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Consumption of artificially sweetened soft drinks and risk of gastrointestinal cancer: a meta-analysis of observational studies

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Abstract

Objective: There remain inconclusive findings from previous observational epidemiological studies on whether consumption of artificially sweetened soft drinks (ASSD) increases the risk of gastrointestinal (GI) cancer. We investigated the associations between the consumption of ASSD and the risk of GI cancer using a meta-analysis.

Design: Systematic review and meta-analysis.

Setting: PubMed and EMBASE were searched using keywords until May 2020 to identify observational epidemiological studies on the association between the consumption of ASSD and the risk of GI cancer.

Subjects: Twenty-one case–control studies and seventeen cohort studies with 12 397 cancer cases and 2 474 452 controls.

Results: In the random-effects meta-analysis of all the studies, consumption of ASSD was not significantly associated with the risk of overall GI cancer (OR/relative risk (RR), 1·02; 95 % CI, 0·92, 1·14). There was no significant association between the consumption of ASSD and the risk of overall GI cancer in the subgroup meta-analyses by study design (case–control studies: OR, 0·95; 95 % CI, 0·82, 1·11; cohort studies: RR, 1·14; 95 % CI, 0·97, 1·33). In the subgroup meta-analysis by type of cancer, consumption of ASSD was significantly associated with the increased risk of liver cancer (OR/RR, 1·28; 95 % CI, 1·03, 1·58).

Conclusions: The current meta-analysis of observational epidemiological studies suggests that overall, there is no significant association between the consumption of ASSD and the risk of GI cancer.

Keywords
Artificially sweetened soft drinks
Gastrointestinal cancer
Observational studies
Meta-analysis

In 2018, the five most common gastrointestinal (GI) cancers, oesophageal, gastric, pancreatic, liver and colorectal cancer, accounted for approximately 25 % of all new cancer cases and 36 % of all cancer deaths worldwide[1].

Artificially sweetened soft drinks (ASSD) are drinks produced with high-intensity synthetic sweeteners with the purpose of reducing or eliminating calories in the products while imitating the sugary sweet taste[2]. The most popular artificial sweeteners used in beverage industries are aspartame (branded as NutraSweet, Sugar Twin or Equal), acesulfame (branded as Sunett or Sweet one), saccharin (branded as Sweet and Low, Sweet Twin, Sweet 'N Low or Necta Sweet), sucralose (branded as Splenda) and neotame (branded as Newtame)[3,4]. The production and consumption of ASSD have increased worldwide, and this popularity has been further triggered by the growing epidemic of obesity in high-income countries and other calorie-related health concerns that arise from consumption of sugar-sweetened beverages[5,6]. The supplies and demands of these soft drinks have established their niches worldwide, including low- and middle-income countries at the peril of whole fruit consumption and home-made real fruit juice. The high sweetness intensity of artificial sweeteners seems also very attractive to ASSD producers.

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Meanwhile, previous laboratory and animal studies have reported that low-dose exposure to aspartame had a significant multi-potential carcinogenicity in colon cell lines and increased the incidence of mammary cancer, lymphoma and leukemia in rats with a dose–response relationship[7,8]. The dose level used in the animal study was close to the acceptable daily intake of aspartame for humans[8,9]. This might indicate that lifespan exposure to aspartame in soft drinks could potentially increase the risk of an individual to cancer, especially GI cancer.

Also, previous observational epidemiological studies have reported inconsistent findings on whether consumption of ASSD increases the risk of GI cancer[10–30]. Ten observational epidemiological studies (four case–control studies and six cohort studies) reported a significant association between the consumption of ASSD and the risk of GI cancer[15,17,18,21,22,24,26], while twenty-eight studies (fifteen case–control studies and thirteen cohort studies) reported no association between them[10–14,16,19,20,23,25,27–30]. However, no meta-analysis on this topic has been published so far.

Thus, we investigated whether the consumption of ASSD increases the risk of GI cancers by using a meta-analysis of observational epidemiological studies such as case–control studies and cohort studies.

