MINERVA CARDIOANGIOL 2008;56(Suppl. 1 to issue N. 5):71-8

Use of polyglucosamine and physical activity to reduce body weight and dyslipidemia in moderately overweight subjects

U. CORNELLI [1], G. BELCARO [2], M. R. CESARONE [2], M. CORNELLI [3]

[1]*Loyola University Medical Center Maywood, IL, USA*
[2]*University of Chieti, Chieti, Italy*
[3]*Corcon s.r.l., Milan, Italy*

Aim. **A low molecular weight chitosan (polyglucosamine, PG) was studied in overweight hyperlipemic patients under physical training. A double blind study was conducted in two groups of 30 patients (M/F; from 25 to 59 years).**
Methods. **Tablets containing PG (2 g/day) or placebo were given for a 4-month period during a physical training (+8 MET-hours/week). Anthropometric measures, caloric intake, blood pressure, LDL and HDL cholesterol, blood glucose and triacylglycerol were measured before and after the treatment. The groups were similar for the caloric intake and expenditure and ended up with positive results in most of the parameters examined.**
Results. **In PG group compared to placebo a more significant (P<0.05, t test) reduction was found for body weight (respectively 6.9±1.87 *vs* 3.0±1.61 kg), waist circumference (7.3±2.49 *vs* 3.1±4.21 cm), LDL cholesterol (44±14.7 *vs* 12.5±12.6 mg/dL), triacylglycerol (52±29.3 *vs* 39±15.2 mg/dL); HDL increase was also higher (6±3.6 *vs* and 3±4.2 mg/dL). At baseline metabolic syndrome (MS) according to ATP III was present in 15 and 14 patients respectively in the group PG and placebo.**
Conclusion. **Unexpectedly, at the end of the treatment MS was reduced in 12 cases of the PG group and in 3 cases only of the placebo group (P<0.05). Results indicate that PG may improve the effect of the physical training in moderately overweight patients with dyslipidemia and may be of some help in the treatment of MS.**

Key words: **Polyglucosamine - Dyslipidemia - Cholesterol - Triacylglycerol.**

Chitosans as a class of products have been studied extensively for biological aspects [1, 2] and toxicology.[3] The use of chitosans for body weight and cholesterol reduction has been debated in literature as there are trials showing positive results [4-9] as well as uncertain or negative results.[10-14]

However, experimental conditions adopted in different trials are not comparable. Obese subjects or simply overweight subjects may be differently affected by chitosans. The type of food in terms of caloric contents, or even the amount of saturated and unsaturated fats in food, can determine different results.

The specific type of chitosans used for the treatment is an important factor also, since it is known that chitosans can differ in

Acknowledgments.—European Society of Biological Nutrition (SENB), Milan, Italy and Cor.Con. Srl, Milan wish to thank the authors for assistance for providing the raw material.

Conflicts of interest.—Prof. U. Cornelli and Dr. M. Cornelli are managers of Corcon, Milan, Italy.

terms of density, molecular weight,[15] derivation (from crab or crayfish chitin), and formulation. The presence in the formula of ascorbic acid or other components may modify the ability of the chitosans to bind water and lipids.[16, 17] Simply comparing aqueous solutions of different chitosans a remarkable variation can be noted in the ability to bind water and to form emulsions with oils of common use (rapeseed oil, extra virgin olive oil).

Attempts to perform a meta-analysis of randomized controlled trials with at least 4 weeks of treatment [18] hesitated in the conclusion that chitosan seems to be more active than placebo, but the activity should be confirmed by more rigorous clinical trials.

Although the various trials considered in this meta-analysis were in the limits of an acceptable methodology, different kinds of chitosan, dosages and formulations were used.

The present trial is based on the activity of polyglucosamine (PG) which is a low molecular weight acidified chitosan. PG has been used in capsule, in a double blind randomized 4-month study to determine its activity on body weight and lipids blood levels in moderately overweight patients suffering from dyslipidemia and under physical training.

Materials and methods

Subjects under treatment and experimental designs

Sixty subjects were enrolled in two cohorts, one cohort between the last week of August and the first two weeks of September and the second between January and March (next year). Calling for enrolment was conducted by posters exposure in two gymnasia provided with medical center asking for people who wished to start a program of physical training. The subjects took part to this experience with informed consent.

