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High Protein Intake Does Not Prevent Low Plasma Levels of Conditionally Essential Amino Acids in Very Preterm Infants Receiving Parenteral Nutrition

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Abstract

Background: We have shown that increasing protein intake using a standardized, concentrated, added macronutrients parenteral (SCAMP) nutrition regimen improves head growth in very preterm infants (VPIs) compared with a control parenteral nutrition (PN) regimen. VPIs are at risk of conditionally essential amino acid (CEAA) deficiencies because of current neonatal PN amino acid (AA) formulations. We hypothesized that the SCAMP regimen would prevent low plasma levels of CEAAs. *Aim*: To compare the plasma AA profiles at approximately day 9 of life in VPIs receiving SCAMP vs a control PN regimen. *Methods*: VPIs (<29 weeks' gestation) were randomized to receive SCAMP (30% more PN AA) or a control regimen. Data were collected to measure parenteral and enteral protein, energy, and individual AA intake and the first plasma AA profile. Plasma profiles of the 20 individual protogenic AA levels were measured using ion exchange chromatography. *Results*: Plasma AA profiles were obtained at median (interquartile range [IQR]) age of 9 (8–10) days in both SCAMP (n = 59) and control (n = 67) groups after randomizing 150 VPIs. Median (IQR) plasma levels of individual essential AAs were higher than the reference population mean (RPM) in both groups, especially for threonine. SCAMP infants had higher plasma levels of essential AAs than did the controls. Median (IQR) plasma levels of glutamine, arginine, and cysteine (CEAAs) were lower than the RPM in both groups. *Conclusion*: Plasma AA levels in PN-dependent VPIs indicate there is an imbalance in essential and CEAA provision in neonatal PN AA formulations that is not improved by increasing protein intake. (*JPEN J Parenter Enteral Nutr*. 2017;41:455-462)

Keywords

parenteral nutrition; preterm; amino acids; arginine; threonine; glutamine

Clinical Relevancy Statement

Parenteral amino acid (AA) intakes at the high end of current recommendations play an important part in minimizing early postnatal growth failure in very preterm infants (VPIs). Despite the increasing use of this strategy, much of the data about plasma AA levels in different parenteral nutrition (PN) AA formulations are from studies with lower AA intakes. The optimal intake of individual parenteral AAs in VPIs is unknown, and many AA formulations in current use are more than 25 years old. The potential deficiency of conditionally essential AAs (CEAAs) in PN-dependent preterm infants is well described. This study shows high AA intakes do not prevent low plasma levels of CEAAs. It provides new data demonstrating the imbalance of essential AAs and CEAAs in the plasma AA profile of VPIs with potential implications for both toxicity and deficiency of individual AAs. There is a clear need to redesign neonatal parenteral AA formulations.

Introduction

Very preterm infants (VPIs) are at high risk of postnatal growth failure in the first month of life.[1] VPIs (infants <29 weeks' gestation) accumulate deficits in actual nutrient intake[2] despite the use of neonatal parenteral nutrition (PN).[3,4] Nitrogen balance in the first week of life can be improved by starting higher amounts of protein, in the form of parenteral amino acids (AAs), immediately after birth.[5–8] Increasing protein and energy intake can improve early postnatal growth,[9,10] ameliorate early head growth failure,[11] and is associated with improved neurodevelopmental outcome.[12,13] Nutrient intake in

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the first week of life appears to be particularly important.[13,14] Studies[15–17] have not demonstrated an association between early nutrition and long-term benefits, although there has yet to be a randomized controlled trial (RCT) adequately powered to investigate neurocognitive outcomes in childhood.

Improving early AA intake requires PN strategies that start parenteral AAs immediately after birth[18] and achieve higher target AA intakes. This approach is supported in national recommendations, and survey data indicate it is being widely adopted. Despite the concern that excess administration of AAs (protein) may result in saturation of both the catabolic and metabolic pathways of protein metabolism in VPIs, relatively few data describe plasma AA profiles. Recent studies investigating plasma AA profiles have tended to focus on the first 72 hours[5,7,8] rather than the second or third week of life when many VPIs are still PN dependent. There is also a wide variation in AA intake, AA formulation, and analytical techniques for measuring plasma AAs. Most AA formulations have not changed during the recent period of increased early AA intake for VPIs, suggesting that the original safety data for these neonatal PN AA formulations need updating. The optimum AA formulation is not known, with some formulations using cord blood AA profiles as a template and others the AA profile of human milk proteins.

