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Essential Nutrients in Paediatric Nutritional Supplementation

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Am J Clin Nutr. 2016;103(3):926-33.

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Effects of supplementation with iron salts on ferritin concentration in schoolchildren
Nutr J. 2014;13:71.

Calcium and vitamin D intakes in children: a randomized controlled trial
BMC Pediatr. 2013;13:86.

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Use of whey protein and whey permeate for the improvement of recovery rates in children with moderate acute malnutrition

Heather C. Stobaugh[1,2], Kelsey N. Ryan[2], Julie A. Kennedy[2], Jennifer B. Grise[2], Audrey H. Crocker[2], Chrissie Thakwalakwa[3], Patricia E. Litkowski[4], Kenneth M. Maleta[3], Mark J. Manary[2,3,6] and Indi Trehan[2,5]

[1]Friedman School of Nutrition Science and Policy. Tufts University. Boston, Massachusetts (USA). | [2]Department of Pediatrics. Washington University in St. Louis. Missouri (USA). | [3]Department of Community Health. University of Malawi. Blantyre (Malawi). | [4]Department of Medicine. Washington University in St. Louis. Missouri (USA). | [5]Department of Paediatrics and Child Health. University of Malawi. Blantyre (Malawi). | [6]Children's Nutrition Research Center. Baylor College of Medicine. Houston, Texas (USA).

Introduction

Moderate acute malnutrition (MAM) in children is treated with a variety of nutritional and dietary supplements based on peanut or soy protein or, more recently, whey proteins.

Although we know that milk protein is important for growth, there is insufficient evidence as to its usefulness for the treatment of MAM. According to the available studies, milk protein produces better results than vegetable proteins, as it increases lean mass, accelerates growth and improves recovery in the malnourished population. In fact, **the use of whey protein has been linked to muscle recovery, bone growth, immune function and intestinal integrity**.

A 12-week randomised double-blind study was carried out to assess the efficacy in the treatment of children with MAM of two nutritional supplements – a soy protein-based treatment and a treatment based on whey protein.

Material and Methods

The main aim of the study was to bring about recovery from MAM, defined as achieving a mid-upper arm circumference of ≥ 12.5 cm without peripheral oedema after 12 weeks of treatment.

The study contained 1086 children who were given soy protein supplements (energy: 559.52 kcal; total protein: 17.06 g; protein digestibility-corrected amino acid score: 0.78), and 1144 who were given whey supplements (energy: 516.34 kcal, total protein: 11.42 g, protein digestibility-corrected amino acid score: 1). It is worth noting that the soy supplement provided approximately 50% more total protein than the whey supplement. The children were monitored every fortnight for 12 weeks.

Whey protein leads to better recovery rates and growth than soy supplements.

Results

The two groups in the study had similar baseline characteristics.

After 12 weeks of treatment, recovery was seen in 83.9% of the children in the whey supplementation group and in 80.5% of those in the soy protein group (relative risk: 1.043; confidence interval of 95%: 1.003, 1.084; $p < 0.04$).

At the end of the study, the whey supplementation group had a significantly better average mid-upper arm circumference than the soy group ($p < 0.009$) (Fig. 1). Furthermore, **the children who had been given whey supplementation gained more weight in the first 2-4 weeks** ($p < 0.05$), reached a higher mean weight-for-height at the end of the study ($p < 0.008$), and their mean weight-for-height improved more than that of the soy supplementation group ($p < 0.02$) (Table 1).

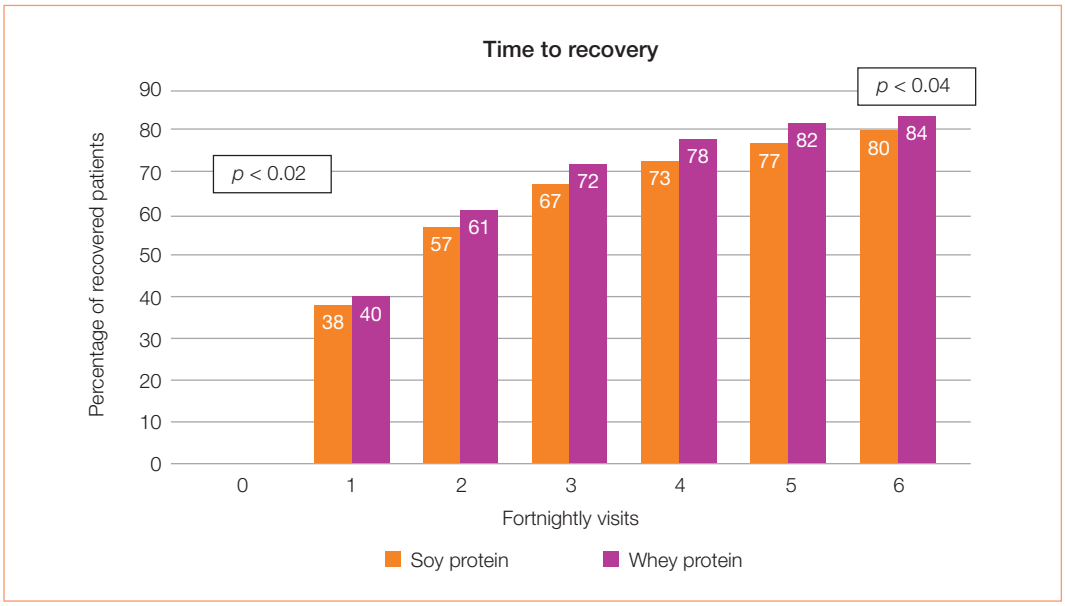

Figure 1. Percentage of recovered patients at each visit.

