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ORIGINAL ARTICLE

Effect of Diamel in patients with metabolic syndrome: A randomized double-blind placebo-controlled study*

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Abstract

Background: The aim of the present study was to determine whether the administration of Diamel, marketed as a food supplement by Catalysis Laboratories (Madrid, Spain) could improve any of the components of metabolic syndrome (MS), as well as insulin resistance and sensitivity.

Methods: In all, 100 patients with MS (19–70 years of age) who satisfied the World Health Organization criteria for MS were included in the study. Participants were randomly assigned to receive either oral Diamel or a placebo (while maintaining a diet appropriate to their weight and physical activity) at a dose of two capsules before each of the three main meals each day for 1 year. Anthropometric indices, blood pressure, fasting plasma glucose, lipid profile, insulin, creatinine, and uric acid (UA) were determined. Insulin resistance (IR) was assessed and three indirect indices were used to calculate insulin sensitivity (IS).

Results: Compared with placebo, Diamel improved fasting insulin concentrations, IS, and IR and reduced UA concentrations from 6 months until the end of treatment ($P < 0.05$ for all). In addition, after 12 months treatment with Diamel, significant changes from baseline were seen for mean fasting insulin ($P < 0.05$), UA ($P < 0.05$), IR ($P < 0.001$), and IS ($P < 0.001$), whereas no such changes were seen in the placebo-treated group. Improvements were noted in body mass index, IR, and IS in both groups.

Conclusions: Long-term Diamel treatment, combined with lifestyle changes, was beneficial for IR and IS, and reduced serum UA levels in patients with MS.

Keywords: Diamel, insulin resistance, insulin sensitivity, metabolic syndrome, uric acid.

Significant findings of the study: Using Diamel as a nutritional supplement for the treatment of MS revealed beneficial effects on insulin resistance and insulin sensitivity, as well as reductions in serum uric acid levels.
What this study adds: Long-term treatment with Diamel appears to provide further health benefits to patients with metabolic syndrome and, as such, represents a new alternative therapy (without adverse effects) for patients with metabolic syndrome, prediabetes, and other diseases characterized by insulin resistance.

Introduction

Metabolic syndrome (MS), one of the most controversial medical entities of recent 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 World Health Organization (WHO) published a working definition of MS meant to facilitate research on the condition and to enable better comparisons between studies rather than to serve as a strict definition.[3] The WHO defined MS as insulin resistance, the presence of impaired glucose tolerance, or type 2 diabetes and at least two of the following: abdominal obesity (i.e. a waist:hip ratio [WHR] >0.90 in men and >0.85 in women or a body mass index [BMI] $\geq$30 kg/m^2); dyslipidemia (serum triglycerides [TG] $\geq$ 1.70 mmol/L or high-density lipoprotein–cholesterol [HDL–C] <0.9 mmol/L in men and <1.0 mmol/L in women), hypertension ($\geq$160/90 mmHg), or microalbuminuria. These core components were considered most suitable for a general definition, although many other disturbances, such as disorders of coagulation and endothelial function, hyperuricemia, and elevated leptin levels, have been associated with MS.[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 (Catalysis Laboratories, Madrid, Spain) arrived on the market. The components of Diamel include trace elements, amino acids, vitamins, and lettuce and blueberry extracts, and are they activated via a magnetization process (Table 1).[11,12] Diamel 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] In turn, these free radicals are responsible, to a considerable extent, for the cell damage and complications associated with MS.[13,14] Diamel has been specially 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 the gastrointestinal absorption of glucose, hopefully making it a nutritional supplement beneficial for MS patients because of its ability to regulate carbohydrate and lipid metabolism[11,12] and its ability to stop diabetes progression.

In 2006, it was 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 glibenclamide alone after 6 months treatment.[12] On the basis of these results, we believe that Diamel could be an effective tool in the treatment of MS.

Table 1 Composition of Diamel,[12] marketed as a food supplement by Catalysis Laboratories (Madrid, Spain; http://www.hipermercado-natural.com/diamel-90-capsulas-de-660-mg-p-897.html, accessed 29 September 2012)

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

The aim of the present study was to assess the effectiveness of Diamel in improving the metabolic and clinical features of MS, as well as insulin resistance and sensitivity (IR and IS, respectively), in a group of MS sufferers treated with the supplement. These parameters were evaluated in the present double-blind placebo-controlled randomized clinical trial over a period of 1 year. The present study is the first clinical trial of Diamel as a nutritional supplement for the treatment of MS.

Methods

Participants

The study subjects were recruited through various methods. For example, people from earlier epidemiologic surveys who were eligible for inclusion were contacted. In addition, subjects were recruited with flyers and by promoting the study through direct communication and via opportunistic population screenings with special emphasis on high-risk groups, such as overweight and/or obese subjects.

