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Original article

Do carnitine and extra trace elements change stability of paediatric parenteral nutrition admixtures?

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SUMMARY

Introduction: High concentrations of trace elements (TE), in particular zinc and selenium, along with carnitine, are often added to parenteral admixtures in paediatric patients on long-term Parenteral Nutrition (PN). We aim to evaluate whether lipid droplet diameters of these admixtures maintain the recommended range of 0.4–1.0 μm.

Materials and methods: Stability studies were carried out on six parenteral admixtures with carnitine, trace elements and electrolytes added in different amounts. Each admixture was formulated with five different lipid emulsions with or without fish oil. Analyses were performed at time 0 (t = 0) and 24, 48, 72, 96 (t = 96) hours after compounding. Droplet diameters were determined by Light Scattering-Reverse Fourier Optics Technique. Samples, stored at 4 °C, were triple tested for a total of 450 analyses. Regression analyses were performed using panel-data techniques.

Results: During the 4 days, lipid droplet diameters were in the expected range of 0.4–1.0 μm regardless of trace element and carnitine amounts in all admixtures apart from those containing fish-oil based emulsions and calcium concentrations equal to 4.5 mmol/L. In these latter admixtures, 12% of droplet diameters were larger than 1.0 μm and 2% exceeded 5.0 μm immediately after compounding.

Conclusion: Carnitine and high concentrations of trace elements do not affect PN admixtures stability and can be safely infused in long-term home-PN paediatric patients and prematures. Only high calcium concentrations in compresence with fish oil based lipid emulsions seem to change PN stability.

1. Introduction

Trace elements (TE), in particular zinc and selenium, along with carnitine, are often added to Parenteral Nutrition (PN) admixtures designed to avoid deficiencies in paediatric and premature patients on long-term PN [1–3]. In clinical practice, any nutrient addition to PN admixtures may change stability, which is an essential requirement for a safe infusion. When lipids are combined with other nutrients, the biphasic characteristic of lipid emulsions (oil/water) may cause admixture physicochemical instability. This occurs especially in the presence of high concentrations of divalent cations (such as magnesium and calcium) which may change the repulsive forces to less negative or positively charged lipid droplets [4,5].

To declare a PN admixture safe for infusion, European Guidelines require that lipid droplet diameters remain between 0.4 and 1.0 μm [2,6–8].

Previously, we reported that high concentrations of calcium in the presence of fish oil, can lead to PN collapse. This is possibly due to the low viscosity of the fat emulsion [9]. To further investigate the effect of other commonly added nutrients to PN stability, we decided to focus on carnitine and TE, which, sometimes, need to be generously added to cover intestinal losses or to reach the recommended levels. They are essential in long-term and home-PN patients and in neonates or, more specifically, in prematures. However, no one has verified the impact of carnitine and TE on PN stability, especially if packed in small volume bags (500 cc). Therefore, the purpose of this study is to understand if PN infusions with carnitine and extra TE can be safely administered to patients.

