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Metabonomics Approach To Determine Metabolic Differences between Green Tea and Black Tea Consumption

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The purpose of this study was to compare the effects of black and green tea consumption on human metabolism. Seventeen healthy male volunteers consumed black tea, green tea, or caffeine in a randomized crossover study. Twenty-four-hour urine and blood plasma samples were analyzed by NMR-based metabonomics, that is, high-resolution ^{1}H NMR metabolic profiling combined with multivariate statistics. Green and black tea consumption resulted in similar increases in urinary excretion of hippuric acid and 1,3-dihydroxyphenyl-2-*O*-sulfate, both of which are end products of tea flavonoid degradation by colonic bacteria. Several unidentified aromatic metabolites were detected in urine specifically after green tea intake. Interestingly, green and black tea intake also had a different impact on endogenous metabolites in urine and plasma. Green tea intake caused a stronger increase in urinary excretion of several citric acid cycle intermediates, which suggests an effect of green tea flavanols on human oxidative energy metabolism and/or biosynthetic pathways.

KEYWORDS: Tea; metabolism; metabolomics; NMR spectroscopy; multivariate analysis; catechins; flavanols; flavonoids; epigallocatechin gallate

INTRODUCTION

Tea (*Camellia sinensis*) is the second most consumed beverage in the world after water (*1*). Tea is a rich dietary source of flavonoids, which have potential beneficial health effects in humans (*2*−*4*). The flavonoids in green tea, mostly flavanols such as (−)-epicatechin (EG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG), are preserved because oxidative enzymes present in tea leaves are inactivated immediately after picking. The processing of black tea involves mechanical maceration of tea shoots, which triggers enzyme-catalyzed oxidation and partial polymerization of ≈80% of the flavanols. The resulting condensed flavanols, known as theaflavins (dimers) and thearubigins (polymers), are responsible for the characteristic taste and golden orange color of prepared black tea (*1*). Flavonoids constitute 10−12% of the dry leaf weight of black as well as green tea.

The bioavailability of tea flavonoids and their metabolism in humans have been topics of considerable interest, but they remain to be solved. Only a few percent of tea flavanols are directly absorbed in the small intestine and excreted in the urine, and a large fraction of this becomes conjugated (*5*−*9*). Flavonoids that are not absorbed in the small intestine, or that have been excreted into the bile, are metabolized by the bacterial flora in the colon (*10*−*12*). The resulting microbial flavonoid metabolites are absorbed from the colon and the urinary concentrations of some of these metabolites exceed the concentrations of the intact flavonoid (*13*−*15*). Hippuric acid, the conjugate of benzoic acid with glycine, has been identified as the major metabolite of both black (*16*, *17*) and green (*18*) teas.

Nevertheless, the overall metabolic impacts of black and green tea may be different. In this study, we pursued this by applying a nontargeted ^{1}H NMR-based metabolic analysis of the urine and plasma samples collected. Biofluid ^{1}H NMR spectra are extremely rich in composition information and allow for a rapid and nonspecific assessment of a broad range of metabolites. To assess any treatment-related effects on the complex biofluid ^{1}H NMR profiles, multivariate data analysis is required, and this is the essence of metabonomics (*19*−*21*). Recent studies have demonstrated that NMR-based metabonomics was able to establish the metabolic impact of black tea (*22*) and chamomile (*23*). The current study uses a similar metabonomics-type approach to distinguish between green tea, black tea, and caffeine intake in humans.

MATERIALS AND METHODS

Experimental Design. The Medical Ethical Committee of the Nederlandse Unilever Bedrijven BV approved this study. Eighteen nonsmoking male volunteers were recruited. One subject withdrew from

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the study because he judged the taste of the study beverage to be unacceptable. The 17 participants that completed the study had a median age of 63 years (range = 20−70 yrs) and a body mass index of 24.8 ± 2.9 kg/m^2.

The subjects were studied with a randomized, full-crossover design with three treatments, that is, green tea solids, black tea solids, and caffeine (control). Each intervention period consisted of a 2-day run-in period with a low-polyphenol diet followed by a 2-day tea treatment period during which the test products were consumed on top of the low-polyphenol diet. Intervention periods were separated by a washout period of almost 10 days, during which the volunteers consumed their habitual diets. Tea solids were prepared by extracting black or green tea (Lipton Research Blends, Lipton, Englewood Cliffs, NJ) with boiling water and spray-drying the resulting extracts. Details on the composition of the tea solids can be obtained from ref *24*.

Volunteers were asked to consume six portions of 1 g of tea solids dissolved in hot water per day (equivalent to 12 cups of tea per day). During the control intervention, a daily dose of 360 mg of caffeine (Sigma-Aldrich, Zwijndrecht, The Netherlands) was supplemented in six gelatin capsules. This amount of caffeine was equivalent to that in the tea solids. For the low-polyphenol diet, volunteers were instructed to refrain from drinking coffee, tea, fruit juices, beer, or wine and from eating onions, kale, broccoli, applesauce, or chocolate during the treatment periods. To compensate for the low-caffeine content of the low-polyphenol diet, a daily dose of 360 mg of caffeine was also supplemented during the run-in days.

Dietary records were maintained during all intervention periods. Volunteers were asked to replicate the diet that they consumed on the 2 run-in days during the subsequent 2 treatment days. They were also encouraged to repeat their diet from the first intervention during subsequent interventions. A printed copy of their first dietary record was given to them as a reminder. The main hot meals were provided as commercial frozen meals (IgloMora Group, 's-Hertogenbosch, The Netherlands) with low polyphenol contents.

