

Association between urinary biomarkers of total sugars and sucrose intake and BMI in a cross-sectional study

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1 Abstract

2 Obesity is an important modifiable risk factors for chronic diseases. While there is increasing
3 focus on the role of dietary sugars, there remains a paucity of data establishing the
4 association between sugar intake and obesity in the general public. The objective of this
5 study was to investigate associations of estimated sugar intake with odds for obesity in a
6 representative samples of English adults. We used data from 434 participants of the 2005
7 Health Survey of England. Biomarkers for total sugar intake were measured in 24h urine
8 samples and used to estimate intake. Linear and logistic regression analyses were used to
9 investigate associations between estimated intake and measures of obesity (BMI, waist
10 circumference and waist-to-hip ratio) and obesity risk., respectively. Estimated sugars intake
11 was significantly associated with BMI, waist circumference and waist-to-hip ratio, and these
12 associations remained significant after adjustment for estimated protein intake. Estimated
13 sugars intake was also associated with increased odds for obesity based on BMI (OR 1.02;
14 95% CI 1.00; 1.04 per 10 g), waist-circumference (OR 1.03; 95% CI 1.01; 1.05) and waist-to-
15 hip ratio (OR 1.04; 95% CI 1.02; 1.06); all OR estimates remained significant after adjusting
16 for estimated protein intake. Our results show a significant association between biomarker-
17 estimated total sugars intake and both measures of obesity and obesity risk, confirming
18 positive associations between total sugar intake, measures of obesity and obesity risk. This
19 biomarker could be used to monitor the efficacy of public health interventions.

20

1 Introduction

Dietary sugars, in particular free sugars (according to the WHO definition monosaccharides and disaccharides added to foods and beverages by the manufacturer, cook or consumer, and sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates" (1)) have received increasing attention from the WHO¹ as well as the UK government² and the UK's Scientific Advisory Committee on Nutrition (SACN)³. While sugar intake is often associated with an increased risk of obesity⁴, the evidence available from observational studies is more ambiguous and shows significant associations for sugar-sweetened beverages (SSB)^{5,6} only, but fails to show consistent associations for intake of sugars as nutrients⁶⁻⁹. However, in most observational studies, sugar intake was assessed using self-report. It is likely that this has introduced bias, especially as underreporting of diet has been found to be more prevalent among obese people¹⁰⁻¹² and it is sugar-rich foods that are most commonly underreported¹³. It is possible that reporting bias contributes to the observed inverse associations between sugar intake and BMI.

Urinary sugars have been investigated^{14,15} and validated^{16,17} as dietary biomarkers of total sugars (i.e., the sum of intrinsic, milk and free sugars) and sucrose¹⁸ and can help to resolve the discrepancy between self-reported and true intake. This biomarker relies on the total excretion of sucrose and fructose within 24h and therefore requires complete 24h urine samples. While we have been able to show a positive association between the biomarker measured in spot urines and BMI^{16,19}, the lack of validation data on the performance of sucrose and fructose as dietary biomarkers from spot urines weakens these results.

In this study, we have investigated the association between sugar intake and obesity risk using exclusively nutritional biomarkers and not relying on self-reported data. The results of this study will allow us to test the feasibility of applying this biomarker to an existing cohort as an instrument to monitor consumption and to investigate associations between sugar intake and obesity.

47 2 Method

48 2.1 Study population

49 The Health Survey for England is a health examination survey of nationally-representative
50 samples of the general population. A new, random, household-based sample has been
51 selected annually since 1991. Individuals living at the selected private addresses are
52 recruited to the study, answer a questionnaire through face-to-face interview, and have
53 trained interviewers measure height and weight. Nurses take other physical measurements
54 and collect biological samples²⁰. The measurement of height, weight (interviewer), and waist
55 and hip circumference (nurse) followed the protocols of the 2003 Health Survey for England
56²¹. No data on diet, except for fruit and vegetable intake, were collected by interview.

57 We used data of participants from the 2005 Health Survey for England (HSE 2005) with
58 the aim of obtaining a nationally-representative sample of the general population aged 19 to
59 64 years living in England. As a supplement to the main HSE 2005, a sub-sample of adult
60 participants were asked to provide a 24-hour urine sample. Overall, 498 survey participants
61 (200 men, 298 women, Table 1), aged 19 and over, who provided a 24-hour urine sample
62 were identified and included in the study. Data collection took place between October 2005
63 and July 2006, with the majority of fieldwork being completed by March 2006. If more than
64 one 24-h urine sample was available for one participant, the first sample was used.

65 2.2 24-hour urine collection

66 Participants were asked to collect all urine they passed during a 24-hour period starting from
67 the second morning urine void of the 24-hour collection day, and ending with the first urine
68 void the following morning. P-amino-benzoic acid (PABA) was used to test for completeness
69 of 24h urine collection and only complete samples (with >85% PABA recovery in urine) were
70 used for this analysis²². All samples were stored at -20°C until analysis.

71 2.3 Analysis of urinary sucrose and fructose

72 Urine samples were thawed at room temperature, centrifuged to remove protein aggregates
 73 and analysed using an ILAB600 clinical chemistry analyser (Werfen (UK) Limited,
 74 Warrington) with a sucrose, fructose and glucose enzyme kit
 75 (Sucrose/D-Glucose/D-Fructose; Boehringer Mannheim, R-Biopharm, Enzymatic
 76 BioAnalysis/Food Analysis, Darmstadt, Germany). This method determines D-glucose by
 77 measuring NADPH + H⁺ formation following phosphorylation of D-glucose by hexokinase and
 78 subsequent oxidation by NADPH⁺-dependent glucose-6-phosphate dehydrogenase. NADPH
 79 + H⁺ is determined by changes in absorption at 340 nm. Sucrose and D-fructose are
 80 determined indirectly following the conversion of D-fructose into D-glucose by
 81 phosphoglucose-isomerase or β -fructosidase and calculating the difference in D-glucose
 82 concentration before and after conversion. The concentration range for sucrose and
 83 D-fructose was 2.5 to 200 mg/L, for D-glucose it was 2.5 to 150 mg/L; samples exceeding
 84 these concentrations were diluted 1 in 10 with purified water and reanalysed. The intra-assay
 85 CV for a 25 mg/L glucose quality control (QC) sample was less than 2% and the inter-assay
 86 CV was 3.6%. The inter-assay CV was also determined for fructose and sucrose and found
 87 to be less than 7%. All concentrations measured were above the lower-limit of quantification.
 88 24-h urinary sucrose and fructose were calculated based on urinary fructose and sucrose
 89 concentration (mg/L) and 24-h urine volume.

