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Phytosterolaemia associated with parenteral nutrition administration in adult patients

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Abstract

Vegetable lipid emulsions (LE) contain non-declared phytosterols (PS). We aimed to determine PS content depending on the brand and LE batch, and in adult hospitalised patients treated with parenteral nutrition (PN), to establish the association between plasma and administered PS. Part I was the LE study: totals and fractions of PS in three to four non-consecutive batches from six LE were analysed. Part II was the patient study: patients with at least 7 previous days of PN with 0·8 g/kg per d of an olive/soyabean (O/S) LE were randomised (day 0) 1:1 to O/S or 100 % fish oil (FO) at a dose of 0·4 g/kg per d for 7 d (day 7). Plasma PS, its fractions, total cholesterol on days 0 and 7, their clearance and their association with PS administered by LE were studied. In part I, LE study: differences were found in the total PS, their fractions and cholesterol among different LE brands and batches. Exclusive soyabean LE had the highest content of PS (422·36 (SD 130·46) μg/ml). In part II, patient study: nineteen patients were included. In the O/S group, PS levels were maintained (1·11 (SD 6·98) μg/ml) from day 0 to 7, while in the FO group, significant decreases were seen in total PS (−6·21 (SD 4·73) μg/ml) and their fractions, except for campesterol and stigmasterol. Plasma PS on day 7 were significantly associated with PS administered (R^2 0·443). PS content in different LE brands had great variability. PS administered during PN resulted in accumulation and could be prevented with the exclusive administration of FO LE.

Key words: **Parenteral nutrition: Phytosterols: Intravenous fat emulsions**

The use of lipid emulsions (LE) in parenteral nutrition (PN) is a widespread practice. Their energy efficiency is high, their administration is safe and the new generations of LE bring functional advantages; only in very specific cases are LE not administered when PN is required. Parallel to the development of new formulations that improve LE stability has been the increasing accumulation of knowledge in all fields of its use.

First-generation LE are 100 % soyabean oil, and they have been used for decades. After that, next-generation LE firstly included new formulations of 50 % medium-chain TAG in combination with 50 % long-chain TAG and a more efficient metabolic profile[1,2], and secondly an 80:20 mix of olive oil and soyabean oil with a high MUFA content, which is less prone to peroxidation than PUFA[3]. Finally, the last development incorporated into the mix has been fish oil (FO)-based LE which is used as a pharmaconutrient because of its anti-inflammatory activity[2,4,5] and marketed either alone or in combination with other generation LE.

LE administration is not exempt from side effects, especially in certain clinical situations and in certain patient groups. Its use has been associated with hypertriacylglycerolaemia, especially in septic patients, and pancreatitis and renal failure[6] in patients who are under metabolic stress and have systemic inflammatory response syndrome, which needs a high-energy PN contribution. It has also been associated with alterations of liver function parameters in patients on long-term PN and in preterm infants, leading to parenteral-nutrition-associated liver disease (PNALD) with cholestasis or steatosis.

Phytosterols (PS) have been noted as a factor associated with the alteration of liver function parameters and are therefore

Abbreviations: FO, fish oil; LE, lipid emulsion; O/S, olive/soyabean; PN, parenteral nutrition; PNALD, parenteral-nutrition-associated liver disease; PS, phytosterol.

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linked to PNALD[7–9]. PS are plant-derived sterols and undeclared components of vegetable origin in LE. Since all vegetable-origin LE contain PS and their content is not declared, the effect of their administration is unpredictable. PS content and their fractions in different LE have been analysed in a few studies[10]. Nevertheless, the cumulative effect of PS and their plasma clearance are not clearly established, and neither is whether these are dose-dependent or vary according to the different fractions of PS present in each LE.

In this study, we present two complementary approaches aimed at determining the presence of PS and their fractions in commercial LE and in the plasma of patients treated with PN. This has been carried out in such a way that allows us later to study their association in the alteration of liver function parameters.

The first objective of the study was to determine if the presence of cholesterol and PS – total content and their fractions – varied depending on the brand and different batches of LE. The second objective was to study, in hospitalised adult patients treated with continuous total PN, the association of plasma PS values – total content and their fractions – with the type and dose of lipid administered, as well as the association with the amount of PS administered.

