LMU KLINIKUM DER UNIVERSITÄT MÜNCHEN
CAMPUS GROSSHADERN
CAMPUS INNENSTADT
DR. VON HAUNER CHILDREN`S HOSPITAL

ENERGETIC EFFICIENCY OF INFANT FORMULAE

Manja Fleddermann, Hans Demmelmair and Berthold Koletzko

BACKGROUND

- Compared to breastfed infants, formula-fed infants:
  - Have 15.5% (age: 1 month), 7.6% (age:6 months) higher energy requirement (Butte 2005)
  - Consume up to 26% more energy (Butte et al. 1990)
  - Have higher weight gain during the first 6 months (Young et al. 2012, Koletzko et al. 2009)

BACKGROUND

- Parameter for the evaluation of growth and nutritional intake: **Energetic efficiency (EE)**

$$EE_{weight} = \frac{Weight\ gain\ [g/d]}{Energy\ intake\ [kcal/d]}$$

$$EE_{length} = \frac{Length\ gain\ [mm/d]}{Energy\ intake\ [kcal/d]}$$

(Fleddermann et al. 2013)

- Breastfed infants have an up to 15% higher energetic efficiency than formula fed infants (Hanning et al. 1992, Butte et al. 1990)

INFANT FORMULA COMPOSITION AND EE

Randomized control trials

Infant formula composition affects energetic efficiency for growth: The BeMIM study, a randomized controlled trial☆

Manja Fleddermann [a], Hans Demmelmair [a], Veit Grote [a], Tatjana Nikolic [b], Branka Trisic [c], Berthold Koletzko [a,*]

[a] Dr. von Hauner Children's Hospital, University of Munich Medical Centre, Munich, Germany
[b] Institute for Gynecology and Obstetrics, Clinical Centre of Serbia, Belgrade, Serbia
[c] HiPP Study Center, Belgrade, Serbia

Age	n	Infant formulae	Weight gain (g/d)	Length gain (mm/d)	Energy intake (kcal/d)	EE_{weight} (g/100kcal)	EE_{length} (mm/100kcal)
birth to 120 d	103	IF (1.89 g protein/100mL, 3.6g lipids, 7.5 g carbohydrates, higher alpha-lactalbumin content, 10.7 mg/100kcal of ARA and DHA)	30.2	1.1	490	6.45	0.23
	104	CF (2.2 g protein/100mL, 3.3 g lipids, 7.8 g carbohydrates, lower alpha-lactalbumin content, no LC-PUFA)	28.3	1.0	531	5.67	0.20
					ΔEE:	**12.1%**	**13.0%**

OBJECTIVE

- Limited data on the influence of formula composition on the energetic efficiency of infant formulae

- Identification of the relationship between macronutrients in infant formula and energetic efficiency
  - Protein?
  - Carbohydrate?
  - Fat?

