A novel urinary biomarker approach reveals widespread exposure to multiple low-calorie sweeteners in adults

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Running title: LCS exposure in adults via a biomarker approach

Abbreviations: ADI, acceptable daily intake; DNFCS, Dutch National Food Consumption Survey; EU, European Union; IQR, inter-quartile range; LCS, low-calorie sweeteners; LCSB, low-calorie sweetened beverage.

Abstract

Background: Observational investigations into the health impacts of low-calorie sweeteners (LCS) in humans fail to adequately identify or fully characterize LCS consumption.

Objectives: We aimed to utilize a novel biomarker approach to investigate exposure to 5 LCS and to test whether reported LCS beverage (LCSB) consumption effectively identifies exposure to LCS in adults.

Methods: In this cross-sectional analysis, two population studies were conducted in adults. Urinary excretions of 5 LCS, namely acesulfame-K, saccharin, cyclamate, sucralose and steviol glycosides, were simultaneously determined using liquid chromatography tandem-mass spectrometry. In Study 1, previously collected 24-hr urine samples (n = 357) were analyzed. In Study 2, previously collected 24-hr urine samples (n = 79) were analyzed to compare urinary excretions of LCS with self-reported LCSB consumption for identifying LCS exposure. Exposure to LCS was characterized using descriptive statistics and Chi-square tests were performed to assess associations between age-groups and LCS excretion, and to assess the proportion of individuals identified as LCS consumers using biomarker data or reported LCSB consumption.

Results: A total of 341 adults (45% males) and 79 adults (39% males) were included in the final analysis of Studies 1 and 2 respectively. In Study 1, over 96% of samples contained at least one LCS and almost 60% contained three or more LCS. A greater proportion of younger adults (< 40 y) excreted three or more LCS than older adults (> 40 y) ($p < 0.001$). In Study 2, a much higher prevalence of LCS consumption was observed using biomarker data (92%) compared to reported LCSB consumption (6%) ($p < 0.001$).

Conclusions: This work indicates widespread exposure to LCS suggesting that population-based research to date into LCS exposure and health may be flawed. Therefore, a urinary biomarker approach offers considerable potential for more robust investigations in this area.

Keywords: low-calorie sweeteners; non-nutritive sweeteners; biomarkers; acesulfame-K, cyclamates; saccharin; steviol glycosides; steviol glucuronide; sucralose.

INTRODUCTION

Consumption of LCS, which provide a sweet taste with little or no energy, is becoming more widespread across all age-groups in the Unites States (1). Indeed, this is likely to increase further with ongoing international efforts to limit free sugar consumption to 5% (2) or 10% (3) of total energy intake. Although the safety of LCS is established prior to regulatory approval, and current intakes are generally within acceptable limits (4), debate continues around LCS and health. To date, experimental research has tended to yield favorable results whilst observational research has produced a more mixed picture (5).

Most cohort studies investigating LCS in the context of health use low-calorie sweetened beverages (LCSB) intake as a surrogate marker of overall LCS exposure. Such an approach is unlikely to adequately capture LCS exposure given that LCSB are only one of many sources of LCS. Furthermore, LCS may be used at different concentrations within various LCSB and they are often used in combinations within the same product. Therefore, inadequate exposure assessments may be an important contributor to the observed variation in observational data, as has been highlighted elsewhere (6, 7). Most cohort studies also fail to discern intakes of specific LCS despite the different biological fates of the various LCS following ingestion (8) and the potential differential effects within the body (9). As such, alternative approaches, whereby LCS consumption is reliably identified and more effectively characterized, will significantly enhance investigations of the potential health impacts of LCS use.

Biomarker approaches for assessing dietary intakes provide an opportunity to obtain objective intake data (10), thereby facilitating more reliable investigation of diet and health. Five commonly used LCS, namely acesulfame-K, saccharin, cyclamate, sucralose and steviol glycosides, are excreted to varying degrees via the urine following ingestion and a novel liquid

chromatography tandem-mass spectrometry (LC-ESI MS/MS) method of simultaneously determining urinary excretions of these LCS has been developed and validated (11).

