The complex human urinary sugar profile: determinants revealed in the cross-sectional KarMeN study

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ABSTRACT

Background: Although sugars and sugar derivatives are an important class of metabolites involved in many physiologic processes, there is limited knowledge on their occurrence and pattern in biofluids.

Objective: Our aim was to obtain a comprehensive urinary sugar profile of healthy participants and to demonstrate the wide applicability and usefulness of this sugar profiling approach for nutritional as well as clinical studies.

Design: In the cross-sectional KarMeN study, the 24-h urine samples of 301 healthy participants on an unrestricted diet, assessed via a 24-h recall, were analyzed by a newly developed semitargeted gas chromatography–mass spectrometry (GC-MS) profiling method that enables the detection of known and unknown sugar compounds. Statistical analyses were performed with respect to associations of sex and diet with the urinary sugar profile.

Results: In total, 40 known and 15 unknown sugar compounds were detected in human urine, ranging from mono- and disaccharides, polyols, and sugar acids to currently unknown sugar-like compounds. A number of rarely analyzed sugars were found in urine samples. Maltose was found in statistically higher concentrations in the urine of women compared with men and was also associated with menopausal status. Further, a number of individual sugar compounds associated with the consumption of specific foods, such as avocado, or food groups, such as alcoholic beverages and dairy products, were identified.

Conclusions: We here provide data on the complex nature of the sugar profile in human urine, of which some compounds may have the potential to serve as dietary markers or early disease biomarkers. Thus, comprehensive urinary sugar profiling not only has the potential to increase our knowledge of host sugar metabolism, but can also reveal new dietary markers after consumption of individual food items, and may lead to the identification of early disease biomarkers in the future. The KarMeN study was registered at drks.de as DRKS00004890.

Keywords: urinary sugar profile, monosaccharide, disaccharide, polyol, sugar acid, GC-MS, dietary marker, sex, human urine, KarMeN study

INTRODUCTION

A variety of structurally different sugar compounds is present in the human body and even more so in our diet. We use the terms “sugar compounds or sugars” to refer to the following substance classes: mono- and disaccharides, as well as derived compounds thereof like polyols and sugar acids. Currently, sugar compounds are usually analyzed in urine samples with a focus on individual substance classes and, to date, most studies in this area have been performed with only a very limited number of volunteers (summarized in Supplemental Table 1). Combining the results from these studies revealed a quite complex urinary sugar profile consisting of many different sugar compounds. This is surprising because most recent studies have investigated the role of sugar compounds in human body fluids and focused mainly on common and well-known sugar compounds. Sugars and sugar derivatives in urine reflect the sugar compounds consumed within the diet as well as from endogenous sources. Of note is that absolute sugar concentrations in urine are very low because numerous sugars are efficiently reabsorbed in kidney tubular cells. Nevertheless, sugar compounds in human urine appear to be suitable dietary markers and, in the future, may even serve as early disease biomarkers, but knowledge on all this is highly limited.

In a few studies, specific sugar compounds were described as dietary markers for individual food items with examples such as chiro- and scylo-inositol for citrus fruit in serum (1).

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Abbreviations used: CART, classification and regression tree; GC-MS, gas chromatography–mass spectrometry; KarMeN, Karlsruhe Metabolomics and Nutrition; QC, quality control.

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threonate and threitol for fruit and vegetables in serum (2, 3), and some C4 and C5 sugar compounds for broccoli consumption in urine (4). Other studies suggested that a combination of urinary fructose and sucrose may reflect total sugar intake (5–7), which is especially relevant in view of associations between sugar intake and negative health outcomes such as an increased risk for cardiovascular disease mortality (8, 9).

Because sugar compounds are involved in a variety of disease pathways, urinary sugars could also serve as biomarkers in the health-disease trajectory. Abnormal concentrations of urinary sugar compounds have been described for conditions such as diabetes mellitus (10–14), uremia (12, 15), invasive candidiasis (16), enzyme deficiencies like galactosemia (17, 18), pentosuria (19), or other inborn errors of metabolism (20–25). It thus seems reasonable to state that “there is more than glucose to look at” (26, 27).

With respect to the more commonly analyzed sugars, such as fructose and sucrose, there is a need to understand which factors determine their urinary excretion (28). Even less is known about the origin, metabolism, and functions of polyols (10, 23, 25). In a recent study, a new pathway for erythritol production from glucose was described and erythritol excretion was demonstrated to be associated with weight gain (29). This study highlights our current limited knowledge on sugars and sugar derivatives in the human body beyond glucose and fructose.

To bridge this knowledge gap especially from the physiologic and pathophysiologic point of view, new analytical methods offering comprehensive detection of a wide range of major and many minor sugar compounds for nutritional and clinical research are thus urgently needed.

Here, we present a semitargeted gas chromatography–mass spectrometry (GC-MS) profiling method for the detection of >50 known and unknown sugar compounds in human urine and its application to 24-h urine samples derived from the observational KarMeN (Karlsruhe Metabolomics and Nutrition) study with 301 healthy participants (30).

METHODS

Study design and subjects

The cross-sectional KarMeN study was performed at the Max Rubner-Institut in Karlsruhe, Germany, between 2011 and 2013. Details on the study design and examination procedures were previously described by Bub et al. (30). Briefly, a total of 312 healthy participants aged between 18 and 80 y, who gave their written informed consent and were willing and able to perform all examinations, were recruited. Participants were excluded if they had a history of prevalent or chronic disease, were smokers, or took any medication, hormones, or dietary supplements. Women who were pregnant or breastfeeding were also excluded. Eleven participants who completed the study had to be excluded for other reasons, such as diseases requiring treatment, cardiac complications, voluntary dropout, cancer history, and acute cold with medication (30). Thus, a total of 301 participants remained for statistical analysis, 172 of whom were men and 129 were women. The local ethics committee approved the study and it was in accordance with the 1964 Helsinki declaration and its later amendments. The study was registered at the German Clinical Study Register (DRKS00004890) and has the WHO universal trial number U1111-1141-7051.

Participants were asked for a 24-h urine collection. Throughout the collection, bottles were kept in cool bags with cooling units. At the study center, the volume of the received 24-h urine samples was recorded. 2 × 14 mL were centrifuged at 1850 × g at 4°C for 10 min and then separated into aliquots. Samples were initially frozen at −20°C for 1 d and then cryopreserved at −196°C until analysis. A quality control (QC) sample was prepared by pooling 24-h urine samples from KarMeN participants. Osmolality was assessed via freezing-point depression of 24-h urine samples with the use of a micro-osmometer (Advanced Miro-Osmometer model 3MO, Advanced Instruments, Norwood, MA).

For the day of the 24-h urine sample collection, trained study personnel assessed the food consumption of each participant (in grams per day) in a personal interview through the use of a 24-h dietary recall with the software EPIC-Soft (developed by the International Agency for Research on Cancer (IARC) in Lyon) (31, 32), now renamed as GloboDiet. The amount of different foods consumed per day was assessed with the use of a picture booklet providing photographs of portion sizes for various foods as well as household measures and standard portions. For further analysis, the reported foods were summarized into food group variables (see Supplemental Table 2). Additionally, based on the German Nutrient Database “Bundeslebensmittelschlüssel” (BLS, version 3.02) (33), the total energy intake (in kcal per day) and intake of nutrients were calculated.