90 2.4 Analysis of urinary nitrogen

91 We measured 24-h urinary nitrogen, a recovery biomarker for protein intake, to partialy
 92 control for non-sugars energy intake. Urine samples were thawed at room temperature prior
 93 to analysis. Approximately 1 ml of samples was weighed into a tin foil capsule. For Total
 94 Nitrogen (N %) determination, the sample was combusted in oxygen and the nitrogen
 95 released measured with a thermal conductivity cell using a LECO FP-428 Analyser (LECO
 96 Corp., St. Joseph, MI). The coefficient of variation for within-run and within-laboratory

precision was 1.77 and 3.80 %, respectively for an internal quality control sample containing 1 % N. The limit of quantification for the test was 0.018 % N.

2.5 Biomarker-based estimates of total sugars and protein intake

Estimated total sugars intake was calculated based on a calibration equation for the sugars biomarker developed from a feeding study conducted in the UK (16), which describes the association between the biomarker and true intake¹⁷

$$CM_{i,j} = M_{i,j} - 1.67 - 0.02 \times S_i + 0.71 \times A_i$$

where CM is log transformed calibrated biomarker of person i at time point j, i.e. predicted total sugars intake, M is log transformed sum of 24-hour urine fructose and sucrose, S is sex (male: S=0, female: S=1) and A is log transformed age. Estimated protein intake was calculated based on the assumptions that 81% of dietary nitrogen is recovered from urine²³ and an average nitrogen content of proteins is 16% [P: protein intake (g/d), N: total nitrogen excretion (g/d)]:

$$P = N/0.81 \times 6.25$$

2.6 Data handling

Calculated fructose and sucrose concentrations of zero were assigned a value of 0.1 to allow for a log₂-transformation of the data.

2.7 Statistical analyses

All data were processed using R²⁴ version 3.3.2. Urinary fructose, urinary sucrose and the sum of 24-hour urinary fructose and sucrose were skewed to the right and log₂-transformed, whereas biomarker estimates of total sugars and protein intakes were used without transformation. The ratio of urinary sugars and estimated sugar intake, to urinary nitrogen and estimated protein intake, respectively, were log₂-transformed. We used the ratios of estimated total sugars to protein intake or urinary sugars to urinary nitrogen to investigate the effect of sugars while controlling for dietary composition. Unadjusted models were used

121 when investigating associations between estimated total sugars intake and BMI and obesity
 122 risk (based on WHO definition either as BMI ≥ 30 kg/m² or waist-to-hip ratio > 0.85 for
 123 women and > 0.90 for men), given the calibration equation for the sugars biomarker which
 124 we used to estimate total sugars intake included age and sex. Models with uncalibrated
 125 urinary fructose, uncalibrated urinary sucrose or estimated protein intake and BMI and
 126 obesity risk were adjusted for age and sex. Associations with BMI were investigated using
 127 linear regression models; OR for obesity (as estimate of risk) was estimated using logistic
 128 regression. Urinary nitrogen or estimated protein intake was included in the models to control
 129 for protein intake as a contributor to energy intake. P<0.05 was used as threshold for
 130 statistical significance.

131 3 Results

132 3.1 Study population

133 Study population characteristics and description of the analytical sample are shown in Table
 134 1. Complete data on age, sex, BMI, waist-to-hip-ratio and 24h urine volume were available
 135 for 298 women and 200 men (n=498). Due to missing samples or insufficient volume, not all
 136 samples could be analysed for urinary biomarkers; data on urinary sugars and nitrogen are
 137 available for 261 women and 173 men only (n=434).

138 Table 1: Study population characteristics and description of analytical sample. Median and inter-quartile range or absolute number and proportion.

	Women				Men			
n	298	285	261	247	200	189	173	165
Available data								
Age, Sex, BMI, hip-to-waist ratio and urine volume	X	X	X	X	X	X	X	X
Urinary sugars		X	X	X		X	X	X
Urinary nitrogen			X	X			X	X
Excluding extremes [†]				X				X
Age [years]	45 (34 – 56)	45 (36 – 54)	45 (36 – 55)	44 (36 – 55)	48 (37 – 56)	48 (37 – 56)	48 (37 – 56)	48 (36 – 55)
Waist circumference [cm]	85.6 (78.7 – 94.5)	85.6 (78.9 – 94.5)	85.6 (78.5 – 94.6)	85.6 (78.8 – 94.5)	98.4 (90.9 – 108)	98.4 (91.4 – 108)	97.9 (90.4 – 107)	97.7 (90.4 – 107)
Waist-to-hip ratio	0.82 (0.77 – 0.86)	0.82 (0.77 – 0.86)	0.81 (0.77 – 0.86)	0.81 (0.77 – 0.86)	0.93 (0.89 – 0.98)	0.93 (0.89 – 0.98)	0.93 (0.89 – 0.97)	0.93 (0.89 – 0.98)
BMI [kg/m ²]	25.6 (23.1 – 29.4)	25.5 (23.0 – 29.5)	26.0 (23.5 – 29.8)	26.0 (23.5 – 29.7)	27.2 (24.5 – 30.2)	27.2 (24.5 – 30.1)	27.5 (25.3 – 30.4)	27.3 (25.2 – 30.1)
Normal weight	131 (44%)	125 (44%)	108 (41%)	101 (41%)	56 (28%)	52 (28%)	33 (22%)	38 (23%)
Overweight	103 (35%)	99 (35%)	93 (36%)	91 (37%)	90 (45%)	87 (46%)	86 (50%)	84 (51%)
Obese	64 (22%)	61 (21%)	60 (23%)	55 (22%)	54 (27%)	50 (27%)	49 (28%)	43 (26%)
Urinary excretion								
Sucrose [mg/d]	—	26.0 (12.0 – 50.3)	26.4 (11.6 – 50.6)	25.1 (10.7 – 46.1)	—	38.6 (23.8 – 62.7)	38.6 (23.9 – 62.6)	37.2 (23.0 – 59.7)
Fructose [mg/d]	—	17.7 (9.3 – 32.5)	18.1 (9.4 – 33.3)	17.5 (9.2 – 29.8)	—	17.7 (9.3 – 32.5)	18.4 (11.7 – 27.1)	18.1 (11.1 – 26.3)
Nitrogen [g/d]	—	—	10.3 (8.0 – 12.3)	10.4 (8.0 – 12.3)	—	—	13.3 (10.4 – 16.4)	13.3 (10.5 – 16.4)
Estimated intake								
Total Sugars [g/d]	—	122 (66.1 – 216)	127 (66.1 – 219)	117 (62.0 – 201)	—	168 (91.3 – 247)	167 (93.4 – 247)	162 (91 – 227)
Protein [g/d]	—	—	79.4 (61.8 – 94.8)	80.0 (62.0 – 94.7)	—	—	102 (80.4 – 127)	102 (80.6 – 127)

