

## **Closing report on the project**

### ***“Long-chain polyunsaturated fatty acids and prebiotics as nutritional supplements in paediatric prevention and treatment”***

The period to be reported on was burdened in its substantial part with consequences of the COVID-19 crisis. We were unable to work properly from the spring of 2000 onwards, because the Department of Paediatrics of the University of Pécs, i.e. the host institution of the project, became one of the five Regional Centres for caring COVID-19 positive children in Hungary. This transition required substantial modification of many of our activities.

Moreover, the last two years were characterised by the nearly total lack of any opportunity to present our data in prestigious international meetings. However, we would like to follow the traditional order of presenting the data in meetings and submit them for publication only thereafter. Based on this consideration, we exceptionally included the specifications of a single “paper to be submitted” into the list of publication of this report.

Our project goals were divided between research on long-chain polyunsaturated fatty acids and research on prebiotics.

#### **Research on long-chain polyunsaturated fatty acids**

As to the potential role of trans isomeric fatty acids, we analysed the occurrence of trans isomeric fatty acids in various foods. Given the increased formation of TFAs during heating, many studies already investigated compositional changes in oils after prolonged heating or at extremely high temperatures. In contrast, our aim was to measure changes in fatty acid composition and in some health-related indices in edible oils after short-time heating that resembles the conventional household use. Potatoes were fried in palm, rapeseed, soybean, sunflower and extra virgin olive oils at 180 °C for 5 min, and samples were collected from fresh oils and after 1, 5 and 10 consecutive heating sequences. Regardless of the type of oil, the highest linoleic acid and alpha-linolenic acid values were measured in the fresh samples, whereas significantly lower values were detected in almost all samples following the heating sequences. In contrast, the lowest levels of TFAs were detected in the fresh oils, while their values significantly increased in almost all samples during heating. Indices of atherogenicity and thrombogenicity were also significantly higher in these oils after heating. The data obtained in this study indicate that prolonged or repeated heating of vegetable oils should be avoided; however, the type of oil has a greater effect on the changes of health-related indices than the number of heating sequences. We were able to publish these data in *Foods* (IF: 4.121).

As to the follow-up of neurodevelopment in children who participated in our previous nutritional trials using n-3 long-chain polyunsaturated fatty acids supplements in pregnant women, we evaluated the long-term effects of maternal prenatal supplementation with fish-oil (FO), 5-methyltetrahydrofolate (5-MTHF), placebo or FO + 5-MTHF, as well as the role of fatty acid desaturase (*FADS*) gene cluster polymorphisms, on their offspring's processing speed at later school age. There were significant differences in processing speed performance among children at different ages ( $p < 0.001$ ). The type of prenatal supplementation had no effect on processing speed in children up to 9 years. Secondary exploratory analyses indicated that children born to mothers with higher AA/DHA ratio at delivery ( $p < 0.001$ ) and heterozygotes for *FADS1* rs174556 ( $p < 0.05$ ) showed better performance in processing speed at 9 years. These considerations support that maternal prenatal supplementation should be quantitatively

adequate and individualized to obtain better brain development and mental performance in the offspring. We were able to publish these data in *Nutrients* (IF: 4.171).

The associations of foetal fatty acids status to immune-related health parameters later in life are unclear. Our aim was to collect all available information on the relationship between fatty acid status at birth and allergy in childhood. Systematic literature search was performed on Ovid MEDLINE, Cochrane Library and Embase. The search retrieved 897 articles without duplicates; 14 articles remained after excluding those that didn't fit into our inclusion criteria. When the dichotomous parameter of suffering or not from allergic condition in childhood was analysed, cord blood eicosapentaenoic acid (EPA) values proved to be significantly lower in allergic than in non-allergic children in 4 comparisons from 3 studies. When the linear parameters of odds ratios and relative risks for allergy were taken into consideration, high cord blood EPA, but also high docosahexaenoic acid (DHA) and high total n-3 long-chain polyunsaturated fatty acid values were associated to clinically relevant reduction (at least 38%) in 8 comparisons from 5 studies. Within the cord blood samples, higher EPA, docosapentaenoic acid and DHA values were significantly and negatively associated in 8 correlation analyses from 3 studies with laboratory parameters considered to reflect allergic trait. The data reported here may provide information for defining optimal fatty acid intakes for pregnant women. We were able to publish these data in *Life* (IF: 3.817).

