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Role of protein and amino acids in IYCN and relationship with growth

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Introduction

- Amino acids are the building blocks of protein and are required in suitable proportions for growth and all body functions including regulation of gene expression
- There is no storage pool for either protein or amino acids
- Proteins are shown to have the fastest turnover and lowest oxidation rate (compared to fats and carbohydrates)

Adult protein and amino acid requirements (WHO, 2007)

	WHO 2007 Estimates		1985 FAO/WHO/UNU	
	EAR g/kg BW	Safe Level g/kg BW	EAR g/kg BW	Safe Level
Protein	0.66	0.83	0.6	0.75
Amino Acid Requirements	mg/kg per day	mg/g protein	mg/kg per day	mg/g protein
Histidine	10	15	8 to 12	15
Isoleucine	20	30	10	15
Leucine	39	59	14	21
Lysine	30	45	12	18
Sulfur amino Acids (Methionine+ cysteine)	15	22	13	20
Methionine	10	16		
Cysteine	4	6		
Phenylalanine +tyrosine	25	38	14	21
Threonine	15	23	7	11
Tryptophan	4	6	3.5	5
Valine	26	39	10	15
Total Indispensable amino acids	184	277	93.5	141

	WHO 2007 Estimates	
	6-12 months	12-24 months
	EAR g/kg BW (Safe)	EAR g/kg BW (Safe)
Protein	1.12 (1.31)	0.86 (1.05)
Amino Acids	mg/kg per day	mg/kg per day
Histidine	22	15
Isoleucine	36	27
Leucine	73	54
Lysine	64	45
Sulfur amino Acids (Methionine+ cysteine)	31	22
Phenylalanine +tyrosine	59	40
Threonine	34	23
Tryptophan	9.5	6.4
Valine	49	36

Utilizable protein, prevalence of stunting and prevalence of protein inadequacy by region in 2005

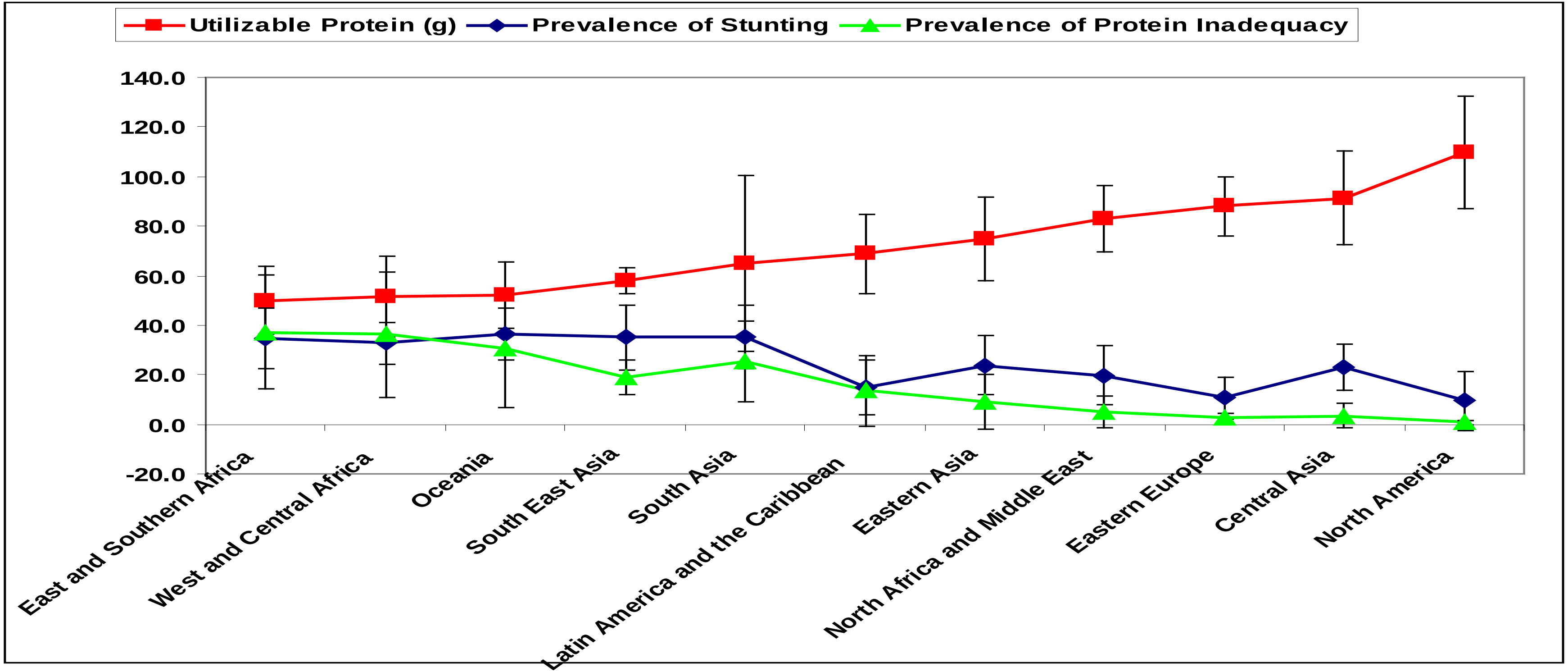


Table 5. Associations (linear regression) between prevalence of stunting and prevalence of protein inadequacy (total protein and utilizable protein with adjusted protein requirements) and energy supply, for 115 countries

