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ORIGINAL CONTRIBUTION

Postprandial glucose, insulin and gastrointestinal hormones in healthy and diabetic subjects fed a fructose-free and resistant starch type IV-enriched enteral formula

Cruz Erika García-Rodríguez · María Dolores Mesa · Josune Olza · Gilda Buccianti · Milagros Pérez · Rosario Moreno-Torres · Antonio Pérez de la Cruz · Ángel Gil

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Abstract
Background Reducing the dietary glycaemic response has been proposed as a means of reducing the risk of diabetes.
Aim To evaluate the effects of a new diabetes-specific formula (DSF) enriched with resistant starch type IV and fructose-free on postprandial glycaemia, insulinaemia and gastrointestinal hormones in healthy volunteers and in outpatient type 2 diabetics.
Methods (1) Twenty-four healthy volunteers were divided into two groups: Group 1 ($n = 10$) was provided 50 g of the carbohydrate (CHO) constituent of the new product and 50 g of glucose separated by 1 week; Group 2 ($n = 14$) was provided 400 ml of the new DSF (T-Diet Plus® Diabet NP) and 400 ml of a control product separated by 1 week. (2) Ten type 2 diabetic patients received 400 ml of the new DSF and two other commercially available DSF (Glucerna® SR and Novasource® Diabet) on three occasions separated by 1 week. Venous blood samples were drawn at time 0 and at different times until 120 min. Glucose, insulin and gastrointestinal hormones were determined. Glycaemic and insulinaemic indices and glycaemic load were calculated.
Results The CHO constituent and the new DSF showed low glycaemic index and glycaemic load. In healthy subjects, insulin and C-peptide release were lower after administration of the CHO constituent as well as after the new DSF ($P < 0.001$). Ghrelin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) production were lower after intake of the CHO constituent (P ranging from <0.001 to 0.019) compared with glucose, and GIP was lower after ingestion of the new DSF ($P = 0.002$) than after the control product. In type 2 diabetic patients, glucose AUC was lower after the administration of the new DSF ($P = 0.037$) compared with the others.
Conclusions Our results indicate that this new product could be beneficial for diabetic patients.

Keywords Enteral nutrition · Diabetes mellitus type 2 · Carbohydrates · Insulin · Blood glucose · Gastrointestinal hormones

C. E. García-Rodríguez (✉) · M. D. Mesa · J. Olza · Á. Gil
Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology "José Mataix", Biomedical Research Centre, University of Granada, Avenida del Conocimiento s/n, 18100 Armilla, Granada, Spain
e-mail: erikagr@ugr.es

G. Buccianti · R. Moreno-Torres · A. Pérez de la Cruz
Clinical Nutrition and Dietetic Unit, University Hospital Virgen de las Nieves, Granada, Spain

M. Pérez
Research and Development Department, VEGENAT, S.A., Pueblonuevo del Guadiana, Badajoz, Spain

Introduction

Diabetes mellitus is a chronic disease with a marked social and economic impact, which is likely to increase over the coming years. More than 230 million people worldwide have diabetes mellitus, predominantly type 2 [1, 2]. Most patients with type 2 diabetes have insulin resistance that alters the metabolism of carbohydrate (CHO) and fat leading to dyslipidaemia and an increased risk of cardiovascular diseases. Tight glycaemic control has been shown to prevent and delay associated acute and long-term complications [3, 4]. A major nutritional goal of diabetes

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therapy is to improve glycaemic control by normalizing blood glucose levels [5, 6], and dietary management plays an important role in achieving it [7].

Recent research has suggested the potential utility of the dietary glycaemic index (GI) for reducing postprandial hyperglycaemia [8, 9]. The GI is a quantitative measure of dietary CHO quality based on the blood glucose response after consumption, in comparison with glucose [10, 13]. In this way, fast digested-CHO foods have high and rapid blood glucose responses and typically high GI values. In contrast, slowly digested-CHO release glucose gradually into blood and have low GI values. Expanding this concept to the insulin levels evoked by foods, the Insulinaemic Index (InI) can also be determined [11]. Glycaemic load (GL) [12, 13] may be of particular use when trying to evaluate the glycaemic response of foods within a meal setting, as it takes into account the relative amount of available CHO within the food.

On the other hand, a recent systematic review showed that the use of diabetes-specific formulas (DSFs) is associated with improved glycaemic control compared with standard formulas (STFs) [14]. DSFs intended for oral and enteral nutrition have been developed to improve glycaemic control in diabetics. These products contain slowly digestible CHOs to induce a delayed and limited rise in postprandial glucose levels. In comparison with STFs, they often have lower CHO/fat ratio as well as further adaptations on macro- and micro-nutrient composition involving fructose, increased amounts of monounsaturated fatty acids (MUFAs), protein and fibre. Accordingly, the proportion of fat, protein and CHO may influence the glycaemic response of DSF, causing increased or prolonged secretion of the gastrointestinal hormones glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP), pancreatic polypeptide (PP), peptide YY (PYY) and cholecystokinin (CCK), all of which have been suggested as potential satiety factors [15–17]. Further, it is well known that GLP-1 and GIP exhibit an incretin role as they enhance insulin secretion. On the contrary, ghrelin increases food intake and enhances appetite [15, 17, 18].

