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Original Article

Influence of an eicosapentaenoic and docosahexaenoic acid-enriched enteral nutrition formula on plasma fatty acid composition and biomarkers of insulin resistance in the elderly

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SUMMARY

Background & aims: *n*-3 Polyunsaturated fatty acids may improve cardiovascular outcomes in elderly. The aim of this study was to determine the effect of feeding elderly patients exclusively with an *n*-3 polyunsaturated fatty acid-enriched diet specifically designed for enteral nutrition for 6 months, evaluating modifications in plasma fatty acid profile and some biomarkers of insulin resistance (IR).

Methods: Thirty-two patients >65 years were fed a new enteral formula (T-Diet Plus®) containing 75 mg/l of eicosapentaenoic acid (EPA) and 35 mg/l of docosahexaenoic acid (DHA) and 33 were fed an enteral diet intended for elderly (Jevity®). Blood samples were drawn at the beginning and after 3 and 6 months of feeding. Plasma lipids, total plasma and lipid fraction fatty acid profiles, and some IR-associated adipokines were analysed.

Results: Feeding on T-Diet Plus® allowed EPA and DHA incorporation into plasma lipids and normalised blood triacylglycerols (TAG) levels after 3 months without major changes in IR, leptin and adiponectin.

Conclusions: Feeding the elderly exclusively with an enteral formula enriched with EPA and DHA improves their plasma lipid fatty acid profile and lowers TAG, a well known cardiovascular risk biomarker, without affecting IR.

1. Introduction

Diet is a useful therapeutic tool that may modulate health outcomes, improving a number of clinical and biochemical indicators of disease. Enteral nutrition (EN) formulae are used when pathological alterations impede normal oral feeding. Prescription of the appropriate formula may contribute to better outcomes. These products usually contain lipid mixtures derived from plant sources and provide a reliable source of energy and essential fatty acids.[1] However, various studies have demonstrated that enteral formulae may alter specific body responses when the balance between *n*-6 and *n*-3 polyunsaturated fatty acids (PUFA) is not appropriate.[2]

The relationship of dietary fatty acids with plasma lipid profile, lipoprotein metabolism and cardiovascular disease (CVD) is well documented.[3] *n*-3 Long-chain polyunsaturated fatty acids (LC-PUFA), namely eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3), can modulate the development and progression of atherosclerosis, including their effects on lowering plasma triacylglycerol (TAG) levels, blood pressure, inflammation, and cardiac excitability, and modulation of platelet function and the stability of atheroma plaques, although the molecular mechanisms are still not fully elucidated.[3,4] Specifically, the beneficial effect induced by dietary *n*-3 LC-PUFA is the reduction in plasma TAG by inhibition of their hepatic biosynthesis.[5,6] In addition, a higher ratio of *n*-6/*n*-3 PUFA leads to an increase in proinflammatory mediators derived from

Non-standard abbreviations: AA, arachidonic acid; CVD, cardiovascular disease; CE, cholesteryl esters; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EN, enteral nutrition; EPA, eicosapentaenoic acid; HDLc, high density lipoprotein cholesterol; HOMA, Homeostasis Model Assessment Index; IR, insulin resistance; LA, linoleic acid; LNA, linolenic acid; LC-PUFA, long-chain polyunsaturated fatty acids; LDLc, low density lipoprotein cholesterol; MS, metabolic syndrome; PL, phospholipids; QUICKI, Quantitative Insulin-Sensitivity Check Index; TEN, total enteral nutrition; TAG, triacylglycerols; MAC, mid-arm circumference; TS, tricipital skinfold.

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arachidonic acid (AA, 20:4*n*-6), while anti-inflammatory mediators are derived from EPA.[7]

Aging is generally characterised by changes in physiological processes, including a reduced ability to respond to stress, increased homeostatic imbalance and higher risk of disease, particularly CDV[8] and this pathology is recognised to be the leading cause of death in the world.[9] Besides genetic predisposition, environmental factors such as central or abdominal obesity, a sedentary life style, fat- and carbohydrate-rich hypercaloric diets and smoking favour the development of CVD.[10] CVD is influenced by lipid alterations, such as hypertriacylglyceridaemia, presence of small type B low density lipoprotein cholesterol (LDLc), decrease in high density lipoprotein cholesterol (HDLc) and apolipoprotein A-I (apoA-I) and higher postprandial lipaemia.[11] Furthermore, all of the conditions associated with postprandial lipid abnormalities are also characterised by insulin resistance (IR) and are known features of the entity designated as metabolic syndrome (MS).[12]

Metabolic alterations and physiological changes in digestive processes during aging may lead to situations of malnutrition that can accelerate the progression of underlying diseases.[13] EN is especially relevant for patients in whom a good nutritional status may help to prevent health impairment. Given the proven beneficial effects of *n*-3 PUFA in CVD, it may be of interest to include these functional ingredients into new EN for the elderly to improve their nutritional status and cardiovascular risk factors. The aim of this study was to determine the effect on cardiovascular risk factors in elderly patients of an exclusive 180-day EN diet that contains EPA and DHA in its fatty composition, evaluating changes in plasma fatty acid and lipid profile and in some biomarkers of IR.