The protocol was submitted to the Ethical Committee of the gymnasia. The suggestion from the Committee was to avoid the treatment with placebo only (as proposed originally) because a 4-month period without any physical or pharmacological therapy was considered a risk for the subjects admitted to the trial.

Admission criteria were the following: males and females in the age between 30 and 60 years with a body mass index (BMI) ranging from 26 to 30 kg/m^2, total cholesterol levels >200 mg/dL and triglycerides levels from 199 to 400 mg/dL. Coherence between the energy intake (through a questionnaire) and body weight were also one of the admission criteria.

The body weight reduction was considered as a main parameter whereas the modification of plasma lipids picture was taken as secondary parameter.

Subjects under therapy with hypolipidemic drugs or allergic to crustacea were not admitted. Exclusion criteria were chronic diseases and any therapy for body weight reduction. Any disease occurring during the study would have caused the exclusion from the trial if lasting more than four days.

No particular alimentary precaution was requested, and subjects were asked to keep the habitual diet with the only variation of hydrating with at least 1.5 litres of water a day in addition to the one taken with the meals.

With the assistance of a professional nutritionist the caloric intake of participants was determined at baseline, and monthly for the following 4 months. A questionnaire was used where 250 different type of food were listed.[18] Subjects were asked also to indicate the number of servings/week for the following types of food: vegetables, fruit, cereals, legumes, meat, fish, dairy products, alcohol and eggs. The amount of each portion as little, medium, and large portion (respectively P, M, G) was recorded. Photographic examples of P, M, G portions and corresponding amount of grams were enclosed to the questionnaire only for those foods having high caloric content (cereals, meat, cheese, legumes).

A part of the physical training, subjects were urged to keep their habitual occupational and leisure activities.

Physical training

A standardized mild physical training was applied to the patients consisting of bicycling (10-13.9 mph), dancing (aerobic modern) and treadmill. Each of these type of exercise was limited to 4-6 MET-hour [19] and the patient had to undergo each of the three activities for 15-25 min in one single session twice/week in the gymnasium. Each session lasted 1 h, no matter how the exercises were combined. Subjects were assisted during the performance by a trainer in order to maintain the MET-hour/day close a total of 8-MET hour/week.

Polyglucosamine

A chitosan with an average molecular weight (MW) between 125 and 145 kD was chosen. The molecular weight reduction was obtained by mechanical milling of chitosan up to a polymer with the desired MW. The molecular weight was determined by Size Exclusion Chromatography (SEC) by Triple Detector Array (TDA) (20). This low MW chitosan was added to L-ascorbic acid and tartaric acid in the proportion of 91% chitosan, 6% L-ascorbic acid, and 3% of tartaric acid. This preparation was called "linear polyglucosamine" (PG) or FF45. The term "linear" means that the negative charges of L-ascorbic acid and tartaric acid reduce the tendency of the polymer to form aggregates and convolutions, which may hidden the positive charges of the glucocamines. For these reasons PG has less tendency than high molecular weight chitosans to form aggregates or convolutions. This PG has was already tested in experimental animals and found active in reducing body weight once added at 2% concentration to the rats diet.[21]

Tablets preparation, administration and distribution

Tablets were produced adding 500 mg of PG (active ingredient) or lactose (placebo) to the usual excipients for pharmaceutical preparations. The two types of products were almost identical and given to subjects in identical boxes containing 4 blisters of 12 tablets each.

Products were distributed according to a randomization list. Subjects were taking 4 tablets/day (2 tablets twice a day) 30 minutes before the two main meals with half a glass of water. The therapy lasted for four months. Immediately after the assignment to the treatment, a product supply for 2 weeks was distributed to the subjects. Subsequent supplies were given after 2 weeks and than monthly for the following three months. Therefore, the subjects referred to the experimental center for the product supply and undergo to an interview about the general tolerability of the treatment. One item of the record form concerned the presence of constipation or diarrhea.

At each control subjects had to give back to the center the empty boxes for the evaluation of the compliance. In some cases (10 cases: 6 with the PG and 4 with the placebo) the product has been sent by mail, following a telephonic agreement with the patients. No other control for compliance was requested.