A number of AAs in VPIs are regarded as conditionally essential: tyrosine, glutamine, arginine, cysteine, glycine, and proline.[19] Several workers have drawn attention to the vulnerability of preterm infants to deficiency in the first 4 of these AAs.[19–21] Some AAs are unstable in parenteral formulations limiting PN content (tyrosine, glutamine, and cysteine), whereas for other AAs, there appears to be a particularly high demand in the PN-dependent VPI (arginine). We have previously published plasma AA data demonstrating this potential deficiency using Primene (AA-P; Baxter, Newbury, UK) as the PN AA source.[22] However, the actual AA intakes in this study fell well below the target AA intakes. More recently, we have performed an RCT demonstrating improved head growth at 28 days[11] with a standardized, concentrated, added macronutrients parenteral (SCAMP) nutrition regimen compared with a control standardized, concentrated PN regimen.[11] This study achieved actual AA intakes much closer to the target AA intakes. The aim of this secondary analysis was to compare the plasma AA profiles of the SCAMP and control groups in the second and third weeks of life. We hypothesized that the increased AA intake achieved with the SCAMP regimen would prevent low plasma levels of the neonatal conditionally essential AA.

Methods

The study (ISRCTN: 76597892) received ethical and regulatory approval and is described in detail with the published primary outcome.[11] Eligible infants were born <29 weeks' gestation, weighed <1200 g, and were admitted to the neonatal intensive care unit (NICU) at Liverpool Women's Hospital (LWH) within 48 hours of birth between October 2009 and July 2012. This was a single-center RCT with blinding of all those involved in care and assessment except for the dispensing pharmacist. Randomization occurred within 120 hours.

Table 1. Comparison of Amino Acid (AA) Content (g/100 g AA) of Human Milk Protein and Manufacturers' Published Data for 3 Commonly Used Parenteral AA Formulations: AA-V (Vanimolact; Fresenius Kabi), AA-P (Primene; Baxter), and AA-T (Trophamine; Braun).

AA	Human Milk[23]	AA-V	AA-T	AA-P
Phenylalanine (Phe)	3.7	4.1	4.8	4.2
Valine (Val)	5.4	5.5	7.8	7.6
Leucine (Leu)	9.9	10.7	14.0	10.0
Isoleucine (Iso)	5.5	4.7	8.2	6.7
Lysine (Lys)	6.9	8.6	12.0	11.0
Methionine (Met)	1.4	2.0	3.4	2.4
Threonine (Thr)	4.5	5.5	4.2	3.7
Histidine (His)	2.2	3.2	4.8	3.8
Tryptophan (Try)	1.7	2.1	2.0	2.0
Tyrosine (Tyr)	4.0	0.8	2.4	0.5
Glutamine (Gln)	0.0	0.0	0.0	0.0
Arginine (Arg)	3.9	6.3	12.0	8.4
Cystine (Cys)	2.1	1.5	0.4	1.9
Glycine (Gly)	2.3	3.2	3.6	4.0
Proline (Pro)	8.2	8.6	4.1	3.0
Glutamate (Glu)	17.2	10.9	5.0	10.0
Aspartate (Asn)	0.0	0.0	0.0	0.0
Asparagine (Asp)	8.9	6.3	3.2	6.0
Alanine (Ala)	3.8	9.6	5.4	8.0
Serine (Ser)	4.7	5.8	3.8	4.0
Ornithine (Orn)	0.0	0.0	0.0	3.2
Taurine (Tau)	0.0	0.5	0.3	0.6