Using this novel methodology, the present work aimed to assess exposure to these five LCS in a free-living adult population and to compare biomarker data with a commonly used surrogate for LCS intake (namely LCSB intake). The main outcome measures were prevalence of exposure, identification of specific LCS being excreted and total excretion of these LCS. It was hypothesized that actual prevalence of exposure to LCS (from urinary biomarker data) would be higher than that reported elsewhere in published exposure assessments as well as observed using LCSB intake data.

METHODS AND PARTICIPANTS

Two separate cross-sectional studies were conducted in adults to address the research questions. To assess LCS intake in a free-living adult population, urine samples previously collected as part of a salt and iodine excretion study in the Netherlands in 2010 (12) were analyzed. To compare biomarker data with self-reported intakes of LCSB for identifying LCS consumption, 24-hr urine samples previously collected as part of a European hydration study (13) were analyzed.

Assessment of LCS exposure in a free-living adult population (Study 1)

24-hr urine samples (n = 357) were collected from free-living adults (aged 19-70 y) as part of a study investigating intakes of salt and iodine (12). In brief, participants collected a single 24-hr urine sample after receiving comprehensive written and verbal instructions: the first void of the morning was discarded and all urine for the subsequent 24-hr period up to and including the first sample of the following day was collected. The completeness of the sample was verified by total creatinine excretion along with verbal confirmation from participants. Renal impairment and

pregnancy were exclusion criteria for the study. Data on general lifestyle factors including level of education and smoking status were also available and used in this analysis. The study was approved by the Medical Ethics Committee of the University Medical Centre Utrecht.

Comparison of LCSB intake with LCS biomarker data for identifying LCS consumers (Study 2)

To compare urinary biomarker data with self-reported LCSB intake data for identifying LCS consumers, a study was conducted to analyze a randomly selected sub-sample of 24-hr urine samples (n = 79) that were previously collected as part of a multi-center European hydration study for which detailed information on the study protocol is reported elsewhere (13). In brief, participants collected urine samples over 7 consecutive 24-hr periods while maintaining a 7-day food diary in which all foods and beverages consumed during that period were recorded. The completeness of the 24-hr collections were determined by total creatinine excretion. For the present work, a single 24-hr urine sample, along with reported intake of LCSB related to the day of urine sampling, were considered for analysis. Participants were categorized as consumers or non-consumers of LCS based on reported consumption of LCSB on the day of the urine collection. However, intakes of specific LCS were not determined using reported LCSB consumption as concentration data for LCS are not available from dietary analysis software packages or food composition databases. The study was approved by the Agricultural University of Athens Research Ethics Committee (Study No. 197/27-02-2012).

Urine sample analysis

All urine samples were stored at -80 ^{0}C until analysis. Samples were analyzed in duplicate using a novel LC-ESI MS/MS methodology to simultaneously determine urinary concentrations of

acesulfame-k, saccharin, cyclamate, sucralose and the excretory metabolite of steviol glycosides, steviol glucuronide as detailed elsewhere (11). Intra-batch and inter-batch % coefficients of variation were below 10% for all compounds of interest.

Prevalence of exposure to LCS and estimated intakes using urinary excretions

An individual was identified as a consumer of LCS if urinary excretions were detected. Total 24-hr urinary excretions of the LCS were used in conjunction with published pharmacokinetic data (14-22) to estimate recent intake of the respective LCS.

To allow comparisons between intakes and acceptable daily intakes (ADI), it is necessary to express intakes in relation to body weight i.e. mg/kg body weight. However, information on body weight was not collected as part of the salt and iodine excretion study (12). Therefore, data from the nationally representative Dutch National Food Consumption Survey (DNFCS) 2007-2010 (23), which reported sex and age-group specific mean self-reported body weight, were used to provide an estimate of exposure within this population. Uncertainties exist when applying such assumptions in relation to body weight; however, it was nevertheless deemed worthwhile to express estimated intake data in this way to aid interpretation of the results. The ADI for steviol glycosides is expressed as steviol equivalents; therefore, steviol glucuronide values were converted to steviol equivalents applying a factor of 0.643 (calculated based on the ratio of the respective molecular weights, namely 317/493) to allow for comparison.

