VASCULAR AND METABOLIC RESEARCH

RICERCHE SPERIMENTALI IN TEMA DI PATOLOGIA VASCOLARE E METABOLICA
Pasta intake to reduce body weight

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Pasta (tagliatelle) enriched with chitosan from shellfish (hereinafter referred to as Polyglucosamine - PG) with standardized molecular weight and deacetylation degree, has been administered in a double-blind controlled experience to over-weight volunteers from the village of Preci (Umbria - Italy) in comparison with normal pasta (PP). Preci is a famous Italian village since it was the seat of the first European School of Surgery (the Abbey of S. Eutizio 800 a.C.) and it is known for the production of ham. Forty volunteers aged between 25 and 70 years (25 for the PG group and 15 for the PP group - control group) were enrolled for a two-month treatment with the two pastas. Both PG and PP group were well balanced for age and sex. Anthropometric measurements, laboratory analysis and caloric intake were recorded at baseline and after two months of treatment.

Twenty-two subjects (8 males and 14 females) from the PG group and 12 subjects (3 males and 9 females) from the PP Group completed the treatment. Drop-outs were determined by the monotony of eating every day the same type of pasta. An average reduction of 2.6±1.81 (SD) kg and 1±0.99 (SD) kg of body weight were respectively found in the PG and in the PP group. The caloric intake of subjects during the study was not modified in the PG group whereas an average decrease of 75 ± 62 (SD) kcal/day was registered in the PP group. This may justify the limited body weight reduction in the PP group. This caloric restriction in the PP group was determined both by a slight reduction of servings/week of cereals and by an increase of fruit servings per week. It is directly correlated (r = 0.887) with the body weight decline. Since subjects affected by hyperli- demia were under pharmacological therapy, a modification of cholesterol and triglycerides parameters was not expected. No side effects were reported. The effect of PG probably is due to its content of fiber which may reduce the glycemic index of pasta. In conclusion, data suggest that the use of pasta including PG is active in the control of body weight without any particular caloric restriction and may help people used to eat pasta frequently.

Key words: Chitosan - Polyglucosamine - Body weight reduction - Pasta.

The demand for weight loss in response to the increased frequency of overweight and obesity stimulates and increased consumption of weight loss supplements. The Federal Trade Commission and the regulatory agencies frequently challenge the efficacy of many dietary supplements used as an aid for weight loss.

Conflicts of interest.—Prof. U. Cornelli and Dr. M. Cornelli are managers of Corcon, Milan, Italy. Prof. U. Cornelli owns a patent application pertaining to pasta enriched with Polyglucosamine.
However, recently, a medical product containing chitosan (capsules or tablets, to be taken before meals) has been admitted by the European Union (EU) as body weight reductor and as a lipid-lowering agent.

A debate is currently reported in the available literature pertaining to the activity of chitosan, such that we find “chitoeaters”, 3-5 “chitoskeptical” 6-9 and even “chitoneutrals”. 10-12 It is a matter of fact that also people who need to reduce body weight and cholesterol levels try to use an available brand for a short period of time and according to its results join one of the mentioned groups. Most of the times, in vitro and in vivo studies and the brands on the market referred to different chitosans and results are hardly comparable due to differences in characteristics among these products.

To demonstrate a simple difference among chitosans it is sufficient to dilute 0.2 g of the raw material (if available) or even some capsules or grinded tablets from the commercially available brands, in 20 mL of water. Results show that each brand has different solubility and viscosity. 13 A more sophisticated analysis will determine that samples have also different degree of deacetylation 14 and molecular weights. Furthermore, according to the formulation (with or without Vitamin C they will show a different capability to emulsify with lipids. 15, 16

In other terms, chitosans are a class of different products and the claimed activity of each of them is determined by several factors such as source, molecular weight and type of end-formulation.

The aim of the present study is to determine the activity on human body weight of a well characterized chitosan which, for the first time, has been added into a flower used to produce a special pasta. The product (β-1,4 Polymer of D-Glucosamine and N-Acetyl-D-Glucosamine) - polyglucosamine (PG) was added to the flour for the preparation of pasta in a way to obtain a defined quantity for a specific weight. This pasta was then administered for a two-months period in a controlled trial (product evaluation) to overweight volunteers leaving in the village of Preci (Italy) in comparison with normal pasta (PP or placebo pasta).

