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# Safety and tolerability of a novel oral nutritional supplement in healthy volunteers

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SUMMARY

*Background and objective:* Foods for Special Medical Purposes (FSMPs) are formulated to support the nutritional needs of subjects with impaired capacity to ingest, digest or absorb ordinary food or nutrients. Polglumyt® is a proprietary highly purified, high quality glycogen obtained from mussels. Here we report the results of a single-center, single dose, open label, single arm study carried out to investigate acceptance (i.e. gastrointestinal tolerance and palatability), metabolic profile and safety of a low osmolarity, high-density energy Polglumyt®-based drink (the investigational product, IP) as a novel FSMP.
*Methods:* Twelve healthy subjects received a single oral administration of the IP under fasting conditions. The study endpoints were: changes in gastrointestinal system tolerability at 3 h, 6 h and 24 h after IP intake; IP palatability evaluation; metabolic evaluation through the kinetic profile of circulating glucose, insulin and C-peptide from 0 h to 6 h after IP intake and changes from baseline in circulating triglycerides at 3 h and 6 h after IP intake.
*Results:* The IP showed a good gastrointestinal tolerability and an acceptable palatability. The IP did not affect the physiological glycemic profile and the triglycerides levels 6 h after the intake. The IP was well tolerated by study subjects, with no or minor adverse events.
*Conclusions:* The study results encourage additional clinical investigations on the IP as a novel FSMP in patients with impaired digestion or gastrointestinal absorption, unable to assume an ordinary diet, e.g. patients undergoing invasive gastrointestinal surgery, elderly or oncological patients, even with certain metabolic disorders.



## 1. Introduction

The preservation of a proper nutritional status can be quite challenging in subjects affected by acute disease, alterations in functional status or severe comorbidities [1]. If individual nutritional needs cannot be reached through usual dietary intake or meal modifications, other interventions are necessary. Foods for Special Medical Purposes (FSMPs) are highly controlled, nutritionally complete or incomplete formulations to be administered under healthcare professional supervision and useful to temporarily or permanently supplement inadequate nutritional intake [2]. FSMPs are formulated to provide sufficient energy and macro

*Abbreviations:* AEs, Adverse Events; BMI, Body Mass Index; EP, Evaluable Population; FSMPs, Foods for Special Medical Purposes; GI, Gastrointestinal; IP, Investigational Product; MDs, maltodextrins; MedDRA, Medical Dictionary for Regulatory Activities; OGTT, Oral glucose tolerance test; PTAEs, pre-treatment adverse events; TEAEs, Treatment Emergent Adverse Events; TP, Treated Population.

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and micronutrients, including carbohydrates, proteins, unsaturated fatty acids, vitamins and minerals at different ranges of concentration, to reduce the risk or the level of malnutrition. Several types of FSMPs are currently commercially available, with different formulations, including additions or restrictions of specific nutrients, depending on the medical conditions of patients for whom they are intended, their age, or whether the product is intended as the sole source of nourishment [3,4]. Oral or enteral administration of FSMPs is indicated in patients who maintain a sufficient function to receive, digest and absorb nutrients through the digestive system.

Glycogen is the main storage polysaccharide in animals and microorganisms. Polglumyt® is a highly purified, high quality glycogen, proprietary of A.C.R.A.F. S.p.A. (International patent publication number WO9403502 "Glycogen Polysaccharides") [5] and extracted from mussels. The chemical-physical properties of Polglumyt® are identical to those of glycogen reported in reference manuals (e.g. Merck Index), including the high molecular weight ($2.5 \pm 0.1 \times 10^6$ Da for Polglumyt®, evaluated by viscometric method, and about $2.7 \times 10^5$ to $3.5 \times 10^6$ for glycogen). The quality of a glycogen depends on the presence of reducing sugars and protein residues after extraction and on the title of glucose after hydrolysis. Polglumyt® is a high-quality glycogen with low nitrogen and reducing sugars content and with a high title of glucose after hydrolysis. The digestion rate of a carbohydrate is determined by the complexity of its chemical structure, and thus by how "resistant" it is to the activity of the enzymes. Polglumyt® is a structurally complex glucose polysaccharide, composed of D-glucose molecules linked by $\alpha(1 \rightarrow 4)$ bonds with branches every 7–11 glucose units, linked by $\alpha(1 \rightarrow 6)$ bonds, resulting in a substantially spherical macromolecular structure, where surface and bulk have an identical chemical composition. The high degree of branching confers to Polglumyt® a high solubility in water (30% W/V), and a very low viscosity ($10^{-3}$ Pa*s), similar to pure water, with a typical Newtonian flow behavior. Furthermore, the high molecular weight confers to Polglumyt® aqueous solutions a very low osmolarity, even at high concentrations (1 mOsm/L at the concentration of 18% W/V). Of major interest, the high degree of branching and the structural complexity of the polysaccharide described above could confer to Polglumyt® a slower enzymatic hydrolysis respect to other carbohydrates, guaranteeing a gradual and more lasting contribution of energy, without triggering a strong response of insulin. Finally, preliminary tests performed by A.C.R.A.F. S.p.A. *in vitro* and in murine models confirmed the safety of Polglumyt® in terms of chemical and microbial degree of purity, toxicity (as single and repeated dose), mutagenicity and sensitivity, supporting the use of Polglumyt® in humans. On these bases, a potential use of Polglumyt® as main carbohydrate source in the formulation of a novel FSMP has been hypothesized.

This study aimed to investigate the acceptance (intended as gastrointestinal [GI] tolerance and palatability), the metabolic profile and the safety after a single oral administration in healthy volunteers under fasting conditions of a Polglumyt®-based drink (here from the "Investigational Product", IP) [5] formulated as a FSMP and never tested in humans before.

## 2. Methods

### 2.1. Study design

The study has a single-center, single dose, open label, single arm design, and has been carried out between January 9th, 2016 and February 6th, 2016 (first subject-in, last subject-out) at the Phase I Unit Policlinico Universitario Campus Bio Medico of Rome, Italy. The final approved protocol and the Informed Consent Form were reviewed by the Ethics Committee of the Università Campus Bio-Medico of Rome, Italy. The protocol (final Version 1/0 dated 03.11.2015) and the Informed Consent Form (Version 1/1 dated 03.11.2015) were approved by the Ethics Committee on December 15th, 2015. The Protocol (Version 1/0 dated 03.11.2015) was notified to the Competent Authority (Agenzia italiana del farmaco, AIFA) on March 03th, 2016.

The objective of the study was to investigate the acceptance (intended as GI tolerance and palatability), the metabolic profile and the safety of the IP Polglumyt®-based drink after a single oral administration to healthy volunteers, under fasting conditions. A total of three visits were scheduled for the study (Table 1). Enrolled subjects were administered in fasting conditions with 200 mL of the IP at 8–9 a.m., to be drunk within 10 min (time limit). To guarantee a standardization of the experimental conditions, starting from 24 h before Visit 1 on Day 1 (Table 1), the subjects had to take a balanced dinner, prescribed by a registered dietitian. After the IP intake, the subjects remained fasted until 6 h post-dose and, for the same time, when not involved in study activities, they remained seated and were not allowed to lie down. During the 6 h of stay at the research facility, it was forbidden to smoke cigarettes and to eat or drink anything else. Only water was allowed, except for the 30 min before and after IP administration. During the 6 h of stay at the research facility, serum, plasma and GI symptoms analyses were performed as detailed below and in Tables 1, and 4-8. A light lunch was provided to the subjects after the 6 h of stay at the research facility, followed by a balanced dinner at the end of Day 1 and by a balanced breakfast on Day 2.