Methods

Study of lipid emulsions

A prospective observational study was conducted to determine the daily exposure to PS of patients treated with lipid PN, by quantification (μg/ml) of cholesterol, total PS and their fractions in commercialised intravenous LE available in the pharmaceutical market (Table 1). At least three batches, corresponding to non-consecutive manufacturing, of each one of the five commercial preparations of vegetable origin LE were studied. The PS fractions studied in the LE were *β*-sitosterol, campesterol, lanosterol and stigmasterol. Cholesterol contained in egg lecithin added to LE as an emulsifier was also studied.

For the quantification of total PS and their fractions, an analytical method of HPLC was developed. This method allowed us to separate PS from the matrix in a simple and efficient way, designed so that in a short time, PS samples were obtained with a high percentage of extraction and good repeatability. Liquid chromatography was carried out in a Dionex Ultimate 3000 chromatograph, as published by our group[11]. PS analyses of the LE were carried out according to the 'European Union Regulation (EEC) Number 2568/91' for liquid chromatography or GC. Each sample was analysed three times, and the CV between the replicated analyses was determined. The sample preparation was adapted and modified (without derivation) using the methods previously described by Xu *et al.*[12]. The tubes were purged with N_2 and subjected to saponification at 100°C for 1 h. Our sample size was established from the results obtained by Xu *et al.*[12] in their study on the amount of PS in different commercial preparations, in which the reproducibility among batches was greatly reduced for all fractions.

We estimated, according to the different standard deviations referenced, a sample size of three samples per batch for a power of 90 % and an error of 5 % and, given the characteristics, without estimated losses. To establish the differences between brands and batches of LE, one-factor ANOVA was carried out using the *post hoc* Scheffé test.

Table 1. Intravenous lipid emulsion composition as declared by the producer

Brand (pharmaceutical laboratory)	Composition
Clinoleic (Baxter)	80 % olive oil and 20 % soyabean oil
Intralipid (Fresenius Kabi)	100 % soyabean oil
Intralipid LCT/MCT (Braun)	50 % soyabean oil and 50 % MCT
Lipoplus (Braun)	50 % MCT, 40 % soyabean oil and 10 % fish oil
Omegaven (Fresenius Kabi)	100 % fish oil
SMOFlipid (Fresenius Kabi)	30 % soyabean oil, 30 % MCT, 20 % olive oil and 15 % fish oil

LCT, long-chain TAG; MCT, medium-chain TAG.

Study in patients

Plasma values of PS, their fractions, total cholesterol, their clearance and their association with those administered via LE were studied by means of a prospective, unicentric, randomised, double-blind study. The selected population corresponded to that included in a clinical trial designed to study the relationship between the type of LE used and the evolution of liver function (EudraCT Number: 2014-003597-17, www.clinicaltrialsregister.eu). We studied if, in patients with *γ*-glutamyltransferase alteration associated with PN containing vegetable origin LE, the strategy of reducing the lipid dose by 50 % by switching to an FO-based LE would reduce plasma levels of PS and *γ*-glutamyltransferase, and whether it is more effective and equally safe as a strategy of reducing the lipid by 50 % while maintaining the same vegetal LE. The patients had received a minimum of 7 d of PN with a lipid intake of 0·8 g/kg per d of an olive/soyabean (O/S) LE, until they were randomised to two LE groups (day 0): O/S *v.* 100 % FO (*n*-3 fatty acids, without PS) at a dose of 0·4 g/kg per d for a minimum of 7 d (day 7).

To determine the plasma values of PS, blood samples were collected in 4 ml tubes of lithium heparin and kept cold at 2–8°C for up to 1 h. They were centrifuged at 2000 ***g*** for 10 min at 4°C and aliquoted in 5 ml plastic tubes that were stored at −80°C until processing. Measurements of different PS concentrations in the plasma were carried out using the UPLC-ACQUITY TQD measurement system, which uses liquid chromatography of high and rapid resolution (UPLC) coupled to tandem MS as a measurement principle (MS/MS). We worked in the reverse phase modality using a C18 UPLC column that allowed a faster and higher resolution of the chromatographic peaks. The mobile phase was composed of two solutions of ammonium acetate and 0·1 % (v/v) formic acid, one in acetonitrile and the other in methanol, using a gradient elution.

Plasma values of total PS, their fractions and cholesterol were measured on days 0 and 7. The differences between O/S and FO groups were calculated. Since the PS and cholesterol content of LE was known, total amount of PS, their fractions and cholesterol administered during the 7 d could be calculated. The PS plasma

fractions studied were β-sitosterol, sitostanol, campesterol, lanosterol and stigmasterol. In these plasma determinations, one more fraction studied was sitostanol, since the developed plasma method was more precise than the one developed to study PS in LE. In addition, the plasma PS values and their fractions were adjusted for the amount of cholesterol, leading to the PS:cholesterol ratio: (phytosterol/cholesterol) × 100. Analysis of plasma PS is included in Appendix I as an online Supplementary material.

