

Metabolic profiling of tryptophan and kynurenine pathway in meconium and first stool

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RECETOX

OVERVIEW

Aim:

Quantify kynurenine and tryptophan metabolites from microbiome of CELSPAC Study participants.

Methods:

Tryptophan metabolites were analyzed using the targeted SRM metabolic profiling in stool.

Achievements:

- Quantification of tryptophan and kynurenine metabolites in stool and meconium
- Significant different bacterial composition in first day of delivery
- Significant difference in stool metabolites, sorted after IgA

INTRODUCTION

- mode of delivery, caesarian section (CS) or vaginal delivery (VD) determines the diversity of the bacterial microflora
 - different microbial composition, has an influence on the health of the newborn¹
- Tryptophan and its metabolites, produced by many different bacterial species, have an important role in the mammalian gut immune homeostasis²
- 134 samples were collected from participants in the CELSPAC Cohort Study. The samples were taken after delivery of the baby (day 0 - day 3)
- stool samples were pre-sorted according to the IgA level into the category's meconium and first stool after the hours of delivery
- groups compared (1) mode of birth (CD vs. ND) in Stool (2) Bacterial composition of stool sorted by day after delivery
- analyzed a panel of tryptophan metabolites in neonatal fecal samples
- tryptophan metabolites were quantified using labeled internal standards.
- tested for significance with 95% confident interval