  } Content and source

- Method: review of published studies

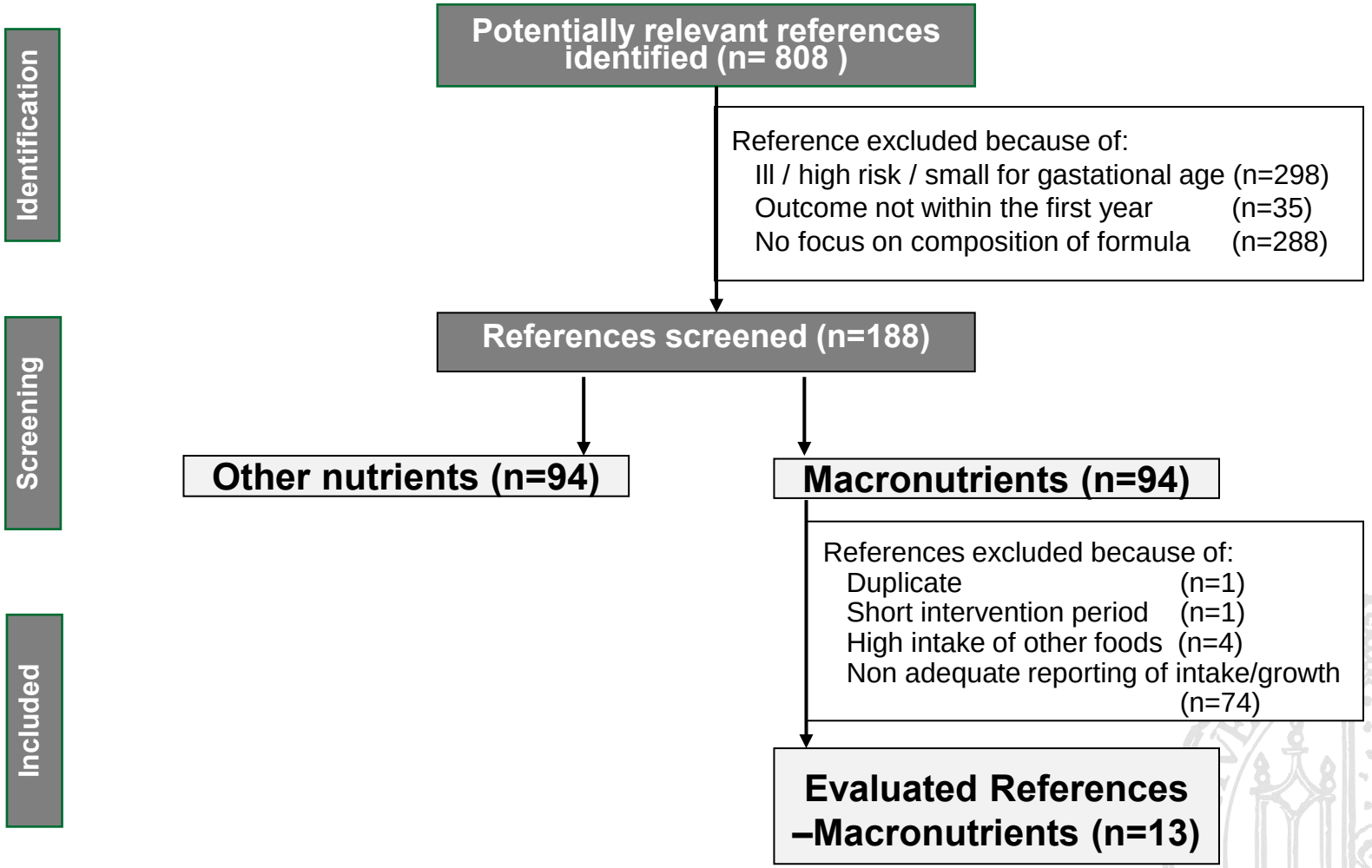
LITERATURE SEARCH

- Pubmed search for: **Clinical trials** in **humans** with focus on **infant formula**, where a **full text** is available in **English**