Using these data, exposure was estimated and expressed as % of the ADI using the following equation:

$$[(\text{Total excretion} \div \% \text{ absorption}) / (\text{Body weight (kg)})] \div \text{ADI} \times 100$$

Statistical analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) (Version 25.0, Chersey, UK). The distribution of continuous variables was assessed using the Shapiro-Wilk test; for data that were not normally distributed, non-parametric tests were utilized. For both Study 1 and Study 2, descriptive statistics were used to assess the general characteristics of the study populations. In Study 1, Chi-square test was used to assess level of education in males and females whilst mean differences in age, urine volume and creatinine excretion between males and females were assessed using independent samples t-test. The main outcome measures related to LCS exposure were prevalence of LCS excretion, the number of LCS excreted, as well as identification of specific LCS in the urine. Associations between the number of LCS detected and age, sex and level of education were assessed using Chi-square test. To assess the relationship between age and exposure to multiple LCS, the cohort was collapsed into three age-groups, defined as: 18-39 y (n = 119), 40-55 y (n = 100) and ≥ 56 y (n = 122). Further analyses were conducted to investigate relationships between sex and age in terms of absolute LCS excretions. LCS excretions and estimated intakes in males and females were compared using Mann Whitney U test. The relationship between age and absolute excretions of each LCS was explored using simple linear regression analysis. In Study 2, Chi-square test was used to assess whether the proportion of those identified as LCS consumers differed between the biomarker data and the surrogate measure i.e. reported LCSB intake. A P value of <0.05 was considered as statistically significant throughout.

RESULTS

Assessment of LCS exposure in a free-living adult population (Study 1)

A total of 357 participants submitted a urine collection in the salt and iodine excretion study. From these, 16 participants were excluded prior to statistical analysis owing to an unknown urine

sample volume (n = 1) or incomplete or incorrect urine collections (n = 15) resulting in a study population of n = 341 (154 males, 187 females) (see Supplemental Fig. 1). The general characteristics of the study participants are presented in **Table 1**. Males and females did not differ in terms of age, level of education or mean volume of urine samples; however, as expected, males excreted more creatinine than females ($P < 0.0001$) (Table 1).

A total of 96% of urine samples (n = 328) contained at least one LCS. The number of LCS detected was not associated with sex ($P = 0.87$) or level of education ($P = 0.51$).

The prevalence of exposure to multiple LCS was high with approximately 60% of urine samples containing three or more LCS. A significant association between age-groups and exposure to multiple LCS was observed with a higher proportion (74%) of those aged 39 y or younger excreting 3 or more LCS than those aged 40-55 y (60%) or 56 y and older (46%) ($P < 0.001$). When stratified by sex, a higher proportion of younger males (76%) exposed to multiple LCS as compared to their older counterparts (40-55 y, 63%; 56 y and older, 36%) ($P < 0.0001$). No significant trend was observed for females (39 y and younger, 70%; 40-55 y, 56%; 56 y and older, 55%) ($P = 0.14$).

Absolute urinary excretions ranged from 0-200 mg/d for acesulfame-K, 0-51 mg/d for saccharin, 0-141 mg/d for cyclamate, 0-2 mg/d for sucralose and 0-15 mg/d for steviol glucuronide, equating to 0-10 mg/d in steviol equivalents (for median, IQR and 95th percentile data, see **Table 2**). No differences were observed in total excretion of acesulfame-K, saccharin, cyclamate, sucralose or steviol between male and female consumers (Table 2). Indeed, age, level of education and sex were not associated with total excretion of acesulfame-K, saccharin, cyclamate and steviol glucuronide; however, age was a significant, albeit weak, predictor of sucralose excretion ($r^2 = 0.07$, $P = 0.037$).