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2. Materials and methods

2.1. Admixtures

Stability studies were carried out on six paediatric PN admixtures (All-in-One) compounded with paediatric multiple trace elements solution (Peditrace®, Fresenius-Kabi, Ganz, Austria, 10 mL), zinc sulphate (S.Orsola-Malpighi Pharmacy Laboratory, Bologna, Italy, 1 mg/mL vials 5 mL), sodium selenite pentahydrate (Selesyn®, Biosyn Arzneimittel GmbH, Fellbach, Deutscheland 100 μg/1 mL vials 10 mL), L-carnitine (Carnitene®, Sigma Tau, Pomezia, Italy 2 g/5 mL vials) along with electrolytes, glucose, paediatric amino acids (TPH®6%, Baxter, Rome, Italy 250 mL) and lipid emulsions added in different quantities (see Table 1). Amounts of TE and carnitine were added to PN based on practical and pharmaceutical reasons following recommendations and gastrointestinal losses. We selected the most common in house prescribed PN formulations designed for paediatric and neonatal critical conditions such as cancer or short-bowel syndrome, and prematures. Some formulations had amounts of calcium and TE which may lead to PN instability. Each of the six admixtures was replicated five times, changing every time the oil based lipid emulsions, some of which contained fish oil: i) soybean oil LCT (Intralipid® 30%, Fresenius-Kabi, Ganz, Austria 330 mL), ii) soybean-olive oil LCT (Clinoleic® 20%, Baxter, Rome, Italy 500 mL), iii) soybean oil LCT + coconut oil MCT (Lipofundin MCT® 10% + 10%, B.Braun, Milan, Italy 500 mL), iv) soybean-olive-fish oil LCT + coconut oil MCT (SMOFflipid® 20%, Fresenius-Kabi, Ganz, Austria 500 mL) and finally v) soybean-fish oil LCT + coconut oil MCT (Lipidem® 20%, B.Braun, Milan, Italy 500 mL). Afterwards, considering that these lipid emulsions have different viscosity, which would affect admixture stability, two oil-based emulsion groups, one with and the other without fish oil, were formed and compared.

All-in-One admixtures were prepared into ethyl-vinyl acetate (EVA) plastic bags by the Parenteral Nutrition Centralized Pharmacy Service - S.Orsola-Malpighi Medical School - Bologna-Italy through an automatic filling up system (Siframix®, Fresenius-Kabi, Ganz, Austria) in a clean room under positive pressure and vertical air flow filtered by High Efficiency Particulate Air (HEPA) absolute filters. Admixture samples were collected and analysed at starting time (t = 0), just after compounding, and then, after a proper bag storage at 4 °C in a refrigerator, at 24, 48, 72, and 96 (t = 96) hours, at room temperature, to reproduce storage and infusion conditions [6–11]. Admixture pH was constantly recorded at 6.5.

2.2. Lipid droplet dimension

Droplet diameters were determined by means of a Laser Droplet Sizer Analysette 22 (Fritsch GmbH, Idar-Oberstein, Deutschland), which uses Laser Diffraction Technique (Light Scattering-Reverse Fourier Optics), as in our previous work [9]. To this end, after setting the granulometer at zero, each sample (5 mL) was combined with distilled water (15 ml) and introduced into the dispersion unit to reach sample/water ratio (1:3 v/v). The standard measuring range was calibrated between 0.16 μm and 1160 μm. To allow a proper cleaning of the measuring cell after each determination, sample turnover averaged 15–20 min. An interfaced computer calculated and presented the results both numerically and graphically. For every admixture sample, parameters d10 (maximum diameter of 10% of droplets) and d90 (maximum diameter of 90% of droplets), were recorded and graphycally converted into droplet distribution areas [10].

2.3. Statistics

In total, 450 analyses (6 admixtures × 5 lipid formulations × 5 points in time × 3 tests) were performed as every sample was triple tested. Results were summarized by arithmetic mean and standard deviation (SD). One-way analysis of variance (ANOVA) with multiple-comparison tests and the Bonferroni correction was used to compare droplet dimensions among groups and panel-data techniques for droplet dimensions over time. Statistical analyses relied on STATA Software 15 (Santa Monica, California).

3. Results

In all the six admixtures, physicochemical stability did not change remarkably between t = 0 and t = 96. Droplet diameters remained in the expected range of 0.4–1.0 μm regardless of trace element and carnitine amounts, although droplet diameters showed increments over four days (d90 at T96 vs T0 p value < 0.001 and d10 at T96 h vs T0 p-value <0.0001). The only physicochemical stability exception occurred in the combined presence of both fish-

Table 1
Composition of tested neonatal and paediatric PN admixtures related to 1000 mL volume[a].