Based on the above, the objectives of the present study were (1) to determine the GI and InI of the CHO constituent of a new fructose-free DSF diet intended for enteral nutrition and characterized by a high content of resistant starch (RS) type IV, a mixture of soluble and insoluble fibre, a high content of MUFAs and enriched with *n*-3 long-chain polyunsaturated fatty acids (T-Diet Plus® Diabet NP); (2) to determine the GI and GL of this product; (3) to evaluate the effects of this new diet on blood glucose, insulin, serum lipid and gastrointestinal hormones in healthy subjects; and (4) to compare the postprandial responses in outpatient type 2 diabetics fed either the new diet or the two other commercially available fructose-containing DSFs (Glucerna® SR and Novasource® Diabet).

Table 1 Baseline characteristics of subjects included in the studies

	Study	
	Healthy volunteers	Type 2 diabetic patients
Subjects	24	10
Age (y)	22–59	37–64
Gender (M/F)	11/13	4/6
Height (cm)	171 ± 2	161 ± 3
Weight (Kg)	68 ± 3	91 ± 28
Body mass index	23.2 ± 0.6	35.5 ± 3.6
Fasting glucose (mg/dl)	86 ± 2	175 ± 18
Fasting insulin (μU/ml)	7.8 ± 0.9	10.6 ± 2.8
HbA1c (%)	Not measured	8.3 ± 0.8
TAG (mg/dl)	97 ± 14	146 ± 17
Cholesterol (mg/dl)	191 ± 8	179 ± 13
LDLc (mg/dl)	115 ± 7	117 ± 9
HDLc (mg/dl)	68 ± 3	60 ± 5
NEFA (pg/dl)	7.1 ± 0.9	8.2 ± 1.9

Values are mean ± SEM. *HbA1c* glycated haemoglobin A1c, *HDLc* high-density lipoprotein cholesterol, *LDLc* low-density lipoprotein cholesterol, *NEFA* non-esterified fatty acids, *TAG* triacylglycerols

Subjects and methods

Healthy volunteers study

Twenty-seven healthy subjects, 14 men and 13 women, aged 22–59 years, with a normal body mass index (BMI) (23.2 ± 0.6) and without drug therapy were recruited for this study. The exclusion criteria for healthy individuals were a BMI >30 kg/m^2. Those individuals with a fasting plasma glucose concentration >110 mg/dl or basal insulin >20 U/l were excluded a posteriori. At the end of the experimental period, a total of 24 subjects, 11 men and 13 women, were eligible for the study (Table 1).

Healthy volunteers were chosen to receive either the glucose or the experimental CHO mixture since ethically diabetic subjects should not receive glucose because of their disease. In addition, healthy subjects were randomized into two groups, in order to avoid several blood extractions to the same volunteers. The first group (group 1, $n = 10$) received 50 g of a CHO constituent of the new product and 50 g of control glucose dissolved in 400 ml of water, on two separate days, 1 week apart. The second group (group 2, $n = 14$) received 400 ml of two different

Table 2 Nutrient composition of formulas used in the study

Nutrient composition/ 100 ml	Products		
	T-Diet Plus® Diabet NP	Glucerna® SR	Novasource® Diabet
Energy (kcal/kJ)	100/422	89/371	100/420
Macronutrients			
Carbohydrate (g (%))	10.00 (40)	11.09 (45.2)	12.80 (51)
Fibre (g (%)[b])	2 (20)	0.76 (6.9)	1.50 (11.7)
Protein (g (%))	3.80 (15)	4.65 (20.8)	4.00 (16)
Fat (g (%))	5.10 (45)	3.38 (34)	3.66 (33)
Fatty acids			
SFA (g (%)[a])	0.96 (18.8)	0.29 (8.6)	0.80 (21.9)
MUFA (g (%)[a])	2.80 (54.9)	2.54 (75.2)	2.06 (56.3)
PUFA (g (%)[a])	1.30 (25.5)	0.42 (12.4)	0.79 (21.6)
Carbohydrate/fat	1.96	3.28	3.50

Where indicated, percentages of total calories are given in parentheses

[a] % of fatty acids on total fat. *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *SFA* saturated fatty acids

[b] % of fibre on total carbohydrate

products (T-Diet Plus® Diabet NP and Control product (CP)) on two separate days, also 1 week apart. The four products were ingested in about 5 min each.

The tested DSF (T-Diet Plus® Diabet NP, Vegenat S.A., Spain) (Table 2) was a normoproteic, normocaloric and nutritionally complete liquid supplement designed to support diabetic patients, in order to improve their nutritional situation. The product provided 402 kcal/845 kJ (400 ml serving) with 32 g as CHO (40 % of energy), 20.1 g as fat (45 % of energy) and 22 g as protein (15 % of energy) and contained a mixture of vegetable and fish oils (20 mg/dl of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA)). It also included 7.2 g (20 % on total CHO) of fibre content (inulin and cellulose 20/80 wt/wt). The CHO in this new DSF was low dextrose equivalent (DE) purified and atomized maltodextrins (5–8 DE) (31.7 %) obtained by the hydrolysis of starch, and RS type IV (53.7 %) obtained from partially hydrolysed maize starch by heating in the presence of food grade acid. This diet was also fructose-free. Proteins consisted of 50 % caseinates, 25 % glycomacropeptide-enriched whey proteins and 25 % pea protein, and fat was comprised of 23.8 % of saturated fatty acids (SFAs), 51.8 % monounsaturated fatty acids (MUFAs) and 24.4 % polyunsaturated fatty acids (PUFAs) (Table 2). The CP was a normoproteic and normocaloric diet which differed to T-Diet Plus® Diabet NP in its CHO composition, containing 18–20 DE purified and atomized maltodextrins (99.4 %) obtained by the hydrolysis of starch, and in its fibre content, that is, inulin- and cellulose-free.