Conclusion

The authors of the study concluded that **the use of whey protein is important for the treatment of MAM, as it achieves better recovery rates and growth than soy supplementation**, and this was the case in spite of the whey supplements used containing less total protein and energy.

Table 1. Results after 12 weeks of treatment

	Baseline soy protein	Soy protein at 12 weeks	Baseline whey protein	Whey protein at 12 weeks	*p* value (12-week visit)
MUAC, cm	12.1 ± 0.27	12.59 ± 0.56	12.10 ± 0.27	12.66 ± 0.53	0.0088
WHZ	–1.88 ± 0.71	–1.18 ± 0.90	1.85 ± 0.73	–1.08 ± 0.86	0.0077
Weight gain		2.79 ± 2.16		2.95 ± 2.04	0.11

MUAC: mid-upper arm circumference; WHZ: mean weight for height.

Comments

Although the children in the whey group received less total protein and energy than those in the soy group, the children who were given whey supplements had better results than those treated with soy protein.

The better results of whey supplementation can be explained by a number of factors. On the one hand, **whey protein is known to be an excellent source of branched-chain amino acids**, which are metabolised by muscles and counteract the wear of lean tissue, a key step for recovering from acute malnutrition. Furthermore, supplementation with whey has been shown to increase fasting insulin and the retention of the amino acids absorbed.

In addition, whey contains bioactive peptides such as α-lactalbumin, β-lactoglobulin, whey proteins, lactoferrin and immunoglobulins, which are **associated with growth and the immune system** (iron-binding, repair of tissue, and resistance to infection).

This is compounded by the fact that soya contains over twice as much phytic acid as whey, which inhibits the digestion of protein and absorption of minerals.

As a result of all this, **nutritional supplementation with whey protein achieves better results than soy protein with less total energy and protein**.

Stobaugh HC, Ryan KN, Kennedy JA, Grise JB, Crocker AH, Thakwalakwa C, et al. Including whey protein and whey permeate in ready-to-use supplementary food improves recovery rates in children with moderate acute malnutrition: a randomized, double-blind clinical trial. Am J Clin Nutr. 2016; 103(3):926-33. Available at: https://academic.oup.com/ajcn/article/103/3/926/4629950

Nutrients. 2020;12(11):3259.

Bifidobacterium longum subsp. *infantis CECT7210* (*B. infantis* IM-1®) displays in vitro activity against some intestinal pathogens

Lorena Ruiz[1,2], Ana Belén Flórez[1,2], Borja Sánchez[1,2], José Antonio Moreno-Muñoz[3], Maria Rodríguez-Palmero[3], Jesús Jiménez[3], Clara G. de Los Reyes Gavilán[1,2], Miguel Gueimonde[1,2], Patricia Ruas-Madiedo[1,2] and Abelardo Margolles[1,2]
[1]Instituto de Productos Lácteos de Asturias (CSIC). Villaviciosa (Asturias). | [2]Instituto de Investigación Sanitaria del Principado de Asturias (ISPA). Oviedo (Asturias). | [3]Laboratorios Ordesa S.L. Barcelona.

Introduction

The gut microbiota and the mucosa themselves act as barriers against potential pathogens, promoting normal intestinal function. It is generally accepted that **nutrition and a balanced gut microbiota in a person's first few years of life can significantly affect their immune development**, with effects on their health and on the risk of developing chronic and inflammatory diseases in the short and long terms. Effects on the gut microbiota in children are currently brought about through the use of probiotics, prebiotics and synbiotics.

Of the prebiotics, fructooligosaccharides and galactooligosaccharides are particularly worth highlighting due to their stimulating effect on the immune system. The most noteworthy probiotics, on the other hand, are the *Bifidobacterium* strains that inhibit the growth of various enteropathogens, and more specifically *B. longum* subsp *infantis CECT7210* (*B. infantis* IM-1®), which has been shown to provide protection against rotavirus infection in both animals and humans.

Aim

The aim of this project was to assess, by means of an enterocyte adhesion assay, the extent to which *B. infantis* IM-1® is able to compete with enteropathogens and displace them.

Bifidobacterium longum subsp. _infantis CECT7210_ (_B. infantis_ IM-1®) has been shown to provide protection against rotavirus infection in both animals and humans.

Material and Methods

Strains and Conditions for Bacterial Growth

The study used *B. infantis* IM-1® strains provided by Laboratorios Ordesa and *Bifidobacterium animalis* subsp. *lactis* Bb12, as well as a variety of enteropathogens (*Escherichia coli*, *Listeria monocytogenes*, *Cronobacter sakazakii*, *Clostridium difficile*, *Salmonella enterica*, *Yersinia enterocolitica* and *Shigella sonnei*).

Pathogen Growth Inhibition

Bifidobacterium infantis IM-1® inhibits the growth of enteropathogens in vitro. The highest inhibition of growth was in *C. difficile*, followed by *C. sakazakii*. No inhibition of *E. coli* or *L. monocytogenes* was found as a result of using *B. infantis* IM-1®.

The growth of *C. sakazakii* was moderately reduced after 8-12 hours in a co-culture with *B. infantis* IM-1®, and this reduction was greater after 24 hours (Fig. 1).

Displacement of pathogens and prevention of pathogen attachment to enterocytes

The study confirmed the ability of *B. infantis* IM-1® to change the extent to which enteropathogens adhere to the HT29 cell line, displacing all the pathogenic strains tested (Fig. 2), particularly *C. sakazakii* and *S. enterica*.

Comments

The *B. infantis* IM-1® strain has been shown in previous studies to prevent episodes of diarrhoea in formula-fed infants and to provide protection against rotavirus infection in various experimental models.

