Impact of optimized protein in infant formula on the metabolic and nutritional health in infants: Systematic review with meta-analysis

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Abbreviations: Standardized mean differences (SMD), Randomized clinical trial (RCT), Primary Reporting Items for Systematic Reviews and Meta-Analyses Statement (PRISMA), Body Mass Index (BMI), Blood Urea Nitrogen (BUN), Insulin Growth Factor type 1 (IGF-1)

Contributors´ Statement

PGC is the main and corresponding author of the manuscript. He assembly the research protocol, contributes analyzing the evidence, write the paper, and review the statistical analysis

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JLC, AAO and LDG participate assembly the protocol, identify the evidence in data bases, and review the quality of evidence
SVC and CJG participate assembling the data base and made the statistical analyzing
GLV participate assembling the research protocol, contributes analyzing the evidence, and assembling the manuscript
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Abstract
Background. Increasing evidence demonstrate that concentration of protein in infant formula >1.9g/100Kcal with high levels of insulinogenic aminoacids is associated with accelerated weight gain, increased fat mass accumulation and risk of adiposity. Purpose of this study was to conduct a systematic review to determine the metabolic effects in infants feed with infant formula optimized in protein. Methods. Systematic review was conducted according PRISMA Statement. RCTs with one intervention group (infant formula with 1.6-1.9gr of protein/100Kcal) and at least one comparative control group (infant formula with >1.9gr of protein/100Kcal) were included. Standardized mean differences (SMD), through random model were calculated. Results. 15 RCT were included. Optimized protein in infant formula was associated with less gain of BMI at 24 months of follow-up (SMD -0.25, $IC_{95\%}$ -0.36 to -0.13, p 0.01) and less fat mass accumulation (SMD -0.68, $IC_{95\%}$ -0.98 to -0.37, p 0.01). Optimized protein was also associated to less gain of weight, weight/age Z-score, weight/length Z-score, BUN (mmol/dL) and IGF–1 (ng/ml). No effect on length/age Z-score was observed. Conclusions. Robust evidence showed optimized protein (1.6gr/100Kcal to 1.9gr/100Kcal) in infant formula is associated with metabolic benefits in infants with less weight gain, BMI and fat mass accumulation.

Key words: Optimized protein in formula, infants, obesity risk reduction.

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Background

Prevalence of overweight and obesity around the world has increased considerably in the last thirty years. As a consequence, Chronic Disease Related to Nutrition (CRN) is one of the most prevalent conditions, which represents a major challenge for public health given the broad spectrum of associated pathologies (Diabetes, Hypertension, others), which are intimately related to the 70% cases of dead in young adults. (1) Considering the unfavorable results obtained in the long term from programs focused on treating individuals when they already present varying degrees of obesity, preventive strategies are essential to avoid the development of obesity during growth. (2-3) The early metabolic programming hypothesis sustain that environmental factors that interact during the early stages of life, with the fetal tissues, particularly nutrition, influence health status during the all life. The evidence indicates that fetal and postnatal nutrition induces changes in gene expression and influences phenotypes through epigenetic modifications. (4) Recent evidence has shown the effects of prenatal programming as a predictor of obesity and NRWD in adulthood. It is known that early and excessive weight gain during the first two years of life are associated with a significant increase in the risk of obesity in later stages. (5-7) Some of these studies showed a lower risk of obesity in breastfeeding babies, which seems to be related to the amount and type of protein intake (8,9) We know breast milk is the ideal food in the first months of life and it has been well documented that the composition of this milk changes considerably in the course of the first year after delivery, mainly in protein composition and concentration. The amount of protein is on average 2.2 to 2.5g/100 kcal during the first days of lactation and decreases to 1.2-1.8g/100 kcal at six months of age. (10) Hester et al reported a protein content for colostrum (1 to 5 days) of 2.5g/100ml; for transition milk (6 to 14 days) of 1.7g/100ml; and for mature milk (> 14 days) of 1.3g/100 ml. (11) Unlike the dynamism observed in the composition of breast milk, infant formula maintains a fixed concentration of proteins, so that infants fed under this modality usually consume from 2.2 to 2.5g of protein/100kcal. (12,13) Since 2002, several researchers have shown that the high amounts of protein in infant formulas (>1.9g/100Kcal) are significantly associated