Outpatient type 2 diabetics study

Ten type 2 diabetic patients, 4 men and 6 women, aged 37–64 years, with a mean BMI of 35.5 ± 3.6, and with good glycaemic control, were recruited. Most of the participants (7 out of 10) had first-degree relatives with diabetes. No patients were excluded from the study (Table 1).

The diabetic patients ingested a single 400 ml serving of three different DSFs: T-Diet Plus® Diabet NP, Glucerna® SR and Novasource® Diabet, which were tested on three separate days, each 1 week apart. The composition and nutrient distribution of the three supplements are shown in Table 2.

T-Diet Plus® Diabet NP (Vegenat S.A., Spain), Glucerna® SR (Abbott Laboratories, Chicago, IL, USA) and Novasource® Diabet (Nestlé Healthcare Nutrition, Switzerland) are commercially available nutritionally complete liquid diets that were designed for diabetic patients (Table 2). The supplements differ primarily in CHO composition. Glucerna® SR contains a complex CHO mixture consisting of fructose (22.9 %) and slowly digestible maltodextrins (49.9 %). It also includes short-chain fructo-oligosaccharides and fibre (6.9 % on total CHO). Starch (84 %) and fructose (24 %) are the sources of CHO in Novasource® Diabet. It also includes fibre (11.7 % on total CHO). T-Diet Plus® Diabet NP composition has been described above. The three supplements are low in SFAs and rich in MUFAs. Compared with Glucerna® SR and Novasource® Diabet, only T-Diet Plus® Diabet NP contains *n*-3 fatty acids from plant (linoleic and linolenic acid) and marine sources (EPA + DHA).

Blood sampling

Following an overnight fast, all subjects (healthy volunteers and diabetic patients) arrived at the University Hospital Virgen de las Nieves (UHVN, Granada, Spain) at 08.00 am. A catheter was placed in the antecubital vein, and a baseline blood sample was collected. After collection of the first blood sample ($t = 0$ min), subjects ingested their appropriate supplement within 5 min. Thereafter, blood samples were taken at $t = 15$, 30, 45, 60, 90 and 120 min. Assessments of feelings of hunger/satiety were performed immediately after each blood sample was taken, according to Haber et al. [19]. The rating scale used was graded from −10, to represent extreme hunger, to +10, to represent extreme satiety. Subjects were asked to indicate how they felt in terms of hunger or satiety by pointing to the appropriate point on the scale. The scale was punctuated with phrases describing various degrees of hunger and satiety, but subjects were free to choose any point along it. Subjects weight and height were measured in light clothing. BMI was calculated as weight (kg) divided by height (m^2).

Biochemical measurements

Blood (3 ml) was collected into EDTA-containing tubes and centrifuged at 1700*g* for 15 min at 4 °C. An aliquot was used for immediate analysis of glucose, triacylglycerols (TAG) and insulin, at the laboratory of the UHVN. Some other aliquots of plasma were frozen immediately and stored at −80 °C until analysis of gastrointestinal hormones and non-esterified fatty acids (NEFAs). Plasma glucose and TAG were measured by standardized spectrophotometric techniques using a Roche Hitachi Modular DDP clinical analyser system (Roche Diagnostics, S.L., Spain). Insulin and C-peptide were analysed by standardized electrochemiluminescence techniques using an E-170 Elecsys Modular Analytics system (Roche Diagnostics, S.L., Spain).

In the Department of Biochemistry and Molecular Biology II, at the University of Granada, gastrointestinal hormones were measured in plasma in their active (ghrelin, GLP-1) or total concentration (GIP, PYY and PP), after previous addition of 30 μl of dipeptidyl peptidase-IV (DPP-IV) inhibitor (Rafer S.L., Spain) and 130 μl of serine protease inhibitor Pefabloc® SC AEBSF (Roche Diagnostics S.L., Spain) per 3 ml of blood before centrifugation. Their determination was performed with a Luminex 200™ System based on xMAP® technology and using MILLIPLEX® kits (Cat.No. HGT-68 K, Linco Research, St. Charles, MO, USA). Plasma active CCK and NEFA concentrations were analysed using enzyme immunoassay and colorimetric kits, respectively: CCK immunoassay kit (Phoenix Pharmaceuticals, Belmont, CA, USA) and Non-Esterified Fatty Acids kit (FA 115, Randox Laboratories, Crumlin Co Antrim, United Kingdom), respectively.

All participants provided signed informed consent to participate in the study, which was approved by the Ethics Committee of the UHVN. All procedures complied with institutional guidelines, following IHC Harmonized Tripartite Guidelines for Good Clinical Practice in accordance with the *Helsinki Declaration of the World Medical Association, Ethical principles for medical research on human beings* (Revised in Edinburgh, October 2000). The study was registered at www.clinicaltrials.gov (NCT 01247714).