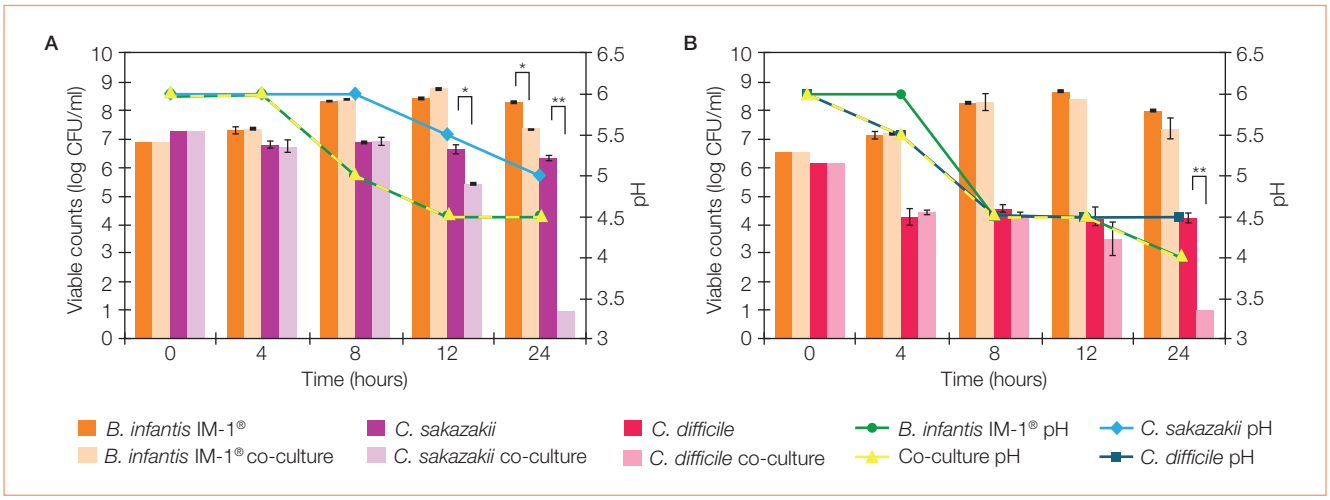

Figure 1. Growth of *C. sakazakii* (A) and *C. difficile* (B) in a co-culture with *B. infantis* IM-1®.

CFU: colony forming units. * $p < 0.001$, ** $p < 0.0001$.

This study shows that ***B. infantis* IM-1® can reduce the growth of various enteropathogens in vitro, as already shown for other Bifidobacterium strains/species**. For example, isolated *Bifidobacterium* strains have been used to treat gastrointestinal diseases caused by *C. difficile*. On the other hand, the results of this study are in contrast with the anti-pathogenic activity against *E. coli* found in other *Bifidobacterium* species.

Probiotics can compete directly for adhesion sites in the gut, displace already-attached pathogens, or even prevent the attachment of pathogens by blocking the adhesion sites. *Bifidobacterium* strains have been shown to be able to act in these three scenarios. However, the range of pathogens whose attachment might be reduced by probiotic Bifidobacteria seems to depend on the strain. In this regard, the *B. infantis* IM-1® strain seems to have a preferential activity over *C. sakazakii*, with a better inhibition effect than for the *B. animalis* Bb12 strain used as a control. **This suggests a promising effect of the *B. infantis* IM-1® strain on *C. sakazakii* infections, which can be particularly concerning in infants.**

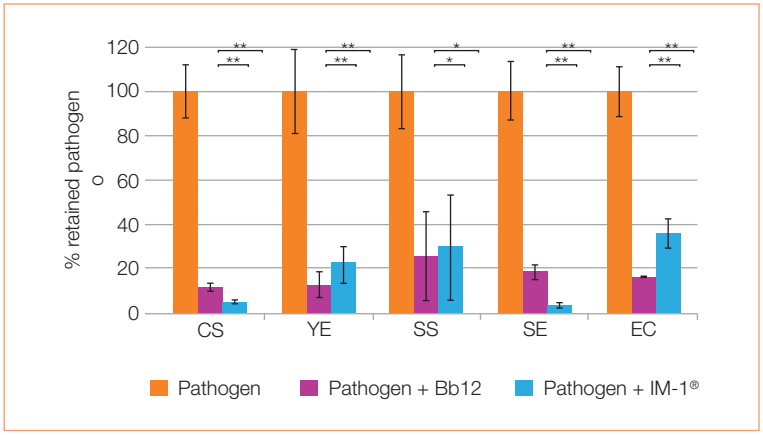

Figure 2. Retained pathogen attachment to HT29 cells after subsequent displacement by exposure to *B. infantis* IM-1®.

CS: *Cronobacter sakazakii*; EC: *Escherichia coli*; SE: *Salmonella enterica*; SS: *Shigella sonneil*; YE: *Yersinia enterocolitica*. * $p < 0.01$, ** $p < 0.001$.

Conclusions

The *B. infantis* IM-1® strain was able to prevent attachment and effectively displace all the pathogens tested, particularly *C. sakazakii*. This research increases the anti-pathogenic potential of bifidobacterial strains isolated from particular population groups (breastfed infants) and justifies the design of new functional foods and synbiotic combinations, including the *B. infantis* IM-1® strain. Thus, the *B. infantis* IM-1® strain, which had already been shown to reduce episodes of diarrhoea in infants and prevent infection from rotavirus, also has the potential to antagonise other enteropathogens in vitro.

