

# A GC–MS-based metabonomic investigation of blood serum from irritable bowel syndrome patients undergoing intervention with acidified milk products

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**Abstract** In the present study, the use of gas chromatography mass spectrometry (GC–MS)-based metabonomics to characterize blood serum in an intervention study of patients suffering from the common gastrointestinal disorder irritable bowel syndrome (IBS) was investigated. The patients included in the study consumed an acidified milk product with ( $n = 30$ ) or without probiotics ( $n = 31$ ) (*Lactobacillus paracasei* F19, *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB-12) for an 8-week period, and blood serum samples were collected before and after the intervention. Acidified milk is commonly used as a delivering vector for probiotics in commercial consumer settings. The serum samples were extracted and derivatized using *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA), and GC–MS analysis was carried out. Multivariate data analysis including principal component analysis (PCA), orthogonal partial least squares-discriminant

analysis (OPLS-DA), and S-plot was applied on the obtained GC–MS data, which revealed higher serum lactate, glutamine, proline creatinine/creatinine, and aspartic acid levels and lower serum glucose levels after the intervention period for both treatment groups. Consequently, the present study indicated an effect of acidified milk consumption on the plasma metabolite profile, which was independent of a concomitant intake of probiotics. In addition, the present study demonstrates that GC–MS is a useful analytical technique for metabonomics studies of blood serum.

**Keywords** GC–MS · Probiotics · Acidified milk products · Irritable bowel syndrome · Metabonomics · Lactate · Derivatization

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## Introduction

Metabolomics is a rapidly growing discipline concerned with analyzing the entire measurable array of low-molecular-weight compounds, metabolites, in a given biological system [1, 2]. Measurements are performed on both biofluids (e.g., plasma/serum, urine, and cerebrospinal fluid) and tissues (liver, brain, gut, and muscles), providing vast amounts of data and highly complex metabolite profiles describing the system. Through the study of time variation of metabolites, complex responses to different perturbations or inputs such as disease [3], stress [4, 5], drug administration [6], environmental stimuli [7] and diets [8–10] can be analyzed, thereby providing very useful knowledge of affected pathways. Nuclear magnetic resonance (NMR) is a widely applied method due to the non-selective detection of multiple metabolites with minimum sample preparation and high reproducibility [11]. Mass spectrometry-based methods in combination with chromatography, such as gas chromatography–mass spectrometry (GC–MS) [12–14] offers, on the other hand, much higher sensitivity and the ability to detect a larger number of metabolites. The main drawback is that metabolites need to be derivatized before GC–MS analysis, which is labor intense. Nonetheless, the low detection limit and small sample volume required make it feasible for analysis of subtle metabolic changes induced by a diet intervention as pursued in this study.

Consumption of a probiotic-containing diet is believed to have beneficial effects on the gastro-intestinal tract and general wellbeing. Indeed, probiotics have been shown to have positive effects on gastrointestinal inflammation, thereby conferring beneficial effects to patients suffering from irritable bowel syndrome (IBS) and inflammatory bowel disease [15–20]. IBS is a common bowel disorder characterized by abdominal pain and disturbed bowel habit, i.e. diarrhea and/or constipation. Many patients can keep symptoms at a minimum by “over-the-counter” medication, control of diet and lifestyle; however, symptoms can be so severe that they in a proportion of patients can have a disabling effect on everyday life [21–23].

The aim of the present study was to elucidate the use of a GC–MS-based metabolomic application to examine the effect of an 8-week intervention with either an acidified milk product or an acidified milk containing probiotics on the blood serum metabolite profile in IBS patients.

## Materials and methods

### Study participants and experimental design

Based on the Rome II criteria [24], an initial group of volunteer IBS patients were enrolled in the study. Patients were then followed during a screening period for 2 weeks