Materials and methods

Literature search strategy

We conducted a literature search in both PubMed and Excerpta Medica dataBASE (EMBASE) databases up to May 2020. We used a combination of the National Library of Medicine Medical Subject Headings (MeSH) terms with a wide range of free-text terms as search terms in order to identify as many relevant articles as possible. A PICO framework was used to determine search terms related with the topic of the current study as follows: P for population is 'general population'; I for intervention (exposure in the current study) is 'consumption of ASSD'; C for comparison is 'little or no consumption of ASSD' and O for outcome is 'incidence of GI cancers'. Additionally, we restricted a study design to case–control study and cohort study for the current study. Thus, by using Boolean operators for all the determined MeSH and free-text terms, we created a combination of search terms as follows: (artificially sweetened beverages or sweetened beverages or carbonated drinks or soft drinks or diet drinks or fizzy drinks or cola or soda or non-alcoholic beverages or non-alcoholic drinks) and (gastrointestinal neoplasms or oesophageal neoplasms or stomach neoplasms or liver neoplasms or pancreatic neoplasms or colorectal neoplasms) and (cohort study or case–control study). Appendix 1 shows the final search strategy for the PubMed example. We further reviewed the reference lists from the identified articles to find relevant studies not identified through this search strategy.

Inclusion criteria

We included observational epidemiological studies that met the following criteria: (1) a case–control study or a cohort study; (2) investigated the associations between the consumption of ASSD and any of the five major types of GI cancer (oesophageal, gastric, pancreatic, liver and colorectal cancer); (3) reported outcome measures with adjusted OR, relative risks (RR), or hazard ratios and 95 % CI. If data were reported in more than one study on the same cancer type, the study presenting the most comprehensive data was included. Studies that were not published in peer-reviewed journals or only presented in conferences were excluded.

Selection of relevant studies

Two authors (Jatho A and Cambia JM) independently selected all the studies retrieved from the databases. We extracted year of publication and first author's name, type of study, country, year of the enrollment of participants, population (number of participants, gender and baseline age range), type of GI cancer, definition of ASSD intake (highest *v.* lowest category), adjusted OR/RR/HR with 95 % CI and adjusted variables for the general characteristics of the included studies.

Assessment of methodological quality

We evaluated the methodological quality of the included studies based on the Newcastle-Ottawa Scale for assessing the quality of case–control studies and cohort studies in the meta-analyses[31]. The Newcastle-Ottawa Scale star system ranges from 0 to 9 representing the three subscales of the study quality dimensions: study selection, comparability and exposure assessment[31]. Because there are no established cut-off criteria for high or low quality of a study, we classified a study with more than the mean score of each study type (case–control studies or cohort studies) into a high-quality study.

Main and subgroup analyses

We investigated the associations between the consumption of ASSD (highest *v.* lowest consumption or never consumed) and the risk of GI cancer for the main analysis. This was followed by subgroup meta-analysis by type of study design (case–control study or cohort study), type of GI cancer (oesophageal, gastric, pancreatic, liver or colorectal cancer), gender (female or male), continental region (Africa, America, Australia, Asia or Europe) and methodological quality of the included studies (high or low quality) in each study type. Also, we conducted subgroup meta-analysis by each factor (type of GI cancer, gender, region and study quality) under each type of study design.

Statistical analyses

We computed the pooled OR, RR or HR with its 95 % CI using the adjusted OR, RR or HR and its 95 % CI from each

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study reporting the association between the consumption of ASSD (highest *v.* lowest consumption or never consumed) and the risk of GI cancer. We further examined heterogeneity across the studies using Higgins I^2[32], which measures the percentage of total variation across the studies[11]. I^2 is calculated as follows:

$$I^2 = 100\ \% \times (Q - df)/Q,$$

where Q is Cochran's heterogeneity statistic, and df indicates the df. Negative values of I^2 were set at zero; I^2 ranges from 0 % (no observed heterogeneity) to 100 % (maximal heterogeneity)[32]. An I^2 value greater than 50 % indicates substantial heterogeneity[32].

