

ORIGINAL ARTICLE

Sugar-sweetened beverage intake in relation to reproductive parameters in young men



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Received 17 October 2020; accepted 16 April 2021

Available online 8 May 2022

KEYWORDS

Sugar-sweetened
beverages;
Semen quality;
Reproductive
hormones

Abstract

Background: There has been a decrease in sperm concentration in recent years. Concurrently, there were important dietary changes, including an increase in sugar-sweetened beverage intake (SSB). The relation between SSB and male reproduction functions in humans are barely described in the literature.

Methods: Cross-sectional study with 209 participants (18–23 years old) recruited during one year in Murcia, Spain. All men provided semen and blood samples the same day. SSB consumption was evaluated using a 101-item validated food frequency questionnaire. Reproductive hormones were analysed from serum samples, obtaining levels of follicle-stimulating hormone, inhibin B, luteinizing hormone, estradiol, and testosterone. The evaluation of semen analysis followed the WHO guidelines and consisted of seminal volume, sperm concentration, total sperm count, percentage of morphologically normal sperm, and percentage of motile sperm. SSB intake association with semen parameters and hormone levels were examined using multiple linear regression.

Results: Men in the highest quartile of the SSB intake had a higher percentage of morphologically normal sperm, 37.4% [6.1, 68.3] (p , trend = 0.047) and higher estradiol levels (9.5% [−3.5, 22.5]) (p , trend = 0.047) than those in the first quartile. SSB intake was unrelated to other semen quality parameters or reproductive hormone levels.

Conclusions: Our results indicate that sperm morphology and estradiol levels may be associated with sugar-sweetened beverage intake. These findings might be explained by physiological metabolism homeostasis, though more studies are required to confirm these results and draw conclusions in other male populations.

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PALABRAS CLAVE

Bebidas azucaradas;
Calidad seminal;
Hormonas
reproductivas

Ingesta de bebidas azucaradas y su relación con parámetros reproductivos en jóvenes**Resumen**

Antecedentes: Se ha observado una disminución en la concentración espermática en estos últimos años. Al mismo tiempo, ha habido cambios dietéticos, incluyendo un aumento en la ingesta de bebidas azucaradas (BA). La relación entre BA y la fertilidad masculina apenas está descrita en la literatura.

Métodos: Estudio transversal con 209 participantes (18-23 años) seleccionados durante un año en Murcia, España. Todos los hombres proporcionaron muestras seminales y sanguíneas el mismo día. El consumo de BA se evaluó utilizando un cuestionario validado de frecuencia alimentaria de 101 ítems. Se analizaron los niveles séricos de hormona foliculoestimulante, inhibina B, hormona luteinizante, estradiol y testosterona. El análisis seminal se realizó siguiendo los criterios de la OMS, analizando el volumen seminal y la concentración, movilidad y morfología espermáticas. La asociación de la ingesta de BA con los parámetros seminales y hormonales se evaluó mediante regresión lineal múltiple.

Resultados: Los varones en el cuartil más alto de ingesta de BA presentaron un mayor porcentaje de espermatozoides morfológicamente normales (37,4% [6,1, 68,3]) (p-tendencia = 0,047) y niveles de estradiol más altos (9,5% [-3,5, 22,5] (p-tendencia = 0,047) que los del primer cuartil. La ingesta de BA no se relacionó con otros parámetros de calidad seminal o niveles de hormonas reproductivas.

Conclusiones: Nuestros resultados indican que la morfología de los espermatozoides y los niveles de estradiol podrían estar asociados con la ingesta de BA. Estos hallazgos podrían explicarse por la homeostasis del metabolismo fisiológico, aunque se requieren más estudios para confirmar estos resultados y sacar conclusiones en otras poblaciones masculinas.