Measurements and lab assessment

After the evaluation of the admission criteria and following an overnight fasting the subjects were submitted to anthropometric test such as height, body weight, waist circumference at the umbilical line (WC). Blood pressure (Max and Min) was also measured, followed by blood sampling for the determination of total cholesterol, LDL, HDL cholesterol, triacylglycerol and blood glucose. All the lab data were determined in the same lab analysis for all the participants after a 5 mL blood sample from the brachial vein. The blood was collected in heparinized test tubes which were immediately centrifuged and submitted to lab assessment.

Standard methods by means of a digital spectrophotometer (Free System Diacron-Grosseto-Italy) and dedicated reagents were used.

Statistical analysis

Groups of 25 subjects each, in case of a difference of body weight reduction of one standard deviation (SD) for 1-β 0.9 and α 0.05, gives to the trial a power >0.9. Considering a

TABLE I.—*Characteristics of the two groups of subjects at baseline.*

	PG Group	Placebo	p[a]
Male/total	16/28	14/28	NS
Female/total	12/28	14/28	NS
Age	39±8.2	40±6.5	NS
Height [m]	1.73±0.07	1.73±0.08	NS
Body weight [kg]	81.6±6.75	81.8±13.06	NS
BMI [kg/m^2]	27.4±1.39	27.4±1.14	NS
MET-hours/week [b]	248±19.0	249±20.0	NS
Waist circumference [cm]	95.2±5.32	95.3±5.55	NS
Systolic BP [mmHg]	128±10.3	124±8.1	NS
Diastolic BP [mmHg]	70±4.7	66±5.2	NS
Total cholesterol [mg/dL]	228±19.5	235±17.6	NS
HDL [mg/dL]	42±6.9	41±8.7	NS
LDL [mg/dL]	143±19.7	147±19.5	NS
HDL/LDL	0.30±0.067	0.29±0.057	NS
Triacylglycerol [mg/dL]	215±30.1	235±29.9	NS
Glucose [mg/dL]	98±11.4	94±10.3	NS
Other characteristics and diseases			
— Blood glucose >110 mg/dL	6/28	3/28	NS
— Smoking	11/28	10/28	NS

[a]Differences in frequency were determined using the χ^2 test (Yates correction); mean differences were tested using the Student's t test for independent data; [b]physical activity.

maximum drop out percentage of 20%, groups of 30 subjects were formed.

The differences among the various groups were computed on the basis of the means and SD. The comparisons were carried out by the Student's t test between the basal values and the ones at the end of the treatment (interdependent two-tailed test), as well as on the differences between before and after the treatment with PG Vs controls (independent two-tailed test). The differences between frequencies have been determined on the base of both exact chi-square test (Fisher) and chi-square with Yates correction. For every test α 0.05 was chosen as limit of statistical significance.

Results

Seventy-five subjects asked to participate to the trial but only 60 subject were fitting all the admission and exclusion criteria. Fifteen cases were excluded due to the history of a recent myocardial infarction and/or a BMI>30.

Among the 60 patients 4 were drop-outs as they did not came to the physical training in the second or third week. They were male, two under treatment with PG and two with placebo, and the reason for the interruption was the lack of time to continue the physical training. Therefore, the assessment of the differential action of the two treatments is limited to 28 cases/group.

All the 58 remaining participants ended the trial period. Two episodes of constipation (one/group) and one episode of transient diarrhea (PG treatment) were reported. Treatment was not suspended in any of the subjects and the symptoms recovered completely in few days. No other episodes of intolerance were reported.

General data concerning the subjects are collected in Table I which shows that at baseline values of the two groups were very similar.

Table II summarizes the data related to the weekly number of servings divided by the nine food categories. For these items also the two groups were very similar. The dimension of the servings (P, M, L) were practically identical in the two groups (data not reported) as evident from the MJ/day which did not differ between groups.