The pooled estimate was computed using the DerSimonian and Laird method[33]. We used a random-effects model because the identified studies were conducted in a wide range of geographical settings and in different populations.

We also evaluated publication bias using the Begg's funnel plot and Egger's test[34]. Publication bias exists when the Begg's funnel plot shows asymmetry or when the *P*-value of the Egger's test is less than 0·05[34] Further, we conducted sensitivity analyses to explore the influence of each study on the pooled estimate by omitting an investigation one by one and re-analysing. We used Stata SE version 16.1 statistical software package (StataCorp) for all the meta-analyses.

Results

Identification of relevant studies

Figure 1 shows a flow diagram of how we selected the relevant studies for the current study. A total of 448 articles were identified by searching two electronic databases (PubMed and EMBASE) and by hand-search. We excluded ninety-two duplicate articles and additional 313 articles based on the predetermined selection criteria. We conducted the full-text review of the remaining forty-three articles. Among these, twenty-two articles were excluded for the following reasons: sugar-sweetened soft drinks (*n* 9); inclusion of sweets, snacks and desserts (*n* 4); undefined cancer sites (*n* 3); report of inflammatory scores or index (*n* 2); biliary track and gallbladder cancer (*n* 2) and irrelevant studies (*n* 2). The remaining twenty-one articles with eleven case–control studies[10–20] and eleven cohort studies[18,21–30] involved individual twenty-one and seventeen studies, respectively, totaling to thirty-eight studies in the main and subgroup meta-analysis.

Characteristics of studies included in the final meta-analysis

We included thirty-eight studies in the final meta-analysis; twenty-one case–control and seventeen cohort studies from twenty-one articles that had 2 486 849 participants (12 397 cancer cases and 2 474 452 controls). The mean age of all the participants was 54 years (range, 18 to 97 years). Table 1 shows the general characteristics of the studies included in the final meta-analysis. The types of GI cancers were as follows: oesophageal[10–13,19], gastric[10,11,14,20,21,25,26], pancreatic[14–16,21–24], liver[18,28] and colorectal cancer[12,17,21,25,27,29,30]. Only six studies reported gender-disaggregated data[11,12,14,15,21,24]. Studies were conducted in Europe[10,12,14,16,18,22,24,27,30], America[11,15,19,20,23,28,29], Australia, other Oceania[13,25,26], Asia[21] and Africa[17].

Methodological quality of studies

We assessed the methodological quality of the included studies based on the Newcastle-Ottawa Scale. The quality scores ranged from 5 to 9; the average score was 7·4 for case–control studies (range 5–9) and 8·1 for cohort studies (range 6–9). Nine case–control studies and ten cohort studies are considered as high-quality studies (scores of 7 or higher in case–control studies and 8 or higher in cohort studies) (Table 2).

Consumption of artificially sweetened soft drinks and risk of gastrointestinal cancer

As shown in Figure 2, the consumption of ASSD was not associated with the risk of GI cancer (OR/RR, 1·02; 95 % CI, 0·92, 1·14). In the subgroup meta-analyses by study design, no significant association between them was observed in both case–control studies (OR, 0·95; 95 % CI, 0·82, 1·11) and cohort studies (RR/HR, 1·14; 95 % CI, 0·97, 1·33).

In the subgroup meta-analyses by type of GI cancer, the consumption of ASSD was associated with a significantly increased risk of liver cancer (OR/RR, 1·28; 95 % CI, 1·03, 1·58; *n* 3), while no association was found in any other types of GI cancers (Fig. 2).