The primary end-points of the study were: the changes from baseline in GI system tolerability at Visit 1, post-dose (3 h and 6 h after IP intake) and the product taste evaluation. Study secondary end-points were the metabolic (glycemic and lipid) profile evaluation through the assessment of the kinetic profile of plasma glucose, serum insulin and serum C-peptide and the changes from baseline in triglycerides values at Visit 1, post-dose (3 h and 6 h after IP intake). Other study parameters were the monitoring of adverse events (AEs) and the physical examination focused on GI system (performed at Follow-up Visit, Table 1).

### 2.2. Study investigational product

The IP has been manufactured by Even Santé Industrie according to the HACCP and UNI ISO 22000. The main complex carbohydrate source in the IP formulation is represented by Polglumyt®, an A.C.R.A.F. S.p.A proprietary highly purified, high quality glycogen $(C_6H_{10}O_5)_n$, extracted from mussels, with a molecular weight of 2500 kDa, and a percentage of α-1-6 glucoside bonds ranging from 7% to 11%, relative to the total number of glucoside bonds. All the raw materials used in the formulation have been supplied by Even Santé Industrie, except for Polglumyt®, provided by A.C.R.A.F. S.p.A.

The IP is a high-energy (1.82 kcal/mL) mixture of proteins (22 g), fats (21.2 g), carbohydrates (total 21.4 g of which 7.6 g of simple sugars) and micronutrients in a 200 mL volume formulation. Its full nutritional profile is summarized in Table 2. The IP contains minerals, vitamins and trace elements in compliance with the regulations for FSMPs (1999/21/EC). The Ω-6 to Ω-3 ratio of fatty acids in the IP is 2.2:1. The osmolarity of the IP formulation is 400 mOsm/L.

The IP stability studies were performed by A.C.R.A.F. S.p.A. for the climatic zone II (sub-tropical and Mediterranean climate, 25 °C/60% relative humidity). According to the analytical results reported in Table 3, the IP is chemically and microbiologically stable after 24 months after its production. The IP was packaged in 200 mL bottles with the following structure and composition: high-density polyethylene (HDPE) 6 layers (white); cap: PE (white); peel off: laminate of aluminum and peelable HDPE.

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**Table 1**
Summary of study steps and procedures per each study visit.

| Visit | Screening Visit | Visit 1 | | Follow-up Visit | ETTV |
|---|---|---|---|---|---|
| Day | Day −10/-5 | Day 1, Pre-dose (before IP uptake) | Day 1, Post-dose (after IP uptake) | Day 2 | |
| Informed Consent | X | | | | |
| Collection of demographic data | X | | | | |
| Lifestyle | X | | | | |
| Medical history | X | | | | |
| Physical examination | X | | | X[a] | X |
| Questionnaire for evaluation of GI symptoms (before IP intake) | | X | | | |
| Questionnaire for tolerance evaluation of GI system (after IP intake) | | | X | | |
| Vital signs | X | | | | |
| BMI calculation | X | | | | |
| ECG | X | | | | |
| OGTT | X | | | | |
| Laboratory analysis | X | X | X | | |
| Laboratory analysis evaluation | | X | | | |
| Urinalysis | X | | | | |
| Pregnancy test | X | X | | | |
| Urine drug test | X | X | | | |
| Alcohol breath test | | X | | | |
| Study diary delivery | X | | X | | |
| Study diary check | | X | | X | X |
| Inclusion/Exclusion criteria | X | X | | | |
| Blood sampling for kinetic profile of plasma/serum glucose, insulin and C-peptide | | X | X | | |
| Laboratory analysis for triglyceride values | | X | X | | |
| IP administration | | X | | | |
| IP taste evaluation | | | X | | |
| IP accountability | | | X | | |
| Previous treatments | X | | | | |
| Concomitant treatments | X | X | | X | X |
| Adverse events monitoring | | X | | X | X |

BMI: Body Mass Index; ECG: electrocardiogram; ETTV: Early Treatment Termination Visit; GI: Gastrointestinal; IP: Investigational Product; OGTT: Oral Glucose Tolerance Test.
[a] Physical examination focused on GI system only.

**Table 2**
Nutritional profile of the IP.

| | | 200 mL | Molarity |
|---|---|---|---|
| **Energy content** | **kcal** | 364 | |
| | **kJ** | 1525.16 | |
| **Proteins** | **g** | **22** | |
| Caseins | g | 16.6 | |
| WP | g | 5.6 | |
| **Carbohydrates** | **g** | **21.4** | |
| Sucrose | g | 7.6 | |
| Polglumyt | g | 13.2 | |
| **Lipids** | **g** | **21.2** | |
| SFA whom MCT | g | 5.8 | |
| | | 4.4 | |
| MUFAs | g | 10.4 | |
| PUFAs | g | 4.8 | |
| **Fiber** | – | 0 | |
| **Salt** | mg | 338 | |
| Mineral salts: | | | |
| Calcium | mg | 400 | 0.050 |
| Potassium | mg | 340 | 0.043 |
| Sodium | mg | 130 | 0.028 |
| Copper | mg | 0.3 | $2.36*10^{-5}$ |
| Magnesium | mg | 32 | 0.0066 |
| Manganese | mg | 0.24 | $2.18*10^{-5}$ |
| Iron | mg | 2.2 | $1.97*10^{-4}$ |
| Phosphorus | mg | 280 | 0.045 |
| Zinc | mg | 2.2 | 0.021 |
| Chromium | mg | 0.01 | $9.6*10^{-7}$ |
| Chlorine | mg | 140 | 0.020 |
| Fluorine | mg | 0.30 | $7.9*10^{-5}$ |
| Molybdenum | mg | 0.022 | $1.15*10^{-6}$ |
| Iodine | mg | 0.026 | |

MCT: Medium Chain Triglyceride; MUFAs: Monousaturated Fatty Acids; PUFAs: Polyunsaturated Fatty Acids; SFA: Saturated Fatty Acid; WP: Whey Protein.
Bold indicates the main categories of macronutrients and micronutrients.

### 2.3. Study populations

A total of 12 subjects were planned to be enrolled. Subjects who fulfilled all of the following criteria were eligible to enter the study: 1) Male and female subjects, 18–55 years of age (limits included), with no limitation of race; 2) Body Mass Index (BMI) $\geq$18.5 and $\leq$ 24.9 kg/m$^2$; 3) vital signs: SBP 100–139 mmHg, DBP 50–89 mmHg, HR 50–90 bpm, measured after 5 min of rest in the sitting position; 4) subjects able to understand the study procedures and to comply with protocol requirements; 5) subjects legally able to give written informed consent to the trial (signed and dated by the subject).

The exclusion criteria were: 1) ECG (12-leads) recording with clinically significant abnormalities; 2) clinically significant abnormal laboratory values indicative of physical illness, except for deviations that, according to the Investigator's judgment, were permissive of recruitment; 3) history of previous allergy or sensitivity to any component of the formulation; 4) use of any oral supplement, herbal medications or product similar to the IP within 2 weeks before the start of the study and during the whole study; 5) use of systemic steroids over the last 3 months before the start of the study and during the whole study; 6) clinically relevant past or present history of organic and/or functional GI diseases, such as functional heartburn or dyspepsia, irritable bowel syndrome and functional vomiting, according to Rome III Criteria [Rome III Diagnostic Criteria 2006]; 7) diabetes and/or alterations of glucidic metabolism (types 1 and 2 diabetes, impaired glucose tolerance); 8) clinically relevant past or present history of renal, hepatic, cardiovascular, respiratory, skin, hematological, endocrine, neurological or psychiatric (in particular mood disorders, anxiety or any primary psychiatric disorder) diseases interfering with the study objectives;

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**Table 3**
Analytical results of IP stability.