The control regimen comprised the standardized, concentrated neonatal PN regimen used in current clinical practice. This was designed to give 2.8 g/kg/protein (3.3 g/kg/d AA) and 85 kcal/kg/d energy. All study infants received the control PN regimen as soon after birth as possible. Following parental consent, VPIs were randomized to either start the SCAMP regimen (designed to give 3.8 g/kg/d protein or 4.3 g/kg/d AA and 105 kcal/kg/d) or continue receiving the control regimen. The regimens have been described in detail previously,[11] and both used Vaminolact (AA-V; Fresenius-Kabi, Runcorn, UK) as the parenteral AA source. The individual AA composition is shown in Table 1 along with that of 2 other contemporary parenteral AA sources: Primene (AA-P; Baxter) and Trophamine (AA-T; Braun, Bethlehem, PA) in current widespread use. There were no differences in the micronutrients, vitamins, or electrolytes provided by the 2 regimens.

All infants received clinical care in accordance with LWH PN protocols, including fluid management; introducing, increasing, and stopping enteral feeds; and biochemical monitoring. The study intervention continued until 28 completed

Table 2. Comparison of Mean (SD) Individual Parenteral and Enteral Amino Acid Intakes (mg/kg/d) in SCAMP and Control Groups.[a]

	Parenteral Values			Enteral Values		
Amino Acid	SCAMP (n = 59)	Control (n = 67)	*P* Value	SCAMP (n = 59)	Control (n = 67)	*P* Value
Phenylalanine	146 (43)	122 (19)	<.001	18 (23)	14 (16)	.25
Valine	197 (58)	164 (26)	<.001	26 (33)	21 (23)	.31
Leucine	382 (113)	318 (50)	<.001	48 (61)	38 (43)	.29
Isoleucine	169 (50)	141 (22)	<.001	27 (34)	21 (24)	.27
Lysine	306 (90)	255 (40)	<.001	34 (43)	27 (30)	.29
Methionine	71 (21)	59 (9)	<.001	7 (8)	5 (6)	.11
Threonine	197 (58)	164 (26)	<.001	22 (28)	17 (19)	.24
Histidine	115 (34)	96 (15)	<.001	11 (14)	9 (10)	.35
Tryptophan	76 (23)	64 (10)	<.001	8 (10)	7 (7)	.51
Tyrosine	27 (8)	23 (4)	<.001	20 (25)	16 (17)	.29
Glutamine	—	—		—	—	
Arginine	224 (66)	186 (30)	<.001	19 (24)	15 (17)	.28
Cystine	55 (16)	45 (7)	<.001	10 (13)	8 (9)	.31
Glycine	115 (34)	96 (15)	<.001	11 (14)	9 (10)	.35
Proline	306 (90)	255 (40)	<.001	40 (51)	32 (35)	.30
Glutamate	388 (114)	323 (51)	<.001	84 (107)	67 (74)	.30
Asparagine	—	—		—	—	
Aspartate	224 (66)	186 (30)	<.001	43 (55)	34 (38)	.28
Alanine	344 (101)	287 (45)	<.001	18 (23)	15 (16)	.39
Serine	207 (61)	173 (27)	<.001	23 (29)	18 (20)	.26

SCAMP, standardized, concentrated, added macronutrients parenteral; —, not present in formulation.
[a]Enteral intake based on average values for human milk protein amino acid profile.[23] *P* values not corrected for multiple testing.

days of life. PN was discontinued once enteral feeds exceeded 75% total. The transition from PN to enteral feeds has been described previously[11] and involved the preferential use of expressed or donor breast milk, which remained unfortified until 150 mL/kg/d enteral feeds.

Patient data were collected from the electronic patient record. Daily enteral and parenteral protein and energy intake data were calculated as described previously for the 48 hours preceding the plasma AA sample. Individual AA intake was calculated from manufacturers' data for composition of PN or formula and estimated from published average values for the protein content of human milk.[23] The plasma profile of 20 protogenic individual AAs (μmol/L) was recorded from the first plasma AA sample obtained for routine clinical monitoring. Our clinical practice guidelines recommend this is approximately 7 days after maximum AA intake has been achieved and only in infants receiving >50% nutrition intake (volume) via the parenteral route. AAs were measured using ion exchange chromatography (IEC) with normal reference ranges obtained from a recent multicenter U.K. study of infants <6 months old (including our own laboratory) and using the same analysis technique.[24]

Results

The demographic data of the SCAMP (n = 74) and control (n = 76) groups have been summarized previously.[11] In the SCAMP group, 4 infants died, 6 infants did not meet criteria for plasma AA analysis, and 5 infants had no sample performed, leaving 59 infants with a plasma AA profile for analysis. In the control group, there was 1 death, 5 infants did not meet criteria, and there were 3 missed samples, leaving 67 infants with a plasma AA profile for analysis.

