Long-Term Treatment of Overweight and Obesity with Polyglucosamine (PG L112): Randomized Study Compared with Placebo in Subjects after Caloric Restriction

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Abstract

Background: Short-term treatment of overweight and obesity with polyglucosamine (PG) was found to be more effective than placebo and orlistat in double-blind clinical studies.

Objective: The aim of the study was to compare the efficacy of long-term (12-mo) treatment of weight loss with PG and placebo (PL).

Methods: This was a double-blind randomized study in 100 participants of both sexes with a body mass index (in kg/m^2) >30 to <35. One group of 50 participants was treated for 1 y with PG at 1.6 g/d and a similar group received PL. PG is a combination of low-molecular-weight chitosan with organic acids. Participants were instructed to reduce their caloric intake by 10% and increase the physical activity level by 9 metabolic equivalent task hours/wk. Dietary compliance was checked every 3 mo by using a weekly questionnaire [food intake assessment (FIA)] based on 25 different food servings. Body weight (BW), waist circumference (WC), blood pressure (BP), glucose, lipids, and high-sensitivity C-reactive protein (hs-CRP) were also monitored.

Results: Ninety-seven participants completed the study (49 in the PG group, 48 in the PL group). The decrease in calories was similar in both groups, as was the change in number of food servings ($P > 0.05$, ANOVA). Decreases in BW and WC were 8.0 kg and 10.2 cm, respectively, in the PL group, whereas they were 12.1 kg and 13.3 cm in the PG group ($P < 0.001$, ANOVA). The decrease in BP, plasma lipids, glucose, and hs-CRP was more evident in the group treated with PG ($P < 0.05$, ANOVA). The intake of lipids was found to correlate directly with hs-CRP, with the exception of extra-virgin olive oil.

Conclusions: PG was found to be more effective than PL in reducing BW, WC, glucose, BP, plasma lipids, and hs-CRP in moderately obese individuals undergoing a 10% caloric reduction and a slight increase in physical activity. Dietary monitoring with the use of an FIA was an effective tool in supporting dietary compliance. This trial was registered at clinicaltrials.gov as U111111292405 (WHO). *Curr Dev Nutr* 2017;1:e000919.

Introduction

Excess weight and obesity are 2 of the biggest public health problems of the 21st century, even though medical associations have been warning for some time that these conditions are becoming epidemic and are often associated with many comorbidities such as cardiovascular diseases and cancer. In 2014, >1.8 billion adults were overweight (1) and ∼3.4 million die each year as a result of this condition, which is worsened by the fact that the necessary pharmacotherapy has many safety and efficacy limitations.

The decade-old statement that "obesity is now on everyone's plate" (2) is still highly relevant, even though it is an oft-forgotten truth. The financial implication of excess

weight is a real concern because it is no longer confined to developed countries. The economic impact of the obesity epidemic is global and is now rapidly penetrating the world's poorest nations.

Physicians usually warn their patients about the risks that excess weight and obesity pose to their health. These problems can usually be tackled by reducing caloric intake; however, what has become a serious issue is the impact of food-related television advertisements (accounting for ~75% of all television commercials).

Nutritionists and dieticians know that food addiction is one of the most difficult to overcome. However, the problem of overeating can be resolved with effective integrated tools. One of these is to make the best eating choices by eating the right amount of high-quality food; another is the use of substances that prevent a certain amount of the ingested calories from being absorbed. Pharmacologic treatment is also an important option, and to date there are agents that have been shown to be effective in long-term treatment (1 y) that allow the maintenance of a ≥5% reduction in body weight (BW) (3).

However, the problem of adverse reactions associated with the use of drugs and food supplements still remains a major concern, although these drugs are becoming increasingly popular due to the general belief that they have fewer side effects (4). One of the many treatments that has been proposed contains chitosan, which is among the most abundant polysaccharides found in insects, fungi, squid, oysters, krill, clams, and shellfish. It is a natural N-deacetylated derivative of chitin (5), which interacts with hydrophobic compounds such as cholesterol, TGs, FAs, and bile acids, reducing their absorption or re-entry into the mucosal cells of animals and humans. In a review that considered clinical trials conducted up to 2008 (6), the authors' conclusion was that despite some evidence that chitosan is more effective than placebo in the short-term treatment of overweight and obesity, the poor quality of the trials indicates that the effect of chitosan is minimal and unlikely to be of clinical significance. However, there was one aspect not taken into consideration in the analysis of these trials, which relates to the molecular weight (MW) of chitosan. It was not reported that the activity in fat binding depends on this variable, because high-MW polymers tend to have a lower fat-binding affinity (7).

Recently, a new product that belongs to the class of medical devices, polyglucosamine (PG) has been shown to have a very efficient fat-binding capacity (8) without causing steatorrhea (9, 10). PG is a low-MW chitosan used in fixed combinations with ascorbic and tartaric acids. Pharmacologic studies have shown that PG reduces BW and increases the glucose elimination in feces (10), and the concomitant oral administration of [9-^{14}C]oleic acid in mini pigs has been found to lead to a consistent decrease in fecal fat content (11). Gut bacteria seem to use the fats bound to PG and released in the large intestine as a source of energy, thus helping to restrict caloric intake and reduce steatorrhea (9, 10).

In short-term double-blind trials (between 12 and 24 wk) designed to compare this product with placebo (12) or orlistat (13), PG was found to be very safe and substantially more reliable than placebo or orlistat in reducing BW, BMI, and waist circumference (WC) when used in combination with caloric restriction (500 kcal/d) and increased physical activity [3–7 metabolic

equivalent task hours (MET-h)/wk]. The 5% decrease in body weight was achieved in a much shorter time than with placebo.

The aim of this trial was to investigate long-term treatment with PG (12 mo) and to determine if the improved outcomes obtained in previous trials are confirmed. As in every trial related to BW reduction, compliance with caloric restriction was one of the most relevant aspects, because individuals tend to abandon their diet when the restriction is too drastic. For this reason, monitoring the participants' diets and their adherence was considered fundamental, and the participants were given dietary counseling to help change their eating habits permanently.

Methods

Study design

This study was conducted at a single center as a randomized, double-blind, placebo-controlled trial. The study was designed and implemented according to UNI EN ISO 14155:2012 and the STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) checklist (14) in conformance with the guidelines laid down in the Declaration of Helsinki and Good Clinical Practice. The Italian Personal Data Protection Code and other applicable laws, regulations, mandatory standards, and recommendations were also taken into consideration.