139

140 [†]excluding the top 5% of estimated total sugar intake

The distribution of estimated dietary sugar intake (median 144 g/d, range 0 – 2777 g/d) was skewed right with some extremely high values. We have therefore truncated the data at the 95th centile of estimated intake (527 g/d). The remaining sample included 247 women and 165 men (n=411). Participants in the top 5th centile (14 women, 8 men) were older (mean age 50.6 years vs 44.8 years, *t*-test: *p*=0.024) and had a higher excretion of sucrose (247 mg/d vs 36.4 mg/d, *t*-test: *p*<0.001) and fructose (84.9 mg/d vs 22.3 mg/d, *t*-test: *p*<0.001) than those in the remaining sample. There were however no statistically significant differences in BMI, waist circumference, waist-to-hip ratio or protein intake.

3.2 Associations between biomarker excretion, measures of obesity and obesity risk

Associations between urinary fructose, urinary sucrose, the sum of 24-hour urinary fructose and sucrose, and 24-hour urinary nitrogen and measures of obesity (BMI, waist circumference and waist-to-hip ratio), adjusted for age and sex, are shown in Table 2. We found a significant positive association for 24h urinary sucrose with all measures of obesity; these associations were strengthened when including 24h urinary fructose and 24h urinary nitrogen in the model. Total urinary sugars were significantly associated only with waist circumference and waist-to-hip ratio, although the former association became non-significant after adjusting for urinary nitrogen. There were no associations between any marker and total urinary fructose, whereas total urinary nitrogen was significantly associated with BMI and waist circumference, but not waist-to-hip ratio.

Table 2: Associations between 24h excretion of sucrose, fructose and nitrogen and BMI, waist-circumference and waist-to-hip-ratio (β and 95% CI). Data were log₂-transformed and models are adjusted for age and sex. Estimates in each column represent a separate model.

Regression coefficient (β and 95% CI per doubling of excretion)									
BMI [kg/m ²]									
Sum of 24-h urinary sucrose and fructose [mg/d]	0.201 (-0.099; 0.501)	—	—	—	—	0.171 (-0.129; 0.471)	—	—	—
24-h urinary sucrose [mg/d]	—	0.243 (0.029; 0.458) [†]	—	0.309 (0.073; 0.545) [†]	—	—	0.236 (0.023; 0.450) [†]	—	0.318 (0.083; 0.553) ^{††}
24-h urinary fructose [mg/d]	—	—	-0.030 (-0.258; 0.199)	-0.166 (-0.416; 0.083)	—	—	—	-0.067 (-0.296; 0.163)	-0.209 (-0.460; 0.042)
24-h urinary Nitrogen [g/d]	—	—	—	—	0.960 (0.077; 1.842) [†]	0.910 (0.024; 1.797) [†]	0.931 (0.052; 1.810) [†]	0.997 (0.105; 1.890) [†]	1.038 (0.151; 1.925) [†]
Waist circumference [cm]									
Sum of 24-h urinary sucrose and fructose [mg/d]	0.754 (0.022; 1.486) [†]	—	—	—	—	0.666 (-0.066; 1.397)	—	—	—
24-h urinary sucrose [mg/d]	—	0.782 (0.259; 1.305) [†] †	—	0.946 (0.370; 1.521) ^{††}	—	—	0.762 (0.242; 1.282) ^{††}	—	0.973 (0.402; 1.544) ^{†††}
24-h urinary fructose [mg/d]	—	—	0.004 (-0.556; 0.563)	-0.415 (-1.024; 0.194)	—	—	—	-0.105 (-0.666; 0.456)	-0.540 (-1.150; 0.070)
24-h urinary Nitrogen [g/d]	—	—	—	—	2.857 (0.702; 5.012) [†] †	2.665 (0.506; 4.825) [†]	2.764 (0.626; 4.901) ^{††}	2.916 (0.736; 5.096) ^{††}	3.041 (0.887; 5.196) ^{††}
Waist-to-hip ratio [x 100]									
Sum of 24-h urinary sucrose and fructose [mg/d]	0.474 (0.081; 0.867) [†]	—	—	—	—	0.458 (0.063; 0.853) ^{††}	—	—	—
24-h urinary sucrose [mg/d]	—	0.415 (0.133; 0.696) ^{††}	—	0.496 (0.186; 0.805) ^{††}	—	—	0.411 (0.129; 0.692) ^{††}	—	0.502 (0.192; 0.812) ^{††}

24-h urinary fructose [mg/d]	—	—	0.015 (-0.286; 0.316)	-0.204 (-0.532; 0.123)	—	—	—	-0.008 (-0.312; 0.296)	-0.233 (-0.564; 0.098)
24-h urinary Nitrogen [g/d]	—	—	—	—	0.627 (-0.540; 1.794)	0.495 (-0.672; 1.662)	0.577 (-0.581; 1.734)	0.632 (-0.549; 1.813)	0.697 (-0.472; 1.865)

[†]p<0.05; ^{††}p<0.01' ^{††}p<0.001

1 Total urinary sucrose was positively associated with obesity risk when using waist
2 circumference as the obesity marker, and this association became stronger when adjusting
3 for 24h urinary fructose, and for both 24h urinary fructose and 24h urinary nitrogen (Table 3).
4 Total urinary sucrose was also positively associated with obesity risk when using waist-to-
5 hip-ratio as obesity marker, but only after adjustment for urinary fructose and urinary fructose
6 and nitrogen. We found no statistically significant increase in obesity risk when using BMI as
7 the obesity marker.