Consumption of artificially sweetened soft drinks and risk of gastrointestinal cancer by gender, region and methodological quality of study

Table 3 shows findings from the subgroup meta-analyses stratified by gender, region and methodological quality. In the meta-analysis of all the studies, overall, no significant association between the consumption of ASSD and the risk of GI cancer was observed except for liver cancer (OR/RR, 1·28; 95 % CI, 1·03, 1·58; *n* 3). The significantly increased risk of liver cancer by consumption of ASSD was found in cohort studies (RR/HR, 1·50; 95 % CI, 1·04, 2·16; *n* 2) as well as case–control studies (OR, 1·18; 95 % CI, 1·04, 1·34; *n* 1).

Heterogeneity, publication bias and sensitivity analysis

Statistical heterogeneity was observed ($I^2 = 64·3$ %) in the meta-analysis of all the included studies. In the subgroup meta-analysis by type of study, case–control studies

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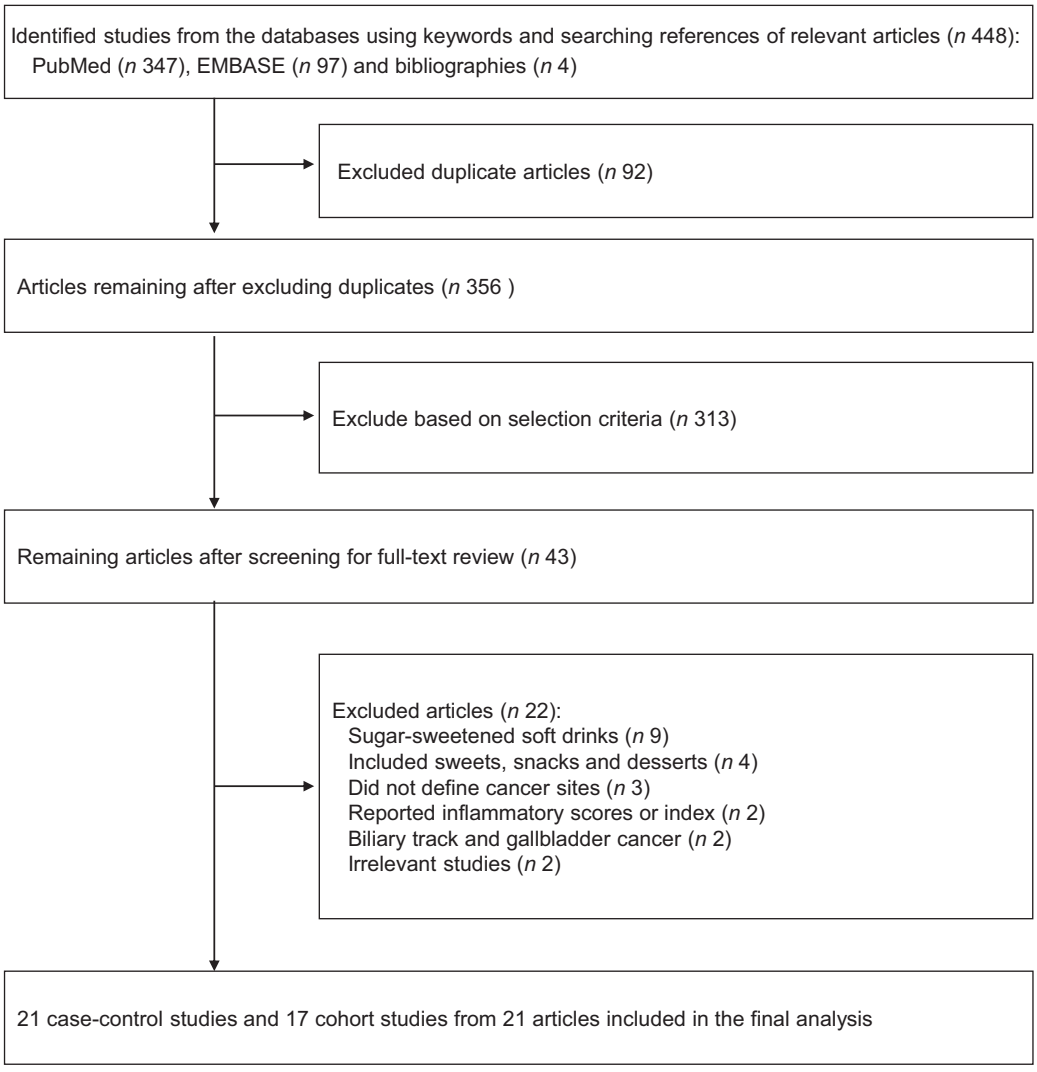

