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ARÁN

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Trabajo Original

Nutrición en el anciano

A new fructose-free, resistant-starch type IV-enriched enteral formula improves glycaemic control and cardiovascular risk biomarkers when administered for six weeks to elderly diabetic patients

Una nueva fórmula enteral enriquecida en almidones resistentes de tipo IV y sin fructosa mejora el control glucémico y los biomarcadores de riesgo cardiovascular cuando se administra durante seis semanas a pacientes diabéticos ancianos

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Abstract

Background: Reducing the dietary glycaemic response has been proposed as a way to reduce the risk of diabetes complications.

Objective: The aim of the present study was to evaluate the glycaemic control and cardiovascular risk biomarkers in fragile, elderly type 2 diabetes patients after the intake of a new fructose-free diabetes-specific formula enriched with resistant-starch type IV and high in monounsaturated fatty acids.

Methods: Forty-one type 2 diabetes patients aged 78.9 ± 2.8 years were fed exclusively with an enteral diabetes-specific formula for 6 weeks. Data were collected at baseline and after 6 weeks of feeding. Carbohydrate and lipid metabolism and inflammatory and cardiovascular risk biomarkers were measured to evaluated the course of diabetes complications.

Results: Blood glycated haemoglobin significantly decreased after the intervention (6.1 ± 0.1 *vs.* 5.8 ± 0.1 %; $p < 0,045$), as well as monocyte chemotactic protein-1 and soluble E-selectin ($p < 0.05$), while soluble vascular cell adhesion molecule and plasminogen activator inhibitor-1 tended to decrease from baseline to 6 weeks ($p = 0.084$ and $p = 0.05$, respectively).

Conclusion: The new product improves glycaemic control and cardiovascular risk without altering lipid metabolism, which is useful for the prevention of diabetic complications. Longer intervention studies are needed in order to validate these results in a larger population.

Key words:

Diabetes *mellitus* type 2. Cardiovascular risk. Enteral nutrition. Glycaemic control. Glycated haemoglobin. Resistant-starch type IV.

Resumen

Introducción: el control de la respuesta glucémica se ha propuesto como un mecanismo útil para reducir el riesgo de las complicaciones en los diabéticos.

Objetivo: evaluar el efecto sobre el control glucémico y el riesgo cardiovascular en ancianos con diabetes de tipo 2 de una nueva fórmula específica para diabéticos, sin fructosa, y que contiene almidones resistentes de tipo IV y un elevado contenido en ácidos grasos monoinsaturados.

Métodos: 41 pacientes con diabetes mellitus de tipo 2 y una edad media de 78,9 ± 2,8 años se alimentaron exclusivamente de forma enteral con la fórmula específica para diabéticos durante 6 semanas. Se tomaron muestras al inicio y al final del periodo de intervención y se determinaron biomarcadores del metabolismo de los carbohidratos y lípidos, así como de inflamación y riesgo cardiovascular, con objeto de evaluar el curso de las complicaciones de la diabetes.

Resultados: la hemoglobina glicosilada en la sangre disminuyó de forma significativa tras la intervención (6,1 ± 0,1 *vs.* 5,8 ± 0,1 %; $p < 0,045$), así como la proteína quimiotáctica de monocitos-1 y la E-selectina soluble ($p < 0,05$), mientras que la molécula de adhesión vascular y el activador del plasminógeno-1 tendieron a disminuir tras las 6 semanas de intervención ($p = 0,084$ y $p = 0,05$, respectivamente).

Conclusión: el nuevo producto mejora el control glucémico y el riesgo cardiovascular sin alterar el metabolismo lipídico, lo que resulta útil para la prevención de las complicaciones de los diabéticos. Se necesitan estudios más prolongados para confirmar este efecto en una población más amplia.

Palabras clave:

Diabetes mellitus de tipo 2. Riesgo cardiovascular. Nutrición enteral. Control glucémico. Hemoglobina glicosilada. Almidones resistentes de tipo IV.

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BACKGROUND

Type 2 diabetes *mellitus* is characterized by altered glucose metabolism secondary to abnormal insulin action or insulin deficiency. Indeed, insulin resistance (IR) leads to hyperglycaemia and dyslipidaemia and increased blood pressure, which are well-known characteristics of metabolic syndrome (1). In addition, IR results into oxidative stress, inflammation and endothelial dysfunction, all of which accelerate atherosclerosis development (2). Hence, cardiovascular disease (CVD) is one of the main complications found in diabetic patients (2).

Uncontrolled post-meal hyperglycaemia has been shown to be an independent risk factor for the development of cardiovascular complications in type 2 diabetes patients (2). Therefore, improved glycaemic control and better management of other identified risk factors for the complications of diabetes and more effective treatment of cardiovascular disease and microvascular complications have resulted in a more optimistic outlook for people with diabetes (3). Zang et al. (4) conducted a meta-analysis of prospective studies to estimate the association of glycosylated haemoglobin level with the risk of all-cause mortality and cardiovascular outcomes among patients with type 2 diabetes. They have concluded that chronic hyperglycaemia is associated with an increased risk for cardiovascular outcomes and all-cause of mortality among patients with type 2 diabetes (4).