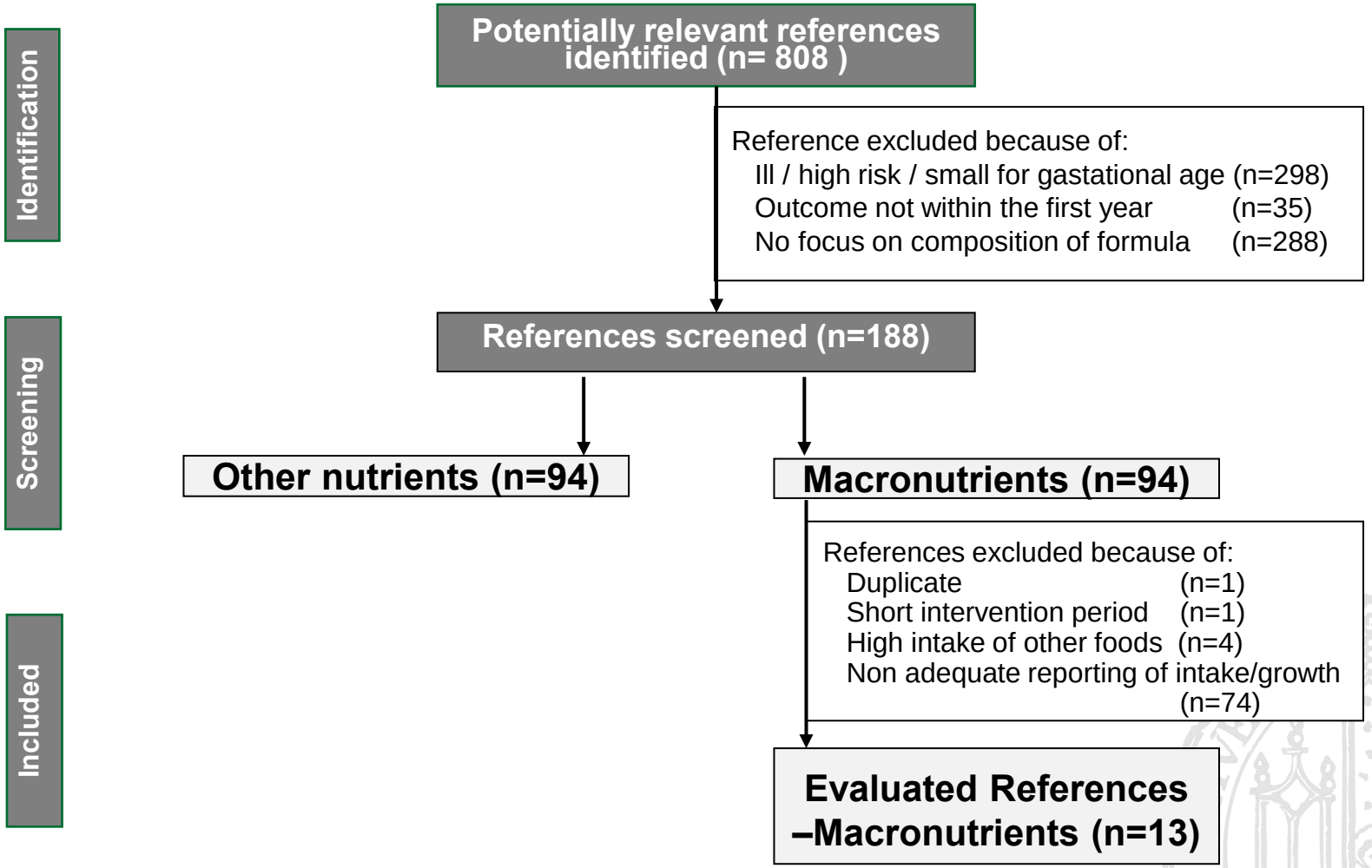
  - Inclusion: term infants,
    normal weight,
    no increased allergy risk
    fully formula-fed (<25% other foods)

  - Exclusion: **premature**/**preterm** infants

LITERATURE SEARCH



LITERATURE SEARCH



PROTEIN COMPOSITION AND EE

α-Lactalbumin-rich infant formula fed to healthy term infants in a multicenter study: plasma essential amino acids and gastrointestinal tolerance

AM Davis[1], BJ Harris[1], EL Lien[2], K Pramuk[1] and J Trabulsi[1]

[1]*Wyeth, Collegeville, PA, USA and* [2]*Department of Food Science and Human Nutrition, University of Illinois, Urbana, IL, USA*

Age	n	Infant formulae	Weight gain (g/d)	Length gain (mm/d)	Energy intake (kcal/d)	EE_{weight} (g/100kcal)	EE_{length} (mm/100kcal)
birth to 8 wk	64	SF (1.5g protein/100mL, alpha-lactalbumin 1.3 g/L)	35.0	1.31	718	4.87	0.18
	64	EF (1.4 g protein/100 mL, alpha-lactalbumin 2.2 g/L)	35.9	1.29	661	5.43	0.20
					ΔEE:	**10.3%**	**10.0%**

CARBOHYDRATE/PROTEIN COMPOSITION AND EE

Randomized Double-Blind Study of the Nutritional Efficacy and Bifidogenicity of a New Infant Formula Containing Partially Hydrolyzed Protein, a High β-Palmitic Acid Level, and Nondigestible Oligosaccharides

*Hansjörg Schmelzle, †Stefan Wirth, ‡Heino Skopnik, §Michael Radke, ‖Jan Knol, ¶Heinz-Michael Böckler, ¶Anja Brönstrup, ¶John Wells, and *Christoph Fusch

*Centre for Paediatric Medicine, University of Greifswald, Greifswald, Germany; †Children's Hospital, Wuppertal, Germany; ‡Municipal Hospital, Worms, Germany; §Ernst von Bergmann Clinic, Hospital for Paediatric Medicine, Potsdam, Germany; ‖Numico Research BV, Wageningen, The Netherlands; and ¶Numico Infant Nutrition Group, Friedrichsdorf, Germany