In the 48 hours preceding the plasma AA sample, the mean (SD) total protein intake was 3.66 (0.63) g/kg/d and 3.03 (0.33) g/kg/d in the SCAMP and control groups, respectively ($P < .001$). Enteral protein intake accounted for <15% total protein, with mean (SD) intakes of 0.49 (0.62) and 0.39 (0.43) g/kg/d in SCAMP and control groups, respectively ($P = .42$). Table 2 shows a highly statistically significant difference in the parenteral intake (mg/kg/d) of all 20 individual protogenic AAs when comparing SCAMP and control groups over the 48 hours. There are no differences in enteral intakes.

The median (interquartile [IQR]) day of the first plasma AA sample was day 9 (8–10) of life in both groups. Table 3 compares the SCAMP and control group plasma AA levels (micromol/L) for the 20 individual protogenic AAs with the reference population described above. In general, plasma essential AA levels are higher than in the SCAMP vs control group, but allowing for multiple comparisons, only valine and leucine are statistically significant. In both groups, the median plasma essential AA levels are above the reference mean, especially for threonine. Among the conditionally essential AAs, there is little

Table 3. Comparison of Median (IQR) Individual Plasma Amino Acid Levels (μmol/L) in SCAMP and Control Groups.[a]

Amino Acid	SCAMP (n = 59)	Control (n = 67)	*P* Value	Reference Range[16]
Phenylalanine	86 (74–95)	78 (71–86)	.06	52 (25–80)
Valine	187 (165–203)	162 (144–184)	.004[b]	146 (65–290)
Leucine	156 (131–169)	136 (122–156)	.004[b]	97 (44–169)
Isoleucine	52 (46–61)	46 (38–54)	.01[b]	50 (20–91)
Lysine	242 (203–314)	218 (194–267)	.17	155 (70–266)
Methionine	31 (26–38)	28 (22–35)	.20	25 (11–49)
Threonine	541 (445–731)	461 (354–619)	.03[b]	97 (39–175)
Histidine	91 (82–107)	87 (78–95)	.05	74 (43–111)
Tryptophan	20 (15–24)	20 (14–24)	.57	15 (10–19)
Tyrosine	63 (36–95)	54 (38–69)	.80	58 (22–103)
Glutamine	495 (392–589)	441 (365–553)	.30	559 (323–810)
Arginine	41 (25–57)	35 (22–46)	.21	57 (12–112)
Cystine	29 (20–37)	35 (22–46)	.39	–
Glycine	398 (344–470)	390 (313–468)	.96	246 (120–436)
Proline	369 (313–455)	308 (262–384)	.001[b]	154 (66–330)
Glutamate	96 (78–113)	94 (74–115)	.70	100 (32–240)
Asparagine	29 (23–41)	26 (21–38)	.91	38 (18–58)
Aspartate	31 (27–40)	30 (25–37)	.46	19 (17–21)
Alanine	379 (314–437)	330 (268–404)	.02[b]	300 (112–592)
Serine	253 (216–307)	236 (190–284)	.27	127 (69–206)
Taurine	142 (89–218)	128 (78–187)	.91	70 (26–169)

IQR, interquartile range; SCAMP, standardized, concentrated, added macronutrients parenteral.
[a]Median (IQR) reference amino acid levels from a population of infants <6 months old[16] shown.
[b]$P < .05$; *P* values not corrected for multiple testing.

difference between SCAMP and control groups except for proline. The median plasma levels of glutamine, arginine, and cysteine are all below the reference mean in both groups.