The prevalence of type 2 diabetes is especially high in the elderly population (5). Many of these patients require nutritional support, as they are often unable to eat and drink sufficiently. Treatment with enteral nutrition diabetes-specific formulas (DSFs) may help to improve glycaemic control in diabetic patients and thus prevent further complications (6). Slowly digestible carbohydrates are combined with specific proteins and fibres to maintain the postprandial glycaemia as low as possible (7). Formulas enriched with monounsaturated fatty acids (MUFA) have been recommended to limit the intake of *n*-6 polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) (8), because high MUFA content may increase high-density lipoprotein cholesterol (HDLc) and decrease low-density lipoprotein cholesterol (LDLc) cholesterol, triacylglycerols (TAG), and total cholesterol concentrations, and thus may improve lipid metabolism (8). Accordingly, we have recently reported the potential benefits of a new fructose-free, resistant-starch type IV-enriched DSF with 52% of MUFA (9). The glycaemic index of the carbohydrates included in this formula was characterized as low compared with glucose (9). This new DSF reduced postprandial insulinaemia in healthy volunteers and postprandial glycaemia in outpatients with type 2 diabetes. Moreover, the new product did not alter postprandial lipid metabolism in either healthy or type 2 diabetic subjects, noting that this new DSF could be beneficial for diabetic patients (9). In the present study, we aimed to evaluate the influence of total enteral feeding with a new fructose-free DSF on the glycaemic control and on diabetes-derived cardiovascular risk biomarkers in fragile elderly type 2 diabetes patients fed enterally for 6 weeks.

MATERIAL AND METHODS

SUBJECTS

Forty-one type 2 diabetes patients requiring total enteral nutritional feeding (32 female, 9 male; mean age 78.9 ± 2.8) from the Clinical Nutrition and Dietetics Outpatient Unit of the University Hospital Virgen de las Nieves (Granada, Spain) participated in the study. The inclusion criteria were as follows: type 2 diabetes *mellitus*, age > 70 years, prescription of total enteral tube feeding nutrition for ≥ 3 months because of malnutrition, risk of malnutrition or incapacity to feed orally mainly due to dysphagia and neurological disturbances, under regular medical supervision and voluntary consent to participate. Total enteral nutrition allows the control of other nutritional factors that may influence the outcomes. The exclusion criteria were as follows: diagnosed with other illnesses, receiving lipid-containing medication, unstable clinical condition, fatal illness, inclusion in another clinical trial, refusal to participate or other criteria, such as social and humanitarian issues or lack of cooperation. The patient or next of kin was informed regarding the purpose and procedures of the study before written consent was obtained. The protocol was performed in accordance with the Helsinki Declaration of the World Medical Association, Ethical Principles for Medical Research on Human Beings (Revised in Edinburgh, October 2000) and approved by the Ethics Committee of the University Hospital Virgen de las Nieves. The present work is part of the study registered at www.clinicaltrials.gov as NCT 01247714. Three people were unable to complete the study due to changes in diet because of tolerance problems, although these complications did not require the withdrawal of the enteral nutrition to be resolved. No adverse events or negative health effects were observed or reported during this study.

STUDY DESIGN

An experimental, prospective, intention-to-treat clinical trial was performed in forty-one elderly type 2 diabetes patients who were fed exclusively a new fructose-free DSF enriched with resistant-starch type IV and MUFA for 6 weeks (mean of 35 days). The main outcome of the present study was to evaluate the glycaemic control of these patients and cardiovascular risk diabetes complications by analysing inflammatory and cardiovascular risk biomarkers (see below). The composition of the DSF (T-Diet Plus® Diabet NP, Vegenat S.A., Spain) is shown in table I. This was a normoproteic, normocaloric and nutritionally complete liquid diet designed for diabetic patients. The product provided 500 kcal/2109 kJ per 500 ml serving, with 50 g carbohydrates (40% of energy), 25.3 g fat (45% of energy) and 18.8 g protein (15% of energy). This DSF did not contain fructose but contained a mix of low-dextrose equivalent maltodextrins (31.7%) and resistant-starch type IV (53.7%) as well as 10 g (20%) of fibre (inulin and cellulose). Proteins consisted of 50% caseinates, 25% glycomacropeptide-enriched whey proteins and 25% pea protein. Its