We used the interest generated for COVID-19 infection to draw the attention to the possible beneficial effect of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) supplementation in SARS-CoV-2 infection and urge the medical community for further investigations and conduction of clinical trials. Evidence suggests that n-3 LC-PUFAs can modulate the immune response and function in many ways. Among these complex immunomodulatory effects, interleukin-6 (IL-6) and interleukin-1 $\beta$  (IL-1 $\beta$ )—because of the suspected central regulatory role in the “cytokine storm”—should be highlighted, and these cytokines can be affected by dietary EPA and DHA intake. In addition, poly(ADP-ribose) polymerase enzymes that have anti-inflammatory properties, translatable to human COVID-19 infection were shown to improve tissue levels of DHA and EPA, as well as the downstream anti-inflammatory metabolites of EPA and DHA. Based on the available data, the supplementation of EPA and DHA in COVID-19 patients appears to have potential beneficial effect in managing the “cytokine storm.” Therefore, the use of EPA and DHA supplementation should be considered as both a supportive therapy and a prevention strategy in SARS-Cov-2 infection. We reported our findings in *Frontiers of Physiology* (IF: 3.160).

### **Research on prebiotics as nutritional supplements**

We aimed to explore whether prophylactic supplementation with prebiotic fructans is able to influence the frequency of infectious diseases in kindergarten children during a winter period. (Inulin-type fructans used in formula have been shown to promote microbiota composition and stool consistency closer to those of breastfed infants and to have beneficial effects on fever occurrence, diarrhoea, and incidence of infections requiring antibiotic treatment in infants.) We also attempted to ascertain the effect on the intestinal microbiota. Altogether 142 boys and 128 girls aged 3-6 years were randomly allocated to consume 6 g/day fructans or maltodextrin for 24 weeks. At baseline, stool samples were collected for microbiota analysis and anthropometric measurements were made. During the intervention period, diagnoses were recorded by physicians, whereas disease symptoms, kindergarten absenteeism, dietary habits, and stool

consistency were recorded by parents. Baseline measurements were repeated at week 24 of the study. In total 219 children finished the study. Both the relative abundance of *Bifidobacterium* and that of *Lactobacillus* were 19.9% and 7.8% higher, respectively, in stool samples of children receiving fructans as compared with those of controls at week 24. This was accompanied by significantly softer stools within the normal range in the prebiotic group from week 12 onwards. The incidence of febrile episodes requiring medical attention [ $0.65 \pm 1.09$  compared with  $0.9 \pm 1.11$  infections/(24 week  $\times$  child)] and that of sinusitis ( $0.01 \pm 0.1$  compared with  $0.06 \pm 0.25$ ) were significantly lower in the prebiotic group. The number of infectious episodes and their duration reported by parents did not differ significantly between the 2 intervention groups. The data mentioned above allowed the conclusions that prebiotic supplementation modified the composition of the intestinal microbiota and resulted in softer stools in kindergarten-aged children. Moreover, the reduction in febrile episodes requiring medical attention supports the concept of further studies on prebiotics in young children. We reported our findings in *The Journal of Nutrition* (IF: 4.398).

By applying quantitative and qualitative microbiota analysis within the aforementioned study, we aimed at characterising the gut microbiota composition and at exploring effects of prebiotic intervention on the gut microbiota during a 24-weeks intervention and during antibiotic treatment in healthy children. Faecal samples were collected and subject to targeted qPCR analysis and phylogenetic profiling by multiplexed high throughput sequencing of the prokaryotic 16S rRNA gene PCR amplicons. The microbiota composition of the cohort could be clustered into three distinct constellations (enterotypes). Prebiotic intake resulted in a selective modulation of the gut microbiota composition. Relative abundance of *Bifidobacterium* was significantly higher in the prebiotic group compared to control group and this effect was found for all three enterotypes. Antibiotic administration decreased the relative abundance of *Bifidobacterium* in both groups. Nonetheless, children of the prebiotic group receiving antibiotic treatment displayed significantly higher levels of *Bifidobacterium* than children receiving the placebo control. Prebiotic supplementation induced specific changes in the gut microbiota composition of children aged 3 to 6 years. Moreover, it attenuated antibiotic-induced disturbances in the gut microbiota composition as shown by higher relative abundance of bifidobacteria at the end of the antibiotic treatment in the prebiotic group. With the previously reported benefits on immune function, the study contributes to the evidence on the immune-modulating effects of prebiotics through gut microbiota modifications. We reported our findings in *Beneficial Microbes* (IF: 2.939).