Semitargeted GC-MS sugar profiling

A Shimadzu GCMS QP2010 Ultra instrument was used in Scan-/SIM (selected ion monitoring)-mode to achieve high selectivity and sufficient sensitivity while at the same time being able to detect a priori unknown sugar compounds. Additionally, some abundant nonsugar compounds could be analyzed via this method. Table 1 and Supplemental Table 3 list all compounds that were detectable via this method, including the target and reference ions used for integration. The structural similarity of sugar compounds enables the usage of only a few selected masses for selective relative quantitation in the urine matrix (see Supplemental Table 3 and Supplemental Figure 1).

Analytical details regarding chemicals, sample preparation, instrument, method, and data processing parameters were described by Rist et al. (34). Briefly, 24-h urine samples were diluted according to osmolality (60 mosmol/kg), 40 µL were evaporated and then derivatized via a 2-step procedure with 15 µL methoxylaminhydrochloride solution (20 mg/mL in pyridine; 30 min, 70°C, 1000 rpm) and 50 µL N,N,N,N,N-methyl-N-(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane reagent (1 h, 75°C) before analysis. To remove slight drift and offset effects, the raw signal intensities were corrected through the use of QC sample-based local linear regression functions (35).

Statistics

For all statistical analyses, the software JMP (version 13, SAS Institute Inc., Cary, NC, 1989–2007) was used.

Association of the urinary sugar profile with sex

A matrix with all known and unknown sugar compounds and the information on sex and age of the KarMeN participants was used to build a decision tree with the CART (classification and regression tree) algorithm. This approach was used to...
| Polyols         | Monosaccharides | Disaccharides | Sugar acids | Other      | Unknown sugar-like | Amino acids | Organic acids | Others       |
|-----------------|-----------------|---------------|-------------|------------|-------------------|-------------|---------------|--------------|
| *meso*-Erythritol| Xylose          | Disaccharide U26 | Erythronic acid | Levoglucosan | Unknown U03 | Serine | Tartaric acid | Creatinine-enol |
| Threitol        | Arabinose       | Sucrose       | Threonic acid | Ethyl-β-glucuronide | Unknown U04 | Threonine | Isocitric acid | Unknown U11 |
| Polyol U02      | Ribose          | Lactose       | Sugar acid U01 | Unknown U05 | Unknown U05 | Cysteine | Hippuric acid | Unknown U16 |
| Xylitol         | Fucose          | Maltose       | Sugar acid U06 | Unknown U12 | Unknown U12 | Phenylalanine | Quinic acid |
| Arabitol        | Psicose         | Disaccharide U27 | Xylonic acid | Unknown U24 | Unknown U24 | Lysine |            |              |
| Ribitol         | Fructose        | Disaccharide U28 | Ribonic acid | Unknown U25 | Unknown U25 | Tyrosine |            |              |
| 1-Deoxysorbitol | Allose          | Disaccharide U29 | Sugar acid U09 |            |            |        |            |              |
| Fucitol         | Galactose       |              | Arabinonic acid |            |            |        |            |              |
| Mannitol        | Glucose         |              | Glucuronic acid |            |            |        |            |              |
| Sorbitol        | Mannose         |              | Mannonic acid |            |            |        |            |              |
| Galactitol      | Mannheptulose   |              | Galactomic acid |            |            |        |            |              |
| *chiro*-Inositol| Sedoheptulose   |              | Gluconic acid |            |            |        |            |              |
| *scyllo*-Inositol| Monosaccharide U21 |         |            |            |            |        |            |              |
| *myo*-Inositol  |                 |              |            |            |            |        |            |              |
| Perseitol       |                 |              |            |            |            |        |            |              |

1 Generally, 2 derivatives are formed for reducing sugar compounds during methoximation and silylation. For reasons of readability, the chemically exact denomination of compounds was deliberately omitted and only the first of 2 derivatives are listed.
uncover associations between the urinary sugar profile and sex. Advantages of the CART algorithm are its ability to cope with missing (not detected) values and its ability to handle categoric and numeric values in parallel. Not detected values (usually the results of signals below detection limits) were treated by the algorithm as a separate level of the variable. Concerning differences in the sugar profiles between men and women, the focus in this work was primarily on sugar compounds that were detected in <75% of the KarMeN participants, and thus, are potentially more sex-specific (in a qualitative sense). Age was included as an additional continuous variable after the first split, thus allowing the observation of associations between age and sugar compounds. Splitting was only allowed when \(-\log_{10}(P\text{ values})\) (calculated after Bonferroni correction) meant a \(-\log_{10}(P\text{ value}) > 3.1206\) for the algorithm as a separate level of the variable.

After CART analysis, the nonparametric Wilcoxon test was generally used to test for significant differences between men and women for the 2 most important metabolites as well as to distinguish between the maltose excretion of pre- and postmenopausal women.

Association of the urinary sugar profile with diet

To assess associations of diet with the human urinary sugar profile, an exploratory correlation analysis was performed with the use of the variables derived from the 24-h dietary recall (food and nutrient intake) with detected urinary sugar compounds (listed in Supplemental Tables 2 and 3, respectively). In a first step (selection of interesting correlations), Spearman rank correlation coefficients were determined by the pairwise method (threshold \(\rho < -0.30\) or \(\rho > 0.30\)) and evaluated in conjunction with scatter plots. In a second step, participants were divided into groups based on consumption of certain food items for promising correlations. A Wilcoxon test was performed to ascertain significance for these groups. If <100 participants consumed a particular food or nutrient, an equally large group of nonconsumers was randomly selected. If >100 participants were consumers, tertiles were built and a Wilcoxon test for the first against the third tertile performed.

Sugar screening in plant materials from fruit and vegetables

To assess the plausibility and specificity of some of the potential dietary markers for food consumption, a screening of sugar compounds in a range of fruit and vegetable varieties was performed with the use of the same GC-MS profiling as for the urine samples. The aim was to screen as many fruit and vegetables as possible, but not to perform a comprehensive evaluation. Thus, only 1 pooled sample for each fruit and vegetable variety was measured.

Sample preparation for fruit and vegetables

Fruit and vegetables were bought from regional producers directly, weekly markets, or supermarkets. Overall, a total of 75 fruit and vegetable varieties (see Table 2) were purchased and, if possible, they were seasonally and regionally produced. The edible plant material of 5–20 fruits or vegetables (depending on fruit or vegetable size) was pooled into 1 sample, frozen in liquid nitrogen, and then coarsely ground and freeze dried for \(\geq 3\) d. The dried material was ground to a fine powder with a ball mill (Retsch MM400, Haan, Germany) for 20–60 s (depending on the consistency of the plant material) at 30 Hz and then stored at \(-80^\circ\text{C}\) until analysis. For each sample, 20 ± 0.1 mg of freeze-dried powder was weighed out and then after addition of 20 µL of internal standard solution (pinitol and phenyl-β-glucopyranoside in water, each 2 mmol/L) extracted twice with 750 µL methanol for 10 min at 35°C and 1400 rpm. The collected supernatant was mixed and then centrifuged for 5 min at 4°C and 16,100 × g. After which, 20 µL of supernatant was evaporated and then derivatized with the use of the same 2-step procedure as for the urine samples, except that 40 µL of methoxyl-aminohydrochloride solution in pyridine (25 mg/mL) and 96 µL of \(N\)-methyl-\(N\)-(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane were used.