	Admixture 1	Admixture 2	Admixture 3	Admixture 4	Admixture 5	Admixture 6
Volume (mL)	1000	1000	1000	1000	1000	1000
Glucose (g)	115	100	100	110	140	120
Lipids (g)	23	19	20	20	20	20
Paediatric amino acids (g)	23	23	25	25	30	24
Sodium (mmol)	38	38	100	120	30	20
Potassium (mmol)	27	23	30	25	20	16
Chloride (mmol)	38	38	100	120	30	20
Calcium gluconate (mmol)	2.7	4.6	3.0	4.5	4.0	5.0
Magnesium (mmol)	2.3	2.3	5	5	2	2
Organic phosphate (mmol)	17	19	15	18	10	10
Zinc[b] (mg)	3.5	3.5	3.5	3.5	3.4	3.4
Selenium[a] (μg)	70	70	60	60	90	90
Copper (μg)	200	200	200	200	200	200
Manganese (μg)	10	10	10	10	10	10
Fluorine (μg)	570	570	570	570	570	570
Iodine (μg)	10	10	10	10	10	10
Carnitine (mg)	100	100	100	100	100	100
Vitamin solution (ml)	10	10	10	10	10	10

[a] *As described in the materials and methods section, each admixture was compounded with 5 different intravenous lipid emulsions. To improve comparability the content of each admixture is shown as 'amounts per 1000 ml'. Admixture 5 and 6 are meant for neonates and prematures with volume of 500 ml.
[b] Zinc and selenium amounts were the combination of 10 ml Peditrace® and extra additions.

oil based emulsion (FO admixtures) and calcium concentrations equal to 4.5 mmol/L (admixtures 2, 4 and 6 in Table 1). In these admixtures, physicochemical stability changed as 12% of droplet diameters exceeded 1.0 μm and 2% exceeded 5.0 μm immediately after compounding (see Tables 2 and 3 and Figs. 1 and 2).

To further investigate the preliminary results, a panel data analysis with random effects was performed on the two subsets d10 and d90, using droplet diameter as the dependent variable, time as well as the concentrations of each nutrient added to PN as independent variables. Calcium was alternatively specified as a continuous variable or as a dichotomous variable for concentrations equal to or below 4.5 mmol/L. Likewise, fish oil was specified as a dummy or as a continuous variable for amounts added to PN. The interaction of fish oil and calcium (both as continuous and dichotomous variables) was systematically positive and statistically significant (p-value $\leq$0.001), strongly suggesting that the joint presence of fish oil and calcium had an incontrovertible effect on droplet dimension at d90 and especially at d10. In the latter, droplet diameters were immediately increased at t = 0. The dichotomous variable for time = 96 was also positive and strongly significant (p-value $\leq$0.001).

None of the other nutrients (amino acid, long-chain fatty acids, medium-chain fatty acids, glucose, sodium, potassium, chloride, magnesium, phosphate, zinc, selenium, and carnitine) appeared to modify droplet dimensions across admixtures or over time (Table 4).

4. Discussion

Admixture stability, intended as maintenance of the same characteristics over time, is a key issue for patients of all ages receiving PN.

In a previous study, we proved that the presence of calcium and fish oil are the two elements at play for droplet instability, beside time after compounding [9]. In line with these results, we wanted to verify whether the addition of extra amounts of micro nutrients would change PN stability. To this aim, we selected the most common in-house prescribed PN formulations with amounts of electrolytes and trace elements that may make them unstable. These PN formulations are commonly used in paediatric and neonatal wards treating critical conditions such as cancer, short-bowel syndrome or very premature babies.

Our results showed that paediatric PN admixtures containing extra concentrations of trace elements and carnitine are stable and suitable for infusion in paediatric patients up to 96 h after compounding, provided calcium concentration is maintained below 4.5 mmol/L in the presence of fish oil formulations. On the fourth day, we observed a slight increase in droplet diameters due to smaller droplets aggregating into larger droplets of lipid emulsion (Ostwald-Ripening phenomena) [12]. However, neither creaming nor flocculation were found to occur. On the contrary, when calcium concentrations reached 4.5 mmol/L and lipid emulsions contain fish oil (irrelevant of the amount), we witnessed droplet diameters that are almost 5–10 time greater for d10 (immediately at t = 0 after compounding) than those in admixtures with calcium below 4.5 mmol/l with fish oil (admixtures 2,4,6 vs 1,3,5 in Table 3) or in admixtures with calcium equal to 4.5 mmol/l with and without fish oil (admixtures 2,4,6 in Table 3 vs admixtures 2,4,6 in Table 2).

