of public health and optimal personalized nutrition, it can be concluded that in subjects at high risk of developing chronic diseases (because of obesity and unhealthy lifestyle), the modification of dietary habits alone, through an isocaloric dietary replacement of refined wheat products with 70 g WG wheat, can boost a positive immune response, thereby possibly reducing the risk of developing obesity-related diseases over the long term.

The authors' responsibilities were as follows—PV and VF: designed the research and had primary responsibility for the final content; PV, IM, AAR, RG, and MAG: conducted the experiments and collected data; FT and SJ: provided whole grain; PV and RF: analyzed bioavailability data; PV and IM: analyzed data of inflammatory and metabolic disease markers; IM and LS: analyzed anthropometric data; ALS, DE, SMG, JAG, and PV: performed microbiological analyses and analyzed the data; PV: wrote the manuscript; and all authors: read and approved the final manuscript. All authors declared no conflicts of interest.

REFERENCES

1. Slavin J, Tucker M, Harriman C, Jonnalagadda SS. Whole grains: definition, dietary recommendations, and health benefits. Cereal Foods World 2013;58:191–8.
2. Jonnalagadda SS, Harnack L, Liu RH, McKeown N, Seal C, Liu S, Fahey GC. Putting the whole grain puzzle together: health benefits associated with whole grains—summary of American Society for Nutrition 2010 Satellite Symposium. J Nutr 2011;141:1011S–22S.
3. Cho SS, Qi L, Fahey GC Jr, Klurfeld DM. Consumption of cereal fiber, mixtures of whole grains and bran, and whole grains and risk reduction in type 2 diabetes, obesity, and cardiovascular disease. Am J Clin Nutr 2013;98:594–619.
4. Lefevre M, Jonnalagadda S. Effect of whole grains on markers of subclinical inflammation. Nutr Rev 2012;70:387–96.
5. Ye EQ, Chacko SA, Chou EL, Kugizaki M, Liu S. Greater whole-grain intake is associated with lower risk of type 2 diabetes, cardiovascular disease, and weight gain. J Nutr 2012;142:1304–13.
6. Pol K, Christensen R, Bartels EM, Raben A, Tetens I, Kristensen M. Whole grain and body weight changes in apparently healthy adults: a systematic review and meta-analysis of randomized controlled studies. Am J Clin Nutr 2013;98:872–84.
7. Fardet A. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutr Res Rev 2010;23:65–134.
8. Belobrajdic DP, Bird AR. The potential role of phytochemicals in wholegrain cereals for the prevention of type-2 diabetes. Nutr J 2013;12:62.
9. Costabile A, Klinder A, Fava F, Napolitano A, Fogliano V, Leonard C, Gibson GR, Tuohy KM. Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. Br J Nutr 2008;99:110–20.
10. Carvalho-Wells AL, Helmolz K, Nodet C, Molzer C, Leonard C, McKevith B, Thielecke F, Jackson KG, Tuohy KM. Determination of the in vivo prebiotic potential of a maize-based whole grain breakfast cereal: a human feeding study. Br J Nutr 2010;104:1353–6.
11. Christensen EG, Licht TR, Kristensen M, Bahl MI. Bifidogenic effect of whole-grain wheat during a 12-week energy-restricted dietary intervention in postmenopausal women. Eur J Clin Nutr 2013;67:1316–21.
12. Martínez I, Lattimer JM, Hubach KL, Case JA, Yang J, Weber CG, Louk JA, Rose DJ, Kyureghian G, Peterson DA, et al. Gut microbiome composition is linked to whole grain-induced immunological improvements. ISME J 2013;7:269–80.
13. Lappi J, Salojärvi J, Kolehmainen M, Mykkänen H, Poutanen K, de Vos WM, Salonen A. Intake of whole-grain and fiber-rich rye bread versus refined wheat bread does not differentiate intestinal microbiota composition in Finnish adults with metabolic syndrome. J Nutr 2013;143:648–55.
14. Tuohy KM, Conterno L, Gasperotti M, Viola R. Up-regulating the human intestinal microbiome using whole plant foods, polyphenols, and/or fiber. J Agric Food Chem 2012;60:8776–82.
15. Walter J, Martínez I, Rose DJ. Holobiont nutrition: considering the role of the gastrointestinal microbiota in the health benefits of whole grains. Gut Microbes 2013;4:340–6.
16. Vitaglione P, Napolitano A, Fogliano V. Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. Trends Food Sci Technol 2008;19:451–63.
17. Adom KK, Sorrells ME, Liu RH. Phytochemical profiles and antioxidant activity of wheat varieties. J Agric Food Chem 2003;51:7825–34.
18. Andreasen MF, Christensen LP, Meyer AS, Hansen A. Content of phenolic acids and ferulic acid dehydrodimers in 17 rye (*Secale cereale* L.) varieties. J Agric Food Chem 2000;48:2837–42.