Semitargeted GCMS sugar profiling and data processing

The method for the measurement of the fruit and vegetable samples was the same as for the urine samples, except that the Rxi-5Sil-MS column was slightly shorter (54 m + 4 m precolumn), and as a result, time frames for SIM had to be adjusted (34). Each day, 30 fruit and vegetable samples, six 24-h urine QC samples, and a solvent blank were prepared and analyzed. Data processing was performed in the same way as for the 24-h urine samples (34).

RESULTS

Analytical performance of the semitargeted GC-MS sugar profiling method

Our newly developed and validated semitargeted GC-MS sugar profiling method (see Supplemental Tables 3 and 4 and Supplemental Figures 1 and 2) enables the sensitive detection and relative quantification of 55 major and minor sugar compounds (see Figure 1 and Table 1) encompassing mono- and disaccharides, polyols, sugar acids, and as yet not identified sugar compounds (see Supplemental Figure 1).

The assignment of the analyzed known and unknown sugar compounds into the different substance classes is shown in Figure 1. If desired, some amino and organic acids can also be analyzed with the method described here and this leads to a total number of 68 integrated analytes (see Table 1 and Supplemental Table 3). All sugar compounds detected via the semitargeted GC-MS method and their signal intensity ranges are listed in Figure 2.

Our method is also characterized by very good long-term repeatability and intermediate precision (see Supplemental Table 4 and Supplemental Figure 2) as proven by measurement series comprising overall 456 runs (312 study samples plus 144 QC samples). Thus, the method is suitable for long-term measurement series of human biofluids in large study cohorts.

Sugar profiling in participants of the KarMeN study

Sugar profile of human urine and biological variability

To determine metabolite-specific differences in the interindividual, i.e., biological variability, the CVs of the measured sugar compounds across all 301 KarMeN participants were
### TABLE 2
Summary of a sugar screening in 75 fruit and vegetable varieties

|                | Psicose | Mannoheptulose | Perseitol | Mannitol | Galactose | Threitol | Xylose | Polyol U02 |
|----------------|---------|----------------|-----------|----------|-----------|----------|--------|------------|
| Eggplant       |         | —              | 5         | tr       | Middle    | —        | Low    | tr         |
| Avocado        | —       | 100            | 100       | tr       | tr        | —        | —      | tr         |
| Leaf spinach   | tr      | 13             | —         | tr       | Low       | —        | —      | tr         |
| Cauliflower    | tr      | 1              | —         | tr       | High      | —        | Middle | tr         |
| Common bean    | tr      | 6              | <0.1      | Low      | —         | Low      | Low    | —          |
| Broccoli       |         | 5              | tr        | Low      | —         | Low      | Low    | —          |
| Iceberg lettuce|         | 3              | <0.1      | Low      | —         | Low      | Low    | —          |
| Peas           | tr      | 2              | <0.1      | Low      | —         | tr       | Low    | —          |
| Lamb’s lettuce | Low     | 27             | —         | tr       | Middle    | —        | Low    | High       |
| Cucumber       |         | 4              | <0.1      | Middle   | —         | —        | —      | —          |
| Carrot         |         | 29             | 2.6       | Low      | —         | tr       | Low    | —          |
| Potato         |         | 5              | <0.1      | Low      | —         | —        | —      | tr         |
| Garlic         |         | —              | tr        | tr       | —         | —        | —      | tr         |
| Kohlrabi       |         | tr             | —         | tr       | Low       | —        | —      | tr         |
| Garden lettuce |         | 11             | tr        | Low      | —         | Low      | Middle | —          |
| Red cabbage    |         | tr             | —         | Middle   | —         | tr       | —      | tr         |
| Pointed cabbage|         | tr             | —         | Low      | —         | tr       | —      | tr         |
| White cabbage  |         | tr             | —         | Low      | —         | tr       | —      | tr         |
| Pumpkin        |         | tr             | <0.1      | Middle   | tr        | tr       | Low    | —          |
| Leek           |         | tr             | —         | Low      | —         | —        | —      | —          |
| Striped lentil |         | —              | —         | —        | tr        | —        | —      | —          |
| Black lentil   |         | —              | tr        | —        | —         | —        | —      | —          |
| Lentil, “Perla”|         | —              | tr        | tr       | —         | —        | —      | —          |
| Corn           |         | —              | tr        | Low      | tr        | —        | —      | —          |
| Green bell pepper| —     | 6              | <0.1      | High     | —         | Low      | —      | tr         |
| Red bell pepper|         | 7              | <0.1      | High     | —         | Low      | —      | tr         |
| Hot pepper     |         | 22             | <0.1      | High     | —         | Low      | —      | tr         |
| Button mushroom|         | 1              | <1        | 100      | tr        | —        | —      | —          |
| Shiitake       |         | <1             | 77.4      | tr       | tr        | —        | —      | —          |
| Small radish   |         | 2              | <0.1      | Low      | —         | Low      | —      | tr         |
| Radish         |         | —              | —         | Low      | —         | tr       | —      | tr         |
| Beetroot       |         | —              | —         | —        | Middle    | —        | —      | tr         |
| Pointed pepper |         | 12             | <0.1      | Middle   | —         | —        | —      | —          |
| Soy            |         | —              | —         | <0.1     | Low       | —         | tr      | tr         |
| Green asparagus|         | —              | —         | <0.1     | Low       | —         | Low    | tr         |
| White asparagus|         | —              | —         | <0.1     | Low       | —         | Low    | tr         |
| Grape tomato   |         | 6              | <0.1      | Low      | —         | Low      | —      | —          |
| Tomato, “Matina”| —    | 11             | <0.1      | High     | —         | Low      | —      | tr         |
| Tomato, “Resi”|         | 7              | <0.1      | High     | —         | tr       | —      | —          |
| Zucchini       |         | —              | <0.1      | High     | —         | Low      | —      | Low        |
| Onion          |         | —              | tr        | Low      | —         | tr       | —      | —          |
| Pineapple      |         | —              | —         | Middle   | —         | —        | —      | —          |
| Apple          |         | 6              | 0.1       | Low      | —         | High     | —      | tr         |
| Apricot        |         | 4              | <0.1      | Low      | —         | Low      | —      | tr         |
| Banana         |         | —              | tr        | Low      | —         | —        | —      | —          |
| Pear           |         | 5              | <0.1      | Low      | tr        | —        | —      | —          |
| Blackberry     |         | 4              | <0.1      | Low      | —         | Low      | —      | —          |
| Clementine     |         | —              | tr        | Low      | —         | —        | —      | —          |
| Strawberry, “Asia”| —  | 4              | —         | Low      | —         | High     | —      | tr         |
| Strawberry, “Elsanta”| — | 2              | —         | tr       | Low      | —         | High   | tr         |
| Pomegranate    |         | 7              | 18.1      | Low      | tr        | —        | —      | tr         |
| Grapefruit     |         | tr             | —         | Low      | —         | —        | —      | —          |
| Blueberry      |         | 10             | tr        | Low      | —         | —        | —      | —          |
| Raspberry      |         | 4              | tr        | Low      | —         | —        | —      | —          |
| Honeydew melon |         | tr             | <0.1      | High     | tr        | Low      | —      | —          |
| Red currants   |         | 6              | tr        | Low      | —         | —        | —      | —          |
| Black currants |         | 14             | <0.1      | Low      | —         | Low      | —      | —          |
| Sour cherry    |         | 7              | <0.1      | Low      | tr        | Low      | —      | tr         |
| Sweet cherry   |         | 1              | <0.1      | Low      | —         | Low      | —      | tr         |
| Kiwi fruit     |         | 5              | <0.1      | Middle   | —         | —        | —      | —          |
| Mango          |         | 4              | —         | Low      | —         | tr       | —      | —          |
| Small yellow plums | —   | 6              | <0.1      | Low      | —         | Low      | —      | —          |