Age	n	Infant formulae	Weight gain (g/d)	Length gain (mm/d)	Energy intake (kcal/d)	EE_{weight} (g/100kcal)	EE_{length} (mm/100kcal)
birth to 3 mo	52	1.5 g protein/100mL (60%whey/ 40%casein), lactose only, 67 kcal/100mL	32.7	1.20	510	6.41	0.24
	49	1.7 g protein/100mL (100% hydrolyzed whey protein), lactose/maltodextrin/ starch, prebiotics, 70 kcal/100mL	35.6	1.30	490	7.26	0.27
					ΔEE:	**11.7%**	**11.1%**

FAT COMPOSITION AND EE

J Pediatr Gastroenterol Nutr. 1996 Dec;23(5):553-60.

Structural position and amount of palmitic acid in infant formulas: effects on fat, fatty acid, and mineral balance.

Carnielli VP[1], Luijendijk IH, Van Goudoever JB, Sulkers EJ, Boerlage AA, Degenhart HJ, Sauer PJ.

Author information

[1]Department of Pediatrics, Sophia Children's Hospital, Rotterdam, The Netherlands.

Age	n	Infant formulae	Weight gain (g/d)	Length gain (mm/d)	Energy intake (kcal/d)	EE_{weight} (g/100kcal)	EE_{length} (mm/100kcal)
birth to 5 wk	n=9 (males)	regular formula (19.9% palmitic acids, 13% esterified to sn-2 position)	26.6	1.59	426	6.24	0.37
	n=9 (males)	beta formula (23.9% palmitic acid, 66% esterified to sn-2 position)	29.2	1.29	417	7.01	0.31
	n=9 (males)	intermediate formula (24% palmitic acid, 39% esterified to sn-2 position)	23.3	1.26	416	5.58	0.30
					ΔEE:	**11.0% 10.6%**	**16.2% 18.9%**

WHICH FACTORS INCREASE THE ENERGETIC EFFICIENCY FOR GROWTH?

-CONTENT OF MACRONUTRIENTS
-PROTEIN QUALITY
-CARBOHYDRATE COMPONENTS
-FAT QUALITY

FACTORS INCREASE THE ENERGETIC EFFICIENCY FOR GROWTH- CONTENT OF MACRONUTRIENTS

Autor /Journal /Year	Focus	ΔEE_{weight} (%)	ΔEE_{length} (%)	Energy	Protein	Carbohydrate	Fat
Fleddermann et al. / Clin Nutr / 2013	Protein	12.1	13	-	↓	↓	↑
Fomon et al. / Am J Clin Nutr /1995	Protein	2.1	-	-	↑	↓	-
Turck et al. / JPGN / 2006	Protein	6.9	-	-	↓	↓	↑
Rozé et al. / Br J Nutr / 2012	Protein	11.1	5	↓	↓	↓	↑
Davis et al. / Eur J Clin Nutr / 2008	Protein	10.3	10	-	↓	-	-
Räihä et al. / JPGN / 2002	Protein	8 1	- 5	-	↓	↑	-
Vandenplas et al. / JPGN / 1993	Protein	11.9	16.6	↑	↑	↓	-
Hanning et al. / Am J Clin Nutr / 1992	Protein	13.8	8.3	↑	↓	NA	NA
Schmelzle H et al. / JPGN / 2003	Protein	11.7	11.1	↑	↑	↑	↓
Kennedy et al. / Am J Clin Nutr / 1999	Fat	2.5	4	↑	-	-	↑
Carnielli et al. /JPGN / 1995	Fat	10.6 11.0	18.9 16.2	-	-	-	↓

