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ARD CHOLESTEROL AND HDL INCREASE

SUMMARY

Patients suffering from Metabolic Syndrome (MS) were treated with two different products containing polyglucosamine (PG): PG (ARD Lipiban) and PG plus phytosterols (ARD Cholesterol).
A double blind trial was conducted on two Groups of 14 patients each (7 M and 7 F, aged between 45 and 64 years) not undergoing dietetic regimen. Treatments were administered for a period of 3 months in single-dose packets.
PG was given twice a day in 2 packets containing 900 mg of PG each, whereas PG with phytosterols (900 mg of PG plus 200 mg of phytosterols) was given twice a day in 2 packets. One of the packets was containing placebo (double dummy).
Patients were analyzed before and after treatment for parameters typical of MS according to ATPIII (abdominal circumference, HDL cholesterol, triglycerides, blood glucose, and blood pressure).
Moreover, patients underwent a dietary intake interview according to a questionnaire based upon weekly servings in order to control possible alterations of diet during treatment. At the end of treatment 9/14 patients recovered from MS using only PG, while 10/14 patients recovered from MS with PG plus phytosterols. The latter treatment was found significantly more effective in increasing HDL cholesterol than PG alone (respectively 9.8 ± 5.24 and 3.6 ± 4.45 mg/dL; t test $p<0.05$) and in reducing blood pressure (respectively 7/14 and 2/14; $p<0.05$ chi square test).
The combination of PG and phytosterols is more effective for controlling MS and can be administered once a day.

KEY WORDS

METABOLIC SYNDROME, POLYGLUCOSAMINE, PHYTOSTEROLS

INTRODUCTION

Metabolic Syndrome (MS) is considered the **disease of the third millennium** [1, 2] in developed countries.
Its incidence is estimated at 30%, varying from 20% to 35% depending upon the country [3].
- MS is held responsible for the development of **three diseases**: cardiovascular disease, type II diabetes and different types of tumors.
Polyglucosamine (PG) administration has been identified as a possible treatment for the MS [5].

PG is a natural fiber (FIG. 1) derived from a high-molecular-weight polymer which has undergone a mechanical depolymerization process (without the use of solvents). The basic unit consists of *glucosamine molecules* joined together by β (1-4) bonds with the positive charge on NH_2 (in the form of NH_3^+), and with a strong ability to join together fatty acid chains together. The β (1-4) bond linking two glucosamine molecules **does not permit** hydrolysis of the gastrointestinal enzymes and hence behaves like a *fiber*.
Some bacteria of the *Bacillus* genus are capable of performing hydrolysis [16]. The reason behind their presence in the human gastrointestinal tract has not yet been fully understood.

PG possesses a high affinity and great selectivity for oxidized lipids, in particular, lipids with the greatest potential to form atherosclerotic plaque, which are implicated in the pathogenesis of chronic inflammatory and degenerative diseases of the stomach and intestines.
Studies [6,9] have shown an average decrease in total cholesterol, LDL cholesterol and triglyceride levels, an increase in HDL cholesterol and an improvement in the HDL-C/LDL-C ratio, in addition to consistent weight loss and reduction in abdominal girth.
PG is administered **twice daily before** the main meals (4 tablets /day).

