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Glucose- and Lipid-Related Biomarkers Are Affected in Healthy Obese or Hyperglycemic Adults Consuming a Whole-Grain Pasta Enriched in Prebiotics and Probiotics: A 12-Week Randomized Controlled Trial

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ABSTRACT

Background: Synbiotic foods, which combine the action of prebiotics and probiotics along the gastrointestinal tract, can affect inflammatory and glucose-related markers.

Objective: The aim of this study was to investigate the effects on inflammatory and glycemia-related markers of a whole-grain pasta containing barley β-glucans and *Bacillus coagulans* BC30, 6086 in healthy overweight or obese volunteers.

Methods: A single-blind, parallel, randomized, placebo-controlled dietary intervention study was carried out. Forty-one healthy sedentary overweight (body mass index [BMI] 25–29.9 kg/m^2) and obese (BMI $\geq$30) volunteers, aged 30–65 y and low consumers of fruit and vegetables, ate 1 serving/d of whole-grain control (CTR) or innovative (INN) pasta for 12 wk and maintained their habitual diets. Biological samples were collected at baseline and every 4 wk for primary (plasma high-sensitivity C-reactive protein [hs-CRP] and fasting plasma lipid profile) and secondary outcomes (glycemia-related markers, blood pressure, fecal microbiota composition, and body weight). Between (CTR compared with INN) and within (among weeks) group differences were tested for the whole population and for subgroups stratified by baseline values of BMI ($\geq$30) and glycemia ($\geq$100 mg/dL).

Results: INN or CTR pasta consumption had no effect on primary and secondary outcomes over time, except for a significant increase in plasma γ-glutamyltransferase (GGT) after 12 wk of CTR pasta consumption. Comparisons between intervention groups revealed differences only at 12 wk: plasma GGT was higher in the CTR group; plasma hs-CRP, plasma LDL/HDL cholesterol ratio, and *Bifidobacterium* spp. were lower in the INN subgroup of obese volunteers; plasma resistin was lower and *Faecalibacterium prausnitzii* abundance was higher in the INN subgroup of hyperglycemic volunteers.

Conclusions: A daily serving of a synbiotic whole-grain pasta had limited effects on primary and secondary outcomes in the entire group of volunteers but affected glycemia- and lipid-related markers and resistin in a subgroup of healthy obese or hyperglycemic volunteers. This trial was registered at clinicaltrials.gov as NCT02236533. *J Nutr* 2019;149:1714–1723.

Keywords: whole grain, randomized controlled trial, β-glucans, *Bacillus coagulans*, inflammation, obesity, gastrointestinal tract, γ-glutamyltransferase, resistin

Introduction

Epidemiologic studies have shown that the consumption of $\geq$90 g/d of whole-grain cereals is associated with a 19% decrease in the risk of developing coronary heart disease, as well as decreases of 12% for stroke and 22% for cardiovascular disease (CVD) (1). A significant linear inverse relationship has also

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been described between whole-grain intake and the occurrence of type 2 diabetes, with an overall absolute disease reduction of 0.3% for each additional intake of 10 g/d, up to 150 g/d (2). However, those findings have not always been supported by the results of whole-grain-based intervention studies (3). The main reason for the incongruence between population-based studies and whole-grain-based randomized controlled trials (RCTs) may be ascribable to the complexity associated with designing and leading effective RCTs to confirm the healthy protective effect of whole grains. The unfamiliar foods provided to study volunteers and their difficulty in maintaining long-term compliance with the treatment, as well as the variety and different daily doses of grains consumed, may partly explain the different findings among RTCs (4). Furthermore, when a healthy effect is found, it is quite difficult to ascribe it to a particular component of the whole-grain cereal, because of the plethora of bioactive compounds contained in these foods. Among these, phenolic compounds, mainly phenolic acids and alkylresorcinols, seem to contribute to the antioxidant and anti-inflammatory effects of cereals (5, 6). However, it has been shown that almost 95% of phenolic compounds are linked to cereal cell wall polysaccharides (7), indicating a pivotal role of fiber in delivering polyphenols into the colon tract, where they are metabolized and absorbed (6, 8). Fiber also contributes to the health effects of whole grains, since it may delay gastric emptying and the glycemic peak, thereby limiting insulin secretion (9). Among fibers, barley β-glucans are able to form a viscous layer in the small intestine, which delays the absorption of sugars and the uptake of dietary cholesterol (10). These mechanisms were at the basis of the positive opinions of the European Food Safety Authority concerning barley β-glucan consumption and reduction of postprandial glycemic responses (11) and lowering of blood cholesterol (12). The latter effect is elicited by short-chain fatty acid formation in the colon via microbiota-mediated fermentation of dietary polysaccharides with prebiotic compounds (13–15). Whole-grain fiber may also activate or potentiate the probiotic effect of bacteria, creating a synergy that may provide further benefits to the gut (16).

"Synbiotic foods" consist of a combination of probiotic strains and prebiotics in a single preparation (17). Although few studies are available on the health effects of synbiotic foods in humans, their consumption has been shown to have protective effects on CVD by boosting the beneficial activities of the gut microbiota and, in turn, by modulating blood levels of cholesterol, high-density lipoprotein, and triglycerides, as well as by increasing satiety sensation (18). In such foods, spore-forming bacteria can be employed to increase the survival of micro-organisms during technologic processes (i.e., heating, drying, cooking) and passage through the gastrointestinal tract (GIT) (i.e., low gastric pH) (19).

We have developed a synbiotic whole-grain pasta enriched with barley β-glucans and the spore-forming *Bacillus coagulans* GBI-30, 6086 (marketed as GanedenBC[30], hereinafter referred to as BC30) to evaluate whether its daily consumption might reduce some markers of CVD risk in a group of subjects at a certain level of risk. Primary outcomes of the study were inflammatory markers, such as plasma high-sensitivity C-reactive protein (hs-CRP), and the fasting lipid profile. Secondary outcomes were glycemia-related markers, blood pressure, fecal microbiota composition, and body weight.

Methods

Food products

A pasta produced from a durum whole-wheat flour (*Triticum durum* L., cultivar Vendetta) rich in polyphenols and supplemented with barley β-glucans and the probiotic strain BC30 was used as the test product (INN). The technologic and composition characteristics of the 2 pasta products have been described previously (20). In particular, the choice of the spore-forming probiotic BC30 strain was supported by its survival through processing and shelf-life and its viability after cooking, as well as by its demonstrated probiotic effects and safety for human consumption (20). The control pasta was made with the same durum whole-wheat flour without the addition of barley β-glucans and BC30 (control pasta, CTR). To check the bacterial concentration in pasta before and after cooking, a quantitative PCR assay with specific DNA primers for *B. coagulans* strains was performed (20). After 7 min of cooking, the INN pasta showed a BC30 concentration of ~7 log CFU/g (10 million CFU/g), whereas the CTR pasta did not contain *B. coagulans* (20). The pasta shape was "penna rigata," with a thickness of 1.12 mm, length of 38–42 mm, and a diameter of 9 mm.