In Table III are summarized the differences between the end of treatment and the base-

TABLE II.—*Characteristics of the two groups of subjects at baseline.*

Food category	N. of servings/week		pa
	PG Group	PP Group	
Vegetables	5.0±1.23	5.2±1.28	NS
Fruit	6.8±3.95	6.9±2.99	NS
Cereals (and potatoes)	23.5±2.03	24.9±2.85	NS
Pulses	1.7±1.24	1.8±0.88	NS
Dairy products	6.0±1.23	5.7±1.41	NS
Meat	5.7±1.49	5.5±1.62	NS
Fish	1.9±0.72	1.8±0.79	NS
Eggs	1.6±0.50	1.7±0.48	NS
Alcohol[b]	6.2±4.90	6.2±3.50	NS
MJ/day	11.5±1.32	11.8±1.64	NS

[a]Student's t test for independent data; [b]one serving correspond to: 120 mL of wine; 330 mL of beer; 40 mL of liquor.

TABLE III.—*Differences between values at the end of the treatment and at baseline; total pasta and energy intakes.*

	PG Group	PP Group	t test[a]
Body weight [kg][c]	-6.9±1.87[b]	-3.0±1.61[b]	P<0.05
BMI [kg/m^2]	-2.3±0.84[b]	-1.0±0.53[b]	P<0.05
MET-hours/day	7.8±2.3[b]	7.9±0.6[b]	NS
Waist circumference [cm]	-7.3±2.49[b]	-3.1±4.21[b]	P<0.05
Systolic BP [mmHg]	-4±4.9[b]	1±3.0	P<0.05
Diastolic BP [mmHg]	-2±2.5[b]	1±3.7	P<0.05
Total cholesterol [mg/dL]	-48±14.2[b]	-19±12.5[b]	P<0.05
HDL [mg/dL]	6±3.6[b]	3±4.2[b]	P<0.05
LDL [mg/dL]	-44±14.7[b]	-12±12.6[b]	P<0.05
HDL/LDL	0.19±0.073[b]	0.04±0.041[b]	P<0.05
Triacylglycerol [mg/dL]	-52±29.3[b]	-39±15.2[b]	NS
Glucose [mg/dL]	-7±8.6[b]	-2±8.9	P<0.05
MJ/day	0.1±0.39	-0.3±1.24	NS

[a]Student's t test for independent data; [b]P<0.05 t-test for interdependent data.

line values for all the parameters under analysis.

The energy intake (MJ/day) was not significantly modified during the treatment in any of the two groups and the physical training in terms of MET-hours/week increase was very similar for both, whereas all the other items showed a favorable change.

Physical training was found significantly effective (t test P<0.05) for the reduction of body weight (3.7%), LDL (8.5%), WC (3.3%), triacylglycerol (18.7%), and for the increased of HDL (7.5%) and HDL/LDL ratio (12%).

More consistent results were obtained by the combination of PG an physical training for the reduction of body weight (8.4%), LDL (30.8%, triacylglycerol (24.3%) and for the increase of HDL (14.9%) and HDL/LDL ratio (38%). All the differences between PG Vs placebo were statistically significant (t test P<0.05) with the exception of triacylglycerol levels.

In the PG treated group BP and blood glucose also were significantly reduced, whereas with the placebo they remained practically unchanged.

When data were analyzed in terms of presence of metabolic syndrome (MS) according to ATPIII,[22] surprisingly it was found that patients of the group treated with training plus PG recover from the MS in a very consistent percentage. The reason of this improvement were due to a consistent reduction of WC concomitant to an increase of HDL

TABLE IV. — *Subjects with metabolic syndrome (ATP III) and frequency of single symptoms before and after the treatment.*

	PG Group		PP Group	
	Before	After	Before	After
Metabolic syndrome	15/28	3/28[a]	14/28	12/28
WC	10/28	0/28[b]	13/28	12/28
HDL	16/28	7/28[b]	16/28	16/28
Tryacilglycerol	28/28	25/28	28/28	28/28
Blood glucose	6/28	2/28	3/28	1/28
BP	11/28	5/28	6/28	6/28
LDL<100 mg/dL	0/28	16/28[b]	0/28	1/28

[a]$P<0.05$ χ^2 test (Yates correction); [b]$P<0.05$ χ^2 test (Fisher).

(Table IV). In the PG group blood glucose and BP also were modified in some case, but the difference in terms of frequency was not statistically significant ($P>0.05$ χ^2 test) compared to placebo. In most of the subjects of this last group despite the modification were some times clinically relevant, the values were not sufficient to reach the cut-off for the exclusion from the MS.