without study intervention to establish whether they truly matched the Rome II criteria and to study the severity of their symptoms. The IBS severity scoring system (SSI) [25] using Visual Analogue Scales (VAS) was used to assess individual symptom severity. VAS measures characteristics that cannot easily be directly measured, such as pain. The scale ranges across a continuum from none to extreme without any discrete jumps. In order to be included in the treatment phase of the study, patients had to score high on at least one of the five questions in the IBS SSI questionnaire. Furthermore, patients had to have a lack of adequate relief of the IBS symptoms the week before inclusion. Patients not fulfilling these criteria were excluded from the study, leaving 74 subjects. Subjects were randomized for one of two treatments. During the intervention period, seven subjects dropped out for reasons unrelated to the study. For different reasons, blood serum samples were not acquired from 6 subjects leaving the final group of subjects completing the study on 61 subjects. The total patient group consisted of 45 women and 16 men aging from 18 to 79 years with a mean of 42.5 years. Patient characteristics are described in previously published material [26, 27]. Informed consent was provided by all patients. The study was performed in accordance with the Declaration of Helsinki II and approved by the regional Ethical Committee at University of Gothenburg. The trial was registered at ClinicalTrial.gov with the registration number: NCT01127828.

### Dietary treatment and sample collection

The two different patient groups were administered two different dietary treatments. One treatment consisted of a fermented dairy product containing probiotics. The probiotic product (Cultura®, Arla Foods a.m.b.a, Denmark) was produced by fermentation of homogenized, high-pasteurized, low-fat (1.5%) milk by the use of two fermentation starter cultures, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* and the inclusion of three probiotic strains, *Lactobacillus paracasei* F19, *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB-12, in concentrations of  $5 \times 10^7$  CFU/mL (Proximate composition: protein 40 g/kg, fat 15 g/kg, carbohydrate 45 g/kg, lactose 35 g/kg). The final probiotic concentration was found to be  $10^7$ – $10^9$  CFU/g. The other treatment was a non-probiotic chemically acidified milk product produced by acidification of homogenized, high-pasteurized, low-fat (1.5%) milk by the addition of D-(+)-gluconic acid  $\delta$ -lactone ( $\geq 99.0\%$ ) (GDL) (Sigma-Aldrich, Seelze, Germany) (Proximate composition: protein 40 g/kg, fat 15 g/kg, carbohydrate 65 g/kg, lactose 45 g/kg). Both products had a pH value of 4.5. Over a period of 8 weeks, the two patient groups were instructed to consume 0.4 L/day,

divided in two intakes per day of either the GDL-acidified milk product ( $n = 30$ ) or the probiotic milk product ( $n = 31$ ). In the study, the two products are referred to as the GDL milk product and the probiotic milk product. Patients were instructed not to consume any other probiotic containing dairy products during the intervention period. One day before the intervention period commenced (baseline), body weight measurements were performed and blood samples were collected from each subject. This procedure was repeated on the last day of the intervention period (post-treatment). Furthermore, dietary intake for each patient in 3 days before both baseline and post-treatment sample collection was recorded. Dietary records and weight measurements are reported in previously published material [26]. Patients had been fasting overnight and then consumed a fiber-rich non-dairy meal (Content: 540 kcal; 36% fat, 15% proteins, 49% carbohydrates; 9.2 g fiber) 1 h before collection of blood. Blood serum for GC–MS analyses was prepared by collecting 5 mL blood from the antecubital vein in silicon-treated Vacutainer<sup>®</sup> tubes. Clotting time was 30 min. followed by 20-min. centrifugation at 1,600g. The serum fraction was collected and stored in aliquots at  $-70^{\circ}\text{C}$ .

#### Chemicals and reagents

Methanol:water (8:1 v/v), methoxyamine, pyridine, MSTFA (*N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) were purchased at Sigma-Aldrich (Germany). Standard compounds for D-glucose, glutamine, aspartic acid, proline, creatinine and lactic acid were purchased at Sigma-Aldrich (Germany). D-proline-1-<sup>13</sup>C was purchased at Cambridge Isotope Laboratories (United Kingdom).

#### Serum derivatization and GC–MS analysis

Extraction of metabolites from serum was performed using a modified method based on Jiye et al. 2005 [12]. From each sample, metabolites were extracted using 100  $\mu\text{L}$  diluted serum (1:4 v/v in ddH<sub>2</sub>O) and 900  $\mu\text{L}$  methanol:water (8:1 v/v). The solution was mixed and followed by a centrifugation at 19,600g for 10 min. at  $4^{\circ}\text{C}$ . 200  $\mu\text{L}$  supernatant was collected, transferred to a GC vial and evaporated to dryness in a vacuum centrifuge. Each residue was then dissolved in 30  $\mu\text{L}$  methoxyamine (15 mg/mL) in pyridine. Methoxymation was performed at room temperature for 16 h. After methoxymation, silylation was carried out by adding 30  $\mu\text{L}$  MSTFA with 1% TMCS to the mixture. This solution was vortexed and incubated for 1 h at room temperature. After silylation, 40  $\mu\text{L}$  heptane was added to each vial. Derivatized serum extracts were analyzed with an Agilent gas chromatograph. A 1- $\mu\text{L}$ -extract