Participants

Volunteers were recruited into the study by public announcements in a local newspaper, through social networks, and among students and staff of the Department of Food and Drugs, University of Parma, Parma. Selection was carried out following an interview that posed questions on health status as well as on behavioral and lifestyle factors, recorded by a validated physical activity questionnaire (21). A physician collected the anthropometric data. A nutritionist recorded the habitual diet relative to the past 12 mo through a validated FFQ (22). A total of 56 subjects were screened and 46 consented to participate in the study. Men and women were eligible if they were between 30 and 65 y old, had a BMI between 25 and 35 kg/m^2, had a habitual diet characterized by a low consumption of fruit and vegetables ($\leq$3 servings/d) and whole-grain cereals (<50 g/wk), were habitual consumers of pasta ($\geq$1 serving/d) and had a low level of physical activity (total physical activity <500 metabolic equivalent of task [MET]-min/wk). Participants were excluded if they had been diagnosed with functional or metabolic disease, including hyperlipidemia and type 1 and 2 diabetes, metabolic syndrome conditions, food allergies, or if they dieted or had been under a controlled dietary regimen in the previous 3 mo, or if they were vegetarians or vegans. Also excluded were persons who had received antibiotics in the past 3 mo, regularly used a prebiotic and probiotic supplement, or if females were pregnant or lactating. Eligible subjects who agreed to participate entered into the study by providing written informed consent.

Study design and protocol

A 12-wk single-blind, parallel, randomized, placebo-controlled dietary intervention study was carried out. Intervention was preceded by a 1-wk baseline period, where participants started to monitor their physical activity level and bowel functions. After the 1-wk run-in period, volunteers were randomly assigned (balanced in terms of sex, age, and BMI) to the intervention groups by means of a computerized random allocation list. At baseline, a nutritionist met each participant

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Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BC30, *Bacillus coagulans* BC30; CVD, cardiovascular disease; CTR, control; GIP, glucose-dependent insulinotropic peptide; GIT, gastrointestinal tract; GLP-1, glucagon-like peptide-1; hs-CRP, high-sensitivity C-reactive protein; INN, innovative; GGT, γ-glutamyltransferase; MET, metabolic equivalent of task; RCT, randomized controlled trial; VCAM-1, vascular cell adhesion molecule-1.

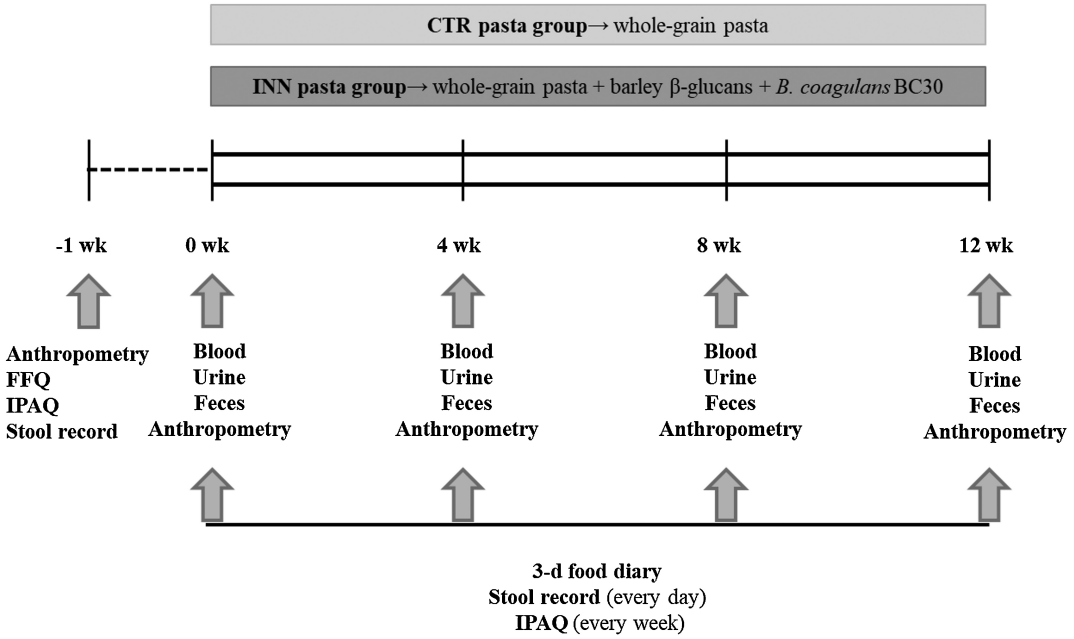

FIGURE 1 Schematic outline of the study protocol. CTR, control; INN, innovative; IPAQ, International Physical Activity Questionnaire.

individually to instruct him/her on how to fill in a 3-d food diary, a 7-d record of physical activity, and a daily bowel function diary. Subjects also received instructions not to use antibiotics, probiotics, prebiotics, and vitamin supplements throughout the study period. At the same meeting, subjects received sterile tubes for urine collection and a refrigerated box to collect and deliver stool samples to the medical facility. For 12 wk, subjects were asked to replace their daily pasta meal with a serving of the INN or CTR pasta. Pasta serving size and seasoning were not established but left to each volunteer's own choice in order to increase the compliance with the study. In addition, volunteers were asked to avoid changing their intake of fruit and vegetables, their body weight, and their lifestyle habits (exercise, alcohol consumption, and smoking) throughout the study.

The study protocol was approved by the local Ethics Committee for Human Research of the City of Parma (protocol no. 13331). The primary and secondary endpoints were those declared when the study was registered at clinicaltrials.gov (as NCT02236533). A scheme of the study design is illustrated in Figure 1.

Eight packs of pasta, 500 g each, were supplied to the study participants at baseline and every 4 wk at the Department of Food and Drug, University of Parma.

Compliance with the dietary treatments was assessed every 4 wk by self-recorded 3-d food diaries (2 working days and 1 weekend day). Moreover, telephone call interviews at 2 and 6 wk were done by an expert dietitian to monitor compliance with the protocol. Physical activity was monitored weekly by the International Physical Activity Questionnaire (21). Daily bowel functions were recorded on a specific stool chart that incorporates descriptors of fecal frequency, weight, consistency, and possible pain records (23). At baseline and after every 4 wk of treatment, fasting participants returned to the medical facility to provide blood and urine samples. During those occasions, they also delivered the fecal sample collected the same morning or the day before and stored at −20°C until arrival. Volunteers also brought their filled diaries, i.e., the 3-d food diary, the physical activity, and the bowel function diaries, which were checked for completeness by a physician and a nutritionist.

Data collection

To avoid subjective errors, all measurements were performed by the same operator following standard procedures. Height was measured during the selection phase at the medical facility to the nearest 0.5 cm with a stadiometer (Seca 220; Seca). Weight was measured at every visit, after voiding, with subjects wearing light clothing and without shoes to the nearest 0.1 kg on a mechanical column scale (Seca 700; Seca). Waist circumference was measured at every visit on undressed subjects at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest while the subject was standing, after a moderate expiration, with a nonstretchable tape. Blood pressure was measured by using a mercury cuff sphygmomanometer after the study participant had been quietly seated for ≥10 min.

Nutritional assessment

An ad-hoc computer program (Nutrition Analysis of Food Frequency Questionnaire), developed by the Epidemiology and Prevention Unit of the IRCCS Foundation, National Cancer Institute of Milan, was used to convert the FFQ dietary data into frequencies of consumption and mean daily quantities of foods (grams per day), energy, and nutrients consumed (22, 24).