	Regression Coefficient					
Dependent variable	Energy (kcal/capita /day)	Prevalence of Protein Inadequacy[1] (%)	Adjusted Prevalence of Protein Inadequacy[2] (%)	Constant	Adjusted R square	F
Stunting[3]			0·330 $p < 0·000$	17·55	0·379	70·52
	− 0·011 $p < 0·001$		0·170 $p = 0·003$	49·83	0·446	46·92
		0·437 $p < 0·000$		21·47	0·198	29·11
	− 0·015 $p < 0·001$	0·102 NS		65·31	0·407	40·18

[1] Prevalence of protein inadequacy calculated using estimates of total protein (g/capita/day) and current protein requirements of 0·66 g/kg for 60 kg adult
[2] Prevalence of protein inadequacy calculated using estimates of utilizable protein (g/capita/day) and protein requirements of 0·66 g/kg for 60 kg adult adjusted for energy deficit and infection needs
[3] Defined as the percentage of children under five years of age in a particular country who fall below −2 standard deviations for height-for-age z-score

Ghosh et al. Assessment of protein adequacy in developing countries: quality matters. British Journal of Nutrition (2012), 108, S77–S87

Type of Dietary Protein and Growth

- Association b/n protein intake, sIGF-I concentrations, and height in 2.5-y-old healthy Danish children
  - Diet (7-d record) and sIGF-I (radioimmunoassay), n=90, multiple linear regressions adjusting for gender and weight
  - Dietary variables: animal protein, vegetable protein, milk and meat

- Animal protein significantly associated with sIGF-1 (p=0.013) and height (p=0.010)

- Specifically milk protein (g/day) was significantly associated with sIGF-1 (p=0.045) and height (p=0.007)

Hoppe et al. Animal protein intake, serum insulin-like growth factor I, and growth in healthy 2.5-y-old Danish children Am J Clin Nutr 2004; 80:447–52.

Type of Dietary Protein and Growth

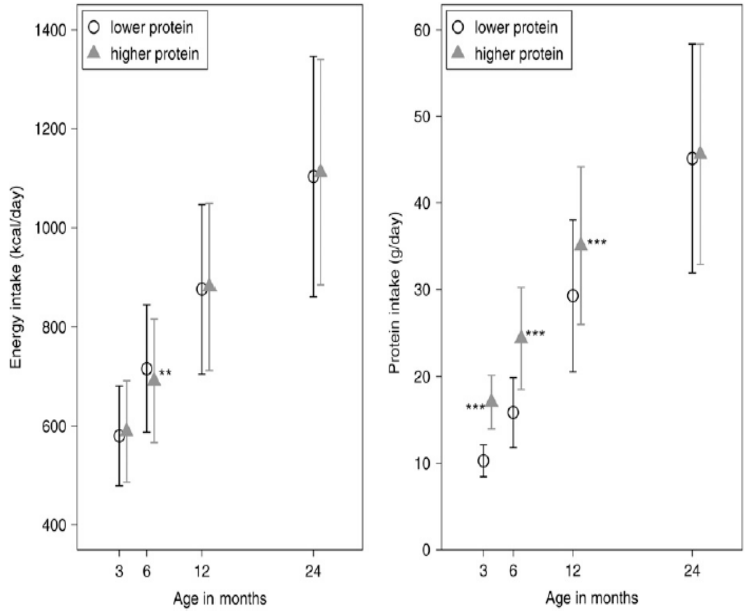
- Eight year old Danish boys receiving a high milk intake had higher IGF-1 levels compared to boys receiving protein from meat (Hoppe C, Molgaard C, Juul A, Michaelsen KF. High intakes of skimmed milk, but not meat, increase serum IGF-I and IGFBP-3 in eight-year-old boys. Eur J Clin Nutr. 2004;58(9):1211-6)

- Amino acids, peptides specific to milk and/or other milk components (e.g. β-lactaglobulin, α-lactalbumin, immunoglobulins, lactoferrin) are likely to be the active stimulating components

Koletzko et al. Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial Am J Clin Nutr 2009;89:1836–45.

High versus low Protein Intake early infancy through 24 months

- 1138 healthy formula fed infants : cow milk based formula with follow on with lower (1.77 and 2.2 g protein/100 kcal) or higher (2.9 and 4.4 g protein/100 kcal)
- Weight, length, weight for length and BMI at inclusion, 3, 6, 12 and 24 months of age
- Estimated difference in Weight for length (WLZ) at 24 months b/n low and high was 0.20 (0.060, 0.34, p=0.005)
- WLZ in lower protein – very close to WLZ in observational breast fed group



Individual Amino Acids and Growth

- Association between intake of dietary protein and high arginine intake and linear growth in pre-pubertal girls at 6 years of age (beta = 1.09 (SE 0.54, p=0.05)

- High arginine combined with high lysine intake (ranging from 3.8-4.6 g/day) at 6 years of age were inversely associated with change in fat mass index in pre-pubertal lean girls at 9 years of age (beta = -0.74, SE 0.60 p=0.03)

- Two animal studies support a shift from fat to muscle gain on arginine supplementation (1.25% to 1.5% of the diet)

- One study looked at single versus multiple restrictions and found lowered IGF-1 with any restriction

Van Vught et al, Public Health Nutrition: 2009, 13(5), 647–653 doi:10.1017/S1368980009991510
Nall et al. J Nutr. 2009;139(7):1279-85, Jobgen et al. J Nutr. 2009;139(2):230-7, Takenaka et al J. Nutr. 130: 2910–2914, 2000

Plasma amino acids and stunting

- Cross sectional study in Malawi, 313 children (12-59 months of age), no severe acute malnutrition, or congenital or chronic disease. No diarrhea reported at enrollment

- Serum samples were analyzed for 139 metabolites (22 amino acids, 6 amino acid metabolites, 3 biogenic amines, 15 sphingolipids, 8 acylcarnitines and 85 glycerophospholipids