2. Material and methods

2.1. Materials and reagents

EDTA-coated tubes were purchased from BD Vacutainer, Plymouth, UK. Fatty acid standards, butylated hydroxytoluene (BHT), acetyl chloride were from Sigma–Aldrich Química S.A., Spain. Hexane, isopropanol, methyl-tert-butyl-ether, acetic acid, methanol, benzene, potassium carbonate were from Panreac Química S.A., Spain. Aminopropyl columns for lipid separation were SepPak Cartridges from Waters, Milford, MA, USA.

2.2. Study subjects

Sixty-five patients (mean age 75 years) from the Clinical Nutrition Outpatient Unit of Virgen de las Nieves University Hospital (Granada) were invited to participate in the study. Inclusion criteria were age >65 years and requirement for total enteral nutrition (TEN) for ≥6 months. Exclusion criteria were unstable clinical situation, fatal illness, refusal to participate in the study or enrolment in another clinical trial. All participants signed their informed consent to participate in the study, which was approved by the Ethics Committee of the University Hospital Virgen de las Nieves from Granada. All procedures complied with institutional guidelines, following IHC Harmonised Tripartite Guidelines for Good Clinical Practice in accordance to *Helsinki Declaration of the World Medical Association. Ethical principles for medical research on human beings* (Revised in Edinburgh, October 2000). Baseline characteristics of the patients are shown in Table 1.

2.3. Study design

A randomised, experimental, prospective, intention-to-treat comparative 6-month study was performed in two parallel groups fed solely by TEN. Patients were randomly divided into two groups (experimental and reference). The experimental group (experimental) ($n = 32$) was fed a novel normoproteic and isocaloric enteral formula (T-Diet Plus® from Vegenat S.A., Spain) containing 75 mg/l of EPA and 35 mg/l of DHA. The reference group (reference) ($n = 33$) was fed a standard normoproteic and isocaloric enteral diet intended for nutrition in adults, including the elderly (Jevity®, from Abbot Laboratories, USA). At the end of the experimental period, only 17 out of the 32 patients enrolled in the experimental group and 20 out of the 33 patients in the reference group remained in the study. In the experimental and reference groups, six and eight patients, respectively, withdrew, four and three died; three and two changed their diet and two experimental group patients no longer required EN.

Patients received 1500 ml of the diet, guaranteeing their daily energetic and nutritional requirements. The overall mean daily intake for T-Diet Plus group was 1266 ± 60 ml/d (mean ± SEM) and for reference group 1362 ± 50 ml/d, with no difference between groups, implying a mean EPA and DHA intake of around 94 and 44 mg/d, respectively, for those receiving the T-Diet Plus. Both products were administered as a bolus, using a nasogastric tube with a large-bore syringe. All patients received 1000–1200 ml of water daily to maintain optimal hydration status. Table 2 shows the

Table 1
Baseline characteristic of the patients receiving T-Diet Plus® and Jevity®.

		T-Diet Plus®	Jevity®
Age (years)		70.1	78.6
Sex	Female	25	27
	Male	7	6
Tricipital skinfold (mm)		17.6	17.5
Mid-arm circumference (cm)		24.3	25.0
Concomitant diseases (%)			
Cognitive deficits and Alzheimer disease		52.9	55.0
Cerebrovascular diseases and cardiovascular events		41.2	45.0
Other causes (cancer. accidents)		17.6	15
Medication (%)			
Gastric protectors		82.3	70.0
Psychodrugs		40.0	41.2
Anticoagulants		41.2	40.0
Antihypertension medication		25.0	29.4
Diuretics		30.0	23.5
Analgesics		20.0	23.5
Antiarrhythmics		15.0	11.8
Antidiabetics		5.0	10.0

Table 2
Nutritional composition of experimental and reference diets.

	Experimental[a]	Reference[b]
Energy (kcal/dl)	100	103
Proteins (g/dl)	4.0	4.0
Carbohydrates (g/dl)	12.3	14.05
Fat (g/dl)	3.9	3.47
Saturated (g/dl)	0.93	0.70
Monounsaturated (g/dl)	2.02	2.00
Essential fatty acids	0.86	0.76
EPA	75	–
DHA	35	–
EPA/DHA	2.14	–
n-6:*n*-3 ratio	6.3:1	10:1
Fibre (g/l)	1.7	1.44
Total minerals (g/dl)	0.70	0.57

Both products contain a vitamin complex to provide 100% of vitamin reference intake for elderly, assuming a daily intake of 1500 kcal (6276 kJ).
[a] T-Diet Plus® (Vegenat S.A.).
[b] Jevity® (Abbot Laboratories).

nutritional composition of the experimental and reference diets and Table 3 shows their fatty acid composition.

2.4. Anthropometric measurements

Mid-arm circumference (MAC) and tricipital skinfold (TS) were measured with a flexible measure tape and a calliper, respectively. The MAC (cm) was taken mid-way between the tip of the acromion and olecranon process. The TS (mm) was taken on the back of the arm and halfway between the point of the acromion and olecranon process. We did not attempt to measure the body mass index (BMI) as the length is usually distortioned in many elderly subjects, which in turn most of them were at bed.

2.5. Blood samples

Blood samples were drawn after 8–10 h overnight fasting at time 0 (baseline) and after 3 and 6 months of TEN. Serum (without anticoagulant) and plasma (EDTA-coated tubes) were separated by centrifugation (15 min at 1750 g).