aliquot of the extracts was injected into a HP-5MS capillary column coated with 5% phenyl cross-linked 95% dimethylpolysiloxane (30 m  $\times$  250  $\mu\text{m}$  i.d., 0.25-mm film thickness; Agilent J&W Scientific, Folsom, CA) in the split mode (10:1). MS quadrupole temperature was set to  $150^{\circ}\text{C}$  with inlet temperature at  $270^{\circ}\text{C}$  and interface temperature at  $280^{\circ}\text{C}$ . Initial GC temperature was  $70^{\circ}\text{C}$ . After injection, the temperature was raised to  $230^{\circ}\text{C}$  with  $15^{\circ}\text{C}/\text{min}$ . Subsequently, the temperature was raised to  $300^{\circ}\text{C}$  with  $10^{\circ}\text{C}/\text{min}$  and held there for 4 min. Flow for helium carrier gas was set at 1.2 mL/min, and measurements were performed with an electron impact ionization (70 eV) in a full scan mode of 50–500  $m/z$ . Reproducibility was tested by running specific samples two times with a span of 12 h between. Also samples were run with a <sup>13</sup>C isotope labeled proline as internal standard.

#### Data processing and multivariate data analysis

Inspection of Total Ion Chromatograms (TIC) showed errors in two GDL milk group samples and two probiotic milk group samples caused by errors in either derivatization or data acquisition. These samples along with their complementary baseline or post-treatment samples were excluded from the study leaving a data set comprised of 28 GDL and 29 probiotic samples. Each sample was represented by a GC–MS–TIC, and peaks with a signal-to-noise ratio higher than 5:1 was detected. Out of 69 initially automatically selected peaks, 14 were excluded from the original selection due to problems with low signal-to-noise ratio or shoulder separation. The intensities of the 55 selected peaks were calculated and used for multivariate data analysis (MVA). Normalization to total intensity of the spectrum was performed before further data analysis. Cross-validated principal component analysis (PCA) was performed on mean centered data to identify discrete patterns within the data set. PCA is an unbiased algorithm that lowers data dimensionality in large data set while retaining the variation within the data set. The algorithm identifies principal components (PC) in which the variation are at maximum, thereby large data sets containing many variables can be explained by a relatively low number of PCs. Analysis of the produced component plots can then identify similarities, differences, and discrete patterns in the data. Patterns detected in PCA are subsequently analyzed by cross-validated orthogonal partial least squares discriminant analysis (OPLS-DA) [28]. For the OPLS-DA, data were scaled using mean centered variable stability (VAST) scaling. VAST was used to obtain unit variance within each treatment [29]. The OPLS-DA algorithm decomposes the variation between two identified groups into three parts; the variation in  $X$  (the data matrix) related to  $Y$  (the discriminant matrix), the systemic variation in  $X$  and the residual

variation in  $X$ . Thereby a model with minimum predictive components defined by the number of degrees of freedom between group variances was produced. S-plots [30] were used to sort out relevant metabolites. The S-plot incorporates both the covariance and the correlation of variables, thereby minimizing the possibility of false positives. MVA was performed using the Simca-P+12.0 software (Umetrix, Umeå, Sweden). One-way ANOVA was performed on principal component scores, and paired Student's  $t$  test was performed on peak areas. All tests were performed on the 0.05 significance level.

## Results

After GC–MS analysis of all samples, the TICs were inspected individually and by overlay plots. No discrepancies in peak appearance were found between different samples. A peak detection limit was imposed on the TIC for selection of metabolites, and 55 peaks were found to have a signal-to-noise ratio within the imposed detection limit. Figure 1a shows a chromatogram for a representative sample. The chromatograms showed a very high degree of alignment. The reproducibility was also found to be very high when comparing chromatograms recorded from a sample that was run twice with 12 h between the analyses (Fig. 1b). The 55 selected peaks were used for the subsequent MVA.