The patients were randomly divided into 2 groups. In addition to a 10% calorie restriction and an increase in physical activity (9 MET-h/wk), 1 group received treatment (PG) and the other received placebo (PL): 2×2 tablets before the 2 main meals for 12 mo. All of the procedures were approved by the Ethics Committee of the Rende Municipality (approval N14 according to section 48 of Italian legislative decree 267/2000 of 28 January 2010). The experiment was conducted in subjects who lived in the geographical triangle between Rende, Rovito, and San Lucido during the MAP (Monitoraggio Alimenti e Patologie) study. The study was conducted between May 2014 and October 2016, and enrollment took 3 mo. The trial was also formally registered at clinicaltrials.gov as U1111111292405. There was no need to make any important changes to the methods after the trial commenced.

Participants

A total of 187 participants were analyzed. The inclusion criteria were as follows: *1)* age between 25 and 65 y, *2)* BMI (in kg/m^2) range of >30 to <35, and *3)* able to complete the food intake assessment (FIA) questionnaire correctly. Of these, only 100 were included in the trial (50 men and 50 women). The exclusion criteria were as follows: *1)* inability to complete the FIA questionnaire and comply with the trial protocol criteria; *2)* pregnancy or breastfeeding; *3)* receiving treatments for BW reduction or metabolic syndrome; *4)* alcohol abuse, drug abuse, or drug addiction; *5)* cancer or malignant tumors; *6)* known hypersensitivity reactions to crustaceans or any of the ingredients in the products; *7)* pre-existence of chronic intestinal disease, such as constipation requiring medical treatment; *8)* postoperative state after gastrointestinal surgery; *9)* metabolic disorders or chronic malabsorption disorder; *10)* current use of medications that decrease intestinal motility, such as opiates; and *11)* long-term use of

medications, with the exception of antihypertensive drugs. Patients undergoing treatment for hypertension were admitted, provided they continued taking the same type of medication during the trial.

Because the treatment continued for 12 mo, any interruption of >4 wk, pregnancy, or missing >2 examinations were considered sufficient to exclude the participant from the final assessment. If acute diseases occurred, all types of treatments were allowed.

The investigators informed the patients about the trial both verbally and in writing. A written informed consent was obtained on a form signed by both the trial participant and the investigators themselves. The patients were insured as specified on the consent form.

FIA questionnaire and dietary suggestions

The FIA is a typical FFQ that considers all of the available foods registered by the Istituto Nazionale Nutrizione Alimenti (INRAN; National Institute for Research on Food and Nutrition). The INRAN lists foods in terms of their caloric content and all other food-related variables (e.g., carbohydrates, lipids, protein, water, vitamins, and trace elements). The INRAN tables contain almost all of the foods available in Italy (15).

The FIA consists of a questionnaire with 250 of the most common foods, which the participant had to complete on a daily basis for 7 consecutive days. An extended version of the FIA was used for this trial: the number of food servings was increased from the usual 9 (16) to 25. The amount of food had to be reported in grams and was subsequently transformed by using an algorithm into "average portions" according to the INRAN tables.

All of the foods were divided into 25 different food servings. For example, milk, eggs, and cheese were counted as 3 different food servings and were not simply defined as "dairy." The first course was treated as a separate category, because it involved the preparation and cooking of food (e.g., pasta, together with a sauce made of sausages, pulses, vegetables, oil or butter, and cheese or other dressings).

Before the baseline assessment, all participants were given instructions on how to complete the questionnaire, and those admitted to the trial were taught how to choose alternative foods with a lower calorie content. A nutritionist was on hand for consultation during the baseline assessment and during the whole trial.

The participants decided mostly for themselves which types of foods to replace and also to plan their calorie restriction because the nutritionist only gave suggestions. In this way, the participants were responsible for their own dietary change and they were given relevant information about common foods and servings with a low calorie content. The main advantage of this method was that it was possible to analyze the efficacy of the product under investigation, PG or PL, and, at the same time, limit any bias linked to dietary compliance. We knew from our previous experiment in the same geographical area that 9 types of servings cover >75% of weekly caloric intake: first course, biscuits (during breakfast, during the day, or both), bread, cheese, vegetables, eggs, spirits, meat, and processed meat. Apart from vegetables and eggs, the nutritionist made an effort to convince the participants to reduce or replace the food servings that mainly contain carbohydrates or fats.

With regard to the modifications made in the first course, the approach was to halve the quantity of pasta (e.g., from 100 to 50 g)

and prepare the serving with 3 times the amount of the usual vegetables (from 20 to 60 g of any given boiled vegetable). In this way, it is possible to maintain more or less the same volume, but the caloric content can be lower by as much as 40%.

Reducing the consumption of biscuits [croissants and rusks (hard, double-baked biscuit) belong to the same category] was more difficult because they are part of the typical Italian breakfast together with milk, coffee, orange juice, and jam. It was suggested to replace them with omelets made with 1 egg and 10 g of any jam. In this way, the volume was almost exactly the same or even higher, but the caloric intake was ~30% lower. To decrease cheese intake, the suggestion was again to make omelets by using 1 egg and 20 g of any cheese. On the whole, the volume of the serving increased, whereas ~30% fewer calories were consumed. The participants were advised to reduce the total amount of spirits consumed or replace drinks containing 42–45% of alcohol with the same volume of those containing <30% of alcohol (e.g., limoncello, mirto, various amaros, dry or sweet marsala). Recommendations were also made to reduce calories from pizza: for example, to cut the crust off the pizza (corresponding to one-quarter of the serving). An Italian pizza weighs ~325 g and provides ~880 kcal. With the proposed suggestion, the serving can be reduced to ~660 kcal.

In addition, participants were given a list of alternative recipes, particularly for first courses, to help reduce the quantity of pasta eaten and to increase the amount of vegetables and pulses in the servings. In this way, it was also possible to minimize the amount of olive oil and bread eaten during meals.

The FIA provided a kind of "dietary fingerprint," in which the amounts in grams of the 25 categories were transformed into average food servings. For this reason, it was very important for the participants to be able to complete the FIA and correctly report the weight of the foods, so that these could be transformed into the number of average weekly servings.

Before starting the treatment, all of the data recorded by the participants were checked twice: first after the initial instructions so as to allow participants to become familiar with the questionnaire and the second time at the baseline examination. A data recording precision cutoff value was set as an admission criterion, on the basis of the Mifflin-St. Jeor (MSJ) equation. Only participants who recorded values with an MSJ score of ≥90% × 1.2 were admitted. The 1.2 factor was arbitrarily chosen to reflect minimal daily activity. The data recorded by the participants were inconsistent in ~13% of cases (25 of 187) because the calculated caloric intake was much lower than the quantity needed to maintain their metabolic rate at their given weight, sex, and age.