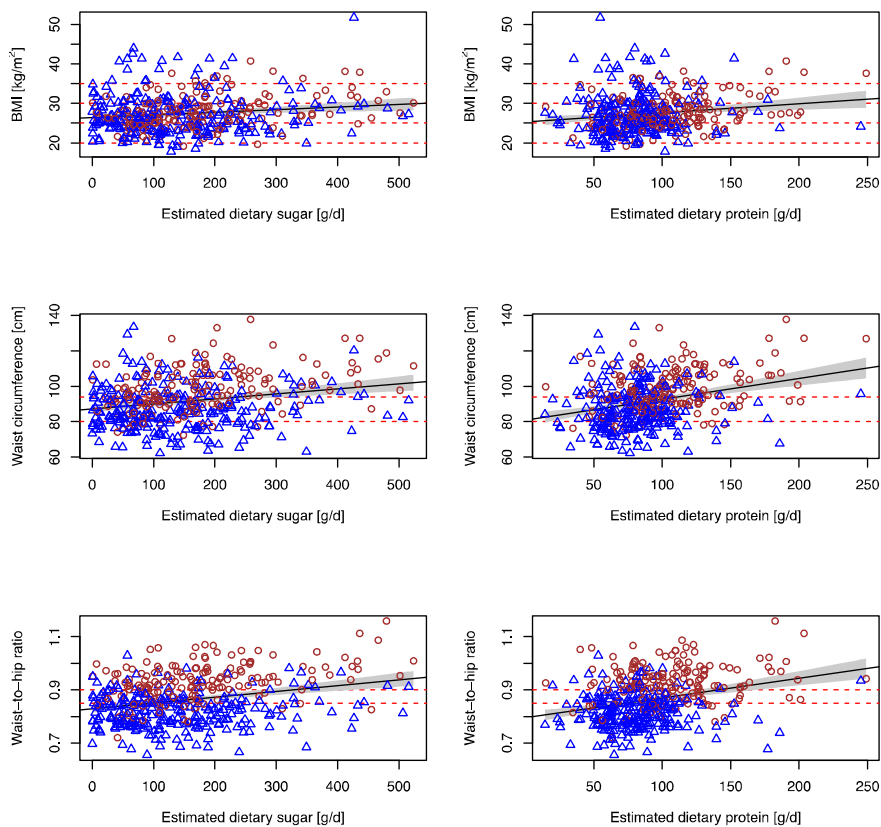
Table 3: Associations between 24h urinary excretion of sucrose, fructose and nitrogen and odds for obesity (OR and 95% CI). Data were log₂-transformed and models are adjusted for age and sex. Estimates in each column represent a separate model.

Obesity risk (OR and 95% CI – per doubling of excretion)									
BMI ≥ 30 kg/m ²									
Sum of 24-h urinary sucrose and fructose [mg/d]	1.04 (0.90; 1.22)	—	—	—	—	1.03 (0.89; 1.21)	—	—	—
24-h urinary sucrose [mg/d]	—	1.08 (0.97; 1.22)	—	1.12 (0.98; 1.27)	—	—	1.08 (0.97; 1.22)	—	1.12 (0.99; 1.28)
24-h urinary fructose [mg/d]	—	—	0.99 (0.89; 1.10)	0.94 (0.84; 1.07)	—	—	—	0.97 (0.87; 1.09)	0.93 (0.82; 1.05)
24-h urinary Nitrogen [g/d]	—	—	—	—	1.53 (0.97; 2.48)	1.52 (0.96; 2.46)	1.53 (0.97; 2.48)	1.56 (0.99; 2.53)	1.60 (1.00; 2.61)
Waist circumference > 85 cm (women) or 94 cm (men)									
Sum of 24-h urinary sucrose and fructose [mg/d]	1.04 (0.90; 1.22)	—	—	—	—	1.13 (0.99; 1.30)	—	—	—
24-h urinary sucrose [mg/d]	—	1.12 (1.02; 1.24) [†]	—	1.16 (1.04; 1.29) [†]	—	—	1.12 (1.02; 1.24) [†]	—	1.16 (1.04; 1.30) [†]
24-h urinary fructose [mg/d]	—	—	0.99 (0.89; 1.10)	0.93 (0.82; 1.04)	—	—	—	0.97 (0.87; 1.09)	0.91 (0.80; 1.03)
24-h urinary Nitrogen [g/d]	—	—	—	—	1.43 (0.97; 2.15)	1.38 (0.93; 2.07)	1.41 (0.95; 2.12)	1.46 (0.98; 2.19)	1.49 (0.99; 2.25)
Waist-to-hip ratio > 0.85 (women) or 0.90 (men)									
Sum of 24-h urinary sucrose and fructose [mg/d]	1.07 (0.93; 1.24)	—	—	—	—	1.07 (0.93; 1.24)	—	—	—
24-h urinary sucrose [mg/d]	—	1.11 (1.00; 1.24)	—	1.15 (1.03; 1.30) [†]	—	—	1.11 (1.00; 1.24)	—	1.16 (1.03; 1.31) [†]
24-h urinary fructose [mg/d]	—	—	0.97 (0.87; 1.08)	0.91 (0.80; 1.03)	—	—	—	0.97 (0.87; 1.08)	0.90 (0.80; 1.02)
24-h urinary Nitrogen [g/d]	—	—	—	—	1.09 (0.71; 1.68)	1.08 (0.70; 1.66)	1.09 (0.71; 1.67)	1.11 (0.72; 1.71)	1.13 (0.74; 1.76)

[†]p<0.05

3.3 Associations between estimated intake, measures of obesity and obesity risk

Estimated total sugars and protein intake were positively associated with BMI, waist circumference and waist-to-hip ratio, both independently and when combined in the same model (Table 4; Fig 1). They were also positively associated with obesity risk when using waist-to-hip-ratio as the obesity marker. However, associations were weaker for BMI and waist circumference. Significant associations were observed only for estimated protein intake (both independently and in the combined model) and estimated sugar intake when using BMI



as the obesity marker, and only for estimated sugar intake in the combined model when using waist circumference.

