Impact of storage conditions on the human stool metabolome

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1. Introduction

Given the fact that fecal samples comprise a rich source of endogenous human metabolites, gut microbiota metabolites and food residuals, the interest in metabolic profiling of fecal samples in the search for (gastrointestinal) disease biomarkers and to better understand host-gut microbiota interactions has increased recently [1,2]. In addition, this biological specimen can be obtained in a non-invasive manner. Up to date, fecal metabolic profiles have already been proven to enable the discrimination of a control group from patients with colorectal cancer [3], inflammatory bowel diseases [2], liver cirrhosis, hepatocellular carcinoma [4], etc. Although direct analysis or preparation is the ideal option for metabolic fingerprinting of biological samples in order to quench biological processes, this is often not feasible in practice, which is especially the case for fecal samples [1,5]. In contrast to blood or urine samples, fecal samples are often collected at home, causing the biological processes to continue during sample collection, ambient storage, and transport [1]. Knowledge about the stability of the fecal metabolic fingerprint upon storage is of crucial importance to avoid erroneous conclusions.

2. Approach

In this study, we investigated the effect of storage duration (0, 1, 2, 4, 8, 18 or 25 weeks), the number of freeze-thaw cycles (0, 1 or 2), aerobic vs. anaerobic storage, and storage temperature (−20 or −80 °C) on the polar metabolic fingerprint of (lyophilized) fecal samples. To this end, an untargeted UHPLC-HRMS analytical method using a Q-Exactive™ instrument [2] was applied in conjunction with univariate and (piecewise) multivariate data processing methods. Fresh fecal samples were received from 4 healthy male and 5 healthy female volunteers (20–50 years) (University Hospital Ghent, EC 2016/0673).

3. Results

The effect of the number of freeze-thaw cycles and storage temperature were studied using pairwise OPLS-DA models and paired *t*-tests, thereby considering the within-person variation (separated from between-person variation using a multilevel approach). One freeze-thaw cycle already induced a significant change in 6.7 to 13.5% of the metabolites, while a second freeze-thaw cycle on top of the first significantly changed 9.2 to 11.1% additional metabolites. Storage at −20 °C significantly altered 15.3 to 21.8% of the metabolic fingerprint compared to −80 °C for both storage durations (0 and 4 weeks), irrespective of the FT cycles, as indicated by the good prediction performance ($Q^2 > 0.6$), R^2Y close to 1 or 1 and $R^2X > 0.5$ of the six models. As a consequence, our recommendation is to store samples at −80 °C and avoid any FT cycle.

For the long-term and (an)aerobic storage conditions, no valid OPLS-DA models ($Q^2 \leq 0.353$, $pQ^2 \geq 0.08$) could be built for discriminating between the aerobic and anaerobic storage condition, within each timepoint and per temperature. Pairwise OPLS-DA models between the two storage temperatures within each timepoint were highly significant, indicating a clear difference between the two temperatures, which was confirmed by additional *t*-tests (7.0 to 10.8% significantly altered). Furthermore, the relative magnitude of change in the metabolic profile between subsequent timepoints was assessed using the piecewise OPLS-DA modelling technique, described by Rantalainen *et al* [6]. This revealed a maximal difference between week 2 and 4 for −20 °C (16.5%) and between week 4 and 8 for −80 °C (15.9%).

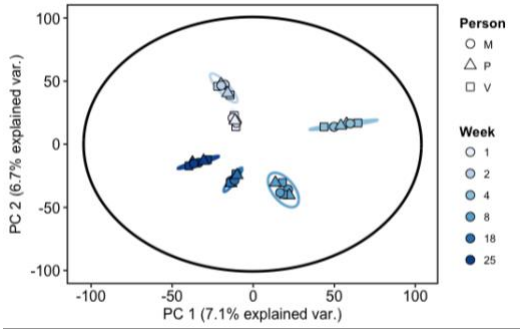

Figure 1. PCA score plot for long-term storage at −20 °C.

4. Discussion

Our study confirms that freeze-thaw cycles, both for storage at −20 °C and −80 °C, should be avoided as much as possible. It should be noted, however, that induced changes are relatively small compared to interindividual variation. For the long-term storage, there was no difference observed between aerobic and anaerobic storage, which may be due to the lyophilization step. Fecal samples should ideally not be stored longer than 2 weeks at −20 °C and not longer than 4 weeks at −80 °C, as indicated by both the piecewise multivariate approach as well as the univariate approach. However, longer storage induces only small additional changes (6.4%).

References

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