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## **Sugar intake among German adolescents: trends from 1990-2016 based on biomarker excretion in 24-h urine samples**

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**Shortened version of the title:** trends in sugar excretion during adolescence

**Keywords:** urinary fructose, urinary sucrose, adolescence, biomarker, trends, sugar intake

### **Ethical standard**

The DONALD Study was approved by the Ethics Committee of the University of Bonn, Germany



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## Abstract

Trend analyses based on dietary records suggest decreases in the intakes of total (TS), added (AS) and free sugar (FS) since 2005 among children and adolescents in Germany. In terms of age trends, TS intake decreased with increasing age. However, self-reported sugar intake in epidemiological studies is criticized, as it may be prone to bias due to selective underreporting. Furthermore, adolescents are more susceptible to underreporting than children. We thus analyzed time and age trends in urinary fructose excretion (FE), sucrose excretion (SE) and the sum of both (FE+SE) as biomarkers for sugar intake among 8.5-16.5-year-old adolescents. Urinary sugar excretion was measured by UPLC-MS/MS in 997 24-h urine samples collected from 239 boys and 253 girls participating in the DONALD study cohort between 1990 and 2016. Time and age trends of log-transformed FE, SE and FE+SE were analyzed using polynomial mixed-effects regression models. Between 1990 and 2016 FE as well as FE+SE decreased (linear time trend:  $p=0.0272$  and  $p<0.0001$ , respectively). A minor increase in excretion during adolescence was confined to FE (linear age trend:  $p=0.0017$ ). The present 24-h excretion measurements support a previously reported dietary-record based decline in sugar intake since 2005. However, the previous seen dietary record-based decrease in TS from childhood to late adolescence was not confirmed by our biomarker analysis, suggesting a constant sugar intake for the period of adolescence.

## Introduction

A high added sugar (AS) or free sugar (FS) intake is discussed to promote the development of numerous diseases like dental caries<sup>(1,2)</sup>, overweight and obesity<sup>(3-5)</sup>, cardiovascular diseases<sup>(6,7)</sup> or metabolic syndrome<sup>(8,9)</sup>. AS is defined as sugars added to foods during processing/preparation at home or in manufacture, including sugars from honey, syrups and fruit juice concentrates<sup>(10)</sup>. To consider sugars from liquid sources, the World Health Organization (WHO) defined FS as “all monosaccharides and disaccharides added to foods by the manufacturer, cook, or consumer, plus sugars naturally present in honey, syrups, and fruit juices”<sup>(11)</sup>. Since 2015 the WHO recommends limiting FS intake to less than 10% of daily energy intake (%E)<sup>(11)</sup>. In 2018, the German Nutrition Society, the German Obesity Society and the German Diabetes Society jointly adopted this recommendation<sup>(12)</sup>.

Data on dietary sugar intake in Germany suggests a high sugar intake especially among children and adolescents<sup>(13-16)</sup>, which is probably due to an innate sweet preference, the strength of which diminishes into adulthood<sup>(17,18)</sup>. In a recent publication from the DONALD

study, children and adolescents exceeded the recommended limit for FS intake set by the WHO <sup>(15)</sup>. Based on dietary records, the median FS intake ranged from 15.2 to 17.5%E across the age groups (3-5, 6-10, 11-14 and 15-18 years) <sup>(15)</sup>. To our knowledge, the DONALD study is the only study so far providing data on free sugar intake among German children and adolescents between 3 to 18 years. A high sugar intake during adolescence is of particular interest, because this age is regarded as a potentially “critical period” for the development of various diseases in later life <sup>(19–21)</sup>. Together with the decline in physical activity, which is often seen in adolescence and changes in the regulation of satiety and appetite <sup>(22)</sup>, a high sugar intake can contribute to a positive energy balance and therefore to the development of overweight. In addition, dietary pattern adopted during adolescence could track into adulthood <sup>(23)</sup>.

Of note, recent time trend analyses based on more than 10,000 dietary records collected in the DONALD study between 1985 and 2016, suggested that the intake of total sugar (TS), FS and AS (as percentage of total daily energy intake [%E]) is generally high in children and adolescents (3-18 years), but has decreased since 2005, most notably from 2010 onwards <sup>(15)</sup>. Age trend analyses in the same study sample suggested that TS intake decreased with increasing age <sup>(15)</sup>. However, self-reported data may be prone to bias due to underreporting <sup>(24–26)</sup>, in particular during adolescence <sup>(27,28)</sup>. This may apply particularly to self-report of socially less desired foods rich in sugar <sup>(26)</sup>. In the ALSPAC study, 10-year-old participants, who were identify as underreporters, recorded consuming fewer sugar rich foods such as biscuits, cakes, chocolate and sweets compared to plausible reporters <sup>(29)</sup>. Hence, information on time and age trends of sugar intake based on biomarkers would be desirable. In addition to potential bias, the estimation of sugar intake from all dietary assessment instruments underlies further measurement errors <sup>(30)</sup>: The intake of processed foods has increased in the last decades <sup>(31)</sup> and the sugar content in products varies depending on the manufacturer. Most of these specific foods are not included in food composition tables. In addition, nutrient data e.g. carbohydrates contents in food composition tables can differ from chemical analysis of foods in the laboratory <sup>(32,33)</sup>.