Table I. Nutrient composition of the studied formula

Nutrient composition/100 mL		T-Diet Plus® Diabet NP
Energy	kcal/kJ	100/422
Carbohydrate	g (%)	10.00 (40)
Fibre	g (%)	2 (20)
Inulin	g (%)	1.2 (12)
Protein	g (%)	3.80 (15)
Caseinates	g (%)	1.9 (7.5)
Whey protein	g (%)	0.9 (3.6)
Glicomacropeptido	g (%)	0.13 (0.5)
Vegetable pea protein	g (%)	0.9 (3.6)
Taurine	mg (%)	8 (0.1)
Fat	g (%)	5.10 (45)
SFA	g (%)*	0.96 (18.8)
Medium Chain TAG	g (%)*	0.50 (9.8)
MUFA	g (%)*	2.80 (54.9)
PUFA	g (%)*	1.30 (25.5)
Linoleic acid	g (%)*	1 (19.6)
Linolenic acid	g (%)*	0.12 (2.4)
EPA + DHA	g (%)*	0.02 (0.4)
Carbohydrate/fat		1.96

*Where indicated percentages of total calories are given in parentheses. *Percentage of fatty acids on total fat. DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; TAC: triacylglycerols; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.*

fatty profile was 23.8% SFA, 51.8% MUFA and 24.4% PUFA (0.2 mg/ml of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) from a mixture of vegetable and fish oils (Table I) (9). Following nursing homes protocols, subjects were provided 1.500 ml of the DSF daily, although the average consumption was 1.266 ± 59 ml. This diet implies a mean EPA and DHA intake of approximately 253 mg/day. This amount is the standard used, taking into account the age, exercise and operability of the technique. A calorimetric calculation could be done by any equation, but it would create a problem for those responsible for patients. In that case a greater dropout would be expected. The product was administered as a bolus using a nasogastric tube with a large-bore syringe. To maintain the optimal hydration state, all patients also received 1,000-1,200 mL of water daily.

BLOOD SAMPLING

Fasting blood samples were obtained from patients between 8:00 and 10:00 am after an 8-10 h overnight fast, at baseline and after 6 weeks of the intervention. Serum and plasma (EDTA coated tubes from BD Vacutainer, Plymouth, UK) were separated by centrifugation (15 min at 1.750 x g) and immediately processed or divided into aliquots and frozen at -80 °C until analysis.

BIOCHEMICAL ANALYSIS

Fasting serum glucose, TAG, HDLc, LDLc, total cholesterol, apolipoproteins (Apo) A1 and B, albumin, prealbumin, transferrin, creatinine, urea, uric acid, glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), and gamma-glutamyl transferase (GGT) were determined by standardised spectrophotometric techniques using a Roche Hitachi Modular DDP clinical analyser system (Roche Diagnostics, S.L., Spain). Plasma non-esterified fatty acid (NEFA) concentrations were analysed using a colorimetric enzymatic kit (FA 115, Randox Laboratories, Crumlin, Co. Antrim, UK). Blood glycated haemoglobin A1c (HbA1c) was analysed by an immunoturbidimetric method (Roche Diagnostics, S.L., Spain), plasma insulin was analysed by standardised electrochemiluminescence using an E-170 Elecsys Modular Analytics system (Roche Diagnostics, S.L., Spain), and serum C-peptide was determined using a chemistry system based on particle-enhanced turbidimetric-immunoassay (PETIA) technology. Insulin resistance was calculated using the Homeostatic Assessment Model Index of Insulin Resistance (HOMA-IR) calculated following the Matthews formula, as the product of fasting plasma glucose (mmol/L) and insulin (mU/L) concentrations divided by 22.5 (10). Peripheral tissue insulin sensitivity was estimated using the Quantitative Insulin-Sensitivity Check Index (QUICKI) using the inverse of the sum of the logarithms of the fasting insulin (μU/mL) and fasting glucose (mg/dL) (11).

PLASMA FATTY ACIDS

Plasma fatty acids were transmethylated using acetyl chloride, according to Lepage and Roy (12). Hexane-resuspended methylated fatty acids were injected into a Hewlett Packard HP 5890 Series II chromatograph (Hewlett Packard, Palo Alto, CA, USA) with a capillary column (60 m x 32 mm inner diameter; 20 mm film thickness) impregnated with SP 2330 FS (Supelco, Bellefonte, CA, US). The results were expressed as the percentage of the total fatty acids analysed.

INFLAMMATORY AND CARDIOVASCULAR RISK BIOMARKERS

Plasma oxidized LDL (oxLDL) was analysed using an enzyme immunoassay kit BI-20042 (Oxystat, Biomedica Gruppe, Austria). MILLIPLEX® kits (Linco Research, MO, US) were used on a Luminex 200™ System (Luminex Corporation, TX) to determine: soluble intercellular adhesion molecule (sICAM)-1 (CV: 7.9%), soluble vascular cell adhesion molecule (sVCAM)-1 (CV: 4.5%),

soluble endothelial selectin (sE-selectin) (CV: 11.2%), matrix metalloproteinase (MMP)-9 (CV: 6.8%) and myeloperoxidase (CV: 12.3%) (Cat. #HCVD1-67AK); interleukin (IL)-6 (CV: 7.8%), IL-8 (CV: 7.9%), tumour necrosis factor (TNF)-α (CV: 7.8 %), monocyte chemotactic protein (MCP)-1 (CV: 7.9%) (Cat. #HADK2-61K-B); and adiponectin (CV: 5.6%), resistin (CV: 6.0%) and active plasminogen activator inhibitor (PAI)-1 (6.6 %) (Cat. #HADK1-61K-A).

