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The effect of Diamel on patients with metabolic syndrome: a randomised, double-blinded, placebo-controlled study

Running Title**: Diamel on patients with metabolic syndrome**

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Abstract

Objective: The aim of this study was to assess whether the administration of Diamel could improve any of the components of metabolic syndrome (MS), as well as insulin resistance and sensitivity**.**

Methods: A total of 100 patients with MS, aged between 19 and 70, satisfying the MS criteria established by the WHO, were included in the study. Participants were randomly assigned either oral Diamel or a placebo (while maintaining a diet appropriate to the patient´s weight and physical activity), at a dose of two capsules before the three main meals of each day for one year. In addition to anthropometric measures and blood pressure, fasting plasma glucose, lipid profile, insulin, creatinine and uric acid (UA) were determined. Insulin resistance (IR) was assessed. Three indirect indexes were used to calculate insulin sensitivity (IS).

Results: Diamel improved fasting insulin concentrations, IS, IR, and reduced UA concentrations from month six to the end of treatment as compared to placebo (***P*** <0.05). In addition, after the 12^{th} month of treatment the only significant changes from baseline in the mean of fasting insulin (*P* <0.05), UA (*P* <0.05), IR (*P* <0.001) and IS (*P* <0.001) variables occurred in the patients assigned to Diamel, as compared with the patients treated with placebo. The body mass indexes**,** IR and IS were found to be improved in both groups.

Conclusion: Long-term Diamel treatment, combined with lifestyle changes, was beneficial for insulin resistance, insulin sensitivity and reduced serum uric acid levels in patients with MS.

The significant finding(s) of the study: The novelty of this trial was the use, for the first time, of Diamel as a nutritional supplement to treat MS. Diamel was beneficial for insulin resistance, insulin sensitivity and reduced serum uric acid levels in patients with MS.

This study adds: Long-term treatment with Diamel appears to additionally benefit the health of patients with MS. It represents a new alternative therapy (without adverse effects) in patients with MS, pre-diabetes and other insulin-resistance diseases.

Keywords: Diamel, Insulin resistance, Insulin sensitivity, Metabolic Syndrome, Uric acid

Introduction

Metabolic syndrome (MS), one of the most controversial medical entities of the last few years, has aroused increasing interest within the scientific community. It has been defined as a combination of various risk factors and precursors of cardiovascular disease (CVD) and type 2 diabetes.[1,2]

The WHO published a working definition meant to facilitate research on the metabolic syndrome and aid comparability between studies rather than serve as a strict definition.[3]

The metabolic syndrome was defined as insulin resistance or the presence of impaired glucose tolerance or type 2 diabetes and the presence of at least two of the following: abdominal obesity (waist-hip ratio > 0.90 in men or >0.85 in women or body mass index ≥ 30 kg/m2), dyslipidaemia (serum triglycerides ≥ 1.70 mmol/litre or high density lipoprotein (HDL) cholesterol < 0.9 mmol/litre in men or <1.0 mmol/l in women), hypertension (≥160/90 mmHg), or microalbuminuria. These core components were considered most suitable for a general definition, although many other disturbances - for example, disorders of coagulation and endothelial function, hyperuricaemia, and elevated leptin levels - have been associated with the metabolic syndrome.[4,5] Various drugs are currently used to treat MS and do so by targeting the specific disorders that the disease causes in affected patients. Examples of such drugs include: metformin, orlistat, statins, fenofibrate, gemfibrozil, thiazolidinediones, exenatide, acarbose, captopril and enalapril.[6-10]

Recently, a nutritional supplement known as "Diamel" has arrived on the market. Its components include: trace elements, amino acids, vitamins, as well as lettuce and blueberry extracts, and are activated via a magnetisation process (Table 1).[11,12] The product acts on the pancreas, gastrointestinal tract, kidneys and the intracellular environment – areas often rich in free radicals produced secondarily to the massive oxidative stress caused by diabetes.[11,12] These free radicals, in turn, are responsible to a great extent for cell damage and complications associated with MS.[13,14] Diamel is especially designed to stimulate pancreatic β-cells and to act on the digestive tract. Its natural ingredients act like biocatalysts and antioxidants, and its lettuce extract reduces gastrointestinal absorption of

glucose, hopefully making it a nutritional supplement beneficial to MS patients via its regulation of carbohydrate and lipid metabolism[11,12] and its ability to stop diabetes progression.

In 2006**,** Hernández Yero and Vargas found that, when Diamel was used with glibenclamide to treat patients with type 2 diabetes, it improved metabolic control and β-cell function beyond levels achieved by the use of glibenclamide alone at the six month point in the study.[12] Taking these results into account, we believe that Diamel could be an effective tool in the treatment of MS.

This study was aimed at assessing the effectiveness of Diamel for the metabolic and clinical features of MS, as well as insulin resistance and sensitivity, in a group of MS sufferers treated with the supplement. We therefore decided to carry out a double-blind, placebo-controlled, random clinical trial to study these points further during the follow-up period for one year. This is the first clinical trial that makes use of Diamel as a nutritional supplement to treat MS.

Methods

Participants

The study subjects were recruited through various methods. People who in earlier epidemiologic surveys had been found eligible were contacted. Subjects were also recruited with flyers and by promoting the study through direct communication and via opportunistic

population screenings with special emphasis on the high risk groups such as overweight and/or obese subjects.

The inclusion criteria accepted individuals of both genders, aged between 19 and 70 years, fulfilling the WHO diagnostic criteria for metabolic syndrome (MS) and having no history of previous or current use of oral antidiabetic agents. Patients who conformed to the above conditions and who agreed to take part in the study were asked to confirm this in writing.