2.6. Isolation of plasma lipids and separation of lipid fractions

After mixing 0.5 ml of plasma with 0.5 ml of water, lipids were extracted with 4 ml of hexane:isopropanol (3:2 v/v) containing 25 mg/l of BHT. The organic layer was evaporated to dryness under vacuum and the isolated lipids were dissolved into 200 μl of hexane:methyl-tert-butyl-ether:acetic acid (100:3:0.3). TAG, phospholipids (PL) and cholesteryl ester (CE) fractions were separated by using aminopropyl columns (SepPak Cartridges; Waters, Milford, MA, USA), as described elsewhere.[14] Fractions obtained were evaporated to dryness under vacuum and dissolved in 100 μl hexane. This method yields 96–100% recovery of lipid fractions.

2.7. Quantification of fatty acids in total plasma lipids and plasma lipid fractions

Fatty acids from total plasma and different hexane fractions were identified and quantified after methylation by gas–liquid chromatography using a 60-m long capillary column (32 mm internal diameter and 20 mm film thickness) impregnated with SP 2330 FS (Supelco, Bellefonte, CA, USA). Fatty acid methyl esters from plasma lipids were obtained as previously reported by Lepage and Roy.[15] Briefly, the hexane extracts of total plasma and lipid fractions were dissolved into 2 ml methanol:benzene (4:1 v/v). Methylation was carried out at 100 °C for 1 h by adding 200 μl acetyl chloride. After cooling, 5 ml of 0.43 M K_2CO_3 was added to stop the reaction and neutralise the mixture. Tubes were then shaken and centrifuged and the benzene upper phase was dried under N_2 and re-suspended with 100 μl hexane.

Table 3
Fatty acid analysis of experimental and reference diets.

Fatty acids	Experimental[a]	Reference[b]	*P* value
Caprylic (8:0)	2.04 ± 0.12	2.21 ± 0.63	0.833
Capric (10:0)*	3.25 ± 0.35	6.28 ± 0.29	0.004
Lauric (12:0)*	0.10 ± 0.01	0.05 ± 0.01	0.010
Myristic (14:0)*	0.47 ± 0.03	0.13 ± 0.00	0.001
Palmitic (16:0)*	12.90 ± 0.04	5.40 ± 0.20	0.013
Palmitoleic (16:1*n*-9)*	0.15 ± 0.01	0.00 ± 0.00	<0.001
Stearic (18:0)*	2.96 ± 0.03	5.12 ± 0.07	<0.001
Oleic (18:1*n*-9)	50.86 ± 0.15	54.50 ± 0.78	0.124
Linoleic (18:2*n*-6)*	23.17 ± 0.09	21.59 ± 0.14	0.001
Arachidic (20:0)*	0.36 ± 0.02	0.48 ± 0.03	0.037
c-11-Eicosenoic (21:1)*	1.68 ± 0.05	3.02 ± 0.16	<0.001
α-Linolenic (18:3*n*-3)	0.34 ± 0.02	0.41 ± 0.05	0.178
c-8,11,14-Eicosatrienoic (20:3*n*-6)*	0.00 ± 0.00	0.21 ± 0.09	0.021
Behenic (22:0)	1.28 ± 0.08	0.45 ± 0.00	0.003
Eicosapentaenoic (20:5*n*-3)*	0.19 ± 0.01	0.00 ± 0.00	0.001
Lignoceric (24:0)	0.17 ± 0.01	0.17 ± 0.01	0.820
Docosahexaenoic (22:6*n*-3)*	0.09 ± 0.01	0.00 ± 0.00	0.001

Values are expressed as percentages (mean ± SEM) of total fatty acids, measured in triplicate. *Mean values were significantly different from those of the reference group ($P \leq 0.05$).
[a] T-Diet Plus® (Vegenat S.A.).
[b] Jevity® (Abbot Laboratories).

2.8. Biochemical parameters and hormones

Plasma TAG, total cholesterol (TC) apoB, HDLc, LDLc and apoA-I, glucose and uric acid concentrations were determined by standardised espectrophotometric techniques using a Roche Hitachi Modular DDP clinical analyser system (Roche Diagnostics España, S.L., Barcelona). Fasting insulin and C peptide were analysed by standardised electrochemiluminescence techniques using an E-170 Elecsys Modular Analytics system (Roche Diagnostics España, S.L., Barcelona). All parameters were analysed at the laboratory of the Virgen de las Nieves University Hospital (Granada).

The Homeostasis Model Assessment Index (HOMA) of IR was calculated, following the Matthews formula, as the product of fasting plasma glucose (mmol/l) and insulin (mU/l) concentrations divided by 22.5.[16] The Quantitative Insulin-Sensitivity Check Index (QUICKI) was derived using the inverse of the sum of the logarithms of the fasting insulin (μU/ml) and fasting glucose (mg/dl). This index provides a solid and reproducible estimation of peripheral tissue insulin sensitivity and is negatively related to the body mass index.[17]

Leptin, adiponectin and resistin were analysed by using LINCO*plex*™ kits with a Luminex 200 System built on xMAP technology.[18]

2.9. Statistical analysis

All data are presented as mean values with standard error of the means (SEM). Before any statistical analysis, all variables were checked for normality and homogeneous variance by using the Kolmogorov–Smirnov and the Levene tests, respectively. When a variable did not follow normality, log transformations were performed. An unpaired Student *t* test was performed to assess differences between EN products for their fatty acids composition.