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Introduction

Overweight and obesity have been increasing in Spain for the last 30 years. Nowadays, the prevalence of overweight and obesity is around 40% and 20%, respectively,¹ which represents an almost 3-fold increase when compared with about 7% of obesity in the 1980s. In parallel, there has been a nutritional transition taking place in Mediterranean countries, including Spain.² As an integral part of that nutritional transition, there was a considerable increase during the last 50 years concerning sugar intake, consumption increased more than five times which has resulted in an addition of 450 kcal daily to the diet.³

During that period, there has been a decrease in male fertility for the last 50 years,^{4,5} that has been also clearly documented for Southern Spain.⁶ No clear explanation had been found for the decrease in male fertility, though several hypotheses have been tested.^{7,8}

Overweight and obesity have been both related to low sperm count. Obesity alters the hypothalamic-pituitary-gonadal axis, influencing sperm production.⁹ Besides, this effect is also described by insulin resistance and systemic inflammation due to overweight.¹⁰

Several systematic reviews and meta-analysis relate total sugar and sugar-sweetened beverage (SSB) consumption to overweight and obesity.¹¹ It is known that the consumption of these beverages has metabolic consequences as they simulate a corporal obesogenic state,¹² associated with diabetes, cardiovascular disease, and insulin resistance, which

could influence sperm quality through oxidative stress.¹³ In addition, lower fecundability status in couples had been associated with SSB beverages in a pre-conception cohort study.¹⁴

There are very few studies evaluating SSB intake with sperm quality, reproductive hormones, and male fertility, and only one human study addresses this association.¹⁵ In this study, it was found that increased SSB consumption was associated with lower progressive sperm motility. Nevertheless, there are strong methodological concerns, with that study as differences could also be attributed to other differences in nutritional habits in the population that were not properly accounted for.

The objective of this study is to evaluate the associations between SSB intake and male reproductive outcomes – semen parameters and reproductive hormones – in young Spanish men

Methods**Study population**

It is a cross-sectional study enrolling healthy men between 18 and 23 years old in the Murcia Region (Southern Spain) during 2010–2011 (The Murcia Young Men's Study – MYMS). This study, used in previously published works,^{16–18} takes part as one of the international studies (Spain, USA, Denmark, Finland) concerning environmental factors, semen

quality, and reproductive hormones, undergoing a physical examination and completing dietary, lifestyle and personal history questionnaires. Inclusion criteria were to be university students, born in Spain after 31st December 1987, and able to contact their mother and ask her to complete a questionnaire. Participants received a 50€ gift card for their participation. Of 215 men selected with the established criteria, six were excluded in the analysis due to excessive energy intake (>5000 kJ/d), obtaining analytical samples of 209 men. Written informed consent was obtained by all students being this study approved by the Research Ethics Committee of the University of Murcia.

Physical examination

All physical parameters (weight and height) were measured using a digital scale (Tanita SC 330-S) and the body mass index (BMI) was calculated by its formula (weight in kilograms divided by squared height in meters). Hydrocele, varicocele, or other scrotal abnormalities were evaluated and recorded, previously measuring testicular volume with an orchidometer, being all these examinations performed by the same investigator (J.M.).

Dietary assessment

Diet was assessed using a previously validated 101-item food frequency questionnaire (FFQ) used in the Spanish adult population (available at <http://bibliodiet.umh.es/files/2011/07/CFA101.pdf>). Men reported, over the past year, how often, on average, they had consumed each type of food and supplements. Food frequency options were divided into nine different categories, ranged from never to more than six times per day. Concerning beverages, multiple definitions for one serving were used: one bottle, one can, or one glass, assuming a volume equal to 330 ml. Total SSB was obtained adding up serving intakes of carbonated SSB with and without caffeine (e.g. Coke, Fanta) sugar-free carbonated SSB, and bottled fruit juice.

Semen analysis

Semen and blood samples were provided simultaneously. Semen samples were collected on-day after abstinence for at least 48 h. Semen was obtained in the clinic, with no use of lubrication. Sperm motility was calculated and described under the WHO criteria.¹⁹ The ejaculated volume was considered the sample's weight (considering a density of 1.0 g/ml) and sperm concentration was assessed using a hemocytometer. Beyond these parameters, the total sperm count (TSC) were also calculated multiplying volume times sperm count. To evaluate sperm morphological characteristics, the sample was firstly dried and fixed, dyed with Papanicolaou, and eventually observed. Semen preservation with azide solution (10 µl of 3 M for each ml of ejaculation) made possible quality control testing. Inter-laboratory variations were measured with samples sent from Copenhagen's to Murcia's laboratory during the study and with an internal validation inter-examiner with a 4.0% coefficient of variation [1.7%, 7.1%].