STATISTICAL ANALYSIS

Values are presented as the mean ± standard error of the mean (SEM). Prior to statistical analyses, all variables were checked for normality using the Kolmogorov-Smirnov test. The homogeneity of variances was estimated using Levene's test. To evaluate differences over time, Student's *t*-test for paired samples was performed. p values < 0.05 were considered statistically significant. All statistical analyses were performed with the Statistical Package for the Social Sciences 15.0 software for Windows.

RESULTS

BIOCHEMICAL ANALYSIS

Fasting serum glucose, insulin, C-peptide and the HOMA-IR and QUICKI indexes remained unchanged after the 6 weeks of enteral feeding with the DSF. However, blood HbA1c percentage was lower at the end of the intervention (Table II). Specifically, eight volunteers (19.5%) had a percentage of HbA2 > 7% at base line, and only three (7.3%) remained > 7% at the end of the intervention. Serum concentrations of lipids, albumin, prealbumin, transferrin, urea, uric acid, and hepatic transaminases were unmodified by feeding the enteral formula, while creatinine values

Table II. Plasma biochemical parameters in elderly diabetic patients before and after 6 weeks of enteral feeding with the experimental formula

	Baseline (mean ± SEM)	6 weeks (mean ± SEM)	p-value
Glucose (mmol/L)	6.05 ± 0.28	6.11 ± 0.28	0.814
Insulin (µU/mL)	6.0 ± 0.6	6.8 ± 0.6	0.359
C-peptide (ng/mL)	2.7 ± 0.1	2.8 ± 0.1	0.385
HbA1c (%)	6.1 ± 0.1	5.8 ± 0.1	0.045*
HOMA-IR	1.66 ± 0.32	1.89 ± 0.32	0.611
QUICKI	0.38 ± 0.01	0.37 ± 0.01	0.408
TAG (mmol/L)	1.45 ± 0.06	1.36 ± 0.06	0.227
NEFA (pg/mL)	0.17 ± 0.01	0.17 ± 0.01	0.345
Total cholesterol (mmol/L)	4.19 ± 0.02	4.32 ± 0.08	0.212
HDLc (mmol/L)	1.16 ± 0.03	1.19 ± 0.03	0.221
LDLc (mmol/L)	2.33 ± 0.03	2.38 ± 0.05	0.455
GOT (U/L)	22 ± 1	20 ± 1	0.05
GPT (U/L)	16 ± 1	14 ± 1	0.071
GGT (U/L)	40 ± 5	33 ± 5	0.402
Albumin (g/L)	35.1 ± 0.6	35.6 ± 0.6	0.619
Prealbumin (mg/dL)	19.9 ± 0.4	19.1 ± 0.4	0.180
Transferrin (mg/dL)	214 ± 3	213 ± 3	0.683
Creatinine (mmol/L)	58.3 ± 0.9	62.8 ± 0.9	0.026*
Urea (mmol/L)	7.17 ± 0.33	7.83 ± 0.33	0.120
Uric acid (mmol/L)	297 ± 6	291 ± 6	0.633
ApoA1 (mg/dL)	127 ± 2	126 ± 2	0.969
ApoB (mg/dL)	77 ± 1	79 ± 1	0.282

*Values are mean ± SEM. *Statistically significant differences (p < 0.050) over time using a Student t-test for paired data. Apo: apolipoprotein; GGT: gamma-glutamyl transaminase; GOT: glutamate oxaloacetate transaminase; GPT: glutamate pyruvate transaminase; HbA1c: glycated haemoglobin; HDLc: high-density lipoprotein cholesterol; HOMA-IR: homeostatic model assessment-insulin resistance; LDLc: low-density lipoprotein cholesterol; NEFA: non-esterified fatty acids; QUICKI: quantitative insulin sensitivity check index; TAG: triacylglycerols.*

increased from 58.3 ± 0.9 mg/dl at baseline to 62.8 ± 0.9 mg/dl after 6 weeks of feeding (p = 0.026) (Table II).

PLASMA FATTY ACIDS

The plasma oleic acid percentage decreased from baseline (31.8 ± 0.8 %) to 6 weeks (28.7 ± 1.0 %) (p= 0.014), while the linoleic acid percentage increased from baseline (22.7 ± 0.5 %) to 6 weeks (27.2 ± 0.6 %) (p = 0.001). In comparison to baseline values, the plasma MUFA percentage was lower (p = 0.016) and both the plasma PUFA and *(n-6)* PUFA were higher (p = 0.001 in both cases) after 6 weeks of treatment (Table III).