(Continued)
determined. Some sugar compounds were excreted with a narrow concentration range, for example glucuronic acid with a CV of 29.8%, whereas others showed a huge biological variability, such as lactose with a CV of 294.5% (see Figure 2). In addition, the relative frequency of occurrence of individual sugar compounds in the 24-h urine samples of KarMeN participants is listed in Supplemental Table 3. To further assess factors underlying the huge biological variability, analyses focused on sex as a determinant and on dietary intake reconstructed from dietary intake measures.

**Association of the urinary sugar profile with sex**

To identify sugar compounds associated with sex, a decision tree using the CART algorithm was built (see Figure 3). In Table 3, possible candidates for a split are given for the first 3 nodes and for leaves. Evidently, the most relevant sugar compound separating men and women was maltose, which was detected in 78.3% of women but only in 35.5% of men. In addition, the urinary maltose concentration was significantly higher in women ($P < 0.0001$, see Figure 3). Other important metabolites were gluconic acid, fructose, and an unknown sugar compound, which were found in $>75\%$ of the study samples and recently discussed by Rist et al. (34). In the second node, where age was included as an additional potential splitting candidate, the 3 top determinants for separating men and women were age, gluconic acid, and sedoheptulose. Interestingly, splitting on the basis of sedoheptulose would have been similar to splitting on age as a result of the close association between age and sedoheptulose concentration in 24-h urine samples (34). The second split was done based on age as a top determinant, thereby indicating a close association between urinary maltose, sex, and age. The cut point for age was 45 y, thus suggesting that sugar excretion patterns change with menopause in women (see Figure 3). Gluconic acid was the only possible candidate metabolite for the third and last split (see Figure 3 and Table 3); however, to prevent overfitting, no further splitting was done. Boxplots of the 2 most important sugar compounds that separate men and women and the interaction between maltose excretion, sex, and age (menopausal status) are shown in Figure 3.

**Association of the urinary sugar profile with diet**

A correlation analysis was performed based on 24-h urinary sugar profiles with the food consumption and nutrient intake data and a heat map generated on the basis of the Spearman rank correlation coefficients (Figure 4). The Spearman rank correlation coefficients with $\rho > 0.30$ are listed in Table 4; no correlations with $\rho < -0.30$ were observed. Significant correlations were observed for 1) avocado consumption with perseitol, 2) dairy product consumption with galactose and lactose, 3) alcoholic beverage consumption with xylitol and ethyl-\(\beta\)-glucuronide, 4) mushroom consumption with mannitol,
5) fruit consumption with threitol, xylose, and an unknown polyol, 6) citrus fruit juice and fruit drink consumption with *chiro*-inositol and galactonic acid, and 7) sucrose intake with fructose and sucrose (see Table 4 and Figure 4). In the case of avocado, in addition to perseitol, mannoheptulose presented itself as a potential dietary marker although the correlation coefficient was slightly below our threshold of 0.30 (ρ = 0.2704; see Figure 4). To verify this observation despite the low number of avocado consumers (n = 9), Spearman rank correlation coefficients were calculated for the avocado consumers and 18 randomly chosen nonconsumers (n = 27) (mannoheptulose: ρ = 0.7748, perseitol: ρ = 0.8713; see Figure 5). For some of these potential dietary markers box and scatter plots as well as their origin and potential confounders or other interferences are shown in Figure 5. A second line of evidence that those metabolites may be potential dietary markers for distinct foods/products was provided by analyzing the sugar profiles of 75 selected fruit and vegetable varieties (see Table 2).

**DISCUSSION**

Sugar profiling in participants of the KarMeN study

Sugar profile of human urine and biological variability

With the analytical method described here, we provide a straightforward and reliable tool to obtain sugar profiles and
DETERMINANTS OF THE HUMAN URINARY SUGAR PROFILE

FIGURE 3 Identification of urinary markers discriminating sex via the CART approach. (A) Decision tree with splitting rules, the number of men or women, and the ratio between men and women for each branch; (B) box plots for the 2 top sugar compound candidates to differentiate sex; (C) association of age with maltose excretion in women (pre- and postmenopausal). Significance was established with the use of the Wilcoxon test, with participants excluded where the sugar compound was not detected. CART, classification and regression tree.

TABLE 3
Results of building a decision tree (CART) for the identification of possible markers to differentiate sex. Possible candidate sugar compounds for splitting are listed only if significant $P$ values were achieved after Bonferroni correction.

| Sugar compound | Candidate $G^2$ | $-\log_{10}$ ($P$ value) | Cut point |
|----------------|-----------------|--------------------------|------------|
| First node: split candidates | | | |
| Maltose | 152.6 | 55.8316 | 6.50E+03 |
| Gluconic acid | 56.6 | 17.0557 | 1.05E+05 |
| Unknown U05 | 37.8 | 9.1285 | 6.66E+05 |
| Fructose | 27.1 | 5.0777 | 2.25E+05 |
| Second node: split candidates | | | |
| Age | 34.7 | 8.6471 | 45.36 |
| Gluconic acid | 27.1 | 5.8762 | 1.38E+05 |
| Sedoheptulose | 25.3 | 5.2497 | 1.32E+05 |
| Unknown U05 | 19.8 | 3.4428 | 7.07E+05 |
| Third node: split candidates | | | |
| Gluconic acid | 20.0 | 4.0476 | 1.38E+05 |
| First leaf: split candidates | Other sugar compounds | | |
| Second leaf: split candidates | Other sugar compounds | | |
| Third leaf: split candidates | Other sugar compounds | | |
| Fourth leaf: split candidates | Other sugar compounds | | |

$1$G$^2$, likelihood ratio chi-square; highest values indicate best split.
$2$Best value for splitting the variables (cut point).
$3$For reasons of readability, only the higher-ranking derivative was listed.
$4$Candidate $P$ values were below the significance level.