Fig 1: Associations between estimated sugars and protein intake and obesity markers.

- 12 Associations between estimated sugars and protein intake and BMI, waist circumference and
- 13 waist-to-hip ratio in men (brown circles) and women (blue triangles)

Table 4: Associations between estimated total sugars and protein intake and BMI (β and 95% CI per 10 g) and obesity risk (OR and 95% CI per 10 g). Estimates in each column represent a separate model.

	Marker of obesity							
	BMI		Waist circumference			Waist-to-hip ratio [x 100]		
	Regression coefficient (β and 95% CI per 10 g/d)							
Total estimated sugars intake [10 g/d]	0.066 (0.024; 0.108) ^{††}	—	0.055 (0.012; 0.097) [†]	0.281 (0.165; 0.396) ^{†††}	—	0.220 (0.106; 0.333) ^{†††}	0.219 (0.145; 0.292) ^{†††}	0.182 (0.109; 0.254) ^{†††}
Estimated protein intake [10 g/d]	—	0.229 (0.095; 0.365) ^{†††}	0.197 (0.061; 0.333) ^{††}	—	1.180 (0.818; 1.543) ^{†††}	1.049 (0.686; 1.412) ^{†††}	0.745 (0.511; 0.979) ^{†††}	0.636 (0.404; 0.868) ^{†††}
	Obesity risk [‡] (OR and 95% CI per 10 g/d)							
Total estimated sugars intake [10 g/d]	1.02 (1.00; 1.04) [†]	—	1.02 (1.00; 1.04)	1.03 ^{††} (1.01; 1.05)	—	1.03 (1.01; 1.05) ^{††}	1.04 (1.02; 1.06) ^{†††}	1.03 (1.01; 1.05) ^{†††}
Estimated protein intake [10 g/d]	—	1.08 (1.02; 1.15) [†]	1.07 (1.01; 1.14) [†]	—	1.05 (0.99; 1.12)	1.03 (0.97; 1.10)	1.12 (1.05; 1.19) ^{†††}	1.10 (1.03; 1.17) ^{††}

16

17 [†]p<0.05; ^{††}p<0.01; ^{†††}p<0.001; [‡] BMI \geq 30 kg/m²; waist circumference > 85 cm (women) or 94 cm (men); waist-to-hip ratio > 0.85 (women) or 0.90 (men)

18 (women) or 0.90 (men)

19 3.4 Associations between ratio of sugar-to-protein intake, BMI and obesity risk

20 We investigated the ratios of estimated total sugars to protein intake or urinary sugars to
 21 urinary nitrogen to investigate the effect of sugar intake while controlling for dietary
 22 composition (Table 5). These data showed a positive association between the urinary
 23 sucrose-to-nitrogen ratio and measures of obesity, especially after adjustment for urinary
 24 fructose-to-nitrogen ratio. The increase in urinary sucrose to nitrogen ratio was associated
 25 with statistically significant increased risk of obesity (waist circumference and waist-to-hip
 26 ratio) after adjusting for urinary fructose to nitrogen ratio in the model. We found no
 27 statistically significant increase in obesity risk with estimated total sugars to nitrogen intake,
 28 urinary sugars to nitrogen ratio or urinary fructose to nitrogen ratio.

29 Table 5: Associations between ratio of sugars and protein intake, and ratio of urinary sugars and nitrogen and BMI (β and 95% CI) and obesity risk (OR and 95% CI).
30 Estimates in each column represent a separate model.

		Regression coefficient(β and 95% CI) [‡]					Obesity risk [#] (OR and 95% CI) [‡]				
		BMI [kg/m ²]					BMI \geq 30 kg/m ²				
Estimated intake	Total Sugars/Protein	0.108 (-0.187; 0.403)	—	—	—	—	1.01 (0.88; 1.17)	—	—	—	—
	Sum sucrose and fructose/Nitrogen	—	0.087 (-0.206; 0.380)	—	—	—	—	1.00 (0.87; 1.15)	—	—	—
24h excretion in urine	Sucrose/Nitrogen	—	—	0.178 (-0.032; 0.389)	—	0.265 (0.032; 0.497) [†]	—	—	1.05 (0.95; 1.17)	—	1.08 (0.96; 1.23)
	Fructose/Nitrogen	—	—	—	-0.094 (-0.323; 0.135)	-0.218 (-0.470; 0.035)	—	—	—	0.96 (0.87; 1.07)	0.93 (0.82; 1.05)
		Waist circumference					Waist circumference > 85 cm (women) or 94 cm (men)				
Estimated intake	Total Sugars/Protein	0.628 (-0.191; 1.448)	—	—	—	—	1.08 (0.95; 1.22)	—	—	—	—
	Sum sucrose and fructose/Nitrogen	—	0.406 (-0.311; 1.124)	—	—	—	—	1.09 (0.95; 1.25)	—	—	—
24h excretion in urine	Sucrose/Nitrogen	—	—	0.586 (-0.072; 1.100)	—	0.811 (0.243; 1.379) ^{††}	—	—	1.10 (0.99; 1.21)	—	1.14 (1.02; 1.27) [†]
	Fructose/Nitrogen	—	—	—	-0.187 (-0.748; 0.374)	-0.566 (-1.183; 0.050)	—	—	—	0.96 (0.86; 1.07)	0.91 (0.80; 1.02)
		Waist-to-hip ratio [\times 100]					Waist-to-hip ratio > 0.85 (women) or 0.90 (men)				
Estimated intake	Total Sugars/Protein	0.596 (0.070; 1.122)	—	—	—	—	1.08 (0.96; 1.23)	—	—	—	—
24h excretion in urine	Sum sucrose and fructose/Nitrogen	—	0.384 (0.000; 0.769)	—	—	—	—	1.06 (0.92; 1.22)	—	—	—