Calculations and statistical analysis

Plasma glucose, insulin and gastrointestinal hormones responses were calculated as the positive area under the curve (AUC) above baseline levels ($t = 0$ min) except for ghrelin that was calculated below baseline level. The AUC was calculated according to the trapezoidal method [20]. GI, InI and GL were calculated according to the following formulae: GI of CHO = (AUC of blood glucose response after consuming the equivalent of 50 g of the CHO constituent/AUC of blood glucose after administration of 50 g of glucose) × 100; InI = (blood insulin AUC after consuming 50 g of the CHO constituent/AUC of blood insulin after administration of 50 g of glucose × 100); GL = (GI of the CHO mixture/100 × 32 g of available CHO per 400 ml serving of the product).

Values are presented as mean ± SEM. Prior to the statistical analyses, all variables were checked for normality using the Kolmogorov–Smirnov test. The homogeneity of variances was estimated using Levene's test. A general linear model of variance for repeated measures was performed to assess differences between times, between diets and the interactions diets per time. When Mauchly's test indicated that the assumption of sphericity was violated, the Greenhouse-Geisser correction was applied for univariate analysis. When the Greenhouse-Geisser correction was less than 0.05, we used multivariate ANOVA statistic tests, which do not depend on the assumption of sphericity. A general linear model of variance for repeated measures was applied to evaluate the effects of time (0, 15, 30, 45, 60, 90 y 120 min) within each group, and a posteriori Bonferroni tests were used for the comparison of multiple means. When the initial values ($t = 0$ min) were different (NEFA), the statistical analyses were corrected using baseline values as a covariate. AUC was analysed using a student t test for paired data. P values <0.05 were considered to be statistically significant. All statistical analyses were performed with the Statistical Package for Social Science (SPSS) 15.0 for Windows.

Results

Safety and tolerance

The new DSF was well tolerated and no patients withdrew from the study due to adverse events or intolerance.

Satiety

The sensation of satiety was similar for all CHOs and products in both healthy volunteers and type 2 diabetic patients, analysed by the Haber scale [19].

Postprandial responses in healthy volunteers

GI, GL and InI

The GI was 54.8 % for the new CHO mixture and 26.9 % for the new DSF. The GL was 4.4 g for the new product, and the InI reached a value of 35.7 % (Table 3). Based on previous bibliography [22], the GI was reported to be

Table 3 GI and InI of the CHO mixture, GI and GL of T-Diet Plus® Diabet NP and GI of two commercially available DSFs (Glucerna® SR and Novasource® Diabet)

	Carbohydrate constituent		
GI	54.8 %		
InI	35.7 %		
	T-Diet Plus® Diabet NP	Glucerna® SR	Novasource® Diabet
GI	26.9 %	23 %	26 %
GL	8.6 g		

GI glycaemic index, *GL* glycaemic load, *InI* insulinaemic index

GI, InI and GL were calculated for the experimental product according to the following formulae: GI of CHO = (AUC of blood glucose response after consuming the equivalent of 50 g of the CHO constituent/AUC of blood glucose after administration of 50 g of glucose) × 100; InI = (blood insulin AUC after consuming 50 g of the CHO constituent/AUC of blood insulin after administration of 50 g of glucose × 100); GL = (GI of the CHO mixture/100 × 32 g of available CHO per 400 ml serving of the product)

23 ± 5 for Glucerna® SR and 26 ± 5 for Novasource® Diabet. No data have been found for these two commercially available DSFs in relation to their GL and InI.

Plasma glucose, insulin, C-peptide, TAG and NEFA

Figure 1 shows the postprandial responses to the CHO constituent of the new product versus glucose. Figure 2 depicts the postprandial plasma glucose, insulin, C-peptide, TAG and NEFA responses for the new DSF versus CP. Table 4 summarizes the AUC responses for these parameters.

A trend for a lower blood glucose concentration was found for the CHO constituent of the new product versus glucose ($P = 0.066$). Plasma insulin concentration was significantly affected by diet ($P < 0.001$) being lower for the CHO constituent of the new product compared with glucose. Plasma insulin concentration differed significantly between groups over time (P ranging from <0.001 to 0.022). Indeed, insulin AUC was significantly lower after intake of the CHO constituent of the new product than glucose ($P < 0.001$). Plasma C-peptide concentration was also significantly affected by diet ($P < 0.001$) being lower for the CHO constituent of the new product compared with glucose, and significant differences were found between groups over time (P ranging from <0.001 to 0.033). In addition, C-peptide AUC was significantly lower after intake of the CHO constituent of the new product than glucose ($P < 0.001$). NEFA concentrations at different times were similar between groups and NEFA AUC.

Comparison of the new DSF versus CP showed no statistically significant differences in blood glucose. Plasma insulin concentration was significantly affected by diet ($P < 0.001$) being lower for the new DSF compared with CP. Plasma insulin concentration differed significantly between groups over time (P ranging from <0.001 to 0.003). Indeed, insulin AUC was significantly lower after intake of the new DSF than CP ($P < 0.001$). Plasma C-peptide concentration was significantly affected by diet ($P < 0.001$) being lower for the new DSF compared with CP, and significant differences were found between diets over time ($P < 0.001$–0.010). C-peptide and TAG AUC were also significantly lower after intake of the new DSF than CP ($P < 0.001$). NEFA concentrations at different times were similar between groups and NEFA AUC.

