Clinical research project HUB2025312

Dietary supplement, gut microbiota and ADHD

Statistical analysis

Sponsor: Hôpital Erasme – Cliniques universitaires de Bruxelles – Department of Infant, Child, Adolescent and Young Adult Psychiatry – H.U.B – Université Libre de Bruxelles

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Investigators: Barbara Luyens; Dr François Felgueroso-Bueno; Prof. Dr Isabelle Massat

Microbiota and biostatistical analyses: Prof. Dr Amandine Everard, Louvain Drug Research Institute, Metabolism and Nutrition (UCLouvain/LDRI/MNut), within an academic collaboration

Research project number: HUB2025312

Related document: Clinical research protocol HUB2025312, version signed on 22/08/2025

Statistical analysis

Software and descriptive statistics

Statistical analyses will be performed using R software (version 4.6.1; R Core Team, R Foundation for Statistical Computing, Vienna, Austria). Variables will be described as mean and standard deviation, or as median and interquartile range in the case of skewed distributions.

Assessment of normality

The normality of the distribution of variable of interest will be assessed in each of the two groups using the Shapiro–Wilk test and visual inspection of quantile–quantile (Q–Q) plots.

Between-group comparison

The comparison between the ADHD group and the control group will be performed using Welch's independent-samples t-test, which does not assume equal variances. In the event of a marked departure from normality, the non-parametric Mann–Whitney U test (Wilcoxon rank-sum test) will be used instead. Results will be expressed as the difference in means between the two groups, together with its 95% confidence interval and an effect size estimate (Cohen's d).

Significance level

All tests will be two-sided, and a p-value < 0.05 will be considered statistically significant.

Gut microbiota: laboratory, bioinformatic and biostatistical analyses

The laboratory analyses of the gut microbiota and the statistical analyses will be carried out within the framework of an academic collaboration with Prof. Dr Amandine Everard. Metagenomic DNA will be extracted from faeces using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, with modifications. The V1–V3 region of the 16S rRNA gene will be amplified using the following universal eubacterial primers: 27Fmod (5'-AGRGTTTGATCMTGGCTCAG-3') and 519Rmodbio (5'-GTNTTACNGCGGCKGCTG-3'). The purified amplicons will be sequenced on an Illumina MiSeq. Sequence reads will be demultiplexed and processed using the QIIME2 pipeline. Biostatistical analyses will be performed within this laboratory by bioengineers with expertise in this specific field.