INFLAMMATORY AND CARDIOVASCULAR RISK BIOMARKERS

Adiponectin, MCP-1 and sE-selectin decreased from baseline to 6 weeks (p = 0.003, p = 0.011 and p = 0.001, respectively). A tendency to decrease from baseline values was observed for sVCAM-1 (p = 0.084) and PAI-1 (p = 0.05). The remaining biomarkers were unchanged during the study period (Table IV).

DISCUSSION

The present work evaluates the evolution of the glycaemic control and biomarkers of diabetes-derived cardiovascular comorbidities after feeding a new DSF during 6 weeks. We have demonstrate that the administration of this new fructose-free and resistant-starch type IV-enriched DSF resulted in a better glycaemic control over the 6 weeks of intervention by decreasing HbA1c in blood. Indeed, the nutritional intervention improved MCP-1, sE-selectin, sVCAM-1 and PAI-1 plasma concentrations, which are well-known biomarkers of diabetes-derived cardiovascular risk.

The main therapeutic goal for diabetes is to improve glycaemic control. Glycaemic control has been shown to prevent and delay associated acute and long-term complications in type 2 diabetes patients (4). HbA1c has now been recommended by an International Committee and by the ADA as a means to diagnose diabetes (13). HbA1c reflects average plasma glucose over the previous eight to 12 weeks (13), avoiding the inconvenience of the plasma glucose day-to-day variability. The risk of diabetic macrovascular complication has been associated with hyperglycaemia. Each 1% increase in HbA1c poses 15-18% relative risk of cardiovascular disease in diabetic patients (14). Indeed, it has been demonstrated a 21% reduction in the risk of diabetes related deaths and 14% reduction in the incidence of myocardial infarction with only a 1% reduction in HbA1c in type 2 diabetic patients (15). In the present study, fasting serum glucose, insulin, C-peptide, and, therefore, the HOMA-IR and QUICKI indexes, remained unchanged after the 6 weeks of intervention. However, blood HbA1c decreased after this short period, indicating an improvement of the glycaemic control through that time. We want to point out that a 0.3% HbA1 reduction represent an improvement of the glycaemic control in well-controlled diabetics patients feed the DSF during 6 weeks. Similar results were observed in a longer-term trial that evaluated a different enteral formula specific for diabetic patients (16). This improvement may be caused by the presence in the new formula of slowly digestible carbohydrates, low-dextrose equivalent malto-

Table III. Percentages of plasma fatty acids in elderly diabetic patients before and after 6 weeks of enteral feeding with the experimental formula

	Baseline (mean ± SEM)	6 weeks (mean ± SEM)	p-value
Palmitic C16:0	17.8 ± 0.3	18.8 ± 0.4	0.058
Stearic C18:0	6.4 ± 0.2	6.6 ± 0.2	0.524
Oleic C18:1n-9	31.8 ± 0.8	28.7 ± 1.0	0.014*
Linoleic C18:2n-6	22.7 ± 0.5	27.5 ± 0.6	< 0.001*
Arachidonic C20:4n-6	6.88 ± 0.16	6.99 ± 0.19	0.652
EPA C20:5n-3	0.65 ± 0.04	0.75 ± 0.05	0.108
DHA C22:6n-3	1.85 ± 0.17	1.86 ± 0.20	0.982
SFA	35.3 ± 1.0	34.0 ± 1.2	0.443
MUFA	34.8 ± 0.8	31.6 ± 1.0	*0.016
PUFA	30.4 ± 0.7	34.3 ± 0.9	*0.001
n-6 PUFA	26.9 ± 0.5	31.1 ± 0.6	*< 0.001
n-3 PUFA	3.43 ± 0.45	3.26 ± 0.53	0.811

*Values are mean ± SEM. *Statistically significant differences (p < 0.050) over time using a Student t-test for paired data. DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.*

Table IV. Plasma inflammatory and cardiovascular risk biomarkers in elderly diabetic patients before and after 6 weeks of enteral feeding with the experimental formula[Δ]

	Baseline (mean ± SEM)	6 weeks (mean ± SEM)	p-value
OxLDL (ng/mL)	1.31 ± 0.10	1.31 ± 0.10	0.980
Myeloperoxidase (ng/mL)	180 ± 21	153 ± 24	0.393
Adiponectin (pg/mL)	27 ± 2	17 ± 2	0.003*
IL-6 (pg/mL)	10.8 ± 1.9	12.0 ± 2.2	0.672
IL-8 (pg/mL)	6.4 ± 0.5	6.9 ± 0.6	0.598
TNF-α (pg/mL)	8.2 ± 0.5	8.6 ± 0.6	0.580
MCP-1 (pg/mL)	199 ± 5	178 ± 6	0.011*
MMP-9 (ng/mL)	136 ± 9	127 ± 10	0.512
PAI-1 (ng/mL)	14 ± 1	12 ± 1	0.05
Resistin (ng/mL)	59 ± 6	66 ± 7	0.417
sE-selectin (ng/mL)	37 ± 1	32 ± 1	0.001*
sICAM-1 (ng/mL)	75 ± 3	78 ± 3	0.401
sVCAM-1 (ng/mL)	961 ± 17	916 ± 20	0.084