Twenty-four-hour urine samples were collected both during the second day of the run-in period and during the second day of the treatment period (last day of the intervention). Urine was collected into 500-mL polyethylene flasks containing 3.5 mL of a 50% (by vol) solution of metaphosphoric acid. The pH of the pooled 24-h urine samples was adjusted to a value between 2.0 and 3.0 with 50% (by vol) metaphosphoric acid solution. Samples of the acidified urine were stored at −20 °C. Blood samples were collected in vacuum tubes containing EDTA as anticoagulant, late in the afternoon on day 4. Plasma was obtained by centrifugation and stored at −70 °C.

Screening of Urine and Plasma Samples by ^{1}H NMR Spectroscopy. Urine samples were prepared for ^{1}H NMR spectroscopy by adjusting the sample pH to 2.5 with a minimal volume of 1 M HCl and adding 10% ^2H_2O (final volume = 0.5 mL). ^{1}H NMR spectra were acquired at 600.13 MHz on a Bruker Avance 600 spectrometer. Spectra of all urine samples were acquired in a single run, and samples were randomized prior to NMR analysis. A Bruker Efficient Sample Transfer (BEST; Bruker Analytische GmbH, Karlsruhe, Germany) flow injection accessory was employed for urine sample delivery to the 4-mm outer diameter ^{1}H−^{13}C Z-gradient probe. Urine NMR spectra were measured at 303 K using a standard water presaturation pulse sequence, that is, RD−90°−t_1−90°−t_m−90°−acquire. Here, RD is a relaxation delay of 1.5 s during which the water resonance was selectively irradiated, and t_1 corresponds to a fixed delay of 0.15 s. A total of 128 transients were collected into 32K data points, with a spectral width of 7000 Hz. Prior to Fourier transform (FT) the free induction decays (FIDs) were multiplied by a 0.3-Hz exponential line-broadening function and zero-filled by a factor of 2.

Plasma samples were prepared for NMR by diluting 300 μL of plasma 1:1 with ^2H_2O, followed by spinning the samples for 10 min at 14000 rpm in an Eppendorf centrifuge. ^{1}H NMR spectra of plasma were measured at 310 K using a 5-mm triple-resonance (TXI) probe. Plasma NMR spectra were measured using a 1D Carr−Purcell−Meiboom−Gill (CPMG) sequence with a total of 256 transients, 32K data points, a pulse train length of 178 ms, and a relaxation delay of 3 s. An exponential window function of 0.5 Hz was applied to the FID prior to Fourier transformation.

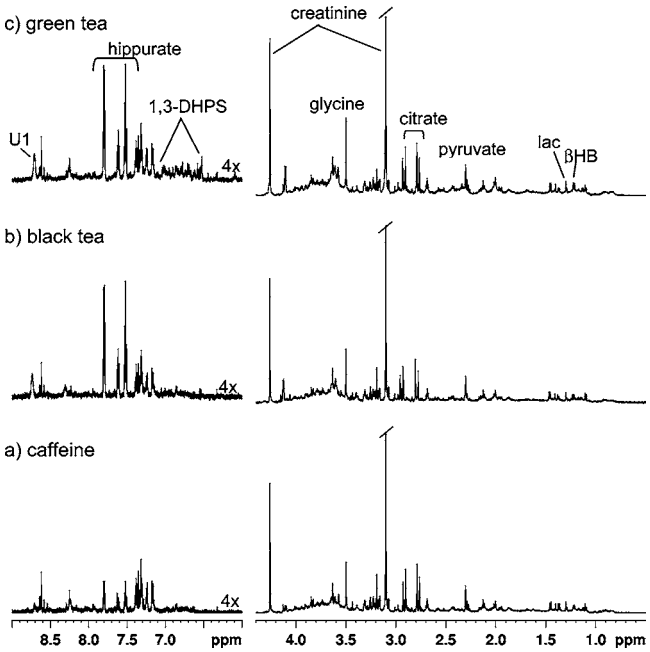

Figure 1. 600-MHz ^{1}H NMR spectra of human urine after 2-day consumption of (**a**) caffeine, (**b**) black tea, and (**c**) green tea. The aromatic region of the NMR spectra (6–9 ppm) was magnified 4 times as compared to the aliphatic region (0.4–4.5 ppm). Abbreviated assignments: βHB, β-hydroxybutyrate; 1,3-DHPS, 1,3-dihydroxyphenyl-2-*O*-sulfate; lac, lactate.

All spectra were manually phase- and baseline-corrected using XWINNMR (Bruker GmbH). Urine spectra were referenced internally to the creatinine methyl peak at δ 3.10, and plasma NMR spectra were referenced to the lactate−CH_3 signal at 1.33 ppm. Metabolites were identified using NMR spectral assignments available in the literature (*25*). To aid spectral assignment, two-dimensional *J*-resolved (J-Res) and ^{1}H−^{1}H total correlation (TOCSY) NMR spectra were measured with solvent presaturation.