| TEST | Unit | Specification | Range Accettability | STORING CONDITIONS (ACCELERATED) 25 °C ± 2°C- 60% ± 5% R.H.; Packaging: bottle 200 mL HDPE 6 layers (white) - Cap: PE (white) - TIME POINTS (months) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 | 1 | 2 | 3 | 5 | 6 | 12 | 18 | 24 |
| Organoleptic | | Slightly yellow creamy liquid with intensive vanilla smell: vanilla taste | | complies | complies | complies | complies | | complies | complies | complies | complies |
| Polglumyt | g/100 mL | 6.6 | 5.28–7.92 | 7.35 | | | 7.29 | | 5.80 | 6.19 | 6.04 | 6.38 |
| Vitamin A (retinol activity equivalents RAE) | µg/100 mL | 200 | 160–260 | 214.3 | 230,0 | 192 | 209 | | 207 | 169 | 180 | 186 |
| Vitamin E | mg/100 mL | 2.0 | 1.6–3 | 2.57 | 3.08 | 2.38 | 2.56 | | 2.74 | 2.17 | 2.61 | 2.58 |
| Vitamin C | mg/100 mL | 12.0 | 9.6–24 | 19.6 | 17.7 | 15.6 | 16.4 | | 17.6 | 14.7 | 12.64 | 11.85 |
| Protein | g/100 mL | 11.0 | 9.35–12.65 | 10.7 | | | 10.6 | | 11.0 | 10.7 | 10.31 | 10.97 |
| Osmolality | mOsm/kg | ≈400 only control in development | / | 394 | 382 | 380 | 420 | 419 | 405 | 410 | 398 | 405 |
| pH | | 6.7 | 6.0–7.0 | 6.67 | 6.58 | 6.65 | 6.67 | | 6.69 | 6.61 | 6.56 | 6.50 |
| Density | g/mL | 1.06 | 1.00–1.10 | 1.061 | 1.080 | 1.069 | 1.044 | | 1.066 | 1.057 | 1.063 | 1.097 |
| Viscosity | | | | 100 rpm:217CP<br>60 rpm: 220CP<br>50 rpm: 223CP<br>30 rpm:234CP | 100 rpm:242CP<br>60 rpm: 245CP<br>50 rpm: 249CP<br>30 rpm:267CP | 100 rpm:220CP<br>60 rpm: 222CP<br>50 rpm: 224CP<br>30 rpm:237CP | 100 rpm:281CP<br>60 rpm: 292CP<br>50 rpm: 301CP<br>30 rpm:324CP | | 100 rpm:277CP<br>60 rpm: 295CP<br>50 rpm: 305CP<br>30 rpm:332CP | 100 rpm: 533CP<br>60 rpm: 600CP<br>50 rpm: 645CP<br>30 rpm: 796CP | 60 rpm: 1419CP<br>50 rpm: 1550CP<br>30 rpm: 1968CP<br>20 rpm: 2429CP | 60 rpm: 3421CP<br>50 rpm: 3685CP<br>30 rpm: 5010CP |
| Vitamina B1 | mg/100 mL | 0.25 | 0.20–0.325 | 0.28 | | | | | | | | |
| Vitamina B2 | mg/100 mL | 0.30 | 0.24–0.39 | 0.33 | | | | | | | | |
| Niacin (Vit PP) | mg/100 mL | 2.3 | 1.84–2.99 | 2.79 | 2.72 | 2.41 | 2.57 | | 2.38 | 2.51 | 2.33 | 2.58 |
| Pantothenic Acid | mg/100 mL | 0.9 | 0.72–1.17 | 0.95 | | | | | | | | |
| Vitamin B6 | mg/100 mL | 0.4 | 0.32–0.52 | 0.41 | 0.41 | 0.42 | 0.38 | | 0.42 | 0.38 | 0.38 | 0.38 |
| Biotin | µg/100 mL | 12.5 | 6.25–18.75 | 15.1 | | | | | | | | |
| Folic Acid | µg/100 mL | 30.0 | 15–45 | 32.0 | | | | | | | | |
| Vitamin B12 | µg/100 mL | 0.5 | 0.25–0.75 | 0.44 | | | | | | | | |
| Vitamin D3 | µg/100 mL | 1.4 | 0.7–2.1 | 1.70 | | | | | | | | |
| Vitamin K1 | µg/100 mL | 9.0 | 4.5–13.5 | 10.6 | | | | | | | | |
| Sodium (Na) | mg/100 mL | 65.0 | 48.75–81.25 | 60.5 | | | | | | 60.6 | | |
| Calcium (Ca) | mg/100 mL | 200.0 | 150–250 | 210.1 | | | | | | 215.0 | | |
| Potassium (K) | mg/100 mL | 170.0 | 127.5–212.5 | 162.3 | | | | | | 170.0 | | |
| Magnesium (Mg) | mg/100 mL | 16.0 | 13.6–20 | 16.1 | | | | | | 15.75 | | |
| Water | g/100 mL | | | 72.4 | | | | | | 71.9 | | 74.37 |
| Dry residue | g/100 mL | 33.0 | 28.05–37.95 | 33.7 | | | | | | | | |
| Ashes | g/100 mL | | | 1.10 | | | | | | 1.046 | 1.094 | 1.075 |
| Fiber | g/100 mL | 0 | | <0.5 | | | | | | | | |
| Fat | g/100 mL | 10.6 | 9.01–12.19 | 10.9 | | | 10.5 | | 11.0 | 10.8 | 10.7 | 11.08 |
| Sugars | g/100 mL | | | 1.87 | | | 1.94 | | 1.91 | 1.78 | 1.80 | 1.94 |
| TYMC | UFC/g | NMT 1000 | | <10 | <10 | <10 | <10 | | <10 | <10 | <10 | <10 |
| TAMC | UFC/g | NMT 100 | | <10 | <10 | <10 | <10 | | <10 | <10 | <10 | <10 |
| Pathogen | /25 g | Absent | | Absent | / | / | / | | / | / | / | Absent |

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9) blood donations within 3 months before the study; 10) history of drug, alcohol [>2 standard drinks/day for men and >1 standard drink/day for women, defined according to USDA Dietary Guidelines 2010] [6], caffeine (>5 cups of coffee-tea/day) or tobacco abuse (≥10 cigarettes/day); daily use of distilled spirits; 11) pregnancy, lactation or female with a positive or missing urine pregnancy test result at Screening Visit or Visit 1; 12) positive urine drug test for cocaine, amphetamine, methamphetamine, cannabinoids (delta-9-tetrahydrocannabinol), opiates and ecstasy at Screening and/or Visit 1; 13) positive alcohol breath test at Visit 1; 14) abnormal diet or substantial changes in eating habits and weight in the 4 weeks before the study, vegetarians; 15) positive test for human immunodeficiency virus antibodies; 16) positive Hepatitis B-virus antigen and Anti-hepatitis C-virus antibodies tests; 17) participation in another trial involving any investigational drug during the past 3 months; 18) subjects involved in the conduct of the study.

All subjects gave written informed consent to participate in the study and were aware that they could stop their participation at any time. During the study, any withdrawal or dropped out subject was replaced in order to reach the planned sample size. After an initial screening of 24 subjects, 12 of them were selected as eligible on the basis of the inclusion/exclusion criteria, and 12 subjects were finally administered with the IP. All the 12 subjects completed the study and were considered suitable for final analysis. The disposition of subjects enrolled in the study is summarized in Fig. 1.

### 2.4. Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was performed in fasting conditions as described by the World Health Organization [7], using a glucose load containing 75 g anhydrous glucose dissolved in 250–300 mL of water, and blood samplings before and 2 h (±5 min) after the 75 g-glucose load.

### 2.5. Blood and urine analysis

The blood laboratory analyses performed at Screening Visit included haematology, clinical chemistry (including triglycerides), serum virology. Urinalysis performed at Screening Visit included color, appearance, pH, specific gravity, sediment examination, proteins, glucose, urobilinogen, bilirubin, hemoglobin, ketones.