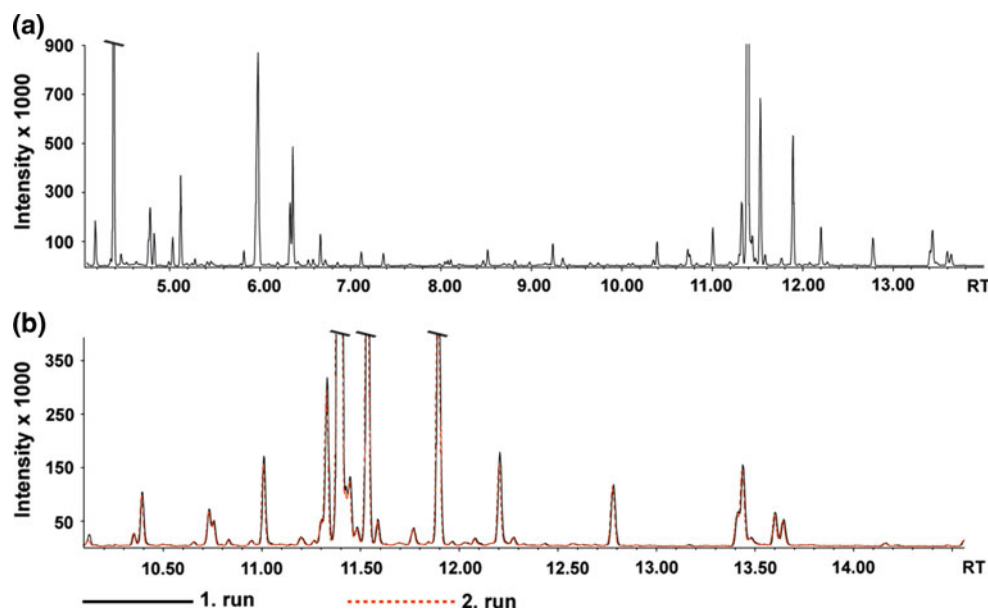
Peak intensity was calculated, and data were normalized to the total intensity of each spectrum. Baseline and post-treatment samples were analyzed by PCA for groupings caused by age, body mass index, gender, response to treatment, and type of IBS, respectively. No groupings

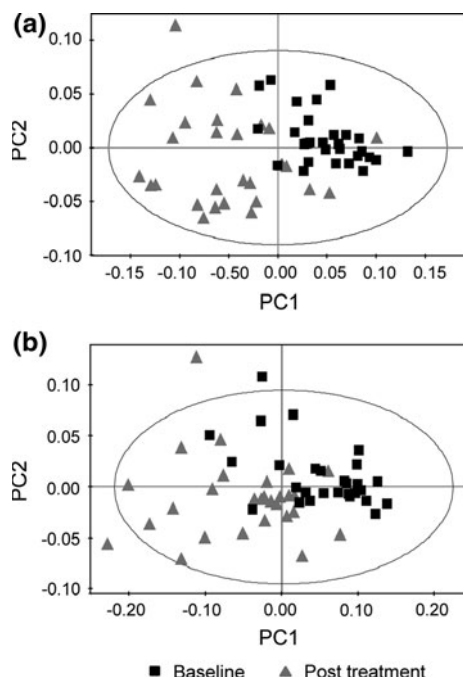
were found within either the GDL-milk-treated patient group or the probiotic-milk-treated group of patients (data not shown). We then tested for groupings in PCA when comparing baseline and post-treatment samples for each treatment (Fig. 2) and found that there was a distinct separation between baseline and post-treatment for both GDL-milk (Fig. 2a)- and probiotic-milk (Fig. 2b)-treated patient groups.

Both PCA models, composed of 4 principal components for the GDL patient group and 5 principal components for the probiotic group, resulted high  $R^2(X)$  values and  $Q^2(cum)$  values indicating that nearly all the variation in the spectra were explained, and that the predictability of the models were good. Furthermore, one-way ANOVA on principal component scores showed a significant difference between baseline and post-treatment samples for PC1 for both groups and PC4 for the GDL patient group. The differences between baseline and post-treatment samples were identified using OPLS-DA. OPLS-DA models explained 42.5% (GDL,  $n = 28$ ) and 48.2% (probiotic,  $n = 29$ ) of the total variance with  $Q^2$  values of 0.708 (GDL) and 0.767 (probiotic).  $Q^2$  is the cross-validated predictive ability which is related to the statistical robustness of the model. Models were constructed with one predictive component and 4 orthogonal components. Consequently, both PCA and OPLS-DA showed a difference between baseline and post-treatment for both treatment groups. A PCA model was also constructed on post-treatment samples including both the probiotic-milk-treated patient group and the GDL-milk-treated patient group; however, no difference between the two groups could be observed (data not shown).