Plasma gastrointestinal hormones (ghrelin, GLP-1, GIP, PYY, PP and CCK)

Plasma AUC for ghrelin, GLP-1, GIP, PYY, PP and CCK responses is shown in Table 4.

Ghrelin, GLP-1 and GIP AUC were significantly lower after intake of the CHO constituent of the new product than glucose (P ranging from <0.001 to 0.019). PYY, PP and CCK AUC were unaffected after administration of the CHO constituent of the new product or glucose.

GIP AUC was significantly lower after intake of the new DSF than CP ($P = 0.002$). Ghrelin, GLP-1, PYY, PP and CCK AUC were unaffected after administration of the new DSF or CP.

Postprandial responses in outpatient type 2 diabetics

Plasma glucose, insulin, C-peptide, TAG and NEFA

Plasma AUC for glucose, insulin, C-peptide, TAG and NEFA responses is shown in Table 5.

Glucose AUC was significantly lower after intake of the new DSF than one of the two other commercially available formulas tested ($P = 0.037$). Insulin, C-peptide, TAG and NEFA AUC were unaffected after administration of the three DSF.

Plasma gastrointestinal hormones (ghrelin, GLP-1, GIP, PYY, PP and CCK)

Plasma AUC for ghrelin, GLP-1, GIP, PYY, PP and CCK responses is shown in Table 5.

AUC of all gastrointestinal hormones was unaffected after administration of the three DSF.

Discussion

The most relevant findings of the present study were that the new fructose-free and RS type IV-enriched DSF as well

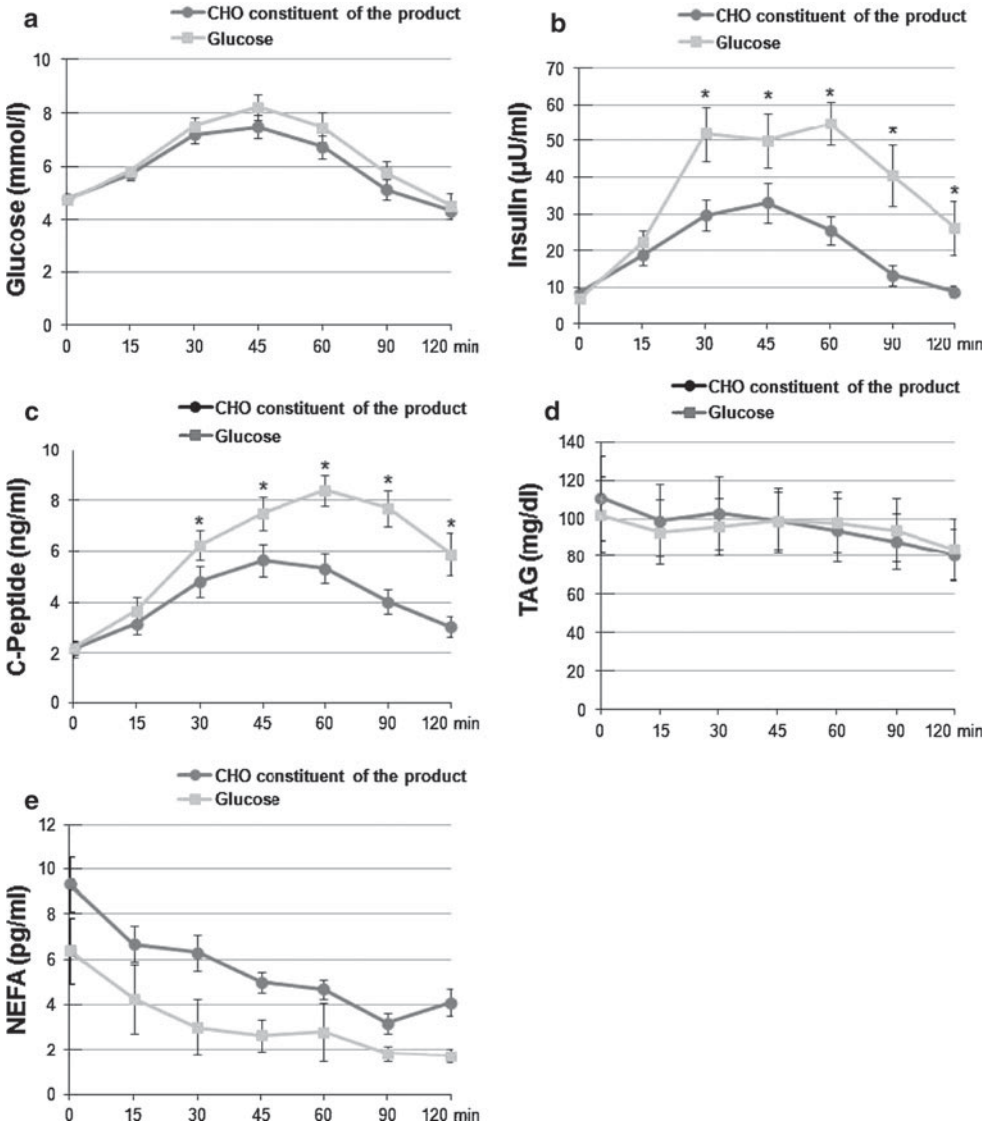

Fig. 1 Postprandial plasma glucose (**a**), insulin (**b**), C-peptide (**c**), TAG (**d**) and NEFA (**e**) in healthy volunteers following an intake of 50 g of CHO contained in the new product or 50 g of glucose. Values are mean ± SEM, $n = 10$. *Significant differences at each time after administration of the CHO constituent of the new product versus glucose, $P < 0.05$. The interaction diet × time for plasma NEFA postprandial concentrations was not significant, and when the baseline concentration was included as a covariate, diets did not differ ($P = 0.14$). *CHO* carbohydrate, *NEFA* non-esterified fatty acids, *TAG* triacylglycerols