Reproductive hormones measurement

Blood samples, from the participants' cubital veins, were drawn on the same day and at the same time as the semen sample. Blood was centrifugated and serum was frozen, stored at -80°C . Some samples were shipped to Copenhagen at -20°C for parallel analysis. Hormones were analyzed at the same time, decreasing intra-laboratory variations. Serum levels of luteinizing hormones (LH), follicle-stimulating hormone (FSH), and sex hormone-binding globulin (SHBG) were obtained using immunofluorometric assays (DELFA: PerkinElmer, Skovlunde, Denmark). In each of the three assays, variations were < 5%. Testosterone levels (T) were determined using a fluoroimmunoassay method (DELFA; PerkinElmer) with an intra- and inter-assay variation < 8%. Estradiol (E2) was determined by radioimmunoassay (Pantex, Santa Monica, CA) with an intra-assay variation < 8% and an inter-assay variation < 13%. Inhibin B levels were determined by a specific two-sided enzyme immunometric assay (Oxford Bio-Innovation Ltd, Bicester, UK) with an intra- and inter-assay variation of 13% and 18%, respectively. Free testosterone (FT) was calculated using the equation of Vermeulen et al.,²⁰ assuming fixed albumin of 43.8 g/L. Hormone ratios were also calculated to assess potential hormonal dysregulation.

Statistical analyses

Log-transformation of sperm morphology, semen volume, total sperm count, sperm concentration, FSH, and estradiol were used due to their non-normal distribution, later transformed back for normal interpretation. Quartiles of SSB intake were calculated. Linear regression models with 95% confidence interval (CI) were used in reproductive and semen quality parameters in increasing quartiles of SSB. Continuous variables were analyzed using the Kruskal–Wallis test, while Chi-squared tests were undertaken for categorical variables. The first quartile was used as a reference while adjusting for potential confounders. Linear trend tests were calculated using the median values of each quartile as a continuous variable while semen parameters as the response variable.

Possible confounders were considered if previously have been related to semen quality parameters,^{18,21} and were included in the multivariable analysis if previously have been associated with SSB intake in the univariate analysis. We used analysis of covariance (ANCOVA) to calculate multivariable-adjusted semen quality and reproductive hormone levels for each quartile. The potential effect of abstinence time (hours), smoking status (yes or no), total protein and fat intake (g), physical activity (hours/week), total antioxidants intake (g), and the Western dietary score were assessed using linear regression models (*p*-trends across quartiles). Sperm motility analyses were additionally adjusted for the time between ejaculation and the start of semen analysis¹⁵ and hormonal parameters for the hour of blood sampling.

All tests were performed with the statistical package IBM SPSS 24.0 (IBM Corporation, Armonk, NY, USA) and the level of significance was set at 0.05.

Table 1 Characteristics of the Murcia Young Men's Study population according to quartiles of sugar-sweetened beverage (SSB) intake.