Food and nutrient intake recorded by the 3-d food diaries was calculated with an in-house Microsoft Access application (Microsoft Corp.) (25) linked to the European Institute of Oncology's food database, covering the nutrient composition of >900 Italian foods (26). Food items consumed were grouped into the following food categories: whole-grain pasta, other cereals, sweets and desserts, potatoes, fruit, vegetables, legumes, meat and processed meat, fish and seafood, eggs, milk and dairy, and oils and fats.

Biochemical analyses

Blood was obtained from an intravenous catheter into tubes containing EDTA, heparin, or nothing depending on the analysis. Analyses were performed in a single-blind manner by the personnel of the Laboratory of Clinical Chemistry and Hematology, University Hospital of Parma. Fasting plasma glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), total cholesterol, HDL cholesterol, triglycerides, and homocysteine were assayed by an automated analyzer (AU5800; Beckman Coulter); plasma hs-CRP was measured by means of immunoturbidimetry assay (Dimension Analyzer; Siemens). From serum samples, insulin was assayed by means of immunometric assay (Liaison Analyzer; Diasorin) and total folates by means of chemiluminescence assay (DXI; Beckman Coulter). LDL cholesterol was calculated from the Friedewald formula.

Determination of metabolic and inflammatory markers

Metabolic and inflammatory markers were determined in duplicate in 25 μL plasma with the use of Bio-Plex Pro human immunoassay multiplex kits (Bio-Rad) and Luminex technology (Bio-Plex; Bio-Rad), according to the manufacturer's instructions. Blood samples were collected into EDTA-coated tubes, to which was immediately added dipeptidylpeptidase IV inhibitor (Millipore) and phenylmethanesulfonyl fluoride (Sigma-Aldrich). After centrifugation at 2400 × *g* for 10 min at 4°C, supernatants were stored at –80°C before analysis (5).

The Bio-Plex Pro immunoassay kit allows the quantification of the following biomarkers: C-peptide, ghrelin, glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide-1 (GLP-1), leptin, plasminogen activator inhibitor-1, resistin, visfatin, IL-6, IL-10, TNF-α, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 (VCAM-1).

The sensitivity levels of the assay (in pg/mL) were as follows: C-peptide, 14.3; ghrelin, 1.2; GIP (total), 0.8; GLP-1 (active), 5.3; leptin, 3.1; plasminogen activator inhibitor 1, 2.2; resistin, 1.3; visfatin, 37.1; IL-6, 2.6; IL-10, 0.3; TNF-α, 6.0; intercellular adhesion molecule 1, 2.4; and and vascular cell adhesion molecule 1, 0.6. The percentage CV interassay variation was 4%, whereas the percentage CV intra-assay variation was 5%.

Microbiological analysis

Upon arrival in the laboratory, an aliquot of fecal samples was stored at 4°C for culture-dependent analyses, whereas further aliquots were immediately mixed with RNA*later* solution (Sigma-Aldrich) and frozen at –20°C until DNA extraction and molecular analysis were performed.

Enumeration of BC30 was conducted by mixing 1 g of fecal sample with 9 mL sterile peptone water (0.1%, w/v) and stirring until homogenization. Appropriate dilutions were heat treated (75°C for 30 min), immediately cooled, and pour plated in duplicate onto glucose yeast extract agar. Colony counts were performed after incubation at 37°C for 48 h in aerobiosis.

DNA was extracted from 200-mg fecal samples using the QIAamp DNA stool Mini Kit (Qiagen), as per the manufacturer's instructions, with a lysis temperature of 95°C.

qPCR was used to detect BC30, *Faecalibacterium prausnitzii*, *Bifidobacterium* spp., and *Prevotella* spp. in fecal samples.

We selected *F. prausnitzii*, *Bifidobacterium* spp., and *Prevotella* spp. because previous RCTs have shown that the separate administration of β-glucans or BC30 or fibers, which here were combined in the INN pasta, leads to an increase in these micro-organisms (27–30).

Primers and reference strains used for the construction of standard curves are listed in **Supplemental Table 1**. In particular, the primers GBI-30F and GBI-30R were newly designed, based on comparative analyses of available *B. coagulans* genomes. These primers recognized a region located in a sequence (ID JPSK01000058.1, NCBI) coding for a hypothetic protein, and their specificity was assessed on a collection of *B. coagulans* strains. qPCRs were performed with a LightCycler Nano System (Roche), with the use of the FastStart DNA Master SYBR Green kit (Roche), in a final volume of 20 μL, containing 5 μL of DNA sample, 0.25 μM of each primer, MasterMix buffer 1×, and PCR-grade water. The qPCR conditions consisted of 1 cycle at 95°C for 10 min, and 45 cycles of denaturation at 95°C for 15 s, annealing at the temperature reported in Supplemental Table 1 for 15 s, and extension at 72°C for the time indicated in Supplemental Table 1. The cycle threshold of each sample was compared with standard curves made by diluting genomic DNA of known concentration from the reference strains (Supplemental Table 1). Cell numbers of bacteria in samples were expressed as genomes per gram of feces. Each qPCR reaction was performed in triplicate.

Statistical analysis

The sample size was calculated on the primary outcome hs-CRP. From post-hoc analysis of a previous study (25), we calculated that 18 participants in each treatment group would give sufficient power (α error of 0.05, 80% power, and 2-sided testing) to detect a 1.2-mg/L decrease in plasma hs-CRP concentration, assuming an average value of 2.5 mg/L and an SD of 2 mg/L. Considering possible dropouts, the participant number was increased to ≥20/group.

Statistical analyses were performed for the whole population and by subdividing the study participants into 2 subgroups based on *1*) baseline BMI cutoff value between overweight (BMI 25–29.9) and obese condition (BMI ≥30), or *2*) baseline plasma glycemia cutoff value identified as threshold for impaired fasting glucose (100 mg/dL) (31).

Values are reported as means ± SEMs. The Kolmogorov-Smirnov test was used to evaluate the normality of distribution of all monitored variables, and logarithmic transformation was applied to nonnormally distributed data.

A repeated-measurement general linear model was performed to assess the effect of time, time × intervention, and time × diet groups on energy, nutrients (total carbohydrate, dietary fiber, protein, total fat, saturated fat, monounsaturated fat, and polyunsaturated fat), food groups (whole-grain pasta, other cereals, potatoes, fruit, vegetables, legumes, meat and processed meat, fish and seafood, eggs, milk and dairy, oils and fats, sweets and desserts), anthropometric measurements and metabolic and inflammatory markers, testing the assumption of sphericity through the Mauchly's test, and applying Greenhouse-Geisser corrections if ε was <0.75 or Huynh-Feldt corrections if ε was >0.75, when the assumption of sphericity was violated. In addition, if a main effect of time × diet groups was registered, Bonferroni post-hoc tests were used for multiple comparisons.