We aimed to corroborate our data obtained in 2015-2018 and carried out a novel, double-blind, randomized placebo-controlled, multi-centre trial investigating the effect of prebiotic inulin-type fructans on infectious disease incidence in children aged 3 to 6 years. From the 698 children finishing the study per protocol, 231 children were included in Pécs, 60 in Szekszárd, 67 in Mohács, 180 in Győr and 160 in Szeged. Stool consistency was softer already after few days of product consumption in the prebiotic compared to the placebo group and remained softer during the whole study. The numbers of acute upper respiratory tract infections, lower respiratory tract infections, ear infections, gastrointestinal infections and any other infections did not differ between the prebiotic and the placebo groups. Though in this multicentre trial we were unable to confirm our previous finding on the beneficial effect of prebiotics in reducing febrile episodes requiring medical attention supports, the extent and quality of this second clinical study on prebiotics will most probably allow to publish our data at the same level as the first study, i.e. in *The Journal of Nutrition* (IF: 4.398).

While inulin-type fructans are considered to stimulate the growth of beneficial microorganisms, like *Bifidobacterium* in the gut and support health. However, both the fructan source and chemical structure may modify these effects. A systematic review was conducted to assess the effects of chicory-derived inulin-type fructans consumed either in specific foods or as dietary supplements on abundance of *Bifidobacterium* in the gut and on health-related outcomes. Three electronic databases and two clinical trial registries were systematically searched until January 2021. Two authors independently selected randomized controlled trials that investigated with a protocol of minimum seven days supplementation the effect of chicory-derived inulin-type fructans on *Bifidobacterium* abundance in any population. Meta-analyses with random-effects model were conducted on *Bifidobacterium* abundance and bowel function parameters. We evaluated risk of bias using Cochrane RoB tool. Chicory-derived inulin-type fructans at a dose of 3-20 g/day significantly increased *Bifidobacterium* abundance in participants with an age range from 0 to 83 years (standardized mean difference: 0.83, 95% CI: 0.58-1.08;  $p < 0.01$ ; 50 studies; 2525 participants). Significant bifidogenic effects were observed in healthy individuals and in populations with health impairments, except gastrointestinal disorders. Significant beneficial effects on bowel function parameters were observed in healthy subjects. Chicory-derived inulin-type fructans may have significant bifidogenic effects and may beneficially influence bowel function in healthy individuals. We published our data in Crit Rev Food Sci Nutr (IF: 12.260).

#### **Additional research carried out under the project umbrella**

We utilised our skills obtained in evidence-based medicine (EBM) to perform an online survey in all medical and health sciences faculties in Hungary. In total, 1080 students of medicine and 911 students of health sciences completed the online questionnaire. The attitude towards EBM was generally positive; however, only a small minority of students rated their EBM-related skills as advanced. EBM trained medical students were more likely to choose online journals (17.5% compared to 23.9%,  $p < 0.05$ ) and professional guidelines (15.4% compared to 6.1%,  $p < 0.001$ ) instead of printed books (33.6% compared to 52.6,  $p < 0.001$ ) as the main source of healthcare information retrieval and used Pubmed/Medline, Medscape and the Cochrane Library to a significant higher rate than students without any previous EBM training. Healthcare work experience (OR = 1.59; 95% CI = 1.01-2.52), conducting student research (OR = 2.02; 95% CI = 1.45-2.82) and upper year university students (OR = 1.65; 95% CI = 1.37-1.98) were other factors significantly influencing EBM-related knowledge. We conclude that the majority of students of medical and health sciences faculties are keen to acquire EBM-related knowledge and skills during their university studies. Significantly higher EBM-related knowledge and skills among EBM trained students underline the importance of targeted EBM education, while parallel increase of knowledge and skills with increasing number of education years highlight the importance of integrating EBM terminology and concepts also into the thematic of other courses. We reported our findings in PLOS One (IF: 2.740).