- Lower serum concentrations of all nine essential amino acids (tryptophan, isoleucine, leucine, valine, methionine, threonine, histidine, phenylalanine, lysine) ($p < 0.01$)

- Significantly lower serum concentrations of conditionally essential amino acids (arginine, glycine, glutamine), non-essential amino acids (asparagine, glutamate, serine), and six different sphingolipids

Semba, R.D., et al., Child Stunting is Associated with Low Circulating Essential Amino Acids, EBioMedicine (2016), http://dx.doi.org/10.1016/j.ebiom.2016.02.030

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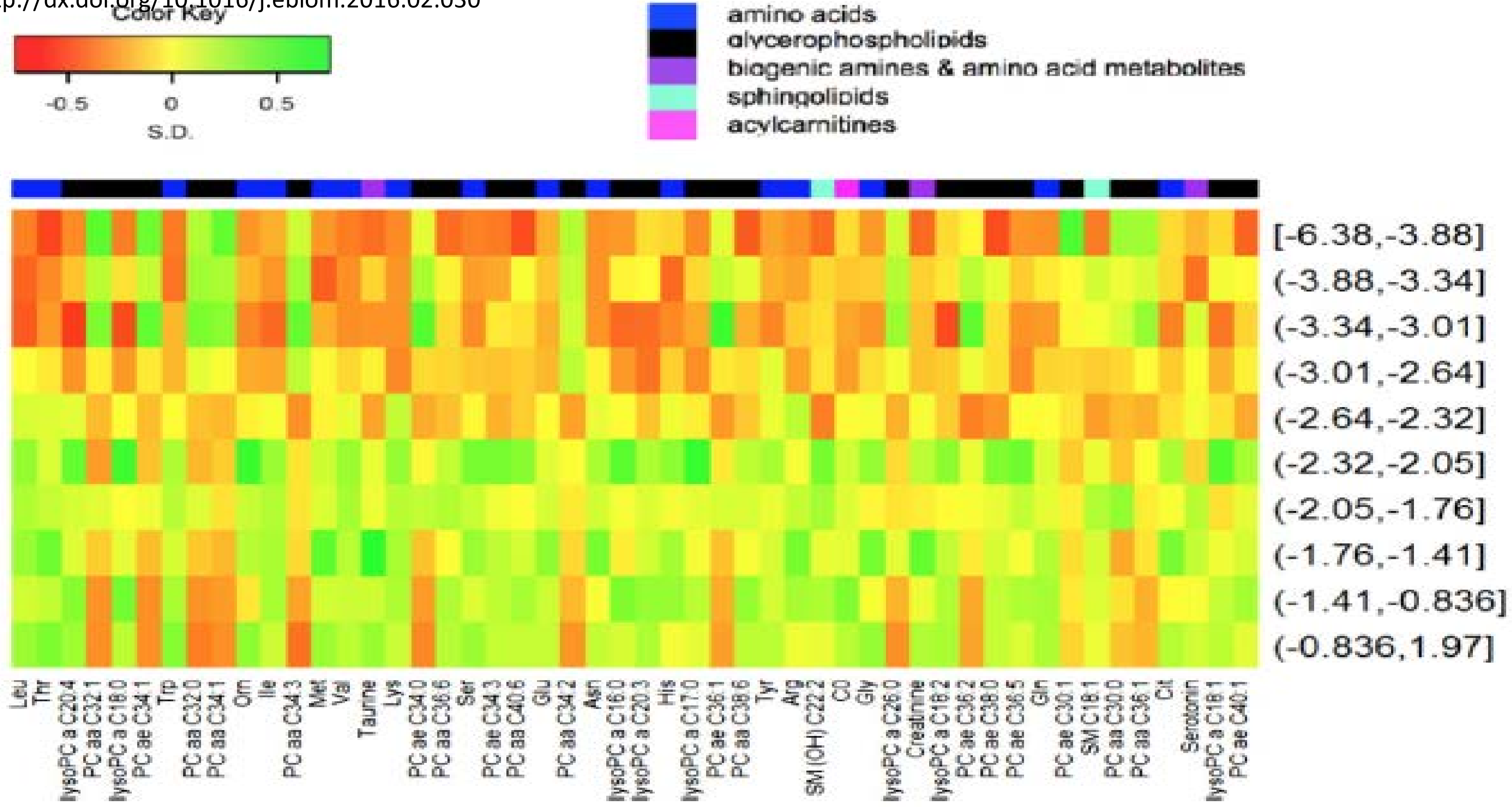


Fig. 1. Heat map showing the relationship of the fifty most significant serum metabolites by HAZ, adjusted by age and gender. HAZ divided into deciles. Metabolites are ordered by p-values, with the lowest p-values on the left side. Standard three-letter abbreviations used for amino acids. Abbreviations for lipid nomenclature are described in the methods section. Other abbreviation: carnitine (C0).