Independent-sample *t* tests were executed to perform between-group comparisons for all nutritional variables (energy, nutrients, and food groups), anthropometric measurements, and metabolic and inflammatory markers at each time point (4, 8, and 12 wk) and for the comparison between BC30 quantification values obtained with plating and qPCR at 12 wk, after checking for the homogeneity of the variance through Levene's test. Mann-Whitney and Wilcoxon tests were performed in order to evaluate differences between intervention groups and intervention times, respectively, in fecal strains (Bifidobacteria, *F. prausnitzii* and *Prevotella*) of subgrouped volunteers. All reported *P* values are 2 factor, with a *P* value <0.05 indicating statistical significance. Statistical analyses were performed with IBM SPSS 24.0 Statistics (IBM SPSS, Inc.).

Results

Recruitment and baseline characteristics

The recruitment and the enrollment of the volunteers occurred between March and July 2014 and the study was completed in December 2014. No adverse events were identified in the INN and CTR pasta groups during the study period.

Ten out of the 56 screened participants were excluded before being allocated to the intervention for not meeting the inclusion criteria or after making a personal decision to not join the study. The remaining 46 volunteers were enrolled and randomly assigned to a dietary intervention (23 for CTR pasta and 23 for INN pasta). Of these, 2 volunteers dropped out because of family reasons and 3 because they had started antibiotic therapy (**Supplemental Figure 1**). Therefore, 41 volunteers completed the study, 20 in the CTR pasta group (12 females/8 males) and 21 in the INN pasta group (13 females/8 males) (Supplemental Figure 1). Their general characteristics at baseline are reported in Table 1.

Energy and nutrient intakes for monitoring compliance with the dietary treatment

Analysis of the food diaries over the study period showed that there was no main effect of time or time × intervention on energy and nutrient intakes (Table 2). In both the CTR and INN pasta groups, there were no significant differences among the 3 time points for any of the nutritional variables, except for energy intake in the INN pasta group, which was lower at 12

TABLE 1 Baseline anthropometric measurements and nutritional assessment of the healthy overweight or obese participants who completed the study[1]

	Mean ± SEM
n	41
Gender, F/M	25/16
Age, y	53 ± 2
BMI, kg/m^2	30.9 ± 0.8
Pasta consumption, g/d	69.4 ± 7.0
Whole-grain cereal consumption, g/d	5.7 ± 0.9
Fruit consumption, g/d	266 ± 22.8
Vegetable consumption, g/d	240 ± 15.2
Total physical activity, MET-min/wk	249 ± 37.9

[1] MET, metabolic equivalent of task.

wk than at 8 and 4 wk ($P = 0.045$ and $P = 0.016$, respectively). No significant differences were observed between the dietary intervention groups for any of the nutritional variables at any time point; the only exception was that saturated fat intake was found to be lower in the CTR pasta group at 8 wk ($P = 0.036$) than in the INN pasta group.

No main effect of time or time × intervention was observed on food group intake. Similarly, no changes in the food groups eaten were observed within either the CTR or INN groups over the 3-mo study period. In addition, no significant difference in the food groups eaten was observed between the intervention groups at any time point, except the higher fruit intake ($P = 0.048$), and the lower milk and dairy product intakes ($P = 0.047$) at 8 wk in the CTR compared with the INN group (**Supplemental Table 2**).

Anthropometric, blood pressure, and blood biomarkers

No significant variations in body weight or BMI were observed during the study period within or between study groups. A significant effect of time, irrespective of the CTR or INN pasta intervention, was observed on waist circumference during the 12-wk intervention ($P = 0.012$) (Table 3). No significant variations in systolic and diastolic blood pressure were found within or between groups, although a significant time × treatment effect was found for diastolic blood pressure ($P = 0.042$). Concerning the transaminases, a main effect of time ($P = 0.000$) and of time × treatment ($P = 0.050$) was observed for plasma GGT. Comparing the 2 treatment groups, plasma GGT was significantly lower in the INN pasta group compared with the CTR pasta group at the 12-wk time point ($P = 0.033$). Moreover, the data show that, within the CTR pasta group, there was a main effect of time ($P = 0.000$) on plasma GGT that resulted in significantly higher levels at 12 wk compared with baseline ($P = 0.002$), 4 wk ($P = 0.000$), and 8 wk ($P = 0.044$). A main effect of time was found for plasma resistin ($P = 0.042$), GLP-1 ($P = 0.036$), and GIP ($P = 0.012$), independent of the dietary intervention. Finally, there was a main effect of time on plasma IL-6 ($P = 0.020$) within the INN pasta group, for which a significant increase in plasma IL-6 was found after 4 wk of INN consumption compared with baseline ($P = 0.024$). None of the other analyzed markers were significantly influenced by the treatments (Table 3).

After subdividing the study participants using a glycemia cutoff value of 100 mg/dL at baseline, the results showed a significantly lower value of plasma resistin concentration at 12 wk in the 8 participants belonging to the subgroup with high glycemia and who had consumed the INN pasta compared with the 10 hyperglycemic subjects consuming the CTR pasta ($P = 0.025$; Figure 2A). Furthermore, by subdividing the study participants into overweight (BMI 25–29.9) and obese (BMI ≥30 kg/m^2) groups, significantly lower values of the plasma LDL/HDL cholesterol ratio ($P = 0.038$) and plasma hs-CRP ($P = 0.040$) were observed among obese subjects in the INN pasta group ($n = 12$) compared with the CTR pasta group ($n = 7$) after 12 wk of pasta consumption (Figure 2B, C). When the plasma cytokines and other plasma inflammatory markers were assayed in the enrolled participants subdivided using the glycemia cutoff value or the BMI, no differences were found during the study period within or between the CTR and INN pasta groups.

TABLE 2 Energy and nutrient intakes by the healthy overweight or obese participants consuming CTR or INN pasta over the 12-wk study period[1]

	4 wk		8 wk		12 wk	
Nutritional information	CTR	INN	CTR	INN	CTR	INN
Energy, kcal/d	2007 ± 77.9	2077 ± 56.5[a]	1970 ± 65.3	2063 ± 62.6[a]	1982 ± 65.8	1994 ± 59.1[b]
Carbohydrates						
Total, g/d	237 ± 13.7	258 ± 10.9	237 ± 8.1	251 ± 12.0	229 ± 9.5	240 ± 10.1
Dietary fiber, g/d	24.7 ± 1.30	26.0 ± 1.2	25.8 ± 1.2	24.6 ± 0.8	24.0 ± 0.9	23.8 ± 1.0
% energy	46.9 ± 1.8	49.6 ± 1.5	48.1 ± 1.4	48.7 ± 1.5	46.5 ± 1.4	47.9 ± 1.2
Proteins						
Total, g/d	74.9 ± 2.5	78.9 ± 2.9	74.4 ± 3.9	78.5 ± 3.3	78.5 ± 3.5	80.2 ± 3.8
% energy	15.2 ± 0.5	15.3 ± 0.4	15.1 ± 0.5	15.3 ± 0.5	15.9 ± 0.5	16.2 ± 0.6
Fats						
Total, g/d	79.0 ± 3.4	79.6 ± 2.9	76.3 ± 3.7	81.2 ± 3.1	78.2 ± 3.8	78.7 ± 2.7
Saturated, g/d	24.8 ± 1.4	25.9 ± 1.5	22.8 ± 1.4	27.2 ± 1.5*	24.9 ± 1.4	25.5 ± 1.6
Monounsaturated, g/d	38.9 ± 1.8	36.9 ± 1.2	38.3 ± 2.0	37.1 ± 1.5	38.5 ± 2.2	37.3 ± 1.4
Polyunsaturated, g/d	9.6 ± 0.6	10.9 ± 0.7	9.9 ± 0.6	10.9 ± 1.1	9.5 ± 0.5	10.1 ± 0.6
% energy	35.8 ± 1.4	34.6 ± 0.9	35.0 ± 1.1	35.5 ± 1.2	35.5 ± 1.3	35.8 ± 1.0
Alcohol, g/d	15.7 ± 4.5	11.1 ± 2.6	14.0 ± 3.4	10.8 ± 1.9	15.1 ± 3.6	9.2 ± 1.9

[1] All values are reported as means ± SEMs, $n = 41$. CTR, control; INN, innovative. Different letters in the same row indicate differences within the same intervention group among the 3 time points ($P < 0.05$ from a repeated-measures general linear model with Bonferroni post-hoc test). * in the same row indicates a difference between intervention groups (CTR compared with INN) at the specific time point (*$P < 0.05$ from independent-sample *t* test).