Pasta (in the form of tagliatelle) was chosen because in Italy is an essential component of the normal diet and every diet-free of pasta is hardly maintained for a long period of time. The hypothesis was to substitute pasta with pasta with chitosan to manage body weight without altering the dietary habits.

Material and methods

Calling for enrolment

Preci is a village located in the region of Umbria (Central Italy) with a population of some 450 people. It is historically known for the Abbey of San Eutizio, in which the first European school of surgery was born (800 a.C.), and, more recently, for the production of ham.

Tranquillity and good food are such that people are frequently overweight, sometimes with high levels of cholesterol or tryglicerides. Diabetes and hypertension are also frequent. Most important, people are used to pasta. As soon as some people from the village knew that we were studying a new type of pasta they asked the major to test it and taste it.

The protocol for the evaluation of pasta - enriched with 2% of chitosan (PG) - was proposed as in a preliminary study 13 doctors and nurses, who were eating for 10 days both pasta and bread containing 2% PG showed significant body weight and cholesterol reduction.

The protocol was discussed and approved by the Committee of the village which was composed by the major, the physician of the National Health Service (ASL) and by representatives of the community. The decision was to test pasta containing chitosan and not the combination of bread and pasta. After the approval, the study was presented to the people of the village during an ad-hoc community meeting calling for volunteers. The local newspaper published an article on the study such that many people were asking to be enrolled in the trial.

A mobile lab unit was used to stimulate the enrolment of subjects. Since it was a 13
were composed by 25 subjects treated with PG and 15 subjects treated with PP. Subjects were randomly allocated to one group or the other. In the PP group any significant difference of anthropometric parameters and lab assessment was expected and consequently this group was composed by less cases. The study was two months long, and volunteers were divided in two groups: the first group (10 males and 15 females) received “tagliatelle” (‘flat’ noodles) containing 2% of PG; the second group (14 males 11 females) was administered with normal pasta PP (placebo pasta). PG and PP were prepared by the same producer. (Trevi - Italy)

Volunteers were instructed not to take vitamins, minerals, or other dietary supplements during the study and to maintain the usual physical activity.

In order to determine possible variations of the diet, total energy intake of all subjects was measured at baseline and at the end of treatment. The energy intake was calculated through a register which was filled up by a dietician during the interviews with subjects. Interviews were based upon a questionnaire in which the number of servings/week were recorded together with the dimension of the servings (small, medium, large and very large) which corresponded to different amounts according to the type of food. The amount of Kilo Joules (KJ) was determined through a standard reference.

Physical activity was also recorded through a four point scale (sedentary; normally active; moderately active; very active).

Daily KJ intake was calculated at baseline and at the end of treatment. Subjects were controlled for body weight, Body Max Index (BMI) and measure of the abdomen (umbilical line). After overnight fasting blood was collected from all subjects to determine cholesterol, triglycerides, glucose, creatinine and transaminases before treatment and immediately after.

Participants who were suffering from any disease during the trial had to stop the administration of pastas and in case the interruption was more than five days long they were excluded from the evaluation.
The experience was completed in about three months, from April to July 2005.

**Characteristics of the pasta used in the study**

PG and PP servings were given weekly to subjects. Pasta was prepared weekly and the standard packaging weighted 600 g/bag. Each bag reported the number of the subject and the dietician distributing pasta was not aware of the content of each bag.

**Chitosan**

The chitosan was selected among different raw materials. We chose a chitosan with a degree of purity >90%, a degree of deacetylation >85%, and a molecular weight (MW) of 125-145 KD. MW was determined using a triple detector. The capability to create a complete emulsion with extra virgin olive oil in the ratio 1:10:10 (respectively chitosan, water, and extra virgin olive oil) was the most important factor. The complete emulsion (emulsion test) was obtained in a cuvette (1 cm x 1 cm x 5 cm) after 90 shakings of the mixture composed by 1 mL of the suspension containing 100 mg/mL water and 1 mL extra virgin olive oil. The product with these characteristics was a mixture of 91% chitosan, 6% L-ascorbic acid, and 3% tartaric acid. This product was called polyglucosamine (PG). The same raw material without the presence of ascorbic and tartaric acid was inactive in the emulsion test.

**Statistical analysis**

All data were analyzed using mean and standard deviation (SD) and an ANOVA. Differences before and after each treatment were determined using the t-test for interdependent data. Differences between treatments were determined comparing the differences before and after each treatment using the t-test for independent data. Differences among frequencies were evaluated using the Chi-square test. Correlation coefficient (r) was also calculated in order to determine a relationship between body weight and some of the other parameters.