In 1996 urinary fructose and sucrose were first proposed as biomarkers for sucrose intake <sup>(34)</sup>. Since 2005 total excretion of urinary sucrose and fructose in 24-h urines, as well as the sum of both, have been validated as biomarkers of dietary sugar intake in different study populations <sup>(35–39)</sup> and have been successfully used in epidemiological studies <sup>(40–43)</sup>.

Therefore our aim was to analyze time and age trends in 24-h-excretion of fructose (FE), sucrose (SE) and the sum of fructose and sucrose (FE+SE) among children and adolescents (8.5-16.5 years) between 1990 and 2016.

## Methods

### Study population

For the present analyses 24-h urine samples (n=997) were selected from participants of the DONALD study, an ongoing, open cohort study conducted in Dortmund, Germany. This study collects data on diet, growth, development and metabolism of healthy children and adolescents since 1985. In the first few study years approximately 300 participants >2 years old were also recruited. Since then, approximately 35-40 infants are newly recruited every year. The regular visits begin at 3 months of age. The participants return for three more visits in the first year, two in the second year and thereafter annually until young adulthood. Yearly examinations include 3-day weighed dietary records, anthropometric measurements, collection of 24-h urine samples, interviews on lifestyle and medical examinations. Parental examinations (anthropometric measurements, lifestyle interviews) take place every four years. Further details on the DONALD study were described elsewhere<sup>(44)</sup>. The study was approved by the Ethics Committee of the University of Bonn according to the guidelines of the Declaration of Helsinki. Parental and later on children's written consent was obtained for all examinations.

### Sample selection

For this evaluation 997 urine samples were selected from the DONALD urine biobank. Only complete and plausible 24-h urine samples, as validated by 24-h urinary creatinine excretion<sup>(45)</sup>, were included. Uncooled (>-12°C) and contaminated (blood, faeces) samples were not selected. Urine samples were selected in a 2-stage process. Firstly, 464 urine samples available from a previous study<sup>(46)</sup>, which had included participants with at least two 24-h urine samples during adolescence and a blood measurement in young adulthood were chosen. Secondly, a 533 additional urine samples were selected as to include at least 4 urine samples per year of adolescence (boys: 9.5-16.5 years; girls: 8.5-15.5 years) in every study year (1990-2016). If more than 4 urine samples were available for a specific study year or year of adolescence, additional urine samples were randomly selected from those available.

## 24-hour urine collection

Annual 24-h urine collections are scheduled in participants older than 3 or 4 years. Collections follow a standardized procedure after detailed instruction of the families. The participants are asked to void their bladders upon getting up in the morning; this micturition is completely discarded. This sets the start of the collection and which ends with voiding the bladder in the next morning<sup>(38)</sup>. During the collection period at home, the participants store the micturitions in preservative-free, Extran-cleaned (Extran, MA03; Merck, Darmstadt, Germany) 1-liter plastic containers at less than -12 °C. After the transfer to the study institute by a dietitian they are stored at -22 °C until thawed.

## Urinary measurements and laboratory analyses

Urinary creatinine and urea excretion were measured in the DONALD laboratory. 24-h creatinine excretion was measured by a creatinine analyzer (Beckman-2; Beckman Instruments, Fullerton, CA, USA) using the kinetic Jaffe' procedure. 24-h urea excretion was determined by Urease-Berthelot-Method.