The general linear model of the variance for repeated measures (GLMRM) was performed to assess differences among times and between groups as well as interaction of groups per time. We performed GLMRM analysis using the *n* of subjects who completed the study ($n = 17$ for T-diet Plus and $n = 20$ for Jevity). When Maunchy's test indicated that the assumption of sphericity was violated, the Greenhouse–Geisser test was used to correct it for univariate analysis. When the Greenhouse–Geisser correction was lower than 0.5 we used MANOVA multivariate test statistics as they are not dependent upon the assumption of sphericity.

For variables that were non-normally distributed, differences between groups were carrying out by the Friedman test. A *post hoc* comparison using the Wilcoxon test was used to analyse specific differences among times. A value of $P \leq 0.05$ was considered significant. Data were analysed by using a statistical software package (SPSS for Windows, 15.0, 2005, SPSS Inc. Chicago, IL, USA).

3. Results

At baseline, no significant differences between patients receiving T-Diet Plus® and Jevity® were found for age, sex, anthropometric characteristics, concomitant diseases and medication, and the average number of drugs used in both groups were

Table 4
Percentages of main fatty acids in total plasma of elderly patients fed exclusively by total enteral nutrition with the experimental (T-Diet Plus®) and reference (Jevity®) diets for 6 months.

Fatty acids (%)	Experimental ($n = 17$)			Reference ($n = 20$)			*P* value		
							Source of variation		
	Baseline	3 Months	6 Months	Baseline	3 Months	6 Months	Group (G)	Time (T)	Interaction G × T
Palmitic (16:0)	21.7 ± 0.7	21.2 ± 0.5	20.7 ± 0.5	21.2 ± 0.7	21.8 ± 0.5	21.4 ± 0.5	0.148	0.425	0.212
Stearic (18:0)	6.05 ± 0.23	6.20 ± 0.20	6.23 ± 0.19	6.15 ± 0.22	6.35 ± 0.20	6.45 ± 0.18	0.493	0.138	0.889
Oleic (18:1*n*-9)	32.9 ± 1.3[a]	29.9 ± 0.9[b]	29.0 ± 0.9[b]	31.6 ± 1.3	30.4 ± 0.8	30.1 ± 0.8	0.004	<0.001	0.110
Linoleic (18:2*n*-6)	21.9 ± 1.1[a]	25.4 ± 0.8[b]	25.8 ± 0.6[b]	22.33 ± 1.0	22.6 ± 0.7	22.2 ± 0.6	0.054	0.001	0.001
α-Linolenic (18:3*n*-3)	0.18 ± 0.02	0.17 ± 0.01	0.19 ± 0.01	0.18 ± 0.02[ab]	0.17 ± 0.01[a]	0.22 ± 0.01[b]	0.232	0.021	0.224
Arachidonic (20:4*n*-6)	6.68 ± 0.48	6.81 ± 0.50	6.89 ± 0.46	7.70 ± 0.45	7.82 ± 0.47	7.78 ± 0.45	0.110	0.817	0.960
Eicosapentaenoic (20:5*n*-3)	0.45 ± 0.07[a]	0.58 ± 0.05[b]	0.66 ± 0.05[c]	0.67 ± 0.06	0.58 ± 0.05	0.61 ± 0.05	0.344	0.027	0.023
Docosapentaenoic (22:5*n*-3)	0.41 ± 0.04[a]	0.41 ± 0.03[a]	0.55 ± 0.03[b]	0.44 ± 0.04	0.40 ± 0.03	0.48 ± 0.03	0.642	<0.001	0.223
Docosahexaenoic (22:6*n*-3)	1.56 ± 0.14	1.64 ± 0.10	1.74 ± 0.09	1.58 ± 0.13	1.34 ± 0.10	1.36 ± 0.08	0.103	0.440	0.013

Data are expressed as mean ± SEM. For each group, mean values within the same row with unlike superscript letters were significantly different for time ($P < 0.05$).

close to five (Table 1). In addition, MAC and TS did not change significantly over the intervention period in any of the groups.

Haematological and biochemical parameters were measured in all participants at baseline and after 90 and 180 days. No changes in white blood cells, coagulation indicators or plasma electrolytes were observed (data not shown).

3.1. Total plasma and plasma lipid fraction fatty acid profiles

Fatty acid determinations were performed to evaluate the incorporation of dietary essential and *n*-3 LC-PUFA into total plasma lipids and lipid fractions. Intake of the experimental diet increased total plasma percentages of EPA at 3 and 6 months, docosapentaenoic acid (DPA, 20:6*n*-3) from 3 to 6 months and linoleic acid (LA, 18:2*n*-6) at 3 months and, reduced the percentage of oleic acid at 3 months. In patients receiving the reference diet, all plasma fatty acids remained unchanged at 3 and 6 months (Table 4). LA, EPA and DHA exhibited different pattern behaviours in the experimental compared with the reference group, as assessed by the interaction of group × time. In fact, while LA and EPA increased in the experimental group, in the reference group did not change over the time.