as its CHO constituent had a low GI and GL in healthy volunteers. Moreover, in type 2 diabetic patients, the new DSF exhibited significantly lower AUC compared with another classic product designed for diabetics. Likewise, the new DSF showed significantly lower insulin concentration in healthy volunteers compared with the CP. On the other hand, ghrelin, GLP-1 and GIP showed lower AUC after administration of the new DSF and the CHO constituent of the new product, compared with CP and glucose, respectively.

Our data indicated that the CHO constituent included in the new product had a GI of 54.8 % and, the new DSF, a GI of 26.9 % compared with glucose. Differences in glucose response to the CHO constituent of the new product and DSF may be a result of other components such as protein and fat present in the new product that may also influence the glycaemic response [21]. The GI found for the new DSF (26.9 %) is consistent with previous findings on specific formulas for diabetic patients, demonstrating that DSFs are characterized by a lower GI than STFs [22]. Evidence suggests that a clinically useful effect of low GI diets on glycaemic control can be obtained in type 2 diabetic patients, with an improvement in lipid metabolism and glycaemic control [23, 24]. Improving glucose control and lipid metabolism by using a DSF instead of a STF has also a beneficial effect on clinical outcome in diabetic patients in need of nutritional support [14, 25, 26].

Comparison of the composition of enteral formulas with a low GI (DSF) and those with a high GI (STF) showed that, in general, a low GI formula is characterized by a lower available CHO content, the presence of fructose and a higher fat content containing more MUFA [14, 22, 26]. The CHO blend of the new DSF consists of slowly digestible CHO, mainly RS type IV. DSFs provide CHO