Discussion

In the present trial a light physical training has determined some positive effect of the body weight and lipid profile of subjects suffering from dyslipidemia.

Physical activity has shown a protective effect in many epidemiological and clinical studies since it is among the tools to reduce cardiovascular disease.[23] However, physical exercise can cause stress, particularly when its intensity requires a substantial energy expenditure. A way to limit the stress could be to follow a slow and light training adjusted to the subject and performed with continuity. Even a modest MET-hour/day increase may affect body weight [19, 24] particularly when associated to dietetic adjustments. However, some times it is hard to achieve a reduction of the caloric intake, particularly when energy expenditure is increased. It is a common observation that people increasing energy expenditure tend to increase the caloric intake, and can be considered a success just to maintain the same quantity of food. Restrained eating may have a counterproductive effect and eventually be followed by weight gain due to disinhibition which lead to a vulnerable weight cycle.[25, 26]

For this reason a combination of a soft physical training and PG could be a new approach for the body weight and lipids reduction with minimal changes of the food intake.

The results of this study show that this can be possible in subjects with moderate body weight increase (BMI<30) and dyslipidemia.

The reduction of about 31% of LDL cholesterol and 24% of triacylglycerol obtained in 4 months in subjects treated with the combination of PG and physical training is an important achievement. With light physical training only the results obtained although significant were much less than 50% of those obtained with the combination with PG . None of the subject in the two groups was in the optimal level of <100 mg/dL of LDL (Table IV) before the treatment.

With the combined treatment 57% (16/28) of the cases obtained this reduction of LDL, while with physical training only 4% (1/28) could reach the same result. Longer treatments and/or some minimal dietetic restriction could improve further the lipid profile and body weight loss. Following the combined treatment, both body weight and WC declined of about 8% compared to 3-3.5% with physical training only ($P<0.05$).

Similar results were obtained with chitosan by other authors also [9] due mainly to a loss of the fat mass with preservation of lean mass.

PG performs *in vitro* a more efficient action than high molecular weight chitosan in "sequestering" lipids, and in theory make them less available for absorption.

In a trial on obese patients the fat increase in feces following chitosan treatment was minimal.[12] However, a small reduction of lipids absorption may have an impact on the FFA levels, which are known to produce insulin resistance [27] that is a quite common feature in these patients and is responsible for fat accumulation. A low molecular weight polymer such as PG is more efficient in lipid binding and could end up with a more consistent improvement of insulin sensitivity.

Recently has been shown that basic polysaccharides such as PG can reduce the pancreatic lipase activity [28] and suppress dietary fat absorption from the small intestine.

Furthermore, bacteria of the colon could use fats as fuel, and consequently a much higher quantity of lipids could be distracted from the absorption than the one recovered in feces. In other terms, steatorrhea is not the only mirror of the reduction of fats absorption.

Most probably a decrease of triacylglycerol levels is a much more reliable marker of fats availability and metabolism than steathorrea which is very complex to evaluate due to the collection of feces and lab analysis determination.

The reduction of the WC and triacylglycerol levels together with the increase of HDL have certainly an impact on SM, since they are three of the five items that characterize the disease. In particular, WC, BMI and tryacilglycerol levels are also considered as directly relate to insulin resistance which may increase the risk of some adverse outcome [29, 30] particularly when they are concomitantly present in the same subject.

Blood glucose and blood pressure reduction shown with the combination of PG and physical training could be due to an indirect effects of body weight loss and they give more chance to the patients to "get out" from MS.

However, the observation of the activity of PG on MS was only occasional and found in subjects whose clinical history of MS was not properly defined before this first observation.

Consequently, more complex and *ad hoc* clinical trials in subjects with a stable MS have to be planned to confirm the activity of PG on this syndrome.

The combination of PG and physical training may create a good starting base to prevent the development of MS and reduce the incidence of cardiovascular and metabolic complications related to this disease.

Conclusions

In conclusion, PG showed to improve substantially the efficacy to the physical training as an example of synergism between different measures that taken separately may not end up with the desirable clinical result. Long term trials with more cases are needed to define the activity of PG on MS.