After identifying the significant variables, we tested them both as continuous and as dichotomous. Dichotomous variables, in particular, are used to verify whether a specific threshold plays a particular role. For fish oil, the dichotomous variable is used to check if the very presence of fish oil makes a difference, independent of its quantity. For calcium, the dichotomous variable is used to check if exceeding the recommended threshold of 4.5 mmol/L has a peculiar impact on particle dimension. From another viewpoint, the use of dichotomous variables can also be viewed as a way of testing the robustness of the results obtained from the corresponding continuous variables.

All the other fats (long-chain or medium-chain) did not interfere with PN stability as well as fish oil with calcium at 4 mmol/L We believe that calcium interferes with fish oil conformation. Dose-effects of calcium on lipids have extensively been presented in the literature. It is known that calcium rigidifies and arranges lipid membranes and that it contributes to conformational changes. It is also known that lipid membranes have calcium-binding capacity through a negative charge in lipid membrane itself and positive on calcium [13–20].

FO being less viscous than other lipids can be more unstable. Although data on TE are limited, they may be strong fasteners to lipid membranes and responsible for PN instability because they may collapse the z potential of admixtures [21]. This may depend on the type of cations (divalent and trivalent) and their quantity as it exists a critical number above which lipid droplets aggregate. Copper and selenium, having high cation valence, have greater potential for admixture destabilization. None was found significant in our case maybe because the added extra amount did not reach

Table 2
Dimensional droplet parameter (mean ± standard deviation) of paediatric PN Admixtures 1–6 compounded without fish oil based emulsions [soybean, olive and medium-chain tryglicerides, or emulsions i), ii), and iii) as reported in the text].

Time	Admixture 1	Admixture 2	Admixture 3	Admixture 4	Admixture 5	Admixture 6
t = 0	d10 = 0.33 μ ± 0.001 μ d90 = 0.20 μ ± 0.001 μ	d10 = 0.40 μ ± 0.002 μ d90 = 0.25 μ ± 0.001 μ	d10 = 0.25 μ ± 0.001 μ d90 = 0.19 μ ± 0.001 μ	d10 = 0.35 μ ± 0.001 μ d90 = 0.29 μ ± 0.001 μ	d10 = 0.45 μ ± 0.001 μ d90 = 0.37 μ ± 0.001 μ	d10 = 0.45 μ ± 0.001 μ d90 = 0.36 μ ± 0.001 μ
t = 24 h	d10 = 0.33 μ ± 0.001 μ d90 = 0.20 μ ± 0.001 μ	d10 = 0.40 μ ± 0.002 μ d90 = 0.25 μ ± 0.001 μ	d10 = 0.25 μ ± 0.001 μ d90 = 0.19 μ ± 0.001 μ	d10 = 0.35 μ ± 0.001 μ d90 = 0.29 μ ± 0.001 μ	d10 = 0.45 μ ± 0.001 μ d90 = 0.37 μ ± 0.001 μ	d10 = 0.45 μ ± 0.001 μ d90 = 0.36 μ ± 0.001 μ
t = 48 h	d10 = 0.33 μ ± 0.001 μ d90 = 0.20 μ ± 0.001 μ	d10 = 0.40 μ ± 0.002 μ d90 = 0.25 μ ± 0.001 μ	d10 = 0.25 μ ± 0.001 μ d90 = 0.19 μ ± 0.001 μ	d10 = 0.35 μ ± 0.001 μ d90 = 0.29 μ ± 0.001 μ	d10 = 0.45 μ ± 0.001 μ d90 = 0.37 μ ± 0.001 μ	d10 = 0.45 μ ± 0.001 μ d90 = 0.36 μ ± 0.001 μ
t = 72 h	d10 = 0.33 μ ± 0.002 μ d90 = 0.20 μ ± 0.001 μ	d10 = 0.40 μ ± 0.002 μ d90 = 0.25 μ ± 0.001 μ	d10 = 0.25 μ ± 0.002 μ d90 = 0.19 μ ± 0.001 μ	d10 = 0.35 μ ± 0.001 μ d90 = 0.29 μ ± 0.001 μ	d10 = 0.45 μ ± 0.002 μ d90 = 0.37 μ ± 0.001 μ	d10 = 0.45 μ ± 0.002 μ d90 = 0.36 μ ± 0.001 μ
t = 96 h	d10 = 0.35 μ ± 0.002 μ d90 = 0.40 μ ± 0.001 μ	d10 = 0.45 μ ± 0.002 μ d90 = 0.30 μ ± 0.001 μ	d10 = 0.28 μ ± 0.002 μ d90 = 0.25 μ ± 0.001 μ	d10 = 0.40 μ ± 0.001 μ d90 = 0.35 μ ± 0.001 μ	d10 = 0.48 μ ± 0.002 μ d90 = 0.39 μ ± 0.001 μ	d10 = 0.48 μ ± 0.002 μ d90 = 0.39 μ ± 0.002 μ