NMR Spectral Data Reduction. The NMR spectra were automatically reduced to discrete regions of equal width (0.02 ppm), and the integral of each region was determined using Analysis of Mixtures (AMIX; Bruker GmbH). For urine, the region from 4.3 to 6.0 ppm was excluded from analysis to remove variations in the suppression of the water resonance and variations in the urea signal. Urine profiles were normalized to the peak integral of creatinine at 3.10 ppm to correct for concentration effects. For plasma, the spectral region of water between 4.4 and 5.0 ppm was excluded, and plasma profiles were then normalized to the total sum of the spectral regions. The resultant data sets were then imported into Excel (Microsoft Excel 97) software. For urine, the spectral bins between 2.74 and 2.82 ppm and between 2.88 and 2.96 ppm were combined to account for slight pH-related shifts in the complex citrate signal. To reduce the effects of inherent day-to-day variation of the urine composition, the "pretreatment" urine profile was subtracted from the "test day" profile. The resultant set of difference spectra were then imported into the software package SIMCA-P version 10.5 (Umetrics, Umeå, Sweden). For plasma, no pretreatment NMR profiles were available to calculate difference spectra. Therefore, to minimize intersubject variation as much as possible, data were mean-centered per person, as described below.

Multivariate Data Analysis. Multivariate data analysis was carried out using mean-centered data and Pareto-scaling, in which each variable is weighted by the square root of its standard deviation. The plasma profiles were mean-centered per person to minimize the dominant intersubject variation. Principal component analysis (PCA), an unsupervised pattern recognition method, was performed to examine the dominant intrinsic variation in the dataset. Next, a supervised method, partial least-squares discriminant analysis (PLS-DA), was performed to maximize the separation between samples from different treatments. PCA and PLS-DA models are depicted as complementary score plots (PC1, PC2), which display any intrinsic grouping of the samples based

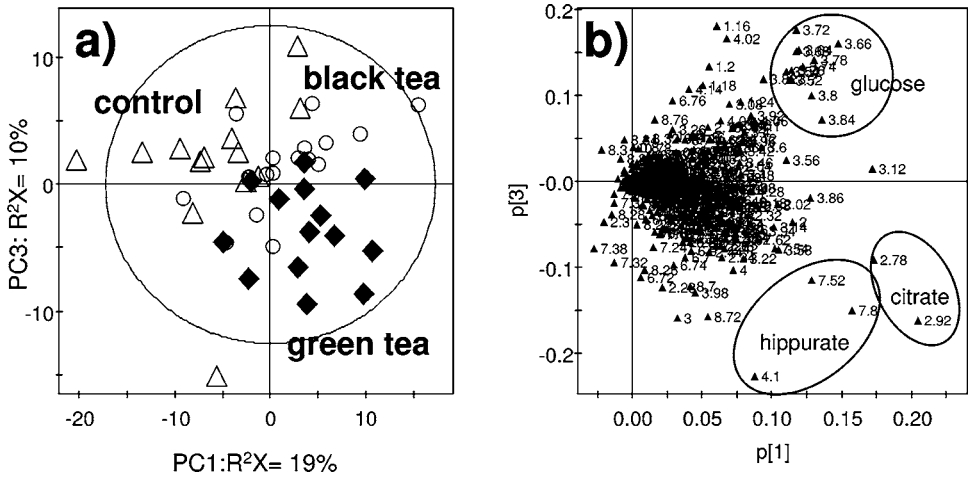

Figure 2. PCA of the urine NMR profiles. PCA shows a separate clustering of profiles due to black tea (○) and green tea (◆) intake versus caffeine (△): (**a**) PCA score plot of the first and third PCs; (**b**) complementary PCA loading plot, in which each symbol represents an NMR variable (labeled as chemical shift in ppm). The position of each (NMR) variable on the loading plot relates to the strength and direction of its contribution to the clustering in the score plot. Circles indicate groups of variables assigned to metabolites that were increased after tea consumption. R^2X describes the fraction of the variance in the NMR dataset (X) that is explained by each component.

on spectral variation, and loading or coefficient plots (p[1] or CoeffCS-[1]), which show the contribution of each spectral variable to the score formation. PLS-DA models comparing black tea versus controls and green tea versus controls were calculated in a pairwise fashion. The quality of the various models is described by the parameters R^2X, R^2Y, and Q^2. Parameters R^2X and R^2Y give for each of the model components the fractional explained variation in the X-space (NMR data) or Y-space (treatment), respectively. The validity of the models was tested by seven rounds of internal cross-validation, and the parameter Q^2 gives the fraction of the variation in Y that can be predicted by one or all model components (Q^2_{cum}).

Statistics. Descriptive statistics of selected metabolite levels was performed on the basis of their peak integrals relative to that of creatinine in the urine NMR profile. The difference in levels of metabolites in urine was tested using one-way analysis of variance (ANOVA), with Tukey's post test. P values of <0.05 were considered to be statistically significant.

RESULTS

Analysis of Urine ^{1}H NMR Profiles. Figure 1 displays three representative urine ^{1}H NMR spectra. The most obvious change was the appearance of characteristic NMR signals of hippuric acid after tea consumption. To be able to detect more subtle treatment-related metabolic differences, pattern recognition techniques were applied.

PCA was performed on all urinary metabolite profiles (**Figure 2**). Four urine samples were classified as outliers and were removed from the analysis: three samples because of spectral baseline problems and one because of the presence of a substantial signal of ethanol. The PCA scores plot (**Figure 2**) showed a clustering of urine samples related to black tea, green tea, and caffeine intake in the first and third PCs. The corresponding loadings plot (**Figure 2b**) showed that increased urine NMR peaks from hippuric acid, citrate, and glucose contribute most to the separate clustering of the groups.