*Values are mean ± SEM. *Statistically significant differences ($p < 0.050$) over time using a Student t-test for paired data. [Δ]There are no reference values for inflammatory and cardiovascular disease biomarkers in healthy adults. IL: interleukin; MCP-1: monocyte chemotactic protein-1; MMP-9: matrix metalloproteinase-9; oxLDL: oxidized LDL; PAI-1: plasminogen activator inhibitor-1; sE-selectin: soluble endothelial selectin; sICAM-1: soluble intercellular adhesion molecule-1; sVCAM-1: soluble vascular cell adhesion molecule-1; TNF-α: tumour necrosis factor-α..*

dextrins and resistant-starch type IV, which are characterized by a low GI (9). A limitation of the present study is that most of the patients included were bedridden, and was impossible to determine theirs weights and calculate the evolution of the body mass index. We are aware that the loss of weight may lead to a better metabolic control. For this reason, we cannot exclude the potential influence of this factor in our data. Longer studies are needed to validate these results.

The new formula was fructose-free. There is a great controversy related to the effect of fructose on the development of diabetes, obesity, hypertension and dyslipaemias, and it seems that a high intake of fructose, particularly if combined with a high energy intake in the form of glucose/starch, may have negative health effects by increasing TAG, LDLc, uric acid and blood pressure (17). The absence of fructose may be implicated in the fact the new DSF did not increased plasma lipids despite the high fat content (45%), maintaining an adequate plasma lipid status in our patients. In addition, the liver transaminases tended to decrease after the enteral feeding with the DSF which, in addition, may reflect an improvement of the liver status. Another additional reason for the maintenance of normal plasma lipid concentrations could be due to the relatively high levels of MUFA and PUFA in the DSF. Several intervention studies have shown that diets high in MUFA and low in carbohydrates improve glycaemic control as well as lipoprotein status (8). However, we did not observe plasma fatty acids changes, except for a decrease in oleic acid and an increase in linoleic acid. A limitation of the present study is that we did not record any data about the dietary fat intake previous to the inclusion in the study. In fact, all volunteers were from a geographical area where olive oil is the main dietary fat, and probably, the intervention did not really modify the intake of oleic acid. Therefore, we cannot reach any conclusion about the different fatty acid profile found in plasma that usually reflects dietary fatty acid profile (18). The EPA and DHA content of the new formula was 0.2 mg/ml that contribute to the intake of a mean of 253 mg/day, while the average daily intake of long-chain PUFA (LC-PUFA) was estimated at 300 mg/day. This may be the reason that (*n*-3) LC-PUFA were not modified after the intake of the DSF for 6 weeks. The recommended intake of (*n*-3) LC-PUFA to treat type 2 diabetic patients remains to be further elucidated.

Many studies suggest that hyperglycaemia might trigger oxidative stress resulting in endothelial dysfunction and inflammatory processes that may contribute to CVD development (19). In fact, oxLDL and inflammatory biomarkers are increased in patients with type 2 diabetes (20,21). Therefore, avoiding oxidative and inflammatory processes in diabetic patients may help to the prevention of cardiovascular complications. Macrophages generate lipid-laden foam cells due to uncontrolled uptake of modified LDL, especially oxLDL (19). In this sense, feeding the DSF during 6 weeks did not alter plasma oxLDL, indicating that oxidative stress is not increased during the intervention. Myeloperoxidase is an enzyme linked to both oxidative stress and inflammation, and may promote atherogenesis (22). Patients with type 2 diabetes have shown elevated levels of these molecules compared with non-diabetic population (23), and increasing myeloperoxidase levels were associated with greater progression of atherosclerosis

in diabetic patients (24). In fact, our type 2 diabetic patients had elevated plasma levels of myeloperoxidase, but its concentrations did not change with the intervention.