### 2.6. Evaluation of glucose and hormones kinetics

Venous blood samples (14 mL) were collected for metabolic profiling (glucose, insulin, C-peptide kinetics) at Visit 1, pre-dose (baseline), and then post-dose, i.e. at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4 and 6 h after the IP intake. Pre-dose sampling was collected before the IP administration and differed from the time zero sampling considered as the end of the IP administration. The following events were considered reasons to exclude a subject from data analysis for possible alterations of the glucose, insulin and C-peptide plasma/serum concentration–time profiles: vomiting and diarrhea after the IP intake; concomitant medications; AEs; administration errors or others.

The following kinetic parameters were measured and/or calculated (when feasible) for the observed plasma glucose, serum insulin and serum C-peptide levels, with a non-compartmental analysis (linear trapezoidal linear extrapolation method) by the A.C.R.A.F. Pharmacokinetics Department, using the validated software Phoenix WinNonlin v. 6.3 or higher (Certara, St. Louis, MO, USA):

$C_{max}$: Maximum observed plasma/serum concentration ($C_{max}$ in Phoenix WinNonlin output);

$t_{max}$: Time at which $C_{max}$ is observed ($T_{max}$ in Phoenix WinNonlin output);

$AUC_{(0\text{-}t)}$: Area under the concentration–time curve from the time of IP administration to the last sampling time, calculated with the linear trapezoidal method (AUC last in Phoenix WinNonlin output).

Calculations of the above parameters, particularly of $C_{max}$ and $AUC_{(0\text{-}t)}$, were performed without subtracting any baseline level to the sequent readings of glucose and insulin levels and, hence, considering the 'total concentration' at $t_{max}$ and the 'total area' under the curve. Moreover, the calculations of the above parameters were based on the actual sample timing *vs.* time zero, defined as the end of IP administration for each subject.

### 2.7. Triglyceride levels

Triglyceride parameters were evaluated as changes from baseline (Visit 1, pre-dose) to post-dose (3 h and 6 h after IP intake), and were summarized as mean change and 95% confidence intervals. The number and percentage of subjects with out of range triglyceride values at Visit 1, post-dose (3 h and 6 h after IP intake), if any, were not evaluated, since there are no recognized reference normal ranges of triglycerides related to a post-ingestion condition.

### 2.8. GI tolerability test

A tolerance evaluation focused on the GI system, intended as the assessment of presence and/or occurrence of GI symptoms after IP intake, was performed through the completion of questionnaires. The questionnaires used to assess the presence and occurrence of GI symptoms after the IP intake are a brief and study-specific version of the "Rome III Diagnostic Questionnaire for Adult Functional GI Disorders", a validated questionnaire developed for the identification of subjects who have one or more functional GI disorders. The questionnaires were proposed as interview by the Investigator to the subject at Visit 1, pre-dose, and post-dose (3 h and 6 h after IP intake). An additional evaluation of GI system has been performed at the Follow-up Visit, 24 h after the IP intake (Table 1).

### 2.9. Palatability test

The IP taste was assessed on a five-point scale (1 = dislike; 2 = moderately dislike; 3 = neutral opinion; 4 = moderately liked; 5 = liked) and was presented in terms of number and percentage of subjects' responses. The palatability test was administered to subjects at Visit 1, immediately after the IP intake.

### 2.10. Safety

All adverse events (AEs) registered during the study were coded using the Medical Dictionary for Regulatory Activities (MedDRA) and were listed providing separate listing of pre-treatment (PTAEs) and after treatment AEs (Treatment Emergent AEs, TEAEs). TEAEs were summarized by numbers and percentages of TEAEs and by number and percentages of subjects reporting TEAEs, showing the description reported by the Investigator, the MedDRA Preferred Term and the System Organ Class. Changes from baseline (Visit 1, pre-dose) to each post-baseline available visit in clinical evaluation of GI system were listed.

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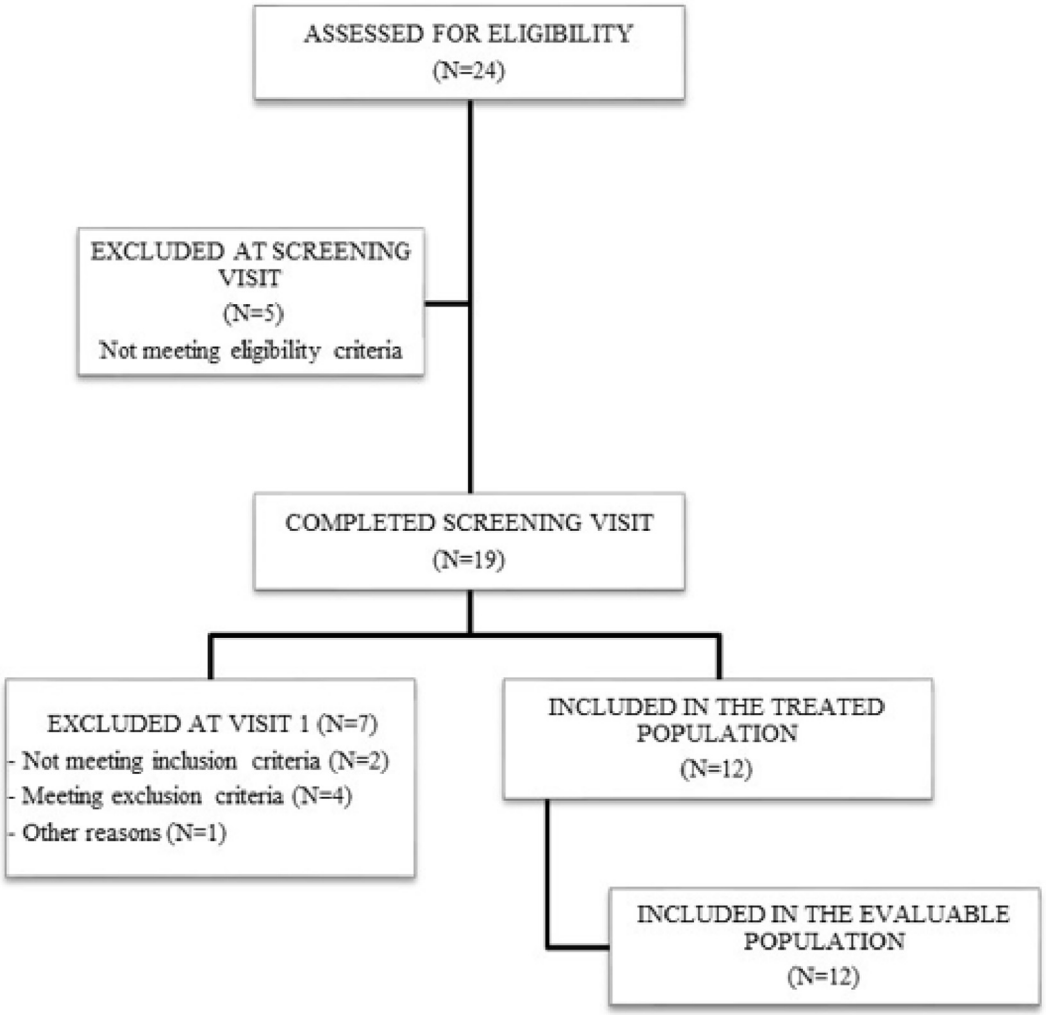


**Fig. 1.** Disposition of subjects enrolled in the study.

### 2.11. Statistical analyses

Since this was an open-label, single arm exploratory study, no formal calculation of the population sample size was performed. The data were summarized using descriptive statistics (arithmetic mean, SD, CV (%), minimum, median and maximum values for quantitative variables, and frequencies for qualitative variables). In more detail, mean and median values have been used to provide a proper distribution index for continuous (i.e. $C_{max,}$ AUC) and discrete (i.e. $t_{max}$) variables. Repeated-measures ANOVA was conducted to analyze any change in time when appropriate. The statistical analyses were performed using the SAS version 9.4 software package. The following populations were defined for statistical analysis: Treated Population (TP), defined as all subjects who took the study IP, even partially; Evaluable Population (EP), defined as all subjects who took the entire dose (200 mL) of the IP, completed all the procedures foreseen at Visit 1 (Table 1) and did not present any of the exclusion criteria listed above. Subjects with major protocol deviations were excluded. All study parameters were evaluated on the TP and EP populations on the available data set. ANOVA repeated-measures analyses were performed to assess whether metabolic changes after the IP intake *vs.* the basal level were statistically significant. The significance level of the statistical tests used was 5%: the statistical tests that gave p values $< 0.05$ were considered statistically significant.