19. Garcia-Conesa MT, Plumb GW, Waldron KW, Ralph J, Williamson G. Ferulic acid dehydrodimers from wheat bran: isolation, purification and antioxidant properties of 8-O-4-diferulic acid. Redox Rep 1997;3:319–23.
20. Sun RC, Sun XF, Zhang SH. Quantitative determination of hydroxycinnamic acids in wheat, rice, rye, and barley straws, maize stems, oil palm frond fiber, and fast-growing poplar wood. J Agric Food Chem 2001;49:5122–9.
21. Adom KK, Sorrells ME, Liu RH. Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. J Agric Food Chem 2005;53:2297–306.
22. Mattila P, Pihlava JM, Hellström J. Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. J Agric Food Chem 2005;53:8290–5.
23. Klepacka J, Fornal Ł. Ferulic acid and its position among the phenolic compounds of wheat. Crit Rev Food Sci Nutr 2006;46:639–47.
24. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003;35:1381–95.
25. Vitaglione P, Barone Lumaga R, Ferracane R, Sellitto S, Morelló JR, Reguant Miranda J, Shimoni E, Fogliano V. Human bioavailability of flavanols and phenolic acids from cocoa-nut creams enriched with free or microencapsulated cocoa polyphenols. Br J Nutr 2013;109:1832–43.
26. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 2012;6:1621–4.

27. Piombino P, Genovese A, Esposito S, Moio L, Cutolo PP, Chambery A, Severino V, Moneta E, Smith DP, Owens SM, et al. Saliva from obese individuals suppress the release of aroma compounds from wine. PLoS ONE 2014;9:e85611.
28. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7:335–6.
29. McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archea. ISME J 2012;6:610–8.
30. Lozupone C, Lladser ME, Knights D, Jesse Stombaugh J, Knight R. UniFrac: an effective distance metric for microbial community comparison. ISME J 2011;5:169–72.
31. Andersson A, Tengblad S, Karlstrom B, Kamal-Eldin A, Landberg R, Basu S, Aman P, Vessby B. Whole-grain foods do not affect insulin sensitivity or markers of lipid peroxidation and inflammation in healthy, moderately overweight subjects. J Nutr 2007;137:1401–7.
32. Katcher HI, Legro RS, Kunselman AR, Gillies PJ, Demers LM, Bagshaw DM, Kris-Etherton PM. The effects of a whole grain-enriched hypocaloric diet on cardiovascular disease risk factors in men and women with metabolic syndrome. Am J Clin Nutr 2008;87:79–90.
33. Giacco R, Clemente G, Cipriano D, Luongo D, Viscovo D, Patti L, Di Marino L, Giacco A, Naviglio D, Bianchi MA, et al. Effects of the regular consumption of whole meal wheat foods on cardiovascular risk factors in healthy people. Nutr Metab Cardiovasc Dis 2010;20:186–94.
34. Price RK, Wallace JM, Hamill LL, Keaveney EM, Strain JJ, Parker MJ, Welch RW. Evaluation of the effect of wheat aleurone-rich foods on markers of antioxidant status, inflammation and endothelial function in apparently healthy men and women. Br J Nutr 2012;108:1644–51.
35. Arranz S, Silván JM, Saura-Calixto F. Nonextractable polyphenols, usually ignored, are the major part of dietary polyphenols: a study on the Spanish diet. Mol Nutr Food Res 2010;54:1646–58.
36. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, et al. Heart disease and stroke statistics—2013 update: a report from the American Heart Association. Circulation 2013;127:143–52.
37. Del Rio D, Costa LG, Lean ME, Crozier A. Polyphenols and health: what compounds are involved? Nutr Metab Cardiovasc Dis 2010;20:1–6.
38. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA 2010;107:14691–6.
39. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011;334:105–8.
40. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, et al. Human gut microbiome viewed across age and geography. Nature 2012;486:222–7.
41. Williamson G, Clifford MN. Colonic metabolites of berry polyphenols: the missing link to biological activity? Br J Nutr 2010;104(Suppl 3): S48–66.
42. Poquet L, Clifford MN, Williamson G. Transport and metabolism of ferulic acid through the colonic epithelium. Drug Metab Dispos 2008; 36:190–7.
43. Stalmach A, Mullen W, Barron D, Uchida K, Yokota T, Cavin C, Steiling H, Williamson G, Crozier A. Metabolite profiling of methyl, glucuronyl and sulfate conjugates in plasma and urine derived from chlorogenic acids following the ingestion of coffee by humans: identification of biomarkers of coffee consumption. Drug Metab Dispos 2009;37:1749–58.
44. Renouf M, Guy PA, Marmet C, Fraering AL, Longet K, Moulin J, Enslen M, Barron D, Dionisi F, Cavin C, et al. Measurement of caffeic and ferulic acid equivalents in plasma after coffee consumption: small intestine and colon are key sites for coffee metabolism. Mol Nutr Food Res 2010;54:760–6.