Effects of Green and Black Tea on the Aromatic Profile of Human Urine. PLS-DA improved the discrimination between the ^{1}H NMR urine profiles of the three treatment groups. Separate PLS-DA models were calculated for the aliphatic region (0.5−4.5 ppm) and the aromatic/phenolic region (6−9 ppm) of the urine NMR profiles. The aromatic region of the urine profile focuses specifically on phenolic metabolites, that is, the metabolic breakdown products of tea polyphenols. The aliphatic region of the urine profile contains mostly signals from endogenous human metabolites, which may indicate effects on human intermediary metabolism. **Figure 3** shows the pairwise comparison of the black tea, green tea, or caffeine impact on the urine phenolic profiles. All three PLS-DA models displayed a treatment-related clustering of urine phenolic profiles (**Figure 4**), with Q^2_{cum} values of 0.80, 0.78, and 0.19 for the black tea versus caffeine control, green tea versus control, and green tea versus black tea intake models, respectively.

The PLS-DA coefficients for the relative impact of black tea or green tea intake versus caffeine showed a significant ($P < 0.001$) increase in hippuric acid levels (**Figure 3b**,**d**), but the difference in excreted hippuric acid levels between black and green tea intake was not significantly different. The PLS-DA loadings also showed elevated urine NMR signals at 6.52 and 7.02 ppm after both black and green tea intake (**Figure 3b**,**d**), and these have been recently assigned to 1,3-dihydroxyphenyl-2-sulfate (1,3-DHPS) (*26*). Although the loadings of **Figure 3f** suggest a stronger increase in 1,3-DHPS excretion after green tea intake than after black tea intake, ANOVA analysis did not reach significance.

Green tea intake caused a stronger increase in urinary levels of several unidentified aromatic compounds that resonate between 6 and 7 ppm (**Figure 3e**,**f**). In addition, green tea caused an upfield shift in NMR signals (4.1 and 8.74 ppm), with respect to black tea, which suggests either a different conjugation of this unknown compound (U1) or pH effects. An extended list of urine metabolites changing after tea consumption, most of which are yet unidentified, is given in **Table 1**.

Effects of Green and Black Tea on Endogenous Metabolites in Urine. The aliphatic region (0−4.5 ppm) of the urine NMR profiles contains signals mostly from endogenous metabolites, like (nonaromatic) amino acids, sugars, and organic acids. PLS-DA of the urine endogenous metabolic profiles (**Figure 4**) also provided models showing the metabolic difference between black tea intake and control ($Q^2 = 0.30$), between green tea intake and control ($Q^2 = 0.27$), and between green tea and black tea intake ($Q^2 = 0.55$).

The PLS-DA models indicate that green tea intake had a different impact on urinary excretion of endogenous metabolites from that of black tea. The observed changes in endogenous metabolites in urine are summarized in **Table 2**.