Physical activity

Physical activity was measured by using a simple questionnaire on daily activity, which was transformed into MET-h per day. Sedentariness (<35 MET-h/d) was very common; the participants were asked to spend just 1 h walking briskly every day, divided into 4 sessions to fit their daily activity. Considering that 1 h of slow walking corresponds to 2 MET-h and 1 h of brisk walking corresponds to 3.3 MET-h, the proposed increase in physical activity was equivalent to ~9 MET-h/wk. The time the participants spent walking was

reported (in hours) for 1 specific week every 3 mo, usually the week before their dietary check, and in the week before the end of the trial. Additional physical activity was not required, and if there had been any, it had to be reported together with their brisk walking time.

Other variables

BW, WC, blood pressure (BP; minimum and maximum), plasma lipids, glucose, and high-sensitivity C-reactive protein (hs-CRP) were measured at baseline and after every 3 mo (at 3, 6, 9, and 12 mo). BW was measured with the participants wearing light clothing and no shoes after voiding urine and feces and a 12-h overnight fast. If possible, the measurement was taken before blood sampling for laboratory analysis. A Tecnilab 2 scale with 50-g accuracy was used. If the participant suffered from temporary constipation, the BW check was postponed until the problem had been solved with increased water intake. WC was measured at the umbilical line by 2 different investigators, and the average value was recorded. BP was measured in both arms after the participant had been sitting for ≥10 min by using an A&D UA-851 digital blood pressure automated monitor, and the average value of the 2 measurements was recorded.

Plasma sampling for glucose, hs-CRP, and total, LDL, and HDL cholesterol was performed after a 12-h overnight fast and just after the BW measurement. A sample of 15 mL blood was obtained and divided into 3 aliquots of 5 mL each. All of the samples prepared for analysis were kept at 4°C until the analysis was carried out. The laboratory tests were performed with a Beckman Coulter AU 500 analyzer.

Procedures: treatment regimens and dosing schedule

The following products were used: PG (Formoline L112; manufactured by Certmedica International GmbH); this group was referred to as the PG group. The other group received a placebo consisting of excipients and gum arabic in the form of tablets that were identical to those of the PG group (referred to as the PL group). Each patient took 2 tablets/d with the 2 meals containing the highest fat content, which meant 4 tablets/d with 400 mg PG L112 or 400 mg placebo in the PL group. If the patients were receiving other treatments that consisted of lipophilic medications, they were asked to take them ≥2 h apart.

The trial medications provided by the manufacturer were packaged in identical blister packs. A double label with the randomization code was affixed to the blister packs by an independent, certified service provider. One of the labels was attached to the patient's case report form by the investigator at trial admission.

Treatment started immediately after admission to the trial. The participants were given 3 packs containing 48 tablets each, sufficient for 12 d of treatment. They were asked to return for more at the end of each month. Each participant was told that he or she could stop taking part in the trial at any time, without giving any reason, and without any negative consequences. They were asked to report adverse events or reactions at each examination, and their comments were recorded. After enrollment, 4 examinations were performed at 3, 6, 9, and 12 mo; and the FIA was carried out at baseline and at 3, 6, and 9 mo.

Sample size

The sample size was calculated on the basis of changes in the hs-CRP concentrations for PL and PG groups and not changes in BW. The measurements were taken at baseline and at 3, 6, 9, and 12 mo. We assumed an autocorrelation of the covariance equal to 0.7, a difference of ≥20% between the 2 groups, and a difference of ≥15% during the period of observation. The experiment should also detect an interaction effect of the same dimension of the "factor time." For this aim, the Geisser-Greenhouse corrected F test was used.

Considering a baseline value of hs-CRP equal to 5 with an SD of 0.12, a 95% power with 0.05, an α level of significance will be obtained by enrolling 50 participants/treatment group. In case of 25% dropout rate, 40 participants allowed a power of ≥80%.

Randomization

The randomization list was prepared by using JMP software (SAS Institute) before enrollment started and sent directly to a certified clinical trial logistics company for the final packaging of the samples. The 2 products were assigned to consecutive patients in chronological order of enrollment. No randomization number was omitted. Once a randomization number was assigned, it could not be reassigned, even if the participant could not actually take part in the clinical trial. The participants were not aware of the treatment group they belonged to. The following people or groups were also blinded to the treatment groups: investigators, staff (nutritionists and technical staff), laboratory workers, sponsors, and biostatisticians.

Compliance

Dietary compliance was measured by using an FIA questionnaire, and the treatment was checked every month by counting the remaining tablets. Physical activity was measured on the basis of the hours of brisk walking the participants reported during the test week (the week before the FIA).

Statistical methods

The procedure used involved a mixed ANOVA (split-plot design or between-within subjects ANOVA). The between factor was the 2 groups compared (PL and PG), whereas the within factor was the 4 or 5 examinations (baseline and 3, 6, 9, and 12 mo). The procedure was not only correct methodologically (simultaneous analysis of data for each variable) but also provided detailed information and considerably reduced the uncontrolled variability of the responses, which led to greater sensitivity or power of the analysis itself. Tukey's test was applied to determine differences between baseline and 12 mo, and also between products. Correlation coefficients were calculated between hs-CRP and the main components of food (protein, carbohydrates, lipids, sugars). The hs-CRP variable was analyzed by using a multiple linear regression model or standard least-squares model in order to look for hs-CRP predictors.

Other model information was obtained by graphical estimation, and a prediction profiler was used to examine the response. These options were chosen because several effects were analyzed with the use of few observations, and the aim was to find a strong effect rather than test for significance. The chi-square or Fisher's chi-square tests were used for frequency analysis.

Results

In total, the cohort of participants consisted of 187 cases, 87 of whom were excluded for the following reasons: 62 because their BMI was not in the range of the admission criteria and 25 because their recorded values on the FIA reported a caloric intake <85% than the value based on the MSJ equation. Of the 100 participants admitted, only 3 dropped out (2 in the PL group and 1 in the PG group), because they moved out of the area. A total of 97 participants completed the experiment: 50 men and 47 women (see **Figure 1**).

The general characteristics of the participants are reported in **Table 1**. No differences were found between the groups in terms of age distribution ($\chi^2 = 0.2240$), hypertension ($\chi^2 = 0.749$), smoking ($\chi^2 = 0.7666$), education ($\chi^2 = 0.749$), or physical activity ($\chi^2 = 1.000$).

The treatments were well tolerated, and no complaints of side effects were reported, apart from a few cases of constipation that were equally distributed between the 2 groups. This problem was solved by advising the patients to increase fluid intake.