Ruiz L, Flórez AB, Sánchez B, Moreno-Muñoz JA, Rodríguez-Palmero M, Jiménez J, et al. *Bifidobacterium longum* subsp. *infantis CECT7210* (*B. infantis* IM-1®) displays in vitro activity against some intestinal pathogens. Nutrients. 2020;12(11):3259. Available at: https://www.mdpi.com/2072-6643/12/11/3259

East Mediterr Health J. 2014;20(6):391-6.

Effect of zinc supplementation in children with asthma

J. Ghaffari[1], A. Khalilian[2], E. Salehifar[3], E. Khorasani[1] and M.S. Rezaii[1]

[1]Department of Paediatrics. Antimicrobial Resistant Nosocomial Infection Research Center. Sari (Islamic Republic of Iran). | [2]Department of Statistics. Mazandaran University of Medical Sciences. Sari (Islamic Republic of Iran). | [3]Department of Pharmacology. Mazandaran University of Medical Sciences. Sari (Islamic Republic of Iran).

Introduction

Asthma is a chronic inflammatory process of the respiratory system. It is estimated to have a prevalence of 7.6% in the Islamic Republic of Iran, although this goes up to 12% in the north of the country.

Although we do not know the exact aetiology of asthma, current hypotheses on the rise in its prevalence include low antioxidant food intake or increased oxidative stress. In fact, patients with asthma have been found to have low serum, hair and sputum zinc levels.

Trace elements such as selenium and zinc are key components of antioxidant enzymes and are necessary for inhibiting the production of the free radicals believed to aggravate asthma. Furthermore, zinc is an immune system modulator that reduces the inflammatory response. An Egyptian study Egypt showed that **consumption of omega-3 fatty acids, vitamin C and zinc, either alone or in combination, led to significant improvements in pulmonary function and sputum inflammatory markers compared to placebo in children with asthma**.

Patients with asthma have low zinc levels in the serum, hair and sputum.

Design and Aim

A double-blind, randomized, placebo-controlled trial on the effect of zinc supplementation on clinical symptoms in zinc-deficient children with moderate and/or partly controlled asthma was carried out in northern Islamic Republic of Iran.

Material and Methods

The study included 300 patients split into the study group (n = 155), whose members were given 50 mg/day of zinc supplements, and the control group (n = 145), which received a placebo for 8 weeks. All the patients in the study were using fixed inhaled steroids (a moderate dose of fluticasone). However, due to some patients being lost to follow-up or discontinuing the treatment, the results of only 144 patients in the zinc group and 140 in the control group were analysed.

Clinical symptoms (coughing, wheezing, dyspnoea), spirometry indices and zinc and Immunoglobulin E (IgE) levels were measured before and after supplementation.

Results

Following treatment, serum zinc levels rose from the baseline level of 61.8 µg/dL to 129 µg/dL in the zinc group, and from 60.9 to 63.8 µg/dL in the control group ($p < 0.001$). No significant differences in IgE levels were found between the two groups ($p < 0.05$).

The patients who had been given zinc supplements showed significant improvement in all the clinical symptoms under assessment compared to the control group: cough (relative risk [RR]: 2.3; confidence interval of 95% [IC: 95%]: 1.3 - 4 .1; p = 0,003), wheezing (RR: 3.6; IC: 95%: 1.8 - 7.4; p < 0,001) and dyspnoea (RR: 4.2; IC: 95%: 1.9 - 8.7; p < 0,001) (Fig. 1A).

The zinc-supplemented group experienced a significant effect on the spirometry parameters that included forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1) and the FEV1/FVC ratio (Fig. **compared to the control group** (Fig. 1B)).

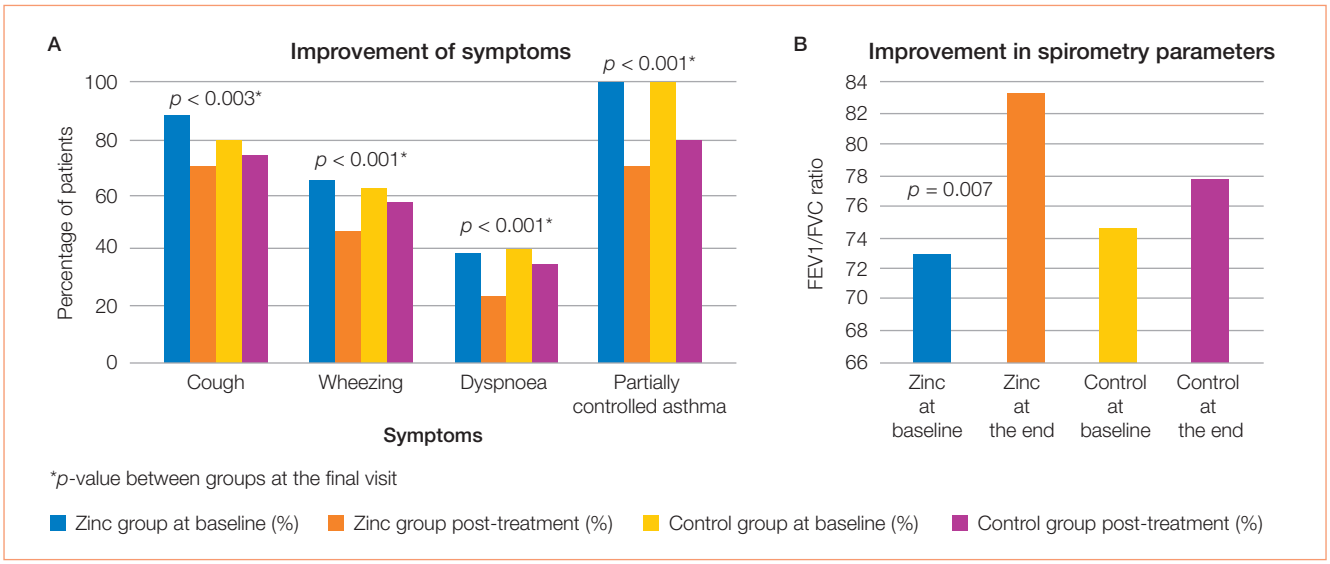

Figure 1. Evolution of zinc-supplemented patients.

FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity.

Comments

Zinc and copper are necessary for the proper operation of the immune system, and low levels of these elements have been observed in acute and chronic inflammatory conditions such as bronchial asthma.

This study shows that zinc supplementation at 50 mg/day for 8 weeks has a beneficial effect on both clinical symptoms and lung function in asthma patients. These results are consistent with other studies showing **the beneficial effect of zinc, omega-3 fatty acid and vitamin C supplements in children with moderate asthma**, also noting that **they are more effective when used in combination than individually**.

Furthermore, some studies have suggested that allergic diseases such as asthma, leukaemia and coronary artery ectasia are associated with deficiencies in trace elements such as selenium and zinc. In addition, zinc deficiencies can reduce antioxidant function and exacerbate asthma, while **normal zinc levels reduce the incidence and prevalence of acute respiratory infections and the severity of common cold symptoms**.

Finally, zinc has also been shown to decrease oxidative stress and the activation of nuclear transcription factor NF-κB in isolated mononuclear cells through the induction of zinc finger protein A20.

Conclusion

Zinc supplementation at 50 mg/day in zinc-deficient children with moderate asthma significantly improves their clinical symptoms and lung function.

Ghaffari J, Khalilian A, Salehifar E, Khorasani E, Rezaii MS. Effect of zinc supplementation in children with asthma: a randomized, placebo-controlled trial in northern Islamic Republic of Iran. East Mediterr Health J. 2014;20(6):391-6. Available at: https://applications.emro.who.int/EMHJ/V20/06/EMHJ_2014_20_6_391_396.pdf

Nutr Neurosci. 2013;16(4):183-90.

Effects of docosahexaenoic acid on brain structure and function in children

Robert K. McNamara[1], Ronald Jandacek[2], Patrick Tso[2], Wade Weber[1], Wen-Jang Chu[1], Stephen M. Strakowski[1], Caleb M. Adler[1] and Melissa P. DelBello[1]

[1]Department of Psychiatry and Behavioral Neuroscience. Center for Imaging Research. University of Cincinnati College of Medicine. Cincinnati, Ohio (USA). | [2]Department of Pathology. University of Cincinnati. Ohio (USA).

Introduction

Docosahexaenoic acid (DHA) is the main long-chain omega-3 fatty acid in the grey matter of the mammalian brain cortex, accounting for around 15-20% of all fatty acids in the adult frontal cortex. It is associated with better neurocognitive development and IQ in childhood.

Proton magnetic resonance spectroscopy (1H MRS) is a non-invasive imaging technique for measuring concentrations of chemical indices of the cortical metabolism, such as myo-inositol (ml), which is found mainly in astrocytes, and N-acetyl-aspartate (NAA) in neurons.

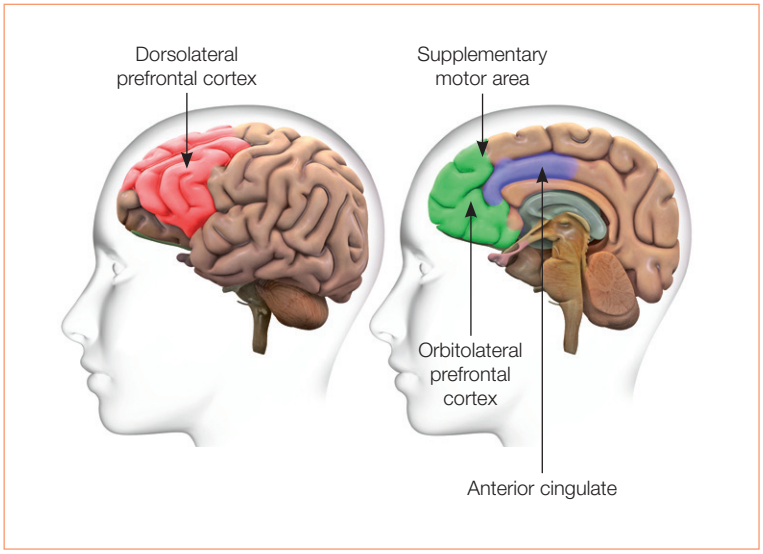

Figure 1. Anatomical location of the cingulate and prefrontal cortex.

Aim

To evaluate the link between the amount of DHA in erythrocytes and the various chemical indices in the left and right dorsolateral prefrontal cortex (R/L-DLPC, BA9) and bilateral anterior cingulate cortex (ACC, BA32/33) of healthy male children (Fig. 1).

Material and Methods

The study included healthy male children aged 8 to 10. Their socio-economic status and the amount of time during which they had been breastfed were recorded. The parents were given a validated omega-3 dietary intake questionnaire.

The identical-pairs version of the continuous performance task (CPT-IP) was used to assessed subjects' sustained attention.

Vocabulary and matrices scores were assessed using the Kaufman Brief Intelligence Test.

Magnetic resonance imaging scans (MRI) and one 1H MRS were carried out.

DHA is associated with better neurocognitive development and IQ in childhood.

Results

The study included 38 subjects, who underwent an MRI and 1H MRS procedure and were split into two groups based on erythrocyte DHA composition: the low-DHA group (low DHA; $n = 19$) and the high-DHA group (high DHA; $n = 19$). **Mean erythrocyte DHA was significantly lower in the low-DHA group (2.5 ± 0.2%) than in the high-DHA group (4.1 ± 0.2%) ($p < 0.0001$).**

The fatty acid composition of the subjects' erythrocytes can be observed in Figure 2.