Likewise, feeding with the experimental diet increased EPA and LA and reduced oleic acid in the plasma PL fraction at 3 months. The reference diet leaded to diminished α-linolenic acid (LNA, 18:3*n*-3), at 6 months. In that fraction, the experimental group showed higher percentage of LA and lower percentage of AA *versus* the reference group at all time points (Table 5). The interaction of

Table 5
Percentages of main fatty acids in plasma phospholipids, cholesteryl ester and triacylglycerols of elderly patients fed exclusively by total enteral nutrition with the experimental (T-Diet Plus®) and reference (Jevity®) diets for 6 months.

Fatty acids (%)	Experimental ($n = 17$)			Reference ($n = 20$)			*P* value		
							Source of variation		
	Baseline	3 Months	6 Months	Baseline	3 Months	6 Months	Group (G)	Time (T)	Interaction G × T
Phospholipids									
Palmitic (16:0)	27.2 ± 0.6	27.4 ± 0.5	27.0 ± 0.5	27.5 ± 0.5	27.3 ± 0.5	27.6 ± 0.4	0.678	0.917	0.563
Stearic (18:0)	13.5 ± 05	13.6 ± 0.4	13.5 ± 0.5	13.6 ± 0.5	14.0 ± 0.4	14.1 ± 0.5	0.535	0.559	0.576
Oleic (18:1*n*-9)	15.7 ± 0.9[a]	13.6 ± 0.5[b]	14.0 ± 0.5[b]	15.4 ± 0.8	14.4 ± 0.5	14.5 ± 0.5	0.720	0.001	0.269
Linoleic (18:2*n*-6)	16.6 ± 0.7[a]	18.2 ± 0.5[b]	18.1 ± 0.4[b]	15.4 ± 0.6	15.6 ± 0.5	15.9 ± 0.4	0.004	0.016	0.164
α-Linolenic (18:3*n*-3)	0.24 ± 0.02	0.24 ± 0.02	0.23 ± 0.01	0.30 ± 0.02[a]	0.25 ± 0.02[ab]	0.23 ± 0.02[b]	0.326	0.030	0.102
Arachidonic (20:4*n*-6)	10.5 ± 0.6	10.2 ± 0.6	10.2 ± 0.6	11.8 ± 0.6	11.8 ± 0.6	12.3 ± 0.5	0.039	0.634	0.213
Eicosapentaenoic (20:5*n*-3)	0.68 ± 0.07[a]	0.81 ± 0.06[b]	0.92 ± 0.06[b]	0.77 ± 0.07	0.86 ± 0.06	0.84 ± 0.07	0.807	0.001	0.080
Docosapentaenoic (22:5*n*-3)	0.70 ± 0.05	0.68 ± 0.05	0.78 ± 0.05	0.77 ± 0.05	0.77 ± 0.05	0.71 ± 0.05	0.661	0.799	0.018
Docosahexaenoic (22:6*n*-3)	3.51 ± 0.28	3.96 ± 0.24	3.94 ± 0.22	3.52 ± 0.26	3.22 ± 0.23	3.15 ± 0.20	0.104	0.795	0.013
Cholesteryl ester									
Palmitic (16:0)	12.2 ± 0.4[a]	10.9 ± 0.3[ab]	10.9 ± 0.3[b]	11.5 ± 0.4	11.1 ± 0.3	11.5 ± 0.3	0.900	0.007	0.045
Stearic (18:0)	0.78 ± 0.04	0.71 ± 0.05	0.62 ± 0.04	0.68 ± 0.04	0.78 ± 0.05	0.73 ± 0.04	0.562	0.144	0.020
Oleic (18:1*n*-9)	29.3 ± 1.4[a]	23.5 ± 0.7[b]	25.1 ± 0.9[b]	26.8 ± 1.4	24.3 ± 0.7	25.8 ± 0.9	0.772	<0.001	0.045
Linoleic (18:2*n*-6)	39.4 ± 1.7[a]	46.7 ± 0.9[b]	46.4 ± 0.9[b]	41.2 ± 1.7	42.6 ± 0.9	42.7 ± 0.9	0.145	<0.001	0.008
Arachidonic (20:4*n*-6)	7.62 ± 0.59	7.86 ± 0.63	7.54 ± 0.60	9.18 ± 0.59	9.38 ± 0.63	9.83 ± 0.60	0.022	0.689	0.493
Eicosapentaenoic (20:5*n*-3)	0.74 ± 0.07[a]	0.95 ± 0.08[b]	1.00 ± 0.08[b]	0.97 ± 0.07	1.07 ± 0.08	0.95 ± 0.08	0.234	0.030	0.079
Docosapentaenoic (22:5*n*-3)	0.83 ± 0.07[a]	0.55 ± 0.06[b]	0.50 ± 0.05[b]	0.65 ± 0.07	0.66 ± 0.06	0.77 ± 0.05	0.249	0.039	<0.001
Docosahexaenoic (22:6*n*-3)	0.64 ± 0.06	0.83 ± 0.06	0.75 ± 0.04	0.68 ± 0.06	0.66 ± 0.06	0.60 ± 0.04	0.074	0.137	0.069
Triacylglycerol									
Palmitic (16:0)	21.2 ± 1.2	19.7 ± 0.7	20.1 ± 0.8	21.8 ± 1.2	21.5 ± 0.7	21.8 ± 0.8	0.204	0.253	0.661
Stearic (18:0)	2.09 ± 0.16[a]	2.27 ± 0.15[ab]	2.64 ± 0.15[b]	2.22 ± 0.16[a]	2.77 ± 0.16[b]	2.76 ± 0.15[b]	0.161	<0.001	0.180
Oleic (18:1*n*-9)	49.9 ± 2.3	51.07 ± 1.1	50.8 ± 0.9	50.8 ± 2.3	49.1 ± 1.1	49.5 ± 0.9	0.760	0.824	0.233
Linoleic (18:2*n*-6)	16.7 ± 2.2	17.2 ± 0.9	17.8 ± 0.7	14.2 ± 2.2	14.8 ± 0.9	15.1 ± 0.7	0.114	0.496	0.950
α-Linolenic (18:3*n*-3)	0.34 ± 0.04	0.29 ± 0.03	0.31 ± 0.03	0.32 ± 0.04	0.38 ± 0.03	0.31 ± 0.03	0.441	0.624	0.164
Arachidonic (20:4*n*-6)	1.28 ± 0.14	1.40 ± 0.16	1.53 ± 0.18	1.53 ± 0.14	1.80 ± 0.16	2.00 ± 0.18	0.069	0.002	0.529
Eicosapentaenoic (20:5*n*-3)	0.26 ± 0.04	0.27 ± 0.04	0.30 ± 0.03	0.26 ± 0.04	0.34 ± 0.04	0.29 ± 0.03	0.640	0.207	0.297
Docosapentaenoic (22:5*n*-3)	0.30 ± 0.05	0.33 ± 0.04	0.39 ± 0.03	0.48 ± 0.05	0.47 ± 0.04	0.39 ± 0.03	0.023	0.952	0.013
Docosahexaenoic (22:6*n*-3)	0.46 ± 0.07	0.53 ± 0.07	0.54 ± 0.05	0.52 ± 0.07	0.60 ± 0.07	0.51 ± 0.05	0.615	0.277	0.507