Patients who refused to take part in the study, as well as any patient exhibiting one or more of the following contraindications were not considered eligible: type 1 diabetes, type 2 diabetes treated with antidiabetic agents at any time before the trial, any clinical disability, use of special diets, history of chronic medication use, use of mineral and/or vitamin supplements, pregnancy, breastfeeding, chronic disease, history of any acute infection, and use of immunosuppressant drugs.

Patients that failed to complete the minimum treatment time (three months) were also excluded. Subjects who followed the treatment for at least three months but later discontinued the treatment were only partly analysed in each of the corresponding three month periods in which the clinical trial groups were compared.

Definition of metabolic syndrome

	World Health Organization (1998)[3]
Required factor	• Type 2 diabetes mellitus (DM) or impaired fasting glucose (IFG) [≥ 110 mg/dl (≥ 6.1 mmol/litre)] or impaired glucose tolerance (IGT), and/or insulin resistance (> 75th percentile HOMA-IR).
Additional factors	Plus two or more of the following six factors: • **Central obesity:** waist-to-hip ratio >0.9 in men; waist-to-hip ratio >0.85 in women, and/or **BMI** ≥ 30 kg/m^2 • **Raised plasma triglycerides:** TG ≥ 150 mg/dl (1.7 mmol/litre) or treatment • **Low HDL–cholesterol:** <35 mg/dl (<0.9 mmol/litre) in men; or <39 mg/dl (<1.0 mmol/litre) in women or treatment • **Raised arterial pressure:** systolic BP ≥ 160 or diastolic BP ≥ 90 mmHg; later modified as ≥ 140/90 mmHg or treatment.

Sample size estimation

The estimation of the sample size was based on a 17.5% decrease in the insulin resistance in the Diamel group. As such, the investigation was calculated at a total of 100 subjects with 80% power to detect any 17.5% decrease in insulin resistance in the Diamel group at a significance level of 0.05. The expected dropout rate was 5%. A sample size resultant of 50 eligible subjects for each group was targeted for recruitment.

Ethical considerations

The study was conducted in accordance with the declaration of Helsinki and its amendments. The protocol was approved by the corresponding ethical and research committee of the National Institute of Endocrinology of Cuba. Written informed consent was obtained from each patient. The Clinical trial had been registered at ClinicalTrials.gov (NCT01025115). Available at http://clinicaltrials.gov/ct2/show/NCT01025115 (accessed February 2012)

Study design and dietary supplement regimen

This study is a mono-centre, randomised, double-blind, parallel-group, placebo-controlled, phase III trial to investigate whether daily oral administration of Diamel can improve any of the components of metabolic syndrome (MS), as well as insulin resistance and sensitivity**.** The length of the study was 24 months (from March 2009 to March 2011).

After initial evaluation, all subjects who met the eligibility criteria and wished to participate in the study were consecutively enrolled. They were randomly assigned to receive: Diamel (n=50) or a placebo (n=50) at a dose of two capsules before the three main meals of each day for one year while maintaining a diet appropriate to their weight and level of physical activity and appropriate hypertensive drugs (ACE inhibitors) for hypertension patients.

A maximum maintenance dose of Diamel 3960 mg (6 capsules) was used and administered two capsules thrice daily - before breakfast, lunch and dinner.[12]

All subjects received advice and counselling on diet and nutrition at the Dietetic Department of the Diabetes Care Centre of the National Institute of Endocrinology, where their *personal* diets were drawn up against their daily calorie intake requirement per Kg of body weight, in accordance with the level of physical activity taken. They were then provided with diets consisting of the following proportion of nutrients: 55-60% of carbohydrates, 15-20% of protein and 20% fat. Diets ranging from 1 200 to 1 500 calories were used.[12]

Patients of both groups were also verbally encouraged to increase physical activity (walking for 30-45 minutes a day 3-4 days a week).[12]

Randomisation within the study was generated using a computerised random number generator. All personnel involved in the study remained unaware of the correspondence between codes and the content of the pills. The treatments used for the study (Diamel and placebo) were supplied by the Catalysis laboratories, labelled with the randomisation code only. Code-to-pill content association was kept in a sealed envelope under custody of the head of the Research Methodology Department of the National Institute of Endocrinology. Seal and envelope integrity were checked every three months and the latter was opened at the end of the study.

The treatment was administered for 12 months from initial patient screening. The effects of Diamel were evaluated at three, six, nine and 12 months from commencement of the treatment and then compared to the effects of the placebo on the other group of subjects.

Adverse effects

Every three months, a clinical examination of the participants was performed to see if they experienced any adverse effects. Height, weight, adverse events such as rashes, dyspepsia, and hepatotoxicity were recorded. For the latter, blood samples were withdrawn from participants during the first three visits and hepatic enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatise) were measured.

Procedure

The medical histories of all individuals satisfying the inclusion criteria were recorded and eligible candidates were then examined by an endocrinologist every three months. The concentrations of fasting glucose and insulin, cholesterol, triglycerides, HDL-cholesterol, uric acid and creatinine were recorded during the aforementioned period for all those taking part.

The concentrations of fasting glucose and insulin were calculated for each subject on two separate occasions: at baseline and at 5 minutes. To calculate the insulin resistance index (HOMA-IR) and insulin sensitivity (IS), the averages of the fasting glucose and insulin values were obtained at baseline and after 5 minutes.

Physical examination

Physical examination included assessments of height, weight, waist and hip circumferences and blood pressure.