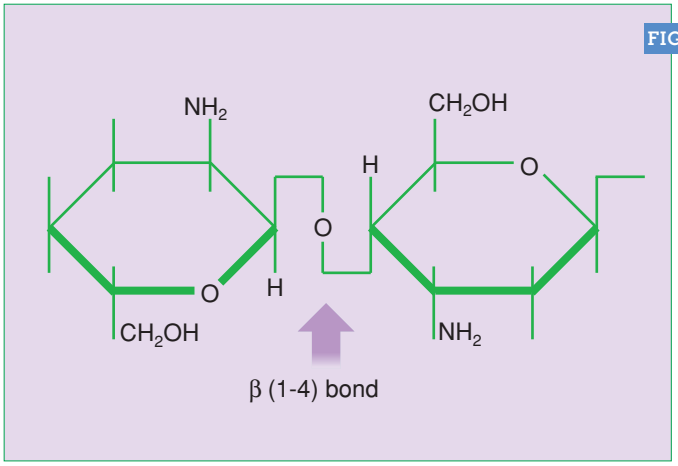


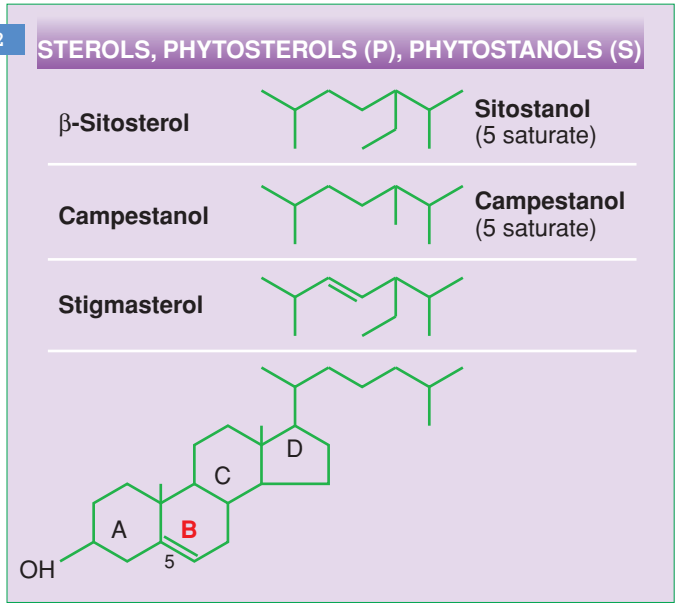
FIG. 1

The basic *polyglucosamine* dimer

More recently, attention has turned to the use of naturally occurring ***phytosterols***, the botanical equivalent of cholesterol in mammals. Over 40 *phytosterols* have been identified.
The main ones are *sitosterol*, *campesterol* and *stigmasterol* (FIG. 2).

FIG. 2

Some *phytosterols* and *phytostanols*. Note position 5 on ring B; in *stanols* it is saturated.



These compounds may be present with their corresponding saturated *phytostanols* compounds (in position 5 on the B ring).
Clinical studies show that *phytosterols/phytostanols* (P/S) play an **active role** in the reduction of cholesterol levels [10, 13].
Thanks to their natural character, they have been introduced into numerous food products (*novel foods*) and consumed widely in an uncontrolled manner. Consequently, dangerous doses are easily reached.
The main characteristic of P/S is **competition** with cholesterol in the absorption process.
P/S have greater affinity compared to cholesterol for micelles that form in the first part of the gastrointestinal tract.
P/S replaces cholesterol that is diverted from absorption (FIG. 3). The micelles (*carousel-shaped* in FIG. 3) are formed by cohesion of bile salts and lipids (monoglycerides, triglycerides, cholesterol, etc.). The enterocytes absorb the individual micelle components through an *active* and *selective* process.
- When *phytosterols* reach the enterocyte membranes they are actively absorbed via intervention of transport protein NPC1 L1 (Niemann-Pick C1-like 1 protein) and are subsequently expelled from the cells into the lumen by way of a mechanism regulated by another

FIG. 3

Micelle formation and their decomposition in the enterocytes.