D10 is the maximum diameter of 10% of droplets.
D90 is the maximum diameter of 90% of droplets.
μ: micrometres.

Table 3
Dimensional droplet parameter (mean ± standard deviation) of paediatric PN Admixtures 1–6 compounded with lipid emulsions with fish oil [soy, olive oil or coconut oil or emulsions iv), and v) as reported in the text].

Time	Admixture 1	Admixture 2	Admixture 3	Admixture 4	Admixture 5	Admixture 6
t = 0	d10 = 0.35 μ ± 0.001 μ d90 = 0.23 μ ± 0.001 μ	d10 = 2.90 μ ± 0.002 μ d90 = 0.50 μ ± 0.001 μ	d10 = 0.30 μ ± 0.001 μ d90 = 0.44 μ ± 0.001 μ	d10 = 3.00 μ ± 0.001 μ d90 = 0.50 μ ± 0.001 μ	d10 = 0.45 μ ± 0.001 μ d90 = 0.29 μ ± 0.001 μ	d10 = 2.95 μ ± 0.001 μ d90 = 0.70 μ ± 0.001 μ
t = 24 h	d10 = 0.35 μ ± 0.001 μ d90 = 0.23 μ ± 0.001 μ	d10 = 2.90 μ ± 0.002 μ d90 = 0.50 μ ± 0.001 μ	d10 = 0.30 μ ± 0.001 μ d90 = 0.44 μ ± 0.001 μ	d10 = 3.00 μ ± 0.001 μ d90 = 0.50 μ ± 0.001 μ	d10 = 0.45 μ ± 0.001 μ d90 = 0.29 μ ± 0.001 μ	d10 = 2.95 μ ± 0.001 μ d90 = 0.70 μ ± 0.001 μ
t = 48 h	d10 = 0.35 μ ± 0.001 μ d90 = 0.23 μ ± 0.001 μ	d10 = 2.90 μ ± 0.002 μ d90 = 0.50 μ ± 0.001 μ	d10 = 0.30 μ ± 0.001 μ d90 = 0.44 μ ± 0.001 μ	d10 = 3.00 μ ± 0.001 μ d90 = 0.50 μ ± 0.001 μ	d10 = 0.45 μ ± 0.001 μ d90 = 0.29 μ ± 0.001 μ	d10 = 2.95 μ ± 0.001 μ d90 = 0.70 μ ± 0.001 μ
t = 72 h	d10 = 0.35 μ ± 0.002 μ d90 = 0.23 μ ± 0.001 μ	d10 = 2.90 μ ± 0.002 μ d90 = 0.50 μ ± 0.001 μ	d10 = 0.30 μ ± 0.002 μ d90 = 0.44 μ ± 0.001 μ	d10 = 3.00 μ ± 0.001 μ d90 = 0.50 μ ± 0.001 μ	d10 = 0.45 μ ± 0.001 μ d90 = 0.29 μ ± 0.001 μ	d10 = 2.95 μ ± 0.001 μ d90 = 0.70 μ ± 0.001 μ
t = 96 h	d10 = 0.39 μ ± 0.002 μ d90 = 0.26 μ ± 0.001 μ	d10 = 3.00 μ ± 0.002 μ d90 = 0.70 μ ± 0.001 μ	d10 = 0.35 μ ± 0.002 μ d90 = 0.51 μ ± 0.001 μ	d10 = 3.10 μ ± 0.001 μ d90 = 0.65 μ ± 0.001 μ	d10 = 0.48 μ ± 0.001 μ d90 = 0.45 μ ± 0.001 μ	d10 = 3.15 μ ± 0.001 μ d90 = 0.85 μ ± 0.001 μ