Height and weight were measured, and BMI was calculated as weight (kg) divided by the square of height (m^2) (kg/m^2). Waist circumference (WC) was measured with the patient standing with a nonelastic tape at midway between the lower margin of the rib cage and the superior iliac crest during mild expiration. Waist-hip ratio was defined as the ratio of waist girth to the circumference of the hips measured at the trochanter major. BMI superior to 30 is considered as obese.

Blood pressure was measured 3 times using standard mercury sphygmomanometers after a 5-minute rest in the sitting position. The readings at the first and fifth Korotkoff phase were taken as systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively. The average of the three blood pressure measurements was recorded and included in the analysis.

Laboratory tests

Fasting plasma glucose (FPG) and a lipid profile, including total cholesterol, triglycerides and HDL-cholesterol, in addition to creatinine and uric acid (UA) were measured enzymatically by autoanalyzer (Elimat, France) using commercial kits from Cpm diagnostic research (Italy) http://www.cpmsas.it/catalogo.php?categoria=6

The fasting plasma insulin concentration was measured by immunoradiometric assay (IRMA, Izotop, Hungary) http://www.izotop.hu/pdf/immuno/rk400ct_a.pdf.

The insulin resistance index was calculated using the Matthews homeostasis model assessment (HOMA-IR) (fasting insulin μU/mL x fasting glucose mmol/L]/22.5).[15] The IR was taken to be the total HOMA-IR value equal or higher than 2.6.[16]

Three indirect indexes were used to calculate the insulin sensitivity (IS). They were calculated according to the following formulae:

- QUICKI index (Quantitative insulin sensitivity check index) (QUICKI) = [1/[log $insulin_0$ + log $glucose_0$]][17]
- Bennett index (BEN) = 1/(log $insulin_0$ x log $glucose_0$)[18]
- Raynaud index (RAY) = [40/$insulin_0$][19]

Outcomes

The primary outcomes included: changes in the glucose, insulin and lipid concentrations, in addition to variations in the creatinine and uric acid concentrations. The primary end point was also to define if long-term treatment with Diamel was able to improve insulin resistance and sensitivity in this person. Decreases in insulin resistance (HOMA-IR) as well as increases in insulin sensitivity (QUICKI, Bennett and Raynaud indexes values) indicate the amelioration of insulin resistance. Secondary outcomes comprised variations in the blood pressure, body mass index, waist circumference and waist-to-hip index.

Moreover, changes were considered to be differences between the measurements obtained from the two groups in each three-month period, as well as those obtained at baseline and at the end of each treatment (12 months).

Statistical analysis

All statistical analyses were done with the SPSS statistical software package for Windows (version 11.5, SPSS; Chicago, USA). In general, significant statistical differences, and resulting *p*-values were two-sided, with an alpha level of 0.05.

Descriptive data was expressed as means ± SD after assessment of the normal distribution by the Kolmogorov-Smirnov test. The differences between the values obtained from each group were compared by means of the Student's t-test for those variables with a normal distribution and the Mann-Whitney U test for variables that did not have a normal distribution. Proportions were compared using the chi-squared test or Fisher exact test, as appropriate.

The differences in the effects of the treatments on metabolic, biochemical and clinical indicators during the follow-up stage per data pair of the groups were evaluated using the Wilcoxon signed-rank test to compare changes between baseline and at the end of the treatment (12 months). Correlation analysis was performed by calculating Spearman's rho statistic.

Results

Participants

Of the 267 overweight and obese subjects screened between 2009 and 2011 for the study at the National Institute of Endocrinology, one hundred and ten met MS criteria for study entry and underwent randomisation. For this analysis, 100 were randomly assigned to two groups of

equal number, one group (50) received Diamel and the other group (50) received a placebo for one year. Figure 1 shows group distribution of participants.

In the Diamel and placebo groups, 23 and 19 subjects respectively, gave up treatment after a year of the clinical trial (Fig. 1).

One individual was removed from the Diamel group a month after the trial had started due to their metformin therapy. In the same group, two subjects were excluded from the study at the third month: a 22-year-old woman who lost 8.5 kg during the study and later fell pregnant, and a second patient, who began treatment with prednisone for bronchial asthma (Fig. 1). In the group assigned to the placebo, one subject began treatment with metformin six months into the trial, once diet and physical activity failed to reduce the patient´s fasting glucose concentrations (9.62 mmol/L) (Fig. 1).

Baseline clinical and biological characteristics of the subjects

The characteristics of metabolic syndrome patients recruited for this study at baseline are given in Table 2. There was no significant difference in any clinical and biochemical variables at baseline.

Clinical, anthropometric, and biochemical evaluations during treatment period

From the second semester on, fasting plasma glucose decreased significantly: more in patients of the Diamel group than in those allocated a placebo. However, no significant changes in fasting plasma glucose were observed with either of the two types of treatment as compared to baseline values after months of therapy (Table 3). In turn, by the sixth

month, the administration of Diamel reduced patient serum insulin concentrations below the values noted at baseline and achieved by the control (placebo) group in the same time period (Table 3). The drops in serum insulin values by the 12th month were also significantly greater in patients on Diamel than in those on placebo (P= 0.025) (Table 3).

Treatment with Diamel diminished the HOMA-IR index below values obtained with the placebo during months six, nine and 12, both in terms of absolute values and also when analysing change from baseline (Fig. 2a).