Urinary fructose and sucrose were measured in the laboratory of the Department of Food & Nutritional Sciences at the University of Reading using LC-MS and quantified using stable-isotope labelled internal standards (<sup>13</sup>C<sub>12</sub>-sucrose and <sup>13</sup>C<sub>6</sub>-fructose, Sigma Aldrich, Gillingham, UK): After shipping on dry ice, urine samples were stored at -80°C until analysis and thawed at 4 °C. 100 µL of urine was combined with 100 µL acetonitrile containing the internal standards, vortex-mixed, centrifuged at 13000g for 10 min and the supernatant transferred into a 96-well plate for LC-MS/MS analysis. Samples were separated by HPLC (Acquity BEH Amide 2.1 x 50 mm, 1.7 µm column (Waters, Milford, MA, USA), kept at 35°C), using 80/20 (v/v) acetonitrile/water with 0.2 % NH<sub>4</sub>OH as mobile phase (250 µL/min) using an Acquity UPLC binary solvent manager, sampler manager and column manager (Waters, Milford, MA, USA), and detected by tandem mass spectrometry using a Quattro Ultima tandem quadrupole mass spectrometer (Micromass, Manchester, UK). The mass spectrometer was operated with electrospray ionisation (ESI) in positive ion mode in multiple reaction monitoring (MRM) mode. Nitrogen was used as the desolvation gas and argon was used as the collision gas. The following generic source conditions were used: capillary voltage, 3.6 kV; cone voltage, 35 V; desolvation temperature, 400 °C; source temperature, 120 °C, desolvation gas flow, 500 L/hr; cone gas flow, 100 L/hr. The concentration range was 0.1 to 500 µmol/L (Fructose: 0.02 - 90.1 mg/L; sucrose: 0.03 - 171.2 mg/L). To calculate

daily excretions concentrations were converted to mg/d by using the molar mass of fructose or sucrose and multiplied with the 24-h urine volume.

### **Dietary assessment**

Dietary intake in the DONALD study is assessed using 3-day weighed dietary records. Participants are asked to collect the 24-h urine on the third day of recording at each visit. For the present analyses, those dietary records were used, which were provided at the same age as the 24-h urines were collected (n=969). 91 % of the dietary records included the day of urine collection. All foods and beverages consumed by the child, as well as leftovers, are weighed and recorded over 3 consecutive days by the parents or by the older participants themselves with the use of electronic food scales ( $\pm 1$  g). The participants choose the day of the beginning of dietary recording within a given period of time. A trained dietitian checks the dietary records for accuracy and completeness. Subsequently, energy and sugar intakes are calculated using our continuously updated in-house nutrient database LEBTAB <sup>(47)</sup>. Data on the composition of unprocessed foods was based on the German food composition tables BLS 3.02. The BLS contains information on total sugar (sum of mono- and disaccharides) as well as individual mono- and disaccharides (<https://www.blsdb.de/>). Energy and nutrient contents of commercial food products, i.e., processed foods and ready-to-eat-meals or snack foods are estimated by recipe simulation. Trained dietitians use the labelled ingredients and nutrients to estimate the product recipe and based on this simulation calculate the energy and nutrient content, including total and added sugar from the ingredients. Added sugar content of a product is estimated summing up the carbohydrates stemming from caloric sweeteners, e.g. sugar, honey or syrup, according to the definition in Cummings & Stephen, 2007 <sup>(10)</sup>. For longitudinal analysis, LEBTAB is continuously being updated with any new foods recorded by the participants. A new food or a commercial food product that already exists in the database but has undergone a change in composition (e.g., new ingredients, fortification) leads to a new entry with a new simulation if necessary and a new food code <sup>(47)</sup>. Energy and nutrient intake were calculated as individual means of three days of recording. FS was calculated for the current analyses according to the definition by SACN <sup>(48)</sup>, including added sugars plus sugars from fruit juices, vegetable juices, juice spritzers and smoothies.

### **Anthropometric measurements**

Height, weight and skinfold measurement are performed by trained nurses according to standard procedures. From the age of 2 years onwards, standing height is measured to the

nearest 0.1 cm using a digital stadiometer (Harpندن, Crymych, UK). Body weight is measured to the nearest 100 g using an electronic scale (Seca 753E; Seca Weighing and Measuring System). Overweight was defined according to International Obesity Task Force's (IOTF) BMI cutoff values for children and adolescents <sup>(49,50)</sup>. Triceps and subscapular skinfolds were measured on the right side of the body using a skinfold calliper (Holtain Ltd, Crosswell, Dyfed, UK). The sum of both skinfolds was used for the estimation of percentage body fat according to the equations of Slaughter <sup>(51)</sup>. Body surface area was calculated according to DuBois and DuBois <sup>(52)</sup>. Data on height were used to estimate the age at take-off (ATO). Methods for determining ATO are described elsewhere <sup>(53,54)</sup>.

### **Family characteristics**

Maternal body weight and height is measured with the same equipment as for the participants. Maternal overweight was defined as a BMI  $\geq 25$  kg/m<sup>2</sup>. Maternal education and employment are inquired with a standardized questionnaire in 4-year intervals. High maternal educational status ( $\geq 12$  years of schooling) and maternal employment were used as socioeconomic characteristics.

### **Statistical analysis**

The present investigation was carried out as an exploratory analysis of a long-term observational study (DONALD study). Therefore, no calculations related to sample size or statistical power were done.