Neurocognitive Performance

The CPT-IP revealed **significantly slower reaction times in the low-DHA group than in the high-DHA group (p = 0.007)**.

1H MRS

The low-DHA group had significantly lower concentrations of ml (-22%; $p = 0.007$; $d = 1.0$), NAA (-18%; $p = 0.007$; $d = 1.0$), choline (-21%; $p = 0.009$; $d = 0.9$) and creatinine (Cr) (-17%; $p = 0.01$; d = 0.9) in the ACC than the high-DHA group.

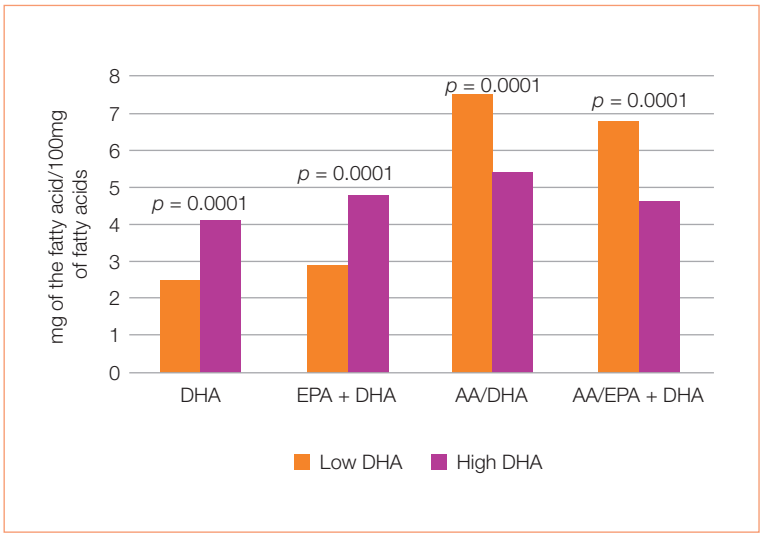

Figure 2. Erythrocite fatty acid composition

Discussion

The study found significantly lower ml concentrations in the CCA of low-DHA subjects than in the high-DHA subjects.

In addition, low-DHA subjects had shorter reaction times in the CPT-IP than high-DHA subjects. Overall, there was a positive correlation between erythrocyte DHA and ml in the ACC, and an inverse correlation with reaction time for both erythrocyte DHA and ACC ml.

Overall, the data suggests that **low erythrocyte DHA levels are associated with lower ACC metabolic function indices and slow reaction times during sustained attention periods in male children**.

The mean erythrocyte DHA level found in the low-DHA group was approximately half the level seen in the high-DHA group, a difference that is similar in magnitude (-39%) to that found in the erythrocyte DHA deficit of children with ADHD.

Subjects in the low-DHA group had lower ACC NAA concentrations than the high-DHA group. NAA is mainly found in neurons and has a positive correlation with mitochondrial metabolism. **High DHA levels have been found to have a neuroprotective effect against ischaemia-induced cortical atrophy.** Structural MRI studies have shown that a high habitual intake of long-chain omega-3 fatty acids is associated with a higher volume of ACC grey matter, which is associated with faster reaction times.

Taken together, these results suggest that DHA level has a positive correlation with the structural and functional integrity of the ACC.

Conclusion

The data from this study supports the hypothesis that low DHA levels are associated with cortical metabolism deficits in developing children, as shown by low NAA, choline and creatinine concentrations in the ACC of subjects with low DHA levels compared to those with high DHA levels.

The use of **dietary DHA supplementation can therefore be expected to improve brain function in developing children**.

McNamara RK, Jandacek R, Tso P, Weber W, Chu WJ, Strakowski SM, et al. Low docosahexaenoic acid status is associated with reduced indices in cortical integrity in the anterior cingulate of healthy male children: A 1H MRS Study. Nutr Neurosci. 2013;16(4):183-90. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4101902/

Nutr J. 2014;13:71.

Effects of supplementation with iron salts on ferritin concentration in schoolchildren

Ximena Duque[1], Homero Martínez[2,3], Jenny Vilchis-Gil[3], Eugenia Mendoza[1], Sergio Flores-Hernández[4], Segundo Morán[1], Fabiola Navarro[1], Victoria Roque-Evangelista[1], Anayeli Serrano[1] and Robertino M. Mera[5]

[1]Mexican Institute of Social Security. Mexico City (Mexico). | [2]RAND Corporation. Santa Monica, California (USA). | [3]Hospital Infantil de México Federico Gómez. Mexico City (Mexico). | [4]National Institute of Public Health. Cuernavaca, Morelos (Mexico). | [5]Vanderbilt University Medical Center. Nashville, Tennessee (USA).

Introduction

Iron deficiency is one of the most common nutritional deficiencies in the world, and it is more widespread where iron requirements are higher, such as in the case of growing children. Anaemia is caused by a negative iron balance, which can arise, among other reasons, from an insufficient iron intake. Furthermore, mineral and vitamin deficiencies can also cause anaemia.

Before a person is diagnosed with anaemia, **the iron deficiency they are suffering from can affect their immune response, their physical ability to work and their intellectual functions, such as attention span**. All this makes children particularly vulnerable to iron deficiency. Actions to correct an iron deficiency should thus focus more on the prevention of anaemia than on its treatment. Furthermore, iron is needed to synthesise haemoglobin and transport oxygen, for the development of the nervous system and for muscle function.