Statistical analysis

Continuous variables were expressed as mean values and standard deviations and the categorical ones as percentages. To study the variation of plasma values between O/S and FO groups, a Student's *t* test was applied. To establish the differences between the baseline values at day 0 and day 7 in each group, mean values were compared by a paired samples *t* test. Simple linear regression tests were applied to study the association between plasma values and administered amounts. The data were processed with the IBM SPSS 22.0 statistical package, and the level of statistical significance was established at $P < 0{\cdot}05$ with a two-tailed test.

Results

Study of lipid emulsions

Contents of sterols in the studied LE are depicted in Table 2, as well as comparisons with the corresponding ANOVA.

Statistical differences were found in the content of total PS, their fractions and cholesterol content among the different LE. Intralipid, exclusive soyabean oil-derived LE, had the highest total PS content (422·36 (SD 130·46) μg/ml).

Among PS fractions, β-sitosterol was found in greater amounts in all vegetable LE, although with variations between the different LE. Clinolenic (76·38 %) and SMOFlipid (74·82 %) had the highest percentage, followed by Lipofundin medium-chain TAG/long-chain TAG (67·3 %) and Lipoplus (67·4 %); Intralipid had the lowest percentage (57·03 %). Stigmasterol was found at its highest percentage in Intralipid (27·29 %), followed by Lipofundin (21·81 %) and Lipoplus (20·63 %). Clinolenic (9·51 %) and SMOFlipid (12·23 %) had the lowest percentage.

There were also differences between different batches of each vegetable LE. Lipofundin was the LE with the least stigmasterol and campesterol inter-lot differences. Clinolenic and SMOF showed no significant differences for stigmasterol.

In Lipoplus, lanosterol was not detected in any of the batches analysed. In FO LE (Omegaven), as expected, no PS fractions were detected.

Cholesterol percentages were very different between LE and different batches of each LE. Omegaven, SMOFlipid and Intralipid had high cholesterol content, while Lipofundin and Clinolenic had the lowest content.

Study in patients: sterol plasma values

We studied nineteen patients, 73·7 % men, 66·74 (SD 11·39) years and 74·92 (SD 15·00) kg. The mean number of days of PN administration prior to inclusion in the study was 9·47 (SD 4·01), and during this period, the patients received an O/S LE at 0·8 g/kg per d. All patients had a digestive pathology with 73·7 % of them suffering from cancer, mostly rectal (*n* 5) and gastric (*n* 4) cancer. The rest of the patients suffered from one case of the following pathologies: adhesions, mesenteric ischaemia, morbid obesity, occlusion and intestinal volvulus.

Table 3 shows the baseline values of the patients on day 0, when no statistically significant differences were found between the group of patients, neither in sterol plasma values nor in demographic parameters.

Plasma PS levels on days 0 and 7 had a normal distribution, according to the Kolmogorov–Smirnov test ($P = 0{\cdot}200$). Table 4 shows sterol variations in the two study groups between day 7 and day 0. Intragroup variation (*t*-student) and intergroup variation (*t*-pairs) were analysed. In the O/S LE, a lipid dose reduction, from 0·8 g/kg per d to 0·4 g/kg per d for 1 week, was not associated with a significant decrease in total plasma PS or any of its fractions; this was also true for cholesterol. However, the change from a dose of 0·8 g/kg per d of O/S LE to 0·4 g/kg per d of FO LE for 1 week was associated with a statistically significant decrease in total PS and their fractions, except for sitostanol, campesterol and stigmasterol.

In the FO group, significant decreases in PS were seen, while in the O/S group, PS levels were maintained. Additionally in the FO group, the most significant decreases during the 7 d of the study were total PS 31·49 %, β-sitosterol 55·70 % and lanosterol 72·11 %, while smaller decreases were seen with stigmasterol and campesterol at 45·07 and 20·73 %, respectively.

Table 5 shows the same temporal comparison as Table 4, adjusting total PS values and their fractions for cholesterol by means of the PS:cholesterol ratio. A decrease was seen compared with the values not adjusted for cholesterol. In the FO group, total PS adjusted for cholesterol decreased by 36·38 %, while for those not adjusted, the decrease was 31·49 %.