	Sugar-sweetened beverage					<i>p</i> -Value ^a
	Total cohort (209)	Q1	Q2	Q3	Q4	
<i>N</i>	209	53	57	56	43	
<i>Median, serving/day</i>	0.86	0.21	0.57	1.18	2.64	
<i>Range</i>	0.1–3.39	0.0–0.4	0.5–0.9	0.9–1.4	1.5–5.8	
<i>Demographics</i>	<i>Median (IQR) or n (%)</i>					
Age, years	20.5 (18.3–22.8)	20.2 (18.4–22.9)	20.2 (18.2–23.0)	20.8 (18.0–23.0)	20.9 (18.6–22.8)	0.42
BMI	23.7 (19.4–30.0)	23.2 (19.6–29.8)	23.9 (19.4–29.9)	23.3 (18.7–29.2)	23.6 (18.9–33.2)	0.58
Current smoker, <i>n</i> (%)	66 (32)	13 (25)	16 (28)	18 (32)	19 (44)	0.21
Moderate-vigorous exercise, h/w	5.0 (0.0–16.1)	5.0 (0.0–17.0)	4.0 (0.0–14.5)	6.0 (0.0–20.0)	6.0 (0.2–13.0)	0.001
Abstinence time, hours	71.0 (39.0–136.5)	72.0 (40.8–188.2)	67.0 (38.7–157.5)	72.0 (38.4–123.1)	67.0 (28.8–113.6)	0.58
<i>Diet</i>						
Alcohol, g/d	6.8 (0.0–24.9)	6.3 (0.4–26.6)	6.5 (0.0–28.5)	7.7 (0.0–21.1)	7.1 (0.9–31.81)	0.83
Caffeine, g/d	77.0 (7.5–398.0)	31.7 (5.6–412.2)	37.8 (5.5–405.8)	85.2 (11.6–390.3)	96.7 (23.0–396.9)	0.001
Total sugar intake, g/d	1.8 (0.0–10.4)	1.8 (0.0–10.4)	1.8 (0.0–18.8)	1.8 (0.0–10.4)	1.8 (0.0–17.1)	0.64
Total carbohydrate, % energy	41.7 (29.8–54.4)	38.6 (23.9–48.8)	42.9 (30.6–54.5)	41.5 (30.9–56.0)	45.0 (35.5–59.3)	<0.001
Total protein, % energy	18.9 (13.2–24.9)	19.6 (15.4–27.2)	18.9 (13.1–24.4)	19.0 (13.2–23.6)	17.2 (11.0–22.3)	<0.001
Total fat, % energy	38.0 (27.7–48.4)	40.0 (30.7–49.7)	38.0 (28.2–50.7)	37.9 (27.1–47.3)	35.6 (24.6–44.1)	0.003
Total energy intake, kcal/day	2278(1292–3840)	2196 (1145–3219)	2090 (1266–3642)	2393 (1476–3672)	2826 (1693–4497)	<0.001
Antioxidants ^b , mg	120.4 (51.4–234.0)	113.6 (48.5–261.1)	111.1 (54.5–218.0)	124.2 (51.0–235.8)	139.4 (35.8–221.9)	0.05
Prudent pattern score ^c	−0.2 (−1.2–1.9)	−0.1 (−1.1–2.2)	−0.2 (−1.2–2.0)	−0.1 (−0.1–1.3)	0.5 (−1.5–3.1)	0.52
Western pattern score ^c	−0.1 (−1.2–2.0)	−0.4 (−1.8–1.0)	−0.1 (−1.2–1.4)	−0.2 (−1.2–2.6)	0.3 (−1.0–2.5)	<0.001
<i>Reproductive history</i>						
Self-reported history of cryptorchidism, <i>n</i> (%)	4 (2)	1 (2)	1 (2)	1 (2)	1 (2)	0.99
Genital disease, <i>n</i> (%)	16 (8)	4 (8)	6 (11)	3 (5)	3 (7)	0.77
Varicocele	32 (15)	9 (17)	9 (16)	9 (16)	5 (12)	0.90

IQR: interquartile range.

^a From Kruskal–Wallis test for continuous variables and Chi-squared test for categorical variables.^b Antioxidants including cryptoxanthin, vitamin C, lycopene, and beta-carotene as described in Minguez-Alarcón et al. (2012).^c Dietary patterns were constructed by factorial analysis as describes in Gaskins et al. (2012). A higher score indicates a higher adherence to the Prudent or Western dietary pattern.

Table 2 Semen quality and reproductive hormones of the Murcia Young Men's Study population according to quartiles of sugar-sweetened beverage (SSB) intake.