D10 is the maximum diameter of 10% of droplets.
D90 is the maximum diameter of 90% of droplets.
μ: micrometres.

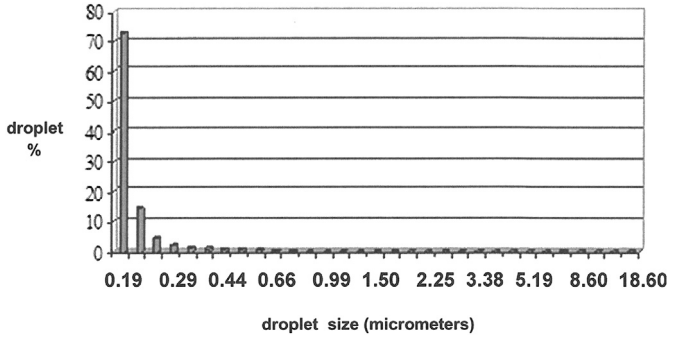

Fig. 1. Droplet size distribution area of admixtures containing carnitine, high concentration of trace elements and intravenous lipid emulsions with fish oil with Ca^{++} < 4.5 mmol/L at t = 0 an t = 96 h and those without fish oil with any calcium content.

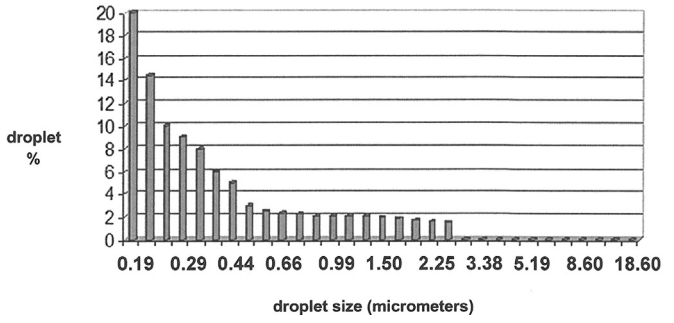

Fig. 2. Droplet size distribution area of admixtures containing carnitine and high concentration of trace elements, and soybean-olive-medium chain trygliceride-fish oil- and soybean-medium chain trygliceride-fish oil based emulsion with Ca^{++} ≥ 4.5 mmol/L at t = 0 an t = 96 h.

the critical concentration. This result is reassuring because it implies that admixtures with extra amounts of zinc and selenium can safely be used in children and neonates with critical situations such as cancer or short-bowel syndrome and especially in prematures for whom small PN volumes are a critical factor.

Amino acids along with glucose may shift lipid surface potential due to their acidity. The use of branched-chain amino acids in prematures and neonates is even more responsible for admixture instability [22]. In our study, carnitine was not identified as a risk component. It did not influence PN stability and this could be due to its chemical structure. Being a primary amine, carnitine behaves as basic amino acids that exert a protective effect on lipid droplet aggregation. In this way, it may have contributed to stabilize admixtures.