Association of the urinary sugar profile with sex

The most important metabolites to differentiate between male and female sugar profiles were maltose and gluconic acid. Maltose has been reported to be present in very low concentrations in human urine, but no differences with respect to sex have been described so far (10, 12, 23, 36–39). We hypothesize that the maltose excretion seen in women may be associated with the vaginal microbiota (dominated by lactic acid-producing Lactobacillus species). Spear et al. (40) demonstrated that vaginal fluid possesses $\alpha$-amylase activity, and thus is able to degrade free glycogen to maltose, maltotriose, and maltotetraose, which can then be utilized by Lactobacillus species (41, 42). This degradation pathway of free glycogen released from the vaginal epithelium might be responsible for the higher excretion rate of maltose in female urine.
We observed a significantly lower maltose content in the urine of postmenopausal women in comparison with premenopausal women (see Figure 3). Postmenopausal women have significantly lower amounts of glycogen and Lactobacilli counts in the vaginal fluid as a result of reduced estrogen concentrations (41, 43, 44). Collectively, these observations concur with our finding of reduced maltose concentrations in postmenopausal women and add plausibility to a link between maltose excretion and the vaginal microbiota.

For gluconic acid and fructose we could not find a plausible biological explanation for the observed sex-dependent differences in urine.

Association of the urinary sugar profile with diet

Based on the correlation analysis, potential dietary markers for the consumption of various food items as well as food groups were identified (Figure 5 and Table 4). This analysis suggests the following sugar compounds to serve as specific dietary markers: mannoheptulose and perseitol for avocado and galactose and lactose for dairy products. These sugars are known constituents of these respective foods (45–50). Although an increase of mannoheptulose and perseitol excretion in urine after avocado consumption (25) has been described in an intervention with 3 volunteers, it was not specifically defined as a dietary marker. An increase of galactose and lactose after pure lactose ingestion has been observed (49, 51–53). Moreover, in a recent intervention study with milk, both galactose and lactose were suggested as specific dietary markers for milk consumption (54).

Although mannitol appears to be a plausible dietary marker for mushroom consumption (55), its specificity is questioned because there are many other sources of mannitol in the human diet (48) (see also Table 2) — a fact that confounds the aforementioned identified association (see Figure 5).
| Sugar compounds       | Dietary intake       | Participants | Spearman   |
|-----------------------|----------------------|--------------|------------|
|                       |                      | n_{exc.} | n_{ing.} | ρ    |
| Perseitol             | Avocado              | 219      | 9        | 0.3388 |
| Galactose<sup>3</sup>  | Milk sum             | 301      | 234      | 0.6644 |
| Galactose<sup>3</sup>  | Dairy products sum   | 301      | 279      | 0.6082 |
| Galactose<sup>3</sup>  | Milk                 | 301      | 174      | 0.4779 |
| Galactose<sup>3</sup>  | Yoghurt              | 301      | 84       | 0.3017 |
| Lactose<sup>3</sup>    | Milk sum             | 300      | 234      | 0.4204 |
| Lactose<sup>3</sup>    | Dairy products sum   | 300      | 279      | 0.3364 |
| Lactose<sup>3</sup>    | Milk                 | 300      | 174      | 0.3180 |
| Galactonic acid       | Milk sum             | 296      | 234      | 0.3403 |
| Galactonic acid       | Dairy products sum   | 296      | 279      | 0.3005 |
| Xylitol               | Alcoholic beverages  | 301      | 96       | 0.6379 |
| Xylitol               | Beer                 | 301      | 47       | 0.4667 |
| Xylitol               | Wine and sparkling wine | 301 | 55       | 0.4531 |
| Ethyl-β-glucuronide   | Alcoholic beverages  | 144      | 96       | 0.3885 |
| Ethyl-β-glucuronide   | Wine and sparkling wine | 144 | 55       | 0.4446 |
| Ethyl-β-glucuronide   | Beer                 | 144      | 47       | 0.3457 |
| Sorbitol              | Alcoholic beverages  | 299      | 96       | 0.4948 |
| Sorbitol              | Wine and sparkling wine | 299 | 55       | 0.4496 |
| Allose                | Alcoholic beverages  | 301      | 96       | 0.3425 |
| Allose                | Wine and sparkling wine | 301 | 55       | 0.3128 |
| Arabitol              | Alcoholic beverages  | 301      | 96       | 0.3340 |
| Mannitol              | Mushrooms            | 301      | 35       | 0.3633 |
| Mannitol              | Button mushroom      | 301      | 32       | 0.3489 |
| chiro-Inositol        | Citrus fruit juices and drinks | 260 | 65       | 0.4941 |
| chiro-Inositol        | Citrus fruit         | 260      | 37       | 0.3000 |
| Galactonic acid       | Citrus fruit juices and drinks | 296 | 65       | 0.3825 |
| Threitol              | Fruit sum            | 301      | 228      | 0.4904 |
| Threitol              | Apple                | 301      | 106      | 0.4359 |
| Xylose<sup>3</sup>     | Fruit sum            | 301      | 228      | 0.4768 |
| Xylose<sup>3</sup>     | Apple                | 301      | 106      | 0.4736 |
| 1-Deoxy-sorbitol      | Apple                | 299      | 106      | 0.4653 |
| 1-Deoxy-sorbitol      | Fruit sum            | 299      | 228      | 0.3504 |
| 1-Deoxy-sorbitol      | Fruit juice and juice drink | 299 | 131      | 0.3130 |
| 1-Deoxy-sorbitol      | Fruit juices         | 299      | 123      | 0.3097 |
| Sugar acid U09        | Fruit juice and juice drink | 301 | 131      | 0.3649 |
| Sugar acid U09        | Fruit juices         | 301      | 123      | 0.3395 |
| Polyol U02            | Fruit sum            | 301      | 228      | 0.3516 |
| Galactonic acid       | Fruit juices         | 296      | 123      | 0.3498 |
| Galactonic acid       | Fruit juice and juice drink | 296 | 131      | 0.3320 |
| Xylose acid           | Fruit juice and juice drink | 301 | 131      | 0.3408 |
| Xylose acid           | Fruit juices         | 301      | 123      | 0.3107 |
| Xylose acid           | Fruit sum            | 301      | 228      | 0.3091 |
| chiro-Inositol        | Fruit juices         | 260      | 123      | 0.3350 |
| chiro-Inositol        | Fruit juice and juice drink | 260 | 131      | 0.3119 |
| 1-Deoxy-sorbitol      | Monosaccharides      | 299      | 301      | 0.4676 |
| Unknown U24           | Polysaccharides      | 301      | 301      | 0.4477 |
| Unknown U24           | Bread sum            | 301      | 293      | 0.4463 |
| Unknown U24           | Carbohydrates        | 301      | 301      | 0.3949 |
| Fructose<sup>3</sup>   | Monosaccharides      | 301      | 301      | 0.3966 |
| Fructose<sup>3</sup>   | Sucrose              | 301      | 301      | 0.3581 |
| Fructose<sup>3</sup>   | Disaccharides        | 301      | 301      | 0.3192 |
| Threitol              | Monosaccharides      | 301      | 301      | 0.3955 |
| Xylose acid           | Monosaccharides      | 301      | 301      | 0.3919 |
| Xylose<sup>3</sup>     | Monosaccharides      | 301      | 301      | 0.3785 |
| Sucrose               | Sucrose              | 301      | 301      | 0.3620 |
| Sucrose               | Disaccharides        | 301      | 301      | 0.3243 |
| Sucrose               | Candy                | 301      | 104      | 0.3132 |
| Mannoheptulose        | Monosaccharides      | 300      | 301      | 0.3135 |

1_{exc.}, number of participants who excreted a certain sugar compound; n_{ing.}, number of participants who ingested individual foods or food groups.