By the sixth month, improvements in insulin sensitivity were also more significant in patients treated with Diamel than those allocated a placebo. This result was obtained by using the QUICKI (Fig. 2b) and Bennett (Fig. 2c) sensitivity indexes. However, the Raynaud index highlighted an increase in insulin sensitivity only at the 12th month in patients receiving Diamel when compared with the patients treated with placebo (0.53 ± 0.19 vs. 0.42 ± 0.20; P= 0.025).

There were no differences between the cholesterol, triglyceride and HDL-C concentrations, or the blood pressures and BMIs, of Diamel and placebo patient groups on completion of the 12-month follow-up (Tables 4 and 5).

Compared to patients assigned to the placebo group, subjects treated with Diamel presented lower levels of Uric acid (UA) from the sixth month of the study (both in absolute values and also when the change from baseline was analysed) (Table 4 and 5).

Treatment with Diamel also reduced the abdominal circumference, creatinine and UA concentrations of patients treated with the supplement below baseline values by the 12th

month of treatment (Table 5). Both treatments (Diamel and placebo) helped reduce patient BMI and insulin resistance through the action of physical exercise and diet themselves; however, they also increased insulin sensitivity and decreased the concentration of insulin as compared to baseline values after 12 months of therapy (Table 5).

The proportion of participants of Diamel and placebo groups who met the goal of at least 150 minutes of physical activity per week (assessed on the basis of logs kept by the participants) was 54.2% (13/24) and 53.3% (16/30) at the 12^{th} month, respectively. Nevertheless, the dietary achievement at the end of the study did not differ between the Diamel and placebo groups (70.4% (17/24) and 70.0% (21/30), respectively).

In addition, after the 12^{th} month of treatment the only significant changes from baseline in the mean of fasting insulin, UA, insulin resistance and insulin sensitivity index parameters occurred in the patients assigned to Diamel as compared with the patients treated with placebo (Table 5).

At the end of the study, there were negative correlations of the changes from baseline of QUICK and Bennett indexes with weight and BMI in the patients treated with Diamel (QUICK index: r= -0.51; *P*= 0.011 and r= -0.53; *P*= 0.008, respectively and for Bennett index: r= -0.49; *P*= 0.015 and r= -0.52; *P*= 0.010, respectively).

No adverse effects were seen in any of the participants during the clinical trial.

Discussion

The present study demonstrated that long-term Diamel treatment, together with changes in lifestyle, significantly affected fasting insulin concentrations, IS, IR, and decreased UA concentrations. This is the first prospective intervention to test the outcome of Diamel in MS.

Several large-scale studies in different populations have highlighted the benefit of a lifestyle modification program including weight-reducing diets and moderate intensity exercise, in both treating components of MS and also decreasing the risk of progression of diabetes.[20-27] Our study confirms this data, as we found that lifestyle changes (appropriate diet according to patient's weight combined with physical activity) during the clinical trial improve some MS components *per se*.

For several years, placebo has been defined by its inert content and use as a control in clinical trials and treatments in clinical practice. Recent research shows that placebo effects are real psychological events attributable to the overall therapeutic context, and that these effects can be robust in both laboratory and clinical settings.[28]

The members of the control group that were treated with placebo also had to change their lifestyles. Perhaps the decrease in BMI and IR, the improvement in insulin concentration and that of IS at 12 months of treatment, compared to the initial values, may have been due to the change in their lifestyle and the characteristic psychological effects of placebo itself. This interpretation is supported by the fact that various patients from the placebo control group improved the aforementioned parameters, supposedly because they satisfied the

physical exercise and diet requirements; and at the end of the clinical trial, when the type of treatment for each of the participants was revealed, they were surprised that they had not been given Diamel.

The insulinaemia, uricaemia values and insulin resistance index in the group of patients treated with Diamel were seen to decrease and there was an improvement in the insulin sensitivity 6 months after starting treatment. This therefore implies that the effectiveness of this food supplement is accumulative and gradual.

The reduction of fasting plasma glucose in the Diamel group, when compared with those allocated a placebo (Table 3), does not mean that Diamel reduced the glucose concentration in this study: firstly because the concentration of glucose was essentially surrounded by normal values in the majority of cases, and secondly because we did not find any difference in glucose concentration at the end of the study when analysing from baseline.

Hernández Yero and Vargas found that treatment with Diamel combined with glibenclamide, in subjects with type 2 diabetes, improved the fasting and 2 hour postprandial glucose concentrations and glycated haemoglobin (HbA1c) at the 6 month point of the study, compared with when only glibenclamide was used.[12] The authors of this first controlled clinical trial also noticed an average drop in cholesterol and triglyceride values at six months, in the group being treated with Diamel and glibenclamide, compared with when they were treated with glibenclamide alone (control group).[12] This coincides with the findings of Cheta and Trifan,[11] although theirs was an open and uncontrolled study. The authors explained that the positive results in lipid concentrations can be justified by

better blood glucose control, as well as the fact that Diamel contains amino acids and vitamins, among other components, that could affect lipid metabolism.

The results obtained in this randomised, double-blinded, placebo-controlled clinical trial on patients with MS fit in with the effect that Diamel has on uric acid concentration[11] and also provide new evidence of how Diamel can reduce IR and improve IS. Furthermore, we have shown an inverse association of the changes from baseline of some insulin sensitivity indexes (QUICK and Bennett) with weight and BMI in the patients treated with Diamel; these results confirm that reduction of body weight increased insulin sensitivity;[10,27] likewise, they demonstrate an additional beneficial effect of Diamel.