FACTORS INCREASE THE ENERGETIC EFFICIENCY FOR GROWTH- CONTENT OF MACRONUTRIENTS

Autor /Journal /Year	Focus	ΔEE_{weight} (%)	ΔEE_{length} (%)	Energy	Protein	Carbohydrate	Fat
Fleddermann et al. / Clin Nutr / 2013	Protein	12.1	13	-	↓	↓	↑
Fomon et al. / Am J Clin Nutr /1995	Protein	2.1	-	-	↑	↓	-
Turck et al. / JPGN / 2006	Protein	6.9	-	-	↓	↓	↑
Rozé et al. / Br J Nutr / 2012	Protein	11.1	5	↓	↓	↓	↑
Davis et al. / Eur J Clin Nutr / 2008	Protein	10.3	10	-	↓	-	-
Räihä et al. / JPGN / 2002	Protein	8 1	- 5	-	↓	↑	-
Vandenplas et al. / JPGN / 1993	Protein	11.9	16.6	↑	↑	↓	-
Hanning et al. / Am J Clin Nutr / 1992	Protein	13.8	8.3	↑	↓	NA	NA
Schmelzle H et al. / JPGN / 2003	Protein	11.7	11.1	↑	↑	↑	↓
Kennedy et al. / Am J Clin Nutr / 1999	Fat	2.5	4	↑	-	-	↑
Carnielli et al. /JPGN / 1995	Fat	10.6 11.0	18.9 16.2	-	-	-	↓

FACTORS INCREASE THE ENERGETIC EFFICIENCY FOR GROWTH- CONTENT OF MACRONUTRIENTS

Autor /Journal /Year	Focus	ΔEE_{weight} (%)	ΔEE_{length} (%)	Energy	Protein	Carbohydrate	Fat
Fleddermann et al. / Clin Nutr / 2013	Protein	12.1	13	-	↓	↓	↑
Fomon et al. / Am J Clin Nutr /1995	Protein	2.1	-	-	↑	↓	-
Turck et al. / JPGN / 2006	Protein	6.9	-	-	↓	↓	↑
Rozé et al. / Br J Nutr / 2012	Protein	11.1	5	↓	↓	↓	↑
Davis et al. / Eur J Clin Nutr / 2008	Protein	10.3	10	-	↓	-	-
Räihä et al. / JPGN / 2002	Protein	8 1	- 5	-	↓	↑	-
Vandenplas et al. / JPGN / 1993	Protein	11.9	16.6	↑	↑	↓	-
Hanning et al. / Am J Clin Nutr / 1992	Protein	13.8	8.3	↑	↓	NA	NA
Schmelzle H et al. / JPGN / 2003	Protein	11.7	11.1	↑	↑	↑	↓
Kennedy et al. / Am J Clin Nutr / 1999	Fat	2.5	4	↑	-	-	↑
Carnielli et al. /JPGN / 1995	Fat	10.6 11.0	18.9 16.2	-	-	-	↓

FACTORS INCREASE THE ENERGETIC EFFICIENCY FOR GROWTH- CONTENT OF MACRONUTRIENTS

Autor /Journal /Year	Focus	ΔEE_{weight} (%)	ΔEE_{length} (%)	Energy	Protein	Carbohydrate	Fat
Fleddermann et al. / Clin Nutr / 2013	Protein	12.1	13	-	↓	↓	↑
Fomon et al. / Am J Clin Nutr /1995	Protein	2.1	-	-	↑	↓	-
Turck et al. / JPGN / 2006	Protein	6.9	-	-	↓	↓	↑
Rozé et al. / Br J Nutr / 2012	Protein	11.1	5	↓	↓	↓	↑
Davis et al. / Eur J Clin Nutr / 2008	Protein	10.3	10	-	↓	-	-
Räihä et al. / JPGN / 2002	Protein	8 1	- 5	-	↓	↑	-
Vandenplas et al. / JPGN / 1993	Protein	11.9	16.6	↑	↑	↓	-
Hanning et al. / Am J Clin Nutr / 1992	Protein	13.8	8.3	↑	↓	NA	NA
Schmelzle H et al. / JPGN / 2003	Protein	11.7	11.1	↑	↑	↑	↓
Kennedy et al. / Am J Clin Nutr / 1999	Fat	2.5	4	↑	-	-	↑
Carnielli et al. /JPGN / 1995	Fat	10.6 11.0	18.9 16.2	-	-	-	↓

WHICH FACTORS INCREASE THE ENERGETIC EFFICIENCY FOR GROWTH?