Figure 1A compares the plasma AA data in the control group with the reference population by converting the individual median plasma AA levels and IQR to percentages of the corresponding reference plasma AA mean (reference mean = 100). This clearly illustrates the imbalance between essential plasma AAs (particularly the high threonine levels) and the lower plasma levels of the conditionally essential AAs: tyrosine, glutamine, arginine, and cysteine. Figure 1B shows the same data for SCAMP infants and indicates the imbalance is not improved by increasing total AA intake. We have previously published plasma AAs[22] from a similarly designed RCT in the same infant population: the ExPN study.[25] The median (IQR) sampling period was also day 9 (8–10) in both groups, and IEC was used for the plasma AA analysis. The PN AA formulation was Primene (AA-P) described in Table 1. Figure 1C (control) and 1D (intervention) uses the published median (IQR) plasma AA levels to compare with the same reference population using the same approach as in Figure 1A,B. Although this study achieved lower total protein intakes, plasma essential AA levels are still generally higher than the reference population. Plasma threonine levels are high but much closer to the reference mean than the plasma threonine levels described in the current study (AA-P has a lower threonine content). The imbalance between essential plasma AAs and the lower plasma levels of the conditionally essential AAs (tyrosine, glutamine, arginine, and cysteine) with the AA-P formulation is similar to that described for the AA-V formulation in our study (tyrosine levels are lower, as is AA-P tyrosine content). Again, the imbalance is not improved by increasing total AA intake (Figure 1D).

Discussion

This study provides the only published plasma AA data for the PN AA-V formulation in a contemporary VPI population. It shows that low plasma levels of conditionally essential AAs in PN-dependent VPIs are not prevented by PN strategies that increase early protein intake. Moreover, this approach leads to plasma levels of essential AAs at or above the upper limit of the reference range, with hyperthreoninemia particularly marked in this study. This imbalance in essential AA vs conditionally essential AA provision using the PN AA-V formulation is also apparent from comparable data from studies using AA-P[22] and AA-T[26] formulations. These studies[22,26] have plasma AA data obtained in the same patient group, at the same postnatal age, and analyzed using IEC. The AA imbalance increases the risk of both toxicity and deficiency of individual AAs (see below). Both have the potential to undermine strategies to improve early growth and ultimately the key outcome measure, long-term neurodevelopmental outcome. The original primary outcome of this