To sort out which metabolites were responsible for the observed differences between baseline and post-treatment

**Fig. 1** Total ion chromatograms of representative human serum samples. **a** TIC from RT 4.0–14.0 min retention time (RT) of a post-treatment sample. **b** Overlay plot from 10.0–14.5 min RT of two spectra collected from two identical post-treatment samples from a probiotic-milk-treated IBS patient run with a time span of 12 h in between





**Fig. 2** PCA of baseline and post-treatment samples. **a** GDL milk group treated IBS patients ( $n = 28$ ). Model composed of 4 principal components.  $R^2X(1) = 0.630$ ,  $R^2X(2) = 0.172$  and  $Q^2(\text{cum}) = 0.658$ . **b** Probiotic milk group treated IBS patients ( $n = 29$ ). Model composed of 5 principal components.  $R^2X(1) = 0.736$ ,  $R^2X(2) = 0.133$  and  $Q^2(\text{cum}) = 0.745$ . One-way ANOVA was performed on baseline and post-treatment samples in all PC's showing  $P \leq 0.01$  for PC1 in both the GDL- and the probiotic-milk-treated patient groups and PC4 for the GDL-milk product-treated patient group. The ellipse indicates the Hotelling T2 95% limit

groups, we investigated the S-plots obtained for both OPLS-DA analyses (Fig. 3). The resulting S-plots show the covariance and correlation of the different TICs included in the model. The two S-plots displayed a high degree of similarity. Metabolites showing a correlation of  $|R| > 0.5$  and a covariance above 0.18 or below  $-0.18$  were selected. This selection was based on the appearance of the S-plot, where three metabolite clusters were separated from the rest. Identification of the peaks revealed that the same metabolites were affected in both treatment groups. Even though metabolite no. 63 appeared to have a higher covariance and correlation in the probiotic-milk-treated patient group than in the GDL-milk-treated patient group, the difference was not high enough to discriminate between the two treatments. Metabolites with negative correlations and covariances had lower levels in the post-treatment samples, and those with positive values had higher levels.

The changes in the peak areas of the selected peaks (Table 1) between baseline and post-treatment levels were significant ( $P < 0.04$ ). The mass spectra of the selected peaks were compared with spectra in the National Institute of Standards and Technology (NIST) and metabolite

identifications obtained from NIST were confirmed by using chemical standards for the specific metabolites (Table 1). We found several derivatives originating from D-glucose to decrease with the treatments, and lactate, aspartic acid, proline, creatinine, glutamine, and one unidentified metabolite to increase. The creatinine found encompasses both blood serum creatinine and creatine, since the condition during derivatization causes creatine to form the cyclic creatinine [31].

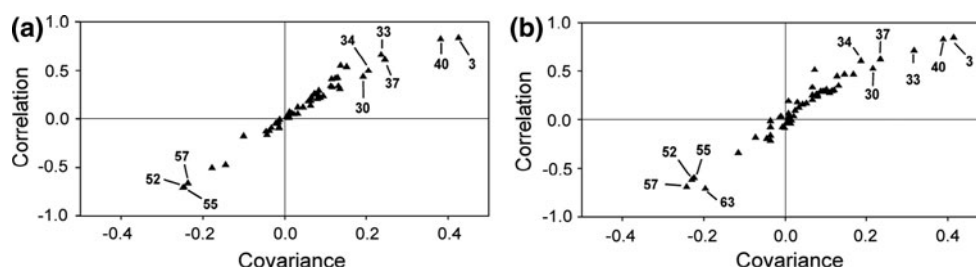
## Discussion

In the present study, the use of GC-MS for a metabolomics application on human serum was explored. Blood serum samples from irritable bowel syndrome (IBS) patients collected before and after an 8-week intervention period with a daily consumption of 0.4 L acidified fermented milk products with or without probiotics were examined. Independent of the fermented milk product contained probiotics or not, higher levels of lactate, glutamine, proline, creatine/creatinine, and aspartic acid were detected in the serum after the diet intervention period. In addition, comparison of pre- and post-intervention serum samples revealed a concomitant lower level of serum glucose for both treatment groups after the intervention period.