Inflammatory cytokines released by adipose tissue may confer insulin resistance in liver, skeletal muscle and vascular endothelial tissue, ultimately leading to type 2 diabetes and CVD (23). These adipokines include mainly adiponectin, leptin, TNF-α, IL-6, resistin, MCP-1, PAI-1, and others, which are usually altered in patients with established type 2 diabetes (14,25-27). In fact, the levels of plasma adipokines may help to establish the risk to develop cardiovascular comorbidities in diabetics (23). Macrophage-derived foam cells accumulate in atheromatous plaques and lead to inflammation by secreting cytokines *e.g.,* IL-6 and TNF-α (28). MCP-1 and IL-8, chemokines abundantly expressed in atherosclerotic lesions, attract monocytes into the plaque and potentially contribute to inflammation enhancement and atherosclerotic plaques development (29). Foam cells in vulnerable regions of atherosclerotic plaques produce MMP, which may mediate extracellular matrix degradation and consequent plaque destabilization. Diabetic environment stimulates the secretion of several MMPs that are considered to participate in its complications (30-32). However, conflicting results have been reported in type 2 diabetes since MMP levels have been shown to increase (30), remain the same (31), and decrease (32). In addition, resistin is an adipokine that up-regulates the expression of adhesion molecules in human endothelial cells, suggesting its potential role in atherosclerosis (33), and adhesion molecules, such as sE-selectin, sICAM-1 and sVCAM-1 are critical mediators of endothelial dysfunction (25). On the other hand, adiponectin is an insulin-sensitising adipokine that is inversely proportional to adiposity and to the risk of cardiovascular disease development (27). To the best of our knowledge, this is the first intervention trial evaluating the influence of enteral administration of a DSF on diabetes-derived cardiovascular risk consequences. Some cardiovascular risk biomarkers such as IL-6, IL-8, TNF-α, MMP-9, sICAM-1 and resistin were not modified after 6 week of enteral feeding with the DSF. However, we have observed that plasma concentration of MCP-1, E-selectine, sVCAM and PAI 1 decreased after feeding the enteral formula during 6 weeks. MCP-1 links macrophage infiltration into adipose tissue to insulin resistance (34) and is involved in diabetic nephropathy (35). Therefore, the decrease of plasma MCP-1 plasma levels may indicate an improvement of the inflammatory progression and diabetes comorbidities. Regarding diabetes-derived endothelial dysfunction, a recent systematic review and meta-analysis has found evidence for macrovascular endothelial dysfunction during acute hyperglycaemia (36). The better glycaemic control found in our patients feeding this DSF may have influenced the improvement of the endothelial function after the intervention.

On the other hand, inhibition of fibrinolysis, which is attributable to elevated concentrations of the acute-phase response protein PAI-1, is associated with insulin resistance and type 2 diabetes, and predicts myocardial infarction and stroke (37). Indeed, subjects with type 2 diabetes have shown elevated levels of PAI-1 (38). Recently, a positive correlation between PAI-1 levels and blood HbA1c has been described (39). Therefore, the better glycaemic control found in our type 2 diabetic patients may explain the decrease of plasma levels of PAI-1 after the 6 weeks of enteral feeding with the DSF, and this fact may indicate a better fibrinolitic response of these patients.

We have not found a variation of traditional plasma biomarkers of renal and nutritional status except an increase of plasma creatinine concentrations. However, creatinine plasma concentration is influenced by multiple non-renal factors, such as age, gender, muscle mass, muscle metabolism, medications, hydration status. Therefore, more studies are needed to reach final conclusions.

CONCLUSION

Our results demonstrate that enteral feeding with the new DSF improves glycaemic control and cardiovascular risk biomarkers without altering lipid metabolism, which is useful for the nutritional treatment of fragile elderly type 2 diabetes patients. Longer intervention studies are needed in order to validate these results in larger populations.

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REFERENCES

1. Kaur JA. Comprehensive Review on Metabolic Syndrome. Cardiol Res Pract 2014;9431-62.
2. Madsbad S: Impact of postprandial glucose control on diabetes-related complications: How is the evidence evolving? J Diabetes Complications 2015;S1056-8727(15)00390-6. DOI: 10.1016/j.jdiacomp.2015.09.019.
3. Nathan DM. Diabetes: Advances in diagnosis and treatment. JAMA 2015;8:1052-62.
4. Zhang Y, Hu G, Yuan Z, et al. Glycosylated hemoglobin in relationship to cardiovascular outcomes and death in patients with type 2 diabetes: a systematic review and meta-analysis. PLoS ONE 2012;7:8 e42551.
5. Chen LK, Chen YM, Lin MH, et al. Care of elderly patients with diabetes mellitus: a focus on frailty. Ageing Res Rev 2010;1:S18-S22.