Phosphate did not affect admixture stability because we used fructose 1,6-bisphosphate, which, in its organic state, is very much soluble and very unlikely to precipitate with calcium as shown in a recent study without lipid addition [23]. Using fructose 1,6-bisphosphate, we could add more calcium in the admixtures without the risk of precipitation. However, it is possible that this calcium amount was all available for reaction with FO.

Laser diffraction was our technique of choice due to the superiority in measurements of solid droplets and droplets dispersed in different settings from food to chemical or aerospatial industry. This technique does not seem to disrupt the solution flow and is able to determine an ample range of droplets and droplets diameter range from 0.16 to 1160 μm in very low sample dilution. In addition, it can determine different shapes either spherical or not and droplet distribution [24,25]. No other technique can identify small droplets, making it the perfect technique for PN admixtures evaluation. In addition, using the same technique allows us to compare these results with our previous findings as well as validate the latter. Unfortunately, comparisons with other stability studies cannot be made because such studies used other techniques such as Light Obscuration Technique [26]. In future, the use of nanodroplets may help understand the mechanisms underlying PN instability, as proven in a recent study on structural properties of colloids [27].

As a final thought, we are conscious that we analysed personalized, although commonly used, PN admixtures, which may limit the generalizability of our observations in terms of practice. However, our amounts of TE such as zinc and selenium or carnitine, mandatory in long-term patients who cannot eat or lose extra amounts through diarrhoea, cover recommendations and sometimes exceed them. Therefore, those amounts can safely be added to PN of any volume given some of our admixtures were prepared in 500 ml to accomplish needs of prematures. Nevertheless, further investigations are required to reach an adequate level of generalizability and transferability. Possible tests may include use of

Table 4
Results from the panel analysis.

Variable	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8	Model 9	Model 10	Model 11	Model 12
	Dimension 90						Dimension 10					
Calcium (continuous)	0.125**	0.122**			0.133**	0.088*	0.814*	0.782***			0.878*	0.291
	(0.036)	(0.015)			(0.021)	(0.088)	(0.098)	(0.007)			(0.068)	(0.277)
Calcium (dummy)			0.175**	0.078					1.153*	0.053**		
			(0.021)	(0.164)					(0.068)	(0.048)		
Fish oil (continuous)	0.058***	0.061***					0.423***	0.458***				
	(0.001)	(0.000)					(0.002)	(0.000)				
Fish oil (dummy)			0.272*	0.094	0.272*	0.208*			2.041*	0.016	2.041*	1.213*
			(0.058)	(0.377)	(0.058)	(0.095)			(0.089)	(0.752)	(0.089)	(0.060)
Interaction calcium/fish oil (continuous)		0.040***						0.480***				
		(0.002)						(0.000)				
Interaction calcium/fish oil (dummy)				0.224***						2.543***		
				(0.000)						(0.000)		
Interaction calcium/fish oil (cont/dummy)						0.100***						1.296***
						(0.003)						(0.000)
Time 24	−0.000	0.000	0.000	−0.000	−0.000	−0.000	0.000	−0.000	0.000	0.000	0.000	−0.000
	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)
Time 48	−0.000	0.000	0.000	−0.000	−0.000	−0.000	0.000	−0.000	0.000	0.000	0.000	−0.000
	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)
Time 72	−0.000	0.000	0.000	−0.000	−0.000	−0.000	0.000	−0.000	0.000	0.000	0.000	−0.000
	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)
Time 96	0.093***	0.093***	0.093***	0.093***	0.093***	0.093***	0.056***	0.056***	0.056***	0.056***	0.056***	0.056***
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
Glucose	0.002	0.002	−0.007	−0.003	−0.004	0.000	0.018	0.019	−0.044	0.003	−0.025	0.025
	(0.757)	(0.697)	(0.485)	(0.703)	(0.693)	(1.000)	(0.667)	(0.439)	(0.583)	(0.361)	(0.756)	(0.572)
Amino acids	−0.011	−0.012	0.025	0.010	0.009	−0.005	−0.093	−0.103	0.170	−0.000	0.062	−0.117
	(0.441)	(0.325)	(0.459)	(0.670)	(0.794)	(0.859)	(0.419)	(0.130)	(0.548)	(0.973)	(0.823)	(0.433)
Phosphate	−0.038	−0.036	−0.006	−0.017	−0.024	−0.034	−0.084	−0.069	0.145	0.015	0.028	−0.105
	(0.280)	(0.217)	(0.871)	(0.491)	(0.555)	(0.325)	(0.769)	(0.685)	(0.628)	(0.197)	(0.933)	(0.555)
Zinc	2.714	2.615	−1.641	−0.166	0.895	2.213	13.053	11.866	−18.173	−1.396	−1.469	15.653
	(0.470)	(0.411)	(0.686)	(0.953)	(0.846)	(0.576)	(0.674)	(0.518)	(0.593)	(0.306)	(0.970)	(0.445)
Long-chain tryglicerides	−0.002	−0.002	0.035	0.010	0.035	0.011	−0.016	−0.016	0.275	−0.004	0.275	−0.031
	(0.591)	(0.523)	(0.506)	(0.781)	(0.506)	(0.806)	(0.635)	(0.417)	(0.528)	(0.840)	(0.528)	(0.895)
Medium-chain tryglicerides	0.000	0.000	0.033	0.010	0.033	0.011	0.000	0.000	0.265	−0.003	0.265	−0.030
	(.)	(.)	(0.512)	(0.784)	(0.512)	(0.809)	(.)	(.)	(0.534)	(0.842)	(0.534)	(0.896)
Constant	−8.386	−8.049	5.396	0.898	−2.955	−6.996	−42.607	−38.575	55.866	4.693	0.840	−51.625
	(0.513)	(0.459)	(0.682)	(0.922)	(0.845)	(0.589)	(0.687)	(0.538)	(0.612)	(0.287)	(0.995)	(0.442)