**Results**

Of the 40 subjects that have been enrolled 34 completed the study (22 treated with PG and 12 treated with PP). Six subjects were considered as drop-outs (3 using PG and 3 PP). The cause for leaving the study was the monotony of eating the same pasta every day. These subjects left the study between the second and forth week of treatment.

The characteristics of the groups of volunteers who concluded the experience are reported in Table 1.

The groups of subjects are similar for all parameters with the exception of body weight that is significantly higher for the PG group. Unfortunately this occurred despite randomization. Among the subjects with hyperlipidemia (class IIa) 4 were under treatment with lipid-lowering drugs (one in the PG group and three in the PP group).

Table II summarizes the data concerning the number of servings/week. Quantities were also determined for each serving in terms of small, medium, large, very large portion. The number of daily servings is similar for both groups. Differences in dairy products, fruits and vegetables consumption are not statistically significant. However, in the PG group the caloric intake is higher than in the PP group and even though not statistically significant it can partially justify, the difference in body weight between the groups.

The average amount of servings was comparable in both groups. The differences in energy intake seem to be determined by the number of servings, particularly by dairy products and fruit which have a higher average value in the PG group. Physical activity was comparable in the two groups.

Anthropometric, vital and laboratory parameters are reported in Table III as differences between the values determined at the end of treatment and at baseline.

Both groups showed significant differences between before and after the treatment. Body weight, BMI and abdominal circumference were affected by both treatments. However the PG group had a more relevant impact on these measures than the PP group (t-test p<0.05)
The number of servings/week is reported in Table IV together with the differences in terms of calories intake.

The PG group showed no differences in the number of servings/week, whereas in the PP group a significant difference was registered as an increase in the intake of fruits and as a reduction in cereals consumption. These differences determined a statistically significant modification of the caloric intake in terms of reduction and can be determined for the body weight reduction in this group. Body weight reduction in the group treated with PG does not have a concomitant modification of the caloric intake. The dimension of servings was not modified in both groups (data not shown) and consequently the differences found belong to the number of servings.
Discussion

The small community of Preci is mainly composed by elderly people such that volunteers enrolled in the trial represented around the 40% of the population between 25 and 70 years of age leaving in the village. Only a small food store is available with a limited variety of foods, and fruits and vegetables are those strictly related to the season. Therefore Preci can be defined as a relatively stable community. The results of this trial have to be carefully considered, due to the variability of parameters and the peculiarity of the environment. Data showed that the use of PG pasta as a substitute of normal pasta is determining a body weight reduction.

A first comment has to be addressed to baseline data which show that the PG group is characterized by a significantly higher body weight than the PP group. Despite randomization the groups, at inclusion, were different in weight. However, the BMI of both groups at baseline was comparable.

If we consider the reduction in body weight as a percentage of base line values, the PG group showed a 2.9% decrease whereas the control group registered only a 1.3% decrease. There is no significant direct correlation between body weight at baseline and body weight reduction in any group (PG group r=0.24 P>0.05; PP group r=0.28 p>0.05).

To confirm the activity of PG pasta, a statistical sub-analysis was carried over for those cases with a BMI between 25 and 35 only. Following these criteria the baseline difference between groups disappeared but still the body weight reduction and the abdominal circumference reduction in the PG group are significantly higher than in the PP group (more than the double).

The PP group was probably psychologically influenced by its participation in the trial, since all anthropometric parameters were favourably modified. This seems to be determined by the reduction in servings of cereals associated with the increase in fruit consumption.

The average caloric intake of the PP group decreased of 75 kcal/day and this auto-restriction could justify the body weight decrease found in the group, since average daily physical activity was not modified. This hypothesis is supported by the correlation between body weight reduction and caloric intake reduction (r = 0.874 and p<0.05). In the PG group the caloric intake remained constant, and this confirms that the activity on body weight is due to the use of pasta containing PG.

Cholesterol and triglycerides modification were not expected because few subjects only were hyperlipidemic, and some of them were already under treatment with drugs. Furthermore, a total amount of 1.6 g/day chitosan was administered to the PG group which is a relatively low dosage. However, in another study (in press) a slight but significant reduction of total cholesterol and triglycerides levels was shown even with this dosage of PG.