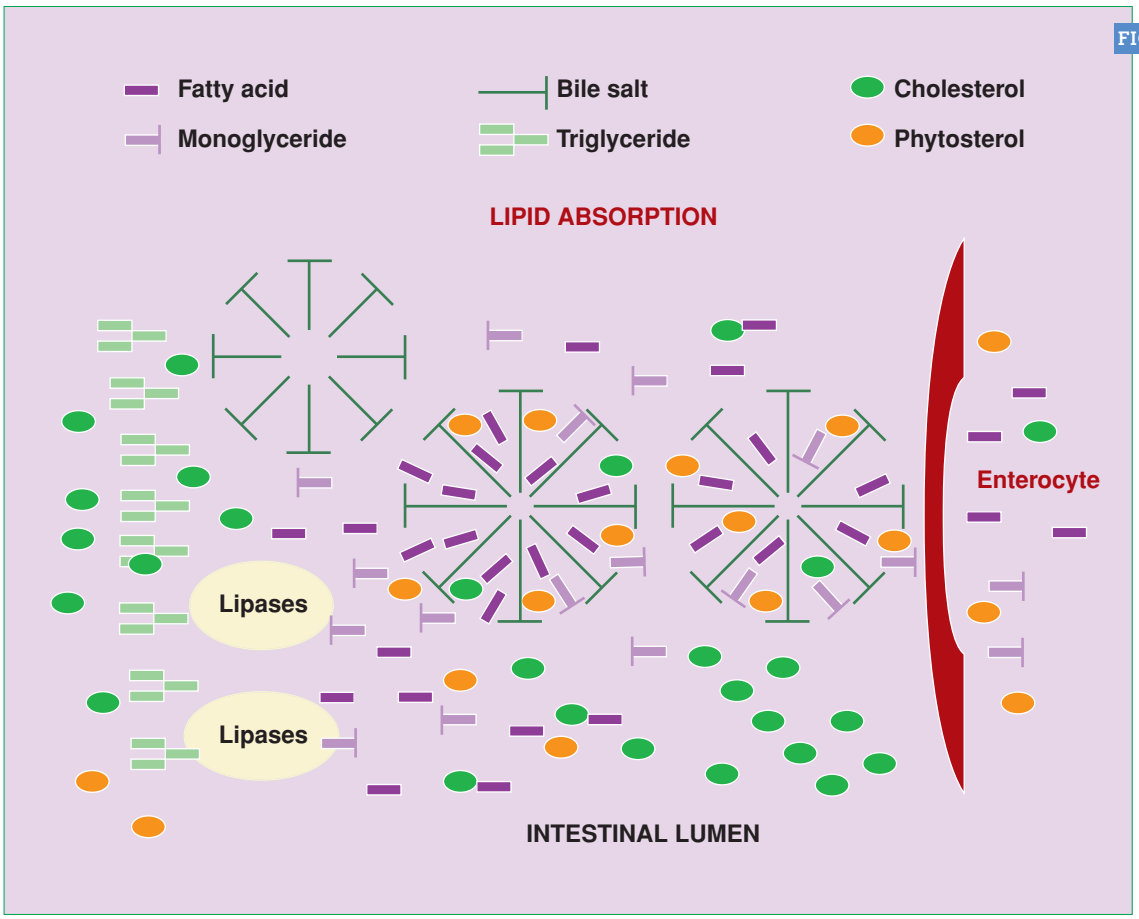
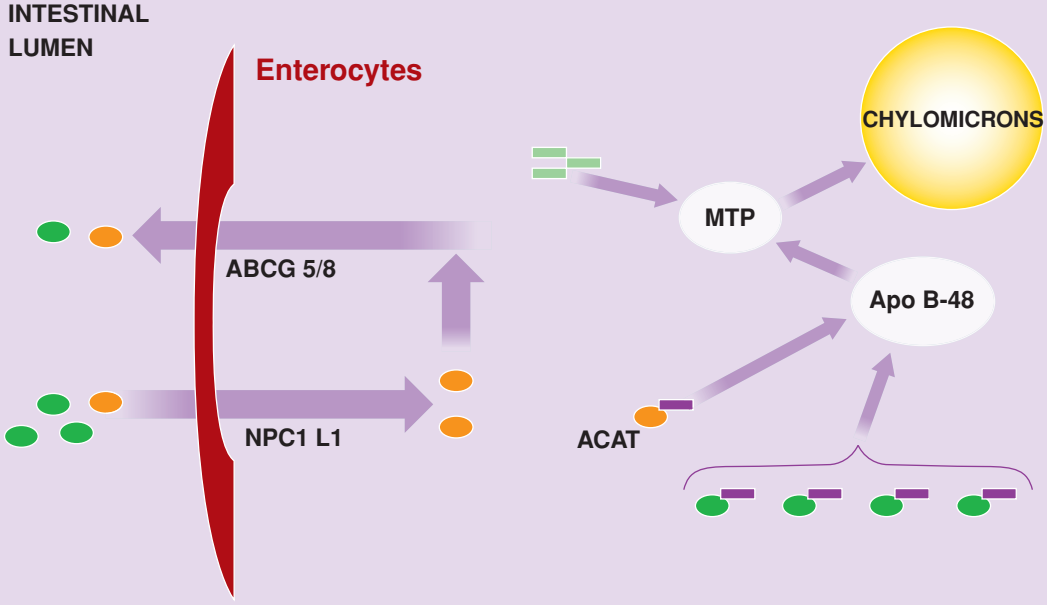


FIG. 4

Control of *phytosterol* absorption.



MTP = microsomal trygl. transport protein
Apo B-48 = apolipoprotein
NPC1 L1 = Niemann - Pick C1-Like 1 Protein
ACAT = acyl CoA colesterol acytranspherase
ABCG 5/8 = ATP binding cassette G5-G8

transport protein known as ABCG 5/8 (ATP *binding cassette protein*) which selectively pumps them out (FIG. 4).