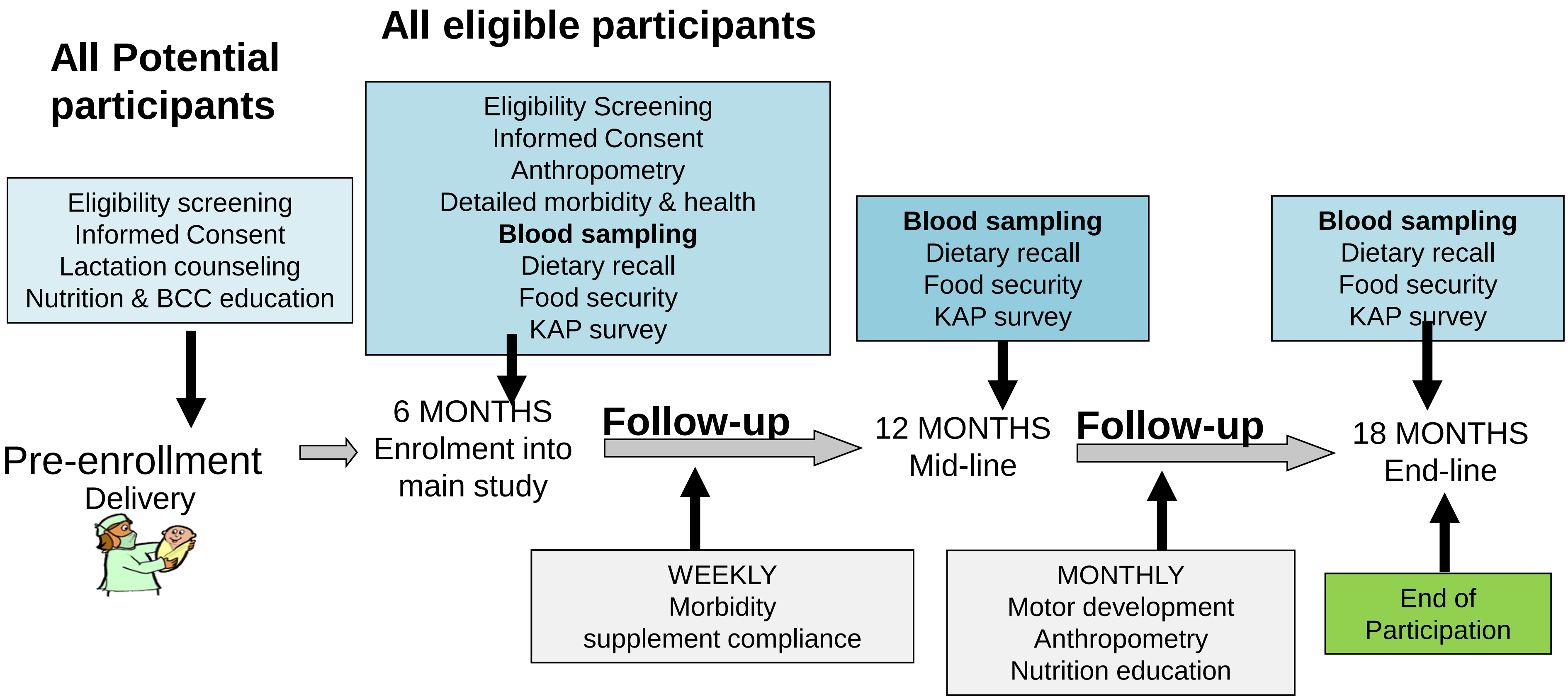
Effect of a macronutrient- micronutrient supplement on linear growth

- Single blind community cluster randomized trial, no SAM, no severe anemia, no congenital conditions, singleton at birth

- 38 communities randomly assigned
  - Macro and Micronutrient powder (+Nutrition Education)- KokoPlus
  - Micronutrient Powder (+ Nutrition Education)
  - Nutrition Education
- Sample size= 903 (301 per group)

http://cdn.ghanaweb.com/imagelib/pics/59387424.gif

Data collection points



Macronutrient Composition

Nutrient	Amount	Percentage in Sachet	Complementary Food Reqt*	% Req Met
Amount per sachet (g)	15.0			
Total Energy (Kcal)	66.5		220	
Protein (g)	2.9	18%		
Utilizable protein (g)	2.6	16%	4.25	62%
Carbohydrate (g)	8.0	48%		
Fat (g)	2.6	36%	6.4	41%
PUFA				
Omega-6 (18:2)	0.9	12%		
Omega 3 (18:3)	0.1	2%		
Proportion of Omega 6 to 3		8.12		

* Requirement for complementary food: Guidelines for Complementary Foods- WHO 2003

Amino Acid Composition

Amino Acids	Amount	Requirement*	% Met	Mg/g protein	AA Score
Tryptophan g	0.04	0.08	54%	16.2	2.19
Threonine g	0.13	0.29	45%	48.4	1.79
Isoluecine g	0.14	0.32	45%	54.01	1.74
Leucine g	0.24	0.64	38%	90.68	1.44
Lysine g	0.31	0.55	56%	116.59	2.24
SAA (Meth +Cys)	0.09	0.27	33%	32.94	1.27
Histidine g	0.08	0.19	43%	30.06	1.67
Valine g	0.15	0.43	35%	55.6	1.32

* Requirement for the day for infants aged 6-24 months (WHO 2007)