	Sugar-sweetened beverage					<i>p</i> -Value ^a
	Total cohort (209)	Q1	Q2	Q3	Q4	
<i>N</i>	209	53	57	56	43	
<i>Median, serving/day</i>	0.86	0.21	0.57	1.18	2.64	
<i>Range</i>	0.1–3.39	0.0–0.4	0.5–0.9	0.9–1.4	1.5–5.8	
<i>Semen parameters</i>	<i>Median (IQR) or n (%)</i>					
Seminal volume (ml)	3.0 (1.0–6.5)	3.2 (0.9–6.7)	2.9 (1.0–6.4)	3.1 (0.8–8.2)	3.0 (1.0–5.6)	0.62
Sperm concentration (mill/ml)	43.3 (8.8–130.2)	39.5 (5.1–127.5)	38.1 (10.0–87.5)	39.4 (7.5–151.6)	52.3 (8.9–148.0)	0.39
Total sperm count (mill)	120.2 (17.4–401.0)	120.4 (10.8–414.6)	95.5 (22.5–348.5)	130.7 (11.6–446.2)	158.4 (18.8–442.7)	0.47
% motile sperm (S + PR + NP)	57.1 (38.8–74.2)	58.3 (43.074.5)	58.0 (35.1–74.8)	56.3 (38.9–75.3)	54.9 (25.0–71.4)	0.25
% morphologically normal sperm	9.0 (2.5–23.0)	7.0 (2.0–23.0)	10.0 (2.9–23.1)	9.0 (1.9–28.0)	11.0 (3.0–24.7)	0.09
<i>Reproductive hormones</i>						
FSH (IU/L)	2.2 (0.9–5.4)	2.3 (1.0–8.9)	2.4 (1.0–5.1)	2.1 (0.9–5.5)	2.1 (0.8–5.8)	0.33
Inhibin B (pg/ml)	193.0 (101.0–336.5)	175.0 (95.4–318.8)	190.0 (100.7–392.7)	193.5 (90.3–336.0)	208.0 (127.4–310.2)	0.37
LH (IU/L)	4.0 (1.9–7.2)	4.3 (2.5–8.1)	4.0 (2.2–7.3)	3.9 (1.7–6.9)	4.2 (2.0–7.1)	0.37
Testosterone (nmol/L)	21.2 (11.5–34.2)	21.5 (9.7–31.9)	18.6 (13.2–34.4)	23.0 (11.3–36.3)	22.0 (9.7–37.5)	0.23
SHBG (nmol/L)	30.0 (16.0–54.5)	31.0 (15.7–59.0)	29.0 (14.9–56.2)	32.0 (15.1–52.0)	29.0 (15.2–53.6)	0.60
Calculated FT (pmol/L)	482.0 (274.3–848.1)	476.0 (241.0–831.5)	460.0 (321.1–841.0)	518.0 (292.0–855.0)	472.0 (216.4–972.8)	0.32
Estradiol (pmol/L)	76.0 (48.5–117.0)	76.0 (45.6–111.3)	69.0 (47.3–102.1)	77.0 (49.7–128.2)	77.0 (48.6–134.4)	0.17
cFT/LH ratio	122.6 (58.0–263.0)	109.1 (45.8–252.5)	124.5 (62.7–224.9)	130.2 (64.9–323.8)	138.4 (46.8–299.5)	0.04
E2/T ratio	3.6 (2.4–5.8)	3.6 (2.2–6.1)	3.5 (2.4–5.1)	3.6 (2.3–5.7)	3.6 (2.3–7.1)	0.96
T/LH ratio	5.3 (2.7–10.8)	5.0 (2.2–8.8)	5.2 (2.4–10.0)	5.9 (3.3–14.3)	5.8 (2.5–11.8)	0.07
Inhibin B/FSH ratio	83.4 (20.6–284.5)	76.6 (13.4–278.3)	81.3 (21.8–352.8)	82.6 (21.0–339.2)	93.2 (27.3–306.0)	0.33

IQR: interquartile range.

^a From Kruskal–Wallis test for continuous variables and Chi-squared test for categorical variables.

SHBG: sex hormone-binding globulin.

cFT/LH: the ratio of calculated free testosterone (pmol/L) to luteinizing hormone (IU/L).

E2/T: ratio of estradiol (pmol/L) to testosterone (pmol/L).

T/LH: the ratio of testosterone (pmol/L) to luteinizing hormones (IU/L).

Inhibin b/FSH: the ratio of inhibin B (pg/ml) to follicle-stimulating hormone (IU).

Table 3 Associations between semen quality parameters and SSB intake [reported as percentage (%) change or untransformed model coefficients (β), with 95%CI].