with a higher deposit of fat mass and with an increased risk of adiposity, due to an increase in insulinogenic aminoacids (valine, leucine and isoleucine) which favors the greater deposition of tissue, predominantly of fatty tissue; and insulin-growth factor type 1 (IGF-1), which favors by epigenetic mechanisms the greater expression of genes like MTORc, which are directly associated with an accelerated weight gain. (14-20) In 2002, Räihä et al., in a randomized controlled trial (RCT) using an optimized protein (modified in protein concentration and amino acid profile), demonstrated that infant feeding with this type of infant formula had weight gains similar to that of breastfed infants. (14) In 2009, B. Koletzko et al. Published a controlled clinical trial (RCT) with 1,678 infants from Belgium, Italy, Poland and Spain, who were fed with an infant formula with optimized protein, with concentrations of 1.77g/100 Kcal, demonstrating that at two years of follow-up, the Z-value for height and the body mass index were significantly lower in relation to the control group. (15) In 2011, P. Socha et al. showed that IGF-1 (0.600.16ng / mL vs. 0.430.13ng / mL, p <0.01) and C-peptide (26,916.0 vs 19,510.0) were significantly higher in children fed with high protein formulas compared to the values of the children fed formula with optimized protein. (16) In 2012 J. Escribano in a similar RCT showed that the high protein concentration in the infant formula was associated with a higher concentration of total fat mass evaluated by impedanciometry (fat mass Z value of 0.620.1) as contrasted with the infants fed with formula with modified protein or with breast milk (-0.050.1 and -1.00.2, respectively), p <0.05. (17) In 2014 M. Weber in a five-year follow-up of children included in the RCT by Koletzko et al. showed a greater risk of obesity of 2.43 (95% CI 1.9 to 2.7) in children fed infant formula with high protein (2.2g/100Kcal). (18) In 2014, J. Inostroza in a subsequent RCT with a protein value even lower than the previous trials (1.5g/100Kcal), showed a lower body mass index at 2 years of follow-up than that observed in infants fed with concentrations high protein levels (2.2g/100Kcal). (19) Considering this evidence, the objective of the present review was to evaluate, through the methodology of the systematic review, the existing publications to demonstrate the effect of optimized protein (1.5 to 1.9g / 100Kcal

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and modified aminoacids profile) compared to the use of high protein (> 1.9g / 100Kcal) in infant formulas on the metabolic and nutritional health of infants.

Methods

This study was conducted and reported according to the general guidelines recommended by the Primary Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) Statement. (Table 1) (20)

Study eligibility criteria

The *a priori* inclusion criteria for this meta-analysis were as follows: (1) randomized controlled trials with the unit of assignment at the participant level, (2) one intervention group (use of infant formula with 1.5 to 1.9gr of protein/100Kcal), (3) at least one comparative control group (infant formula with >1.9gr of protein/100Kcal), (4) use of intervention for at least one year (5) children less than one month at the moment they start to be feeded with the infant formula under research, (6) studies published in full in English or Spanish and any source (journal articles, dissertations, etc.) between January 1, 1988 and December 31, 2016, and (6) with at least one of metabolic or nutritional outcome [weight gain, lenght gain, body mass index (BMI) changes, fat mass, blood urea nitrogen (BUN) or IGF-1.