Plasma sterols according to the sterol content of the lipid emulsions administered

Plasma PS on day 7 were significantly associated with the amount of PS administered during the overall study period with a determination coefficient of R^2 0·443 (Table 6). When studying the PS fractions, both β-sitosterol and lanosterol were significantly associated with the respective fractions administered, with high determination coefficients (R^2) 0·657 and 0·557, respectively. Plasma levels of campesterol and stigmasterol showed no significant association with the amounts administered and showed a low coefficient of determination. Plasma cholesterol was also not associated with administered cholesterol and had low R^2 level.

Discussion

Study of lipid emulsions

There are few publications[10,12–14] that study the content of PS in LE, despite the increasingly relevant evidence of the impact that PS have on hepatic damage. In this study, we show that the approach is not simple because of the significant differences

Table 2. Total phytosterol content, fractions and cholesterol in different brands and batches (Mean values and standard deviations)

			Total phytosterols (μg/ml)		β-Sitosterol (μg/ml)		Campesterol (μg/ml)		Lanosterol (μg/ml)		Stigmasterol (μg/ml)		Cholesterol (μg/ml)	
Fat emulsion	ID	Batch	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Clinoleic 20 %	1 (*n* 3)	14H29N30	231·87^{3}	15·66	172·66^{3}	10·32	18·14^{2,3}	1·77	13·98^{2}	2·01	27·07	3·15	51·04^{2}	4·12
	2 (*n* 6)	15F15N31	227·17^{3}	21·04	171·57^{3}	6·27	9·52^{3}	2·13	23·32^{1,3}	5·22	22·75	12·61	67·16^{1,3}	2·73
	3 (*n* 3)	16F22N30	148·99^{1,2}	3·99	122·16^{1,2}	2·11	7·47^{1}	0·67	12·49^{2}	0·64	6·89	0·76	45·84^{2}	1·08
	Total (*n* 12)		208·80^{II,V,VI}	39·52	159·49^{II,IV,V,VI}	23·35	11·16^{II}	4·60	18·28^{II,III,IV,V,VI}	6·42	19·87^{II}	11·79	57·80^{II,V,VI}	10·29
*P intrabatch			<0·001		<0·001		<0·001		0·006		0·059		<0·001	
Intralipid	1 (*n* 3)	10HB3671	451·34^{2,3}	23·24	276·54^{3}	2·21	32·66^{2}	3·89	13·21^{3}	0·58	128·92^{3}	17·77	360·92^{3}	7·09
	2 (*n* 3)	10IK7012	554·10^{1,3}	36·49	283·03^{3}	17·12	99·97^{1,3}	6·61	13·09^{3}	0·91	158·00^{3}	12·38	368·68^{3}	23·50
	3 (*n* 3)	10KC3584	261·64^{1,2}	12·85	163·10^{1,2}	9·13	32·89^{2}	0·90	6·84^{1,2}	0·37	58·81^{1,2}	2·52	212·20^{1,2}	10·88
	Total (*n* 9)		422·36^{I,III,IV,V,VI}	130·46	240·89^{I,III,IV,V,VI}	59·22	55·17^{I,III,IV,V,VI}	33·82	11·05^{I,III,IV,V,VI}	3·21	115·25^{I,III,IV,V,VI}	45·48	313·94^{I,III,IV,V}	77·55
*P intrabatch			<0·001		<0·001		<0·001		<0·001		<0·001		<0·001	
Lipofundin	1 (*n* 3)	143 638 082	178·84^{3}	3·71	119·70^{3}	2·37	17·67	0·68	2·50^{3}	0·53	38·95	1·16	63·59^{2,3}	2·41
MCT/LCT	2 (*n* 3)	144 718 082	189·75	9·37	125·63^{3}	4·53	19·88	1·73	1·82	0·02	42·42	3·73	82·88^{1}	4·41
	3 (*n* 3)	154 818 081	195·36^{1}	3·97	134·22^{1,2}	2·04	18·03	0·84	1·46^{1}	0·05	41·66	1·32	76·19^{1}	0·18
	Total (*n* 9)		187·99^{II,VI}	9·07	126·52^{II,VI}	6·90	18·53^{II}	1·45	1·93^{I,II}	0·53	41·01^{III,VI}	2·59	74·22^{II,V,VI}	8·85
*P intrabatch			0·045		0·004		0·123		0·016		0·250		0·001	
Lipoplus	1 (*n* 3)	144 538 082	145·88^{2,3}	6·06	102·10^{3}	4·95	17·29^{2,3}	0·59	0·00		26·47^{2}	0·79	182·48^{3}	8·91
	2 (*n* 3)	153 938 083	160·53^{1,3}	1·50	107·88^{3}	1·19	19·48^{1,3}	0·41	0·00		33·16^{1,3}	0·49	176·22^{3}	4·17
	3 (*n* 3)	160 128 082	113·78^{1,2}	1·61	73·25^{1,2}	0·39	13·47^{1,2}	0·52	0·00		27·06^{2}	0·88	112·73^{1,2}	0·40
	Total (*n* 9)		140·06^{II,VI}	20·96	94·41 ^{I,II,VI}	16·27	16·74 ^{II}	2·67	0·00^{I,II,V}		28·90 ^{II,VI}	3·27	157·15 ^{II,V,VI}	11·26
*P intrabatch			<0·001		<0·001		<0·001		–		<0·001		<0·001	
SMOFlipid	1 (*n* 3)	16IF1650	137·64^{3,4}	2·95	99·99^{3,4}	1·36	13·38^{3,4}	1·26	7·41$^{2–4}$	0·56	16·86	1·38	420·95$^{2–4}$	4·67
	2 (*n* 3)	16HI2073	138·94^{3,4}	7·57	99·58^{3,4}	1·18	12·63^{3,4}	2·37	10·27^{1,3,4}	1·56	16·46	3·07	399·49^{1,3,4}	2·68
	3 (*n* 3)	16IG1719	121·12^{1,2,4}	9·29	93·53^{1,2,4}	1·65	7·21^{1,2}	1·68	2·78^{1,2}	0·51	15·78	6·08	578·92^{1,2,4}	6·15
	4 (*n* 3)	16KG5043	102·35$^{1–3}$	3·23	74·50$^{1–3}$	2·06	7·62^{1,2}	0·35	4·79^{1,2}	1·37	15·45	2·24	300·75$^{1–3}$	13·41
	Total (*n* 12)		124·23^{II,VI}	15·28	92·96^{I,II,VI}	9·88	9·61^{II}	3·21	5·60^{I,II,IV,VI}	0·80	16·06^{II,III,IV}	3·9	455·80^{I,II,III,IV}	112·39
*P intrabatch			<0·001		<0·001		<0·001		<0·001		0·976		<0·001	
Omegaven	1 (*n* 3)	16H60131	0·00		0·00		0·00		0·00		0·00		400·39^{2,4}	1·79
	2 (*n* 3)	16IG1719	0·00		0·00		0·00		0·00		0·00		507·35^{1,3,4}	4·42
	3 (*n* 3)	16IE1319	0·00		0·00		0·00		0·00		0·00		408·67^{2,4}	8·90
	4 (*n* 3)	16KF4268	0·00		0·00		0·00		0·00		0·00		348·23$^{1–3}$	3·42
	Total (*n* 12)		0·00^{I,II,III,IV,V}		0·00^{I,II,III,IV,V}		0·00^{I,II,III,IV,V}		0·00^{I,II,III,IV,V}		0·00^{I,II,III,IV,V}		416·16^{I,III,IV}	60·25
*P intrabatch			–		–		–		–		–		<0·001	
†P intrabrands			<0·001		<0·001		<0·001		<0·001		<0·001		<0·001	