Estimated intakes of the LCS were within the stated limits with respect to the European Union ADI (as summarized in **Table 3**). No significant differences were observed in estimated intakes in relation to ADI between males and females for acesulfame-K, saccharin, sucralose and steviol; however, for cyclamate, females consumed a higher % of the ADI than males ($P = 0.017$).

Comparison of LCSB intake with LCS biomarker data for identifying LCS consumers (Study 2)

In Study 2, 79 participants (31 males, 48 females) with a mean age of 36.7 ± 13.7 y were included in the analysis. BMI of the sample was 24.5 ± 4.0 kg/m^2 and the mean volume of the 24-hr urine sample was 1363 ± 549 mL/d. A total of 6% ($n = 5$) reported consumption of LCSB on the day of the urine collection. However, a significantly greater proportion of individuals (92%, $n = 73$) were identified as LCS consumers when urinary biomarker data were considered ($P < 0.001$) (**Figure 1**). The most commonly detected LCS were saccharin (82%, $n = 65$), acesulfame-k (51%, $n = 40$) and cyclamates (34%, $n = 27$). In relation to sucralose and steviol glycosides, 30% ($n = 24$) and 11% ($n = 9$) of participants excreted these LCS respectively. Again, prevalence of exposure to multiple LCS was high with 62% of participants excreting two or more LCS and 42% excreting three or more LCS. To account for the potential contribution of non-dietary sources of saccharin, non-saccharin urinary excretion was also considered; when saccharin excretion was excluded 63% ($n = 50$) were still identified as LCS consumers, a percentage which was still significantly more than that identified via the self-reported data ($P < 0.001$).

DISCUSSION

Building upon existing evidence (7), the present work has utilized a novel biomarker approach to demonstrate for the first time that exposure to LCS is much more widespread than previously

reported. Such findings, which support the use of alternative methodologies for exposure assessments, and promote more robust measurement of LCS exposure, will have potentially important implications for research in this area. A further significant and novel finding, considering recently published work that suggests differential effects of specific LCS in relation to body weight (9), is that exposure to multiple LCS is also highly prevalent.

Study 1 was conducted to investigate how widespread actual exposure to LCS might be based on urinary excretions compared to published exposure data, which relies on self-reported dietary intake data. Furthermore, by analyzing urinary excretions, exposure to the specific LCS of interest can be established. Study 1 found that, within a Dutch adult population, 96% of urine samples contained at least one LCS, which is well in excess of the 59% of the Dutch population aged 7 y and older reported to be consuming "artificially sweetened" products in the DNFCS at that time (23). This may be partly explained by the detection of saccharin, which is commonly used in oral hygiene products in Europe, in approximately 90% of urine samples. Interestingly, most formal exposure assessments of LCS do not consider non-dietary sources of LCS such as oral hygiene products, e-cigarette products and supplements (24-30), potentially resulting in underestimation of exposure within the population. This may have significant implications for research in the area of LCS and health given that the potential differential effects of individual LCS has increasingly gained attention in recent times (9, 31). Indeed, a recent RCT found that a saccharin-sweetened beverage, unlike other LCSB, had similar effects as a sucrose-sweetened beverage on body weight and therefore research that discerns intakes of individual LCS seems warranted (9). Of the other LCS investigated, acesulfame-K and cyclamate were the most commonly detected, found in 74% and 68% of samples respectively, which was still higher than that reported in the DNFCS (23). A lower prevalence of exposure has been reported elsewhere,