## 3. Results

### 3.1. Subjects characteristics

A total of 12 subjects were selected to participate in the trial and were included in the TP. All the 12 subjects included in the TP took the entire dose of the IP and consequently were included in the EP. No major protocol deviation was observed during the study. GI tolerance, palatability and metabolic profile assessments were carried out on both the TP and EP study populations. The mean (SD) age of subjects was 31.9 years (9) (range: 19–50). The majority of subjects were male (58.3%) and all the subjects were Caucasian. The proportion of subjects with a current medical history condition at the Screening Visit was as follows: drug hypersensitivity (25%), iodine allergy (25%), uterine leiomyoma (25%) and anxiety (25%). Concerning the vital signs, the overall mean (SD) diastolic blood pressure was 76.7 (4.9) mmHg (range 70–80), the mean (SD) systolic blood pressure was 112.9 (9.4) mmHg (range 100–130) and the mean heart rate (SD) was 64.2 (5.1) bpm (range 54–75). At the Screening Visit, 9 out of 12 subjects reported normal ECG evaluation and 3 out of 12 subjects reported abnormal but not clinically significant alterations in the ECG; no subject reported abnormal and clinically significant alterations. Concerning the blood and urine analysis, all the 12 subjects had values in the normal range (Tables 4–6). All volunteers had negative response to the multi-

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drug test at the Screening Visit and at Visit 1, and negative alcohol breath test at Visit 1. All the 5 women enrolled in the study resulted negative at the pregnancy test performed at the Screening Visit and at Visit 1.

At OGTT, all the subjects had blood glucose levels in the normal range in fasting conditions and 2 h after the glucose load, confirming that no subject suffered from any type of diabetes mellitus, even in the early stages of disease.

*3.1.1. Primary study end-points*

*3.1.1.1. Gastrointestinal tolerance.* The tolerance evaluation of GI system, was presented as a list of the changes from baseline (nVisnnaire for evaluation of gastrointestinal symptoms at Vit 1, pre-dose) to Visit 1, post-dose (3 h and 6 h after IP intake) in the evaluation of GI system status, collected through the completion of specific questionnaires by the EP group. All the subjects (100%) confirmed that they had no GI discomforts at Visit 1, before IP administration, as well as 3 h and 6 h after IP intake (Table 8). A physical examination focused on the GI system has also been performed at the Follow-up Visit, 24 h after the IP intake (Table 1), and did not reveal any pathological alteration or change in GI conditions.

*3.1.1.2. Palatability.* Palatability is an important factor for long-term use of prescribed FSMPs. In this study, the palatability was measured at Visit 1, post-dose, in terms of distribution of the subjects' responses to the IP taste evaluation at the end of IP administration, through the completion of the following five-point scale: 1 = dislike; 2 = moderately dislike; 3 = neutral opinion; 4 = moderately liked; 5 = liked. The majority of subjects (66.7%) answered “moderately liked” (point 4). Two out of 12 subjects (16.7%) had a neutral opinion (point 3), and two out of 12 subjects (16.7%) answered “liked” (point 5). No subject rated the IP taste as “dislike” and “moderately dislike” (points 1 and 2 of the scale).

*3.1.2. Secondary study end-points*

*3.1.2.1. Kinetic profile of plasma glucose, serum insulin and serum C-peptide.* After a single oral administration of the IP, the following mean kinetic parameters were achieved: mean glucose $C_{max}$ of 96.667 mg/dL, considering a median $t_{max}$ of 0.642 h; mean insulin $C_{max}$ value of 53.808 μUI/mL, considering a median $t_{max}$ of 1.00 h and mean C-peptide $C_{max}$ value of 5.319 ng/mL, considering a median $t_{max}$ of 1.025 h. The mean values of $AUC_{(0\text{-}t)}$ were: 480.375 mg/dL*h (glucose), 71.578 μUI/mL*h (insulin) and 13.747 ng/mL*h (C-peptide).

The kinetic curves showed a rapid glucose peak (Fig. 2A) and a consequent rapid insulin peak (Fig. 2B), reaching the maximum level in the first hour after the IP intake. Between the first and the second hour after IP intake, a progressive reduction of the glucose and insulin concentration, followed by a plateau, was observed. The plateau remained stable up to 6 h. Statistically significant differences were observed in correspondence of the lowest glucose mean values (in the 1.5–2.5 h time range) and after 0.5 h *vs.* the pre-dose mean value ($p < 0.05$). Insulin mean values were significantly higher at 0.5 h, 0.75 h and 1 h *vs.* the mean pre-dose mean value ($p < 0.05$). The C-peptide kinetic profile was consistent with the insulin profile. C-peptide mean values differed significantly from the pre-dose value in the 0.75–2.5 h time range (Fig. 2C).

*3.1.2.2. Lipid profile.* For what concerns the lipid profile, the arithmetic mean of triglycerides levels at baseline was 85.4 ± 20.6 mg/dL, i.e. within the normal range established by the ATP III Guidelines (normal triglycerides <150 mg/dL). Triglycerides mean levels were 107.3 ± 41.0 mg/dL at 3 h and 81.8 ± 27.2 mg/dL at 6 h after IP intake respectively. The slight increase of triglycerides levels at 3 h after the IP intake was statistically significant ($p < 0.05$). The mean change from baseline (Visit 1, pre-dose) to Visit 1, post-dose (3 h and 6 h after IP intake) is summarized in Table 7. The confidence intervals were established at 95%. It should be underlined that the changes in triglyceride levels we detected were expected from a physiological point of view, as they followed the intake of an IP containing lipids (Table 2). Most importantly, from a clinical point of view, the triglyceride values returned to basal levels after IP digestion, 6 h after its intake.

*3.1.3. Safety assessment*

AEs reported after IP intake were classified as TEAEs on the basis of their definition as any AE started on or after the IP administration date, and that was not a pre-existing medical condition. No deaths, serious TEAEs or other significant TEAEs occurred during the study. Throughout the study, no PTAE was reported. Eight TEAEs occurred in 4 out of 12 subjects after the IP intake, with a single subject reporting myalgia, vertigo, vomiting and nausea, 2 subjects reporting abdominal distension, with or without nausea, and a single subject reporting abdominal discomfort. The intensity of all TEAEs was judged mild by the Investigator on the basis of a clinical evaluation. Only a single TEAE (abdominal distension) was judged as possibly related to IP intake by the Investigator on the basis of a clinical evaluation. The correlation of the other 7 TEAEs with IP intake was assessed as unlikely by the Investigator. No action was taken as all the TEAEs resolved, all the subjects recovered and none of them discontinued the study due to TEAEs. The TEAEs have not been reported by subjects during the questionnaire-based evaluation of GI discomfort at 3 h and 6 h after the IP intake, as they occurred at a later time. However, none of the subjects reported pathological alterations of the GI system during the physical examination performed at the Follow-up Visit (24 h after the IP intake).