-CONTENT OF MACRONUTRIENTS
-PROTEIN QUALITY
-CARBOHYDRATE COMPONENTS
-FAT QUALITY

FACTORS INCREASE THE ENERGETIC EFFICIENCY FOR GROWTH- PROTEIN QUALITY

Autor /Journal /Year	Focus	ΔEE_{weight} (%)	ΔEE_{length} (%)	Protein quality
Fleddermann et al. / Clin Nutr / 2013	Protein	12.1	13	alpha-lactalbumin, Trp, Phe
Turck et al. / JPGN / 2006	Protein	6.9	-	whey
Rozé et al. / Br J Nutr / 2012	Protein	11.1	5	whey, alpha-lactalbumin
Davis et al. / Eur J Clin Nutr / 2008	Protein	10.3	10	alpha-lactalbumin
Räihä et al. / JPGN / 2002	Protein	8 1	- 5	modified sweet whey
Vandenplas et al. / JPGN / 1993	Protein	11.9	16.6	protein hydrolysate
Hanning et al. / Am J Clin Nutr / 1992	Protein	13.8	8.3	casein, Trp
Schmelzle H et al. / JPGN / 2003	Protein	11.7	11.1	hydrolyzed whey
Koo et al. / Pediatr / 2003	Fat	6.0	-	whey protein concentrate

PROTEIN AND ENERGETIC EFFICIENCY

- Changed protein composition enhance energetic efficiency:
  - Higher whey : casein ratio
  - Higher alpha-lactalbumin content
  - Higher tryptophan content

    (Tryptophan influence satiation and sleep-wake rhythm (Heine 1999))

WHICH FACTORS INCREASE THE ENERGETIC EFFICIENCY FOR GROWTH?

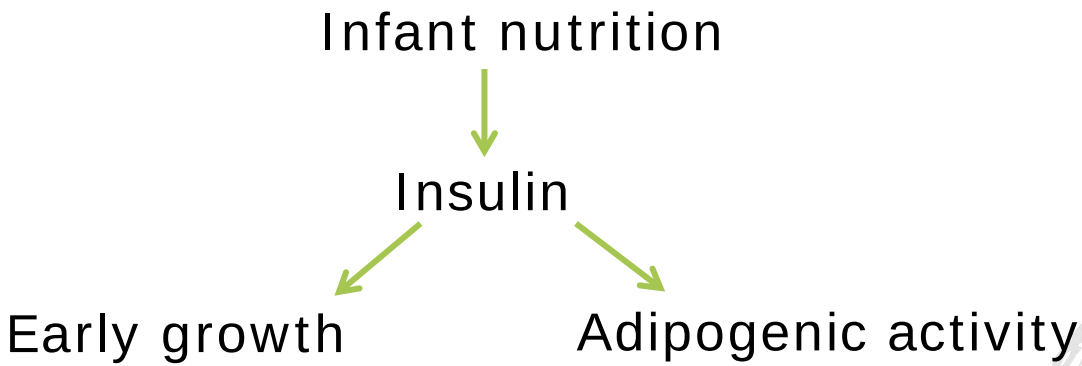
-CONTENT OF MACRONUTRIENTS
-PROTEIN QUALITY
-CARBOHYDRATE COMPONENTS
-FAT QUALITY

FACTORS INCREASE THE ENERGETIC EFFICIENCY FOR GROWTH- CARBOHYDRATE TYPE

Autor /Journal /Year	Focus	ΔEE_{weight} (%)	ΔEE_{length} (%)	Carbohydrate type
Turck et al. / JPGN / 2006	Protein	6.9	-	lactose
Schmelzle H et al. / JPGN / 2003	Protein	11.7	11.1	maltodextrin, starch
Huebi et al. / J Am Diet Assoc / 2000	Carbohydrate	2.2	4.3	maltodextrin, sucrose
Lasekan et al. / Clin Pediatr / 2011	Carbohydrate	3.1	-	cornsyrup, sucrose

CARBOHYDRATE AND ENERGETIC EFFICIENCY

- Low/no effects in studies with focus on carbohydrate composition
- Maltodextrin (Glycemic index (GI): 80-105), cornsyrup (GI: 115), sucrose (GI: 65) and starch (GI: 100) result in higher blood glucose levels than lactose (GI: 46)



Modified from Koletzko B et al. 2005

WHICH FACTORS INCREASE THE ENERGETIC EFFICIENCY FOR GROWTH?