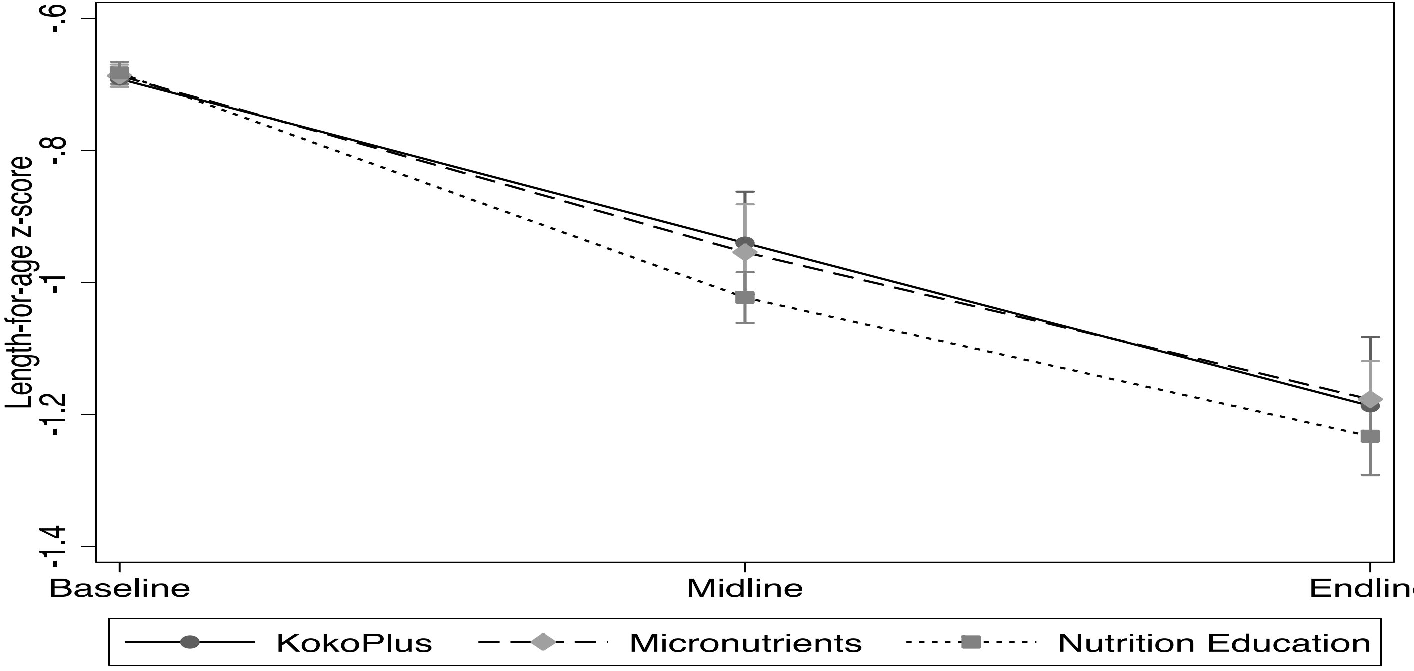
Micronutrient Composition

Micronutrient Composition	Amount	Requirement	% Met
Vitamin A µg RE	200	400	50%
Folic acid µg	45	90	50%
Niacin mg	3.1	6	52%
Riboflavin mg	0.3	0.5	63%
Thiamin mg	0.3	0.5	63%
Vitamin B 6 mg	0.3	0.5	56%
Vitamin B 12 µg	0.5	0.9	50%
Vitamin C mg	30.4	30	101%
Calcium mg	220	500	44%
Iron mg	7	11.6	60%
Phosphorus mg	154	100	154%
Zinc mg	2.4	4.1	59%
Choline Mg	62.5	45.9	136%
Vitamin D µg	2.5	5	50%
Vitamin E mg	2.7	5	54%
Iodide mg	0	0.1	50%
Vitamin K µg	11	15	73%

Baseline descriptives

- No significant difference
  - Anthropometry: Length for age, weight for length and weight for age at baseline
  - Biochemistry: Serum ferritin (adjusted for inflammation), serum retinol binding protein, hemoglobin, serum zinc, plasma amino acids
  - Inflammation: C-reactive protein and Alpha glycoprotein
  - Growth biomarkers: serum IGF-1 and serum cortisol

Mean length-for-age z-scores of children at baseline (6 months), midline (12 months) and endline (18 months), by study group[1]



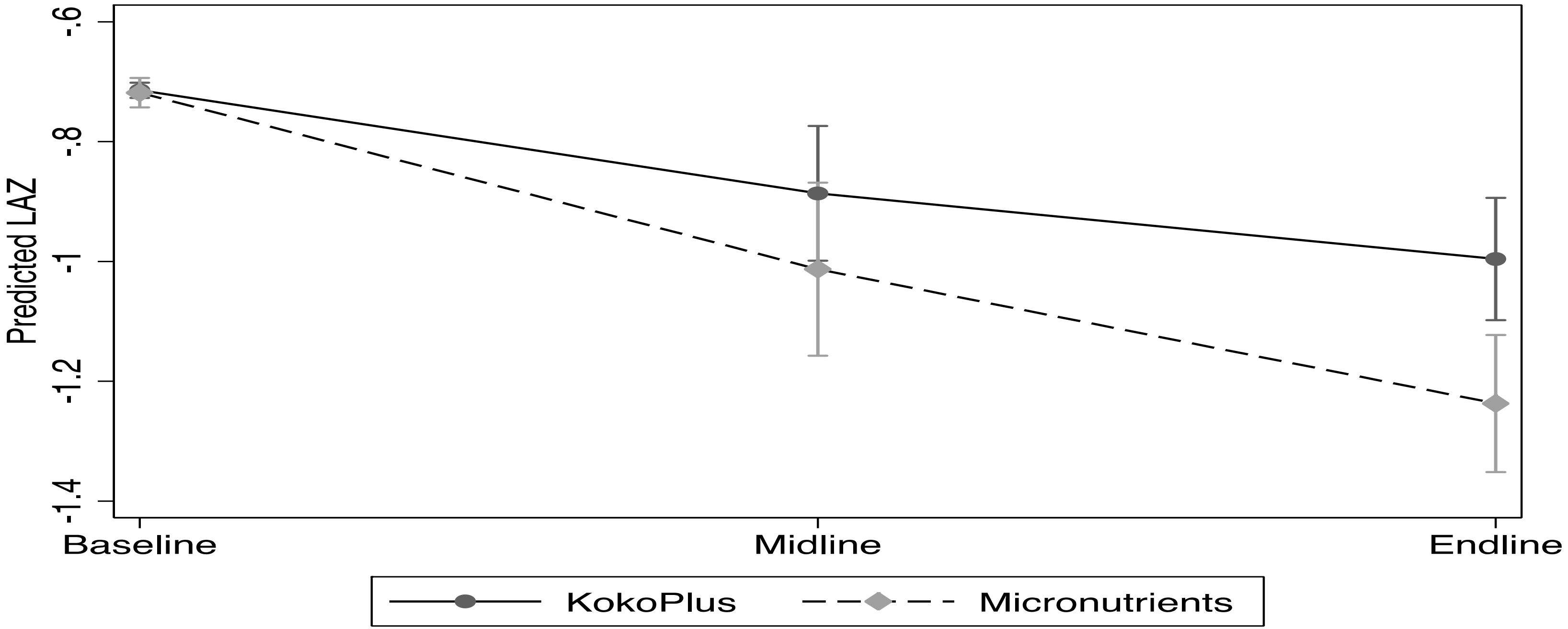
[1] Mixed effects linear model adjusted for fixed effects of baseline LAZ and mother's height, and random effects of study cluster and subject to account for repeated measures