997 24-h urine samples from 492 participants during puberty (girls: 8.5-15.5 years; boys: 9.5-16.5 years) were analyzed. Per participant, one (n=170), two (n=186), three (n=99), four (n=27) or five (n=10) urine samples were available. All statistical analyses were performed using SAS ® procedures (version 9.4; Cary, NC, USA). The significance level was set to  $p < 0.05$ . Descriptive data (Table 1) are presented as medians with their interquartile range or frequencies and percentages. If more than one measurement per participant was available, the individual means of the respective variables were calculated. To visualize the observed changes in sugar excretion over time and age in figure 1 and 2, sugar excretion in mg/d was standardized to 1900 kcal/d (i.e. the rounded median total energy intake (TEI) of the total sample).

Time and age trends in urinary FE, SE and FE+SE were analysed using polynomial mixed-effects regression models including both fixed and random effects (PROC MIXED in SAS®).

Outcome variables were log<sub>10</sub> transformed due to lack of normal distribution of the model residuals. Individual outliers (n=5 for fructose and sucrose measurements, respectively) of the outcome variables (fructose  $\geq 117.1$  mg/d; sucrose  $\geq 287.7$  mg/d) were winsorized, i.e. outliers were replaced by the closest value fitting the distribution. Age and time - continuously in years - were the principle fixed effects of the models. The first included urine sample considered in this evaluation was the baseline time, i.e., time = 0. Therefore, time ranged between 0 and 26 years (1990-2016). Quadratic and cubic terms for age (age<sup>2</sup>, age<sup>3</sup>) and time (time<sup>2</sup>, time<sup>3</sup>), as well as an interaction term of linear age and time (age  $\times$  time), were considered as additional explanatory variables if they improved the fit statistics [Akaike information criterion (AIC)] by more than two points or significantly predicted the respective outcome<sup>(55)</sup>. Interactions between sex and age as well as sex and time were tested and included in the model if the interaction was significant.

A linear trend reflects a constant increase or decrease in the respective outcome variable over the years or with age. Quadratic and cubic trends indicate that the magnitude of the trend changes over time or with age. A repeated statement was considered so as to account for the lack of independence between repeated measures from the same person. Random effects were considered to allow variation between individuals and families with respect to the initial level (intercept) as well as linear, quadratic and cubic age trends of the respective outcome. The AIC was also used to select the covariance structure that best describes the variances and covariances of the initial level, the linear and quadratic trend among persons, and the covariance structure that best describes the correlated nature of the repeated measurements. Variables that were considered in the final models either (1) modified regression coefficients in the basic models by  $\geq 10$  % (2), had a significant and independent association with the outcome variable, or (3) led to an improvement of the AIC by more than two points<sup>(56)</sup>. The single effect estimates of polynomial models cannot be interpreted, i.e. if the analyses render significant results for a combination of linear, quadratic, and cubic trends, the single beta values do not reflect the true age and time trends.

For the current analysis, the following variables were considered as potentially confounding factors: sex (boy/girl), 24-h creatinine excretion (mmol/d), 24-h urea excretion (mmol/d), 24-h urine volume (liter), body fat (%), body surface area (age corrected residuals), TEI (kcal), collection day=weekday (yes/no); as well as maternal overweight (yes/no), high maternal educational status (yes/no) and maternal employment (yes/no). For missing values, the respective median of the total sample was used (n=4 for 24-h urea excretion, n=28 for TEI,

n=14 for maternal overweight). Since there were many missing values for ATO (n=182), a separate sensitivity analysis was performed with ATO as an additional potential confounding factor. However, ATO wasn't relevant in all tested models.

## Results

### *Sample characteristics*

While sucrose excretion could be measured in all 997 urine samples, fructose excretion was below the level of detection in 32 missing samples. These values were regarded as missing and not considered in the analysis of time and age trends in fructose excretion. Participant's characteristics stratified by sex are shown in Table 1. Among boys, median FE+SE was 49.8 mg/d, median FE was 21.1 mg/d and median SE was 26.7 mg/d. Among girls, median FE+SE was 46.7 mg/d, median FE was 20.8 mg/d and median SE was 22.7 mg/d. The percentage of participants with overweight was 18.0% among boys and 15.8 % among girls. Maternal characteristics reflect the high socioeconomic status of DONALD participants: While around 60 % of mothers had a high educational status among both sexes, 70.7 % and 66.8 % of mothers were in employment among boys and girls, respectively. Energy-standardized median sugar excretion in mg/1900kcal/d as well as median TS, AS and FS intake in %E stratified by time periods and age groups are shown in Fig. 1 and 2.