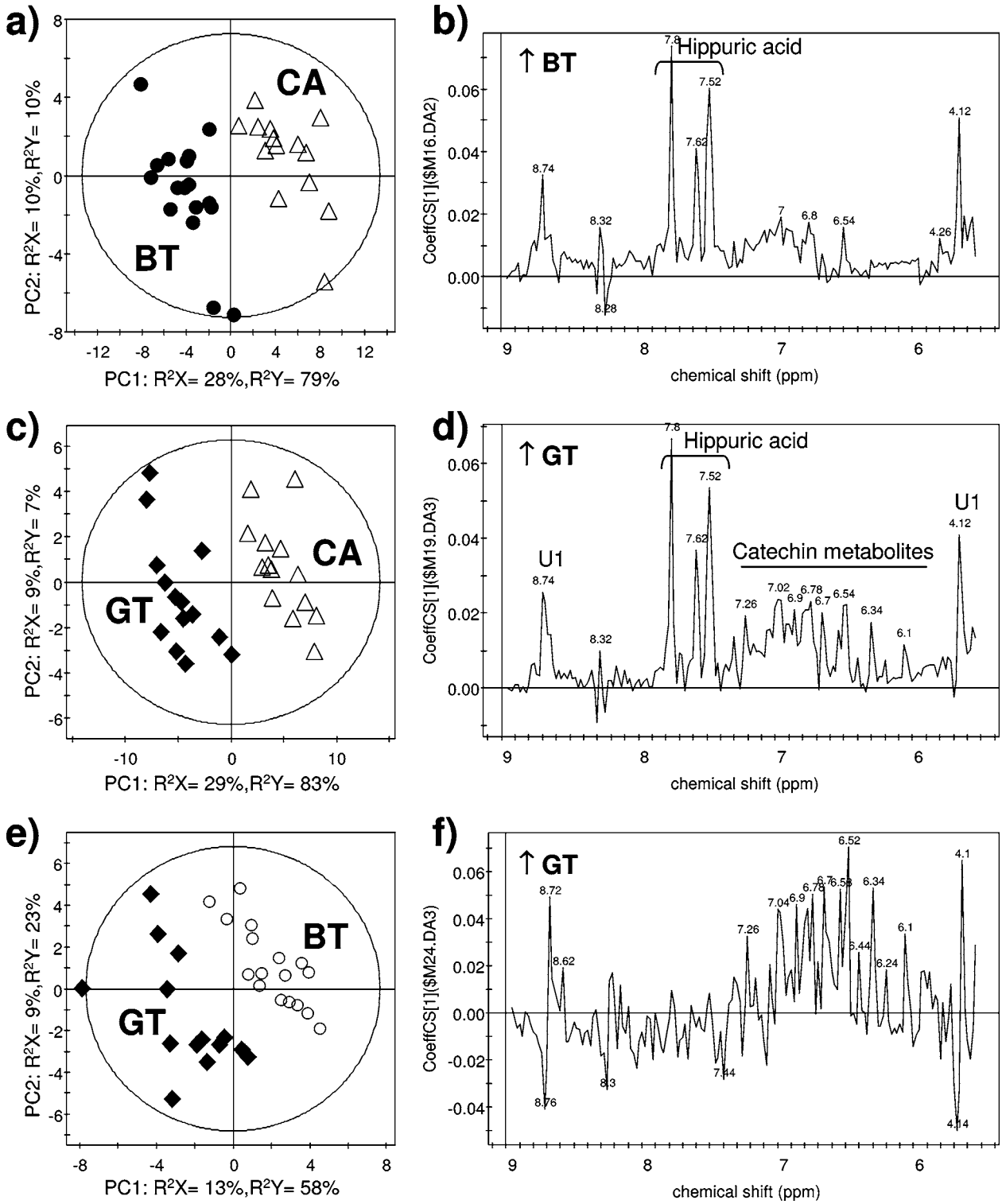

Figure 3. PLS-DA of phenolic NMR profiles of urine shown as pairwise comparison of the urine phenolic profiles after caffeine (CA), black tea (BT), or green tea (GT) intake: (**a**) PLS-DA score plot separating black tea (●) intake from caffeine (△); (**b**) complementary coefficient plot of the first component; (**c**) PLS-DA score plot separating green tea intake (◆) from caffeine (△)l (**d**) coefficient plot of the first component, GT versus CA; (**e**) PLS-DA score plot separating green tea (◆) from black tea intake (○); (**f**) first coefficient for GT versus BT. The peaks in the coefficient plots relate to the contribution of NMR variables to the clustering. R^2X and R^2Y describe how much of the variance in the NMR spectra (X) and treatment class (Y), respectively, is explained by a component, whereas Q^2 describes the part of the variance that is predicted by the model after cross-validation.

Effects of Green and Black Tea on Endogenous Metabolites in Plasma. Plasma NMR data from the run-in periods were not available to calculate difference spectra, as was done for urine. To minimize the intersubject variation, the data were mean-centered per person. PCA of the plasma profiles did not display intrinsic clustering related to tea intake in the first three PCs. PLS-DA was used to maximize the separation between the ^{1}H NMR plasma profiles after green tea, black tea, or placebo ingestion (**Figure 5**). The first coefficient of the PLS-DA model ($Q^2 = 0.25$) showing the impact of black tea consumption on the plasma profile (**Figure 5b**) showed a shift in the lipoprotein distribution and a reduction in plasma glucose