## 4. Discussion

Malnutrition has been documented in approximately one-third of patients admitted to hospital in developed Countries and is associated with negative clinical outcomes, requiring a prompt intervention in at-risk patients [8,9]. Patients' malnutrition can be related to several factors, including illness-induced loss of appetite, GI symptoms, reduced or missing ability to chew or swallow, diagnostic and therapeutic procedures. In addition, inflammation, infection, or other catabolic conditions often contribute to the development of malnutrition [10]. These data justify and stimulate a continuous research of differentiated and optimized FSMP formulations aiming at satisfying the nutritional needs of heterogeneous categories of patients. In this context, A.C.R.A.F. S.p.A. has developed a new formulation of FSMP nutritionally complete and

**Table 4**
Haematology at the screening on EP study population.

| Laboratory test | N | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|---|
| Basophils (%) | 12 | 0.6 | 0.3 | 0.1 | 1.5 |
| Eosinophils (%) | 12 | 2.7 | 1.9 | 0.3 | 5.9 |
| Glycated Haemoglobin[a] (%) | 12 | 5.1 | 0.2 | 4.8 | 5.5 |
| Haematocrit (%) | 12 | 43.0 | 3.2 | 37.0 | 46.7 |
| Haemoglobin (g/dL) | 12 | 15.0 | 1.3 | 12.1 | 16.1 |
| Lymphocytes (%) | 12 | 30.0 | 7.2 | 19.1 | 39.1 |
| Monocytes (%) | 12 | 7.2 | 2.1 | 4.6 | 12.8 |
| Neutrophils (%) | 12 | 60.0 | 8.3 | 45.8 | 74.8 |
| Platelets ($10^{-}3/\mu L$) | 12 | 244.0 | 40.0 | 166.0 | 304.0 |
| Rbc ($10^{-}6/\mu L$) | 12 | 5.0 | 0.4 | 4.4 | 5.6 |
| Wbc ($10^{-}3/\mu L$) | 12 | 6.0 | 2.1 | 3.9 | 11.6 |

[a] Converted values from MMOL/MOL to % by the formula: ((mmol/mol)/10.929) +2.15.

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**Table 5**
Clinical chemistry at the screening on EP study population.

| Laboratory test | N | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|---|
| α1-Globulin (%) | 12 | 4 | 0.6 | 3.1 | 5.1 |
| α2-Globulin (%) | 12 | 9.3 | 1.6 | 6.8 | 13.2 |
| Albumin (%) | 12 | 62 | 2.8 | 54.8 | 66.5 |
| Alkaline Phosphatase (U/L) | 12 | 62 | 14 | 43 | 88 |
| Alt (Sgpt) (U/L) | 12 | 27 | 8.4 | 16 | 46 |
| Amylases (U/L) | 12 | 61 | 16 | 37 | 89 |
| Ast (Sgot) (U/L) | 12 | 18 | 4.1 | 13 | 28 |
| β1-Globulin (%) | 12 | 5.9 | 0.7 | 5 | 7.1 |
| β2-Globulin (%) | 12 | 4.6 | 0.8 | 3.4 | 5.9 |
| C-Reactive Protein (mg/L) | 12 | 3.1 | 2.3 | 1.37 | 5.69 |
| Calcium (mg/dL) | 12 | 9 | 0.2 | 8.7 | 9.3 |
| Chloride (mEq/L) | 12 | 104 | 3.1 | 100 | 109 |
| Cholesterol-Hdl (mg/dL) | 12 | 59 | 13 | 39 | 75 |
| Cholesterol-Ldl (mg/dL) | 12 | 112 | 26 | 70 | 149 |
| Creatine Phosphokinase (Cpk) (U/L) | 12 | 129 | 94 | 50 | 412 |
| Creatinine (mg/dL) | 12 | 0.8 | 0.1 | 0.64 | 1.06 |
| Direct Bilirubin (mg/dL) | 12 | 0.2 | 0.1 | 0.1 | 0.3 |
| Fibrinogen (mg/dL) | 12 | 288 | 53 | 216 | 368 |
| Lactate Dehydrogenase-Ldh (U/L) | 12 | 158 | 23 | 119 | 213 |
| Lipase (U/L) | 12 | 135 | 56 | 92 | 269 |
| Magnesium (mg/dL) | 12 | 2 | 0.1 | 1.8 | 2.2 |
| Phosphorus (mg/dL) | 12 | 2.9 | 0.5 | 2.4 | 3.8 |
| Potassium (mEq/L) | 12 | 3.8 | 0.3 | 3.3 | 4.3 |
| Pt (s) | 12 | 11 | 0.6 | 10.1 | 12 |
| Ptt (s) | 12 | 26 | 1.2 | 24.5 | 28.5 |
| Sodium (mEq/L) | 12 | 141 | 1.1 | 139 | 142 |
| Total Bilirubin (mg/dL) | 12 | 0.7 | 0.4 | 0.3 | 1.82 |
| Total Cholesterol (mg/dL) | 12 | 183 | 36 | 133 | 240 |
| Total Protein (g/dL) | 12 | 7.5 | 0.4 | 6.8 | 7.9 |
| Urea (mg/dL) | 12 | 32 | 7.8 | 21 | 46 |
| Uric Acid (mg/dL) | 12 | 3.8 | 0.7 | 2.6 | 5 |
| γ-Globulin (%) | 12 | 14 | 1.5 | 11.9 | 16.4 |
| γ-Gt (U/L) | 12 | 17 | 17 | 7 | 63 |

**Table 6**
Urinalysis at the screening on EP study population.

| Laboratory test | N | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|---|
| Bacteria | 12 | 59 | 180 | 2 | 631 |
| Epithelial Cells | 12 | 24 | 42 | 0 | 130 |
| Erythrocytes | 12 | 32 | 36 | 7 | 109 |
| Leukocytes | 12 | 8.2 | 14 | 1 | 50 |
| pH | 12 | 6.3 | 1.1 | 5 | 8 |
| Specific Gravity | 12 | 1 | 0 | 1.01 | 1.03 |
| Urobilinogen | 12 | 0.2 | 0 | 0.2 | 0.2 |

with at least a 24 months shelf life, containing a branched glucose polysaccharide named Polglumyt® that, due to its structural complexity, can guarantee a high and long-lasting energy supply.

This study showed that a single oral administration of a Polglumyt®-based drink is well tolerated, with no or minor TEAEs and a good palatability. Moreover, the IP did not affect the physiological glycemic profile (Fig. 2) in healthy volunteers and did not impact on the triglycerides profile after the digestion, 6 h after its intake. It should be underlined that the changes in insulin, glucose, and C-peptide levels we detected were expected from a physiological point of view, as they followed the intake of an IP containing both simple and complex carbohydrates (Table 2). Most importantly, from a clinical point of view, all the plasma/serum parameters returned to basal levels after IP digestion, 6 h after its intake (Fig. 2).