even in younger populations, which are often considered to have potentially high intakes (25, 28, 29). Previous research suggests that individuals aged 45 y or older are more likely to use LCS (32); however, our work suggests that younger adults (39 y and younger) excreted a higher number of LCS, albeit in similar quantities to those aged over 39 y. Further work should investigate this finding in nationally representative sample. Intakes of LCS in US children and adults were recently assessed using data from the National Health and Nutrition Examination Survey and it was found that the prevalence of LCS consumption increased from 6.1 to 12.5% for children and 18.7 to 24.1% for adults (1). Although this prevalence is much lower than the findings of the present study, it suggests an upward trend in LCS use, which is not surprising given the increasingly widespread application of LCS and global efforts to reduce free sugar consumption. It is feasible that, given the ubiquity of LCS in today's market, even though the use of LCS in products must be clearly labelled (33), inadvertent consumption of LCS may be occurring. The French Agency for Food, Environmental and Occupational Health and Safety (ANSES) (34) suggested the use of better designed questionnaires in future cohort studies so that intakes of LCS, individually and in combinations, could be better understood; a urinary biomarker approach potentially addresses this important research need.

Whilst the current work reliably indicates that exposure to LCS is more widespread than previously reported, it was important to also attempt to quantitate intakes using the excretion data to aid further interpretation. Owing to the lack of body weight data in Study 1, assumptions were made based on published age- and gender-specific mean body weight for a Dutch population from this time (23). Published pharmacokinetic data (14-22) were then used to generate estimated intakes and compare it to assigned ADIs. Given that the application of a biomarker approach to assess LCS intake is relatively novel, there is currently an acknowledged

inherent uncertainty in using such an approach to estimate intake. However, this approach makes it possible to provide estimated data on intakes that is more informative than the current reliance on surrogate measures of LCS intake such as self-reported LCSB consumption. Estimated intakes in the present work are within the respective European ADIs and agree with the findings of a recent comprehensive review of global intakes (4). Intake data relating to the Dutch population have been presented in a few studies (35, 36). Van Rooij-van den Bos et al. (35) reported average intakes of <0.5%, 1.0% and 0.4% of the ADI for acesulfame-K, cyclamate and saccharin respectively and our estimates yielded similar results (0.7%, 0.6% and 0.2% of the ADI). Hendriksen et al. (36) implemented a "worst-case" scenario to generate estimates for young males and females who participated in the DNFCS 2007-2010 with high intakes (defined as 95th percentile) in relation to the ADI being 29% and 27% for acesulfame-K, 37% and 29% for cyclamate and 4% and 3% ADI for saccharin respectively. The present study estimated that high intakes (also defined as 95^{th} percentile) were much lower for acesulfame-k (males, 9%; females 11%) and similar for cyclamates (males, 16%; females 27%) and saccharin (males, 5%; females, 7%). It should be noted that LCS exposure studies often focus on groups expected to have high intakes such as children/teenagers (25, 27, 29, 30) or those living with diabetes mellitus (26, 28), and therefore, utilizing a urinary biomarker approach to investigate these populations may yield different results. In 2010 sucralose was a relatively new LCS on the EU market, having received a favorable opinion from the Scientific Committee on Food (SCF) in 2000 (37), and it evidently was not as commonly consumed in this population as acesulfame-K, saccharin and cyclamate at this time. However, a study of LCS exposure in the Belgian population (38) covering approximately the same period as the present study calculated median exposure of 0.39 mg/kg body weight, equating to 3% of the ADI which is significantly higher

than our estimate of 0.5% of the ADI. An interesting finding, which would support integrating biomonitoring within formal exposure assessments to ensure comprehensive and accurate data, was that steviol glucuronide was detected in approximately 6% of the urine samples indicating exposure to steviol glycosides prior to its approval for use in the EU in 2011 (39, 40) (the urine samples from the present study were collected a year before EU approval of steviol glycosides). Our data indicate that median intake in consumers equated to 0.2% of the ADI, which was similar to that reported by Chung et al., (41) in a Korean population and lower than the more recent study conducted by Ha et al. (42) who reported a mean intake of 6.5%.