TABLE 3 Effect of the consumption of the CTR and INN pasta on anthropometric, lipid, glycemia, and inflammatory outcomes in healthy overweight or obese participants[1]

	0 wk		4 wk		8 wk		12 wk			
Characteristic	CTR	INN	CTR	INN	CTR	INN	CTR	INN	*P* time[2]	*P* time × treatment[3]
Body weight, kg	86.5 ± 3.5	88.2 ± 3.1	86.4 ± 3.5	87.9 ± 3.7	86.5 ± 3.5	87.6 ± 2.9	86.6 ± 3.8	87.2 ± 2.9	0.447	0.613
Waist circumference, cm	104 ± 1.9	106 ± 2.5	104 ± 2.1	105 ± 2.6	103 ± 2.1	104 ± 2.7	103 ± 1.7	103 ± 2.9	0.012	0.631
BMI, kg/m^2	31.3 ± 1.4	30.6 ± 0.7	31.2 ± 1.4	30.5 ± 0.8	31.3 ± 1.4	30.7 ± 0.8	30.9 ± 1.4	30.3 ± 0.7	0.458	0.717
Systolic blood pressure, mm Hg	122 ± 3.7	124 ± 2.7	124 ± 3.8	125 ± 31	122 ± 3.2	125 ± 2.7	122 ± 3.5	125 ± 2.5	0.910	0.919
Diastolic blood pressure, mm Hg	79.0 ± 1.7	77.1 ± 1.3	77.3 ± 2.0	78.3 ± 1.2	76.0 ± 1.8	79.1 ± 1.2	77.7 ± 1.8	78.3 ± 1.7	0.762	0.042
Plasma glucose, mg/dL	102 ± 2.5	102 ± 2.9	103 ± 3.3	101 ± 2.3	99.6 ± 2.5	97.5 ± 3.2	101 ± 3.2	97.9 ± 2.4	0.146	0.710
Serum insulin, μIU/ml	12.7 ± 1.5	10.8 ± 1.0	12.5 ± 1.3	11.6 ± 0.8	12.7 ± 1.3	12.7 ± 1.3	11.2 ± 1.4	11.4 ± 1.4	0.332	0.846
Plasma ALT, IU/L	28.8 ± 3.9	30.4 ± 4.0	27.2 ± 3.3	31.1 ± 3.3	26.5 ± 2.6	26.1 ± 2.3	27.8 ± 2.8	26.9 ± 2.4	0.776	0.443
Plasma AST, IU/L	25.2 ± 1.5	25.7 ± 2.2	26.4 ± 1.4	25.5 ± 1.8	24.4 ± 1.4	23.7 ± 1.3	25.0 ± 1.3	23.7 ± 1.3	0.571	0.870
Plasma GGT, IU/L	27.8 ± 4.9^{b}	17.7 ± 1.8	30.6 ± 5.6^{b}	19.1 ± 1.9	32.3 ± 5.4^{b}	19.1 ± 1.4	38.8 ± 6.9^{a}	22.1 ± 2.4*	0.000	0.050
Plasma total cholesterol, mg/dL	219 ± 8.5	215 ± 6.7	222 ± 7.8	215 ± 7.5	219 ± 7.6	209 ± 6.9	220 ± 9.3	212 ± 6.7	0.992	0.622
Plasma LDL cholesterol, mg/dL	136 ± 7.2	137 ± 5.5	137 ± 6.5	134 ± 6.1	135 ± 6.5	131 ± 5.5	138 ± 7.2	132 ± 6.3	0.839	0.454
Plasma HDL cholesterol, mg/dL	59.3 ± 2.6	55.6 ± 1.6	60.3 ± 2.9	55.3 ± 1.7	59.3 ± 2.5	53.1 ± 2.1	57.8 ± 2.6	55.5 ± 2.2	0.884	0.313
Plasma triglycerides, mg/dL	116 ± 12.0	110 ± 9.2	120 ± 13.3	124 ± 12.9	119 ± 17.0	124 ± 12.1	122 ± 16.4	120 ± 16.6	0.566	0.829
Plasma homocysteine, μmol/L	11.5 ± 0.8	11.1 ± 0.8	12.1 ± 0.9	11.6 ± 1.1	13.6 ± 1.8	11.2 ± 0.8	12.6 ± 1.2	13.6 ± 1.7	0.106	0.266
Plasma hs-CRP, mg/L	3.9 ± 1.3	3.7 ± 1.2	3.6 ± 1.2	3.3 ± 1.1	3.5 ± 1.2	3.4 ± 1.1	8.0 ± 3.9	2.7 ± 0.6	0.889	0.510
Serum total folates, ng/mL	5.7 ± 0.5	7.1 ± 0.7	5.9 ± 0.5	6.6 ± 0.7	5.8 ± 0.5	6.4 ± 0.9	5.9 ± 0.56	6.2 ± 0.8	0.430	0.401
Plasma C-peptide, μg/mL	2.5 ± 0.6	1.8 ± 0.2	2.2 ± 0.4	1.8 ± 0.1	2.2 ± 0.5	2.0 ± 0.2	1.9 ± 0.3	1.8 ± 0.2	0.160	0.052
Plasma resistin, μg/mL	7.5 ± 0.7	7.5 ± 0.8	9.0 ± 0.9	9.6 ± 1.0	8.7 ± 0.9	8.1 ± 0.7	7.5 ± 0.5	7.3 ± 0.8	0.042	0.672
Plasma GLP-1, pg/mL	192 ± 13.5	209 ± 13.9	209.5 ± 12.9	228 ± 13.2	200 ± 12.9	216 ± 13.3	207 ± 11.5	209 ± 11.3	0.036	0.674
Plasma visfatin, μg/mL	21.3 ± 17.3	5.4 ± 0.5	26.4 ± 21.6	6.2 ± 0.8	13.3 ± 7.5	6.2 ± 1.1	21.8 ± 16.9	5.6 ± 0.8	0.108	0.451
Plasma leptin, μg/mL	27.5 ± 3.0	31.0 ± 3.9	25.8 ± 2.3	30.3 ± 3.6	25.3 ± 2.5	30.6 ± 4.0	24.9 ± 2.4	27.9 ± 3.7	0.144	0.397
Plasma PAI-1, μg/mL	48.9 ± 7.3	47.4 ± 7.3	48.0 ± 7.5	50.9 ± 6.8	50.3 ± 7.1	42.7 ± 4.9	45.1 ± 5.3	38.4 ± 5.6	0.060	0.385
Plasma ghrelin, μg/mL	1.6 ± 0.2	2.1 ± 0.5	1.9 ± 0.2	2.9 ± 1.1	1.9 ± 0.2	2.8 ± 1.2	1.7 ± 0.2	2.4 ± 0.6	0.072	0.855
Plasma GIP, μg/mL	4.6 ± 4.1	0.6 ± 0.1	4.7 ± 4.1	0.6 ± 0.1	7.7 ± 6.9	1.1 ± 0.3	5.2 ± 4.3	0.8 ± 0.2	0.012	0.850
Plasma IL-6, pg/mL	11.7 ± 1.0	11.6 ± 1.3^{b}	12.2 ± 0.9	14.7 ± 1.5^{a}	12.2 ± 0.9	13.5 ± 1.2^{ab}	12.9 ± 2.3	12.2 ± 1.6^{ab}	0.129	0.293
Plasma IL-10, pg/mL	23.8 ± 13.1	17.3 ± 5.7	25.1 ± 13.7	20.0 ± 4.9	20.2 ± 7.9	20.3 ± 4.9	20.2 ± 9.9	17.9 ± 5.0	0.053	0.069
Plasma TNF-α, pg/mL	22.9 ± 4.4	23.4 ± 3.9	28.2 ± 6.3	39.3 ± 6.9	29.2 ± 6.3	38.3 ± 7.9	23.7 ± 5.8	30.7 ± 6.2	0.237	0.469
Plasma VCAM-1, μg/mL	245 ± 20.6	220 ± 17.6	248 ± 20.7	232 ± 16.6	246 ± 23.9	237 ± 16.6	259 ± 26.6	217 ± 15.7	0.914	0.176
Plasma ICAM-1, μg/mL	132 ± 9.9	137 ± 12.1	125 ± 8.7	144 ± 11.1	142 ± 15.1	146 ± 13.8	124 ± 8.9	135 ± 12.3	0.351	0.487