6. Elia M, Ceriello A, Laube H, et al. Enteral nutritional support and use of diabetes-specific formulas for patients with diabetes: a systematic review and meta-analysis. Diabetes Care 2005;28:2267-79.
7. Frid AH, Nilsson M, Holst JJ, et al. Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects. Am J Clin Nutr 2005;82:69-75.
8. Ros E. Dietary cis-monounsaturated fatty acids and metabolic control in type 2 diabetes. Am J Clin Nutr 2003;78:617S-625S.
9. García-Rodríguez CE, Mesa MD, Olza J, et al. Postprandial glucose, insulin and gastrointestinal hormones in healthy and diabetic subjects fed a fructose-free and resistant-starch type IV-enriched enteral formula. Eur J Nutr 2013;52:1569-78.
10. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentration in man. Diabetologia 1985;28:412-41.
11. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402-10.
12. Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res 1986;27:114-20.
13. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009;32:1327-34.
14. Syed IAA, Khan WA. Glycated haemoglobin - a marker and predictor of cardiovascular disease. Journal of the Pakistan Medical Association 2011;61: 690-5.
15. Stratton IM, Adler AI, Andrew WN, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000;321:405-11.
16. Vaisman N, Lansink M, Rows CH, et al. Tube-feeding with a diabetes-specific feed for 12 weeks improves glycaemic control in type 2 diabetes patients. Clin Nutr 2009;28:549-55.
17. Kolderup A, Svihus B. Fructose Metabolism and Relation to Atherosclerosis, Type 2 Diabetes, and Obesity. J Nutr Metab 2015;2015:823081. DOI: 10.1155/2015/823081.
18. Kuriki K, Nagaya T, Tokudome Y, et al. Plasma concentrations of (n-3) highly unsaturated fatty acids are good biomarkers of relative dietary fatty acid intakes: a cross-sectional study. J Nutr 2003;133:3643-50.
19. Kayama Y, Raaz U, Jagger A, et al. Diabetic Cardiovascular Disease Induced by Oxidative Stress. Int J Mol Sci 2015;16:25234-63.
20. Scheffer PG, Bos G, Volwater HG, et al. Associations of LDL size with in vitro oxidizability and plasma levels of in vivo oxidized LDL in type 2 diabetic patients. Diabet Med 2003;20:563-67.
21. Freitas-Lima LC, Braga VA, do Socorro de França Silva M, et al. Adipokines, diabetes and atherosclerosis: an inflammatory association. Front Physiol 2015;6:304. DOI: 10.3389/fphys.2015.00304.
22. Van der Zwan LP, Scheffer PG, Dekker JM, et al. Hyperglycemia and oxidative stress strengthen the association between myeloperoxidase and blood pressure. Hypertension 2010;55:1366-72.
23. Wiersma JJ, Meuwese MC, Van Miert JNI, et al. Diabetes mellitus type 2 is associated with higher levels of myeloperoxidase. Medical Science Monitor 2008;14:CR406-CR10.
24. Kataoka Y, Shao M, Wolski K Uno K, et al. Myeloperoxidase levels predict accelerated progression of coronary atherosclerosis in diabetic patients: insights from intravascular ultrasound. Atherosclerosis 2014;232:377-83.
25. Van de Voorde J, Boydens C, Pauwels B, et al. Perivascular adipose tissue, inflammation and vascular dysfunction in obesity. Curr Vasc Pharmacol 2014;12:403-11.
26. Blüher M. Clinical relevance of adipokines. Diabetes Metab J 2012;36:317-27.
27. Fisman EZ, Tenenbaum A. Adiponectin: a manifold therapeutic target for metabolic syndrome, diabetes, and coronary disease? Cardiovasc Diabetol 2014;13:103. DOI: 10.1186/1475-2840-13-103.
28. Spranger J, Kroke A, Möhlig M, et al. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 2003;52:812-7.
29. Wiesner P, Tafelmeier M, Chittka D, et al. MCP-1 binds to oxidized LDL and is carried by lipoprotein(a) in human plasma. J Lipid Res 2013;54:1877-83.
30. Derosa G, D'Angelo A, Tinelli C, et al. Evaluation of metalloproteinase 2 and 9 levels and their inhibitors in diabetic and healthy subjects. Diabetes Metab 2007;33:129-34.
31. Papazafiropoulou A, Perrea D, Moyssakis I, et al. Plasma levels of MMP-2, MMP-9 and TIMP-1 are not associated with arterial shiftness in subjects with type 2 diabetes mellitus. J Diabetes Complications 2010;24:20-7.
32. Lewandowski KC, Banach E, Bieńkiewicz M, et al. Matrix metalloproteinases in type 2 diabetes and non-diabetic controls: effects of short-term and chronic hyperglycaemia. Arch Med Sci 2011;7:294-303.
33. Kawanami D, Maemura K, Takeda N, et al. Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine-endothelial cell interactions. Biochem Biophys Res Commun 2004;314:415-9.
34. Kanda H, Tateya S, Tamori Y, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J Clin Invest 2006;116:1494-505.
35. Panee J. Monocyte Chemoattractant Protein 1 (MCP-1) in obesity and diabetes. Cytokine 2012;60:1-12.
36. Loader J, Montero D, Lorenzen C, et al. Acute Hyperglycemia Impairs Vascular Function in Healthy and Cardiometabolic Diseased Subjects: Systematic Review and Meta-Analysis. Arterioscler Thromb Vasc Biol 2015;35:2060-72.
37. Chudý P, Kotuličová D, Staško J, et al. The relationship among TAFI, t-PA, PAI-1 and F 1+2 in type 2 diabetic patients with normoalbuminuria and microalbuminuria. Blood Coagul Fibrinolysis 2011;22:493-8.
38. Madan R, Gupt B, Saluja S, et al. Coagulation profile in diabetes and its association with diabetic microvascular complications. J Assoc Physicians India 2010;58:481-4.
39. Adly AA, Elbarbary NS, Ismail EA, et al. Plasminogen activator inhibitor-1 (PAI-1) in children and adolescents with type 1 diabetes mellitus: relation to diabetic micro-vascular complications and carotid intima media thickness. J Diabetes Complications 2014;28:340-7.