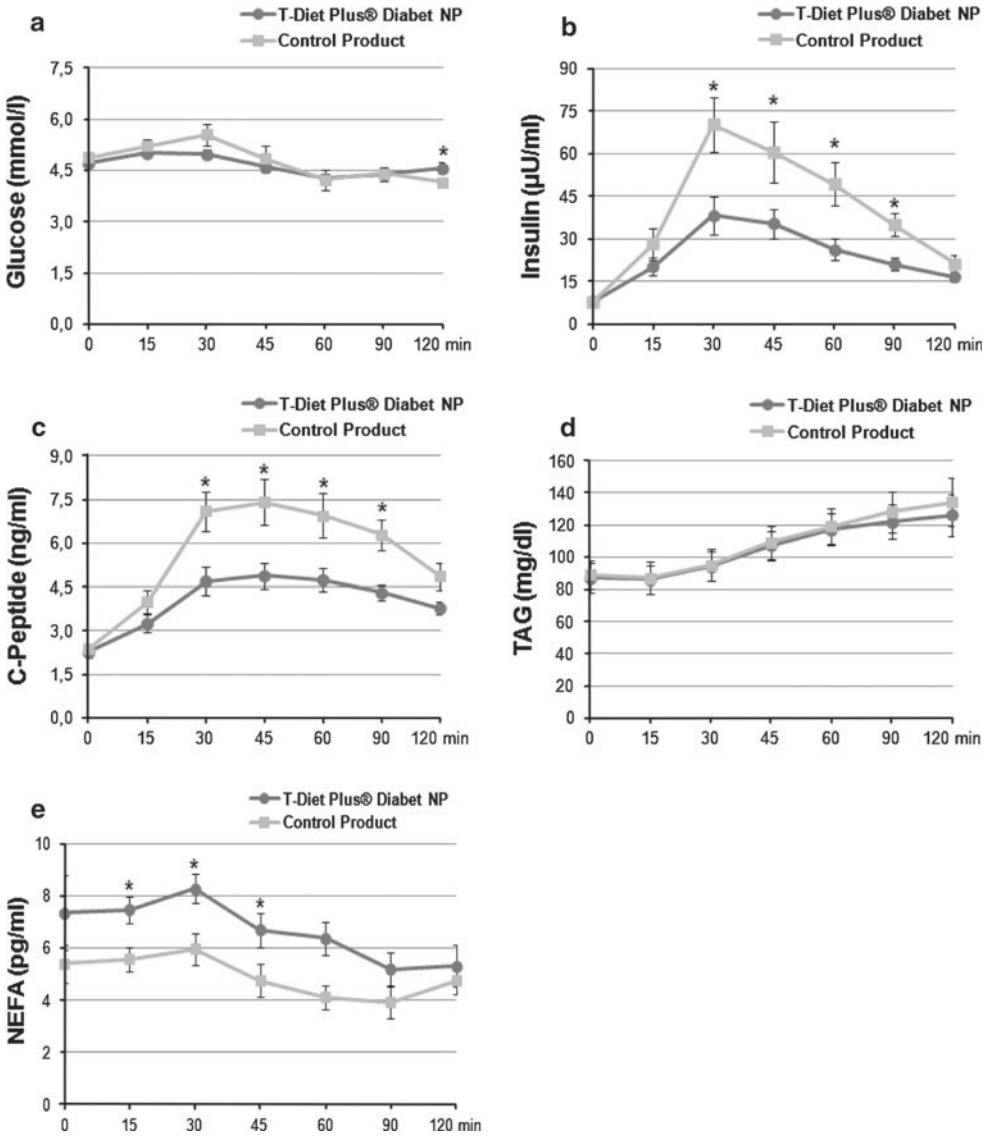

Fig. 2 Postprandial plasma glucose (**a**), insulin (**b**), C-peptide (**c**), TAG (**d**) and NEFA (**e**) in healthy volunteers following an intake of 400 ml of a new DSF or CP. Values are mean ± SEM, $n = 14$.
* Significant differences at each time after administration of the new DSF versus the other two DSF, $P < 0.05$. The interaction diet × time for plasma NEFA postprandial concentrations was not significant, and when the baseline concentration was included as a covariate, diets did not differ ($P = 0.45$). *NEFA* non-esterified fatty acids, *TAG* triacylglycerols

energy in a form which is more slowly digested and absorbed with significantly lower postprandial glycaemic responses compared with STFs [27, 28]. Ingestion of slowly digestible starch resulted in a significant attenuation of the postprandial plasma glucose and insulin responses and a lower concentration of blood lipids compared with maltodextrin in healthy rats [28, 29]. Our results showed a lower glucose AUC after taking the new product when comparing the intake of this new DSF with two other commercially available formulas in type 2 diabetic subjects. This indicates that the CHO composition of this new DSF may have contributed to the improved glycaemic response after consumption, and hence that it may have a clear advantage over the other two classical products. A more obvious effect was seen in terms of insulin response, with significantly higher insulin concentration after taking CP than the new DSF and with C-peptide values varying in the same way, despite no differences were found between the three different DSF tested in type 2 diabetic patients for insulin or C-peptide values. The slowly digestible CHO contained in this new product may produce a slow, sustained release of glucose into the circulation with a corresponding demand for insulin, which may reduce the risk of hypoglycaemia. In addition, in type 2 diabetic patients, AUC insulin values were higher than those observed in healthy volunteers. A possible explanation for this may be the different action of medication in type 2 diabetic subjects like sulfonylureas that stimulate the pancreas to produce more insulin [30]. Fibre, present in this new DSF as inulin and cellulose, may influence the absorption of macronutrients, especially CHO, by delaying gastric emptying and/or shortening the small-intestinal transit time. Colonic fermentation of fibre also produces short-chain carboxylic acids (acetate, butyrate and propionate), lactate and gases

Table 4 Area under the curve (AUC) for biochemical parameters and gastrointestinal hormones in healthy volunteers

	Area under the curve (AUC)					
	CHO constituent	Glucose	*P* value	T-Diet Plus® Diabet NP	Control product	*P* value
Glucose (mmol/l)	148 ± 19	209 ± 32	0.066	14.86 ± 2.77	27.34 ± 7.26	0.102
Insulin (μU/ml)	1430 ± 227	3927 ± 563	<0.001*	1969 ± 209	3961 ± 435	<0.001*
C-Peptide (ng/ml)	245 ± 23	523 ± 38	<0.001*	232 ± 18	430 ± 44	<0.001*
TAG (mg/dl)	129 ± 91	279 ± 129	0.103	2696 ± 347	5550 ± 815	<0.001*
NEFA (pg/ml)	1.4 ± 1.1	4.4 ± 3.3	0.222	158.8 ± 65.9	75.2 ± 25.8	0.267
Ghrelin (pg/ml)	1176 ± 458	3619 ± 780	0.009*	4838 ± 1159	4289 ± 822	0.413
GLP-1 (μg/ml)	1.25 ± 0.44	6.53 ± 1.85	0.019*	4.73 ± 0.87	3.85 ± 0.93	0.285
GIP (μg/ml)	4.41 ± 0.81	11.35 ± 11.57	<0.001*	19.47 ± 2.68	26.91 ± 3.60	0.002*
PYY (μg/m)	0.23 ± 0.16	2.86 ± 1.92	0.207	12.77 ± 2.83	12.45 ± 3.13	0.881
PP (μg/ml)	1.64 ± 0.58	2.32 ± 0.59	0.141	18.45 ± 3.21	22.45 ± 4.56	0.125
CCK (pg/ml)	0.38 ± 0.15	0.34 ± 0.15	0.882	1.28 ± 0.48	1.38 ± 0.280	0.821

Values are mean ± SEM

* Statistically significant differences ($P < 0.05$) between diets using a student *t* test for paired data. *AUC* area under the curve, *CCK* cholecystokinin, *CHO* carbohydrate, *GIP* glucose-dependent insulinotropic peptide, *GLP-1* glucagon-like peptide-1, *NEFA* non-esterified fatty acids, *PP* pancreatic polypeptide, *PYY* peptide YY, *TAG* triacylglycerols