The table presents several models according to whether.
1. The dependent variable was d10 (maximum diameter of 10% of droplets) and d90 (maximum diameter of 90% of droplets).
2. Fish oil is treated as dichotomous and continuous variable. When treated as a dichotomous variable, fish oil is set = 1 when present in any amount.
3. Calcium is treated as dichotomous and continuous variable. When treated as a dichotomous variable, calcium is set = 1 when exceed the threshold of 4.5 mmol/L.
4. The interaction between calcium and fish oil is introduced among the regressors.
Time 0 is the reference time period and thus omitted. Selenium, magnesium, and chlorate were omitted due to collinearity.
p-values in parentheses: *p < 0.10; **p < 0.05; ***p < 0.01.

different amino acids solutions which may change solution's pH or single-use ready-to-use PN products.

5. Conclusion

The results of this study are clinically relevant because carnitine and extra amounts of trace elements did not alter paediatric and premature PN admixtures over four days. These nutrients are not critical and can be safely infused. However, this does not hold for calcium in amounts of 4.5 mmol/L (quantity often reached to cover neonatal or paediatric needs) and for FO (regardless of the amount). The combination of the two destabilizes the admixture just after compounding. Conversely, destabilization did not occur when other LCT or LCT/MCT were used at any calcium level or when FO was compresent with calcium up to 4 mmol/L.

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None declared.

CRediT authorship contribution statement

M.L. Forchielli: Conceptualization, Data curation, Investigation, Formal analysis. **A. Bonoli:** Methodology. **A. Stancari:** Resources. **L.L. Bruno:** Resources. **F. Piro:** Resources. **G. Piazza:** Resources. **C. Albertini:** Writing - review & editing. **A. Pession:** Writing - review & editing. **C. Puggioli:** Writing - review & editing. **G. Bersani:** Conceptualization, Data curation, Investigation, Formal analysis.

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