Study 2, which compared self-reported intake data on LCSB and biomarker data, also suggests higher prevalence of exposure within the free-living population than previously reported. Importantly, it suggests that using LCSB as a surrogate for overall LCS intake may not effectively identify LCS consumers and therefore, alternative, more comprehensive methods of investigating intakes are required for population-based studies to assess the health impacts of LCS use more effectively. Furthermore, the present study demonstrates that exposure to multiple LCS is common and, given the chemical diversity of LCS, obtaining qualitative data in relation to exposure to specific LCS is essential for the reliable assessment of the benefits and risks of LCS.

The strengths and limitations of the present work should be discussed. A significant strength of the current work is that a highly specific and sensitive methodology has been utilized to identify and characterize exposure to LCS by determining urinary excretions of the respective LCS. Whilst the excretion metabolites of these LCS are highly specific to the respective LCS, as previously alluded to, further work is needed to fully characterize the relationship between ingestion and urinary excretion, thereby facilitating extended validation of this biomarker

approach. Parameters of interest include the investigation of variation between individuals as well as within individuals under different consumption conditions and in specific population groups of interest; for example, children or individuals living with obesity or diabetes. Utilizing such a biomarker approach can overcome several important limitations of self-reported intake data by generating objective measures of intake; however, a recognized limitation of this approach is that it cannot identify exposure to all approved LCS or the source of LCS within the diet. Aspartame, which is a commonly used LCS in the US, is metabolized to its constituent parts, phenylalanine, aspartic acid and methanol following ingestion (8). Given that all three constituents of aspartame are commonly found elsewhere in the diet, no specific biomarkers for determining exposure exist. Therefore, to gain a comprehensive picture of LCS exposure, a combination of a biomarker approach and self-reported intake data may yield the most useful data in future research. For Study 2, a relatively small sample was investigated, and LCSB intake data were specific only to the day of urine sample collection, thereby likely limiting the number of LCS consumers identified. Analysis of dietary intake data covering a longer duration may have identified more LCS consumers but is unlikely to have reversed the finding. The completeness of the 24-hr urine collections was assessed in both studies based on the total excretion of creatinine which may not be a reliable measure of compliance (43, 44); the gold standard being the use of para-aminobenzoic acid (45). It has been suggested that future research should attempt to discern the intakes of individual LCS so that the health impact of consumption, both individually and in combinations, can be investigated (34). A major strength of utilizing a biomarker approach, is that exposure to specific LCS can be assessed, which significantly improves the overall exposure assessment both quantitatively and qualitatively. If the findings of

Study 2 are replicated in larger populations, doubt must be cast on the findings of many cohort studies, which have generally used LCSB as a marker overall LCS consumption.

To conclude, a urinary biomarker approach has demonstrated that exposure to LCS is much more widespread in adults than previously reported. It is essential that more reliable and comprehensive methods of assessing LCS intakes are developed and utilized so that the potential health impacts can be assessed more effectively. The biomarker approach presented in the current work overcomes several important limitations with current research approaches by generating objective and more comprehensive data on exposure to five commonly used LCS. Therefore, future research should incorporate enhanced methodologies such as this in order to help assess LCS intake more reliably and comprehensively.

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316 designed the research project, C. L., M. K., A. A. and M. o'M. conducted the practical aspects of
317 the research (sample collection and sample analysis), M. K. and A. A. provided essential study
318 materials, C. L. analyzed the data, and, along with A. M. G., had primary responsibility for
319 writing the paper. C. L. is responsible for the final content of the paper. All authors have read
320 and approved the final version.

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TABLES

Table 1. General characteristics of adults who participated in the salt and iodine excretion study (Study 1)[1]

	Overall *n* = 341	Females *n* = 187	Males *n* = 154	*P* value[3]
Age (y)	46 ±15	46 ±14	47 ±15	0.43
Level of education[2]				0.10
Low	62 (18)	26 (14)	36 (23)	
Medium	174 (51)	104 (56)	70 (46)	
High	99 (29)	53 (28)	46 (30)	
Other	6 (2)	4 (2)	2 (1)	
Urine volume (mL/d)	2002 ±787	1973 ±721	2036 ±862	0.47
Creatinine (mmol/d)	12.0 ±4.0	9.8 ±2.2	14.7 ±4.0	**<0.001**

[1] Values are means ± SDs [for age, urine volume and creatinine] or n (%) [for level of education]

[2] Level of education defined as; Low, including primary school, lower vocational, low or intermediate general education; Medium, including intermediate vocational education and higher general education; High, including higher vocational education and university; Other, level of education not defined.