Data sources

Studies from January 1, 1988 and up to December 31, 2016 were retrieved using the following 11 electronic databases: (1) Medline, (2) CINAHL, (3) Scopus, (4) Academic Search Complete, (5) Educational Research Complete, (6) Web of Science, (7) Sport Discus, (8) ERIC, (9) LILACS, (10) Cochrane Central Register of Controlled Trials (CENTRAL) and (11) Proquest. All electronic searches were conducted by the second author with assistance from a Health Sciences librarian at Library in The Hospital General Dr. Manuel Gea Gonzalez, & National Pediatric Institute. Ministry of Health. Mexico. While the search strategies used varied per the requirements of the different databases searched, keywords centered around

the terms “low and protein and infant and formula” or “modified and protein and infant and formula”. The search strategies for all databases searched can be found in Additional file. (Appendix 1) After removing duplicates, the overall precision of the searches was calculated by dividing the number of studies included by the total number of studies screened. (21) In addition to electronic database searches, cross-referencing for potentially eligible meta-analyses from retrieved reviews was also conducted. All studies were stored in EndNote X8. (22)

Study selection

All studies were selected by two authors (LDG, AAO), independent of each other. Disagreements regarding the final list of studies to include were resolved by consensus. If consensus could not be reached, a third author (PGC) acted as an arbitrator. After an initial list of included studies was developed, the third author, an expert in nutrition and infant formula, reviewed the list for completeness. All included studies as well as a list of excluded studies, including reasons for exclusion, were stored in EndNote X8. (22)

Data abstraction

Prior to data abstraction, a detailed codebook that could hold at least 242 items per study was developed by all three members of the research team in Microsoft Excel 2016. (23) The major categories of variables that were coded included: (1) study characteristics, (2) subject characteristics, (3) level of protein (g/100Kcal), (4) primary outcomes and (5) secondary outcomes. The primary outcomes for this study was BMI z-score and fat mass accumulation. Secondary outcomes included weight gain, weight for age Z score, length for age Z score, weight for length Z score, BUN (mmol/dL) and IgFb1 (ng/ml) in serum. Missing primary outcome data were requested from the author(s). Publication bias was avoided by including data from all published study. Data abstraction was performed using the same procedure as the selection of studies.

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Risk of bias

The Cochrane Collaboration risk of bias instrument was used to assess bias across six categories: (1) random sequence generation, (2) allocation concealment, (3) blinding of participants and personnel, (4) blinding of outcome assessment, (5) incomplete outcome data, and (6) selective reporting. Each item was classified as having either a high, intermediate or low risk of bias. Assessment for risk of bias was limited to the primary outcomes of interest. (24) All assessments were performed by two authors (GLV, JLC), independent of each other. Both authors then met and reviewed every item for agreement. Disagreements were resolved by consensus.

Statistical analysis

Effect size (ES) changes in BMI z-score and fat mass were calculated by subtracting the difference in the optimized protein group from the difference in the control group and reported as standardized mean differences (SMD). Variances were calculated from the pooled standard deviations in the intervention and control groups. If standard deviations were not available, these were calculated from reported 95% confidence intervals (CI) or standard deviation (SD). All secondary outcomes were calculated using the same approach as for BMI primary outcomes. Random models that incorporate heterogeneity into the overall estimate were used to pool results for primary and secondary outcomes from each study. (25) Non-overlapping 95% CI were considered statistically significant. Heterogeneity of results between studies was examined using I^2. (26) Small-study effects (publication bias, etc.) were examined using the regression approach of Egger et al. (27,28)

Results

27 studies were evaluated. (Fig 1) (14-19, 29-37) After evaluation of bias, only 15 studies were included in meta-analysis. (Table 1) Causes for exclusion are summarized in table 2. The level of protein on included studies range from