MCT, medium-chain TAG; LCT, long-chain TAG.

^{I,II,III,IV,V,VI} Roman numbers in superscript are the result of the *post hoc* Scheffé analysis between lipid emulsions identified as I, Clinoleic; II, Intralipid; III, Lipofundin MCT/LCT; IV, Lipoplus; V, SMOFlipid and VI, Omegaven ($P < 0·05$).

^{1,2,3,4} Arabic numbers in superscript are the result of the *post hoc* Scheffé analysis between batches identified as 1, 2, 3 and 4 for each fat emulsion ($P < 0·05$).

* One-factor ANOVA between batches of every fat emulsion, Snedeckor *F* and significance (*P*).

† One-factor ANOVA between fat emulsions, Snedeckor *F* and significance (*P*).

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Table 3. Demographics and baseline (day 0)* values of patients (Mean values and standard deviations)

	Olive/soya (*n* 10)		Fish oil (*n* 9)		
Parameter	Mean	SD	Mean	SD	*P*†
Men					0·089
n	9		5		
%	90		56		
Age (years)	65·7	13·58	67·88	8·29	0·681
Weight (kg)	80·54	8·92	68·68	18·26	0·085
Cholesterol (μg/ml)	1022·96	261·26	954·76	389·83	0·657
Total phytosterols (μg/ml)	22·19	6·40	19·72	6·61	0·420

* Day 0 means that the patients had received a minimum of 7 d of parenteral nutrition with a lipid intake of 0·8 g/kg per d of an olive/soyabean lipid emulsion.
† Significance for the difference between the two groups.