Nonetheless, no changes in lipid metabolism were observed with either of the two types of treatment. What we did find interesting was that there were no significant changes in lipid variables. The reason may be that in the other clinical trials Diamel appeared to be associated with hypoglycaemic drugs.[11,12]

Consequently, the reason Diamel might not have helped reduce the triglyceride concentrations could be the prevalence of obesity (BMI >30 kg/m^2) among patients with MS being treated with Diamel and placebo, both at the start and end of the clinical trial, even though both groups lost weight after 12 months (91.17%, 31/34 vs. 89.47%, 34/38 at the start and 79.16%, 19/24 vs. 80.0%, 24/30 of those completing the 12 months, respectively). However, Diamel does manage to reduce waist circumference (WC) after a year of treatment in relation to the baseline recorded for this parameter. This variation did not manage to decrease triglyceride concentrations either, probably because the majority of

patients with MS were still obese. The drop in WC implies that Diamel might be involved in the distribution of abdominal fat.

We find these results extremely interesting because they show that some of the components in Diamel are directly involved in improving insulin secretion in patients with type 2 diabetes[12] and insulin sensitivity. One possible interpretation is related to the components of Diamel, whereby the molecular activation process through electromagnetic procedures used by Catalysis may help to explain these results. Lettuce extract reduces the amount of glucose absorbed in the intestines, while blueberry extract improve microcirculation. L-carnitine, arginine and ornithine mobilise fat, help to turn it into energy, and partly stimulate the secretion of insulin. Glycine helps to release glucagon and also stimulates insulin secretion.[12] Diverse reports described how several components of Diamel such as L-arginine, carnitine, cysteine, glycine and zinc significantly decreased HOMA-IR as well as improving insulin sensitivity and β-cell function.[29-33]

Recently, Stull et al. (2010) reported that daily dietary supplementation with bioactives from whole blueberries improved insulin sensitivity and reduced glucose concentrations over time in obese, nondiabetic, and insulin-resistant participants.[34] Therefore, these results support the effectiveness of a particular component of Diamel (blueberry extract).[35]

Different studies have identified an association between hyperuricaemia and a moderate increase in glucose concentrations, IR, hyperinsulinaemia, creatinine, early kidney damage, obesity, type 2 diabetes and CVD.[36-42] This data is very interesting because treatment with Diamel from 6 months onwards simultaneously improves insulin concentration and UA

concentrations, in addition to IR and IS. After 12 months of treatment creatinine and UA concentrations improve and waist circumference, compared to the initial value, decreases. This implies that, by improving insulin concentration and enhancing IR and IS with Diamel, we are helping to decrease the hyperuricaemia that affects the concentration of creatinine; therefore, we help to prevent early damage to the kidneys.[40-42] As we reduce IR and hyperuricaemia, we consequently delay the onset of diabetes and CVD.[37,40,42]

The Diamel and placebo groups are the same in terms of creatinine concentrations at month 12 of the clinical trial, but they are much lower than the baseline concentration of creatinine in the group administered Diamel. This, is not, however, the case for the placebo set. Consequently, we should be cautious about highlighting Diamel's ability to reduce concentrations of creatinine because we found no significant changes from baseline between study groups.

On the other hand, no adverse effects were identified during the therapy period, confirming the safety of the use of Diamel for human trials at a daily dose of 3960 mg. After comparing the beneficial effects of Diamel to treat MS with the effectiveness and adverse events reported for other drugs used (Metformin, Acarbose, Thiazolidinediones and Orlistat) on MS, and the prevention of type 2 diabetes in high-risk populations with prediabetes,[6,7,10,43-46] we recommend Diamel as an alternative therapy for MS and the prevention or delay of the onset of type 2 diabetes. Although these drugs and Diamel are effective, they are not sufficient, and lifestyle intervention should be combined.

In the last few months, our group reported that Diamel reduces insulin resistance and improved insulin sensitivity in a small sample of women with polycystic ovary syndrome.[47] However, based on our data it is not possible to draw conclusions regarding the mechanisms by which Diamel therapy could induce a long-lasting improvement in insulin sensitivity and reduce insulin resistance; however, this important issue requires further investigation.

Strengths of our study include its randomised, placebo-controlled, double-blind design, and its follow-up period of one year. In addition, the results were consistent across the different statistical analyses used. The novelty of this trial was the use, for the first time, of Diamel as a nutritional supplement to treat MS.

A possible limitation of our clinical trial is the relatively small sample size at the end of the study; consequently, limited power may have obscured smaller treatment effect. Perhaps the sample reduction was due to the fact that patients should have been told that Diamel could affect weight loss, an argument that may have contributed a little to the strength of the clinical trial. It is important to notice that, according to the sample size, our trial is capable of detecting differences equal to or higher than 32% between treatment groups, keeping its statistical power up to 80%. In fact, we see that there is slightly more than a 55% decrease in insulin resistance (HOMA-IR) in the Diamel group at the end of the study (5.67 ± 3.15 to 2.52 ± 0.93).

In conclusion, long-lasting treatment with Diamel, combined with lifestyle changes, appears to add an additional benefit to the health of patients with MS. Diamel represents a new alternative therapy in patients with MS, pre-diabetes and others insulin-resistance diseases.

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Disclosure

The authors have no conflicts of interest to declare.

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Figure 1 Flow of participants through the study.

Figure 2 Changes in the mean values in the HOMA-IR index of the participants treated with Diamel (a). Changes in the mean values of QUICKI (b) insulin sensitivity index and in the BENNETT index (c) of the participants treated with Diamel. Data are given as mean and standard deviation.