METHODS

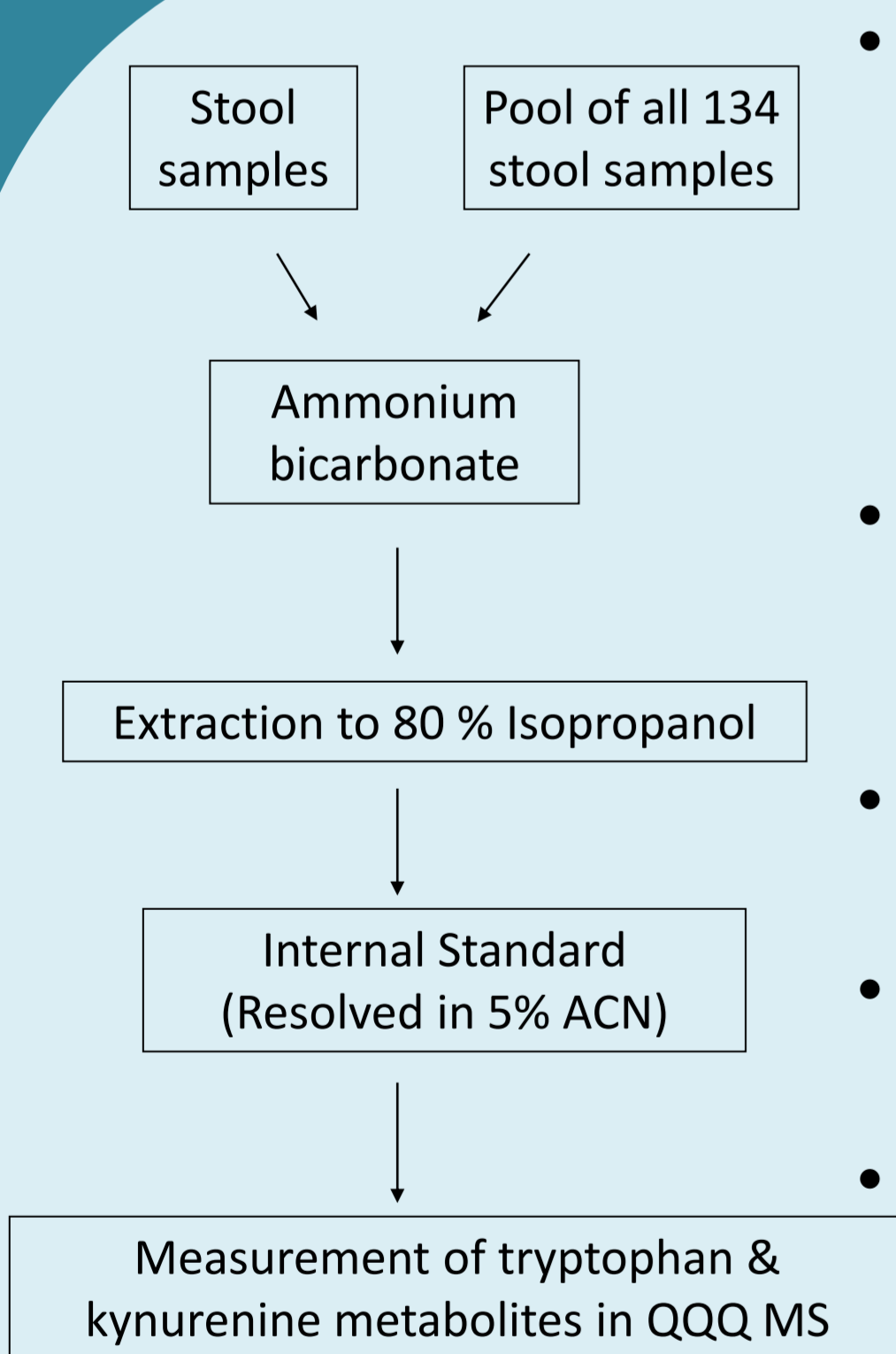


Figure 1: Sample preparation

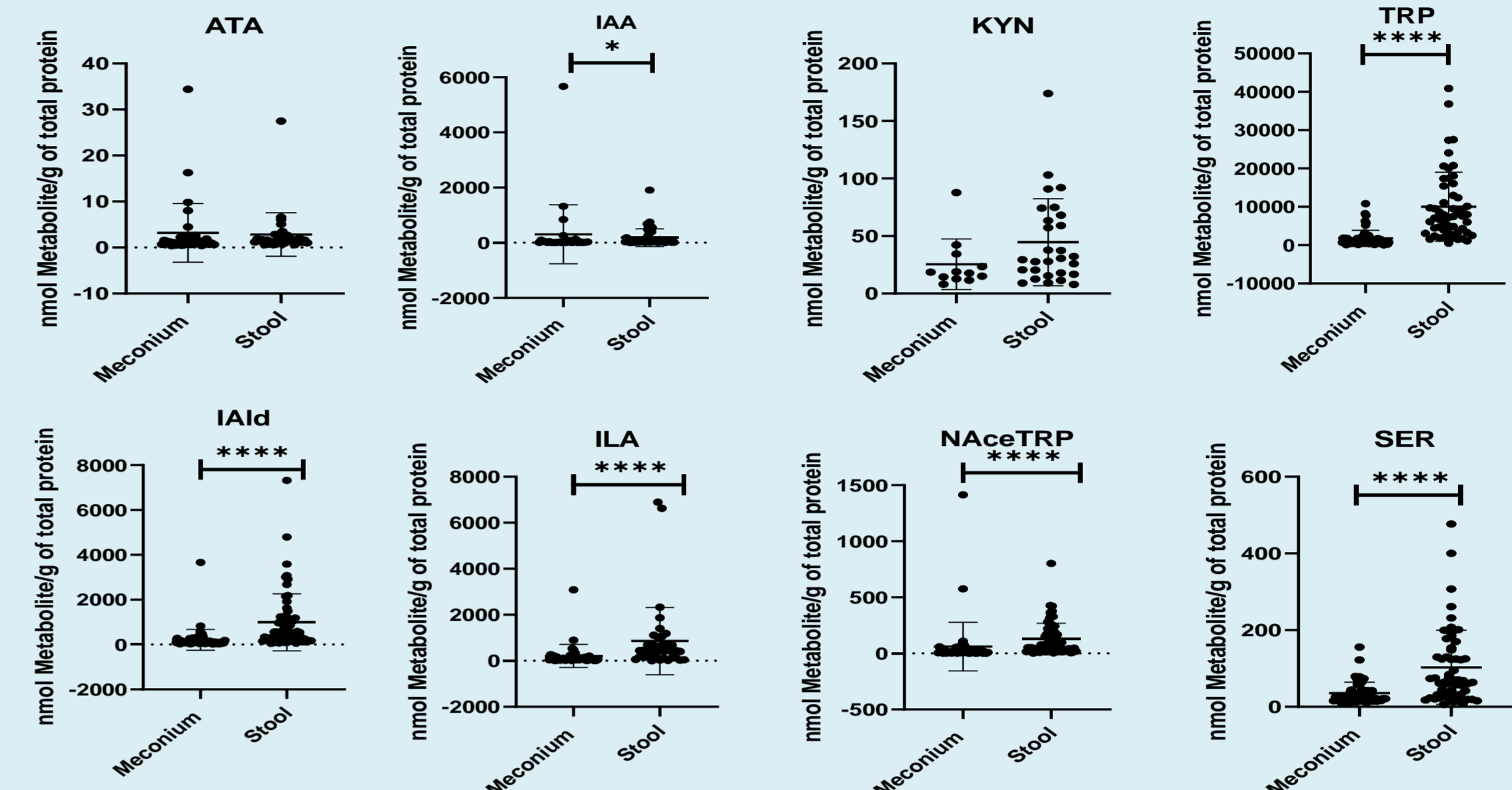


Figure 3: Stool sample collected on a swab

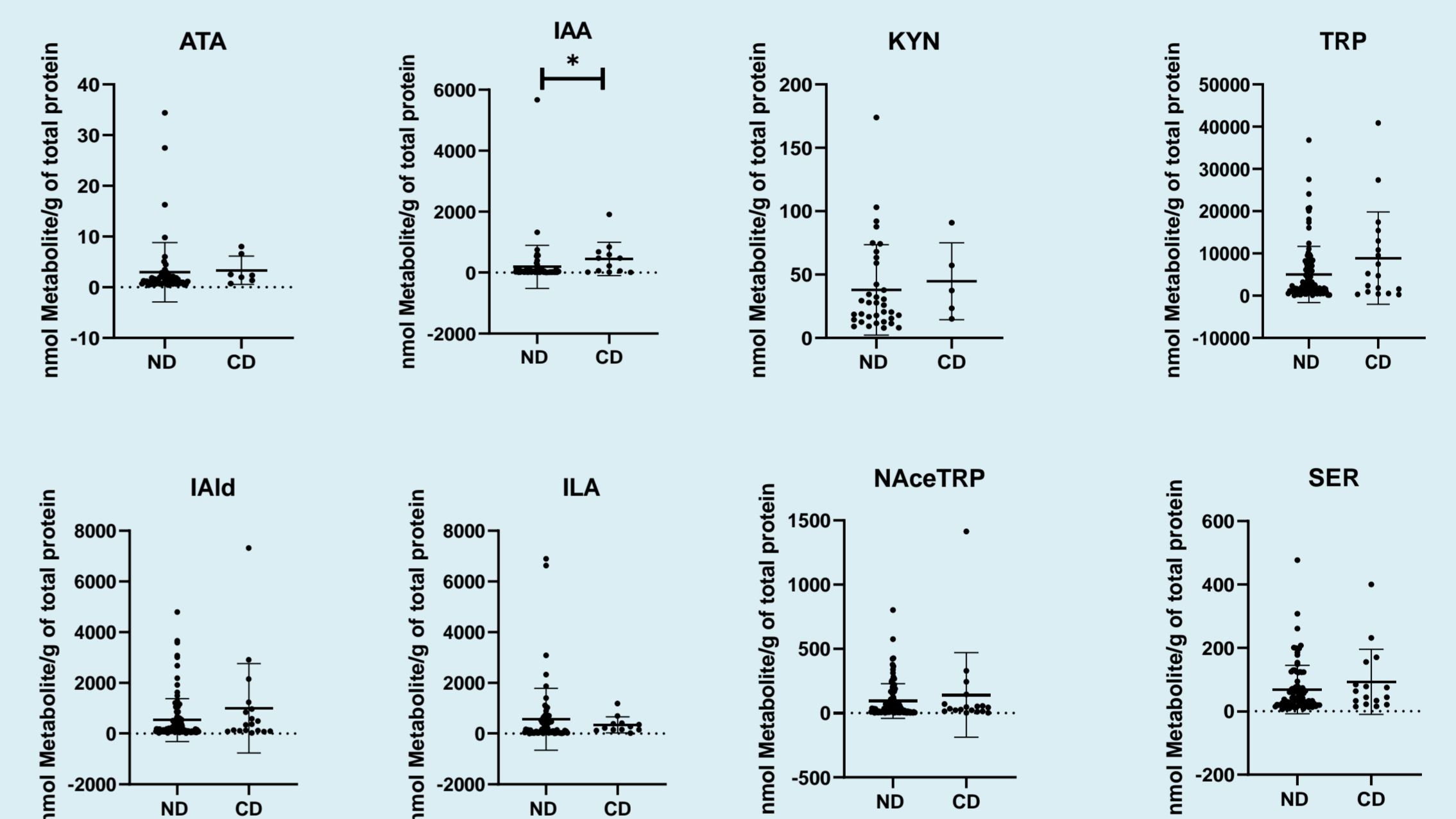
- Quantified metabolites are: Anthranilate (ATA), Indole-acetic acid (IAA), Kynurenine (KYN), Tryptophan (TRP), Indole-3-Aldehyde (IAld), Indole-lactic acid (ILA), N-Acetyl-tryptophan (NAceTRP), Serotonin (SER)
- stool samples were collected from 134 healthy children at the University Hospital Brno. The sample preparation protocol in Figure 1.
- a quality control, a pool of all samples was measured
- samples were injected in 5% Acetonitrile with spiked-in internal standard
- Extracted metabolites were analysed in the Triple-Quad Mass Spectrometer
 - mobile phase contained a gradient with 0.1 % formic acid, Samples injected with UPLC analytical column (C18 Peptide, CSH Column, 1.7 μ M, 2.1x 100 mm)
 - The tryptophan and kynurenine metabolites were measured with the UHPLC-SRM-MS positive ion mode. All data were acquired in SRM mode
- calculation of the concentration of the analytes:
 - transition with the highest intensity for each metabolite \rightarrow also to control the SRM Assay method
 - quantification of all samples with mixed internal standard (labeled and unlabeled)Semi-quantitative quantification with Response Factor-method, according to Pavlova *et al.*, 2017³