- The result is a **limited absorption** of *phytosterols*.

Phytosterols (like *phytostanols*) replace the cholesterol and are absorbed by the enterocytes through NPC1 L1.

The majority is expelled along with the ABCG 5/8.

However, the P/S that doesn't expel nor replace cholesterol - as present in abundance - follows the metabolic pathways of cholesterol. They are esterified through the action of ACAT (acyl-CoA: cholesterol acyltransferase) and subsequently assembled with Apo B-48 and triglycerides made available by MTP (microsomal triglyceride transfer protein), finally forming chylomicrons.

This tendency for expulsion from the cell also becomes apparent in the hepatocytes, which are able to secrete the P into the bile more efficiently.

Consequently, it undergoes hepatobiliary recycling, which favours expulsion of P/S compared to cholesterol.

This mechanism keeps the levels of *phytosterol low* (FIG. 5) and at the same time prevents absorption of one part of the dietary cholesterol. The body limits absorption of the latter because the *phytosterols* presumably have a reduced ability to resist the Na^+ gradient.

They enable an easy entry, causing cellular oxidative stress (due to the increased ATP energy needed to expel the Na via the Na^+/K^+ pump).

In P/S-rich plants, cell membranes effectively counteract the hydrogen gradient. It is logical to presume that an excessive use of P/S could alter the efficacy of cell membranes.

Vegetarians, who consume higher quantities of P and S, usually - and in consequence - have higher blood levels. From the amounts consumed by vegetarians and the finding that they are less affected by certain diseases (dyslipidemias), there does not appear to be any advantage in exceeding the average doses of 200 mg consumed by persons on such diets.

FIG. 5

Average amounts consumed and average blood levels of *phytosterols* and *phytostanols*.

STEROLS, PHYTOSTEROLS (F), PHYTOSTANOLS (S)

Average amounts consumed

Cholesterol	>50 mg (vegetarians) – 750 mg (average 400 mg)
Phytosterols	150 mg – 400 mg (vegetarians)
Phytostanols	50 mg

Blood levels

Cholesterol	150-200 mg/dL
Sitosterol	0.25-2.5 mg/dL
Campesterol	0.25-2.5 mg/dL
Stigmasterol	0.001-0.02 mg/dL
Sitostanol	0.001-0.02 mg/dL
Campestanol	0.0001-0.006 mg/dL

For instance, higher doses above 2 g/day could cause serious problems [14, 15].

In view of these facts, the aim of this study was to compare the effect of PG and the combination of PG and *phytosterols* in low doses (200 mg), in persons suffering from MS, in order to establish **which** of the two treatments offers a greater possibility of controlling the parameters indicative of Metabolic Syndrome.

PATIENTS AND METHODS

Twenty-eight patients (14 male; 14 female), aged between 45 and 64 years, suffering from MS according to ATPIII [2] were enrolled in the trial. The syndrome was to be stable for at least 9 months (clinical tests had to confirm MS stability over a period of at least 3 consecutive tests carried out over the period of 9 months). All the patients attended the Epidemiology Centre PAP/PEA of San Valentino and Spoltore (PE).
Exclusion criteria included the presence of other major chronic diseases not therapeutically controlled, tumors and the use of oral contraceptives.
Suitable patients underwent an overall check-up including anthropometry (body weight, height, body mass index) and blood pressure measurement while lying down.
The following day, after fasting for at least 12 hours, blood tests measured the laboratory parameters of MS.
On the same morning a dietary interview determined the number of portions consumed weekly of: vegetables, fruits, cereals, legumes, milk products, meat, fish, alcohol and eggs and their respective sizes (large, medium, small).
Subsequently, the patients accepted into the clinical trial were randomly assigned to one of two treatment groups for a period of 3 months.
Patients were asked to make no dietary changes throughout the duration of the study to avoid variations that could interfere with results.