Sucrose/Nitrogen	—	—	0.362 (0.085; 0.637) †	—	0.457 (0.151; 0.762) ^{††}	—	—	1.10 (0.99; 1.22)	—	1.14 (1.02; 1.29) [†]
Fructose/Nitrogen	—	—	—	-0.027 (-0.329; 0.275)	-0.240 (-0.572; 0.091)	—	—	—	0.96 (0.86; 1.08)	0.90 (0.80; 1.02)

31 [†] p<0.05; [‡]log₂ transformed and adjusted for age and sex; ^{‡*}BMI ≥ 30 kg/m²; waist circumference > 85 cm (women) or 94 cm (men); waist-to-hip ratio > 0.85
32 (women) or 0.90 (men)

4 Discussion

In this study, we have used exclusively biomarker and biomarker-estimated data and not self-reported data to investigate associations between sugar intake and obesity risk. In our study population, using biomarker-based intake estimates, sugars were significantly associated with BMI, waist circumference and waist-to-hip ratio, and these associations remained significant after adjustment for biomarker-based protein intake. Estimated sugars intake was also associated with increased odds for obesity as measured by BMI, waist-circumference and waist-to-hip ratio. The association between sugar intake and obesity risk in the general public is difficult to investigate because of the known limitation of self-reported dietary assessment, in particular the tendency to underreport the intake of perceived unhealthy foods and foods with high sugar content, especially among overweight individuals¹¹. Indeed, observational studies relying on self-reported intake have long produced inconsistent results and generated controversy. Consistent data are available only for an association between obesity and sweetened beverages but not total sugar intake⁹. The objective assessment of sugar intake using a dietary biomarker^{16,17} relies on total daily sucrose and fructose excretion and therefore the availability of 24h urine samples, which are often not available. Previously, this biomarker has been adapted for use with spot urine samples, showing a significant association between sucrose intake and BMI in two subsets of a cohort study, EPIC Norfolk^{4,19}. However, while the biomarker measured in 24-h urine samples has been thoroughly validated, this is not the case for the biomarker measured in spot urine samples. Controlled feeding studies are needed to investigate and characterize the use of sucrose and fructose from spot urine as a biomarker for sugars¹⁸.

Total sugars and protein intake in our women (117 g/d, 80 g/d) and men (162 g/d; 102 g/d) estimated using biomarkers was higher than in the 2008/9 UK National Diet and Nutrition Survey (NDNS) (women: 78 g/d men, 66 g/d; men: 107 g/d, 89 g/d)²⁵. An explanation for this discrepancy, in particular for total sugars intake, is that the NDNS relies on self-reported data and underreporting, in particular of sugar intake, is likely.

Our data showed a significant association between biomarker-estimated total sugar intake and both measures of obesity and obesity risk, confirming positive associations between total sugar intake, measures of obesity and obesity risk. The main strengths of this study are that the samples are a representative selection of the English population and that 24h urine samples were available. Moreover, the calibration equation that was used to calibrate the biomarker and generate estimate of sugars intake was developed in a UK feeding study under a UK diet. Even though no data on energy intake were available in the study, we were able to partially control for non-sugars energy using an objective measure of protein intake. Limitations include the small sample size; many associations were of borderline statistical significance and a larger study would allow further exploration. Furthermore, the application of biomarkers assumes an equilibrium, i.e., that participants do not change their body composition²⁶, which information was not available, and could have introduced bias.

There was no information about stomach ulcers – which increase gastrointestinal permeability for (unhydrolyzed) sucrose – or impaired kidney function, for example as a result of type 2 diabetes, which could affect urinary fructose and sucrose excretion. Previous research has shown that neither obesity nor stomach ulcers have a significant impact on the biomarker used^{19,27}, but there is a paucity of data investigating the effect of impaired renal function. As sucrose is excreted rapidly and almost completely in urine²⁸, it is unlikely that diabetic kidney disease affects urinary sucrose concentrations. The physiological processes are more complex for fructose as it involves active reabsorption in the kidney²⁹ and higher urinary fructose concentrations have been observed in patients with diabetes³⁰, although it is not clear whether this is due to impaired kidney function. This would result in an overestimation of sugar intake in those participants.

While BMI is commonly used to diagnose obesity, there are some limitations due to its inability to discriminate between fat and lean mass³¹. We have therefore also included waist circumference and waist-to-hip ratio in our analyses and the results are comparable. Indeed, associations between estimated sugar intake and obesity risk are stronger when using waist-circumference and waist-to-hip ratio as measures of obesity.

A possible explanation for the association between sugar intake and measures of obesity could be that sugar intake is an important contributor of energy intake. The paucity of validated recovery biomarkers for fat and total carbohydrate intake makes it difficult to assess total energy intake without double labelled water³² or retrospectively. Protein is the only macronutrient for which intake can be estimated reliably with a dietary biomarker, total urinary nitrogen excretion^{12,23}. In the UK, protein contributed approximately 15% to 20% of total daily energy intake²⁵ and we have therefore used biomarker-estimated protein intake to partially adjust for non-sugar energy intake. Urinary nitrogen excretion was positively associated with BMI and waist circumference, but not waist-to-hip ratio. Independently, estimated protein intake was also associated with many measures of obesity and obesity risk based on BMI and waist-to-hip ratio. These associations remained significant when combining sugar and protein in the same model, although both became slightly attenuated (Fig 2).