2Spearman rank correlation coefficients $< -0.30$ or $>0.30$. All listed correlations had significant $P$ values $< 0.0001$.

3For reasons of readability, only the higher-ranking derivative was listed.
We also identified xylitol as a potential dietary marker for alcoholic beverage consumption. An increase of xylitol in urine after administration of ethanol (56) has been described before, but the causality underlying the relation between alcohol consumption and urinary xylitol output (56, 57) warrants further research. Ethyl-β-glucuronide has already been described as a dietary marker for alcoholic beverage consumption (58–60); we observed a moderate association (see Figure 5 and Table 4). In
FIGURE 5  Overview of potential dietary markers (includes results of the KarMeN study and sugar screening of fruit and vegetables as well as literature data). Potential dietary markers of food consumption with the strongest associations in the correlation analysis, their plausibility in terms of origin, and their specificity in terms of potential confounders or other interferences. Shaded violet: food/nutrient level; shaded light blue: interfering sources for potential dietary markers (foods, drugs, results from sugar screening of fruit and vegetables); shaded red: metabolization in human; shaded yellow: results in 24-h urine samples. Colors of boxes and arrows as follows: green (part 1): consumption of avocado; grey (part 1): consumption of dairy products; dark blue (part 2): consumption of alcoholic beverages; brown (part 2): consumption of mushrooms; light blue: interfering sources for potential dietary markers (foods, drugs, results from sugar screening of fruit and vegetables). Check: compound occurs in specific food; cross: compound does not occur in specific food. The amount of consumed food is given in g per day. Significance was established by use of the Wilcoxon test. Spearman rank correlation coefficients were calculated using all 301 participants, except for avocado, where the 9 avocado consumers plus 18 randomly selected participants were used.
light of the many other potential confounders and interferences for ethyl-β-glucuronide detection (61–64) (see Figure 5), we recommend to use measurements of additional metabolites such as ethylsulfate (65) or in combination with xyitol.

It would be highly desirable to use some of these dietary markers in future as an objective measure of food consumption in comparison with self-reported consumption, where biases such as under- or over-reporting in cases of perceived unhealthy or healthy foods often occur (64). Objective dietary markers would allow more reliable insights into health aspects, and thus, relations between diet and health could be more accurately ascertained.

In more general terms, a dietary marker should fulfill a number of criteria such as its specificity, the dose-response relation, plausibility of origin, and suitability in free-living populations, and, importantly, analytical robustness (66). Questions around the quality of dietary markers also cover issues on whether a metabolite is a short-term marker of intake over a 24–36 h period or whether it can also serve as a long-term reporter molecule especially in epidemiologic studies (67). Moreover, whether there are saturation effects and whether the dietary marker can quantitatively assess consumption are also important issues (66).

Specificity and dose-response effect, plausibility, and suitability in a free-living population as in our KarMeN population on an unrestricted diet as well as methodological validity were all addressed in the present study. The main limitations in our approach were 1) the low number of participants consuming some specific food items such as avocado, 2) the potential bias through the use of self-reported food consumption data for the correlation analysis, and 3) owing to our study design so far only a conclusion about metabolites’ usefulness as short-term markers can be drawn. Other limitations might be that only a single urine collection was measured potentially leading to exaggerated interindividual variation and that only fruit and vegetables, but not processed food and beverages, were screened during the sugar profiling of food. However, our aim is that the developed analytical method and the approaches used to identify some crucial sugar compound determinants will be taken into larger and more diverse cohorts as the next step to deriving quantitative dietary markers and to shedding light on the diet-health relation for one of the most important food substrates in the human diet and metabolism, namely the sugars.

In conclusion, we have demonstrated that the human urinary sugar profile is complex and comprises many more compounds than previously anticipated. With the large number of sugar compounds detected, we identified also a huge gap in knowledge regarding the metabolism of most of these sugar compounds, in particular along the diet-health-disease trajectory. We therefore suggest that future research should not only encompass analyzing common and well-known sugar compounds, but rather strive for a more comprehensive view on sugar compounds. However, the data from our study can be used as a reference for normal sugar profiles of healthy humans with respect to the occurrence of individual sugar compounds along with variances in excretion. For some sugars, we identified crucial determinants such as sex and pre- compared with postmenopausal women. However, these determinants need further study. We also identified a considerable number of sugar compounds as potential dietary markers for individual food items and groups (see Figures 4 and 5), for which confirmation and assessment of their quantitative dimension and their usability as long-term markers in epidemiologic studies are required in future studies. Although our newly developed semitargeted GC-MS method is only semiquantitative, it clearly offers a rapid and cost-effective strategy to obtain comprehensive insights into the sugar profile by detecting not only numerous known, but also some unknown sugar-like compounds that also deserve identification. Our analytical method may also be useful in identifying the underlying physiologic processes that allow assessing determinants for absorption/permeation from the intestine into blood circulation as well as for renal secretion/reabsorption. Ultimately, this analytical method may not only help to identify dietary markers, but also to identify disease biomarkers in the future.