The main advantages of the formulation tested in the study are a low osmolarity (400 mOsm/L), and a high energy density (1.82 kcal/mL), obtained through the macro and micronutrients contained in the volume formulation of 200 mL, without an excessive amount of triglycerides (Table 2). Complex carbohydrates, once ingested, undergo enzymatic hydrolysis, provide a slow release of glucose and can have a wide range of applications when a controlled release of glucose is necessary. They can be used to deliver to a diabetic patient sufficient carbohydrates without a significant raise of the serum glucose level, or for subjects with a reduced glucose tolerance. Several marketed FSMPs include maltodextrins (MDs) as the main source of complex carbohydrates. In clinical nutrition, MDs have been applied, for example, in enteral and parenteral nutrition in combination with proteins for use as preoperative feeding instead of the conventional method of preoperative fasting in patients undergoing GI surgery [11], or in patients with liver cirrhosis, leading to an improvement of short-term clinical outcome [12]. MDs are partially hydrolyzed starch products and consist of D-glucose units linked primarily by α(1 → 4) bonds and containing branched segments linked by α(1 → 6) linkages. Differently from Polglumyt®, MDs with the same dextrose equivalency (DE) can even have differences in their molecular composition and, consequently, different properties [13]. Such differences can be related to the nature of the starch hydrolysis and to the processes used, which include acid hydrolysis, enzymatic hydrolysis or a combination of both methods, that can influence the composition and the properties of the final product [14]. A direct comparison

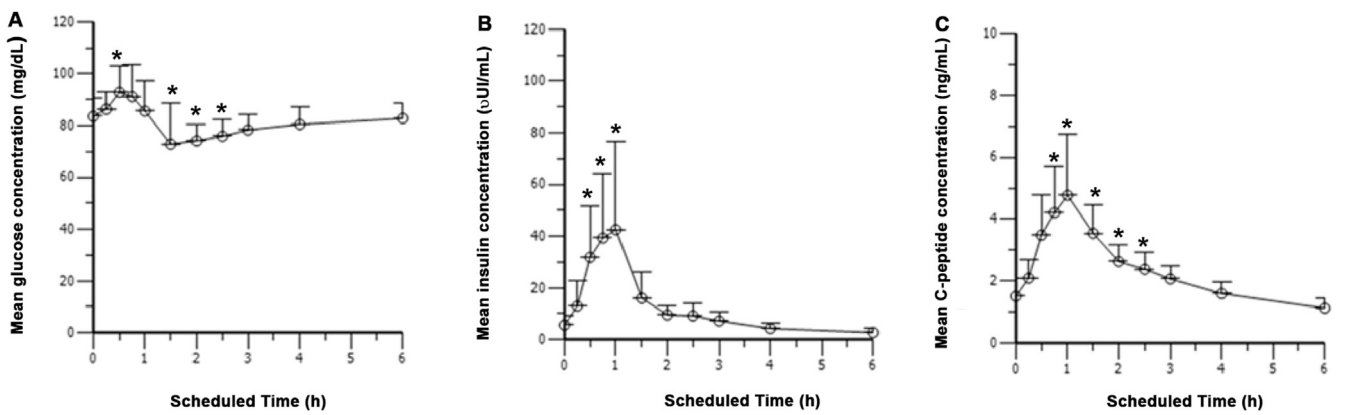


**Fig. 2.** **A**: Mean glucose plasma concentration after a single administration of the IP; **B**: Mean insulin serum concentration after a single administration of IP; **C**: Mean C-peptide serum concentration after a single administration of IP. Venous blood samples were collected at Visit 1, pre-dose (baseline), and then at Visit 1, post-dose, i.e. at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4 and 6 h after the IP intake. Results are expressed in mg/dL, μUI/mL and ng/mL for glucose, insulin and C-peptide, respectively. Data are reported as mean ± SD. Linear scale. *: $p < 0.05$ *vs.* pre-dose value.

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**Table 7**
Changes in triglycerides *vs.* baseline at Visit 1, post-dose (3 and 6 h after IP uptake).

| Change *vs.* baseline | N | Mean | SD | Median | Minimum | Maximum | CI 95% mean change |
|---|---|---|---|---|---|---|---|
| 3 h after IP uptake *vs.* baseline | 12 | 21.9 | 30.9 | 21 | −17 | 99 | (−4.8–48.3) |
| 6 h after IP uptake *vs.* baseline | 12 | −3.7 | 19.4 | −3 | −45 | 26 | (−22.2–14.5) |

**Table 8**
Questionnaire for evaluation of gastrointestinal symptoms at Visit 1, pre-dose, and at Visit 1, post-dose (3 and 6 h after IP uptake).

| Gastrointestinal symptoms Test | Visit 1, pre-dose | Visit 1, post-dose (3 h after IP uptake) | Visit 1, post-dose (6 h after IP uptake) |
|---|---|---|---|
| | 'No' n (%) | 'No' n (%) | 'No' n (%) |
| Do you feel a sense of early satiety? | 12 (100) | 12 (100) | 12 (100) |
| Do you feel bothersome nausea? | 12 (100) | 12 (100) | 12 (100) |
| Do you feel like vomiting? | 12 (100) | 12 (100) | 12 (100) |
| Do you have bowel movements changes? | 12 (100) | 12 (100) | 12 (100) |
| Do you have discomfort or pain anywhere in your abdomen? | 12 (100) | 12 (100) | 12 (100) |
| Do you have pain or burning in the middle of your abdomen? | 12 (100) | 12 (100) | 12 (100) |
| Do you often feel an uncomfortable fullness in your abdomen? | 12 (100) | 12 (100) | 12 (100) |
| Do you often have heartburn? | 12 (100) | 12 (100) | 12 (100) |
| Do you vomit? | 12 (100) | 12 (100) | 12 (100) |

performed in A.C.R.A.F. S.p.A. laboratories demonstrated an overlap of the kinetic profiles of plasma glucose in mice administered with Polglumyt® or with commercially available MDs (unpublished data). A similar controlled glucose release rate from Polglumyt® or MDs has been revealed also in an *in vitro* dynamic digestion test developed by A.C.R.A.F. S.p.A. based on the combination of different models reported in literature [5,15,16]. These data are consistent with the results from independent studies conducted in BALB/c mice administered with enzymatically synthetized glycogen, showing a slow elevation rate of plasma glucose and its maintenance for a prolonged time [17]. These results were interesting, considering the greater molecular weight and structural complexity of Polglumyt® *vs.* MDs. Moreover, Polglumyt® solutions have a remarkably lower osmolality, ranging between 1 and 7 mOsm/Kg, *vs.* the osmolality values of MD solutions, comprised between 100 and 350 mOsm/Kg, in the same range of concentrations [5]. The higher molecular weight of Polglumyt® *vs.* MDs is at the basis of the different osmolarity of their solutions. This observation implies a lower osmolarity of the IP *vs.* other FSMPs containing lower molecular weight polysaccharides for the same quantity of glucose ingested. Osmolality regulates the rate of gastric emptying and the rate of intestine adsorption and also influences the rate of adsorption/secretion of water in the small intestine. High osmolality has been identified as one of the causes of the diarrhea that frequently occurs in patients taking enteral nutrition [18]. In more detail, the higher osmolality of high-energy formulation can result in significant fluid shifts in the stomach and proximal small bowel. The resulting excessive intraluminal volume may accelerate small intestinal transit resulting in a greater fluid load on the large bowel and subsequent diarrhea, with concomitant loss of fluids and electrolytes [19]. Higher volumes administered, or high infusion rates, increase the risk of such AEs. The lower osmolality of Polglumyt® could help maintaining the water/saline balance reducing the phenomena of vomiting and/or diarrhea, an aspect which is of particular interest in inflammatory bowel diseases, thus encouraging the patient compliance, as supported by the GI tolerability data we reported (Table 8). Indeed the osmolarity of the IP tested in the present investigation (400 mOsm/L) is comparable or even lower than other available products [20]. Slowly digestible carbohydrates, providing a controlled release of glucose, are suitable for subjects affected by metabolic disorders such as diabetes, or with a reduced glucose tolerance [21].

The Ω-6 to Ω-3 ratio of fatty acids in the IP is 2.2:1. Researchers agree that the modern Western diet is imbalanced in favour of Ω-6 and that this imbalance may cause or facilitate different pathological conditions. A lower Ω-6/Ω-3 ratio could prevent the pathogenesis of many diseases induced by today's Western diets, a target of 1:1 to 2:1 appears to be consistent with studies on evolutionary aspects of diet, neurodevelopment, and genetics. A balanced ratio of Ω-6/Ω-3 fatty acids is important for health and in the prevention of cardiovascular disease and, possibly, other chronic diseases [22]. Several marketed products have a Ω-6/Ω-3 ratio ranging from 2:1 to 5:1. The Ω-6/Ω-3 2.2:1 ratio in the IP is consistent with the composition of similar products already on the market and may represent a good balance.