		n	KokoPlus	Micronutrients	Nutrition Education
Acute Infection, prevalence (SE)		2345	**0.18 (0.031)**	0.215 (0.018)	**0.249 (0.021)**
	Difference (95% CI)			0.035 (-0.031, 0.101)	**0.069* (0.002, 0.136)**
Adjusted Ferritin, mean (SE)		2298	34.52 (1.712)	35.82 (2.83)	30.649 (2.192)
	Difference (95% CI)			1.295 (-5.17, 7.77)	-3.873 (-9.333, 1.587)
Adjusted Hb, mean (SE)	No Acute infection	**2178**	**114.02 (1.87)**	**107.791 (2.52)**	***109.88 (0.99)***
	Difference (95% CI)			**-6.228* (-12.37, -0.09)**	***-4.14# (-8.29, 0.012)***
	With acute infection	2178	102.18 (3.4)	102.853 (3.5)	103.901 (2.1)
	Difference (95% CI)			0.672 (-8.91, 10.26)	1.72 (-6.13, 9.57)

Delivery and reported consumption of supplement and overall compliance during the trial period, in the KokoPlus and Micronutrient Powder study arms

Supplement exposure	KokoPlus		Micronutrients	
	Mean (SD)	Median	Mean (SD)	Median
Number of supplements delivered to subjects	225.1 (77.6)	238	225.9 (79.5)	231
Percent of intended delivery (365 supplements)	61.7	65.2	61.9	63.3
Number of supplements reportedly consumed	196.1 (81.9)	207	201.9 (81.7)	201
Percent compliance (number of supplements consumed as a percent of those delivered)	86.2 (17)	93.3	88.4 (15)	93.6

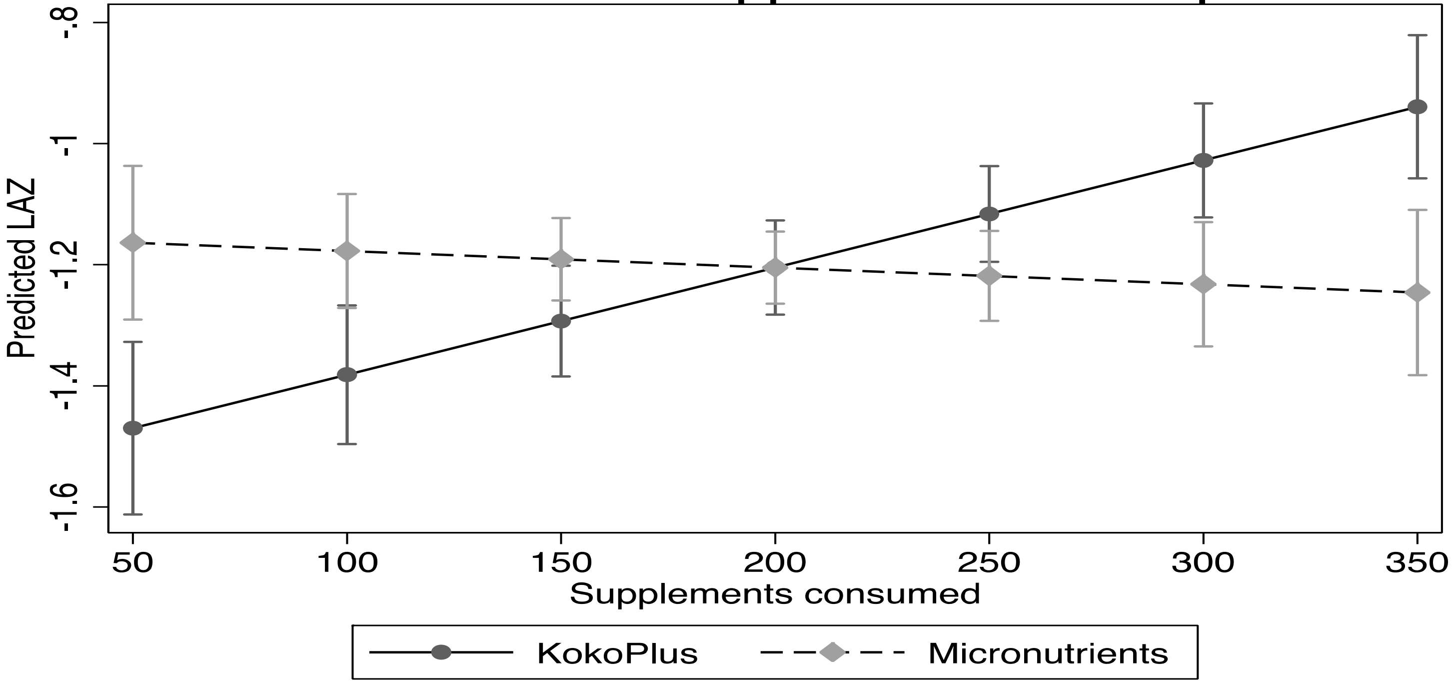
Predicted length-for-age z-scores of children in KokoPlus versus Micronutrient study arms at baseline (6 months), midline (12 months) and endline (18 months), modeling a scenario in which a mean of 314 supplements were consumed [1,2]



[1] Mixed effects linear model adjusted for fixed effects of baseline LAZ and mother's height, and random effects of study cluster and subject to account for repeated measures.