The experience was concluded with the enthusiasm of people loosing weight, and the request to continue for those that could not reach a satisfactory weight reduction. No side effects such as constipation or diarrhoea were observed and, excluding from the relative monotony of eating the same type of pasta for two months, no other complaints were reported.

At the end of the trial suggestions to prepare at least five or six different type of pasta were given by volunteers. Some other indications can be drawn from this experience.
which can be used for further trial in this area.

The first indication is related to the importance of the Community representatives in obtaining the full support of the population. In these small communities it is sufficient to explain the problems related to body weight and show the importance in controlling it to immediately obtain an effect within the community. In fact volunteers that were eating PP showed a significant body weight reduction which is clearly related to a reduction of the daily caloric intake.

In previous animal studies the same raw material administered to rats (2% of pellets) for a period of 2 months was found to reduce their body weight despite food and water consumption were the same. No effects on cholesterol or triglycerides levels were shown. The same product administered in capsules, with similar daily dosages and for the same period of time, showed to reduce body weight, cholesterol and triglycerides levels in hyperlipemic subjects (type IIa) when given 30 minutes before main meals (Cesarone MR: Data on file).

PG in pasta could act as a fiber reducing the glycemic index of pasta. Consequently the released reduction in insulin probably exerts a favourable metabolic effect for body weight reduction.

In conclusion, even though the admission criteria to this experience were wide, to simulate the condition of people eating pasta, the body weight reduction in the group treated with pasta containing PG was significant.

If this specific chitosan and PG showed to be active this does not mean that every chitosan can be similarly active in reducing body weight.

In vitro tests supporting the activity of chitosan may only give few indications, sometimes misleading, which need to be confirmed with specific experiences in humans.

**Riassunto**

**Mangiare pasta per ridurre il peso corporeo**

In uno studio controllato, in doppio cieco, a volontari in soprappeso del paese di Preci (Umbria, Italia) è stata fatta mangiare pasta (tagliatelle) arricchita con chitosano ottenuto da crostacei (d’ora in poi poliglucosamina, PG) con peso molecolare e grado di desacetilazione standardizzati, confrontando i risultati con quelli ottenuti mangiando pasta normale (PP). Preci è un paese famoso, essendo stata la sede della prima Scuola Europea di Chirurgia (Abbazia di S. Eutizio, 800 a.C.) ed è noto per la produzione di prosciutti. Sono stati arruolati 40 volontari di età compresa tra 25 e 70 anni (25 si sono nutriti con PG e 15, il gruppo di controllo, con PP) che sono stati trattati per due mesi con i due tipi di pasta. Entrambi i gruppi erano ben bilanciati per quanto riguarda l’età e il sesso. Le misurazioni antropometriche, le analisi di laboratorio e l’apporto calorico sono stati registrati al baseline e alla fine dei due mesi di trattamento. Hanno completato il trattamento 22 soggetti del gruppo PG (8 di sesso maschile e 14 di sesso femminile) e 12 del gruppo PP (3 di sesso maschile e 9 di sesso femminile). Le uscite dallo studio sono state determinate dalla monotonia derivata dal dover mangiare tutti i giorni lo stesso tipo di pasta. Rispettivamente, nel gruppo PG e in quello PP si è avuta una riduzione media del peso corporeo pari a 2,6±1,81 kg (SD) e di 1±0,99 kg (SD). Durante lo studio l’apporto calorico dei soggetti non è stato modificato nel gruppo PG, mentre si è avuta una diminuzione media di 75±62 (SD) kcal/die nel gruppo PP. Questo può giustificare la limitata riduzione del peso corporeo nel gruppo PP. Questa restrizione calorica nel gruppo PP è stata determinata sia da una leggera riduzione delle portate/settimana di cereali, sia dall’aumento della frutta mangiata ogni settimana. Essa è direttamente correlata (r=0,887) alla diminuzione del peso corporeo. Dal momento che i soggetti affetti da iperlipidemia erano in trattamento farmacologico, non ci si attendeva una modifica dei livelli di colesterolo e di trigliceridi. Non sono stati osservati effetti collaterali. L’effetto di PG è probabilmente dovuto al suo contenuto in fibre che può ridurre l’indice glicemico della pasta. In conclusione, i dati suggeriscono che il PG sia attivo nel controllo del peso corporeo, senza particolari restrizioni caloriche, e che può essere utile per le persone che mangiano frequentemente pasta.

Parole chiave: Chitosan - Poliglucosamina - Riduzione del peso corporeo - Pasta

**References**

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