We utilized S-plots for the identification of significant metabolites, and the identification of the responsible metabolites for discrimination between baseline and post-treatment in both treatment groups was quite unambiguous with only one glucose derivative in one treatment group falling outside the selection criteria. Lowering the covariance limit could induce the risk of identifying metabolites varying only because of analytical variation and noise [30]. The simple data analysis method used here is useful when performing studies with a large number of samples with good stability and few variables. Correlation and covariance depends on both sample amount and stability; thus, analyses such as the one performed here, with many stable samples, offers a setup ideal for employing OPLS-DA coupled with S-plots for metabolite detection.

Symptom evaluation is subjective and is based on the patient's own perception of discomfort, and in studies concerning bowel disorder, a high degree of placebo response has been reported [32–34]. The present GC-MS analysis of blood serum revealed that the GDL and the probiotic-milk-treated patient groups responded in a very similar way to the intervention. Although the evidence for efficacy of probiotic treatment in IBS patients is weak, overall symptom improvements have been shown using approximately the same intervention time and probiotic load as we have used in the present study [35]. We therefore believe that the similar response from the two





**Fig. 3** Identification of metabolites important for baseline and post-treatment discrimination. **a** S-plot for VAST scaled OPLS-DA of baseline and post-treatment samples from GDL-milk-treated IBS patients. **b** S-plot for OPLS-DA of baseline and post-treatment

samples from probiotic-milk-treated IBS patients. Metabolites having a correlation to the discrimination between baseline and post-treatment of  $|R| > 0.5$  coupled with a covariance either above 0.18 or below  $-0.18$  are marked

treatment groups indicates an independency of the probiotics added to one of the products. During the intervention period, a subgroup of the probiotic-milk-treated patients reported a faster symptom relief compared with the GDL-treated patients. However, at the end of the diet intervention period, symptom relief was balanced out between the two treatment groups [27]. Comparing this with our results could indicate that the change in the blood serum metabolite profile is correlated with symptom relief. However, based on our analysis, it is not possible to conclude whether the blood serum metabolic response is directly linked to symptom relief, and to further investigate this, samples should be acquired during the intervention period.

In accordance with a previous NMR-based metabonomic study [26], we were not able to detect any influence of the probiotics administered to the patients. Irrespective of probiotics we can conclude that both responders and non-responders in both treatment groups displayed a similar blood serum metabolite response after 8 weeks of intervention.

We have previously used  $^1\text{H-NMR}$  metabonomics and a standard enzymatic assay to identify lactate and 3-hydroxybutyric acid as metabolites involved the separation between baseline and post-treatment samples [26]. However, by utilizing the higher sensitivity of the GC-MS analysis, we could also identify glucose, glutamine, proline, aspartic acid, and creatine/creatinine to be affected by intake of the fermented milk products. Glucose signals are evident in the  $^1\text{H NMR}$  spectrum, in particular the anomeric CH protons from  $\alpha$ - and  $\beta$ -glucose give rise to characteristic signals. However, unfortunately, the residual water signal interferes with these signals, which may be the reason that glucose was not identified by  $^1\text{H NMR}$ -based metabonomics. In addition to anomeric glucose signals, the  $^1\text{H NMR}$  spectrum contains a large region around  $\sim 3\text{--}4$  ppm, where various CH protons arising from carbohydrates and amino acids are overlapping. This may distort the detection of proline, which appear in the same region. Aspartic acid and creatine/creatinine were less concentrated, and therefore, the higher sensitivity in

metabolite detection together with a better resolution offered by GC-MS probably explains the reason for detection of changes in these metabolites by GC-MS and not by NMR. In contrast to the  $^1\text{H-NMR}$  analysis, we were not able to detect 3-hydroxybutyrate by the applied GC-MS method. The reason for this remains unknown.