The inclusion criteria accepted individuals of either gender aged between 19 and 70 years who fulfilled the WHO diagnostic criteria for MS and had no history of previous or current use of oral antidiabetic agents. Patients who met these criteria and who agreed to take part in the study were asked to provide written informed consent.

Patients who declined to take part in the study were excluded, as were those who exhibited one or more of the following contraindications: type 1 diabetes, type 2 diabetes treated with antidiabetic agents at any time before the trial, any clinical disability, the use of special diets, a history of chronic medication use, the use of mineral and/or vitamin supplements, pregnancy, breast-feeding, chronic disease, a history of any acute infection, and the use of immunosuppressant drugs.

Patients who failed to complete the minimum treatment time (3 months) were also excluded from the study. Data from subjects who adhered to the treatment for at least 3 months but later discontinued treatment were only analyzed for each of the corresponding 3 month periods in which the clinical trial groups were compared.

Definition of MS used in the present study was based on the WHO working definition.[3] Specifically, in addition to having type 2 diabetes, impaired fasting glucose (IFG; $\geq$110 mg/dL; $\geq$6.1 mmol/L), impaired glucose tolerance (IGT), and/or insulin resistance (>75th percentile homeostasis model assessment of insulin resistance [HOMA-IR], patients were required to have at least two of the following four factors: (i) central obesity, defined as a WHR of >0.9 in men and >0.85 in women and/or BMI $\geq$30 kg/m^2; (ii) increased plasma TG levels, defined as $\geq$150 mg/dL ($\geq$1.7 mmol/L), or be receiving treatment; (iii) low HDL–C, defined as <35 mg/dL (<0.9 mmol/L) in men and <39 mg/dL (<1.0 mmol/L) in women, or be receiving treatment; and (iv) increased arterial pressure, initially defined as systolic blood pressure (SBP) $\geq$160 mmHg or diastolic blood pressure (DBP) $\geq$90 mmHg, but later modified to $\geq$140/90 mmHg or to be receiving treatment.

Sample size estimation

The estimation of the sample size was based on a 17.5% decrease in IR 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 IR in the Diamel group at a significance level of 0.05. The expected dropout rate was 5%. Thus, a target of recruiting 50 eligible subjects for each group was set.

Ethical considerations

The present study was conducted in accordance with the Declaration of Helsinki and its amendments (http://www.wma.net/en/30publications/10policies/b3/17c.pdf, accessed 26 September 2012). The study protocol was approved by the Ethics and Research Committee of the National Institute of Endocrinology of Cuba. This study has been registered at ClinicalTrials.gov (registration no. NCT01025115).

Study design and dietary supplement regimen

The present study was a randomized double-blind parallel-group placebo-controlled Phase III trial performed at a single center (National Institute of Endocrinology, Havana, Cuba) to investigate whether daily oral administration of Diamel could improve any of the components of MS, as well as IR and IS. The duration of the study was 24 months (from March 2009 to March 2011).

After initial evaluation, all subjects who met the eligibility criteria and wanted to participate in the study were enrolled consecutively. Subjects were randomly assigned to receive either Diamel (n = 50) or a placebo (n = 50) at a dose of two capsules before each of the three main meals each day for 1 year while maintaining a diet appropriate to their weight and level of physical activity, as well as appropriate hypertensive drugs (angiotensin-converting enzyme inhibitors) in the case of subjects with hypertension. A maximum maintenance dose of Diamel 3960 mg (six capsules) was used.[12]

All subjects received advice and counseling regarding 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 based on their daily calorie intake requirements per kg body weight and their level of physical activity. Subjects were provided with diets with the following proportion of nutrients: 55–60% carbohydrates, 15–20% protein, and 20% fat. Diets ranged from 1200 to 1500 calories.[12] Patients in both groups were also encouraged to increase their physical activity (e.g. walking for 30–45 min/day 3–4 days/week).[12]

Randomization within the study was generated using a computerized random number generator. The treatments used in the study (i.e. Diamel and placebo tablets) were supplied by the Catalysis Laboratories and were labeled with the randomization code only. All personnel involved in the study remained unaware of the association between the codes and the contents of the pills. Code-to-pill content associations were kept in a sealed envelope by the Head of the Research Methodology Department of the National Institute of Endocrinology. Seal and envelope integrity were checked every 3 months. At the end of the study, the envelope was opened.

The treatment (Diamel or placebo) was administered for 12 months from initial patient screening. The effects of Diamel were evaluated at 3, 6, 9, and 12 months from commencement of treatment and compared with the effects of the placebo.