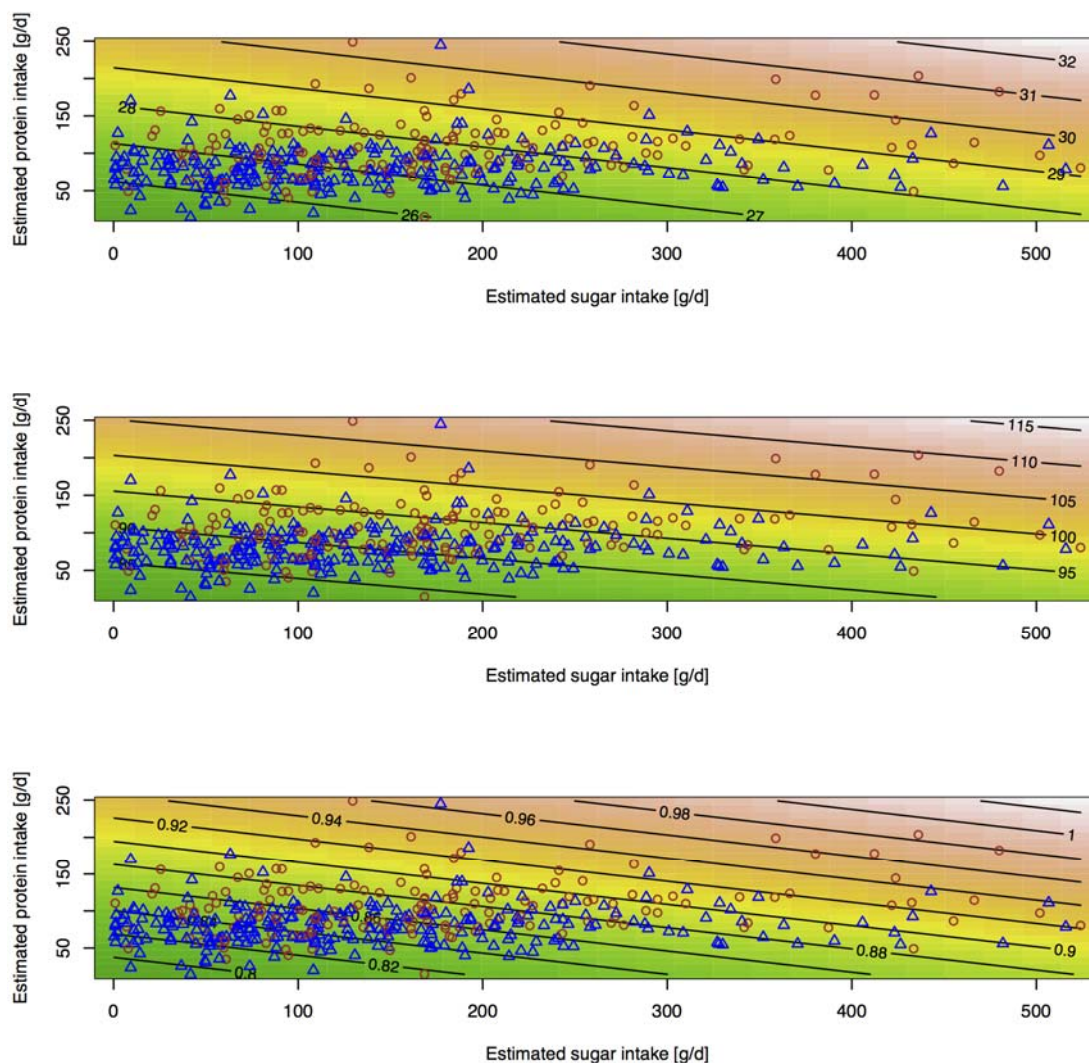


Fig 2: Association between estimated sugar, protein intake and obesity risk markers using a response surface model

Association between estimated total sugars and protein intake and (a) BMI [kg/m^2], (b) waist circumference [cm] and (c) waist-to-hip ratio in women (blue triangles) and men (brown circles) using a response surface model. Points show data for individual participants, contour lines and colours estimated BMI, waist circumference and waist-to-hip ratio of linear regression mode respectively.

We have explored these relationships further by using uncalibrated biomarker data. Our data show a strong association between urinary sucrose and measures of obesity, as well as

obesity risk based on waist circumference and waist-to-hip ratio. These associations were generally strengthened when including sucrose and fructose in the same model. Conversely, there were no significant associations for urinary fructose and only few associations were significant for total urinary sugars.

These results suggest that the associations between sugar intake and measures of obesity are mainly driven by sucrose. In contrast to fructose, which is derived from dietary fructose and hydrolysed sucrose and extensively metabolised, the only source of urinary sucrose is dietary sucrose^{14-16,33}, making it more sensitive to changes in sucrose intake, the main contributor to intake of free sugars in the UK. Furthermore, high-fructose corn syrup (HFCS) or isoglucose was not commonly used in England at the time of the study as import and production was tightly controlled as part of the European Union sugar regime (Commission Regulation (EC) No 314/2002). Therefore the main source of dietary fructose were fruit and fruit products, such that fructose was most likely a surrogate marker of their intake.

Our results show that urinary sugars can be used to estimate sugar intake in the general population when 24h urine samples are available. In the context of current discussions regarding sugar intake and the recently updated WHO recommendations on sugars intake (1), the biomarker could be used to monitor the efficacy of public health interventions. Furthermore, we showed significant associations between sugar intake and BMI, confirming results of previous observations in EPIC Norfolk^{4,19}. It is the first time that such an association has been shown in a nationally-representative sample of the general population using a validated biomarker. Our data also show significant associations between protein intake and measures of obesity and risk, however, in contrast to protein, sucrose is not an essential part of the human diet and intake can be reduced without adverse effects.