[1] All values are means ± SEMs, $n = 41$. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CTR, control; GGT, γ-glutamyltransferase; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; hs-CRP, high-sensitivity C-reactive protein; ICAM-1, intercellular adhesion molecule-1; INN, innovative; PAI-1, plasminogen activator inhibitor-1; VCAM-1, vascular cell adhesion molecule-1. Data were $\log_{10}$ transformed before analysis except for body weight, BMI, waist circumference, and systolic and diastolic blood pressure. Different letters in the same row indicate a significant difference within the same intervention group among time points (repeated-measures general linear model with Bonferroni post-hoc test), $P < 0.05$. * in the same row indicates a difference between intervention groups (CTR compared with INN) at the specific time point (* $P < 0.05$ from independent-samples *t* test).

[2] Significant main effect of time ($P < 0.05$) from a repeated-measures general linear model.

[3] Significant main effect of time × intervention interaction ($P < 0.05$) from a repeated-measures general linear model.

Detection of BC30 and selected bacterial groups

The presence of the probiotic strain BC30 was assessed in all fecal samples from subjects who consumed the INN pasta, but not in those who consumed the CTR pasta. BC30 was absent in fecal samples before pasta consumption, whereas a BC30 concentration of >6 log CFU/g (1 million CFU/g) was found by plating counts throughout the intervention period (**Figure 3**). The strain-specific qPCR performed after 12 wk of pasta consumption confirmed the presence of BC30 and showed significantly higher values than plating counts ($P < 0.05$; Figure 3). Furthermore, the strain was found in the fecal samples of a subgroup of individuals ($n = 5$) who were analyzed 5 d after the end of the treatment with the INN pasta. In these subjects, BC30 was detected at concentrations >1000 CFU/g (data not shown).

Based on qPCR quantification, the levels of *Bifidobacterium* spp., *F. prausnitzii*, and *Prevotella* spp. did not significantly differ between the 2 study groups at baseline or after 12 wk of pasta consumption (**Figure 4**A).

Study participants were subdivided into 2 groups based on their BMI (cutoff value of 30). In the overweight group (BMI 25–29.9), significantly lower concentrations of *Bifidobacterium* spp. were observed in INN subjects at 12 wk ($P = 0.032$; Figure 4B); in the obese group (BMI ≥30), *Bifidobacterium* concentrations were significantly lower in INN subjects than in CTR subjects, at baseline and after 12 wk of pasta consumption ($P = 0.009$ and $P = 0.011$, respectively; Figure 4B).

In addition, after subdividing the study participants based on a 100 mg/dL glycemia cutoff value, subjects in the subgroup with high glycemia and eating the INN pasta showed higher levels of *F. prausnitzii* at 12 wk than those consuming the CTR pasta ($P = 0.043$; Figure 4C).

Discussion

To the best of our knowledge, this is the first RCT aiming to evaluate the effect of the consumption of a synbiotic pasta on