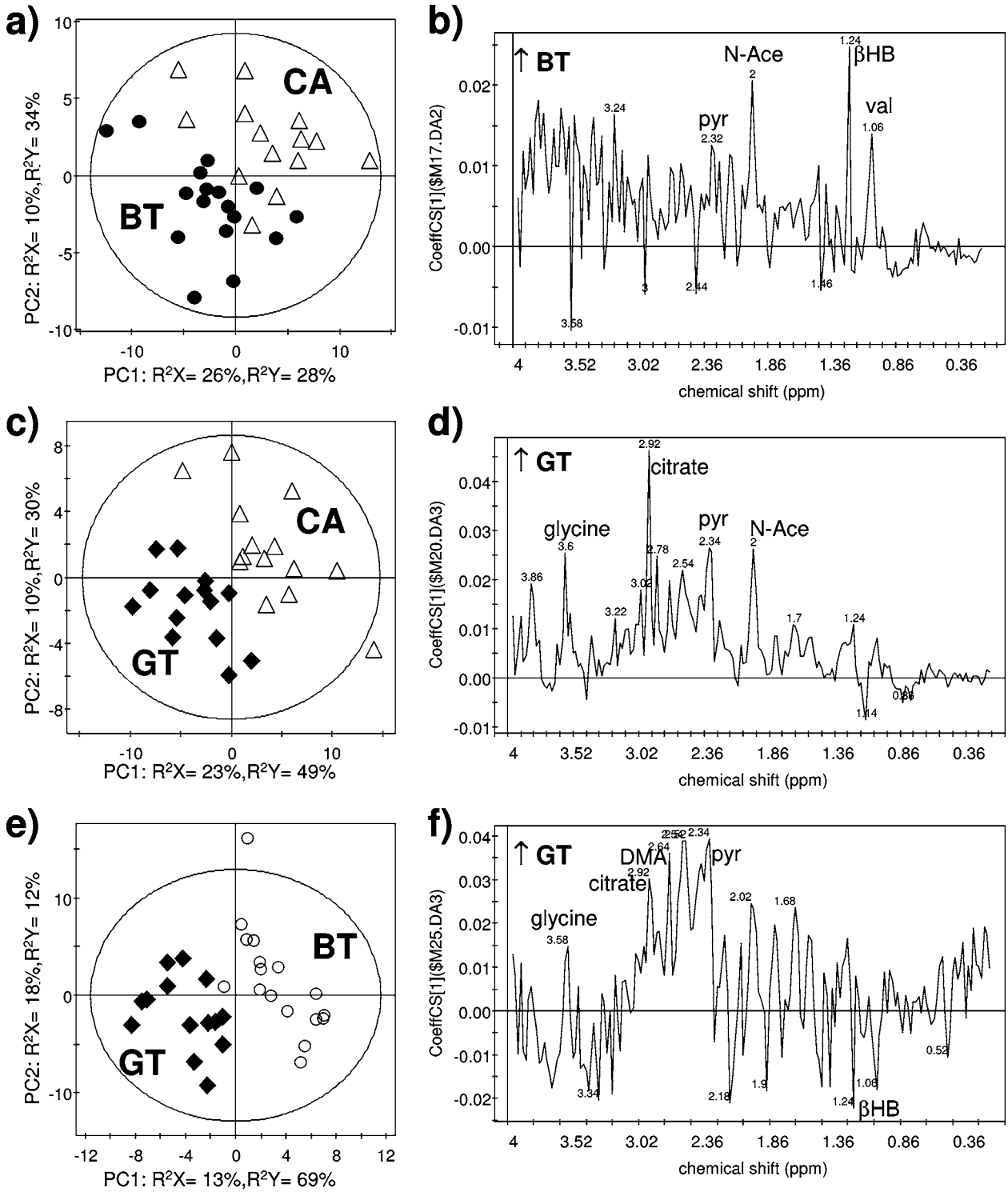

Figure 4. PLS-DA of endogenous metabolite profiles in urine shwon as pairwise comparison of the urine aliphatic metabolite profiles after caffeine (CA), black tea (BT), or green tea (GT) intake: (**a**) PLS-DA score plot separating black tea (●) intake from caffeine (△); (**b**) complementary coefficient plot of the first component; (**c**) PLS-DA score plot separating green tea intake (◆) from caffeine (△); (**d**) coefficient plot of the first component, GT versus CA; (**e**) PLS-DA score plot separating green tea (◆) from black tea intake (○); (**f**) first coefficient for GT versus BT. The peaks in the coefficient plots relate to the contribution of NMR variables to the clustering. Abbreviated assignments: βHB, β-hydroxybutyrate; DMA, dimethylamine; glx, glutamine + glutamate; pyr, pyruvate; N-Ace, N-acetylated glycoproteins.

and acetate. Green tea consumption also showed a reduction in plasma glucose and in specific lipoproteins as compared to caffeine intake (first coefficient, $Q^2 = 0.25$). The PLS-DA model discriminating between green and black tea intake **(Figure 5e**,**f)** was not significant ($Q^2 < 0$). However, some visual clustering in the second PLS-DA dimension ($R^2Y = 27\%$, $Q^2 = -0.03$) suggests an association between green tea intake and lower levels of plasma lactate and alanine and higher levels of acetate and β-hydroxybutyrate (**Figure 5f**).

DISCUSSION

In this study, an NMR-based metabonomics analysis was used to demonstrate that there is a clear metabolic impact of tea in

Table 1. Change in Aromatic Metabolite Levels Excreted into Urine after Consumption of Black or Green Tea, with Respect to a (Caffeine) Control[a]

chemical shift[b]	metabolite	direction of change versus caffeine: black tea	direction of change versus caffeine: green tea
4.12 (d), 7.54 (t), 7.62 (t), 7.78 (d)	hippuric acid	↑	↑
6.54 (d), 7.02 (t)	1,3-dihydroxyphenyl-2-sulfate	↑	↑
3.06 (dd),[c] 3.24 (dd),[f] 3.92 (dd), 6.92 (d), 7.20 (d)	tyrosine[d]	↓	–
4.1, 8.74 (d, J1.4)	U1[e]	↑	↑
7.14–7.38 (m), 3.98 8.34–4.34, 8.62	U2	↓	↓
6.7, 6.94	U3	↑	–
6.94, 7.3,[f] 7.8[f]	U4	–	↑
6.90 (t), 7.20[f]	U5	–	↑
6.82, 7.15[f]	U6	–	↑
6.7, 7.60[f]	U7	–	↑
6.1 (d), 6.26 (t) 6.34 (s?), 6.5 (t), 6.78 (s), 7.1	U8	–	↑
4.0, 7.34	U9	–	↑
6.86	U10	↓	–

[a] Metabolites marked with ↑ are increased and those marked with ↓ are decreased in comparison to control. A dash (–) indicates that metabolites show no change in one of the tea groups. [b] d, doublet; dd, double doublet; m, multiplet; s, singlet; t, triplet. [c] Resonance obscured by overlapping peaks of higher intensity in the PCA loadings plot. [d] Aromatic metabolites not derived from tea polyphenols but from endogenous amino acid metabolism. [e] U1, U10 represent as yet unidentified aromatic metabolites. [f] Coupling to other, unobserved resonances established by 2D TOCSY NMR.

Table 2. Change in Endogenous Metabolites Excreted in Urine after Tea Consumption, with Respect to a (Caffeine) Control[a]

chemical shift[b]	metabolite	direction of change versus caffeine: black tea	direction of change versus caffeine: green tea	function
1.48 (d), 3.72 (q)	alanine	↓	–	amino acids
2.12 (m), 2.44 (m), 3.76 (t)	glutamine/glutamate	↓	↓	
3.28 (t), 3.40 (t)	taurine	↑	–	
3.56 (s)	glycine	↑	↑↑	
1.06 (d)	valine	↑	↑	
2.18 (s), 3.88 (t)	methionine	↑	–	
2.34 (s)	pyruvate	↑	↑↑	energy metabolism
2.40 (s)	oxaloacetate	–	↑	
2.48 (t), 3.02 (s)	α-ketoglutarate	↑	↑	
2.54 (s)	succinate	↑	↑	
2.80 and 2.92 (dd)	citrate	–	↑↑	
3.24 (dd), 3.66 (m), 3.88 (m)	glucose	↑	–	
1.24 (d)	β-hydroxybutyrate	↑↑	–	
3.22 (s)	betaine	–	↑	others
2.64 (s)	dimethylamine	↑	↑↑	
2.0 (s), 2.04 (s)	*N*-acetyl (glycoproteins)	↑	↑↑	