▶ The trial provided **two treatments**: **ARD Lipiban** supplied in packets (each containing 900 mg of PG + excipients) to be taken twice a day or **ARD Cholesterol** to be taken as 2 packets/day. However, **ARD Cholesterol** was supplied in double packets, with one containing 900 mg of PG + 200 mg of phytosterols (also containing a minimum quantity of phytostanols) and the other containing only excipients.
This clinical test is a double blind study. ARD Lipiban was taken twice a day and ARD Cholesterol once a day. The patients treated with ARD Lipiban constituted **group L** while those treated with ARD Cholesterol constituted **group C**.
Each patient was given 30 small containers numbered from 1 to 30, each containing 2 packets.
Each patient was provided with medication for one month's therapy and attend for follow-up in order to receive the next month's supply. Therefore, it was possible to regularly check whether patients adhered to the treatment protocol, and to repeat the discussion of dietary habits, thus monitoring possible changes. Overall, in addition to a baseline interview, three further interviews were conducted with patients at 1, 2 and 3 months after the first treatment.
At the end of the three months patients underwent anthropometric review, measurement of blood pressure and blood parameters, and dietary review. The last review was compared to prior reviews. The average value was considered indicative of diet during the treatment.

Parameter	Group L	Group C
Age	57 ± 5,5	56 ± 5,6
Sex	7 M; 7 F	7 M; 7 F
Height (cm)	172 ± 8,7	174 ± 8,2

TAB. 1
General characteristics of patients in Groups L and C.

Parameter	Group L		Group C	
	Before	After 3 months	Before	After 3 months
P mx [mm Hg]	152 ± 11,5	144 ± 10,6	149 ± 7,7	136 ± 5,7
P min [mm Hg]	83 ± 4,1	79 ± 2,8	83 ± 7,9	78 ± 6,8
Glycaemia [mg/dL]	103 ± 6,8	98 ± 5,9	99 ± 8,6	96 ± 6,0
CT [mg/dL]	248 ± 24,4	229 ± 21,1	233 ± 21,5	202 ± 16,5
LDL [mg/dL]	143 ± 25,8	134 ± 23,3	132 ± 24,2	101 ± 17,8
HDL [mg/dL]	41 ± 4,3	45 ± 4,53	41 ± 6,2	51 ± 3,4
Triglycerides [mg/dL]	194 ± 30,8	158 ± 14,4	181 ± 29,1	153 ± 10,7
Body weight [kg]	83 ± 10,3	81 ± 10,4	82 ± 11,6	80 ± 11,3
BMI [kg/m^2]	28,1 ± 1,27	27,3 ± 1,33	27,1 ± 2,06	26,5 ± 2,04
CA [cm]	98 ± 5,2	96 ± 2,61	99 ± 3,0	98 ± 2,3

Legend: **TC** = total cholesterol; **BMI** = body mass index; **AG** = abdominal girth

TAB. 2
Main parameters of MS before and after 3 months of treatment with the two therapies (average values ± Standard Deviation).

Mean and standard deviations of the collected data were calculated. The evaluation of differences between the two groups was carried out using Student's t test with $\alpha = 0.05$. It also determined the number of subjects who had recovery or partial recoveries from MS and normalization of each of the main parameters of the condition (hypertension, HDL cholesterol, glycaemia, abdominal girth, triglycerides).
For this final evaluation, the differences between the two treatments were calculated using a chi-square test (2x2), maintaining the critical value of $\alpha = 0.05$.

Because of the small sample size (14 cases vs. 14 cases), the experience obtained does not lend itself to a differential assessment of the effect on the MS, but only to a **diversification of individual parameters**, by taking into account the values before and after treatment (*delta*). A difference in *delta* >1 DS (combining both groups), resulting from 14 cases per group, allows one potency comparison of >0, 80 (known maximum is 1).

RESULTS

All enrolled patients completed the trial. TAB. 1 shows the indicative data for the two groups. No significant differences were noted between the two trial groups. TAB. 2 shows information regarding the main parameters of the MS according to ATPIII criteria.
TAB. 3 shows the differences between baseline and final values (following 3 months of therapy), comparing the two treatments, in order to evaluate the **differential efficacy** of the treatments.
Overall, the parameters in both groups underwent similar changes with the exception of **LDL** and **HDL** cholesterol levels. Therefore treatment used in-group C (ARD Cholesterol) was **significantly more effective**.
In both cases, the power of comparison was >0.8. The difference is relevant.
Patients in Group L showed a greater tendency to weight reduction, although the difference was not statistically significant compared with group C.
Assessment of weekly food intake before and during the treatment (as the average of the 3 assessments which followed the baseline one) is shown in TAB. 4.
The variations are similar in both groups and tend to go in the same direction.