Adverse effects

Every 3 months, subjects underwent a clinical examination to determine whether they had experienced any adverse effects. Height, weight, adverse events (e.g. rashes, dyspepsia, and hepatotoxicity) were recorded. To evaluate hepatotoxicity, blood samples were withdrawn from participants during the first three visits and hepatic enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase 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 3 months. Concentrations of fasting glucose and insulin, cholesterol, TG, HDL–C, uric acid (UA), and creatinine were recorded during the aforementioned period for all those taking part.

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

Physical examination

Physical examination included determination of height, weight, waist and hip circumferences, and blood pressure. Height and weight were measured and BMI was calculated as weight (kg) divided by height squared (m^2). Subjects with a BMI >30 kg/m^2 were considered obese. Waist circumference (WC) was measured in standing subjects using a non-elastic tape midway between the lower margin of the rib cage and the superior iliac crest during mild expiration. The WHR was defined as the ratio of waist girth to the circumference of the hips measured at the trochanter major.

Blood pressure was measured three times using a standard mercury sphygmomanometer after a 5-min rest in seated subjects. The readings at the first and fifth Korotkoff phase were taken as SBP and DBP, respectively. The average of the three blood pressure measurements was recorded and included in the analyses.

Laboratory tests

Fasting plasma glucose (FPG) levels and lipid profile, including total cholesterol, TG, and HDL–C, in addition to creatinine and UA, were measured enzymatically using an autoanalyzer (Elimat, Labarthe-Inard, France) using commercially available kits (Cpm Diagnostic Research, Rome, Italy). Fasting plasma insulin concentrations were measured by an immunoradiometric assay (IRMA; Izotop, Budapest, Hungary). The insulin resistance index was calculated according to Matthews et al.[15] as HOMA-IR = fasting insulin [μU/mL] × fasting glucose [mmol/L]/22.5. In the present study, HOMA-IR values ≥ 2.6 were taken to indicate IR.[16]

Three indirect indices were used to calculate IS, including quantitative insulin sensitivity check index (QUICKI),[17] the Bennett Index[18] and the Raynaud index,[19] as follows:

$$\text{QUICK} = 1/(\log \text{insulin}_0 + \log \text{glucose}_0)$$

$$\text{Bennett Index} = 1/(\log \text{insulin}_0 \times \log \text{glucose}_0)$$

$$\text{Raynaud Index} = 40/\text{insulin}_0.$$

Outcomes

The primary outcomes included changes in glucose, insulin, and lipid concentrations, as well as variations in creatinine and UA concentrations. The primary aim of the study was to determine whether long-term Diamel treatment was able to improve IR and IS in subjects with MS. Decreases in HOMA-IR as well as increases in insulin sensitivity (QUICKI, Bennett and Raynaud indices) indicate the amelioration of IR. Secondary outcomes included variations in the blood pressure, BMI, WC, and the WHR.

Moreover, "changes" were considered as differences between the measurements obtained in each of the two groups in consecutive 3-month periods, as well as those between baseline and after 12 months treatment.

Statistical analysis

All statistical analyses were performed using SPSS for Windows (version 11.5; SPSS, Chicago, IL, USA). Two-sided $P < 0.05$ was considered significant. Descriptive data are expressed as the mean ± SD after confirmation of normal distribution by the Kolmogorov–Smirnov test. Differences between mean values in each group were compared by Student's *t*-test for those variables with a normal distribution and by the Mann–Whitney *U*-test for variables that did not have a normal distribution. Proportions were compared using the Chi-squared test or Fisher's exact test, as appropriate.

Differences in the effects of 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 period (12 months). Correlation analyses were 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, 110 met the MS criteria for study entry and underwent randomization, as indicated in Fig. 1. For this analysis, 100 were randomly assigned to two groups of equal number, one group (n = 50) received Diamel and the other group (n = 50) received a placebo for 1 year. In the Diamel and placebo groups, 23 and 19 subjects, respectively, gave up treatment after 1 year of the clinical trial (Fig. 1). One individual was removed from the Diamel group 1 month after the trial had started due to metformin therapy. In the same group, two subjects were excluded from the study at the 3rd month: a 22-year-old woman who lost 8.5 kg during the study and later fell pregnant and a second patient who started prednisone treatment for bronchial asthma (Fig. 1). In the group assigned to the placebo, one subject began treatment with metformin 6 months into the trial once diet and physical activity had failed to reduce fasting glucose concentration (9.62 mmol/L; Fig. 1).

Baseline clinical and biological characteristics of the subjects

The baseline characteristics of the MS patients recruited to the present study are given in Table 2. There was no significant differences between the placebo and Diamel groups for any clinical or biochemical variables at baseline.