	Sugar-sweetened beverage				<i>p</i> -Trend ^a
	Q1	Q2	Q3	Q4	
<i>N</i>	53	57	56	43	
<i>Median, serving/day</i>	0.21	0.57	1.18	2.64	
<i>Range</i>	0.0–0.4	0.5–0.9	0.9–1.4	1.5–5.8	
<i>Sperm motility (% A + B + C)^c</i>					
Crude		–2.7 (–6.9, 1.4)	–2.8 (–6.9, 1.3)	–5.0 (–9.5, 0.6)	0.046
Adjusted ^b	Reference	–1.9 (–6.2–2.4)	–2.3 (–6.7, 2.1)	–4.4 (–9.5, 0.7)	0.120
<i>Sperm concentration (mill/ml)</i>					
Crude		–3.2% (–39.9, 33.5)	–6.9% (–43.8, 29.9)	7.9% (–31.5, 47.4)	0.64
Adjusted ^b	Reference	3.0% (–33.8, 39.8)	1.3% (–36.8, 39.4)	32.1% (–12.0, 76.3)	0.124
<i>Sperm morphology (% normal)</i>					
Crude		18.9% (–6.4, 44.2)	7.7% (–17.7, 33.0)	29.7% (2.4, 57.1)	0.078
Adjusted ^b	Reference	21.7% (–4.1, 47.6)	9.5% (–17.3, 36.2)	37.2% (6.1, 68.3)	0.047
<i>Semen volume (ml)</i>					
Crude		–5.6% (–29.1, 17.9)	–2.4% (–26.1, 21.3)	–8.6% (–33.9, 16.6)	0.580
Adjusted ^b	Reference	–2.8% (–26.6, 21.1)	1.0% (–23.8, 25.9)	–4.7% (–33.3, 24.0)	0.789
<i>Total sperm count (millions)</i>					
Crude		–8.8% (–49.1, 31.5)	–10.3% (–50.9, 30.4)	–0.7% (–44.0, 42.7)	0.910
Adjusted ^b	Reference	0.3% (–39.7, 40.3)	1.5% (–40.1, 43.1)	27.5% (–20.5, 75.5)	0.200

IQR: interquartile range.

^a Estimated using median intake in each quartile as a continuous variable. The *p*-value for trend < 0.05 compared with men in the lowest quartile of SSB intake.^b Adjusted for abstinence time, smoking status, antioxidant intake, total protein intake, total fat intake, physical activity, and Western dietary patterns.^c Additionally adjusted for the time from current ejaculation to start of semen analysis.

Results

The median age of the participants was 20.5 (18–23) years, being most of them non-smokers (68%) with a median of 5 h/week of exercise. Median BMI was 23.7 kg/m² and no variation between quartiles was found. The median (5th, 95th percentile) SSB intake was 0.86 servings/day [0.1–3.39]. There is an increase in total caffeine, carbohydrate, and antioxidant intake between quartiles, and we can appreciate a decrease in protein and fat consumption amongst quartiles. Men who consumed more SSB had higher Western pattern scores and calorie intake (Table 1).

The median (5th, 95th percentile) values of sperm parameters were 3 ml [1.6.5] for seminal volume, 43.3 × 10⁶/ml [8.8, 130.2] for sperm concentration, 120.2 × 10⁶ [17.4, 401.0] for total sperm count, 57.1% [38.8, 74.2] for motility and 9.0% [2.5, 23.0] for normal morphology (Table 2).

Normal morphology percentage was positively related to SSB intake after adjustment for potential confounders (Table 3). Men in the top SSB intake quartile had 37.2% [6.1, 68.3] higher normal morphology than men in the first quartile, reinforced by its incremental trend (*p*-trend = 0.047).

Concerning other semen parameters, SSB had no relation with them, observing a decrease in sperm motility of –4.4 units [–9.5, 0.7] but with no statistical significance amongst quartiles (*p* trend = 0.12).

Lastly, we assessed the relation between SSB intake and reproductive hormone levels (Table 4). Concerning estradiol levels, there is a no significant correlation with men in the higher quartile (9.5 [–3.5, 22.5%]) with those in the first quartile, but we see the existence of an increase in estradiol level's trend (*p*-trend = 0.047). SSB had no relation with other reproductive hormones. We observed that T/LH ratio and cFT/LH ratio had an increase between quartiles with statistical significance in its crude model but losing its trend after adjusting for its possible confounders.

Discussion

Higher intake of SSB was associated with more morphological normal sperm percentage and an increase in trend of serum levels of estradiol in young men after adjusting for its potential confounders. No relation was found between other quality parameters or hormone levels.

Table 4 Associations between reproductive hormone levels and SSB intake [reported as percentage (%) change or untransformed model coefficients (β), with 95%CI].