Table 5 Area under the curve (AUC) for biochemical parameters and gastrointestinal hormones in type 2 diabetic patients

	Area under the curve (AUC)			
	T-Diet Plus® Diabet NP	Glucerna® SR	Novasource® Diabet	*P* value
Glucose (mmol/l)	298 ± 62	415 ± 71	374 ± 70	0.020*
Insulin (μU/ml)	2372 ± 1084	1968 ± 491	4048 ± 2522	0.393
C-Peptide (ng/ml)	153 ± 39	177 ± 54	166 ± 41	0.591
TAG (mg/dl)	2433 ± 613	1136 ± 371	1975 ± 525	0.066
NEFA (pg/ml)	213 ± 192	431 ± 290	500 ± 469	0.859
Ghrelin (pg/ml)	1436 ± 1015	1564 ± 624	1827 ± 784	0.294
GLP-1 (μg/ml)	4.79 ± 1.18	5.25 ± 1.49	5.03 ± 0.85	0.935
GIP (μg/ml)	17.38 ± 3.24	14.15 ± 3.88	13.51 ± 1.88	0.316
PYY (μg/ml)	3.38 ± 1.39	3.10 ± 1.11	4.85 ± 1.21	0.237
PP (μg/ml)	20.25 ± 3.96	12.88 ± 3.43	12.82 ± 2.60	0.167
CCK (pg/ml)	2.31 ± 0.79	7.33 ± 2.52	3.57 ± 1.19	0.228

Values are mean ± SEM

* Statistically significant differences ($P < 0.05$) between diets using a general linear model for repeated measures. *AUC* area under the curve, *CCK* cholecystokinin, *GIP* glucose-dependent insulinotropic peptide, *GLP-1* glucagon-like peptide-1, *NEFA* non-esterified fatty acids, *PP* pancreatic polypeptide, *PYY* peptide YY, *TAG* triacylglycerols

as products of digestion that might influence gluconeogenesis and lipid metabolism [31, 32]. Moreover, fructose, which is often used in diabetic formulas [22] due to its low GI, increases the level of low-density lipoprotein cholesterol and may also increase serum TAG and cause diarrhoea [27, 28]. By lacking fructose, the new DSF avoids its potential harmful effects on lipid metabolism. On the other hand, the new DSF was characterized by a high MUFA content like the other two formulas analysed, which may improve lipid and glycaemic control [5, 7]. The addition of MUFAs provide an alternative energy source to CHO, and consequently, leads to a lower insulin response. The present study showed that NEFA concentrations remained similar in both healthy and type 2 diabetic groups. In addition, TAG AUC was lower in healthy volunteers after intake of the new DSF compared with CP. Hence, it is clear that the new product did not influence the lipid metabolism in a negative way, despite its high fat content.

Another aim of the present study was to analyse different gastrointestinal hormones such as ghrelin, GLP-1, GIP, PYY, PP and CCK. It is well known that ghrelin stimulates appetite and food intake in humans [33]. Furthermore, glucose and insulin may modulate ghrelin response [18, 34, 35]. In addition, complex CHO and fibre

lessen hunger satiety [36]. Therefore, the reduced insulin levels found after the intake of the CHO constituent of the new product might be related with the delayed rise in ghrelin concentration, showing that the CHO constituent of this new DSF, that is, 5–8 DE maltodextrins and RS type IV, could have delayed hunger feeling. Moreover, these results are consistent with those showing that ghrelin participates in the mechanism leading to the appetite signal [15, 17]. Although no differences were found in satiety scores, it is important to emphasize that both internal (physiological and psychological) and external (physical activity, temperature, weather) elements interact with sensations of hunger and satiety [37]. Those factors might explain that satiety and satiation were similar between diets. On the other hand, the incretin hormones GLP-1 and GIP are potent inducers of glucose-dependent insulin release [15–17]. In addition, GLP-1 decreases appetite and food intake. In the present study, we observed that GLP-1 AUC was significantly lower following intake of the CHO constituent of the new product compared with glucose intake. Concerning GIP, it showed significantly lower AUC after the CHO constituent of the new product and also after the new DSF administration when CHOs or products were compared in healthy volunteers. Therefore, incretin hormones decreased insulin secretion after ingestion of the CHO content of the new product, and this decline was independent of glucose. Moreover, our results emphasize the clear importance of the quality of the CHO on insulin control. On the contrary, in type 2 diabetic subjects, incretins were unaffected. Nonetheless, in this kind of subjects, the effect of GLP-1 and GIP is reduced or even absent [15, 16]. In our study, there were no significant differences in healthy volunteers or diabetic subjects in CCK, PP or PYY AUC. These peptides are associated with reduced appetite and food intake [15, 17]; however, no significant differences were found in satiety scores. The limitation of the measurement of hunger, satiety and satiation by using the numerical Haber scale [19] is the difficulty to indicate a precise feeling, while the use of a visual analogue scale would have allowed a better indication of the real state of the subjects [38].

In conclusion, the new DSF tested may improve glycaemic control and reduce insulin requirements without altering lipid metabolism or affecting satiety. Therefore, this study clearly demonstrates that this new DSF could be useful in patients in need of nutritional support of diabetes.

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Conflict of interest The authors declare that they have no conflict of interest.

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