The total caloric intake and main components of the food servings were recorded at baseline and at 3, 6, and 9 mo. Because the data between the individual intermediate examinations did not change substantially, only the averages of the 3 examinations (average over 12 mo) are shown in **Table 2**.

The weekly reduction in caloric intake was almost identical in both groups and slightly exceeded 10%. This was the aim of the diet. The average decrease in calories per week was 1667 kcal for the PG group and 1688 kcal for the PL group. This was mainly due to a decrease in carbohydrate intake (decrease of >860 kcal/wk for PG and PL groups), followed by lipids, alcohol, and protein, for which the reduction in both groups ranged between 240 and 300 kcal/wk. The change in the main dietary components was significant within the groups ($P < 0.05$) and was very similar for both groups ($P > 0.05$). The decrease in protein consumption

was almost identical in the 2 groups (49 g/wk for the PG group and 50 g/wk for the PL group), consisting of reductions of 7.5% and 7.8%, respectively.

The reductions in both carbohydrate and alcohol intakes were the most substantial in terms of percentage: carbohydrate intake was reduced to 221 and 215 g/wk for PG and PL groups, respectively, whereas alcohol intake was reduced to 43 and 37 g/wk respectively. In both cases, the difference between treatments was not significant ($P > 0.05$).

The water content in food was reduced to 499 mL in the PG group and 586 mL in the PL group, but again the difference between treatments was not significant ($P > 0.05$). Extra-virgin olive oil (EVOO) was considered separately, but the difference at baseline was not significant for either of the groups ($P > 0.05$). Its intake during the trial in terms of calories increased slightly in both groups, but again the values were not significant ($P > 0.05$). The decrease in lipid intake was more consistent for the PL group than for the PG group (33 and 27 g/wk, respectively) due to decreased cheese, processed meat, and milk consumption (see **Table 3**), but the differences were not significant ($P > 0.05$). Fiber intake was also lower in both groups. However, this was compensated for in the PG-treated group by the administration of 11.2 g PG/wk, because PG is a polycation fiber. The number of portions before and during the treatment is reported in Table 3.

The "dietary fingerprint" of the 2 groups was not identical; however, looking at the overall diet these differences may be considered as marginal or in the range of normal variability. The differences between treatments were not shown to be significant ($P > 0.05$).

Among the 25 servings, despite some modification in terms of percentages, intakes of 8 servings were found to be not significantly ($P < 0.05$) modified in either of the 2 groups: chocolate, dried fruit, pulses, meat, fish, yogurt, beverages, and chips. Intakes
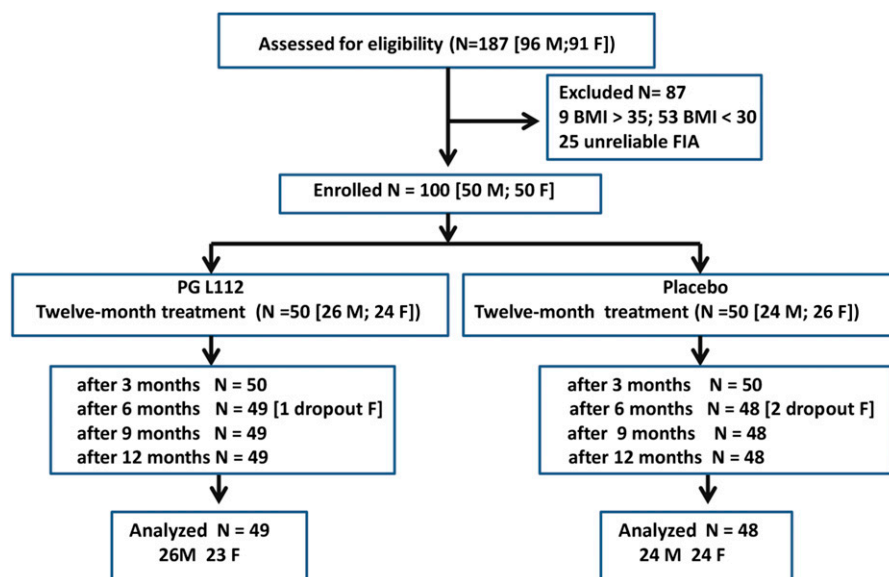

FIGURE 1 Patient recruitment for the single-center, randomized, double-blind, placebo-controlled clinical investigation of PG L112 in overweight and obese participants. FIA, food intake assessment; PG, polyglucosamine.

TABLE 1   General characteristics of the participants undergoing treatment with PG or PL[1]

Treatment	Participants, n	Age, y	BW, kg	Height, m	Hypertension, n	Smoking, n	Degree,[2] n	Physical activity <35 MET-h/d, n
PG	49	47.0 ± 7.75	95.3 ± 6.69	1.68 ± 0.06	8	12	19	16
PL	48	46.4 ± 4.42	95.0 ± 8.27	1.67 ± 0.09	9	14	17	17
PG (M)	26	46.5 ± 7.59	100.3 ± 3.99	1.72 ± 0.03	8	10	10	8
PG (F)	23	47.6 ± 8.07	89.3 ± 3.62	1.63 ± 0.04	0	2	9	8
PL (M)	24	46.9 ± 8.39	101.5 ± 3.66	1.74 ± 0.04	9	11	9	7
PL (F)	24	45.9 ± 10.51	89.6 ± 6.78	1.61 ± 0.06	0	3	8	10

[1] Values are means ± SDs unless otherwise indicated. BW, body weight; MET-h, metabolic equivalent task hours; PG, polyglucosamine; PL, placebo.
[2] Bachelor's or university degree.

of some of the servings (wine and beer) were reduced more consistently in the PL group, whereas there was a higher reduction in the servings of cake, ice cream, mozzarella, and fruit in the PG group. However, with regard to all of these servings, the results were found to be nonsignificant ($P > 0.05$) between both treatment groups.

Six food servings accounted for ~75% of the decrease in calories in both groups: bread, pizza, first course, cheese, biscuits, and spirits. There was a significant decrease in the intake of other food servings (sugar, milk, processed meat, wine, beverages, and cake), but this had a much smaller impact on the total weekly calorie count. Intakes of 2 food servings—vegetables and eggs—increased slightly but significantly ($P < 0.05$). With regard to vegetables, the suggestion to decrease intake of pasta by adding more vegetables to the dish was incorporated by many participants. With regard to eggs, the idea of making omelets was also well received in many cases and was in line with the reduction in carbohydrates (at breakfast) and cheese consumption (during the main meals).

Results of the main variables changed in both groups. However, this change was significantly more substantial in the PG group (see **Table 4**). Table 4 also shows data pertaining to physical activity in terms of average MET-h per day.