1.6gr/100Kcal to 1.9gr/100Kcal in experimental group and 2.2 to 2.7gr/100Kcal in control group. Sample size in studies moves since small sample size cases (n1 10, vs. n2 10) to significant sample size (n1 298 vs. n2 313). On the maxim number of patient comparison, we have 1,074 children in optimized protein and 1,187 in high protein. Considering primary outcome, we identify a significant and positive effect of feeding babies with infant formula with optimized protein in terms of less gain of BMI at 24 months of follow-up (SMD -0.25, $IC_{95\%}$ -0.36 to -0.13, p 0.01) and less fat mass accumulation (SMD -0.68, $IC_{95\%}$ -0.98 to -0.37, p 0.01). In term of secondary outcomes optimized protein in infant formula was associated to less gain of weight (SMD -0.37, $IC_{95\%}$ -0.73 to -0.00, p 0.05), weight for age Z score (SMD -3.09, $IC_{95\%}$ -5.43 to -0.75, p 0.01), weight for length Z score(SMD -1.20, $IC_{95\%}$ -1.37 to -1.02, p 0.01), BUN (mmol/dL) (SMD -0.78, $IC_{95\%}$ -1.17 to -0.39, p 0.01), and IgFb1 (ng/ml) (SMD -1.17, $IC_{95\%}$ -2.18 to -0.16, p 0.01) in serum and with no effect on length for age Z score (SMD -1.17, $IC_{95\%}$ -0.41 to 0.07, p NS),

Discussion and recommendation

It is well established that obesity is a major public health burden in most developed countries, with a foretelling of risk in developing countries worldwide. Mexico is one of the countries with one of the most higher prevalence of obesity in children. (38) Recent evidence shows that in some extent obesity start on the first year of life. (39) Accumulating scientific evidence suggests that excessive protein intake during infancy may increase the subsequent risk of obesity. More than 10 years ago, Koletzko group showed in a large cross-sectional study including more than 9,000 children in Germany that breastfed children have a markedly reduced later risk of obesity at school age than previously bottle (formula)-fed children, with an inverse dose response relationship between breastfeeding duration and the adjusted odds ratios for obesity. (40) This finding was subsequently confirmed in numerous cohort studies and prior meta-analyses. (41-44) The results of this systematic review with meta-analysis where we included 15 RCTs offer robust evidence which support the association of feeding babies with a whey-predominant formula with a lower protein content (1.6 g/100 kcal to 1.9 g/100Kcal), lower than most currently available infant

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formulas) that is closer to that of human milk with an early growth comparable to the WHO growth standards and close to that of breastfed infants and with less BMI and fat mass accumulation. Our evidence is aligned with previous meta-analysis and IPD meta-analysis which had been reported similar results. (45,46) Regarding the mechanisms on how higher content of protein in infant formula increase the risk for adiposity and weight gain, diverse studies had demonstrated a higher formula protein supply to infants induced markedly elevated plasma concentrations of the branched-chain amino acids leucine, isoleucine and valine, along with slight elevations in other indispensable amino acids. (47) Increase of this aminoacids have been shown to be more potent stimulators of IGF-1 release than glucose in fetal rat islets. (48) Studies in 4-week-old rats showed that feeding a diet with 15 instead of 5% protein for only 1 week increased serum IGF-1 more than fourfold. (49) Amino acids also markedly influence insulin secretion with key regulatory roles for anabolic pathways and lipid deposition during early growth. (50,51) As it was already published one pathway through which amino acids and the growth factors insulin and IGF-1 could effectively modulate metabolic response and growth in children is the mammalian target of rapamycin (mTOR), a highly-conserved Ser/Thr kinase present in two structurally and functionally distinct complexes. The growth factors insulin and IGF-1 stimulate mTORC2 via an unknown pathway, and mTORC1 via phosphoinositide 3-kinase (PI3K) and Akt inducing the mTORC1 activator Rheb. Amino acids enhance ATP loading of RAG proteins and RAG-GTPases, which interact with Rheb and activate mTORC1. (52) Of importance, full activation of mTORC1 is only achieved through the synergistic action of both growth factors and amino acids, while a low energy supply downregulates mTORC1. (53) What this systematic review add is the inclusion of recently evidence (36,37) which extends the range of clinically and biological effects of optimized protein from 1.8 to 1.6-1.9g / 100Kcal in infants. While we wait for new evidence to be published, we are able to conclude that feed the infant with 1.6 to 1.9g/100Kcal of protein in infant formulas could be significantly associated with a lower body mass index and lower fat mass gain.

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