Parameter	Group L	Group C	T test
P mx [mm Hg]	-7,9 ± 8,26	-13,0 ± 6,04	Ns
P min [mm Hg]	-4,8 ± 4,71	-5,1 ± 3,68	Ns
Glycaemia [mg/dL]	-4,9 ± 6,05	-2,9 ± 4,50	Ns
CT [mg/dL]	-19,9 ± 19,44	-31,0 ± 19,92	Ns
LDL [mg/dL]	-9,4 ± 17,92	-31,6 ± 23,46	< 0,05
HDL [mg/dL]	3,6 ± 4,45	9,8 ± 5,24	< 0,05
Triglycerides [mg/dL]	-35,9 ± 26,04	-29,2 ± 26,84	Ns
Body weight [kg]	-2,3 ± 1,38	-1,9 ± 0,95	Ns
BMI [kg/m2]	-0,8 ± 0,49	-0,6 ± 0,33	Ns
CA [cm]	-1,9 ± 3,37	-1,6 ± 1,08	Ns

Ns = differences not statistically significant (p>0.05)

TAB. 3
Differences between before and after 3 months of treatment with the two therapies (means ± standard deviation).

Food	Group L		Group C	
	Before	During the 3 months	Before	During the 3 months
Vegetables	5,8 ± 1,76	6,4 ± 1,65	5,1 ± 2,07	6,2 ± 2,39
Fruit	5,6 ± 2,53	6,7 ± 3,56	5,2 ± 2,19	7,2 ± 2,61
Cereals	25,6 ± 4,34	24,8 ± 4,30	26,2 ± 2,97	25,9 ± 2,88
Legumes	1,5 ± 1,22	1,8 ± 1,31	1,6 ± 0,74	1,9 ± 0,83
Milk products	6,8 ± 3,96	6,6 ± 4,01	6,0 ± 2,86	5,9 ± 2,77
Meat	3,6 ± 1,86	3,8 ± 1,76	3,9 ± 1,54	3,7 ± 1,54
Fish	1,3 ± 1,20	1,4 ± 1,09	1,2 ± 0,58	1,4 ± 0,65
Alcohol	7,2 ± 5,21	6,6 ± 4,69	9,5 ± 4,09	8,9 ± 4,29
Eggs	1,3 ± 1,27	1,4 ± 1,28	1,6 ± 0,94	1,6 ± 0,94

TAB. 4
Analyses of weekly food intake before and during the two treatments.

The dietary differences found between the pre-treatment period and during the treatment - shown in TAB. 5 - are very similar.

The slight reduction in the intake of cereals and increased intake of fruit and vegetables are very similar in both groups. No data is shown regarding portion sizes as there were no relevant changes.
Furthermore, it has to be taken into account that the evaluation during the therapy is an average of 3 observations and therefore considerably more accurate compared to the one made prior to the treatment. This difference in the weight of the responses makes comparison between before and after of little relevance.
▶ **Nine out of 14 patients** in Group L (64%) and **10 out of 14 patients** (71%) in Group C recovered from MS.
The parameters altered by the two therapies are shown in TAB. 6.
No statistically significant difference in any of these parameters was seen, even if the incidence of blood pressure reduction is greater in group C compared to group L (in 50% and 14% cases respectively).
Patients treated with ARD Lipiban demonstrated a more consistent (although not statistically significant) weight loss. However, this was not very evident in abdominal girth reduction.

DISCUSSION

The results show both therapies effective in positively altering the parameters of Metabolic Syndrome, with the combination of PG + *phytosterols* achieving greater efficacy in some parameters (lipoproteins and hypertension).
The effects of the two treatments are considered clear-cut and not produced by dietary modifications, which did not alter in such a way as to explain recovery from Metabolic Syndrome.
In fact, the slight increase in the intake of fruit and vegetables (1 or 2 servings/week) and a slight decrease in cereal and alcohol intake (less than one serving/week) provide very limited grounds, both in terms of energy as well as a possible increase in antioxidant production.

It is surprising how a predominantly local GI therapy is able to correct MS.

This is mainly due to the definition of MS according to ATP III, which can be diagnosed in very early stages and therefore controlled more easily with a less drastic therapeutic approach.
Metabolic Syndrome can be controlled with PG alone. This was described in a previous publication [5] and in a multicenter study undergoing of publication.