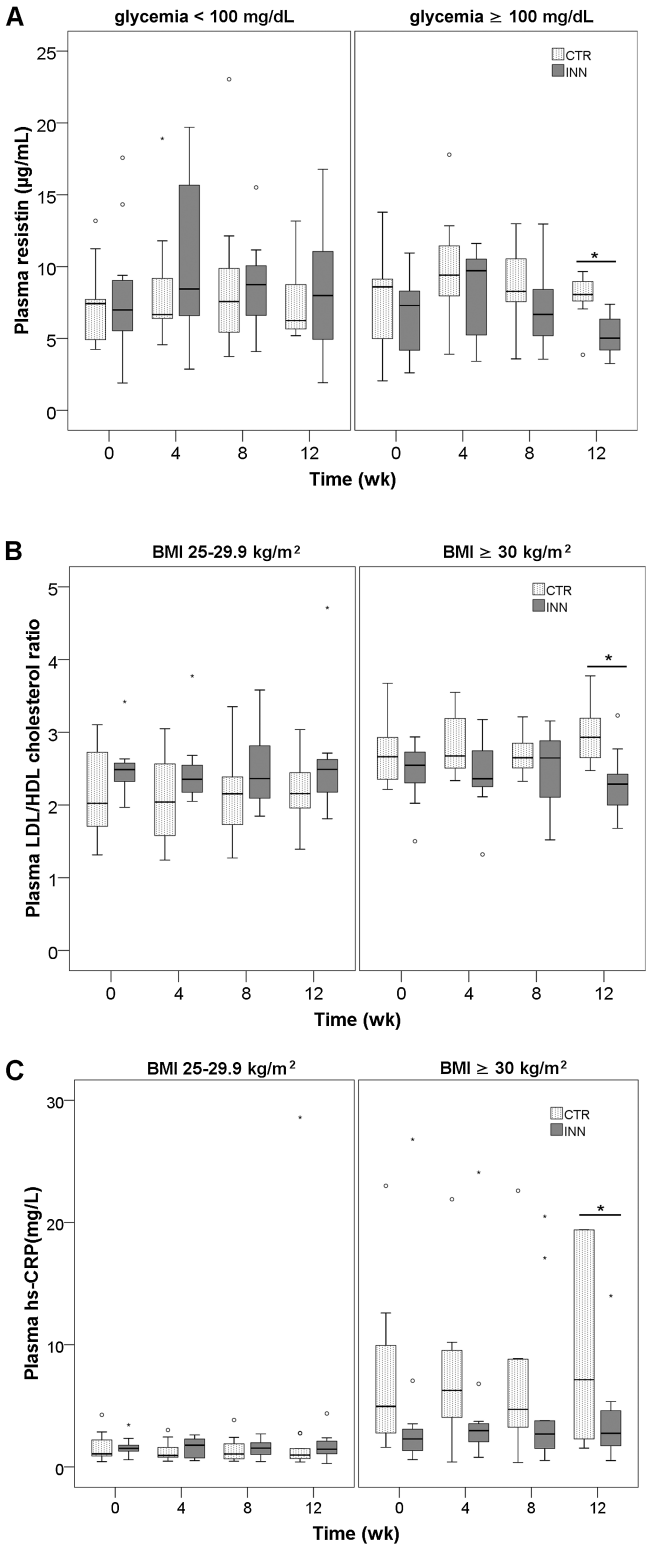

FIGURE 2 Box plot of the plasma resistin level (A), plasma LDL/HDL cholesterol ratio (B), and plasma hs-CRP (C) concentrations in CTR and INN pasta volunteers subgrouped on the basis of glycemia (A) and BMI cutoff values (B, C). Values refers to $n = 41$. ∗Difference between intervention groups (CTR compared with INN) at the specific time point, $P < 0.05$ from independent-sample *t* test. CTR, control; hs-CRP, high-sensitivity C-reactive protein; INN, innovative.

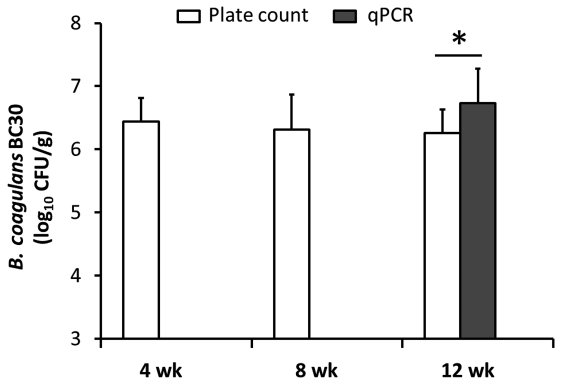

FIGURE 3 Plate counts (log_{10} CFU/g) and qPCR (log_{10} genomes/g) quantification of the probiotic strain *Bacillus coagulans* BC30 in fecal samples of healthy overweight or obese subjects fed with the INN pasta. Values refers to $n = 21$. ∗Difference between plate counts and qPCR quantification at the specific time point, $P < 0.05$ from independent-sample *t* test. INN, innovative.

markers of cardiovascular risk in humans. The CTR pasta was prepared using the same durum whole-wheat flour as the INN pasta, and the 2 pastas were produced in the same industrial plant to ensure similar structural and rheologic properties that are known to affect the health effect of food (32).

The consumption of the tested foods did not influence the dietary habits of the volunteers, except for an increase in whole-grain cereal consumption (which was included in the pasta category). The results also show that INN pasta consumption had no effects on the primary or secondary outcomes compared with the CTR pasta. An unexpected increase in plasma GGT concentration in CTR pasta consumers was found. Such an increase in CTR pasta consumers is in disagreement with previous cross-sectional studies, which have shown significantly lower concentrations of GGT in plasma among higher consumers of whole-grain bread (33, 34). Tovar et al. (35) observed a significant decrease in plasma GGT concentrations after whole-grain barley product supplementation in overweight women, compared with a control diet, probably due to an approximate doubling in total daily fiber intake in the participants (35).

Interesting results emerged when the study participants were subgrouped on the basis of their BMI. Indeed, obese volunteers consuming the INN pasta showed decreased plasma concentrations of hs-CRP and LDL/HDL cholesterol ratio after 12 wk, compared with those consuming the CTR pasta. Regarding plasma hs-CRP, our findings are in agreement with previous interventions with whole-grain-based products in obese individuals (36, 37). Similarly, a reduction in the plasma LDL/HDL cholesterol ratio has also been found in subjects consuming β-glucan-rich products for 8 wk (38) and in overweight or obese subjects eating oat-based products for 12 wk (39).

The delivery of probiotic strains into the GIT may positively affect the growth and metabolism of resident strains known to be short-chain fatty acid producers, mainly propionate and butyrate (40). Among the probiotics, BC30 has recently been shown to exert positive effects on resident microbiota (27, 41), as well as beneficial properties on the host, i.e., improving immunologic parameters, intestinal functionality, and physical performance (42–44). The spore-forming nature of BC30 may allow the micro-organism to survive the technologic processes