	Sugar-sweetened beverage				<i>p</i> -Trend ^a
	Q1	Q2	Q3	Q4	
<i>N</i>	53	57	56	43	
<i>Median, serving/day</i>	0.21	0.57	1.18	2.64	
<i>Range</i>	0.0–0.4	0.5–0.9	0.9–1.4	1.5–5.8	
<i>FSH (UI/L)</i>					
Crude		–9.4% (–30.0, 11.3)	–21.5% (–42.3, –0.8)	–18.8% (–41.0, 3.4)	0.100
Adjusted ^b	Reference	–11.1% (–32.5, 10.4)	–21.2% (–43.3, 1.0)	–20.0% (45.6, 5.6)	0.170
<i>Inhibin B (pg/ml)</i>					
Crude		15.8 (–13.6, 45.1)	2.6 (–26.9, 32.0)	16.7 (–14.9, 48.2)	0.481
Adjusted ^b	Reference	11.6 (–18.2, 41.2)	–6.6 (–37.4, 24.2)	3.8 (–31.8, 39.4)	0.940
<i>LH (UI/L)</i>					
Crude		–0.4 (–1.0, 0.3)	–0.6 (–1.3, 0.0)	–0.5 (–1.2, 0.1)	0.160
Adjusted ^b	Reference	–0.4 (–1.0, 0.3)	–0.5 (–1.2, 0.2)	–0.5 (–1.2, 0.3)	0.330
<i>Testosterone (nmol/L)</i>					
Crude		–0.7 (–3.3, 1.9)	1.2 (–1.5, 3.8)	1.4 (–1.4, 4.2)	0.170
Adjusted ^b	Reference	–0.7 (–3.4, 1.9)	1.2 (–1.5, 4.0)	1.4 (–1.8, 4.6)	0.230
<i>SHBG (nmol/L)</i>					
Crude		–2.2 (–6.6, 2.2)	–1.2 (–5.6, 3.2)	–1.4 (–6.2, 3.4)	0.790
Adjusted ^b	Reference	–1.6 (–6.1, 2.9)	–0.1 (–4.8, 4.5)	0.9 (–4.5, 6.3)	0.520
<i>Calculated FT (pmol/L)</i>					
Crude		–7.1 (–72.0, 57.8)	30.8 (–34.6, 96.3)	50.5 (–19.3, 120.3)	0.080
Adjusted ^b	Reference	–9.4 (–76.0, 57.2)	26.8 (–42.4, 96.0)	35.9 (–43.7, 115.5)	0.260
<i>Estradiol (pmol/L)</i>					
Crude		–6.0% (–16.6, 4.6)	6.6% (–4.1, 17.2)	5.1% (–6.3, 16.5)	0.120
Adjusted ^b	Reference	–4.9% (–15.7, 6.0)	9.1% (–21.0, 20.4)	9.5% (–3.5, 22.5)	0.047
<i>cFT/LH ratio</i>					
Crude		7.4 (–17.1, 32.0)	32.0 (7.2, 56.8)	24.6 (–1.8, 51.0)	0.050
Adjusted ^b	Reference	6.4 (–17.7, 30.5)	24.8 (–0.3, 49.8)	17.7, (–11.1, 46.6)	0.230
<i>E2/T ratio</i>					
Crude		–0.2 (–0.6, 0.3)	–0.1 (–0.5, 0.4)	0.1 (–0.4, 0.6)	0.560
Adjusted ^b	Reference	–0.2 (–0.7, 0.3)	0.0 (–0.5, 0.5)	0.2 (–0.4, 0.8)	0.270
<i>T/LH ratio</i>					
Crude		0.2 (–0.8, 1.2)	1.3 (0.3, 2.3)	0.9 (–0.1, 2.0)	0.050
Adjusted ^b	Reference	0.2 (–0.8, 1.1)	1.1 (0.1, 2.0)	0.8 (–0.3, 1.9)	0.140
<i>Inhibin B/FSH ratio</i>					
Crude		10.2 (–28.1, 48.5)	9.5 (–28.9, 48.0)	15.8 (–25.4, 57.0)	0.510

Table 4 (Continued)

	Sugar-sweetened beverage				<i>p</i> -Trend ^a
	Q1	Q2	Q3	Q4	
Reference					
Adjusted ^b		8.1 (−31.2, 47.3)	1.5 (−39.1, 42.1)	2.7 (−44.2, 49.6)	0.975

IQR: interquartile range.

^a Estimated using median takes in each quartile as a continuous variable. The *p*-value for trend < 0.05 compared with men in the lowest quartile of SSB intake.