▶ The **addition of *phytosterols*** brings further benefits along with the **single** daily dose compared with twice-daily doses of PG alone.
This study has produced two interesting facts:
1) A greater effect on the lipoprotein pattern with a combination of PG + *phytosterols* (increase in HDL and reduction of LDL).
2) A reduction in BP, not so much in terms of consistency, but rather of frequency (evident in a greater number of patients).

As far as the lipoprotein pattern is concerned, the greater efficacy of the PG +

Food	Group L	Group C	T test
Vegetables	0,6 ± 0,93	1,1 ± 1,00	Ns
Fruit	1,1 ± 1,79	2,0 ± 1,96	Ns
Cereals	-0,9 ± 1,17	-0,4 ± 0,63	Ns
Legumes	0,3 ± 0,73	0,3 ± 0,73	Ns
Milk products	-0,2 ± 0,58	-0,1 ± 0,36	Ns
Meat	0,1 ± 0,36	0,2 ± 0,58	Ns
Fish	0,1 ± 0,36	0,2 ± 0,43	Ns
Alcohol	-0,6 ± 0,22	-0,6 ± 1,15	Ns
Eggs	-0,1 ± 0,27	0 ± 0,00	Ns

Ns = differences not statistically significant (p>0.05)

TAB. 5
Differences in weekly food intake before and during the two treatments.

Treatment	BP	Glycaemia	HDL	Triglycerides	AG	*Recovery from MS*
Group L (AR_D Lipiban)	2/14	3/6	7/11	4/14	4/10	**9/14**
Group C (AR_D Cholesterol)	7/14	2/6	7/9	6/13	3/10	**10/14**
Chi square test	Ns	Ns	Ns	Ns	Ns	Ns

Legend: **BP** = blood pressure; **AG** = abdominal girth; **MS** = Metabolic Syndrome.
Ns = differences not statistically significant (p>0.05)

TAB. 6
Frequency of parameters indicating MS after treatment with the two therapies.

phytosterol combination can be explained by considering the role of cholesterol esters.
PG has an adsorbent action on bile salts. It reduces their re-absorption and increases their excretion.
It acts principally as a *fiber adsorbing* lipid or amphoteric substances (with selectivity for oxidized lipids).
The disruption of bile salt re-uptake produces three *phenomena* in the liver:
1) Synthesis of cholesterol and its esters, in order to compensate for the loss of bile salt.
2) Synthesis of LDL receptors in order to recover the circulating cholesterol.
3) Synthesis of HDL cholesterol in order to recover cholesterol from the cells.

The clear effect of PG consists of reduction of LDL cholesterol and triglycerides, an increase of HDL cholesterol and finally, a reduction in body weight.
The effect on body weight results partly from utilisation of omental lipids (abdominal girth reduction) and also by greater utilization of dietary lipids by colon bacteria (carry-over of lipids to the colon).

Phytosterols added to PG enter into competition with cholesterol for the synthesis of bile salts.
In fact, *phytosterols* can enter the hepatocytes much more easily than cholesterol but are immediately expelled by efflux pumps (ATP *binding cassette*).
Therefore, the loss through expulsion from the hepatocytes amounts to the bile salts adsorbed by the PG.
Such a double mechanism produces a further increase in the cholesterol ester synthesis rate. Simultaneously, more HDL and more LDL receptors are produced (to recover the cholesterol).

▶ Consequently, the net effect of PG + *phytosterols* is greater than that of PG alone.

As far as the effect on the blood pressure is concerned, the most credible hypothesis is based on the greater activity of the combination of PG + *phytosterols* on the endothelium in line with improvement in lipoprotein profile. This improvement is reflected in all the reactive/inflammatory phenomena associated with the presence of an excessive amount of oxidized lipoproteins.
Blood pressure levels regarded as the threshold for defining MS are 135/85 and therefore are not yet classified as hypertension.
These blood pressure values assume that the endothelium is not yet excessively "worn" by mechanical tension and, thus, is more easily supported.
This combination also affects body weight although the effect appears to be smaller compared with the effects of PG, in that the amount of adsorbent fiber administered is half that of PG alone.
It appears, however, that the combination of PG + *phytosterols* has greater "metabolic" ability in the lipid order.

▶ In conclusion, the combination of PG + *phytosterols* is **synergistic**, particularly with regard to the lipoprotein pattern and endothelial function. In addition to this - a fact that cannot be overlooked - a single daily dose allows for extremely simple therapy programme.
Longer-term experiences and larger groups are required in order to establish the real limitations of treatment with PG + *phytosterols*. ■

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