Table 4. Variation of sterols between day 0* and day 7† (Mean values and standard deviations)

	Patients with vegetable fat emulsion (*n* 10)							Patients with fish oil (*n* 9)							
	Initial values		Final values		Variations (differences)			Initial values		Final values		Variations (differences)			
Sterol variation	Mean	SD	Mean	SD	Mean	SD	*P*§	Mean	SD	Mean	SD	Mean	SD	*P*§	*P*‡
Phytosterols (μg/ml)	22·190	6·40	23·300	6·91	1·11	6·98	0·621	19·72	6·61	13·51	5·16	−6·21	4·73	0·004	0·016
β-Sitosterol (μg/ml)	13·12	4·11	13·79	4·67	0·67	5·08	0·685	11·50	2·97	5·39	2·05	−6·11	2·20	0·000	0·002
Sitostanol (μg/ml)	0·36	0·15	0·33	0·12	−0·03	0·16	0·560	0·25	0·18	0·14	0·11	−0·11	0·10	0·013	0·223
Campesterol (μg/ml)	2·24	0·69	2·25	0·68	0·01	0·53	0·940	1·93	0·95	1·53	0·68	−0·40	0·73	0·136	0·171
Lanosterol (μg/ml)	1·19	0·68	1·05	0·41	−0·14	0·66	0·536	1·04	0·50	0·30	0·19	−0·74	0·46	0·015	0·035
Stigmasterol (μg/ml)	0·67	0·34	0·68	0·44	0·01	0·44	0·933	0·69	0·53	0·39	0·28	−0·30	0·48	0·101	0·160
Cholesterol (μg/ml)	1022·96	261·27	1145·70	212·80	122·74	234·78	0·133	954·76	389·83	996·24	355·21	41·49	253·91	0·637	0·478

* Day 0 means that the patients had received a minimum of 7 d of parenteral nutrition with a lipid intake of 0·8 g/kg per d of an olive/soyabean lipid emulsion.
† Day 7 means that the patients had received 7 d of parenteral nutrition with a lipid intake of 0·4 g/kg per d of an olive/soyabean lipid emulsion or a fish oil lipid emulsion.
‡ Significance between groups (*P*) for the variable differences (*t*-student).
§ Significance intra-groups (*P*) for initial and final variables (*t*-pairs).

Table 5. Variation of plasma values of phytosterols and fractions adjusted by cholesterol between day 0 and 7 d post-randomisation (Mean values and standard deviations)

	Patients with vegetable fat emulsion (*n* 10)							Patients with fish oil (*n* 9)							
	Initial values		Final values		Variations (differences)			Initial values		Final values		Variations (differences)			
Ratio sterols variation and fractions	Mean	SD	Mean	SD	Mean	SD	*P*†	Mean	SD	Mean	SD	Mean	SD	*P*†	*P**
R_Phytosterols (μg/ml)	2·28	0·78	2·02	0·50	−0·25	0·64	0·243	2·16	0·48	1·37	0·34	−0·79	0·47	0·001	0·057
R_*β*-sitosterol (μg/ml)	1·35	0·51	1·21	0·34	−0·14	0·47	0·367	1·30	0·35	0·56	0·17	−0·74	0·35	0·000	0·006
R_Sitostanol (μg/ml)	0·04	0·02	0·03	0·01	−0·01	0·01	0·119	0·03	0·02	0·01	0·01	−0·01	0·01	0·019	0·606
R_Campesterol (μg/ml)	0·23	0·08	0·20	0·06	−0·03	0·41	0·067	0·20	0·05	0·15	0·04	−0·05	0·05	0·012	0·227
R_Lanosterol (μg/ml)	0·12	0·08	0·09	0·03	−0·03	0·07	0·188	0·12	0·07	0·03	0·02	−0·09	0·06	0·003	0·061
R_Stigmasterol (μg/ml)	0·07	0·04	0·06	0·03	−0·01	0·04	0·537	0·07	0·06	0·04	0·03	−0·03	0·06	0·133	0·301

*R*_, coefficient that results from dividing each phytosterol value by the value of cholesterol expressed as a percentage.
* Significance between groups (*P*) for the variable differences (*t*-student).
† Significance intra-groups (*P*) for initial and final variables (*t*-pairs).

in content between the different commercial brands of LE and, what is also relevant, between batches of the same commercial brand.