-CONTENT OF MACRONUTRIENTS
-PROTEIN QUALITY
-CARBOHYDRATE COMPONENTS
-FAT QUALITY

FACTORS INCREASE THE ENERGETIC EFFICIENCY FOR GROWTH- FAT QUALITY

Autor /Journal /Year	Focus	ΔEE_{weight} (%)	ΔEE_{length} (%)	Fat quality
Fleddermann et al. / Clin Nutr / 2013	Protein	12.1	13	ARA, DHA
Rozé et al. / Br J Nutr / 2012	Protein	11.1	5	ARA, DHA
Koo et al. / Pediatr / 2003	Fat	6.0	-	Low palmitic acid content
Kennedy et al. / Am J Clin Nutr / 1999	Fat	2.5	4	Palmitic acid at sn-2 position
Carnielli et al. /JPGN / 1995	Fat	10.6 11.0	18.9 16.2	Palmitic acid at sn-2 position

FAT AND ENERGETIC EFFICIENCY

No cleavage of sn-2 ester bond during digestion by pancreatic lipase

↓

2-Acylglycerol

↓

Monoacylglycerols are more efficiently absorbed than as free fatty acids

↓

Increase of metabolizable energy

(Carnielli et al. 1995)

CONCLUSION

WHICH FACTORS COULD INCREASE THE ENERGETIC EFFICIENCY FOR GROWTH?

- Energy content of formula seems not to influence for energetic efficiency
- No clear evidence for an influence of protein/carbohydrate/fat content on energetic efficiency
- Small effects of carbohydrate type on energetic efficiency
- Strong indication, that:
  - Higher whey : casein ratio, alpha-lactalbumin, tryptophan enhance the energetic efficiency
  - Improved fat absorption seems to improve the energetic efficiency

				Factors increase the energetic efficiency for growth						
Autor /Journal /Year	**Fokus**	**ΔEE_{weight} (%)**	**ΔEE_{length} (%)**	**Energy content**	**Protein content**	**Protein quality**	**Carbo-hydrate content**	**Carbohydrate quality**	**Fat content**	**Fat quality**
Fleddermann et al. / Clin Nutr / 2013	Protein	12.1	13	-	↓	alpha-lactalbumin, Trp, Phe	↓	-	↑	ARA, DHA
Fomon et al. / Am J Clin Nutr /1995	Protein	2.1	-	-	↑	-	↓	-	-	-
Turck et al. / JPGN / 2006	Protein	6.9	-	-	↓	whey	↓	lactose	↑	-
Rozé et al. / Br J Nutr / 2012	Protein	11.1	5	↓	↓	whey, alpha-	↓	-	↑	ARA, DHA
Davis et / 2008										
Räihä et										
Vanden 1993										
Hanning Nutr / 1992	Protein	13.8	8.3	↑	↓	casein, Trp	NA	NA	NA	NA
Schmelzle H et al. / JPGN / 2003	Protein	11.7	11.1	↑	↑	hydrolyzed whey	↑	maltodextrin, starch	↓	-
Huebi et al. / J Am Diet Assoc / 2000	Carbohydrate	2.2	4.3	-	-	-	-	maltodextrin, sucrose	-	-
Lasekan et al. / Clin Pediatr / 2011	Carbohydrate	3.1	-	-	-	-	-	cornsyrup, sucrose	-	-
Koo et al. / Pediatr / 2003	Fat	6.0	-	-	-	whey protein concentrate	-	-	-	low palmitic acid content
Kennedy et al. / Am J Clin Nutr / 1999	Fat	2.5	4	↑	-	-	-	-	↑	palmitate in sn-2 position
Carnielli et al. /JPGN / 1995	Fat	10.6 11.0	18.9 16.2	-	-	-	-	-	↓	palmitic acid at sn-2 position

Thank you for your attention!

REFERENCES

Butte NF, Smith EO, Garza C. Energy utilization of breast-fed and formula-fed infants. Am J Clin Nutr. 1990;51(3):350-8.

Butte NF. Energy requirements of infants. Public Health Nutr. 2005;8(7A):953-67.

Carnielli VP, Luijendijk IH, Van Goudoever JB, Sulkers EJ, Boerlage AA, Degenhart HJ, et al. Structural position and amount of palmitic acid in infant formulas: effects on fat, fatty acid, and mineral balance. J Pediatr Gastroenterol Nutr. 1996;23(5):553-60.

Davis AM, Harris BJ, Lien EL, Pramuk K, Trabulsi J. Alpha-lactalbumin-rich infant formula fed to healthy term infants in a multicenter study: plasma essential amino acids and gastrointestinal tolerance. Eur J Clin Nutr. 2008;62(11):1294-301.