^b Adjusted for smoking status, antioxidant intake, total protein intake, total fat intake, physical activity, Western dietary patterns, and hour of blood sampling. SHGB: sex hormone-binding globulin; T/LH ratio: the ratio of testosterone to luteinizing hormone; cFT/LH: the ratio of calculated free testosterone to luteinizing hormone, E2/T: the ratio of estradiol to testosterone; Inhibin B/FSH: the ratio of inhibin B to FSH.

Normal sperm morphology seems to follow an increasing pattern amongst quartiles also in the other manuscript evaluating SSB intake and semen quality,¹⁵ but with no significance at all. There is scarce literature explaining this phenomenon, possibly induced by a favorable estrogenic status,²² where estradiol nurtures immature and mature population, supports tight junctions between cells, and nurtures precursor populations while modulating mature ones preventing cellular apoptosis and contributing to a normal cellular genesis. Nevertheless, very high levels of estradiol contribute to negative hormonal feedback, decreasing testosterone and disrupting all the spermatogenesis leading to male infertility.²² We did not observe any relation between SSB intake and sperm motility as seen in other manuscripts,¹⁵ consistent with recent animal experimental data.²³

Regarding reproductive hormone levels, our results are consistent with a recent review concerning animal experimental data.^{24,25} Estrogens are important participants in metabolic regulation. Estradiol may regulate insulin action directly via actions on insulin-sensitive tissues or indirectly by regulating factors like oxidative stress. It is thought that estradiol receptors have a positive effect on insulin signaling and glucose receptor expression, being able to control blood sugar in hyperglycemic status. Higher levels of testosterone are observed while increasing in quartiles, though lacking statistical significance, but could explain the increase of estradiol due to the conversion of it under the intervention of aromatase.²⁶

Knowing the relation between SSB intake and obesity and the association between obesity and semen quality, we pre-assumed that BMI would have an important role.¹⁰ However, we did not observe any changes between assuming BMI as a confounder or not, explained by the scarce difference found between quartiles.

We must emphasize the fact that approximately a quarter of the young population is found in the last quartile, representing a median of 2.64 servings/day which contributes to approximately 90 g of sugar/day, 35% of our daily carbohydrate recommendations. This fact strengthens the importance of reducing these habits, habits that can lead to important future health consequences and that can be easily fought with public health interventions.

Our study has several strong points. Firstly, we are describing a homogeneous young healthy men population. In addition, semen analysis results were not publicly exposed,

making unlikely diet changes in response to fertility issues. As measuring criteria, we used a validated FFQ, bringing us a trustworthy SSB intake measure, being able to adjust for all the other diet variables, reducing all potential confounding. On the other hand, we can't set aside all the important limitations of this study. Starting with the design, we are analyzing a cross-sectional study, reducing the possibility to assess causality but being able to reduce possible bias in all its forms. Another limitation observed was the fact that men only had one sample analyzed, though multiple sample analyses will barely give more information.²⁷

In conclusion, we found that SSB intake was related to an increase in normal sperm morphology and serum levels of estradiol. These findings might be explained by physiological metabolism homeostasis, though more studies are required to confirm these results and draw conclusions in other male populations.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Ethics approval and consent to participate

The Research Ethics Committee of the University of Murcia approved this study (no. 495/2010, approved May 14, 2010), and written informed consent was obtained from all subjects.

Funding statement

This work was supported by Fundación Séneca, Agencia y Tecnología de la Región de Murcia [08808/PI/08, 19443/PI/14]; Consejería de Innovación, Junta de Andalucía

[P09-CTS-5488] and Ministerio de Economía, Industria y Competitividad, Instituto de Salud Carlos III (Acción estratégica en Salud, AES) [PI10/00985, PI13/01237, PI13/02406].

Conflicts of interest

None.

Acknowledgments

A.M.T.C., J.M., and J.J.A.G. were involved in study conception and study design. J.M. was involved in study execution and acquisition of data. J.K.C. performed the statistical analysis.

A.M.T.C., J.M., J.J.A.G., E.A., and J.K.C. contributed to data analysis and interpretation.

J.K.C. drafted the manuscript. A.M.T.C., J.M., J.J.A.G., and E.A. were involved in critical revision for important intellectual contributions and approved the final version of the manuscript.

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