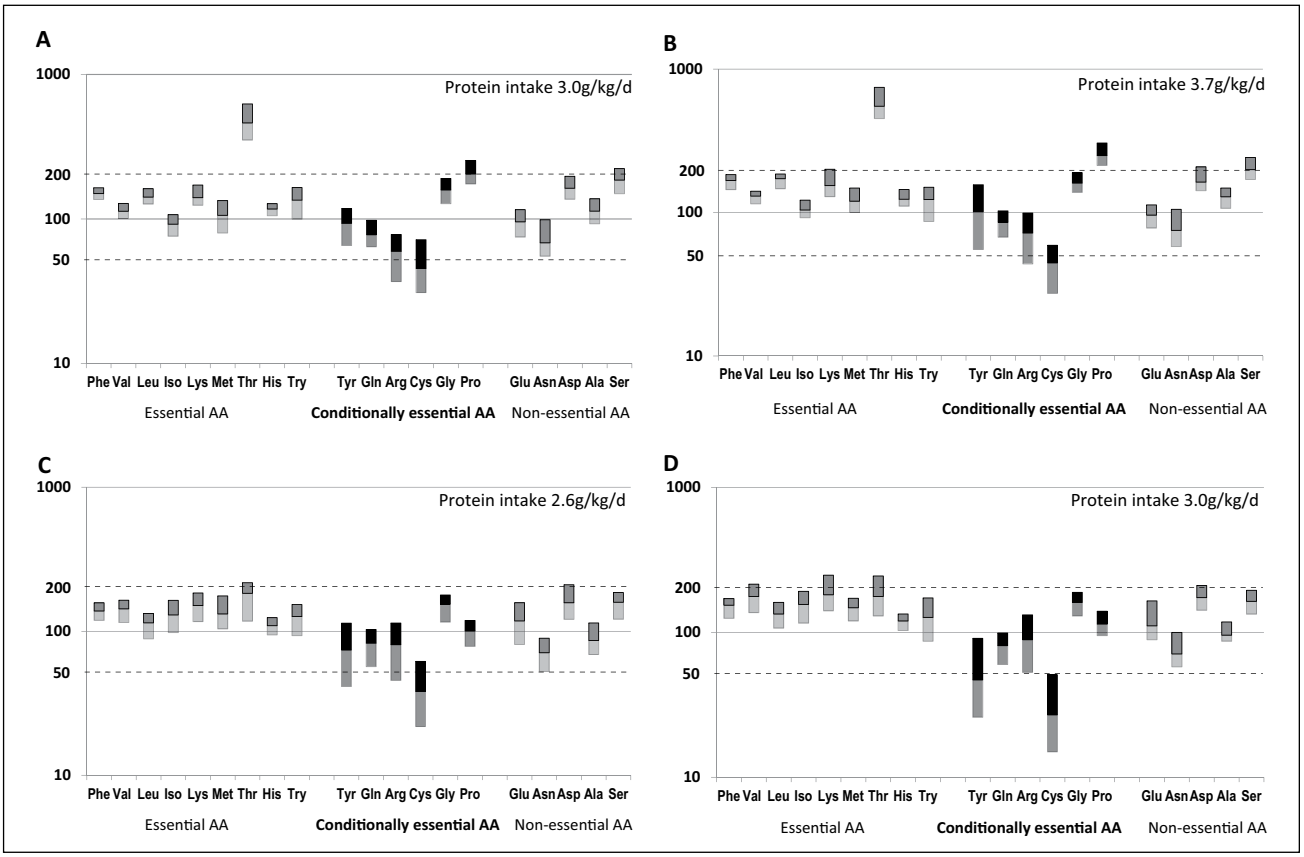

Figure 1. Compares the plasma amino acid (AA) data in the (A) control and (B) standardized, concentrated, added macronutrients parenteral (SCAMP) groups with the (C) control and (D) intervention groups of the similarly designed ExPN study[22,25] using the same reference population. The individual median plasma AA levels and interquartile range have been converted to percentages of the corresponding reference plasma AA mean (reference mean = 100). For definitions of AA abbreviations, see Table 1.

study showed that increased PN protein and energy intake improved early head growth.[11] However, the rates of head growth did not match those achieved after day 28 with lower enteral protein intakes. This is consistent with other published data.[10,27] While early preterm complications may be a factor, a further explanation may be that current PN AA formulations do not provide the optimal balance of individual parenteral AAs required to maximize protein synthesis and tissue growth. This balanced AA formulation will acquire increasing importance as preterm nutrition strategies aim to maximize early protein intake.

There are few plasma AA profile data in contemporary VPI populations receiving current maximum recommended targets for early AA intakes beyond day 3 of life,[22,26,28,29] The 3 neonatal PN AA formulations described in Table 1 are still in widespread use and have not changed in more than 25 years. Whether optimizing the plasma AA profile of VPIs is sufficient to ensure optimal individual parenteral AA intake is unknown, not least because the correct reference population for PN-dependent VPIs cannot be defined. It is an important limitation of this study. However, it would seem a reasonable approach to develop a PN AA formulation that achieves a plasma AA profile as close to the plasma AA profile of a healthy breastfed preterm infant as possible to minimize the risk of toxicity and deficiency. By using data from this study and other comparable studies, it is clear that current PN formulations do not achieve this aim, even though adjusting the balance of essential and conditionally essential AAs appears technologically feasible. It is of concern that newer neonatal PN AA formulations do not appear to address the imbalance between essential and conditionally essential AA provision.[21,30]

There are other limitations of this study. Plasma AA levels were a secondary outcome measure, with sampling determined by routine clinical practice, not a research protocol. This meant missing data for a small number of infants who were mostly enterally fed. This would seem appropriate in a study comparing different parenteral AA intakes, and the small numbers are evenly distributed between the groups. For reasons described below, the plasma AA levels of some AAs (particularly threonine and some conditionally essential amino acids [CEAAs]) may be different in enterally and parenterally fed VPIs. The small number and distribution of these infants means that were

such data achievable, they would not have altered the main study findings. Similarly, the small number of infants with missing plasma data (due to insufficient sampling) is unlikely to have altered the main study findings. The decision to calculate the total protein and individual AA intakes for the 48 hours before the plasma AA sample is a pragmatic one based on previously reported methodology,[22,26] and our previous work suggests little difference in using 48 hours and longer periods[22] when comparing 2 groups with different protein intakes.