Of note, as anticipated above, the stability tests performed by A.C.R.A.F. S.p.A. revealed that the IP is chemically and microbiologically stable at least up to 24 months after its production (Table 3). This observation represents another potential advantage of the IP we tested in this study. FSMP sterility and stability represent a critical point, as these products cannot be sterilized through classical technologies (e.g. thermal sterilization or gamma irradiation). The microbiological stability of the IP tested in this study has been reached through the optimization of the production process, from a careful selection of single components to the choice of IP packaging.

## 5. Conclusions

The IP tested in this study showed a good GI tolerability and safety, and an acceptable palatability. Concerning the glycemic profile, the plateau phase of glucose and insulin observed in the kinetic curves confirmed that IP intake did not affect the physiological glycemic profile expected considering the IP composition. The IP intake did not impact on the triglycerides profile as well.

The high energy, low osmolality formulation we tested in this study could represent a beneficial nutritional support in a population of patients unable to assume an ordinary diet. Additional clinical investigations on the Polglumyt®-based FSMP are thus encouraged, including the evaluation of the outcomes and potential AEs of a longer term administration and the testing in specific categories of patients, in addition to healthy subjects.

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## Author contributions

The authors Annalisa Bonelli, Alessandro Comandini, Vincenzo Russo, Enrica Salvatori and Maurizio Muscaritoli, contributed to the conception/design of the research; Pierantonio Menna, Giorgio Minotti, Silvia Angeletti, Francesca Ferravante, Sara Emerenziani and Michele Cicala contributed to the execution of the research; Rossella Picollo and Elisa Quarchioni contributed to the analysis of the data. All the authors critically revised and approved the final manuscript and agreed to be fully accountable for ensuring the integrity and accuracy of the work.

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## Conflict of interest

MM has served as consultant for A.C.R.A.F. S.p.A.

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## References

[1] Burgos R, Joaquín C, Blay C, Vaqué C. Disease-related malnutrition in hospitalized chronic patients with complex needs. Nutrition 2019 Jun 12:30261–4. https://doi.org/10.1016/j.clnu.2019.06.006. pii: S0261-5614(19).

[2] Cederholm T, Barazzoni R, Austin P, Ballmer P, Biolo G, Bischoff SC, et al. ESPEN guidelines on definitions and terminology of clinical nutrition. Clin Nutr 2017 Feb;36(1):49–64. https://doi.org/10.1016/j.clnu.2016.09.004.

[3] Pradelli L, Mayer K, Klek S, Omar Alsaleh AJ, Clark RAC, Rosenthal MD, et al. ω-3 fatty-acid enriched parenteral nutrition in hospitalized patients: systematic review with meta-analysis and trial sequential analysis. JPEN - J Parenter Enter Nutr 2019 Jun 27. https://doi.org/10.1002/jpen.1672.

[4] Limketkai BN, Shah ND, Sheikh GN, Allen K. Classifying enteral nutrition: tailored for clinical practice. Curr Gastroenterol Rep 2019 Jul 31;21(9):47. https://doi.org/10.1007/s11894-019-0708-3.

[5] European Patent Office, "Food Formulation Comprising Glycogen,". International publication number WO2011015509 (A1). https://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=1&ND=3&adjacent=true&locale=en_EP&FT=D&date=20161028&CC=HU&NR=E027721T2&KC=T2. [Accessed 31 July 2019].

[6] 2010 Dietary Guidelines for Americans. https://health.gov/dietaryguidelines/2010/. [Accessed 6 August 2019].

[7] Adam JM, Tarigan NP. Comparison of the World Health Organization (WHO) two-step strategy and OGTT for diabetes mellitus screening. Acta Med Indones 2004 Jan-Mar;36(1):3–7.

[8] Mogensen KM, Malone A, Becker P, Cutrell S, Frank L, Gonzales K, et al. Malnutrition committee of the American society for parenteral and enteral nutrition (ASPEN). Academy of nutrition and dietetics/American society for parenteral and enteral nutrition consensus malnutrition characteristics: usability and association with outcomes. Nutr Clin Pract 2019 May 10. https://doi.org/10.1002/ncp.10310.

[9] Cederholm T, Jensen GL, Correia MITD, Gonzalez MC, Fukushima R, Higashiguchi T, et al., GLIM Core Leadership Committee, GLIM Working Group. GLIM criteria for the diagnosis of malnutrition - a consensus report from the global clinical nutrition community. Clin Nutr 2019 Feb;38(1):1–9. https://doi.org/10.1016/j.clnu.2018.08.002.

[10] Guenter P, Jensen G, Patel V, Miller S, Mogensen KM, Malone A, et al. Addressing disease-related malnutrition in hospitalized patients: a call for a national goal. Joint Comm J Qual Patient Saf 2015 Oct;41(10):469–73.

[11] Dock-Nascimento DB, Aguilar-Nascimento JE, Linetzky Waitzberg D. Ingestion of glutamine and maltodextrin two hours preoperatively improves insulin sensitivity after surgery: a randomized, double blind, controlled trial. Rev Col Bras Cir 2012 Dec;39(6):449–55.

[12] Cabre E, Gonzalez-Huix F, Abad-Lacruz A, Esteve M, Acero D, Fernandez-Bañares F, et al. Effect of total enteral nutrition on the short-term outcome of severely malnourished cirrhotics. A randomized controlled trial. Gastroenterology 1990 Mar;98(3):715–20.

[13] Chronakis IS. On the molecular characteristics, compositional properties, and structural-functional mechanisms of maltodextrins: a review. Crit Rev Food Sci Nutr 1998 Oct;38(7):599–637.

[14] Takeiti CY, Kieckbusch TG, Collares-Queiroz FP. Morphological and physicochemical characterization of commercial maltodextrins with different degrees of dextrose-equivalent. Int J Food Prop 2010;13(2):411–25. https://doi.org/10.1080/10942910802181024.

[15] Fässler C, Arrigoni E, Venema K, Brouns F, Amadò R. In vitro fermentability of differently digested resistant starch preparations. Mol Nutr Food Res 2006 Dec;50(12):1220–8. https://doi.org/10.1002/mnfr.200600106.

[16] Muir JG, O'Dea K. Measurement of resistant starch: factors affecting the amount of starch escaping digestion in vitro. Am J Clin Nutr 1992 Jul;56(1):123–7. https://doi.org/10.1093/ajcn/56.1.123.

[17] Inagaki K, Ishihara K, Ishida M, Watanabe A, Fujiwara M, Komatsu Y, et al. Rapid rehydration and moderate plasma glucose elevation by fluid containing enzymatically synthesized glycogen. J Nutr Sci Vitaminol (Tokyo) 2011;57(2):170–6.

[18] Barrett JS, Shepherd SJ, Gibson PR. Strategies to manage gastrointestinal symptoms complicating enteral feeding. JPEN - J Parenter Enter Nutr 2009 Jan-Feb;33(1):21–6. https://doi.org/10.1177/0148607108325073. Epub 2008 Nov 21.

[19] Bowling TE, Raimundo AH, Grimble GK, Silk DB. Colonic secretory effect in response to enteral feeding in humans. Gut 1994 Dec;35(12):1734–41.

[20] Zadak Z, Kent-Smith L. Basics in clinical nutrition: commercially prepared formulas. e-SPEN. Eur e-J Clin Nutr Metabol 2009;4:e212–5.

[21] Axelsen M, Arvidsson Lenner R, Lönnroth P, Smith U. Breakfast glycaemic response in patients with type 2 diabetes: effects of bedtime dietary carbohydrates. Eur J Clin Nutr 1999 Sep;53(9):706–10.

[22] Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med (Maywood). 2008 Jun;233(6):674–88. https://doi.org/10.3181/0711-MR-311.

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