One fifth of the world's population is estimated to have a dietary iron deficiency. 46% of children between 5 and 14 years old are anaemic, and the cause of anaemia in 50% of cases is an iron deficiency. **The World Health Organization recommends that school-age children take 30 mg of iron and 250 mg of folic acid a day for three months to prevent iron deficiency anaemia.** A systematic review by the Cochrane Library found improved haematological outcomes in children under 12 that had been given intermittent oral iron supplementation.

Actions to correct an iron deficiency should focus more on the prevention of anaemia than on its treatment.

Aim

To compare the effects on serum ferritin concentration (a marker of iron levels) of daily supplementation with 30 mg of elemental iron (in the form of either ferrous sulphate or iron bisglycinate chelate) in non-anaemic iron-deficient children.

Material and Methods

The children in the study were given **daily supplements of 30 mg of elemental iron + 100 µg of folic acid for 90 days**. Three blood samples were taken during the study (baseline, one week after the end of the supplementation period, and 6 months post-treatment) to establish the children's serum ferritin and haemoglobin concentration. In addition, semi-quantitative 24-hour recall questionnaires were used to assess their intake.

Results

The analysis included 200 non-anaemic children with low iron levels with an average age of 9.3 ± 1.9 years. The subjects had a mean ferritin level of 8.7 ± 2.1 µg/L and a mean haemoglobin level of 140 ± 9.1 g/L.

A significant positive effect of supplementation was seen on ferritin concentration and iron status classification. At baseline, all children had low iron levels as measured by ferritin concentrations. **After a week and at 6 months post-supplementation, around 10% of the children still had low iron levels.**

Compared to the baseline ferritin concentration, a change of 20.16 µg/L was seen one week post-supplementation, and a change of 16.43 µg/L was observed after 6 months (Table 1 and Fig. 1).

Table 1. Results of supplementation on iron levels

	Baseline	1 week post-supplementation	6 months post-supplementation
Mean ferritin level	8.7 ± 2.1 µg/L	28.9 µg/L	25.2 µg/L
Changes to mean ferritin levels		20.16 µg/L	16.43 µg/L
Children with low iron levels	100%	10.5%	11.1%

Comments

The results reveal that, **90 days of supplementation with a daily dose of 30 mg of elemental iron increases ferritin concentration in schoolchildren with low iron levels**, an effect that can be observed as early as one week post-treatment and that remains for 6 months.

An African study showed that iron deficiency anaemia is caused by low iron availability as a result of a pulse- and cereal-based diet. **Iron supplementation can therefore be useful for such subjects where iron-rich foods are not widely available**, improving both haematological and non-haematological outcomes.

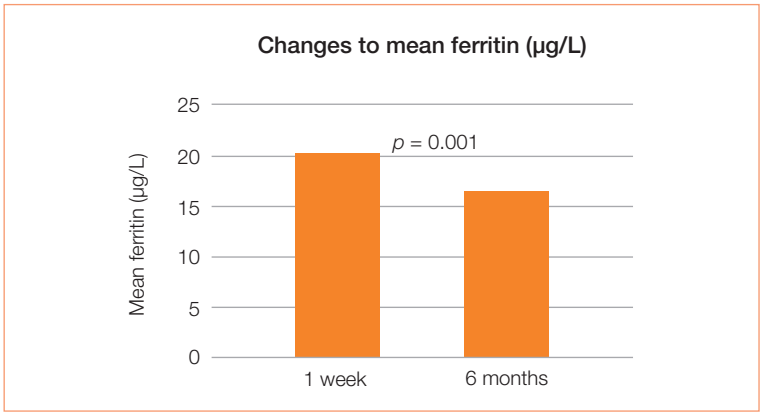

Figure 1. Changes to mean ferritin levels.

Conclusion

Supplementing with low doses of iron (ferrous sulphate or iron bisglycinate chelate) at 30 mg/day for 90 days significantly increases serum ferritin concentration in non-anaemic schoolchildren with low iron levels, with almost no adverse effects. **The effect of the higher iron level continues for up to 6 months post-supplementation**, and both treatments are safe and effective.

These results support the preventive use of low doses of iron to increase serum ferritin levels, something that can be an important tool in cases in which iron deficiency is a public health issue **and when low daily doses of iron supplements can be used for prevention in the school-age population to help maintain an adequate iron nutritional status**.

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Calcium and vitamin D intakes in children: a randomized controlled trial

Linda Cosenza[1], Vincenza Pezzella[1], Rita Nocerino[1], Margherita Di Costanzo[1], Anna Coruzzo[1], Annalisa Passariello[1], Ludovica Leone[1], Marcella Savoia[3], Antonio del Puente[4], Antonella Esposito[4], Gianluca Terrin[5] and Roberto Berni Canani[1,2]
[1]Department of Paediatrics. University of Naples "Federico II". Naples (Italy). | [2]European Laboratory for the Investigation of Food Induced Diseases. University of Naples "Federico II". Naples (Italy). | [3]Department of Molecular Medicine and Medical Biotechnology. University of Naples "Federico II". Naples (Italy). | [4]Department of Clinical and Experimental Medicine. University of Naples "Federico II". Naples (Italy). | [5]Department of Women's Health and Perinatal Medicine. University of Rome "La Sapienza". Rome (Italy).

Introduction

An adequate intake of calcium (Ca^{2+}) and vitamin D (Vit D) in children's diet is key to ensuring normal bone mineralisation and preventing rickets. Ca^{2+} is important mainly due to its structural role, although it also plays a role in other processes, such as coagulation and the secretion of hormones.