The decrease in BW in the PG group was 12.1 kg (−12.7%) compared with 8.0 kg (−8.4%) in the PL group ($P < 0.05$). The BW change with PG was also more rapid ($P < 0.05$), because the weight loss in the first 6 mo was 8.9 kg compared with 5.6 kg in the PL group. The decrease was less evident in both groups (3.2 kg for PG and 2.4 kg for PL) in the second half of the experiment (6–12 mo). However, the decrease in BW in the PG group was again significant ($P < 0.05$, Tukey's test). Only 17% (8 of 49) of patients in the PL group had achieved a reduction in BW of 5% at 3 mo, whereas 55% (27 of 49) in the PG group had achieved this reduction; this difference was significant ($\chi^2 = 16.04$, $P < 0.0001$). After 6 mo, the percentages were 67% and 98%, respectively ($\chi^2 = 16.43$, $P < 0.0001$).

The reduction in BMI was similar to the decrease in BW and was significant ($P < 0.05$) for both treatments. In the first 6 mo, the reduction in BMI in the PG group was −3, followed by a slower rate of decrease, which reached −4.3 after 12 mo. The decrease in BMI was significantly lower in the PL group ($P < 0.05$) and was marked by a flatter curve, which reached a decrease of only −2.8 at 12 mo.

The change in WC reached −13.3 cm in the PG group and −10.2 cm in the PL group ($P < 0.05$). In both cases, the most rapid decrease was recorded during the first 6 mo.

TABLE 2   Intakes of main food components in the week before treatment and during 1 y of treatment in groups treated with PG or PL[1]

Variable	Baseline		Combined values for 3, 6, and 9 mo		Change from baseline, %	
	PG	PL	PG	PL	PG	PL
Total energy, kcal	16,359 ± 1813.3	16,277 ± 1984.4	14,663 ± 1605.4	14,658 ± 1758.6	−10.2[2]	−10.5[2]
Water, mL	7052 ± 959.2	7165 ± 1042.7	6563 ± 748.3	6579 ± 1046.6	−6.2[2]	−8.2[2]
Protein, g	650 ± 78.3	643 ± 73.1	601 ± 66.7	593 ± 66.7	−7.5[2]	−7.8[2]
Lipids,[3] g	609 ± 79.4	602 ± 68.1	582 ± 74.8	570 ± 71.7	−6.1[2]	−5.3[2]
Carbohydrates, g	1836 ± 195.0	1825 ± 228.3	1615 ± 193.6	1610 ± 216.4	−11.5[2]	−11.8[2]
Fiber, g	131 ± 16.2	130 ± 15.5	135 ± 4.6	124 ± 15.9	+3.1[2,4]	−4.6[2]
Alcohol, g	149 ± 82.5	150 ± 88.2	106 ± 75.6	113 ± 82.9	−28.9[2]	−24.7[2]
EVOO, g	352 ± 42.7	347 ± 47.8	343 ± 46.8	340 ± 47.8	−2.6	−2.0
Percentage of total energy						
Carbohydrates	44.8 ± 3.34	44.7 ± 3.40	43.5 ± 3.50	43.8 ± 3.30	−1.8	−2.0
Lipids	33.7 ± 3.22	33.4 ± 2.43	35.2 ± 2.21	34.9 ± 2.66	+3.1	+4.5
Protein	16.2 ± 2.75	15.8 ± 1.18	16.2 ± 1.59	16.3 ± 2.09	0	+2.8
Alcohol	6.3 ± 3.26	6.2 ± 3.33	4.9 ± 3.10	5.2 ± 3.60	−20.6[2]	−16.1[2]
EVOO	19.5 ± 2.75	19.6 ± 2.96	21.1 ± 2.74	21.0 ± 2.47	+8.2	+7.1

[1] Values are means ± SDs unless otherwise indicated; n = 49 and 48 in PG and PL groups, respectively. EVOO, extra-virgin olive oil; PG, polyglucosamine; PL, placebo.
[2] Different between baseline and 12 mo, $P < 0.05$ (Tukey's test).
[3] Also includes EVOO.
[4] Different from PL, $P < 0.05$ (Tukey's test).

TABLE 3 Number of servings at baseline and during 1 y of dietary intervention in participants treated with PG or PL[1]

Type of serving	Baseline		Combined values for 3, 6, and 9 mo		Change from baseline, %	
	PG	PL	PG	PL	PG	PL
Sugar[2]	6.3 ± 2.08	6.4 ± 2.61	4.9 ± 2.51	5.4 ± 2.92	−25[3]	−23[3]
Chocolate	1.6 ± 1.48	1.7 ± 1.44	1.6 ± 1.32	1.8 ± 1.43	−8	+1
Milk	5.5 ± 1.86	5.3 ± 1.99	4.9 ± 2.00	4.6 ± 2.31	−8	−16[3]
Biscuits	5.1 ± 1.76	5.0 ± 2.01	4.6 ± 1.76	4.5 ± 1.43	−11[3]	10[3]
Bread	11.8 ± 2.69	11.9 ± 2.62	9.5 ± 2.18	9.7 ± 2.69	−19[3]	−18[3]
First dish[4]	9.0 ± 1.52	8.9 ± 1.85	8.4 ± 1.34	8.4 ± 1.72	−7[3]	−4
Pizza	1.0 ± 0.89	1.0 ± 0.80	0.8 ± 0.71	0.7 ± 0.67	−29	−18
Vegetables[5]	5.4 ± 1.51	5.4 ± 1.68	5.8 ± 1.57	5.8 ± 1.62	+10[3]	+11[3]
Fruit	11.2 ± 3.34	11.1 ± 3.26	10.0 ± 3.12	11.4 ± 3.03	−11[3]	+3
Dried fruit	1.4 ± 0.98	1.2 ± 0.91	1.4 ± 1.06	1.1 ± 0.97	−1	−13
Pulses	1.9 ± 1.03	1.8 ± 0.82	2.0 ± 0.99	1.7 ± 0.94	−5	+4
Meat	3.1 ± 1.04	2.9 ± 0.95	3.3 ± 0.96	3.0 ± 0.96	+2	+5
Processed meat[6]	4.1 ± 2.06	4.2 ± 1.56	3.7 ± 1.81	3.8 ± 1.32	−6	−11[3]
Fish	1.6 ± 1.02	1.7 ± 0.78	1.6 ± 0.95	1.7 ± 0.76	−5	−6
Cheese	4.9 ± 1.66	4.9 ± 1.92	3.6 ± 1.43	3.9 ± 1.68	−26[3]	−22[3]
Mozzarella	1.5 ± 1.36	1.4 ± 1.35	1.2 ± 1.19	1.2 ± 1.32	−19[3]	−11
Yogurt	3.1 ± 3.43	3.4 ± 3.49	3.2 ± 3.02	3.0 ± 3.05	+2	−13
Wine	7.5 ± 5.09	8.4 ± 5.44	6.6 ± 4.50	7.0 ± 5.01	−13	−16[3]
Beer	1.2 ± 1.89	1.2 ± 1.89	1.0 ± 1.52	0.8 ± 1.54	−14	−48[3]
Spirits	3.3 ± 3.03	3.1 ± 3.28	1.6 ± 2.29	1.10 ± 2.55	−52[3]	−65[3]
Beverages	1.6 ± 2.27	1.7 ± 2.82	1.3 ± 1.71	1.1 ± 1.71	−18	−34
Ice cream	1.3 ± 1.43	1.3 ± 1.67	1.1 ± 1.18	0.7 ± 1.22	−47[3]	−17
Eggs[7]	2.2 ± 1.95	1.8 ± 2.03	2.8 ± 1.85	2.8 ± 1.90	+30[3]	+58[3]
Chips	0.4 ± 0.73	0.50 ± 0.85	0.4 ± 0.75	0.5 ± 0.84	0	0
Cake	1.8 ± 1.59	1.8 ± 1.57	1.3 ± 1.31	1.6 ± 1.46	−29[3]	−11