45. Lang R, Dieminger N, Beusch A, Lee YM, Dunkel A, Suess B, Skurk T, Wahl A, Hauner H, Hofmann T. Bioappearance and pharmacokinetics of bioactives upon coffee consumption. Anal Bioanal Chem 2013;405: 8487–503.
46. Ludwig IA, Paz de Peña M, Concepción C, Alan C. Catabolism of coffee chlorogenic acids by human colonic microbiota. Biofactors 2013;39:623–32.
47. Vardakou M, Nueno Palop C, Gasson M, Narbad A, Christakopoulos P. In vitro three-stage continuous fermentation of wheat arabinoxylan fractions and induction of hydrolase activity by the gut microflora. Int J Biol Macromol 2007;41:584–9.
48. Vardakou M, Palop CN, Christakopoulos P, Faulds CB, Gasson MA, Narbad A. Evaluation of the prebiotic properties of wheat arabinoxylan fractions and induction of hydrolase activity in gut microflora. Int J Food Microbiol 2008;123:166–70.
49. Couteau D, Mc Cartney AL, Gibson GR, Williamson G, Faulds CB. Isolation and characterization of human colonic bacteria able to hydrolase chlorogenic acid. J Appl Microbiol 2001;90:873–81.
50. Hayashi H, Abe T, Sakamoto M, Ohara H, Ikemura T, Sakka K, Benno Y. Direct cloning of genes encoding novel xylanases from the human gut. Can J Microbiol 2005;51:251–9.
51. Chassard C, Goumy V, Leclerc M, Del'homme C, Bernalier-Donadille A. Characterization of the xylan-degrading microbial community from human faeces. FEMS Microbiol Ecol 2007;61:121–31.
52. Dodd D, Mackie RI, Cann IK. Xylan degradation, a metabolic property shared by rumen and human colonic Bacteroidetes. Mol Microbiol 2011;79:292–304.
53. Flint HJ, Scott K, Duncan S, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. Gut Microbes 2012;3: 289–306.
54. Borges A, Ferreira C, Saavedra MJ, Simões M. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. Microb Drug Resist 2013;19:256–65.
55. Brownlee IA, Moore C, Chatfield M, Richardson DP, Ashby P, Kuznesof SA, Jebb SA, Seal CJ. Markers of cardiovascular risk are not changed by increased whole-grain intake: the WHOLEheart study, a randomised, controlled dietary intervention. Br J Nutr 2010; 104:125–34.
56. Tighe P, Duthie G, Vaughan N, Brittenden J, Simpson WG, Duthie S, Mutch W, Wahle K, Horgan G, Thies F. Effect of increased consumption of whole-grain foods on blood pressure and other cardiovascular risk markers in healthy middle-aged persons: a randomized controlled trial. Am J Clin Nutr 2010;92:733–40.
57. Lillioja S, Neal AL, Tapsell L, Jacobs DR Jr. Whole grains, type 2 diabetes, coronary heart disease, and hypertension: links to the aleurone preferred over indigestible fiber. Biofactors 2013;39:242–58.
58. Festa A, D'Agostino R Jr, Tracy RP, Haffner SM. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the Insulin Resistance Atherosclerosis Study. Diabetes 2002;51:1131–7.
59. Kostapanos MS, Florentin M, Elisaf MS, Mikhailidis DP. Hemostatic factors and the metabolic syndrome. Curr Vasc Pharmacol 2013;11: 880–905.
60. Verrijken A, Francque S, Mertens I, Prawitt J, Caron S, Hubens G, Van Marck E, Staels B, Michielsen P, Van Gaal L. Prothrombotic factors in histologically proven nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology 2014;59:121–9.
61. Iacoviello L, Agnoli C, De Curtis A, di Castelnuovo A, Giurdanella MC, Krogh V, Mattiello A, Matullo G, Sacerdote C, Tumino R, et al. Type 1 plasminogen activator inhibitor as a common risk factor for cancer and ischaemic vascular disease: the EPICOR study. BMJ Open 2013;3:e003725.
62. Masters RC, Liese AD, Haffner SM, Wagenknecht LE, Hanley AJ. Whole and refined grain intakes are related to inflammatory protein concentrations in human plasma. J Nutr 2010;140:587–94.
63. Turpeinen AM, Juntunen K, Mutanen M, Mykkanen H. Similar responses in hemostatic factors after consumption of wholemeal rye bread and low-fiber wheat bread. Eur J Clin Nutr 2000;54:418–23.
64. Wiseman H. The therapeutic potential of phytoestrogens. Expert Opin Investig Drugs 2000;9:1829–40.
65. Cerdá B, Tomás-Barberán FA, Espín JC. Metabolism of antioxidant and chemopreventive ellagitannins from strawberries, raspberries, walnuts, and oak-aged wine in humans: identification of biomarkers and individual variability. J Agric Food Chem 2005;53:227–35.
66. Gupta SK. Intention-to-treat concept: a review. Perspect Clin Res. 2011;2:109–12.