[a] Metabolites marked with ↑ are increased and those marked with ↓ are decreased in comparison to control. A dash (–) indicates that metabolites show no change in one of the tea groups. Metabolites marked ↑↑ are higher than in the other tea group. [b] d, doublet; dd, double doublet; m, multiplet; q, quartet; s, singlet; t, triplet.

humans and that the effects of green tea and black tea are subtly different. In urine, the major metabolites resulting from tea intake were hippuric acid and 1,3-DHPS. Both are products of metabolism of tea flavanols and other polyphenols by bacteria in the colon and subsequent conjugation. The majority of the dietary flavonoids are not absorbed in the small intestine, but are metabolized by the gut microflora (*27*−*30*). These microbial flavonoid metabolites are absorbed from the colon and excreted into the urine at levels that may exceed those of the native flavonoid (*31*−*33*). The health benefits of green and black tea are commonly attributed to their high content of flavonoids with potent antioxidant properties (*34*). In view of the low bioavailability of the native tea flavonoids, it is likely that flavonoid metabolites from gut microbial fermentation contribute importantly to the beneficial health effects. This study showed that particularly green tea consumption caused an elevation of several other aromatic compounds (**Figure 4** and **Table 1**), which may be phenolics originating from gut microbial fermentation of green tea flavanols. However, the identification of these phenolics by NMR (**Table 1**) was limited by their low levels in urine and would require more sensitive methods such as GC-MS or LC-MS. Further identification of these compounds was, however, beyond the scope of this study.

The changes in endogenous plasma and urine metabolites, as identified by metabonomics and listed in **Table 2**, stimulate the generation of new ideas on the complex metabolic effects of tea intake. A key observation in our study was the impact of tea on human intermediary metabolism. Several NMR peaks assigned to excreted intermediates of the citric acid cycle (i.e., citrate, succinate, oxaloacetate, and 2-oxoglutarate) were elevated after consumption of black/green tea, as compared to caffeine consumption. This effect was most pronounced after green tea consumption (**Figure 4e**,**f**), and there are several possible explanations for this. First, elevated urinary levels of citric acid cycle intermediates may indicate a stimulation of whole-body oxidative energy metabolism, particularly after green tea consumption. Second, the urinary excretion of citric acid cycle intermediates may signal altered regulation of anaplerosis and cataplerosis pathways, that is, the net synthesis and net removal of citric acid cycle intermediates from mitochondria, respectively. These pathways play a role in biosynthesis routes, such as fatty acid biosynthesis in liver, gluconeo-