[3] Statistical analysis was carried out to investigate differences between males and females. Age, urine sample volume and creatinine excretion were assessed using Independent samples t-test; level of education was assessed using Chi square test. A *P* value of <0.05 was considered statistically significant.

Table 2. Urinary excretion of five low-calorie sweeteners in a free-living adult cohort (n = 341)[1]

	Number of consumers[2] (% of total sample)	Overall (mg/d)	Females (mg/d)	Males (mg/d)	*P*-value[3]
Acesulfame-K	252 (74)	5 (0-21) [61]	6 (0-23) [65]	4 (0-19) [59]	0.60
Saccharin	308 (90)	0 (0-4) [22]	1 (0-4) [23]	1 (0-4) [19]	0.64
Cyclamate	232 (68)	1 (0-11) [53]	3 (0-11) [57]	4 (0-10) [38]	0.05
Sucralose	67 (20)	0 (0-0) [1]	0 (0-0) [1]	0 (0-1) [2]	0.63
Steviol	20 (6)	0 (0-1) [14]	0 (0-1) [-]	0 (0-3) [-]	0.90

[1] Values are median (IQR) [95th percentile] or n (%). IQR, inter-quartile range. '-', not determined owing to insufficient participant data (i.e. $n < 5$).
[2] Percentage of all participants who, based on the biomarker approach, had consumed the given LCS.
[3] Statistical analysis was carried out to investigate differences between males and females. Urinary excretions of the compounds of interest were assessed with Mann-Whitney U Test. A *P* value of <0.05 was considered statistically significant.

Table 3. Estimated intakes of low-calorie sweeteners in relation to acceptable daily intake in free-living adult LCS cosumers[1]

	ADI (mg/kg)	Average absorption (%)[2]	Overall (% ADI)[3]	Females (% ADI)[3]	Males (% ADI)[3]
Acesulfame-K	0-9	90.0	0.73 (0.07-3.30) [9.14]	0.90 (0.08-3.83) [10.57]	0.47 (0.06-2.79) [8.77]
Saccharin	0-5	88.0	0.17 (0.05-1.21) [6.43]	0.18 (0.06-1.33) [7.23]	0.17 (0.04-0.98) [4.95]
Cyclamate	0-7	40.0	0.63 (0.02-5.07) [24.25]	1.34 (0.03-5.40) [27.11]	0.15 (0.01-4.29) [16.04]
Sucralose	0-15	14.5	0.12 (0.04-0.23) [0.80]	0.12 (0.06-0.19) [0.75]	0.14 (0.03-0.33) [1.12]
Steviol	0-4	60.0	0.08 (0.05-0.40) [4.45]	0.10 (0.05-0.44) [-]	0.07 (0.04-1.40) [-]

[1] Values are median (IQR) [95th percentile]. ADI, acceptable daily intake (expressed as mg/kg body weight per day); IQR, inter-quartile range; '-', not determined owing to insufficient participant data (i.e. $n < 5$).
[2] Average absorption based on published pharmacokinetic data (14 – 22).
[3] % ADI determined from average absorption, mean body weight (23) and total urinary excretion of the respective low-calorie sweetener.

LEGENDS FOR FIGURES

Figure 1. Comparison of self-reported LCSB consumption and biomarker data for identifying LCS consumption (n = 79). * Different from LCSB consumption, $P < 0.001$ as tested by Chi-square test. LCS, low-calorie sweeteners; LCSB, low-calorie sweetened beverages.