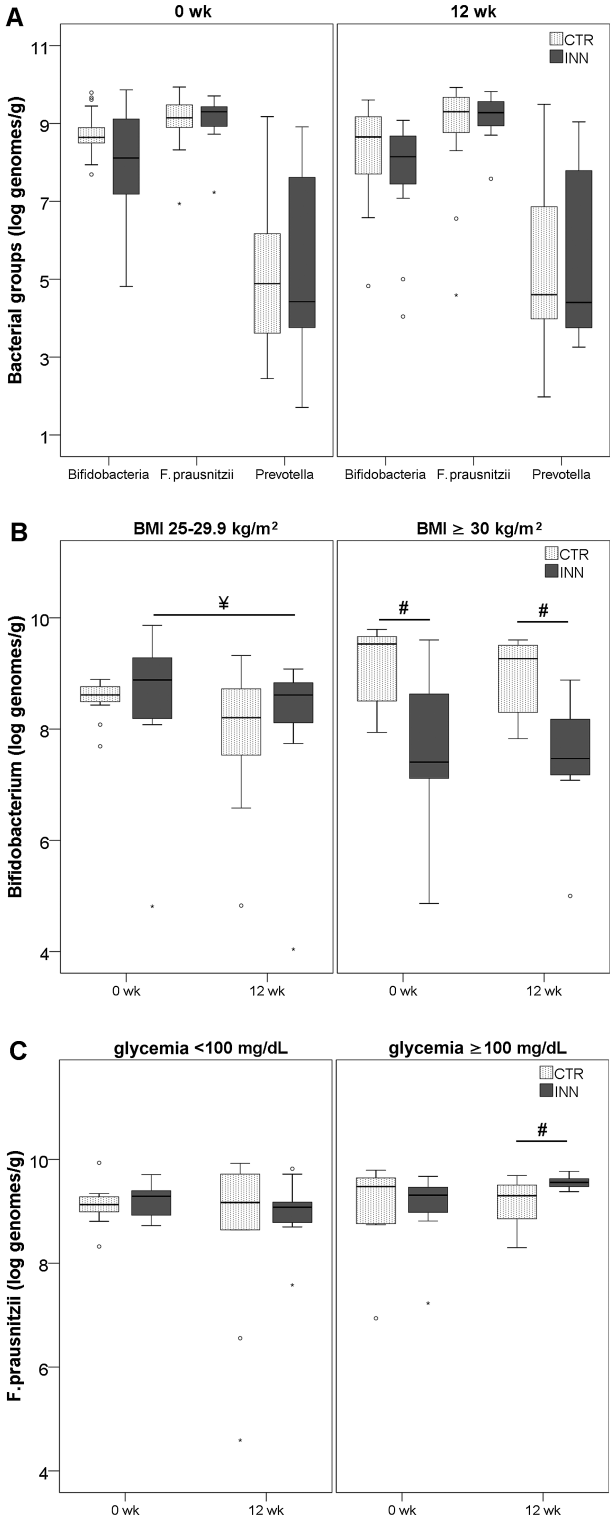

FIGURE 4 Bacterial quantification by qPCR. (A) Quantification (log_{10} genomes/g) of *Bifidobacterium* spp., *Faecalibacterium prausnitzii* and *Prevotella* spp. in the fecal samples of healthy overweight or obese subjects after 0 and 12 wk of consumption of CTR and INN pasta. (B) *Bifidobacterium* spp. and (C) *F. prausnitzii* fecal quantification in volunteers subgrouped on the basis of BMI and glycemia cutoff values, respectively. Values refers to $n = 41$. Labeled values in the same grouped bars with "#" indicate a difference between intervention groups (CTR compared with INN) at the specific time point, $P < 0.05$ from Mann-Whitney test. "¥" indicates a difference between baseline and 12 wks within the same intervention group, $P < 0.05$ from Wilcoxon test. CTR, control; INN, innovative.

of pasta production and enable it to reach the lower GIT unmodified. In the present study, we confirmed that BC30 survives GIT transit, as it was found in fecal samples throughout the intervention period with the INN pasta. Moreover, at 12 wk, significantly higher BC30 levels were observed through qPCR (i.e., quantification of spores and vegetative cells) compared with plate counts after thermal treatment (i.e., viable spore counts), which could be related to the presence of vegetative cells. This suggests that the strain might have germinated and proliferated in the gut of volunteers. Furthermore, the detection of the strain 5 d after the end of the INN pasta intervention, a period longer than the 14–32 h GIT estimated transit time (45), could support the hypothesis of the possible germination of BC30 spores (46).

Although an increase of the genera *Prevotella*, *Bifidobacterium*, and *F. prausnitzii* has been shown in previous RCTs after separate administration of β-glucans or BC30 or fibers (27–30), these taxa were not significantly modulated by the INN or CRT pasta in the whole group of subjects. The high interindividual variability in response to a dietary intervention within a free-living lifestyle might partially explain the absence of gut microbiota modulation observed in the study groups and throughout the study (28, 47). Moreover, the absence of any increase in *Bifidobacterium* suggests that the BC30 spores might have not germinated into a sufficient number of living micro-organisms able to exert their probiotic effect. However, the subdivision of study participants based on a BMI 30 cutoff value revealed a peculiar nonbifidogenic effect of the INN pasta in overweight ($n = 9$) and obese ($n = 12$) subjects. These results are quite surprising because there is evidence of bifidobacteria strains increasing after supplementation with prebiotics, probiotics, or synbiotic foods (48). However, Coyte et al. (49) hypothesized that the introduction of a new species within the gut microbial community leads either to a stabilizing effect of ecologic competition or to a destabilizing effect due to an increase in the number of species. The bacterial community might be slanted towards destabilization by the absence of network cooperation between resident and foreign strains, leading to a decreasing number of strains or microbial counts. These authors concluded that strongly growing communities, i.e., bifidobacteria, are less cooperative towards new strains (49), and their numbers could be reduced due to niche occupation by introduced probiotic micro-organisms.

Another interesting finding was revealed in the subgroup with fasting plasma glucose >100 mg/dL, which is a recognized threshold value for the identification of impaired fasting glucose (48). Fecal samples of individuals with hyperglycemia were found to have higher levels of *F. prausnitzii* after 12 wk of INN consumption ($n = 8$), compared with the CTR pasta ($n = 10$). This species is known to positively influence host metabolism and health status (49). *F. prausnitzii* increases following the consumption of the INN pasta represents a positive effect as its abundance has been demonstrated to be low in subjects with impaired fasting glycemia (50). In the same subgroup, after 12 wk of INN pasta consumption, a lower plasma resistin concentration was observed than in the CTR group. Resistin is a recently discovered compound that is able to impair the action of insulin (51) and has been endorsed as a key factor in the development of endothelial dysfunction and obesity-derived diseases (52). To date, no human intervention studies have shown changes in plasma resistin levels after supplementation with whole-grain-based food or synbiotic foods. It is plausible that INN pasta consumption by hyperglycemic volunteers led to an increase in *F. prausnitzii*, known to influence fasting

blood glycemia (50, 53, 54), which might have reflected a modulation in plasma resistin concentration. These effects may be hypothesized by considering the ascertained glycemia-lowering effects of barley β-glucans (11) and the probiotic evidence of BC30 strains (27) and, more importantly, in terms of the interaction of both these ingredients. However, specific further studies should be carried out to support this hypothesis.

The strength of the present study is that the effects of the substitution of a daily pasta serving with a pasta enriched in fiber and probiotics were observed in healthy volunteers in free-living conditions and having a completely free diet, i.e., without any controlled dietary regimen such as a low-calorie or low-fat diet.

This study also presents some limitations. First, a 12-wk dietary intervention in free-living subjects might be not sufficient to change the gut microbiota composition and blood markers in healthy subjects at risk of disease due to an unhealthy lifestyle. However, extending the duration of the study might affect compliance, thus nullifying any effort. Second, other plasma inflammatory markers, not considered in this work, might be modulated by the 2 pasta dietary interventions, and this aspect should be carefully considered in future studies. Third, the choice of testing whole-grain pasta with the same technologic and rheologic characteristics as a control might have limited the effects of the INN pasta on the primary and secondary outcomes. However, this allowed us to evaluate the effects of β-glucans and probiotics by avoiding possible biases induced by other bioactive compounds present in whole grains as well as by a different food structure that may itself have had an influence on some physiologic effects (32). Fourth, the presence of cultivable BC30 spores in the INN pasta, which may germinate or revert to the spore form in the lower GIT, limited the possibility of ascertaining its specific role in the effects observed in subjects consuming that pasta. Indeed, although the presence of BC30 vital strains was demonstrated in fecal samples, the presence of the bacilli in their living and active form along the GIT tract could not be monitored.

In conclusion, the consumption of the developed synbiotic pasta revealed limited effects on primary and secondary outcomes in the whole group of healthy overweight or obese volunteers. Nevertheless, in the subgroup of obese and hyperglycemic volunteers, in the frame of their habitual dietary and lifestyle habits, a daily serving of the synbiotic pasta had beneficial effects on plasma resistin, plasma LDL/HDL cholesterol ratio, and plasma hs-CRP. These effects were also associated with slight changes in the composition of the gut microbiota, i.e., *Bifidobacterium* and *F. prausnitzii* concentrations. This study for the first time demonstrates that a staple food such as pasta can be modified into a synbiotic food that can have some beneficial effects in subjects at risk of disease due to an unhealthy lifestyle. This will open up the possibility of designing new healthy staple foods.

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