Table 1 Composition of Diamel[12]

Ingredients			
Arginine	35.5 mg	Glycine	7.1 mg
Ascorbic acid	10 mg	Ornithine	17.7 mg
Zinc sulphate	6 mg	Calcium pantothenate	1 mg
Folic acid	33 µg	Blueberry extract	345 mg
Fumaric acid	35.5 mg	Lettuce extract	152 mg
L-Carnitine	35.5 mg	L- Cysteine	14.2 mg
Sodium methylparaben	0.33 mg	Pyridoxal	0.33 mg
Cyanocobalamin	0.16 µg		

This product is marketed as a food supplement by the Catalysis laboratories (Macarena, nº14 28016 Madrid, Spain). Website: http://www.diamel.us/

Table 2 Baseline characteristics of study participants

Characteristics	**Diamel n = 34**	**Placebo n = 38**	***P* value**
	N (%)	**N (%)**	
Gender			
Female	25 (73.5)	30 (78.9)	0.782
Male	9 (26.5)	8 (21.1)	0.782
Skin colour (White)	21 (61.8)	15 (39.5)	0.097
Body Weight (Obese)	31 (91.2)	36 (94.7)	0.661
Smoking	10 (29.4)	8 (21.1)	0.430

Acanthosis Nigricans	26 (76.5)	30 (78.9)	1.000
Impaired fasting glucose (≥ 6.1 and < 7 mmol/L)	5 (14.7)	7 (18.4)	0.758
	Mean ± DS	**Mean ± DS**	
Age (years)	42.12 ± 10.34	45.45 ± 13.85	0.256
Weight (kg)	99.98 ± 17.99	98.62 ± 21.31	0.772
Height(cm)	162.95 ± 8.56	162.69 ± 8.73	0.900
BMI (kg/m^2)	37.64 ± 6.03	37.20 ± 7.38	0.784
WC (cm)	108.26 ± 13.42	107.39 ± 12.84	0.788
WHR	1.105 ± 0.095	1.139 ± 0.096	0.146
Systolic BP (mmHg)	129.24 ± 19.35	127.92 ± 17.40	0.762
Diastolic BP (mmHg)	87.79 ± 14.33	86.45 ± 12.62	0.673
Fasting blood glucose (mmol/L)	4.89 ± 0.85	5.31 ± 1.10	0.077
Fasting insulin (μU/mL)	27.10 ± 16.95	21.72 ± 8.09	0.099
HOMA-IR	5.79 ± 3.58	5.15 ± 2.47	0.377
QUICKI	0.49 ± 0.051	0.50 ± 0.044	0.620
Cholesterol (mmol/L)	4.95 ± 0.88	4.77 ± 0.89	0.395
Triglycerides (mmol/L)	1.68 ± 0.42	1.66 ± 0.37	0.822
HDL-C (mmol/L)	1.15 ± 0.26	1.22 ± 0.34	0.367
Creatinine (mmol/L)	94.82 ± 22.92	90.58 ± 22.27	0.429
Uric Acid (mmol/L)	324.65 ± 64.73	343.50 ± 63.41	0.217

Unless indicated otherwise, data show the number of patients in each group (N), with percentages given in parentheses. n, Total cases studied in each of the groups during the corresponding period analysed. Data are also given as mean and standard deviation.

BMI, Body mass index; WC, Waist circumference; WHR, Waist/hip ratio; BP, Blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; QUICKI (quantitative insulin sensitivity check index), BENNETT and RAYNAUD: insulin sensitivity indexes; HDL-C, High-density lipoprotein cholesterol.

Table 3 Changes in the fasting glucose and insulin concentrations in patients during the follow-up period.

Months of T	Diamel		Placebo			Diamel		Placebo		
	Fasting glucose (mmol/L)					Fasting insulin (μU/mL)				
	n	mean ± SD	N	mean ± SD	p†	N	mean ± SD	n	mean ± SD	*P*†
Baseline	**34**	4.89 ± 0.85	**38**	5.31 ± 1.10	0.077	**34**	27.10 ± 16.95	**38**	21.72 ± 8.09	0.099
3	**34**	4.66 ± 0.71	**38**	4.93 ± 1.19	0.264	**34**	16.82 ± 9.54	**38**	17.96 ± 12.05	0.659
6	**28**	**4.78 ± 0.83**	**34**	5.52 ± 1.38	**0.029**	**28**	**12.89 ± 6.51***	**34**	17.52 ± 9.85	0.057
9	**25**	**4.88 ± 0.75**	**32**	5.54 ± 0.98	**0.011**	**25**	**12.44 ± 6.29****	**32**	15.67 ± 6.80	0.062
12	**24**	**4.85 ± 0.59**	**30**	5.60 ± 1.21	**0.021**	**24**	**11.62 ± 3.83*****	**30**	16.44 ± 8.35	**0.025**

Data are given as mean and SD; T, Treatment; SD, Standard deviation; n, Total cases studied in each of the groups during the corresponding period analysed.

†*P* value of the comparison made between the treatment and control groups for each of the corresponding three month periods. $^{*}P$= 0.043, $^{**}P$= 0.030, $^{***}P$= 0.004 of the comparison between the groups (changes from baseline).