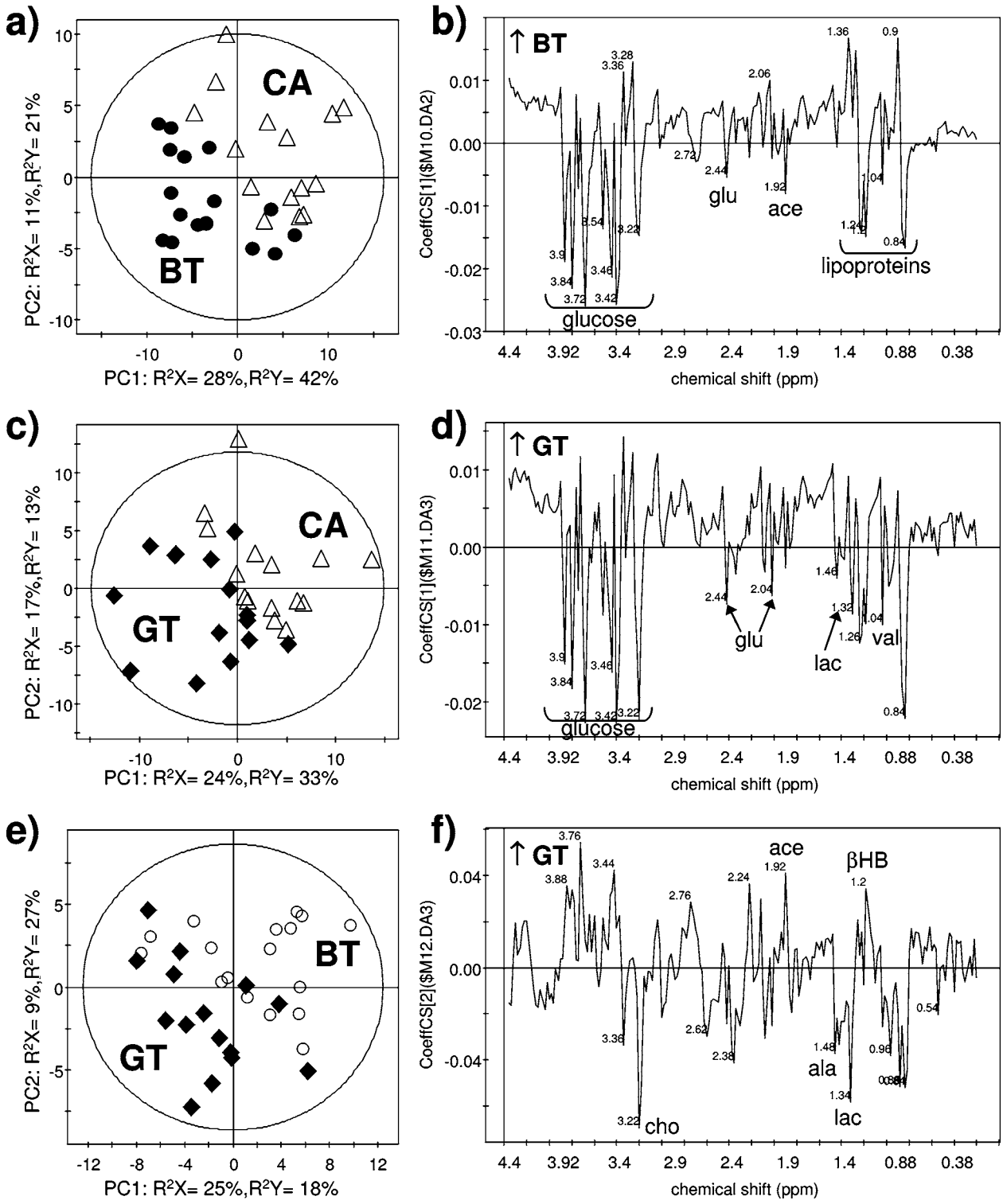

Figure 5. PLS-DA of the plasma metabolite profiles: effect of tea intake on endogenous metabolites in plasma. Pairwise PLS-DA analyses of plasma profiles after caffeine (CA), black tea (BT), or green tea (GT) intake are shown: (**a**) PLS-DA scores after BT versus CA ingestion; (**b**) first coefficient BT versus CA; (**c**) PLS-DA scores, GT versus CA; (**d**) first coefficient GT versus CA; (**e**) PLS-DA scores, GT versus BT; (**f**) first coefficient GT versus BT. Data were mean-centered per person. Abbreviated assignments: ace, acetate; ala, alanine; choline, choline; glu, glutamine; lac, lactate.

genesis in liver and kidney cortex, and glyceroneogenesis in adipose tissue (*35*), as well as in stimulating insulin secretion (*36*). Third, it cannot be ruled out that some of the so-called endogenous urine metabolites have a microbial origin.

It should be noted that in apparent contrast to our human study, a recent study in rats showed a reduced urinary excretion of some citric acid cycle intermediates (citrate and 2-oxoglutarate) after the ingestion of epicatechin (*37*). Possibly, this animal study may have been flawed by the very high epicatechin dose and/or animal conditioning (*37*), as reduced urinary citrate levels have been suggested as indicators of kidney dysfunction (*38*, *39*).

The PLS-DA models of plasma metabolic profiles in this study provide additional support for an effect of tea polyphenols

on energy metabolism. Decreased plasma levels of lactate and alanine were observed after green tea intake, but not after black tea or caffeine intake, suggesting that intake of green tea flavanols may be associated with a reduction in anaerobic glycolyis. However, no change in lactate excretion in urine after green tea consumption was detected. The observed reduction in plasma glucose levels after both green and black tea intake (**Figure 5**) is in line with studies that have shown lower blood glucose levels (*40*) as well as enhanced insulin activity in vitro (*41*), particularly with green tea polyphenols.

Green tea intake has been associated with increased (skeletal muscle) fatty acid oxidation and weight loss (*42*−*45*). Remarkably, our metabonomics analysis showed an increased urinary excretion of the ketone body β-hydroxybutyrate (βHB) after black tea, but not after green tea, consumption. This suggests different impacts of black and green tea on liver ketogenesis and fatty acid oxidation. However, changes in the plasma NMR peak that was (at least tentatively) assigned to βHB were not consistent with the observed changes in urine, and thus this requires a more detailed (targeted) analysis.

The current urine metabonomics analysis shows that both black and green tea have a clearly different impact on human (energy) metabolism when compared to the caffeine placebo. However, this study was limited in that no physiological endpoints or health benefit were measured. An interesting study by Dulloo et al. has shown that green tea intake increases daily energy expenditure and promotes fat oxidation in healthy men and that this is attributed more to EGCg than to caffeine (*46*). More recent human studies have shown, for example, that long-term ingestion of tea flavanols enhances dietary fat oxidation and dietary induced thermogenesis (*47*), whereas combining catechin intake with regular exercise more effectively stimulates body fat utilization for energy expenditure than exercise alone (*48*). Similar studies using mice showed that a diet containing green tea extract caused improved endurance capacity and increased muscle lipid oxidation (*49*) and also reduced plasma lactate levels during exercise (*50*). The molecular mechanism by which tea polyphenols affect physiological changes such as energy expenditure and fat oxidation is not completely understood yet. The proposed mechanism is that flavanols present in green tea contribute to thermogenesis through their inhibition of catechol *O*-methyl-transferase (COMT) (*51*), which prolongs the lifetime of the neurotransmitter norepinephrine.

In conclusion, this study has demonstrated that NMR-based metabonomics analysis can differentiate between the metabolic effects of green tea, black tea, and an appropriate caffeine control. Moreover, metabonomics analysis showed that tea intake may have an effect on human oxidative energy metabolism and biosynthetic pathways. Future studies may be directed toward a mechanistic understanding of the effect of tea flavonoids on human intermediary metabolism.

ABBREVIATIONS USED

BT, black tea; βHB, β-hydroxybutyrate; CA, caffeine; 1,3-DHPS, 1,3-dihydroxyphenyl-2-sulfate; EGCg, epigallocatechin gallate; GT, green tea; PCA, principal component analysis; PLS-DA, partial least-squares discriminant analysis.

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