Both SCAMP and control groups demonstrated hyperthreoninemia in this study. The risk of hyperthreoninemia with neonatal PN, especially in VPIs, has long been recognized.[31] Limited metabolism of excess threonine in the neonatal period has been suggested as the likely mechanism. AA-V has the highest threonine content of any PN AA formulation licensed for neonatal use and is the likely explanation for the hyperthreoninemia seen with a hyperalimentation strategy. However, even the control group had plasma threonine levels well above the reference range, and the increased protein intake in the SCAMP group had a relatively small effect. Hyperthreoninemia is a well-known phenomenon in infants fed a whey protein–predominant formula,[32] with mean plasma levels of 419 μmol reported in infants receiving 60% whey/40% casein.[33] The safe upper limit for neonatal plasma threonine levels is not known, but animal studies have raised concerns about the effect of hyperthreoninemia on the developing brain.[34] Animal studies also indicate that parenteral and enteral threonine have very different metabolic pathways, greatly reducing the need for parenteral threonine intake.[35] This is consistent with data in preterm infants showing that the splanchnic tissues extract a very large amount of the dietary threonine intake.[36] The mean parenteral threonine intakes in our study were similar to those estimated from kinetic studies in animals,[35] although this is a little higher than the original calculation for preterm infants.[31] More recent studies in preterm infants also indicate that the threonine content of current neonatal PN is too high.[37] This highlights the major limitations in using the human milk protein AA profile as the basis for a parenteral AA formulation.

Our study showed that, in contrast to essential AA, the mean plasma levels of the conditionally essential AAs (tyrosine, cysteine, glutamine, and arginine) were below that of the reference range. We had previously observed hypotyrosinemia with neonatal PN using AA-P with a paradoxical fall in association with hyperalimentation.[22] This was not seen in this study, although tyrosine intakes were more than 50% higher, largely because of the higher tyrosine content of AA-V. This suggests that relatively small increases in tyrosine intake greatly reduce the risk of deficiency. As in many previous studies, plasma cysteine levels were much lower than the reference range. Most PN AA formulations (including AA-V) contain little cysteine because it is not stable in solution and rapidly oxidizes to cysteine, which is insoluble. Cysteine is synthesized from methionine (increased in neonatal PN to compensate). It has been suggested that it is a CEAA in preterm infants because of biochemical immaturity of the enzyme cystathionase that is involved in cysteine synthesis.[20,21] There is evidence against this hypothesis,[38] and a review of cysteine supplementation of cysteine-free PN did not find a benefit.[39] However, none of these studies attempted to correct low plasma cysteine levels during high protein intakes to optimize growth.

Poor stability means glutamine is not currently included in neonatal PN formulations despite its presence in human milk. Both SCAMP and control groups showed median plasma glutamine levels just below the reference range. The glutamate content of AA-V is higher than any other neonatal PN AA formulation, suggesting that glutamine deficiency may be ameliorated by glutamate in neonatal PN formulations. Indeed, studies of neonatal PN with a low glutamate content result in very low plasma glutamine levels that are only partly addressed with glutamine supplementation.[26] Recent evidence suggests that neonatal PN containing alanyl-glutamine is an alternative method of improving parenteral glutamine intake.[30] In animal models of experimental enterocolitis, glutamine supplementation has been associated with reduced mucosal injury, lower infection rates, and increased survival.[40] In studies in adult humans, systematic reviews of RCTs in adult surgical or critically ill patients suggest that glutamine supplementation reduces infectious complications and duration of hospital stay but has little effect on mortality.[41] In preterm infants, the available trial data do not provide evidence that glutamine supplementation has clinically important benefits.[42] This meta-analysis is dominated by a single study[26] that only partially corrected low plasma glutamine levels, and the only study[43] investigating glutamine supplementation of the AA-V formulation included few VPIs, the population at greatest risk of infection and necrotizing enterocolitis (NEC).