[1] Values are means ± SDs unless otherwise indicated; n = 49 and 48 in PG and PL groups, respectively. PG, polyglucosamine; PL, placebo.
[2] Includes candies.
[3] Different between baseline and 12 mo, $P < 0.05$ (Tukey's test).
[4] Refers to complete dishes (also containing boiled vegetables/pulses, meat, oil/butter, cheese added to the any pasta or rice, polenta, gnocchi, and tortellini).
[5] Includes oil dressing.
[6] Includes hamburgers, cheeseburgers, and big burgers. Big burgers contain a higher amount of meat and cheese.
[7] Includes omelets and mayonnaise.

The secondary variables also showed progressive improvement, and again the results in participants treated with PG were better than in the PL group (see **Table 5**). Physical activity increased by 1.5 MET-h/d in both groups.

All of the variables improved ($P < 0.05$, Tukey's test) in both treatment groups, apart from HDL cholesterol, for which the increase was only significant in the PG group. Better results were obtained in the PG group for total cholesterol and hs-CRP from the third month, for TGs from the sixth month, and for LDL cholesterol from the ninth month onward ($P < 0.05$).

The minimum and maximum BPs in the PL group decreased significantly (−5.1% and −5.8%, respectively; $P < 0.05$, Tukey's test). Although the decreases were −8.8% and −7.8%, respectively, in the PG group, no significant difference was measured between the 2 groups ($P > 0.05$, Tukey's test).

With regard to plasma lipids, TG concentrations were the most affected by both treatments: −12.2% for the PL group and −17.3% for the PG group. No significant difference was found between the 2 treatments.

The average values of hs-CRP at baseline were close to 5 mg/L in both groups, and at the end of the year they were within the normal range (2.9 mg/L for the PL group and 2.1 mg/L for the PG group). However, only 13 of the 47 cases in the PL group had values within the normal range, compared with 48 of the 49

cases treated with PG (χ^2 = 17.82, $P < 0.001$). This aspect was analyzed by focusing on hs-CRP as the main variable, first considering the baseline data and then pooling the data for the 2 groups. A good correlation was found between hs-CRP and total calories (r^2 = 0.48, $P < 0.0001$); and when single components were considered, protein and carbohydrates were not correlated, whereas lipids were seen to be very well correlated (r^2 = 0.685, $P < 0.0001$). EVOO was treated separately from the other lipids, and no significant correlation was found (r^2 = 0.05, $P > 0.05$).

Multiple correlation values were considered separately for the PG and PL groups. None of the variables were found to be correlated with hs-CRP in the PG group. However, lipids again were shown to be directly correlated in the PL group, with the exception of EVOO.

Discussion

A strong point of this study is the dietary monitoring; however, at the same time, this is also its weak point, because only those who were actually able to understand the instructions provided to complete the rather complex FIA questionnaire were selected. To obtain reliable information about the results achievable with PG, we felt we had to be selective about the cases so as to avoid a large number dropping out, which is common in this type of long-term research,

8   Cornelli et al.

TABLE 4   Primary variables and physical activity at different times during 12 mo of treatment with PG or PL[1]

Time and treatment group	BW kg	BW Change from baseline, %	WC cm	WC Change from baseline, %	BMI kg/m²	BMI Change from baseline, %	Physical activity MET-h/d	PA Change from baseline, %
Baseline								
PG	95.2 ± 6.73	—	115.1 ± 8.65	—	33.9 ± 1.03	—	35.4 ± 1.12	—
PL	95.5 ± 8.07	—	115.2 ± 8.71	—	34.1 ± 1.03	—	35.0 ± 0.83	—
3 mo								
PG	90.5 ± 6.59	−4.9[2]	106.7 ± 7.11	−7.3[2]	32.2 ± 1.01	−5.0[2]	36.9 ± 1.22	+4.2[2]
PL	91.6 ± 7.37	−4.1[2]	109.4 ± 6.84	−5.0[2]	32.7 ± 1.07	−4.1[2]	36.6 ± 1.09	+4.6[2]
6 mo								
PG	86.3 ± 6.52	−9.3[2,3]	104.8 ± 7.70	−8.9[2,3]	30.8 ± 1.05	−9.1[2,3]	36.9 ± 1.32	+4.2[2]
PL	89.9 ± 7.06	−5.9[2]	107.9 ± 6.54	−6.3[2]	32.1 ± 1.14	−5.8[2]	36.5 ± 1.15	+4.3[2]
9 mo								
PG	84.1 ± 6.49	−11.6[2,3]	103.0 ± 8.09	−10.5[2,3]	30.0 ± 1.08	−11.5[2,3]	36.9 ± 1.26	+4.2[2]
PL	88.5 ± 7.04	−7.3[2]	106.1 ± 6.70	−7.9[2]	31.6 ± 1.15	−7.3[2]	36.5 ± 1.15	+4.3[2]
12 mo								
PG	83.1 ± 6.27	−12.7[2,3]	101.8 ± 7.89	−11.6[2,3]	29.6 ± 1.06	−12.7[2,3]	36.9 ± 1.26	+4.2[2]
PL	87.5 ± 6.94	−7.8[2]	105.0 ± 7.02	−8.8[2]	31.3 ± 1.23	−8.2[2]	36.5 ± 1.15	+4.3[2]

[1] Values are means ± SDs unless otherwise indicated; n = 49 and 48 in the PG and PL groups, respectively. BW, body weight; MET-h, metabolic equivalent task hours; PG, polyglucosamine; PL, placebo; WC, waist circumference.
[2] Different between baseline and 12 mo, $P < 0.05$ (Tukey's test).
[3] Different from PL, $P < 0.05$ (Tukey's test).