Data are expressed as mean ± SEM. For each group, mean values within the same row with unlike superscript letters were significantly different for time ($P < 0.05$).

group × time showed that DPA and DHA had different patterns between groups.

In the plasma CE fraction of the experimental group, oleic acid and DPA decreased at 3 months while palmitic acid decreased at 6 months. Conversely, LA and EPA increased at 3 months. In this lipid fraction, the experimental group showed lower AA *versus* the reference group at all time points (Table 5). The interaction of group × time exhibited different patterns for the groups; while in the experimental group palmitic, oleic and docosapentaenoic acids decreased, in the reference group, those fatty acids remained unchanged. On the other hand, LA increased in the experimental group and remained unchanged in the reference.

In plasma TAG, stearic acid increased in the experimental and reference groups, at 3 and 6 months, respectively. In this fraction, DPA showed a different pattern between groups (Table 5).

3.2. Plasma biochemical parameters and hormones

The mean concentration of TAG in the experimental group decreased by 3 months and remained unchanged later on. However, in the reference diet TAG levels did not change over the intervention period. The interaction of group × time showed that the pattern behaviour for TAG differ significantly, decreasing only in the experimental group.

ApoA1 decreased over time when both groups were taken together, but no one showed differences individually. LDLc levels exhibited a differential pattern between groups. TC, apoB and HDLc levels did not change over time and were not different between groups (Table 6).

Glucose, insulin and C peptide levels remained within normal ranges during the study period in both groups. No significant differences between groups or among time points were found in HOMA index values, as an indicator of IR. The QUICKI index, a quantitative indicator of tissue insulin sensitivity, was higher in the experimental than in the reference group but did not significantly change over time in either group (Table 6). Plasma leptin and adiponectin levels did not change after 6 months with any diet. However, resistin concentration was different between groups (Table 6). Uric acid decreased over time when both groups were taken together, but individually, no one showed significant differences.

4. Discussion

Enteral formulas must supply patients with adequate amounts of all essential nutrients, including LC-PUFA. The main findings of the present study were that feeding elderly patients a new EPA- and DHA-enriched EN formula (T-Diet Plus®) allows incorporation of *n*-3 PUFA into total plasma lipids, specifically EPA in PL and CE. Moreover, elevated TAG levels in the experimental group at baseline were normalised at 3 months and remained unchanged until the end of the study. These data represent improvements in some cardiovascular risk factors and indicate the suitability of incorporating EPA and DHA for nutritional treatment of the elderly.

The present results evidence the incorporation of dietary *n*-3 LC-PUFA into plasma lipids, as previously reported in other studies. Garg *et al.*[19] observed significantly higher plasma levels of EPA, DPA and DHA after 6 weeks of oral supplementation with a fish oil concentrate in comparison with a placebo group. Other authors reported that, although supplementation with *n*-3 LC-PUFA modified the fatty acid composition of plasma PL, the effects of EPA differed notably from those of DHA.[20] Enteral feeding trials also demonstrated the incorporation of fatty acids into plasma and cell membranes.[21] Moreover, the receipt by patients of a parenteral lipid emulsion enriched with *n*-3 fatty acids from fish oil after major abdominal surgery increased their plasma levels of EPA,[22] opening up a new therapeutic approach to moderate inflammatory processes and responses to stress in acutely ill patients.