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6 References

- 1 World Health Organization. Guideline: Sugars intake for adults and children. (World Health Organization, Geneva, 2015).
- 2 Public Health England. Sugar Reduction. The evidence for action. (Public Health England, London, 2015).
- 3 Scientific Advisory Committee on Nutrition Carbohydrates and Health. (Public Health England, London, 2015).
- 4 Bingham, S. A. *et al.* Epidemiologic assessment of sugars consumption using biomarkers: comparisons of obese and nonobese individuals in the European prospective investigation of cancer Norfolk. *Cancer Epidemiology Biomarkers & Prevention* **16**, 1651-1654 (2007).
- 5 Hu, F. B. Resolved: there is sufficient scientific evidence that decreasing sugar-sweetened beverage consumption will reduce the prevalence of obesity and obesity-related diseases. *Obes Rev* **14**, 606-619, doi:10.1111/obr.12040 (2013).
- 6 Te Morenga, L., Mallard, S. & Mann, J. Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. *BMJ* **346**, e7492, doi:10.1136/bmj.e7492 (2013).
- 7 Malik, V. S., Pan, A., Willett, W. C. & Hu, F. B. Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. *American Journal of Clinical Nutrition* **98**, 1084-1102, doi:10.3945/ajcn.113.058362 (2013).
- 8 Institute of Medicine (U.S.). Panel on Macronutrients. & Institute of Medicine (U.S.). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*. (National Academies Press, 2005).
- 9 EFSA Panel on Dietetic Products, N., and Allergies (NDA). Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre. *EFSA Journal* **8**, 1462-1539 (2010).
- 10 Johansson, L., Solvoll, K., Bjorneboe, G. E. & Drevon, C. A. Under- and overreporting of energy intake related to weight status and lifestyle in a nationwide sample. *American Journal of Clinical Nutrition* **68**, 266-274 (1998).
- 11 Pryer, J. A., Vrijheid, M., Nichols, R., Kiggins, M. & Elliott, P. Who are the 'low energy reporters' in the dietary and nutritional survey of British adults? *International Journal of Epidemiology* **26**, 146-154 (1997).
- 12 Bingham, S. A. *et al.* Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers. *British Journal of Cancer* **73**, 531-550 (1995).
- 13 Poppitt, S. D., Swann, D., Black, A. E. & Prentice, A. M. Assessment of selective under-reporting of food intake by both obese and non-obese women in a metabolic facility. *International Journal of Obesity and Related Metabolic Disorders* **22**, 303-311 (1998).
- 14 Nakamura, H. & Tamura, Z. Gas chromatographic analysis of mono- and disaccharides in human blood and urine after oral administration of disaccharides. *Clinica Chimica Acta* **39**, 367-381 (1972).
- 15 Luceri, C. *et al.* Urinary excretion of sucrose and fructose as a predictor of sucrose intake in dietary intervention studies. *Cancer Epidemiology Biomarkers & Prevention* **5**, 167-171 (1996).
- 16 Tasevska, N., Runswick, S. A., McTaggart, A. & Bingham, S. A. Urinary sucrose and fructose as biomarkers for sugar consumption. *Cancer Epidemiology Biomarkers & Prevention* **14**, 1287-1294 (2005).
- 17 Tasevska, N. *et al.* Use of the Predictive Sugars Biomarker to Evaluate Self-Reported Total Sugars Intake in the Observing Protein and Energy Nutrition (OPEN) Study. *Cancer Epidemiology Biomarkers & Prevention* **20**, 490-500, doi:10.1158/1055-9965.epi-10-0820 (2011).
- 18 Tasevska, N. Urinary Sugars--A Biomarker of Total Sugars Intake. *Nutrients* **7**, 5816-5833, doi:10.3390/nu7075255 (2015).
- 19 Kuhnle, G. G. C. *et al.* Association between sucrose intake and risk of overweight and obesity in a prospective sub-cohort of EPIC-Norfolk. *Public Health Nutr* (2015).
- 20 Mindell, J. *et al.* Cohort profile: the health survey for England. *Int J Epidemiol* **41**, 1585-1593, doi:10.1093/ije/dyr199 (2012).
- 21 Methodology and documentation. (TSO, London, 2004).
- 22 Bingham, S. A. & Cummings, J. The use of 4-aminobenzoic acid as a marker to validate the completeness of 24 h urine collections in man. *Clinical Science* **64**, 629-635 (1983).

- 23 Bingham, S. A. & Cummings, J. H. Urine nitrogen as an independent validity measure of dietary intake: a study of nitrogen balance in individuals consuming their normal diet. **42**, 1276-1289 (1985).
- 24 R Core Team. R: A Language and Environment for Statistical Computing. (Vienna, Austria, 2015).
- 25 Whitton, C. *et al.* National Diet and Nutrition Survey: UK food consumption and nutrient intakes from the first year of the rolling programme and comparisons with previous surveys. *Br J Nutr* **106**, 1899-1914, doi:10.1017/S0007114511002340 (2011).
- 26 Kuhnle, G. G. C. Nutritional biomarkers for objective dietary assessment. *Journal of the science of food and agriculture* **92**, 1145-1149, doi:10.1002/jsfa.5631 (2012).
- 27 Joosen, A. M. C. P., Kuhnle, G. G. C., Runswick, S. A. & Bingham, S. A. Urinary sucrose and fructose as biomarkers of sugar consumption: comparison of normal weight and obese volunteers. *International Journal of Obesity* **32**, 1736-1740, doi:10.1038/ijo.2008.145 (2008).
- 28 Deane, N. & Smith, H. W. Fate of inulin and sucrose in normal subjects as determined by a urine reinfusion technique. *J Clin Invest* **34**, 681-684, doi:10.1172/JCI103118 (1955).
- 29 Douard, V. & Ferraris, R. P. Regulation of the fructose transporter GLUT5 in health and disease. *Am J Physiol Endocrinol Metab* **295**, E227-237, doi:10.1152/ajpendo.90245.2008 (2008).
- 30 Kawasaki, T., Akanuma, H. & Yamanouchi, T. Increased fructose concentrations in blood and urine in patients with diabetes. *Diabetes Care* **25**, 353-357 (2002).
- 31 Romero-Corral, A. *et al.* Accuracy of body mass index in diagnosing obesity in the adult general population. *International Journal of Obesity* **32**, 959-966, doi:10.1038/ijo.2008.11 (2008).
- 32 Kipnis, V. *et al.* Structure of Dietary Measurement Error: Results of the OPEN Biomarker Study. *American Journal of Epidemiology* **158**, 14-21 (2003).
- 33 Tasevska, N., Runswick, S. A., Welch, A. A., McTaggart, A. & Bingham, S. A. Urinary sugars biomarker relates better to extrinsic than to intrinsic sugars intake in a metabolic study with volunteers consuming their normal diet. *Eur J Clin Nutr* **63**, 653-659 (2009).