Investigators who are familiar with this kind of trial know how difficult it is for participants to maintain a long-term diet. In the light of this, dietary compliance was considered a fundamental aspect.

Many FFQs are available (16–20) that could have been adapted to the trial. However, we chose an FIA, because the investigators were more familiar with this kind of data recording (16, 21, 22).

The diet in this geographical area of southern Italy is generally defined as a Mediterranean diet (23). However, the population is progressively abandoning the foods associated with a healthy diet (24), apart from the use of EVOO. This means that to ensure the suitability of this FFQ in this study, it has to be adapted to the kind of food and cuisine distinctive of this particular region.

TABLE 5   Changes in secondary variables at different times during 12 mo of treatment with PG or PL[1]

Variable and treatment group	Baseline	3 mo Values	3 mo Change from baseline, %	6 mo Values	6 mo Change from baseline, %	9 mo Values	9 mo Change from baseline, %	12 mo Values	12 mo Change from baseline, %
DBP, mm Hg									
PG	75 ± 5.9	72 ± 5.4	−4.0	72 ± 4.5	−4.0	70 ± 4.2	−7.7[2]	68 ± 3.9	−8.8[2]
PL	75 ± 7.1	74 ± 6.4	−1.3	74 ± 6.4	−4.0	71 ± 4.8	−5.3	71 ± 4.0	−5.8[2]
SBP, mm Hg									
PG	139 ± 10.2	134 ± 3.7	−6.6	132 ± 6.6	−5.0	128 ± 5.7	−7.9	128 ± 4.9	−7.8[2]
PL	137 ± 10.1	134 ± 8.8	−3.6	132 ± 5.2	−5.0	130 ± 4.5	−5.1	130 ± 4.0	−5.1[2]
TC, mg/L									
PG	197 ± 10.2	187 ± 7.9	−5.0[2,3]	184 ± 8.1	−6.6[2,3]	179 ± 8.4	−9.1[2,3]	174 ± 7.6	−9.6[2,3]
PL	199 ± 14.7	196 ± 13.6	−1.5	194 ± 12.1	−2.5	192 ± 11.1	−3.5[2]	190 ± 11.4	−4.6[2]
LDL-C, mg/L									
PG	110 ± 15.1	104 ± 12.2	−5.4[2]	101 ± 11.1	−8.8[2]	96 ± 11.2	−12.7[2,3]	93 ± 12.6	−12.9[2,3]
PL	112 ± 18.5	109 ± 16.6	−2.7	109 ± 14	−2.7	107 ± 12.0	−4.5	107 ± 12.0	−5.3[2]
HDL-C, mg/L									
PG	46 ± 9.7	46 ± 7.9	0.0	47 ± 6.6	+2.2	48 ± 5.7	+4.3	48 ± 4.7	+5.4[2]
PL	46 ± 9.4	48 ± 8.0	+4.3	48 ± 6.5	+4.3	48 ± 5.9	+4.3	48 ± 5.1	+3.2
TGs, mg/L									
PG	206 ± 20.6	187 ± 10.2	−9.2[2]	174 ± 9.4	−15.5[2,3]	170 ± 9.1	−17.3[2,3]	170 ± 7.9	−17.3[2,3]
PL	204 ± 23.2	197 ± 20.6	−3.4	187 ± 20.4	−8.3[2]	182 ± 18.4	−10.7[2]	179 ± 17.9	−12.2[2]
Glucose, mg/L									
PG	98 ± 5.2	94 ± 4.6	−4.1[2]	92 ± 3.6	−6.1[2]	90 ± 4.1	−8.2[2]	88 ± 3.9	−8.9[2]
PL	99 ± 5.3	96 ± 4.7	−3.0[2]	96 ± 4.5	−3.0[2]	95 ± 5.1	−4.0[2]	96 ± 3.6	−4.1[2]
hs-CRP, mg/L									
PG	5.0 ± 1.18	3.6 ± 0.60	−28.0[2,3]	3.2 ± 0.47	−36.0[2,3]	2.9 ± 0.43	−42.0[2,3]	2.1 ± 0.51	−58.3[2,3]
PL	4.9 ± 1.11	4.6 ± 0.97	−6.1[2]	3.7 ± 0.69	−24.4[2]	3.4 ± 0.61	−30.6[2]	2.9 ± 0.35	−40.6[2]

[1] Values are means ± SDs unless otherwise indicated; n = 49 and 48 in the PG and PL groups, respectively. DBP, diastolic blood pressure; HDL-C, HDL cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, LDL cholesterol; PG, polyglucosamine; PL, placebo; SBP, systolic blood pressure; TC, total cholesterol.
[2] Different between baseline and 12 mo, $P < 0.05$ (Tukey's test).
[3] Different from PL, $P < 0.05$ (Tukey's test).

In the FIA system, the "cereals" are divided into various food servings (first course, bread, biscuits, and pizza, respectively) as they are eaten, and all of the other servings (vegetables, pulses, meat, and fish) are considered similarly. The validity of an FIA compared with other methods is beyond the scope of this trial, but our experience has taught us that advising people to eat fewer carbohydrates and high-fat foods or less cheese without giving precise information on the "final dish" they should eat leads to a high number of dropouts.

Another point that marked this trial was the focus on a decrease in calories per week, while maintaining the usual balance between carbohydrates, lipids, and proteins, and in particular, a reduced consumption of alcohol. The aim of this was to make changes in amounts of foods consumed without changing the structure of the diet. This would help to prevent any drastic alteration in the participants' eating habits, because the real objective was not to examine a diet but to show the effectiveness of PG while minimizing the dropout rate as much as possible during the long-term trial.

This is the first time, to our knowledge, that a long-term trial comparing PG with placebo has been conducted, and the results confirm what has been shown in previous short-term trials compared with placebo or other treatments (12, 13, 21, 22). Chitosans are a family consisting of a variety of polymers, and one would expect their fat-binding capacities and glucose, biliary salts, and water affinity (19–21, 25) to be different. However, attempting to predict their fat-binding activity on the basis of their physicochemical characteristics is controversial (7, 26). Their activity is limited to the gut, because they are not absorbed as a result of their polyanionic character. This does not mean that they cannot interfere (indirectly) with the metabolic pathways linked to cholesterol, glucose, TGs, and other variables, as was shown in this trial.