Riassunto

Utilizzo della poliglucosamina e dell'attività fisica per ridurre il peso corporeo e la dislipidemia in soggetti moderatamente sovrappeso

Obiettivo. È stato studiato un chitosano a basso peso molecolare (poliglucosamina, PG) in pazienti iperlipemici sovrappeso che svolgevano attività fisica. Lo studio, in doppio cieco, è stato eseguito su 2 gruppi di 30 pazienti (M/F; età compresa tra 25 e 50 anni).

Metodi. Per un periodo di 4 mesi sono state somministrate compresse contenenti PG (2 g/die) o placebo durante l'allenamento fisico (+ 8 equivalenti metabolici standard ore/settimana). Prima e dopo il trattamento sono state valutate le misure antropometriche, l'apporto calorico, la pressione arteriosa, i livelli di colesterolo LDL e HDL, la glicemia e i livelli di triacilglicerolo. I due gruppi erano simili per quanto riguarda l'apporto calorico e la spesa energetica e alla fine dello studio hanno evidenziato risultati positivi per la maggior parte dei parametri esaminati.

Risultati. Il gruppo trattato con PG ha evidenziato, rispetto a quello trattato con placebo, una riduzione più significativa (P<0,05) del peso corporeo (rispettivamente 6,9±1,87 *vs* 3,0 ±1,612 kg), del girovita (7,3±2,49 *vs* 3,1±4,21 cm), del colesterolo LDL (44,0±14,7 *vs* 12,5 ±12,6 mg/dL), del triacilglicerolo (52,0±29,3 *vs* 39,0±15,2 mg/dL), mentre l'aumento del colesterolo HDL è stato maggiore nel gruppo PG (6,0±3,6 *vs* 3,0±4,2 mg/dL). Al baseline la sindrome metabolica secondo l'ATP III era presente in 15 pazienti nel gruppo PG e in 14 pazienti nel gruppo placebo.

Conclusioni. Inaspettatamente, alla fine del trattamento la sindrome metabolica si era ridotta in 12 pazienti del gruppo PG e in soli 3 casi nel gruppo placebo (P<0,05). I risultati indicano che la PG può migliorare l'efficacia dell'attività fisica nei pazienti moderatamente sovrappeso con dislipidemia e che essa può essere utile per il trattamento della sindrome metabolica.

Parole chiave: Poliglucosamina - Dislipidemia - Colesterolo - Triacilglicerolo.

References

1. Singla AK, Chawla M, Chitosan:some pharmaceutical and biological aspects-an update. J Pharm Pharmacol 2001;53:1047-67.