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REFERENCES

1. Guertin KA, Moore SC, Sampson JN, Huang WY, Xiao Q, Stolzenberg-Solomon RZ, Sinha R, Cross AJ. Metabolomics in nutritional epidemiology: identifying metabolites associated with diet and quantifying their potential to uncover diet-disease relations in populations. Am J Clin Nutr 2014;100(1):208–17.
2. Playdon MC, Moore SC, Derkach A, Reedy J, Subar AF, Sampson JN, Albanes D, Gu F, Kontro J, Lassale C, et al. Identifying biomarkers of dietary patterns by using metabolomics. Am J Clin Nutr 2017;105(2):450–65.
3. Pallister T, Jennings A, Moloney RP, Yarand D, Mangino M, Cassidy A, MacGregor A, Spector TD, Menni C. Characterizing blood metabolomics profiles associated with self-reported food intakes in female twins. PLoS One 2016;11(6):e0158568.
4. Lloyd AJ, Favé G, Beckmann M, Lin W, Tailliart K, Xie L, Mathers JC, Draper J. Use of mass spectrometry fingerprinting to identify urinary metabolites after consumption of specific foods. Nutrients 2012;4(4):981–91.
5. Luceiri C, Caderni G, Lodovici M, Spagnesi MT, Monserratt C, Lanceloni L, Dolara P. Urinary excretion of sucrose and fructose as a predictor of sucrose intake in dietary intervention studies. Cancer Epidemiol Biomarkers Prev 1996;5(5):167–71.
6. Tasevska N, Runawick SA, Magee J, A, Bingham SA, Urinary excretion of sucrose and fructose as biomarkers for sugar consumption. Cancer Epidemiol Biomarkers Prev 2005;14(5):1287–94.
7. Tasevska N. Urinary sugars—a biomarker of total sugars intake. Nutrients 2015;7(7):5816–33.
8. Yang Q, Zhang Z, Gregg EW, Flanders WD, Merritt R, Hu FB. Added sugar intake and cardiovascular diseases mortality among US adults. JAMA Intern Med 2014;174(4):516–24.
9. Lustig RH, Schmidt LA, Brindis CD. Public health: the toxic truth about sugar. Nature 2012;482(7383):27–9.
10. Ge S-I, Wang H, Wang Z-F, Cheng S, Wang Q-J, He P-G, Fang Y-Z. Sensitive measurement of polyols in urine by capillary zone electrophoresis coupled with amperometric detection using on-column complexation with borate. J Chromatogr B Analyt Technol Biomed Sci 2013;915–916:39–45.
11. Kawasaki T, Akamura H, Yamanouchi T. Increased fructose concentrations in blood and urine in patients with diabetes. Diabetes Care 2002;25(2):353–7.

12. Pitkänen E. The serum polyl pattern and the urinary polyl excretion in diabetic and in uremic patients. Clin Chim Acta 1972;38(1):221–30.

13. Sim H-J, Jeong J-S, Kwon H-J, Kang TH, Park HM, Lee Y-M, Kim SY, Hong S-P. HPLC with pulsed amperometric detection for sorbitol as a biomarker for diabetic neuropathy. J Chromatogr B Anal Technol Biomed Life Sci 2009;877(14–15):1607–11.

14. Yoshih H, Uchino H, Ohmura C, Watanabe K, Tanaka Y, Kawamori R. Clinical usefulness of measuring urinary polyl excretion by gas chromatography/mass-spectrometry in type 2 diabetes to assess polyl pathway activity. Diabetes Res Clin Pract 2001;51(2):115–23.

15. Niwa T, Yamamoto N, Maeda K, Yamada K, Okhi T, Mori M. Gas chromatography—mass spectrometric analysis of polyls in urine and serum of uremic patients: identification of new deoxyxylitol and xylitol isomers. J Chromatogr B Biomed Sci Appl 1983;277:25–39.

16. Hui M, Cheung S-W, Chin M-L, Chu K-C, Chan RC-Y, Cheng AF-B. Development and application of a rapid diagnostic method for invasive candidiasis by the detection of d-1-arabinotol using gas chromatography/mass spectrometry. Diagn Microbiol Infect Dis 2004;49(4):227–32.

17. Yager C, Wehrli S, Segal S. Urinary galactitol and galactonate quantified by isotope-dilution gas chromatography—mass spectrometry. Clin Chim Acta 2006;366(1–2):216–24.

18. Rakotomanga S, Baillet A, Pellerin F, Baylocq-Ferrier D. Simultaneous determination of gluconolactone, galactonolactone and galactitol in urine by reversed-phase liquid chromatography: application to galactosemia. J Chromatogr B Biomed Sci Appl 1991;570(2):277–84.

19. Hiatt HH. Carbohydrate metabolism in pentosuria. Ann Intern Med 1960;53(2):372–9.

20. Moolenaar SH, Knaap M SvD, Engelke UFH, Pouwels PJW, Janssen-Verhoeven NM, Struys EA, Jakobs C, van der Knaap MMC. Clinical presentations of patients with polyol abnormalities. Neuropediatrics 2004;35(3):167–73.

21. Wamelink MMC, Smith DEC, Jakobs C, Verhoeven NM. Analysis of polyols in urine by liquid chromatography—tandem mass spectrometry: a useful tool for recognition of inborn errors affecting polyol metabolism. J Inherit Metab Dis 2005;28(6):951–63.

22. Wamelink MMC, Struys EA, Jakobs C. The biochemistry, metabolism and inherited defects of the pentose phosphate pathway: a review. J Inherit Metab Dis 2008;31(6):703–17.

23. Wamelink MM, Smith DE, Jakobs C, van der Knaap MS. Profiling of pentose phosphate pathway intermediates in blood spots by tandem mass spectrometry: application to transaldolase deficiency. Clin Chem 2003;49(8):1375–80.

24. Huck JH, Verhoeven NM, van Hagen JM, Jakobs C, van der Knaap MS. Clinical presentations of patients with polyol abnormalities. Neuropediatrics 2004;35(3):167–73.

25. Moolenaar SH, Knaap MSvd, Engelke UFH, Cassano PA. Erythritol is a pentose-phosphate pathway metabolite and associated with adiposity gain in young adults. Proc Natl Acad Sci USA 2017;114(5):E4233–E40.

26. Bub A, Kriebel A, Dörr C, Bandt S, Rist M, Roth A, Himmel E, Kulling S, Hoffmann I, Watzl B. The Karlsruhe Metabolomics and Nutrition (KarMeN) study: protocol and methods of a cross-sectional study to characterize the metabolome of healthy men and women. JMR Research Protocols 2016;3(5):e146.

27. Slimali N, Ferrari P, Ocke M, Welch A, Boeijing H, Liere M, Pala V, Amiano P, Lagiou A, Mattisson I et al. Standardization of the 24-hour diet recall calibration method used in the European Prospective Investigation into Cancer and Nutrition (EPIC): general concepts and preliminary results. Eur J Clin Nutr 2000;54(12):900–17.

28. Slimali N, Dehaveng C, Charonnordie RU, van Kappel AL, Ocke MC, Welch A, Lagiou A, van Liere M, Aguado A, Pala V et al. Structure of the standardized computerized 24-h diet recall interview used as reference method in the 22 centers participating in the EPIC project. European Prospective Investigation into Cancer and Nutrition. Comput Methods Programs Biomed 1999;58(3):251–66.

29. Hartmann BM, Heuer T, Hoffmann I. The German Nutrient Database: effect of different versions on the calculated energy and nutrient intake of the German population. J Food Compos Anal 2015;42:26–9.

30. Rijst MJ, Rooth AF, Frommherz L, Weingert CR, Krüger R, Merz B, Bunzel D, Mack C, Egert B, Bub A et al. Metabolic patterns predicting sex and age in participants of the Karlsruhe Metabolomics and Nutrition (KarMeN) study. PLoS One 2017;12(8):e0183228.

31. Dunn WB, Wilson ID, Nicholls AW, Broadhurst D. The importance of experimental design and QC samples in large-scale and MS-driven untargeted metabolomic studies of humans. Bioanalysis 2012;4(18):2249–60.

32. Shoemaker JD, Elliott WH. Automated screening of urine samples for carbohydrates, organic and amino acids after treatment with urease. J Chromatogr B Biomed Sci Appl 1991;562(1):125–38.