Dietary fatty acids are introduced into plasma as part of the TAG-rich lipoproteins PL and CE, permitting their incorporation into peripheral tissue cell membranes, where they may influence structure and functionality. It is well known that *n*-3 LC-PUFA may exert beneficial health effects, mainly in inflammatory-based diseases such as CVD.[5] The presence of EPA and DHA in cell membranes leads to an increase in EPA-derived anti-inflammatory molecules accompanied by a decrease in AA-derived inflammatory cytokines.[3] This effect derives from the higher affinity of enzymes for EPA rather than AA. Eicosanoids from AA may produce excessive acute stress responses, causing immunosuppression, platelet aggregation and excessive or chronic inflammation. In contrast, *n*-3 PUFA act as precursors of complementary derivatives that counteract the elevated responses of AA-derived eicosanoids.[3] It was recently shown that *n*-3 LC-PUFA are the precursors of resolvins, lipoxins and protectins, which are powerful anti-inflammatory

Table 6
Biochemical parameters and hormones of elderly patients fed exclusively by total enteral nutrition with the experimental (T-Diet Plus®) and reference (Jevity®) diets for 6 months.

(%)	Experimental ($n = 17$)			Reference ($n = 20$)			*P* value		
							Source of variation		
	Baseline	3 Months	6 Months	Baseline	3 Months	6 Months	Group (G)	Time (T)	Interaction G × T
Triacylglycerols (mg/dl)	185.5 ± 24.2[a]	132.6 ± 16.7[b]	124.8 ± 15.9[b]	134.8 ± 23.3	120.7 ± 16.1	122.7 ± 13.5	0.307	0.002	0.043
Cholesterol (mg/dl)	173.2 ± 8.3	168.8 ± 7.8	176.7 ± 7.8	170.4 ± 7.9	154.6 ± 7.4	155.4 ± 9.1	0.236	0.093	0.130
LDLc (mg/dl)	96.0 ± 6.6	98.1 ± 7.0	103.4 ± 8.0	100.0 ± 6.2	90.4 ± 6.6	88.3 ± 7.5	0.495	0.551	0.026
ApoB (mg/dl)	75.2 ± 4.3	84.7 ± 5.5	81.7 ± 5.7	74.5 ± 4.3	73.0 ± 5.3	71.6 ± 5.3	0.271	0.262	0.057
HDLc (mg/dl)	48.8 ± 2.4	49.9 ± 2.8	50.8 ± 3.1	46.9 ± 2.3	46.8 ± 2.6	43.7 ± 3.0	0.227	0.766	0.222
ApoA-I (mg/dl)	132.7 ± 4.7	127.2 ± 5.2	127.0 ± 4.6	131.3 ± 4.6	129.8 ± 5.1	123.4 ± 4.4	0.939	0.042	0.636
Glucose (mg/dl)	85.9 ± 5.4	88.5 ± 5.6	82.3 ± 3.4	87.1 ± 5.1	84.6 ± 5.2	84.9 ± 3.2	0.994	0.159	0.459
Insulin (U/ml)	3.90 ± 0.95	5.77 ± 1.86	4.55 ± 0.80	5.67 ± 0.90	9.32 ± 1.76	6.49 ± 0.75	0.057	0.084	0.784
C peptide (ng/ml)	2.86 ± 0.41	2.86 ± 0.32	2.25 ± 0.29	2.61 ± 0.39	2.71 ± 0.30	2.65 ± 0.25	0.997	0.140	0.160
HOMA	0.97 ± 0.31	1.42 ± 0.48	0.94 ± 0.18	1.28 ± 0.29	2.03 ± 0.45	1.40 ± 0.18	0.189	0.089	0.831
QUICKI	0.43 ± 0.01	0.40 ± 0.01	0.43 ± 0.02	0.39 ± 0.01	0.37 ± 0.01	0.38 ± 0.02	0.026	0.137	0.784
Leptin (ng/ml)	17.9 ± 4.5	20.7 ± 6.1	24.4 ± 7.0	17.1 ± 4.8	33.9 ± 6.5	29.4 ± 8.4	0.462	0.050	0.265
Adiponectin (μg/ml)	18.4 ± 1.4	19.1 ± 1.6	20.1 ± 2.5	20.7 ± 1.3	20.3 ± 1.42	21.0 ± 2.3	0.567	0.848	0.713
Resistin (ng/ml)	18.6 ± 3.0	22.3 ± 4.7	17.6 ± 3.2	18.8 ± 2.9	18.8 ± 4.4	21.7 ± 3.0	0.003	0.558	0.125
Uric acid (mg/dl)	4.91 ± 0.66	4.21 ± 0.46	3.78 ± 0.40	4.54 ± 0.62	3.92 ± 0.45	4.11 ± 0.40	0.870	0.009	0.293

HOMA, Homeostasis Model Assessment Index; QUICKI, Quantitative Insulin-Sensitivity Check Index. For each group, mean values within the same row with unlike superscript letters were significantly different for time ($P < 0.05$).

agents.[23] Hence, the balance between AA and EPA determines the type and biological efficacy of eicosanoids, thereby influencing thrombotic processes and immune and inflammatory responses.[5]