Clinical, anthropometric, and biochemical evaluations during treatment

From the second 3-month period on, fasting plasma glucose decreased significantly more in patients in the Diamel group than in those allocated placebo. However, no significant changes in FPG were observed in either of the two groups compared with baseline after 12 months of therapy (Table 3). By 6 months, Diamel administration had reduced serum insulin concentrations below baseline values and below levels in the placebo group at the same time point (Table 3). Furthermore, by 12 months, the decrease in insulin levels was significantly greater in the Diamel compared with placebo group ($P \leq 0.025$; Table 3).

Diamel treatment decreased HOMA-IR values at 6, 9, and 12 months to levels lower than in the placebo group, in terms of both absolute values and changes from baseline (Fig. 2a).

By 6 months, improvements in IS were more significant in patients treated with Diamel than those allocated

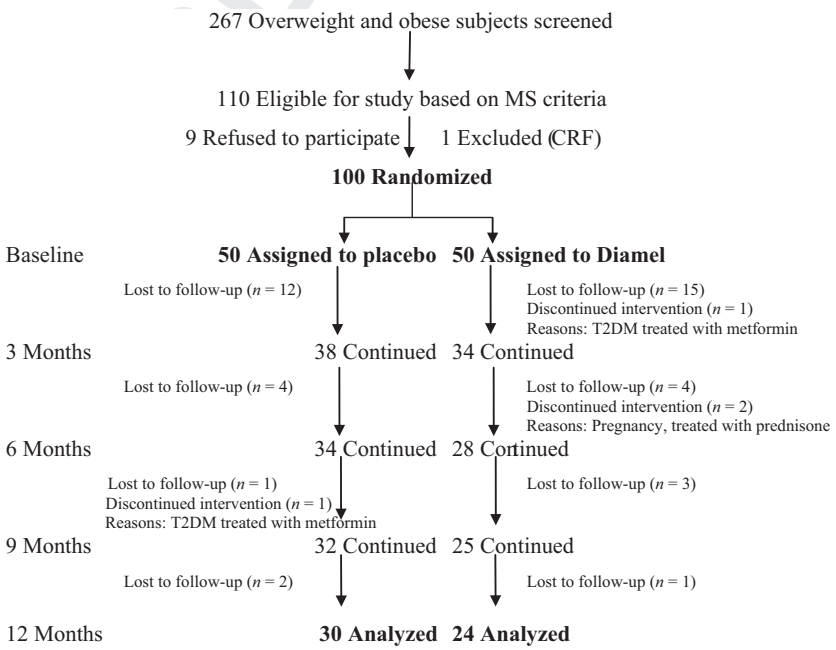

Figure 1 Flow chart showing subject distribution throughout the study. CRF, chronic renal failure; T2DM, type 2 diabetes mellitus.

Table 2 Baseline characteristics of the study participants

	Diamel (n = 34)	Placebo (n = 38)	P-value
No. women (%)	25 (73.5)	30 (78.9)	0.782
No. men (%)	9 (26.5)	8 (21.1)	0.782
No. Whites (%)	21 (61.8)	15 (39.5)	0.097
No. obese individuals (%)	31 (91.2)	36 (94.7)	0.661
No. smokers (%)	10 (29.4)	8 (21.1)	0.430
No. with acanthosis nigricans (%)	26 (76.5)	30 (78.9)	1.000
No. with IFG (6.1–7 mmol/L glucose)	5 (14.7)	7 (18.4)	0.758
Age (years)	42.1 ± 10.3	45.5 ± 13.9	0.256
Weight (kg)	100 ± 18	98.6 ± 21.3	0.772
Height (cm)	163 ± 9	163 ± 9	0.900
BMI (kg/m^2)	37.6 ± 6.0	37.2 ± 7.4	0.784
WC (cm)	108 ± 13	107 ± 13	0.788
WHR	1.105 ± 0.095	1.139 ± 0.096	0.146
SBP (mmHg)	129 ± 19	128 ± 17	0.762
DBP (mmHg)	87.8 ± 14.3	86.5 ± 12.6	0.673
FBG (mmol/L)	4.89 ± 0.85	5.31 ± 1.10	0.077
Fasting insulin (μU/mL)	27.1 ± 17.0	21.7 ± 8.1	0.099
HOMA-IR	5.79 ± 3.58	5.15 ± 2.47	0.377
QUICKI	0.49 ± 0.05	0.50 ± 0.04	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)	94.8 ± 22.9	90.6 ± 22.3	0.429
Uric acid (mmol/L)	325 ± 65	344 ± 63	0.217

Data are given as the number of subjects in each group, with percentages in parentheses, or as the mean ± SD, as appropriate.
BMI, body mass index; WC, waist circumference; WHR, waist:hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HOMA-IR, homeostatic model assessment of insulin resistance; IFG, impaired fasting glucose; QUICKI, quantitative insulin sensitivity check index; HDL–C, high-density lipoprotein–cholesterol.

placebo, as indicated by results of the QUICKI (Fig. 2b) and Bennett Index (Fig. 2c). In contrast, the Raynaud Index highlighted an increase in IS only after 12 months treatment in subjects receiving Diamel compared with those receiving placebo (0.53 ± 0.19 vs 0.42 ± 0.20, respectively; P = 0.025).