33. Bhatti T, Clamp JR. Identification and estimation of monosaccharides and disaccharides in urine by gas-liquid chromatography. Clin Chim Acta 1968;22(4):563–7.

34. Nakamura H, Tamura Z. Gas chromatographic analysis of mono- and disaccharides in human blood and urine after oral administration of disaccharides. Clin Chim Acta 1972;39(2):367–81.

35. Bouatra S, Aziat F, Mandral R, Guo AC, Wilson MR, Knox C, Bjorn Dahl TC, Krishnamurthy R, Saleem F, Liu P et al. The human urine metabolome. PLoS One 2013;8(9):e73076.

36. Spear GT, French AL, Gilbert D, Zarriffard MR, Mirmonef P, Sullivan TH, Spear WW, Landay A, Micci S, Lee BH, et al. Human alpha-amylase present in lower-genital-tact mucosal fluid processes glycoproteins to support vaginal colonization by Lactobacillus. J Infect Dis 2014;210(7):1019–28.

37. Mirmonef P, Hotton AL, Gilbert D, Burgard D, Landay A, Weber KM, Cohen M, Ravel J, Spear GT. Free glycogen in vaginal fluids is associated with Lactobacillus colonization and low vaginal pH. PLoS One 2014;9(7):e102467.

38. Nunn KL, Forney LJ. Unraveling the dynamics of the human vaginal microbiome. Yale J Biol Med 2016;89(3):331–7.

39. Mirmonef P, Hotton AL, Gilbert D, Gioia CJ, Marcia D, Hope TJ, Landay AL, Spear GT. Glycogen levels in undiluted genital fluid and their relationship to vaginal pH, estrogen, and progesterone. PLoS One 2016;11(4):e0153553.

40. Mirmonef P, Modur S, Burgard D, Gilbert D, Golub ET, French AL, McCotter K, Landay AL, Spear GT. Exploratory comparison of vaginal glycogen and Lactobacillus levels in premenopausal and postmenopausal women. Menopause 2015;22(7):1072–9.

41. La Forge FB. D-mannoketoheptose, a new sugar from the avocado. J Biol Chem 1962;237(2):563–9.

42. Bean RC, Barr BK, Welch HV, Porter GG. Carbohydrate metabolism of the avocado. Arch Biochem Biophys 1968;96(3):524–9.

43. Muir JG, Rose R, Rosella O, Liels K, Barrett JS, Gibson PR. Measurement of short-chain carbohydrates in common Australian vegetables and fruits by high-performance liquid chromatography (HPLC). J Agric Food Chem 2009;57(2):554–65.

44. Herrinton LJ, Weiss NS, Beresford SAA, Stanford JL, Wolfla DM, Feng Z, Scott CR. Lactose and galactose intake and metabolism in relation to the risk of epithelial ovarian cancer. Am J Epidemiol 1995;141(5):407–16.

45. Laker MF. Estimation of disaccharides in plasma and urine by gas-liquid chromatography. J Chromatogr B Biomed Sci Appl 1979;163(1):9–18.
51. Sharma SK, Singhal R, Malhotra BD, Sehgal N, Kumar A. Biosensor based on Langmuir–Blodgett films of poly(3-hexyl thiophene) for detection of galactose in human blood. Biotechnol Lett 2004;26(8):645–7.

52. Shinka T, Inoue Y, Peng H, Zhen-Wei X, Ose M, Kuhara T. Urine screening of five-day-old newborns: metabolic profiling of neonatal galactosuria. J Chromatogr B Biomed Sci Appl 1999;732(2):469–77.

53. Arola H. Diagnosis of hypolactasia and lactose malabsorption. Scand J Gastroenterol 1994;29(sup202):26–35.

54. Münger LH, Trimigno A, Picone G, Freiburghaus C, Pimentel G, Burton KJ, Prolong FP, Vionnet N, Capozzi F, Badertscher R, et al. Identification of urinary food intake biomarkers for milk, cheese, and soy-based drink by untargeted GC-MS and NMR in healthy humans. J Proteome Res 2017;16(9):3321–35.

55. Kalač P. A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. J Sci Food Agric 2013;93(2):209–18.

56. Pitkanen E, Sahlstrom K. Increased excretion of xylitol after administration of glucuronolactone and ethanol in man. Ann Med Exp Biol Fenn 1968;46(2):143–50.

57. Rovio S, Sirén K, Sirén H. Application of capillary electrophoresis to determine metal cations, anions, organic acids, and carbohydrates in some Pinot Noir red wines. Food Chem 2011;124(3):1194–200.

58. Weinstam W, Schaefer P, Thierauf A, Schreiber A, Wurst FM. Confirmatory analysis of ethylglucuronide in urine by liquid-chromatography/electrospray ionization/tandem mass spectrometry according to forensic guidelines. J Am Soc Mass Spectrom 2004;15(2):188–93.

59. Wurst FM, Kempter C, Metzger J, Seidl S, Alt A. Ethyl glucuronide—a marker of recent alcohol consumption with clinical and forensic implications. Alcohol 2000;20(2):111–16.

60. Wurst FM, Kempter C, Seidl S, Alt A. Ethyl glucuronide—a marker of alcohol consumption and a relapse marker with clinical and forensic implications. Alcohol Alcohol 1999;34(1):71–7.

61. Helander A, Olsson I, Dahl H. Postcollection synthesis of ethyl glucuronide by bacteria in urine may cause false identification of alcohol consumption. Clin Chem 2007;53(10):1855–7.

62. Politi L, Morini L, Groppi A, Poloni V, Pozzi F, Poletti A. Direct determination of the ethanol metabolites ethyl glucuronide and ethyl sulfate in urine by liquid chromatography/electrospray tandem mass spectrometry. Rapid Commun Mass Spectrom 2005;19(10):1321–31.

63. Helander A, Dahl H. Urinary tract infection: a risk factor for false-negative urinary ethyl glucuronide but not ethyl sulfate in the detection of recent alcohol consumption. Clin Chim Acta 2005;351(1–2):1728–30.

64. Eisinger-Watzl M, Straßburg A, Ramünke J, Krems C, Heuer T, Hoffmann I. Comparison of two dietary assessment methods by food consumption: results of the German National Nutrition Survey II. Eur J Nutr 2015;54(3):343–54.

65. Dresen S, Weinmann W, Wurst FM. Forensic confirmatory analysis of ethyl sulfate—a new marker for alcohol consumption—by liquid-chromatography/electrospray ionization/tandem mass spectrometry. J Am Soc Mass Spectrom 2004;15(11):1644–8.

66. Kuhle GGC. Nutritional biomarkers for objective dietary assessment. J Sci Food Agric 2012;92(6):1145–9.

67. Gao Q, Pratico G, Scalbert A, Vergeres G, Kolehmainen M, Manach C, Brennan L, Afman LA, Wishart DS, Andres-Lacueva C et al. A scheme for a flexible classification of dietary and health biomarkers. Genes Nutr 2017;12:34.