### *Time and age trends*

To adjust for the significant interaction between age and sex in the fructose model ( $p=0.0076$ ), the term age x sex was included in this model. Results of the time and age trend analysis from the polynomial mixed-effects regression models for FE, SE and FE+SE are shown in Table 2. Between 1990 and 2016 FE as well as FE+SE decreased significantly (linear trend:  $p=0.0272$  and  $p<0.0001$ , respectively). SE showed a tendency towards a negative quadratic trend ( $p=0.0644$ ), i.e. SE increased first and decreased again thereafter. In terms of age trends, FE increased significantly with age (linear trend:  $p=0.0017$ ). No age trends were observed for SE and FE+SE in the adjusted models.

We also performed time and age trends of TS, FS and AS intake based on dietary records, among the participants who collected both, urine samples and dietary records (n=478) (see Supplementary material). We found time and age trends similar to those published previously (15).

## Discussion

The present analyses suggest a decrease in dietary sugar intake among adolescents in Germany between 1990 and 2016, indicated by a decrease in urinary FE and FE+SE representing established biomarkers<sup>(30,57)</sup>. The significant increase of FE, but not of SE and FE+SE with age provides an indication of an increase of dietary sugar intake during adolescence (8.5-16.5 years).

These results are partly in line with previous age and time trend analyses based on 10,671 3-day weighed dietary records<sup>(15)</sup>. In these previous analyses, TS, FS and AS (expressed as %E) followed a non-linear course with a decreased intake since 2005, which was most pronounced since 2010. While TS and FS intake increased between 1985 and 2005, AS intake decreased between 1985 and 1995 and increased slightly thereafter until 2005. The median excretion of FE and FE+SE in mg/1900kcal (Figure 1) suggests that sugar excretion followed the same non-linear time trend as self-reported sugar intake<sup>(15)</sup>. However, statistical analysis pointed out differences: the biomarker analysis only confirmed the decline in TS and FS intake since 2005, but failed to confirm the preceding slight increase between 1990 and 2005 as estimated by the dietary assessment<sup>(15)</sup>. This deviation may be due to a lack of power of the biomarker analysis with only one tenth of observations compared to those included in the dietary record analysis. However, overall decrease in sugar intake is confirmed by trend analyses in sugar excretion.

In terms of age trends we observed a few deviations between dietary records and biomarkers: Previous trend analyses among 3-18 year olds based on dietary records showed that %E from TS decreased significantly from early childhood to late adolescence<sup>(15)</sup>, which appears to contradict the results of the present evaluation. Here, energy adjusted FE increased significantly with age during adolescence. Despite the fact that the present analyses cover a smaller age range compared to the previous analysis, differences between age trends in TS intake and age trends in FE point to an increasing selective underreporting of dietary sugars during adolescence, which was already observed among adult study populations<sup>(26,58)</sup> as well as children and adolescents<sup>(29,59)</sup>. However, taking into account all results of the investigation of dietary records<sup>(15)</sup> and sugar excretion, the observed age trend in energy adjusted FE seems to be small and – albeit significant - of less clinical relevance (see Figure 2). Also, age trends of previous self-reported FS and AS intake – i.e. subgroups of TS – were small throughout adolescence<sup>(15)</sup>. In addition, age trends were not confirmed for energy adjusted SE or FE+SE, perhaps also reflecting an overall lack of power. Moreover, the results for SE and FE + SE

would rather suggest a constant excretion of sugar during puberty. Taken together, the results from our trend analyses suggest that sugar excretion as well as sugar intake, with the exception of TS, is relatively constant during adolescence. Observed differences in FE between ages can be regarded as minor.

Unfortunately, FE and SE excretion do not allow conclusions on the amount of the absolute dietary sugar intake. However, the combination of biomarker and dietary record data points to a decreasing dietary sugar intake among adolescents. Nevertheless, free sugar intake based on dietary records<sup>(15)</sup> still exceeding national and international limits<sup>(11,12)</sup>. This underlines the need for obligatory public health measures to further support the observed decline in sugar intake over the study period among children and adolescents in Germany.

The DONALD study and the used biomarkers have some limitations, which have to be discussed. Firstly, genetic, dietary or lifestyle factors as well as physiological or medical conditions could influence individual sugar excretion<sup>(57)</sup>. Thus, “predictive biomarkers contain a certain level of person-specific, intake-related, and covariate-related bias”<sup>(30)</sup>. To solve this problem, Tasevska et al.<sup>(41)</sup> provided an equation for calibrating the 24-h urine biomarker FE+SE based on results from a feeding study conducted among adults in the UK (25-77 years of age)<sup>(35)</sup>. Because this equation was derived for adults, we could not apply it to our adolescent sample. In addition, sugar excretion could show inter-individual variability<sup>(35,57)</sup>. Therefore, we considered within-person variability as well as variability among participants by including random and repeated statements during the model building process using the proc mixed procedure as described in the method section. If the tested variability had an influence on the observed trends, as reflected by changes in the AIC, it was included in the model.