Table 4 Lipid, creatinine and uric acid concentrations in patients with MS treated with Diamel and Placebo during the treatment period

Data are given as mean and SD. †*P* value of the comparison made between the treatment and

Variables	Time (month)	Diamel mean ± SD	Placebo mean ± SD	*P*†
Cholesterol (mmol/L)	**Baseline**	4.95 ± 0.88	4.77 ± 0.89	0.395
	3	4.67 ± 0.95	4.56 ± 0.89	0.613
	6	4.69 ± 0.88	4.78 ± 0.85	0.854
	9	4.67 ± 0.90	4.75 ± 0.76	0.618
	12	4.56 ± 0.93	4.55 ± 0.97	0.747
Triglycerides (mmol/L)	**Baseline**	1.68 ± 0.42	1.66 ± 0.37	0.822
	3	1.58 ± 0.55	1.54 ± 0.57	0.769
	6	1.73 ± 0.70	1.66 ± 0.70	0.810
	9	1.55 ± 0.61	1.82 ± 0.82	0.247
	12	1.67 ± 0.75	1.60 ± 0.60	0.958
HDL-C (mmol/L)	**Baseline**	1.17 ± 0.26	1.22 ± 0.34	0.365
	3	1.17 ± 0.20	1.17 ± 0.28	0.998
	6	1.15 ± 0.27	1.04 ± 0.29*	0.185
	9	1.08 ± 0.17	1.09 ± 0.25	0.376
	12	1.14 ± 0.19	1.16 ± 0.22	0.872
Creatinine (mmol/L)	**Baseline**	94.82 ± 22.92	90.58 ± 22.27	0.429
	3	83.50 ± 21.83	90.21 ± 27.80	0.153
	6	84.71 ± 19.30	84.09 ± 23.92	0.848
	9	87.64 ± 26.43	96.47 ± 23.38	0.139
	12	87.08 ± 17.32	89.10 ± 15.18	0.828
Uric acid (mmol/L)	**Baseline**	324.65 ± 64.73	343.50 ± 63.41	0.217
	3	297.03 ± 56.08	322.84 ± 72.09	0.699
	6	**298.57 ± 68.26**	347.53 ± 82.08	**0.016**
	9	**302.36 ± 69.47****	345.56 ± 87.99	0.077
	12	**281.25 ± 67.44*****	332.00 ± 72.08	**0.011**

control groups for each of the corresponding three month periods. **P*= 0.023; ***P*= 0.040; ****P*= 0.033 of the comparison made between the groups (changes from baseline).

SD, Standard deviation; HDL-C, High-density lipoprotein cholesterol

The total number of cases studied in each period analysed is the same as what is shown in table 3.

