The dynamics of Human Breast Milk composition. Long-term metabolomic analysis of Human Breast Milk.

Human Breast Milk (HBM) is a complex mixture of various macronutrients (carbohydrates, proteins, lipids, vitamins), bioactive compounds and interactive elements (cytokines, chemokines, antimicrobial compounds). Because of its content, HBM is the only food able to satisfy all the needs of the newborn child, by providing all the nutrients required for somatic growth and also important functional factors. Moreover, it is known that HBM vary in composition not only between mothers, but also within an individual mother constantly during lactation period, adapting directly to the individual needs of growing baby. The composition of human breast milk has been extensively studied for several decades. Nevertheless, knowledge about the composition of low molecular compounds such as polar metabolites (amino acids, nucleotides) or lipids still needs to be completed. Especially, in the context of the dynamics of the content of these molecules in human milk throughout the entire lactation period.

The main goal of the project is to monitor and define trends of long-term changes in HBM composition at the molecular level *i.e.* content of metabolites in HBM within the two year period of lactation starting from the labor. The assessment of content changes in the whole period of the lactation will include the analyses of small polar compounds such as aminoacids, nucleotides human milk oligosaccharides and more hydrophobic lipids. In the field of analytical chemistry the aim will be achieved by the use of high performance liquid chromatography coupled with high-resolution mass spectrometry. The analytical methodology developed in our research group allows for comprehensive determination of broad spectrum of metabolites in an untargeted manner, what in practice mean the analysis of hundreds of metabolites resulting in metabolite fingerprints. Human breast milk metabolome dynamics will be assessed through the entire lactation period within individual mother by the comparison of metabolites fingerprints. Time-depended trends will be assessed for hundreds of metabolites what will give more in-depth knowledge about changes in HBM composition. Moreover, the content of metabolites will be compared in 24 periods of lactation as the samples will be collected once a month. Subsequently, results from individual mother will be compared with each other to reveal if the dynamics of HBM composition is similar between studied lactating mothers.

The obtained results will be an original contribution to the knowledge in the field of dynamics of HBM composition within the entire lactation period. The results will also be the base for the further biological interpretation, which can possibly include the relationship between observed differences in metabolites content between HBM samples collected during entire lactation periods with the age of the child. Such an interpretation can contribute to the better understanding of the mechanisms of adaptation of the HBM content in response to the changing, individual nutritional needs of the growing child. Evaluation of the human breast milk molecular composition variance in time within one individual mother and between different lactating mothers can show whether time of lactation or variable external or internal factors impact more on the molecular dynamics of this biofluid.

It should be noted that beneath expanding knowledge about the dynamics of the composition of the breast milk and the mechanism of its adaptation to the individual needs of the breastfed infant, the proposed project has also a valuable social and pro-health impact. The advancement of the knowledge about the uniqueness of HBM and the biological functions of its components is of special importance regarding optimal nutrition of infants and raising public awareness of the benefits of breastfeeding, what is of great importance in regards to infant health and proper growth. Resignation from breastfeeding has impact on infant's health but also is connected with high economical costs of milk formulas, accessories for babie's feeding and health-care associated cost which could be avoided by breastfeeding (i.e. gastrointestinal infections including diarrhea on which breastfeeding has positive health impact). Thus, the benefits of breastfeeding due to the uniqueness of its chemical composition should be widely known and promoted. Expanding knowledge about the unique composition of breast milk and its long-term dynamics will contribute to the increase in knowledge about the benefits of long-term breastfeeding in the community. It is particularly important in the light of numerous statistics on resignation from breastfeeding before the 6^{th} month of child life, despite numerous recommendations from international Pediatric Societies and the World Health Organization, which recommend breastfeeding for 6^{th} month and longer - as long as the mother and the child want it.