There were no significant differences between the Diamel and placebo groups in terms of cholesterol, TG, HDL–C, blood pressures or BMI and the end of the 12-month treatment period (Tables 4,5). However, compared with subjects assigned to the placebo group, those treated with Diamel had lower UA levels from 6 months onwards in terms of both absolute values and changes from baseline (Tables 4,5).

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

Duration of treatment	Fasting glucose (mmol/L)	Fasting insulin (μU/L)
Baseline		
Diamel (n = 34)	4.89 ± 0.85	27.1 ± 17.0
Placebo (n = 38)	5.31 ± 1.10	21.7 ± 8.1
P-value	0.077	0.099
3 months		
Diamel (n = 34)	4.66 ± 0.71	16.8 ± 9.5
Placebo (n = 38)	4.93 ± 1.19	18.0 ± 12.1
P-value	0.264	0.659
6 months		
Diamel (n = 28)	4.78 ± 0.83	12.9 ± 6.5*
Placebo (n = 34)	5.52 ± 1.38	17.5 ± 9.9
P-value	0.029	0.057
9 months		
Diamel (n = 25)	4.88 ± 0.75	12.4 ± 6.3**
Placebo (n = 32)	5.54 ± 0.98	15.7 ± 6.8
P-value	0.011	0.062
12 months		
Diamel (n = 24)	4.85 ± 0.59	11.6 ± 3.8***
Placebo (n = 30)	5.60 ± 1.21	16.4 ± 8.4
P-value	0.021	0.025

Data are given as mean ± SD.
*P = 0.043, **P = 0.030, ***P = 0.004 compared with baseline.

Diamel treatment reduced the abdominal circumference and creatinine and UA concentrations below baseline values after 12 months treatment (Table 5). Reductions in BMI and IR were seen in both groups as a result of increased physical activity and dietary changes. Similarly, increased IS and decreased insulin concentrations compared with baseline values were seen in both groups after 12 months therapy (Table 5).

The proportion of participants in the Diamel and placebo groups at 12 months who met the goal of at least 150 min physical activity per week (assessed on the basis of logs kept by the subjects) was 54.2% (13/24) and 53.3% (16/30), respectively. There was no significant difference between the Diamel and placebo groups in terms of dietary compliance at the end of the study (70.8% [17/24] and 70.0% [21/30], respectively).

After 12 months treatment, only subjects in the Diamel group exhibited significant changes from baseline in mean fasting insulin, UA, IR, and IS (Table 5). At the end of the study, negative correlations were detected in the Diamel-treated group with weight and BMI for changes from baseline for QUICKI (r = −0.51, P = 0.011; and r = −0.53, P = 0.008, respectively) and Bennett Index (r = −0.49,

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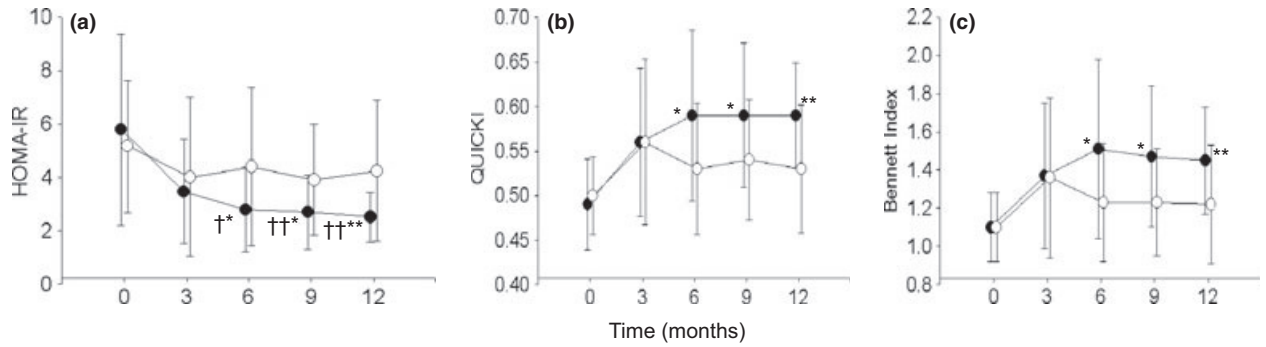

Figure 2 Changes (a) homeostasis model assessment of insulin resistance (HOMA-IR), (b) quantitative insulin sensitivity check index (QUICKI), [10] and (c) Bennett Index of insulin sensitivity in subjects treated with Diamel (●) or placebo (○). Data are given as the mean ± SD. $*P < 0.05$, $**P < 0.01$ compared with placebo; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ compared with baseline. [3]