A further limitation of the present study is the handling of urine samples, which were frozen without preservatives up to 26 years. Little is known about the stability of fructose and sucrose in urine. Luceri et al.<sup>(34)</sup> refer to instability of sucrose in urine samples kept at room temperature. However, our samples were stored at less than -12 °C during the collection period at home and after the transfer to the study institute at -22 °C until thawed. Thus our samples are frozen continuously until use. Since our samples had to be thawed, aliquoted and frozen repeatedly for shipping, we only chose urine samples which were collected after 1990 for the current trend analyses to minimize error due to reduced long-term stability. In addition, the DONALD study population is characterized by a relatively high SES<sup>(44)</sup>, which is known to correlate with lower dietary sugar intake<sup>(60)</sup>. Therefore the generalizability of our

results is limited. Nevertheless, our sugar excretion data seems plausible comparing them to FE and SE levels from other study populations among adults using the sample urine analyses methods<sup>(36,61)</sup>. To our knowledge only three publications include examination data on sugar excretion among children and/or adolescents. The first also included DONALD participants, but using different urine analyses methods<sup>(62)</sup>. In the second evaluation only morning spot urines were collected<sup>(39)</sup>. Nevertheless in both studies sugar excretion levels were similar to the present evaluation. The third study<sup>(38)</sup> reported very low excretion data amounting to less than 1 mg/24-h after a diet with 5% or 25% AS, which seems to be implausible when held against the two other publications and our results. Furthermore, sugar excretion has already been tested as a biomarker for several sugar intakes<sup>(37–39,62)</sup> and it is not clear yet, for which type of sugar the biomarkers are most suitable.

The main strength of the present investigation is the longitudinal design of the DONALD study with a long follow-up and no variation of methods, which enabled trend analyses over more than 25 years. Since 1985, the same methods have been used to collect anthropometric and dietary data, as well as 24-h urine samples. In addition, we used a well-characterized collective and our participants are asked to collect 24-h urines every year. Furthermore the analyses were carried out in an established laboratory by scientists with years of experience in the measurement of sugar excretion in 24-h urine samples.

In conclusion, the biomarker trend analyses of sugar intake suggest a decline in intake among children and adolescents between 1990 and 2016 and confirmed results based on analyses with dietary records. Sugar excretion and sugar intake data seem relatively constant during adolescence. Sugar intake consistently exceeds the given recommendation by the German nutrition society and the WHO.

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### **Conflict of interest**

AEB is a member of the International Carbohydrate Quality Consortium (ICQC) and a member of the Carbohydrate Task Force, ILSI Europe. IP, NG, GKK, TR and UA declare that they have no conflict of interest.

### **Authorship**

The authors responsibilities were as follows: AEB, UA and TR conceived the research project. NG and GKK carried out the sugar analyses of the urine samples. IP conducted the statistical analysis and wrote the manuscript. UA supervised the project and had primary responsibility for the final content. All authors made substantial contributions, critically read and revised the manuscript as well as approved the final version.

### **Informed consent**

All assessments in the DONALD Study were performed with parental and later on participants' written informed consent.

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**Table 1:** Sample characteristics of 492 DONALD study participants (8.5–16.5 years) between 1990 and 2016 stratified by sex

	<b>Boys</b>	<b>Girls</b>
<b>n [%]</b>	239 (48.6)	253 (51.4)
<b>Age [years]</b>	13.4 (12.1; 14.6)	11.6 (10.2; 12.7)
<b>Urinary data<sup>1</sup></b>		
Fructose excretion [mg/d]	21.1 (13.6; 31.2)	20.8 (12.0; 33.4)
Sucrose excretion [mg/d]	26.7 (17.0; 31.2)	22.7 (13.6; 38.8)
Fructose+sucrose excretion [mg/d]	49.8 (34.4; 80.7)	46.7 (30.4; 73.3)
Creatinine excretion [mmol/d]	9.3 (7.4; 11.5)	6.6 (5.6; 8.1)
Urea excretion [mmol/d]	313 (261; 382)	248 (201; 299)
24-h urine Volume [L]	1.0 (0.7; 1.3)	1.0 (0.7; 1.2)
<b>Anthropometrics</b>		
BMI [kg/m <sup>2</sup> ]	19.2 (17.1; 21.2)	17.7 (16.3; 19.8)
Overweight or obese [n (%)] <sup>2</sup>	43 (18.0)	40 (15.8)
Body fat [%] <sup>3</sup>	16.3 (12.0; 21.5)	19.6 (15.9; 24.7)
Body surface area [m <sup>2</sup> ] <sup>4</sup>	1.5 (1.4; 1.7)	1.3 (1.2; 1.5)
ATO [years] <sup>5</sup>	10.3 (9.8; 10.9)	8.7 (8.1; 9.4)
<b>Dietary variables<sup>6</sup></b>		
TEI [kcal/d]	2068 (1840; 2401)	1686 (1510; 1890)
Total sugar		
in g/d	134 (105; 166)	109 (90; 135)
in %E	25.6 (21.1; 29.8)	25.8 (22.0; 30.5)
Added sugar		
in g/d	66.7 (49.6; 89.7)	55.0 (37.9; 70.6)
in %E	13.1 (9.9; 17.6)	12.8 (9.1; 16.3)
Free sugar		
in g/d	88.9 (66.5; 117.0)	71.5 (52.4; 93.8)
in %E	17.6 (13.5; 21.4)	16.7 (12.8; 21.4)
<b>Maternal characteristics [n (%)]</b>		
Overweight or obese <sup>7</sup>	88 (36.8)	91 (36.0)
High educational status <sup>8</sup>	145 (60.7)	152 (60.1)
Employment	169 (70.7)	169 (66.8)