[2] Mean compliance was 86%; for this model it was assumed that if all 365 supplements were delivered average consumption would be 314 sachets (86% of intended delivery).

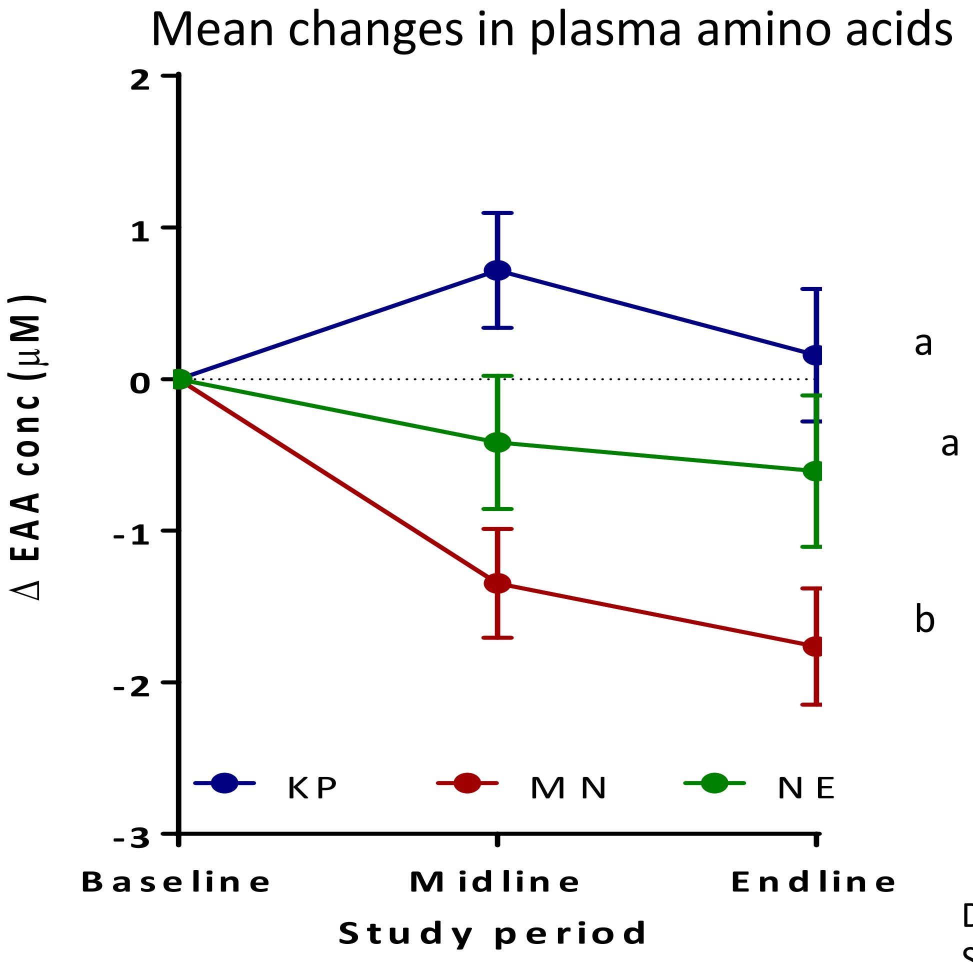
Predicted length-for-age z-scores of children in KokoPlus or Micronutrient study arms at endline (18 months), modeling different levels of supplement consumption[1]



[1] Mixed effects linear model adjusted for fixed effects of baseline LAZ and mother's height, and random effects of study cluster and subject to account for repeated measures.

Essential amino acids (EAAs)