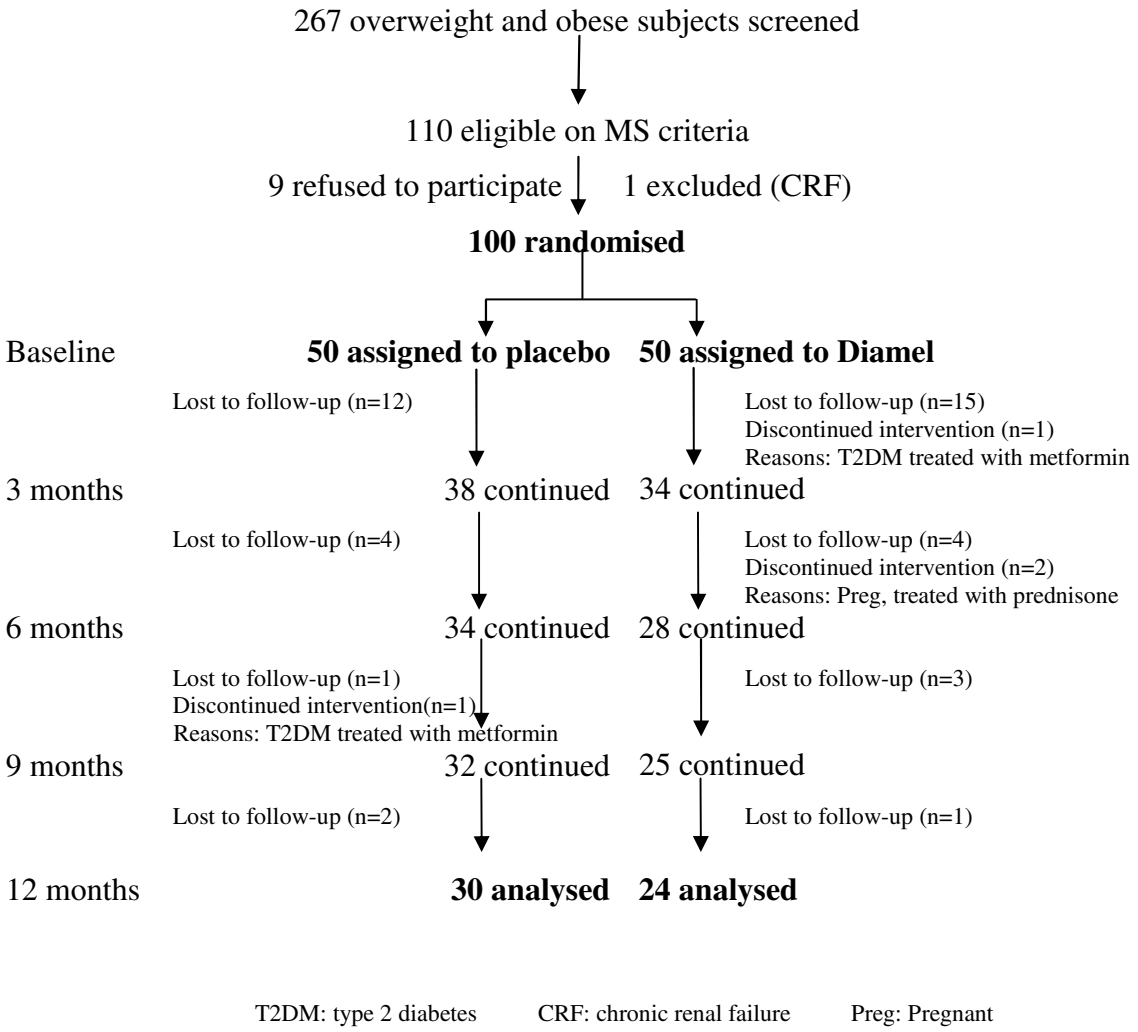
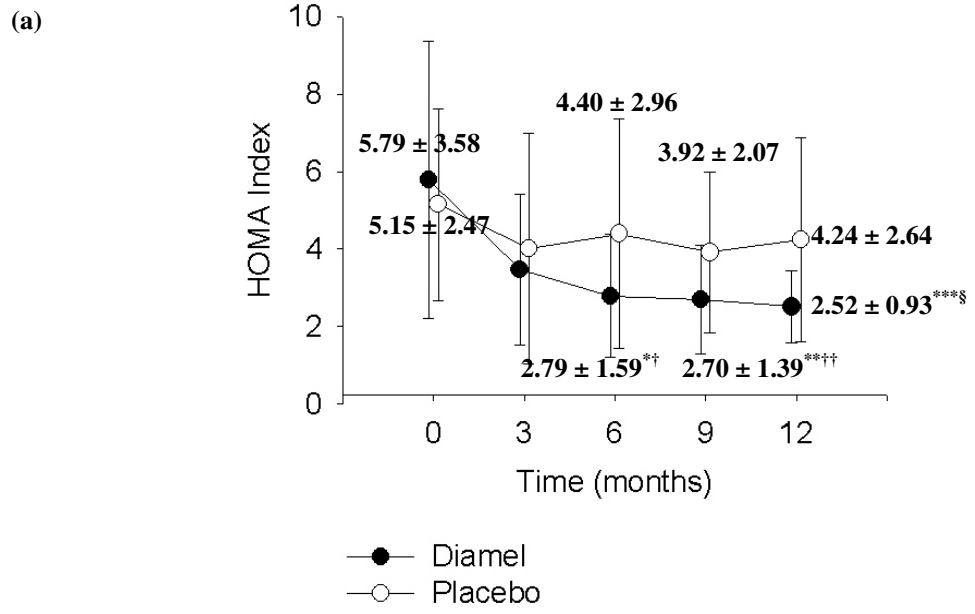
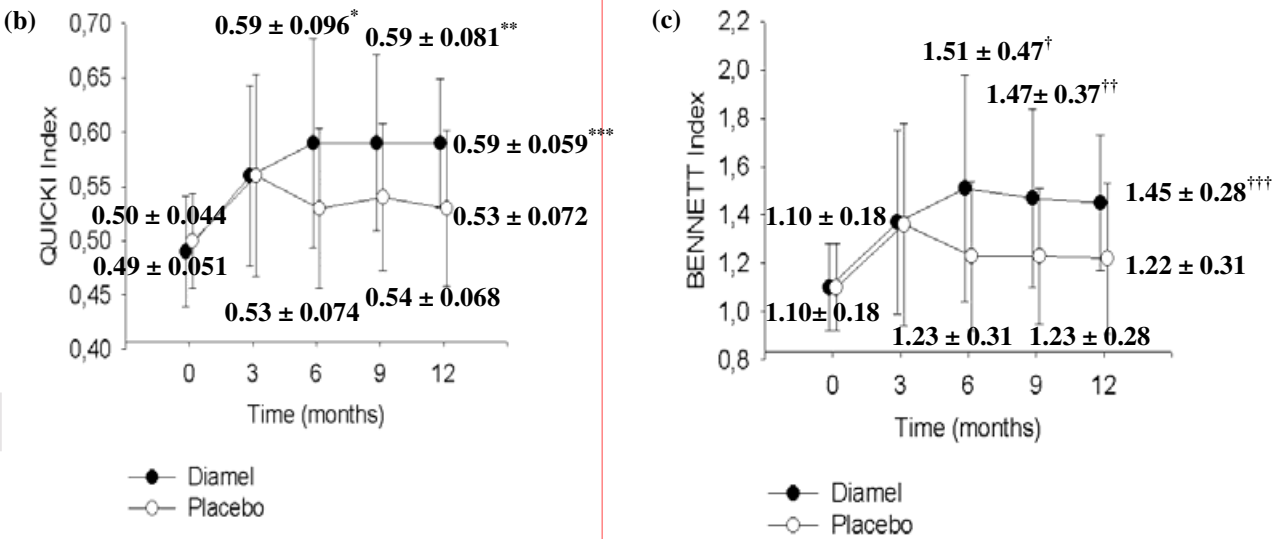


Figure 1 Flow of participants through the study



*P = 0.012 vs. placebo; **P = 0.020 vs. placebo; ***P = 0.008 vs. placebo; †P = 0.038; ††P = 0.007; §P = 0.002 for the comparison between groups (change from baseline)



*P= 0.012 vs. placebo; **P= 0.020 vs. placebo; ***P= 0.008 vs. placebo

†P= 0.012 vs. placebo; ††P= 0.011 vs. placebo; †††P= 0.007 vs. placebo

Figure 2 Changes in the mean values in the HOMA-IR index of the participants treated with Diamel (a). Changes in the mean values of QUICKI (b) insulin sensitivity index and in the BENNETT index (c) of the participants treated with Diamel. Data are given as mean and standard deviation.