2. Muzzarelli RAA. Human enzymatic activities related to the therapeutic administration of chitin derivatives. Cell Mol Life Sci.1997;53:131-40.
3. Kim SK, Park PJ, Yang HP, Han SS. Subacute toxicity of chitosan oligosaccharide in Sprague-Dawley rats. Arzneim Forsh/Drug Res 2001;51:769-74.
4. Bokura H, Kobayashi S. Chitosan decreases total cholesterol in women: a randomized, double-blind, placebo-controlled trial. Eur J Clin Nutr 2003;57:721-5.
5. Giustina A, Ventura P. Weight-reducing regimens in obese subjects: effects of a new dietary fiber integrator. Acta Toxicol Ther 1995;19:199-214.
6. Maezaki Y, Tsuji K, Nakagawa Y, Kaway Y, Akimoto M, Tsugita *et al.* Hypocholesterolemic effect of chitosan in adult males. Biosci Biotech Biochem 1993;571:439-44.
7. Sciutto AM, Colombo P. Lipid-lowering effect of chitosan dietary integrator and hypocaloric diet in obese subjects. Acta Toxicol Ther 1995;16:215-30.
8. Veneroni G, Veneroni F, Contos S, Tripodi S, De Bernardi M, Guarino C *et al.* Effect of a new chitosan on hyperlipemia and overweight in obese patients. Chit Enz 1996;2:1-4.
9. Kaats GR, Michalek JE, Preuss HG. Evaluating efficacy of a chitosan product using a double-blinded, placebo-controlled protocol. Am J Coll Nutr 2006;5:389-94.
10. Pittler MH, Abbot NC, Harkness EF, Ernst E. Randomized, double blind trial of chitosan for body weight reduction. Eur J Clin Nutr 1999;53:379-81.
11. Metso S, Ylitalo R, Nikkila M, Wuolijoki E, Ylitalo P, Lehtimaki T. The effect of long term microcrystalline chitosan therapy on plasma lipids and glucose concentration in subjects with increased plasma total cholesterol: a randomized placebo-controlled double-blind crossover trial in healthy men and women. Eur J Clin Pharmacol 2003;59:741-6.
12. Mhurchu CN, Poppitt SD, McGill AT, Leahy FE, Bennet DA, Lin R B *et al.* The effect of the dietary supplement, chitosan, on body weight: a randomized controlled trial in 250 overweight and obese adults. Int J Obes Relat Metab Disord 2004;28:1149-56.
13. Gades MD, Stern JS. Chitosan supplementation and fat absorption in men and women. J Am Diet Assoc 2005;105:72-7.
14. Pittler E, Ernst M. Chitosan as a treatment for body weight reduction? A meta-analysis. Perfusion 1998;11: 461-5.
15. Chiang MT, Yao HT, Chen HC. Effect of dietary chitosans with different viscosity on plasma lipids and lipids peroxidation in rats fed on a diet enriched with cholesterol. Biosci Biotech Biochem 2000:64:965-71.
16. Kanauchi O, Deichi K, Imasato Y, Kobayashi E. Increasing effect of a chitosan and ascorbic acid mixture on fecal dietary fat excretion. Biosci Biotech Biochem 1994;58:1617-20.
17. Kanauchi O, Deichi K, Imasato Y, Shizukuishi M, Kobayashi E. Mechanism for the inhibition of fat digestion by chitosan and for the synergistic effect of ascorbate. Biosci Biotech Biochem 1994;59:786-90.
18. Ni Murchu C, Dunshea-Mooji CAE, Bennet D, Rodgers A. Chitosan for overweight or obesity. The Cochrane Library 2004;4.
19. Ainsworth BE, Haskell WL, Leon AS, Montoye HJ, Sallis JF, Paffenbarger RS. Compendium of physical activities: classification of energy costs of human physical activities. Med Sci Sport Exerc 1992;25:71-80.
20. Bertini S, Bisio A, Torri G, Bensi D, Terbojevich M. Molecular weight determination of Heparin and dermatan sulfate by Size Exclusion Chromatography with a Triple Detector Array. Biomacromolecules 2005;6:168-73.
21. Bondiolotti G, Bareggi SR, Frega NG, Strabioli S, Cornelli U. Activity of two different polyglucosamines, I.112® and FF45®, on body weight in male rats. Eur J Pharmac 2007;567:155-8.
22. Dandona P, Alijada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome on interaction between obesity, diabetes, and inflammation. Circulation 2005;111: 1448-54.
23. Warburton DE, Nicol CW, Bredin SS. Health benefit of physical activity: the evidence. Can Med Ass J 2006;14: 801-9.
24. Trichoupoulou A, Gnardellis C, Lagiou L, Benetou V, Trichopoulos D. Relation to energy intake and expenditure among adults in Greece. Epidemiology 2000;11: 333-6.
25. Lowe MR. The effects of dieting on eating behaviour: a three-factor model. Psychol Bull 1993;114:100-21.
26. Lauzon-Guillame B, Basdevant A, Romon M, Karlosson J, Borys JM, Charles MA. Is restrained eating a risk factor for weight gain in a general population? Am J Clin Nutr 2006;83:132-8.
27. Wang J, Liu R, Hawkins M, Barzilai N, Rossetti LA. A nutrient sensing pathway regulates leptin gene expression in muscle and fat. Nature 1998;393:68 i-8.
28. Tsujita T, Takaichi H, Takaku T, Sawai T, Yoshida N, Hiraki J. Inhibition of lipase activities by basic polysaccharides. J Lip Res 2007:48:358-65.
29. Farin HMF, Abbasi F, Reaven GM. Body mass index and waist circumference both contribute to differences in insulin-mediated glucose disposal in nondiabetic adults. Am J Clin Nutr 2006;83:47-51.
30. McLaughlin T, Abbasi F, Cheal K, Chu J. Lamendola C, Reaven G. Use of metabolic markers to identify overweight individuals who are insulin resistant. Am Coll Phys 2003;139:802-9.