This study has the lowest reported plasma arginine levels in VPIs, reflecting the fact that AA-V has the lowest arginine content of any neonatal PN AA formulation. The mean level of plasma arginine in our reference population (57 μmol/L) is lower than that (>80 μmol/L) reported in other reference populations, thus potentially underestimating the degree of deficiency.[26] Use of AA-T as the AA formulation (12 g/100 g AA) has resulted in mean plasma arginine levels in 3 separate studies,[26,44,45] with further supplementation (achieving 400 mg/kg/d) doubling plasma arginine levels.[45] In direct contrast to threonine metabolism, intestinal metabolism of arginine precursors during enteral nutrition is essential to meet neonatal arginine requirements in animal[46,47] and human studies.[48,49] Hypoargininemia in PN-dependent neonates leading to hyperammonemia is well described[50–52] and reflects the important role arginine has in the hepatic urea cycle.[53] Hyperammonemia can be severe and is reversed by arginine supplementation.[50] More commonly, asymptomatic hyperammonemia in the preterm infant can occur, and this also responds to arginine supplementation.[52] Plasma arginine levels of 40 μmol/L (as in this study) in VPIs have been associated with hyperammonaemia.[49] While these levels of ammonia are unlikely to have direct clinical

importance, they suggest that the associated hypoargininemia is having a functional effect on important metabolic pathways. Mean plasma arginine levels of >80 μmol/L in VPIs in the second week of life are associated with mean plasma ammonia levels in the normal range.[26,44,45]

Arginine plays a central role in several other metabolic pathways[53] and comprises 14% protein AA, providing further evidence that the arginine requirements of the VPIs may be particularly high.[43] Plasma arginine levels are likely to represent a balance between arginine intake, arginine synthesis, and the demands of protein synthesis and the multiple metabolic pathways for arginine utilization.[53] Arginine is a key substrate for nitric oxide synthesis and may explain the role of low plasma arginine levels in NEC,[44,45] pulmonary disease,[54] and impaired immune function.[55] The risk of NEC is reduced by arginine supplementation,[45] although more evidence is required.[56] Hypoargininemia is also associated with preterm hyperglycemia.[57] Animal models suggest that early postnatal arginine supplementation to correct low arginine intakes improves neonatal growth.[58,59]

The combination of lost enteral arginine synthetic pathways and high arginine demand in VPIs provides the rationale for neonatal PN AAs comprising a significantly higher proportion of arginine than human milk. Human milk contains 3.9 g arginine per 100 g AA.[23] Our study shows that increasing arginine PN by 50% compared with human milk does not avoid hypoarginemia even when overall protein intake is high. Doubling PN arginine content compared with human milk does not avoid hypoargininemia either.[57] Only when PN arginine content approaches 3 times that of human milk do mean plasma arginine levels in VPIs exceed the 80-μmol/L range,[26,44,45] although this is not achieved if total protein intakes are low (that can occur in the first few days of life). The evidence from this study and our previous work[22,57,60] suggests that all currently licensed PN AA formulations in the United Kingdom risk significant hypoargininemia in VPIs.

In conclusion, we have shown that increasing early protein and energy intakes in VPIs does not prevent low plasma levels of CEAAs in the first 14 days of life using AA-V as the parenteral source. There continues to be an imbalance in essential AA and CEAA provision, and this is apparent in other commonly used PN AA formulations. The presence of hyperthreoninemia and hypoargininaemia are important examples of this imbalance and highlight the limitations in using human milk protein AA as the basis for parenteral formulation. Based on the plasma AA data from studies using other PN AA formulations, correcting the AA imbalance appears technologically feasible but is not apparent in the newest PN AA formulations available. Further work is required to explore the optimal doses of CEAA (eg, supplementing existing PN AA formulations with arginine chloride) while minimizing threonine intake.

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Statement of Authorship

C. Morgan and L. Burgess contributed to the conception or design of the research; contributed to the acquisition, analysis, or interpretation of the data; drafted the manuscript; critically revised the manuscript; and agree to be fully accountable for ensuring the integrity and accuracy of the work. Both authors read and approved the final manuscript.

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