Ca^{2+} metabolism relies on Vit D, parathormone and calcitonin levels. In addition to its role in Ca^{2+} homeostasis, Vit D is also relevant for both innate and adaptive immunity and for preventing many diseases. This is why **the American Academy of Pediatrics currently recommends a daily Vit D intake of 400 IU for children and adolescents**. However, there is a high prevalence of vitamin D insufficiency in Western countries' paediatric populations, among other reasons because of an insufficient intake related to their dietary habits.

Aim

To assess the efficacy and applicability of a nutritional intervention to optimise Ca^{2+} and Vit D intake in a population of healthy children.

Methods

All the healthy subjects aged 3 to 17 who took part in a vaccination programme **and had less than 70% of the recommended daily intakes of Ca^{2+} and Vit D were invited to take part in the study**.

The subjects were split into two groups. Group 1 received dietary advice to increase their Ca^{2+} and Vit D intake, as well as commercially available Ca^{2+} and Vit D supplements (400 mg of Ca^{2+} and 400 IU of Vit D). Group 2 only received dietary advice.

The subjects' blood Ca^{2+} and 25-hydroxy vitamin D (25(OH)D) levels were measured at enrolment (T0) and 4 months post-intervention (T1).

Calcium and vitamin D have a structural function and play a role in coagulation, hormone secretion and immunity.

Statistical Analysis

A sample of 12 subjects per group was established to identify a 25% difference in serum 25(OH)D levels between the study groups at T1, with a power of 85% (type 1 error = 0.05; two-tailed test).

Results

The study included the first 24 children with Ca^{2+} and Vit D intakes of < 70% of the recommended daily intakes. The two groups had similar Ca^{2+} and Vit D intakes at enrolment (T0) (Figs. 1 and 2). Adherence was considered optimal, as over 90% of children took their daily dose during the study period. At the end of the study (T1), **the children in group 1 presented an increase in Ca^{2+} and Vit D intake, compared to higher levels of only Ca^{2+} in the group 2 children** (Figs. 1 and 2).

As to 25(OH)D, both groups had below optimal initial baseline levels (≥ 30 ng/mL), while at 4 months all children in group 1 and one child in group 2 were within the normal range.

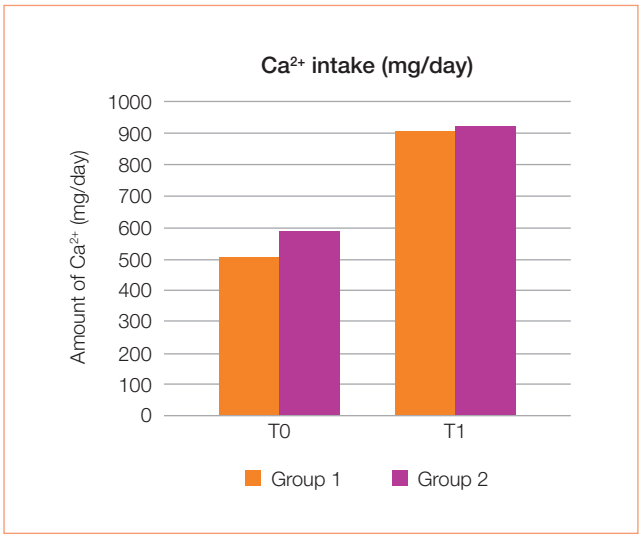

Figure 1. Baseline and end-of-study calcium levels.
Ca^{2+}: calcium; T0: at the start of the study; T1: at the end of the study.

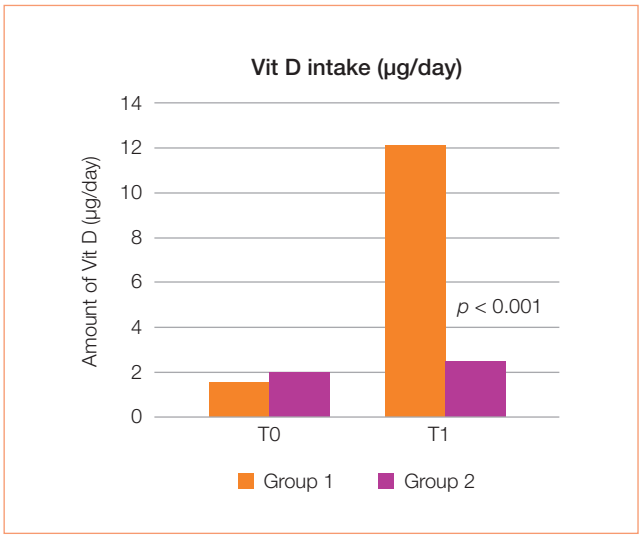

Figure 2. Vitamin D levels at the start and end of the study.
Vit D: vitamin D; T0: at the start of the study; T1: at the end of the study.

Comments

The best Vit D level indicator is 25(OH)D concentration, as it shows the amount of vitamin absorbed from the diet and the amount produced through the skin. Vit D synthesis in the skin takes place all year round, but only near the equator. **In general, insufficient Vit D is synthesised through the skin in winter**. This study thus reinforces the idea that we shouldn't assume that children need less Vit D supplementation simply because they live in a sunny part of the world, **and the results show that dietary advice is not enough to bring Vit D and 25(OH)D to normal levels**.

Conclusion

Vit D deficiency is a global public health issue with serious short- and long-term consequences that affects people of every age. Dietary advice alone is not enough to ensure an adequate Vit D intake, which is necessary for health and to achieve optimal serum levels of 25(OH)D. **A prevention programme involving Vit D-fortified foods and supplements is therefore necessary.**

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Avinguda de la Meridiana, 358, 10ª planta – 08027 Barcelona
Tel. No. +34 932 684 946
E-mail address: informacion@editorialglosa.es

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