The present GC-MS metabonomic approach revealed that glucose levels were lower after the 8-week intervention compared with baseline. Both at baseline and after the intervention, the patients had been fasting overnight and consumed a standardized meal 1 h before sample collection. Consequently, the effect on glucose could indicate a difference in blood serum glucose clearance, since blood serum glucose would increase after the consumed meal before sample collection. The release of insulin following nutrient consumption increases the rate of blood serum glucose removal, and after approximately 2 h, postprandial blood serum glucose should have reached a normal level again [36, 37]. We analyzed 1 h postprandial samples; thus, our measurements were performed as blood serum glucose is being removed. Therefore, the lower level of blood serum glucose could be due to an increased rate of removal [38]. Schenk and colleagues reported a more effective clearance of plasma glucose as an effect of an earlier and marked insulin response in the initial 20-min postprandial period [39], and it has also been shown that addition of fermented products in a mixed meal lowers the postprandial glycemia and insulinemia [40]. Further research is needed to clarify whether short-term acidified milk treatment can lead to an earlier postprandial insulin response.

Glutamine is the major energy source for enterocytes in the intestinal tissue and when diet resources are consumed, intestinal tissues rely on circulating glutamine [41]. Intestinal tissues are affected in IBS patients, and the higher levels of glutamine and proline could indicate a change in the energy status of the intestinal tissue as a result of intake of the acidified milk products, as catabolism of glutamine in the intestine produces proline as an end product [42, 43]. Furthermore, the role of aspartic acid as a precursor for

**Table 1** GC-MS peak identification and mean areas. Difference between post-treatment and baseline was determined using paired Student's *t* test with Holm's sequential Bonferroni

Peak no.	Metabolite	RT	GDL	Probiotic									
				Baseline			Post			<i>t</i> test			
				Mean × 10 <sup>8</sup>	SD × 10 <sup>8</sup>	Mean × 10 <sup>8</sup>	SD × 10 <sup>8</sup>	Mean × 10 <sup>8</sup>	SD × 10 <sup>8</sup>	<i>P</i>	<i>t</i>		
3	Lactate	4.38	1.34	0.46	0.87	2.79	0.87	<0.001	1.42	0.63	2.58	0.88	<0.001
30	Unidentified	8.05	0.03	0.01	0.02	0.05	0.02	<0.001	0.04	0.01	0.05	0.02	0.003
33	Aspartic acid	8.47	0.03	0.01	0.02	0.04	0.02	<0.001	0.03	0.01	0.04	0.01	<0.001
34	Proline	8.52	0.07	0.03	0.05	0.11	0.05	<0.001	0.08	0.03	0.11	0.06	0.012
37	Creatine/creatinine	8.82	0.03	0.01	0.01	0.04	0.01	<0.001	0.03	0.01	0.04	0.01	0.002
40	Glutamine	9.24	0.07	0.02	0.04	0.12	0.04	<0.001	0.07	0.02	0.13	0.03	<0.001
52	D-glucose	11.39	8.14	2.85	2.74	6.60	2.74	0.022	8.49	2.20	6.57	1.92	<0.001
55	D-glucose	11.54	1.46	0.54	0.50	1.18	0.50	NS	1.52	0.42	1.16	0.35	<0.001
57	D-glucose	11.77	0.08	0.02	0.02	0.07	0.02	0.035	0.08	0.02	0.07	0.02	0.003
63	D-glucose	12.28	0.03	0.01	0.01	0.03	0.01	0.007	0.03	0.01	0.03	0.01	<0.001

oxaloacetate, which is an important intermediate in the TCA cycle, and involvement of creatine and creatinine in muscle energy consumption [31], could indicate that intake of the acidified milk products induces a change in metabolism [44, 45].

**Conclusions**

Using GC-MS-based metabolomics, we showed that a short-term dietary intervention with acidified milk products resulted in elevated levels of serum lactate, glutamine, proline, creatine/creatinine and aspartic acid, and a decreased level of serum glucose in patients suffering from IBS. Our results confirm a previous report [26] using <sup>1</sup>H NMR-based metabolomics where lactate was identified as the major metabolite responsible for the difference between baseline and post-treatment samples, but also unravel new information, as effects on glutamine, proline, creatine/creatinine, aspartic acid, and glucose was not identified by <sup>1</sup>H NMR-based metabolomics. Changes in the array of metabolites could indicate a change in the energy metabolism. The effects were observed in both a probiotic and a non-probiotic acidified milk group. This is in good agreement with previously reported symptom relief in both groups after 8 weeks treatment and furthermore indicates that the delivering vector for probiotics, which was acidified milk in this study, can be very important for the study outcome.

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