Values are medians (25th, 75th percentile) or frequencies (%)

BMI, body mass index; ATO, age at take-off; TEI, total energy intake

<sup>1</sup>32 from 997 fructose measurements were excluded

<sup>2</sup>BMI cutoff values for children and adolescents for overweight according to Cole et al. <sup>(49,50)</sup>

<sup>3</sup>percentage body fat according to the equations of Slaughter et al. <sup>(51)</sup>

<sup>4</sup>Body surface area according to the equation of DuBois & DuBois <sup>(52)</sup>

<sup>5</sup>Age at take of according to Buyken et al. <sup>(53)</sup> by using the parametric Preece and Baines model 1 <sup>(54)</sup>

<sup>6</sup>means from dietary records were available from 231 boys and 247 girls

<sup>7</sup>BMI > 25 kg/m<sup>2</sup>

<sup>8</sup>≥12 years of schooling

**Table 2:** Age and time trends in fructose (FE) and sucrose excretion (SE) as well as FE+SE of 997 urine samples (997 sucrose and 965 fructose measurements) of 492 DONALD study participants (9–16 years) between 1990 and 2016

	Age trend per year of age (8.5-16.5 years)	Time trend per study year (1990-2016)	
	Age $\beta$ (p)	Time $\beta$ (p)	Time $\times$ Time $\beta$ (p)
<b>Log (FE)<sup>a</sup></b>			
Unadjusted model	0.06738 (<0.0001)	-0.01020 (0.0117)	-
Adjusted model	0.05460 (0.0017)	-0.00942 (0.0272)	-
<b>Log (SE)<sup>b</sup></b>			
Unadjusted model	0.04948 (<0.0001)	0.006621 (0.6741)	-0.00096 (0.0809)
Adjusted model	-0.01463 (0.4650)	0.01545 (0.3251)	-0.00101 (0.0644)
<b>Log (FE + SE)<sup>c</sup></b>			
Unadjusted model	0.05195 (<0.0001)	-0.01711 (<0.0001)	-
Adjusted model	-0.00762 (0.6491)	-0.01592 (<0.0001)	-

Age and time trends were tested using polynomial mixed-effects regression models.

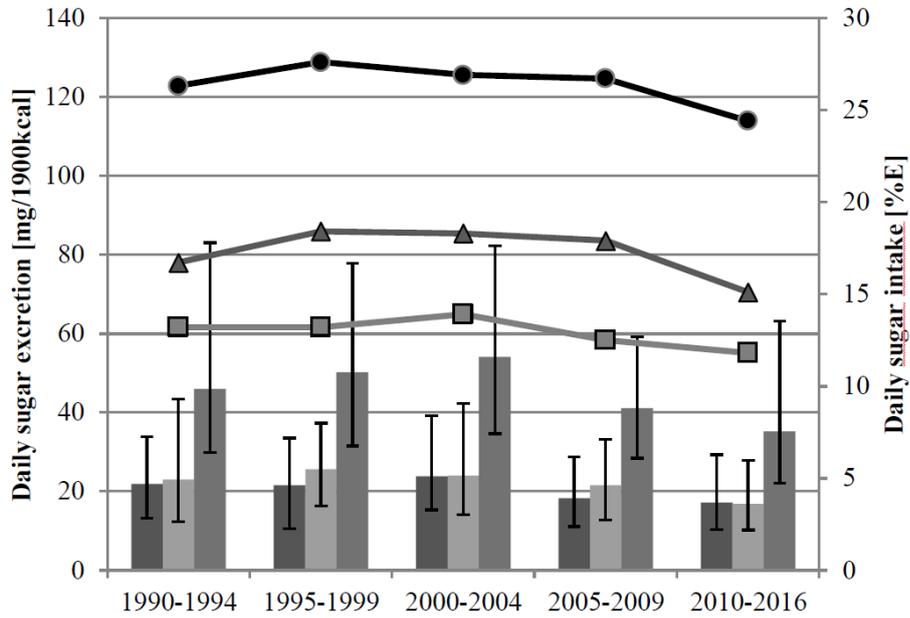
FE, Fructose excretion; SE, Sucrose excretion

<sup>a</sup>Model contains a random statement for the family level with an unstructured covariance structure. Because of a significant interaction between age and sex, the model includes the interaction term age  $\times$  sex. Adjusted for TEI (kcal), urea excretion (mmol/d), urine collection=weekday (yes/no), sex (male/female), maternal employment (yes/no).