In our study, we found that the differences in the amount of total PS (depending on the brand) were within the range described in an update to the American Society of Parenteral and Enteral Nutrition position paper in 2014, which collected data from several studies[12–14] (178·54 (SD 9·56) and 621·85 (SD 7·36) μg/ml). The values we have found are also in line with those reported by Ellegard[9] and Forchielle[15]. The highest concentrations were of *β*-sitosterol, while campesterol and lanosterol had the lowest concentration, even though there were large variations depending on the brand. It has been described previously that stigmasterol is a potent antagonist of some families of hepatic nuclear receptors that trigger biliary disorders, contrary to what happens with *β*-sitosterol and campesterol,

Table 6. Simple linear regressions between plasma phytosterols on day 7 (*y*) and phytosterols administered (*x*) (*n* 19)*
(R^2 coefficients and 95 % confidence intervals)

Sterols	R^2	b_1	95 % CI	*P*
Phytosterol	0·443	0·007	0·003, 0·011	0·002
β-Sitosterol	0·657	0·009	0·006, 0·012	0·000
Campesterol	0·093	0·003	−0·002, 0·008	0·205
Lanosterol	0·557	0·006	0·004, 0·009	0·000
Stigmasterol	0·066	0·001	−0·001, 0·004	0·287
Cholesterol	0·066	0·094	−0·331, 1·051	0·287

R^2, determination coefficient.
* $y = b_0 \pm b_1 x_1$; *y*: plasma phytosterols on day 7; *x*: phytosterols administered.

which have hardly any inhibitory effects on liver cells[16]. On the other hand, it should also be considered that stigmasterol interrupts cholesterol homoeostasis[15].

As far as we know, there are no studies analysing interbatch differences in the sterol content in LE of the same brand. The variability between batches may have its origin in the quality of the oil source, associated with the geographical source, climate, year of harvest and also the extraction and refining process used. Some authors have reported that levels can be further modified by product refinement, with a reduction of free sterols of up to 30 % of their original quantity[17].

Study in patients: sterol plasma values

The values of plasma PS in our series of adult hospitalised patients after at least 7 d with PN were 21 (SD 6·44) μg/ml. These values are considerably different from the 55·4 (SD 6·2) μg/ml result seen with twenty-seven patients with home PN, and reported in our previous publication[18], this was very similar to that found by Ellegard *et al.*[9] – 62·5 (SD 60·3) μg/ml – in sixteen patients diagnosed with short bowel syndrome and treated with long-term PN.

In the previous clinical randomised trial with the same population and design, we found a positive association between plasma values of PS (and their fractions) and values of *γ*-glutamyltransferase and ALT, whereas these associations were not seen in AP[19]. Now our results show that there is an accumulation of PS because their elimination is slower than the usual administration rate. In the group of patients that changed from 0·8 g to 0·4 g of O/S LE, no reduction in administered plasma sterols was observed. On the other hand, in patients treated with FO LE (without PS), after 7 d, an average reduction of 31·5 % to the plasma concentration of 13·51 (SD 5·16) μg/ml was observed, reaching values very close to those obtained in the healthy controls of our previous publication[18] (14·8 (SD 2·3) μg/ml). It has been reported that plasma PS concentrations tend to remain stable in healthy individuals consuming conventional Western diets, ranging from 3 to 17 μg/ml[20], whereas in vegetarians and in patients with hypercholesterolaemia treated with oral PS, higher values of up to 1·5–3 times those of Western diets are expected[21].

In animal models, PS levels increase rapidly following PN initiation, not only in serum, but also in the liver[8,22–24]. In a recent and interesting study in children with intestinal failure, Hukkinen *et al.*[25], through their linear regression model, showed total serum PS to be a robust indicator of accumulated PS liver levels (r 0·83, $P < 0·01$ for absolute concentrations and r 0·98, $P < 0·01$ for ratios to cholesterol) and associated with portal inflammation, biochemical liver injury, liver fibrosis and liver damage. The authors correlated PS levels with increases of *γ*-glutamyltransferase according to other studies[8,18,26–31].

Experimental studies[16,22] confirm that PS inhibit the farnesoid X receptor by decreasing the transcription of target genes involved in the synthesis, uptake and excretion of bile acids, as well as in the excretion of sterol which is linked to cholestasis. Another complementary mechanism that would explain the role of PS in PNALD is the promotion of hepatic inflammation by the activation of hepatic macrophages acting as Toll-like receptor agonists, which activate immune cell responses and promote cytokine production[22]. In a recent work, Guthrie *et al.*[32] concluded that PS alone are not the cause of liver inflammation, but that this occurs in conjunction with sepsis. The study reports that PS have a synergistic inflammatory effect with the experimental administration of lipopolysaccharides in Kupffer cells.