RESULTS

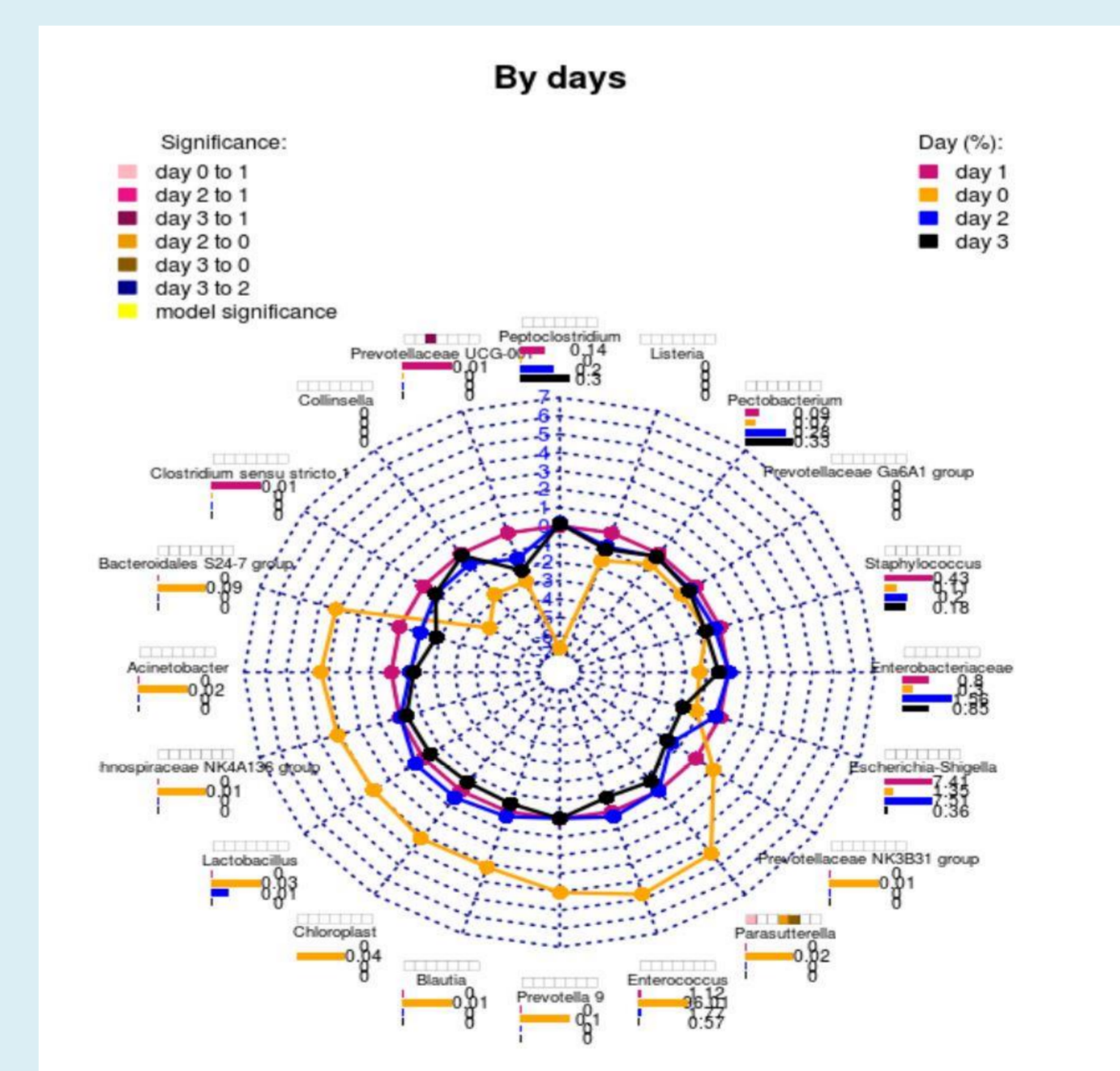
(1) Comparison of metabolites in meconium and stool according to IgA Level. Each quantified metabolite was tested between the two groups meconium (n= 63) and stool (n= 71) for significance (p < 0.05) with Mann-Whitney test. Significant different concentrations between both groups were found for IAA, TRP, IAld, ILA, NAceTRP and SER.



(2) Comparison of metabolites according to mode of Delivery in meconium. 134 samples were divided into the groups ND (n=114) and CD (n= 20). Groups were tested for significance in each metabolites with Mann-Whitney-Test with 95% Confidence-Interval. Significant different concentration between both groups was found for IAA.



(3) Radar chart of the comparison of bacterial composition in meconium and stool according to days after delivery. The bacterial composition all samples was tested for significance (p < 0.05) between the days of delivery (Significance). The Significance between days is shown above the name of bacterial species. The percentage (%) of bacterial composition is shown under the bacteria name.



CONCLUSIONS

- Significant different concentration of metabolites in the comparison of meconium and stool are:
 - IAA (p < 0.05)
 - TRP, SER, IAld, ILA, NAT, SER (p < 0.001)
- Significant different concentration of metabolite in the comparison of the mode of delivery (CD vs. VD):
 - IAA (p < 0.05)
- Significant different percentage of bacteria between days of delivery are:
 - Prevotellaceae UCG-001 (day 3 and 1)
 - Parasutterella (day 0 to 1, day 2 to 0, day 3 to 0)

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