Table 4 Lipid, creatinine and uric acid concentrations in patients with metabolic syndrome treated with Diamel or placebo

Duration of treatment	Diamel	Placebo	*P*-value
Cholesterol (mmol/L)			
Baseline	4.95 ± 0.88	4.77 ± 0.89	0.395
3 months	4.67 ± 0.95	4.56 ± 0.89	0.613
6 months	4.69 ± 0.88	4.78 ± 0.85	0.854
9 months	4.67 ± 0.90	4.75 ± 0.76	0.618
12 months	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 months	1.58 ± 0.55	1.54 ± 0.57	0.769
6 months	1.73 ± 0.70	1.66 ± 0.70	0.810
9 months	1.55 ± 0.61	1.82 ± 0.82	0.247
12 months	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 months	1.17 ± 0.20	1.17 ± 0.28	0.998
6 months	1.15 ± 0.27	1.04 ± 0.29*	0.185
9 months	1.08 ± 0.17	1.09 ± 0.25	0.376
12 months	1.14 ± 0.19	1.16 ± 0.22	0.872
Creatinine (mmol/L)			
Baseline	94.8 ± 22.9	90.6 ± 22.3	0.429
3 months	83.5 ± 21.8	90.2 ± 27.8	0.153
6 months	84.7 ± 19.3	84.1 ± 23.9	0.848
9 months	87.6 ± 26.4	96.5 ± 23.4	0.139
12 months	87.1 ± 17.3	89.1 ± 15.2	0.828
Uric acid (mmol/L)			
Baseline	325 ± 65	344 ± 63	0.217
3 months	297 ± 56	323 ± 72	0.699
6 months	299 ± 68	348 ± 82	0.016
9 months	302 ± 69**	346 ± 88	0.077
12 months	281 ± 67***	332 ± 72	0.011

Data are given as mean ± SD. The total number of individuals studied in each period is as given in Table 3.
$*P = 0.023$, $**P = 0.040$, $***P = 0.033$ compared with baseline. [4]
HDL–C, high-density lipoprotein–cholesterol.

$P = 0.015$; and $r = -0.52$, $P = 0.010$, respectively). No adverse effects were seen in any of the participants during the clinical trial.

Discussion

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

Several large-scale studies in different populations have highlighted the benefits of lifestyle-modification programs including weight-reducing diets and moderate intensity exercise for both treating components of MS and also decreasing the risk of the progression of diabetes.[6,7,20–25] The present study confirms these data, because we found that lifestyle changes (appropriate diet based on the patient's weight and physical activity) during the clinical trial improved some components of MS.

For several years, "placebo" has been defined as containing inert contents and has been used as a control in both clinical trials and treatments in clinical practice. However, recent research shows that the placebo effect is a real psychological event attributable to the overall therapeutic context and that these effects can be robust in both laboratory and clinical settings.[26] In the present study, subjects treated with placebo also had to change their lifestyle. It is possible that, in this group, the decreases in BMI and IR and the improvement in insulin concentrations and IS after 12 months treatment may have been due to lifestyle changes and the characteristic psychological effects of the placebo itself. This interpretation is supported by the fact that various subjects in the placebo group exhibited improvements in the aforementioned parameters, probably because they satisfied the physical exercise and dietary requirements of the study, and, at the end of the clinical trial when the type of treatment for each participant was revealed, they were surprised that they had not been given Diamel.

Table 5 Clinical and biochemical outcomes in patients with metabolic syndrome after 12 months treatment with Diamel or placebo