A factor to take into account is the role of cholesterol, given that during PN administration, the PS:cholesterol ratio is inverted with respect to the usual oral intake. PS in LE can displace cholesterol from cell membranes, which can decrease the elasticity of the tissue and contribute to the damage of hepatocytes[8,33]. As serum and liver sterol proportions are distorted during PN, with high PS and low cholesterol levels reflecting the lipid profile of PN solutions, the ratio of each PS fraction:cholesterol probably mirrors the metabolic effects of PS better than their absolute concentrations. In fact, in our series, when PS fractions were adjusted for cholesterol (Table 5), it was found that the clearance was greater in patients treated with FO. Besides, in the experimental study of Hukkinen *et al.*[25], ratio of plasma PS:cholesterol had a better correlation with the amount of PS in liver tissue than absolute concentrations of PS.

β-Sitosterol was the fraction with the highest concentration in LE administered to patients before starting the study, as well as in the O/S LE group. It has been described that *β*-sitosterol is secreted into bile more effectively than other PS fractions[34], which possibly explains the greater *β*-sitosterol decrease compared with other fractions in the arm treated with FO. In their study, Hukkinen *et al.*[25] explained the decrease in both plasma and hepatic PS after PN interruption. As in our plasma concentrations, in Hukkinen *et al.*[25], stigmasterol and campesterol in the liver did not differ when PN was suspended. In their study, serum and liver stigmasterol were not correlated, suggesting that these fractions are likely to be excreted at a slower rate. The same trend is seen in our series, where after 7 d of PS administration (Table 6), the amount of stigmasterol administrated is not associated with its plasma values so pointing to a slower clearance.

The clinical relevance of LE brand change has been suggested to improve liver function associated with a lower content of plasma PS as well as changes in the profile of PS fractions. In fact, some studies conducted in adult patients show that the transition from PN with soyabean oil to olive oil improves intestinal failure-associated liver disease[28,35], which can be partially explained by the different PS compositions of these LE, especially with stigmasterol which is reduced with the change of LE.

Despite the experimental studies of Carter *et al.*[16] and El Kasmi *et al.*[22] proving stigmasterol as a powerful *in vitro* antagonist of farnesoid X receptor, superior to β-sitosterol and campesterol, there is no clear clinical evidence that stigmasterol itself presents greater hepatotoxicity than other fractions, so it cannot be confirmed that high-stigmasterol LE are critical for the development of PNALD. However, another alternative or complementary mechanism that could explain PNALD improvement when changing brands from soyabean-exclusive LE to O/S LE is that a smaller stigmasterol content would lead to a greater (quicker) clearance of the total PS content.

Study limitations

In the study of PS content of different LE brands, the accepted chromatographic method did not allow us to study small fractions of PS, thus prioritising the implementation of a simple and accessible chromatographic method.

A relevant limitation is that the sample selected corresponds to a substudy of a study designed to evaluate the utilisation of FO LE in the improvement of previously altered liver function parameters. The method for selection of patients did not allow us to establish the basal values of PS, their fractions and cholesterol, before the beginning of the PN, nor to integrate the number of days treatment with PN on the day of onset. The only criterion was that the patients had at least 7 d of PN. Another limitation was the small number of patients included with nutritional support over 7 d, only three cases, which did not allow us to establish statistically significant differences to evaluate cases with more than 7 d of PN, due to lack of statistical power. In the study of plasma values, this is a first approximation that should be extended with subsequent studies.

Conclusion

This study reveals the great variability in the sterol content of LE. The PS content, their fractions and cholesterol vary depending not only on the commercial brand but also between batches of the same commercial brand. In addition, the percentage of different PS fractions varies substantially from one brand to another and is especially relevant in the case of stigmasterol.

The amount of PS administered during PN exceeds the elimination, resulting in accumulation, and this varies depending on the fraction. The administration of FO LE exclusively (without PS) prevents accumulation. It is necessary to complement these data with clinical studies to evaluate the impact of PS levels and the possible therapeutic advantages of the administration of LE without PS.

The results obtained highlight the importance of including the total PS concentration in the technical sheet of each preparation released to the market with the aim of better and safer use in clinical practice.

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Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114520000574

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