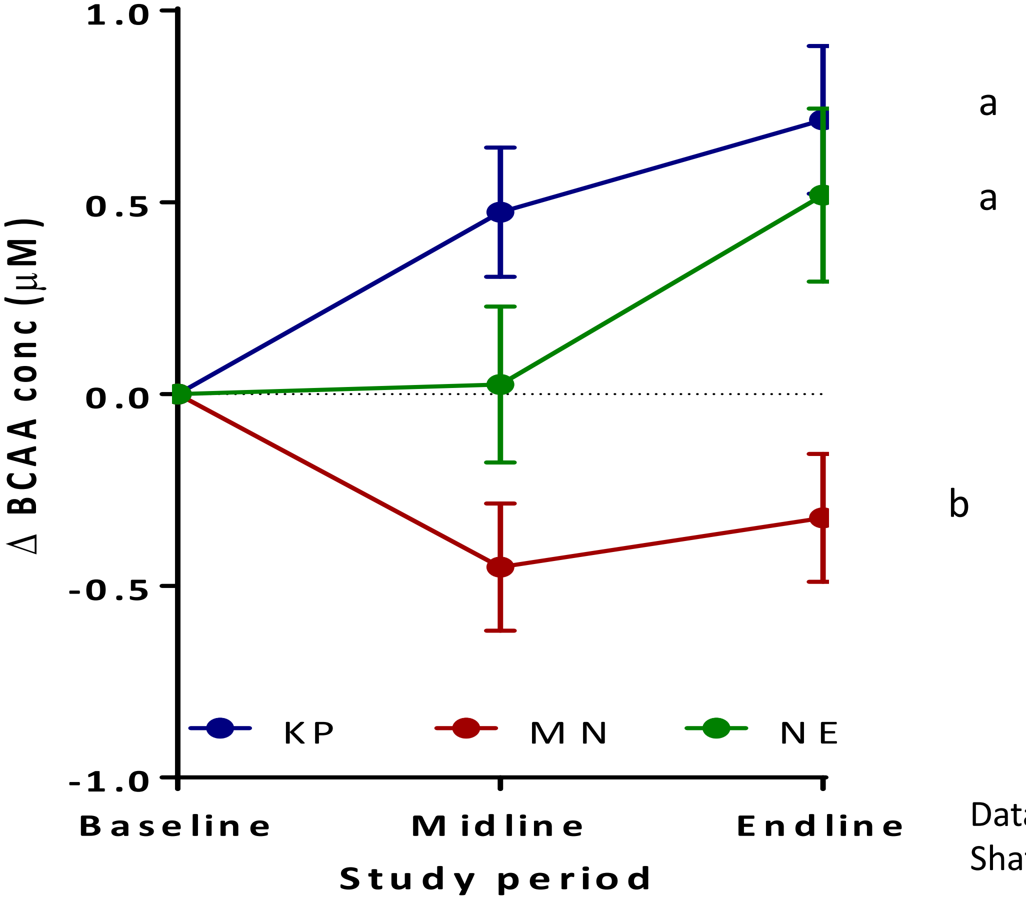
(Lys, BCAAs, Phe, Thr, Trp, His, Met)



Data are expressed as Mean ± SE
Shaffer multiple comparisons test
($p<0.05$: different letter code)

Branched amino acids (BCAAs)
(Val, Leu, Ile)

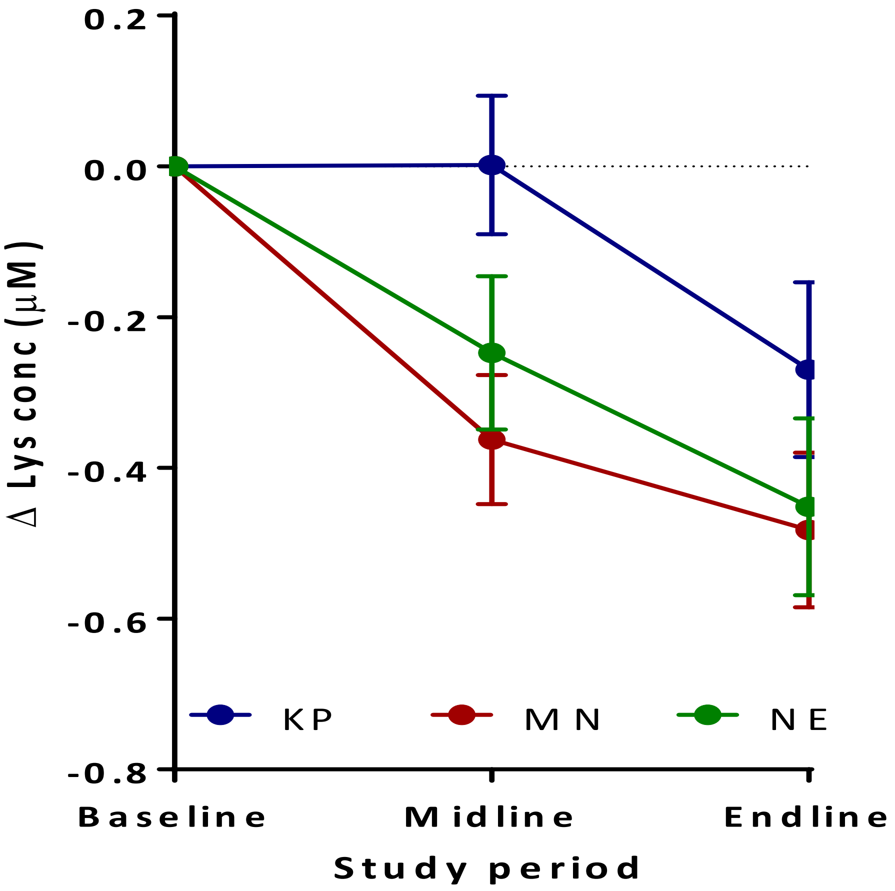
Mean changes in plasma amino acids



Data are expressed as Mean ± SE
Shaffer multiple comparisons test
($p<0.05$: different letter code)

Mean changes in plasma amino acids

Lysine



Data are expressed as
Mean ± SE
Shaffer multiple
comparisons test
($p<0.05$: different
letter code)

KP vs Mn p value=0.053

Postulated pathways

- Amino acids affect growth hormone release, IGF-1
  - Stimulates longitudinal bone growth – proximal growth plate of the tibia and stimulate generation of chondrocytes
- Link to MTORC 1 pathway – a protein complex that functions as a nutrient/energy/redox sensor – facilitates macromolecule biosynthesis, cell cycle progression, growth, metabolism and inhibits autophagy
- General Control non de-repressible 2 pathway- a nutrient sensing pathway that inhibits cellular protein synthesis with AA depletion as a trigger

Conclusion

- Understanding of protein and energy interactions
  - Though not so much about increased protein needs when there is an energy deficit or infection/inflammation

- Better understanding of protein and amino acid requirements- there is need for new data on digestibility (protein quality evaluation)

- Within the context of over and under nutrition, exploring quality over quantity becomes very crucial

- Essential and conditionally essential amino acids and their potential individual functional roles is emerging but amino acid metabolism is complex and their role in growth, metabolism, health and disease is complex

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