	Placebo (n = 30)			Diamel (n = 24)			P-value (change from baseline between groups)	P-value (12 months vs baseline in the Diamel group)
	Baseline	12 months	Change	Baseline	12 months	Change		
Weight (kg)	97.5 ± 20.1	92.0 ± 19.2*	−5.47 ± 1.13	98.6 ± 19.5	91.3 ± 17.9	−7.37 ± 1.25	0.166	<0.0005
BMI (kg/m^2)	37.6 ± 7.3	35.5 ± 7.1*	−2.10 ± 0.43	36.3 ± 5.1	33.6 ± 5.0	−2.68 ± 0.44	0.223	<0.0005
WC (cm)	108 ± 10	105 ± 12	−2.23 ± 1.09	108 ± 15	104 ± 14	−4.39 ± 1.22	0.172	0.002
WHR	0.884 ± 0.061	0.893 ± 0.060	0.01 ± 0.01	0.912 ± 0.090	0.901 ± 0.076	−0.01 ± 0.01	0.088	0.607
SBP (mmHg)	128 ± 18	133 ± 16	4.80 ± 3.22	131 ± 20	128 ± 13	−2.98 ± 4.76	0.146	0.475
DBP (mmHg)	85.3 ± 12.7	88.1 ± 11.1	2.73 ± 2.35	90.0 ± 14.9	87.0 ± 9.7	−2.96 ± 2.84	0.268	0.463
Fasting glucose (mmol/L)	5.25 ± 1.09	5.6 ± 1.2	0.31 ± 0.30	4.85 ± 0.87	4.85 ± 0.59	−0.15 ± 0.17	0.413	0.954
Fasting insulin (μU/mL)	21.7 ± 8.4	16.4 ± 8.4**	−5.71 ± 1.94	27.2 ± 17.3	11.6 ± 3.8	−16.5 ± 4.7	0.004	<0.0005
HOMA-IR	5.05 ± 2.31	4.24 ± 2.64***	−1.02 ± 0.51	5.67 ± 3.15	2.52 ± 0.93	−3.45 ± 0.84	0.002	<0.0005
QUICKI	0.50 ± 0.04	0.53 ± 0.07††	0.03 ± 0.01	0.49 ± 0.05	0.59 ± 0.06	0.09 ± 0.01	<0.0001	<0.0005
Bennett Index	1.11 ± 0.18	1.22 ± 0.31†††	0.12 ± 0.04	1.10 ± 0.17	1.45 ± 0.28	0.35 ± 0.04	0.002	<0.0005
Raynaud Index	0.30 ± 0.15	0.42 ± 0.20‡	0.12 ± 0.03	0.26 ± 0.10	0.53 ± 0.19	0.27 ± 0.04	0.002	<0.0005
Cholesterol (mmol/L)	4.61 ± 0.84	4.55 ± 0.97	−0.11 ± 0.17	4.86 ± 0.82	4.56 ± 0.93	−0.35 ± 0.18	0.356	0.113
Triglycerides (mmol/L)	1.69 ± 0.39	1.60 ± 0.60	−0.27 ± 0.11	1.72 ± 0.47	1.67 ± 0.75	0.11 ± 0.15	0.787	0.530
HDL–C (mmol/L)	1.20 ± 0.29	1.16 ± 0.22	0.00 ± 0.06	1.14 ± 0.27	1.14 ± 0.19	−0.02 ± 0.09	0.781	0.382
Creatinine (mmol/L)	90.8 ± 20.5	89.1 ± 15.2	−2.41 ± 3.83	99.5 ± 21.9	87.1 ± 17.3	−16.9 ± 6.0	0.113	0.031
Uric acid (mmol/L)	349.8 ± 63.8	332 ± 72	0.18 ± 17.00	341 ± 57	281 ± 67	−49.4 ± 20.8	0.033	0.002

Data at baseline and 12 months are given as the mean ± SD. Data for the change from baseline are given as the mean ± SEM.
*P < 0.0005, **P = 0.001, ***P = 0.030, $^{\dagger\dagger}P$ = 0.006, $^{\dagger\dagger\dagger}P$ = 0.027, $^{\ddagger}P$ = 0.002 compared with baseline placebo.
BMI, body mass index; WC, waist circumference; WHR, waist:hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; Bennett Index and Raynaud Index, indirect indices of insulin sensitivity; HDL–C, high-density lipoprotein–cholesterol.

The insulinemia and uricemia values and IR in the Diamel-treated group were seen to decrease and there was an improvement in IS after 6 months treatment. This implies that the effectiveness of the Diamel food supplement is cumulative and gradual.

The reduction in FPG in the Diamel compared with placebo group (Table 3) does not mean that Diamel reduced glucose concentrations because: (i) most subjects had glucose concentrations within the normal range; and (ii) we did not find any difference in glucose concentrations at the end of the study compared with baseline.

It has been reported previously that Diamel combined with glibenclamide treatment in subjects with type 2 diabetes improved fasting and 2-h postprandial glucose concentrations, as well as HbA1c, after 6 months compared with glibenclamide treatment alone.[12] The authors of this first controlled clinical trial also noticed an average drop in cholesterol and triglyceride values at 6 months in the Diamel + glibenclamide-treated group and compared with glibenclamide treatment alone.[12] This coincides with the findings reported by Cheta and Trifan,[11] although theirs was an open and uncontrolled study. The authors of both studies explained that the positive results in lipid concentrations could be explained by better blood glucose control, as well as the fact that, among other components, Diamel contains amino acids and vitamins that could affect lipid metabolism.[11,12]

The results obtained in the present randomized double-blind placebo-controlled clinical trial in subjects with MS concur with the reported effects of Diamel has on UA concentrations[11] and provide new evidence as to how Diamel can reduce IR and improve IS. Furthermore, we have shown an inverse association between changes from baseline of some insulin sensitivity indices (QUICKI and Bennett) with weight and BMI in the Diamel-treated group; these results confirm that a reduction in body weight increased IS.[10,25] Similarly, they demonstrate an additional beneficial effect of Diamel.