BP was reduced as BW decreased, similar to what was shown with hs-CRP. The effect on BP in both PL and PG groups was to be expected because this variable is linked to a decrease in BW. Weight loss was more substantial with PG, and the consequent BP decrease was not linked to any particular mechanism of action of PG.

The change in cholesterol concentrations is well known and well documented for most chitosans. The European Food Safety Authority allows chitosans to be advertised for cholesterol control, provided the daily dosage is ≥3 g, no matter what type of chitosan is concerned. The dosage of PG in this study was much lower (1.6 g/d), and the decrease in cholesterol was ~10%, most probably due to the diet followed at the same time, which is known to decrease cholesterol concentrations. The effect on TGs was also to be expected because dieting can decrease TG concentrations due to a lower intake of food and alcohol. However, PG also has a TG-binding affinity in vitro (20).

The decrease in glucose was to be expected, due to the reduction in BW and because an increase in the fecal excretion of glucose has been observed in rats fed a normal diet with added PG (10). A similar change has also been seen in other clinical trials (16, 21, 22). The mechanism of this effect is not known but may be due to the bacterial hydrolysis of short-chain chitosan, which

is present in a minimal amount in PG (27). Hydrolysis makes some glucosamine available. This, in turn, induces local insulin resistance (28), which prevents some of the glucose from being absorbed by colonic enterocytes.

The effect of PG on hs-CRP was also to be expected; this effect has been previously shown (U Cornelli, unpublished results, 2014) but the relevance of this effect is unknown. In our experiment, it seems that this effect is relevant, because the participants had hs-CRP concentrations out of the normal range before the treatment with PG and only 1 participant had an hs-CRP concentration >3 mg/L at the end of the study.

The activity of fiber on hs-CRP is controversial. An inverse relation with fiber intake was described (29) in the Women's Health Initiative Observational Study (30), but this was not confirmed by the same authors. However, hs-CRP concentrations are considered a good predictor of vascular events (31) and virtually all of the popular diets (Atkins, Ornish, Weight Watchers, and Zone) reduced C-reactive protein concentrations by ~15–20% (although these reductions were not significant, except in the case of the Zone diet) (32).

The participants treated with PL in our experiment showed a fairly substantial decrease in hs-CRP concentrations (−41% compared with baseline values), even though their fiber intake was lower than at baseline. This means that other factors may be important in determining the inflammatory conditions revealed with hs-CRP.

Caloric intake was directly correlated with hs-CRP, and particularly with lipid intake, according to the calculations made on the baseline data by pooling the PG and PL groups. Among the lipids, it was interesting to note that EVOO was not responsible for the increase in hs-CRP, which may show the importance of oxidized lipids. These are found in foods or created during cooking processes, and may be absorbed by enterocytes to form oxidized chylomicrons capable of spreading oxidation in vessels and tissues (32). EVOO limits the oxidative process in the gut due to its antioxidant content. Moreover, PG also has an antioxidant capacity, and one of its characteristics is its particular affinity for oxidized lipids (because they are more polar). This affinity decreases the "explosion" of oxidative stress in the gut. The general conclusion is that PG may protect against inflammatory conditions caused by lipids.

We can make one important observation by considering some comparable clinical trials carried out with PG. Taking the 2 previous double-blind trials with ≥3 mo of treatment at the same dosage, in one trial the average BW loss was 5.6 kg (12) (with an increase in activity of 1 MET-h/d) and in the other the average BW loss was 6.2 kg (13) (with an increase of 3 MET-h/d). In this trial, the weight loss at 3 mo was 4.7 kg (with an increase of 1.3 MET-h/d), which is significantly lower ($P < 0.05$). The reason for this discrepancy may be attributed to the degree of caloric restriction imposed: an average of <250 kcal/d instead of ~500 kcal/d in the 2 previous short-term experiments. However, this trial ended up with a BW reduction of 12.1 kg at 12 mo, which may indicate that PG favors a substantial amount of weight loss, even with relatively mild dietary restrictions. The PL group lost only 8 kg, and the efficacy of the method that was followed and the time needed to reach satisfactory results is debatable.

According to the MSJ equation and considering a factor of 1.2 for daily activity, the theoretical need for calories was a mean $\pm$ SD of 2033 $\pm$ 251.7 kcal/d at baseline for the PL group, whereas the calculated intake was much higher: 2315 $\pm$ 289.4 kcal/d. At the end of the trial, considering an average BW loss of 8.1 kg and a factor of 1.3 for daily activity, the theoretical requirement should have been 2259 $\pm$ 277.6 kcal/d, which is higher than the calculated intake of 2080 $\pm$ 254.7 kcal/d. This means that there is theoretically still room for further BW reduction by continuing with the same diet. The same calculation for the PG group also indicates that by continuing with the same protocol, it should be possible to achieve a further decrease in BW.

On the basis of the present experimental conditions, assuming a linear regression and considering the BW values between 3 and 12 mo (4%) in the PL group, it would be theoretically possible to reach a BMI of 25 in ~4.5 y, whereas with PG, this goal could be obtained in approximately half the time (i.e., after ~2.3 y). The same result is confirmed in the case of quadratic or logarithmic regression.

The only similar long-term experiment that we are aware of compared 4 different diets (Atkins, Ornish, Weight Watchers, and Zone) for a period of 12 mo (33) and followed cohorts of 40 participants who were very similar to ours in terms of BMI (average between 34 and 35). Comparing diets with a similar daily caloric intake decrease (251 kcal/d for the Zone diet and 244 kcal/d for the Weight Watchers diet) with that used in this trial (244 kcal/d), the decrease in body weight was <4 kg, and all of the other variables (physical activity, glucose, lipids, and hs-CRP) were much less affected than in the PL group of our trial. Moreover, the dropout rate was $\geq$35% of the trial enrollment. The reason for such a high number of dropouts was the participants' dislike for the diet or their inability to comply. Only 4% abandoned our trial.

Even though comparing trials carried out in different countries has to be done with care, we may observe that our method, which consists of minimal dietary restriction and light physical activity together with active involvement of the participants, can achieve better results than following common diets with more rigid rules. Practically all of the participants treated with placebo for 1 y achieved an 8.7% reduction in BW. These results were not obtained with the common medications used for the treatment of obesity (3), whereas the use of PG consistently accelerated the achievement of this goal.

In conclusion, treatment with PG for 1 y, combined with caloric restriction and light physical activity, was found to be significantly more effective than placebo, given the same experimental conditions. The use of the FIA questionnaire based on 25 different types of servings, and the adherence of the participants to their own level of caloric restriction, were found to be extremely important to help minimize the dropout rate.

Acknowledgments

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