Depressed levels of tissue *n*-3 LC-PUFA, especially DHA, represent a consistent marker of increased CVD risk. Clinical trials have shown certain beneficial effects of fish oil intake in inflammatory-based diseases,[24] MS[4] and conditions with enhanced platelet aggregation, which is a major contributor to myocardial infarction.[25] The immune function is also improved by dietary *n*-3 LC-PUFA administration, although each fatty acid may have different effects.[20]

In the present study, an exclusive diet of an *n*-3 LC-PUFA-enriched enteral formula normalised plasma TAG levels. In humans, *n*-3 PUFA exert a consistent and dose-dependent hypotriacylglycerolaemic effect[6] in both normolipidaemic and hyperlipidaemic subjects.[26] A number of studies have associated the consumption of *n*-3 fatty acids with a reduced hepatic production of very low density lipoprotein (VLDL) and increased VLDL clearance rates.[6] The regulation of TAG is a complex process that involves several mechanisms in which EPA and DHA exert different hypolipidemic effects.[7] There are three possible mechanisms by which *n*-3 LC-PUFA may reduce TAG synthesis. First, by reducing substrate availability, which in turn may be due to an increase in β-oxidation, a decrease in the delivery of free fatty acids to the liver or a decrease in hepatic lipogenesis; second, by decreasing the activity of TAG-synthesising enzymes, such as diacylglycerol acyltransferase or phosphatidic acid phosphohydrolase; and third, by increasing PL synthesis.[6] It has also been reported that *n*-3 LC-PUFA influence the gene expression of enzymes involved in lipid metabolism,[27] e.g., diacylglycerol acyltransferase. The mechanisms whereby *n*-3 fatty acids affect gene expression are complex and involve multiple processes.[6,28] High plasma TAG levels are a CVD risk factor, therefore their normalisation may reduce this risk.

MS and IR influence CVD outcomes. In the present study, the higher QUICKI scores in the experimental group would indicate higher insulin sensitivity in peripheral tissues, although HOMA values remained unchanged. High-fat diets, especially saturated fatty acid (SFA)-rich diets, adversely affect insulin action and may alter IR and HOMA values,[29] while HOMA index scores decreased in patients orally supplemented with 2 g/d of *n*-3 PUFA for 6 months.[30] Storlien *et al.*[31] observed that a diet rich in SFA, *n*-9 monounsaturated fatty acids (*n*-9 MUFA) or *n*-6 PUFA leads to severe IR that can be prevented by substituting fatty acids with *n*-3 LC-PUFA from fish oils. A recent study concluded that a high-MUFA enteral formula suppresses postprandial hyperglycaemia without elevating insulin secretion in comparison to a high-carbohydrate enteral diet in patients with type 2 diabetes and healthy subjects.[32] Animal studies have demonstrated that steady-state serum concentrations of glucose are significantly higher after a lard-rich diet than after an isocaloric chow diet, attributing differences in dietary fatty acid composition to variations in the induction of IR.[33] However, results on this issue remain inconclusive. Hernandez-Morante *et al.*[34] found significant associations among adipose tissue, adiponectin gene expression and fatty acid composition.

A recent study reported that, although insulin sensitivity is unaffected, a decreased *n*-6/*n*-3 PUFA ratio produces multiple and potentially favourable effects on metabolic and inflammatory profiles, for example by increasing adiponectin[35] which inhibits inflammatory reactions and protects against metabolic and CVDs.[36] In contrast, another recent study reported that dietary *n*-3 PUFA consumed at levels of 3.5% of energy intake did not significantly increase plasma adiponectin concentrations in overweight-to-moderately obese healthy men and women over the course of 14 weeks.[37] The present results showed that incorporation of EPA and DHA into the novel enteral formula did not influence plasma levels of adiponectin or leptin. *n*-3 PUFA prevent the onset of diabetes and IR by activating PPAR expression and influencing lipid oxidation and thermogenesis.[38] Han *et al.*[39] recently demonstrated that a short-term elevation of free fatty acids can induce hepatic and peripheral IR, whereas PUFA induced less hepatic but not peripheral IR compared with MUFA or SFA. Increased intake of or supplementation with *n*-3 marine fatty acids may improve defects in insulin signalling, preventing alterations in glucose homeostasis and consequently the further development of type 2 diabetes.[40] A lower peripheral IR would indicate higher oral glucose tolerance, contributing to better cardiovascular outcomes.

In conclusion, the exclusive feeding of elderly patients with an EN formula enriched with EPA and DHA led to the incorporation of *n*-3 PUFA into plasma lipids, lowered AA levels and improved cardiovascular risk by decreasing plasma TAG without major changes in biomarkers of IR.

Conflict of interest

Mrs. Africa Jiménez, is a member of the Research and Development Department of Vegenat, the company that funded the present study, she was involved in the development of the product T-Diet Plus. Professor Angel Gil has no any contractual relationship with Vegenat; however, he participated in the product design as part of a contract of research between the University of Granada Foundation and Vegenat (contract no. 2388).

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JO carried out the studies and data analyses; MDM was involved in fatty acid analyses, statistical analyses and drafting the manuscript; CMA participated in hormone analyses; RMT was involved in the study design and coordination of institutions; AJ conceived of the study and was responsible for the design of the new product; APC participated in the design of the product and the study; AG participated in the design of the product, was responsible for coordinating the study and collaborated in the data analysis and drafting of the manuscript. All authors read and approved the final manuscript.

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