Fleddermann M, Demmelmair H, Grote V, Nikolic T, Trisic B, Koletzko B. Infant formula composition affects energetic efficiency for growth: The BeMIM study, a randomized controlled trial. Clin Nutr. 2013 doi: 10.1016/j.clnu.2013.12.007. [Epub ahead of print]

Fomon SJ, Ziegler EE, Nelson SE, Frantz JA. What is the safe protein-energy ratio for infant formulas? Am J Clin Nutr. 1995;62(2):358-63.

Hanning RM, Paes B, Atkinson SA. Protein metabolism and growth of term infants in response to a reduced-protein, 40:60 whey: casein formula with added tryptophan. Am J Clin Nutr. 1992;56(6):1004-11.

Heine WE. The significance of tryptophan in infant nutrition. Adv Exp Med Biol. 1999;467:705-10.

Heubi J, Karasov R, Reisinger K, Blatter M, Rosenberg L, Vanderhoof J, et al. Randomized multicenter trial documenting the efficacy and safety of a lactose-free and a lactose-containing formula for term infants. J Am Diet Assoc. 2000;100(2):212-7.

Kennedy K, Fewtrell MS, Morley R, Abbott R, Quinlan PT, Wells JC, et al. Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: effects on stool biochemistry, stool characteristics, and bone mineralization. Am J Clin Nutr. 1999;70(5):920-7.

Koo WW, Hammami M, Margeson DP, Nwaesei C, Montalto MB, Lasekan JB. Reduced bone mineralization in infants fed palm olein-containing formula: a randomized, double-blinded, prospective trial. Pediatrics. 2003;111(5 Pt 1):1017-23.

Koletzko B, von Kries R, Closa R, Escribano J, Scaglioni S, Giovannini M, 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(6):1836-45.

REFERENCES

Koletzko B, Broekaert I, Demmelmair H, Franke J, Hannibal I, Oberle D, Schiess S, Baumann BT, Verwied-Jorky S; EU Childhood Obesity Project. Protein intake in the first year of life: a risk factor for later obesity? The E.U. childhood obesity project. Adv Exp Med Biol. 2005;569:69-79.

Lasekan JB, Jacobs J, Reisinger KS, Montalto MB, Frantz MP, Blatter MM. Lactose-free milk protein-based infant formula: impact on growth and gastrointestinal tolerance in infants. Clin Pediatr. 2011;50(4):330-7.

Lonnerdal B. Infant formula and infant nutrition: bioactive proteins of human milk and implications for composition of infant formulas. Am J Clin Nutr. 2014;99(3):22.

Raiha NC, Fazzolari-Nesci A, Cajozzo C, Puccio G, Monestier A, Moro G, et al. Whey predominant, whey modified infant formula with protein/energy ratio of 1.8 g/100 kcal: adequate and safe for term infants from birth to four months. J Pediatr Gastroenterol Nutr. 2002;35(3):275-81.

Roze JC, Barbarot S, Butel MJ, Kapel N, Waligora-Dupriet AJ, De Montgolfier I, et al. An alpha-lactalbumin-enriched and symbiotic-supplemented v. a standard infant formula: a multicentre, double-blind, randomised trial. Br J Nutr. 2012;107(11):1616-22.

Schmelzle H, Wirth S, Skopnik H, Radke M, Knol J, Bockler HM, et al. Randomized double-blind study of the nutritional efficacy and bifidogenicity of a new infant formula containing partially hydrolyzed protein, a high beta-palmitic acid level, and nondigestible oligosaccharides. J Pediatr Gastroenterol Nutr. 2003;36(3):343-51.

Turck D, Grillon C, Lachambre E, Robiliard P, Beck L, Maurin JL, et al. Adequacy and safety of an infant formula with a protein/energy ratio of 1.8 g/100 kcal and enhanced protein efficiency for term infants during the first 4 months of life. J Pediatr Gastroenterol Nutr. 2006;43(3):364-71.

Vandenplas Y, Hauser B, Blecker U, Suys B, Peeters S, Keymolen K, et al. The nutritional value of a whey hydrolysate formula compared with a whey-predominant formula in healthy infants. J Pediatr Gastroenterol Nutr. 1993;17(1):92-6.

Young BE, Johnson SL, Krebs NF. Biological determinants linking infant weight gain and child obesity: current knowledge and future directions. Adv Nutr. 2012;3(5):675-86.