Nonetheless, no changes were observed in lipid metabolism in either of the two groups. What we did find interesting was that there were no significant changes in lipid variables. The reason for this may be related to the fact that, in the other clinical trials, Diamel appeared to be associated with hypoglycemic drugs.[11,12] Consequently, the reason Diamel may not have helped reduce TG concentrations could be the prevalence of obesity (BMI >30 kg/m^2) among the subjects with MS being treated with Diamel and placebo at both the start (91.2% [31/34] vs 89.5% [34/38], respectively) and end (79.2% [19/24] vs 80.0% [24/30], respectively) of the clinical trial, despite the fact that subjects in both groups lost weight after 12 months. However, Diamel treatment did not reduce WC after 1 year treatment compared with placebo. Nor did Diamel treatment decrease TG concentrations, probably because most of the MS subjects were still obese. The decrease in WC in patients treated with Diamel at the end of the study compared with baseline values implies that Diamel may be involved in the distribution of abdominal fat.

We find the results of the present study 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] as well as IS. One possible interpretation is related to the components of Diamel, whereby the molecular activation process through electromagnetic procedures used by Catalysis Laboratories may help explain these results. Lettuce extract reduces the amount of glucose absorbed in the intestines, whereas blueberry extract improves microcirculation. L-Carnitine, arginine, and ornithine mobilize fat, helping to turn it into energy, and partly stimulate the secretion of insulin. Glycine helps release glucagon and also stimulates insulin secretion.[12] Diverse reports have described how several components of Diamel, such as L-arginine, carnitine, cysteine, glycine, and zinc, significantly decrease HOMA-IR and improve both IS and β-cell function.[27–31]

Recently, Stull et al.[32] reported that daily dietary supplementation with bioactives from whole blueberries improved IS and reduced glucose concentrations over time in obese, non-diabetic, and insulin-resistant subjects. These results support the effectiveness of a particular component of Diamel, namely the blueberry extract.[33]

Different studies have identified an association between hyperuricemia and a moderate increase in glucose concentrations, IR, hyperinsulinemia, creatinine, early kidney damage, obesity, type 2 diabetes, and CVD.[34–40] These data are very interesting because treatment with Diamel from 6 months onwards simultaneously improves insulin and UA concentrations, in addition to IR and IS. After 12 months treatment, creatinine and UA concentrations improved and WC decreased compared with baseline values. This implies that, by improving insulin concentrations and enhancing IR and IS, Diamel helps decrease the hyperuricemia that affects the concentration of creatinine, thus helping to prevent early damage to the kidneys.[38–40] As IR and hyperuricemia are reduced, the onset of diabetes and CVD is consequently delayed.[35,38,40]

Although creatine concentrations at the end of the 12-month treatment period were not significantly different between the Diamel and placebo groups, the change from baseline was much greater in the Diamel-treated group. However, we should be cautious about highlighting the ability of Diamel to reduce creatinine

concentrations because we found no significant changes from baseline between the study groups.

No adverse effects were identified during the treatment period, confirming the safety of Diamel for use in human trials at a daily dosage of 3960 mg. After comparing the beneficial effects of Diamel in MS with the effectiveness and adverse events reported for other drugs used (e.g. metformin, acarbose, thiazolidinediones and orlistat), as well as the prevention of type 2 diabetes in high-risk populations with prediabetes,[6,7,10,41–44] we recommend Diamel as an alternative therapy for MS and the prevention and/or delay of the onset of type 2 diabetes. Although these drugs and Diamel are effective, they are not sufficient and should be combined with lifestyle interventions.

Recently, our group reported that Diamel reduces IR and improves IS in a small sample of women with polycystic ovary syndrome.[45] 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 IS and reduce IR; however, this important issue requires further investigation.

The strengths of the present study include its randomized, placebo-controlled, double-blind design and its treatment period of 1 year. In addition, the results were consistent across the different statistical analyses used. The novelty of this trial was the use of Diamel, for the first time, as a nutritional supplement for MS.

A possible limitation of the present clinical trial is the relatively small sample size at the end of the study; consequently, limited power may have obscured smaller treatment effects. 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 note 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, there was slightly greater than 55% decrease in insulin resistance (HOMA-IR) in the Diamel-treated group at the end of the study (from 5.67 ± 3.15 to 2.52 ± 0.93).

In conclusion, long-lasting treatment with Diamel, combined with lifestyle changes, appears to provide additional health benefits to subjects with MS. Diamel represents a new alternative therapy in patients with MS, prediabetes, and other diseases characterized by insulin resistance.

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Disclosure

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