<sup>b</sup>Model contains a random statement for the family level with a factor analytic covariance structure. Adjusted for TEI (kcal), creatinine excretion (mmol/d), urine volume (liters), high maternal education status (yes/no), urea excretion (mmol/d)

<sup>c</sup>Model contains a random statement for the family level with a factor analytic covariance structure and a random statement for the person level with a factor analytic covariance structure. Adjusted for TEI (kcal), creatinine excretion (mmol/d), urea excretion (mmol/d)

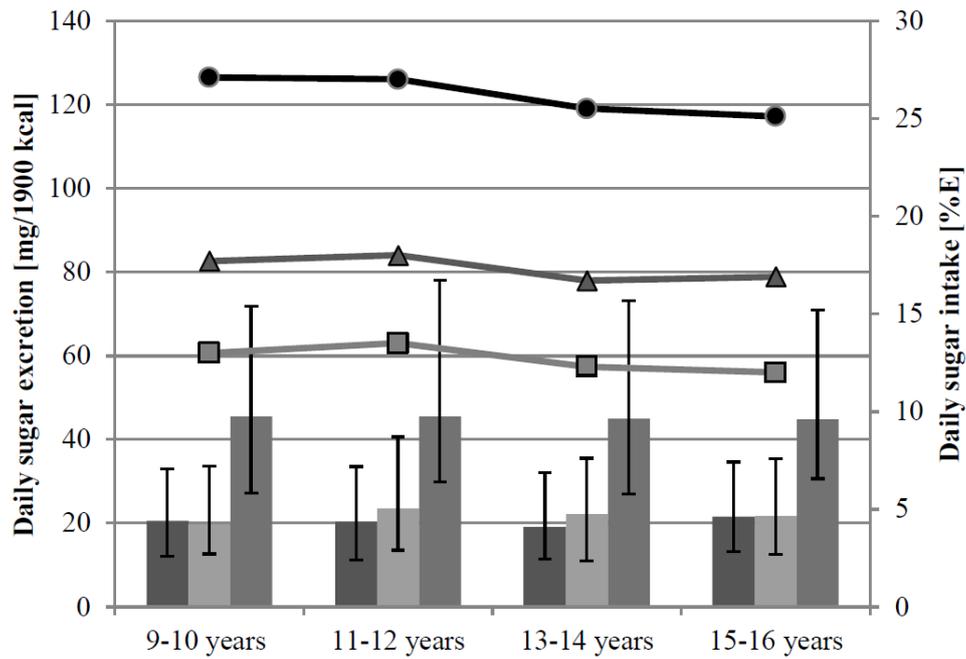
## Figure captions



<b>n</b> <sub>urine samples</sub>					
fructose	144	215	215	173	218
sucrose	152	216	218	188	223
<b>n</b> <sub>records</sub>	145	211	216	186	211

● Total sugar (TS) intake      ▲ Free sugar (FS) intake      ■ Added sugar (AS) intake  
 ■ Fructose excretion (FE)      ■ Sucrose excretion (SE)      ■ Fructose+sucrose excretion (FE+SE)

**Fig. 1** Energy standardized median (25th, 75th percentile) sugar excretion (FE, SE, FE+SE in bars) and median sugar intake as %E (TS in circles, FS in triangles, AS in squares) stratified by time periods (1990-1994, 1995-1999, 2000-2004, 2005-2009, 2010-2016)



<b>n</b> <sub>urine samples</sub>				
fructose	211	305	250	199
sucrose	218	312	263	204
<b>n</b> <sub>records</sub>	209	303	258	199

● Total sugar (TS) intake      ▲ Free sugar (FS) intake      ■ Added sugar (AS) intake  
 ■ Fructose excretion (FE)      ■ Sucrose excretion (SE)      ■ Fructose+sucrose excretion (FE+SE)

**Fig. 2** Energy standardized median (25th, 75th percentile) sugar excretion (FE, SE, FE+SE in bars) and median sugar intake as %E (TS in circles, FS in triangles, AS in squares) from 1990-2016 stratified by age groups (9-10 years, 11-12 years, 13-14 years, 15-16 years)