Fig. 1 PRISMA flow diagram for study selection

showed substantial heterogeneity ($I^2 = 68{\cdot}8\,\%$), while cohort studies showed less heterogeneity ($I^2 = 48{\cdot}3\,\%$). Publication bias was not observed in both Funnel plot (Fig. 3) and Begg's test ($P = 0{\cdot}33$ for all the studies, 0·19 for case–control studies and 0·67 for cohort studies, respectively). Sensitivity analysis to discern the influence of each study did not show any substantial change in the pooled estimate of the effect size and statistical significance (data not shown in figure).

Discussion

Summary of findings

In the current meta-analysis of observational epidemiological studies, we found that the consumption of ASSD was not associated with the risk of overall GI cancer. Also, there was no significant association between them in the subgroup meta-analysis by type of study design. In the subgroup meta-analysis by type of cancer, the consumption of ASSD was significantly associated with the increased risk of liver cancer, which association remained consistent in the subgroup meta-analysis of both case–control and cohort studies.

Possible biological mechanisms

Neoplastic induction by metabolites of artificial sweeteners

Even though we found that there was no significant association between ASSD and the risk of GI cancer, previous laboratory and animal studies have proposed possible biological mechanisms on the association. First, some laboratory and animal studies have showed that the ingredients of ASSD metabolise in the gut into their chemical constituents that could be harmful in long-term exposure[6,7,8]. For example, aspartame is metabolised in the gastric tract into aspartic acid, phenylalanine and methanol[7]. Low-dose exposure to aspartame in both laboratory and animal studies showed a significant multi-potential carcinogenicity in colon cell lines and increased the incidence of mammary

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Table 1 Characteristics of the studies included in the final meta-analysis of artificially sweetened soft drinks (ASSD) and the risk of gastrointestinal (GI) cancer (*n* 21)

Study (reference)	Type of study	Country	Years enrolled	Population (gender, age)	Type of GI cancer	Definition of ASSD intake (highest *v.* lowest category)	OR/RR/HR	95 % CI	Adjusted variables
2004 Khan[21]	Prospective cohort study	Japan	1984–2002	1524 persons and thirty-six cases (men, 40–97 years)	Gastric	Daily drink *v.* never	0·80	0·40, 1·80	Age, health status, health education, health screening, and smoking.
				1524* persons and fifteen cases (men, 40–97 years	Colorectal	Daily drink *v.* never	0·60	0·2, 2·10	
				1524* persons and twelve cases (men, 40–97 years	Pancreatic	Daily drink *v.* never	0·20	0·00, 1·80	
				1634 persons and fifteen cases (women, 40–97 years	Gastric	Daily drink *v.* never	3·90	1·40, 11·10	
				1634* persons and fourteen cases (women, 40–97 years	Colorectal	Daily drink *v.* never	0·80	0·20, 3·00	
				1634* persons and thirteen cases (women, 40–97 years	Pancreatic	Daily drink *v.* never	0·20	0·00, 1·80	
2006 Lagergren[10]	Case–control study	Sweden	1995–1997	262 cases and 820 controls, (men and women, < 80 years)	Gastric	> 6 drinks) *v.* 0 drink (lowest quartile)	1·04	0·60, 1·78	BMI, smoking, alcohol, socio-economic status, fruit, and vegetable intake.
				189 cases and 820* controls, (men and women, < 80 years)	Oesophageal	> 6 drinks) *v.* 0 drink (lowest quartile)	0·80	0·60, 1·90	
2006 Larson[22]	Prospective cohort study	Sweden	1987–2005	77 797 persons, 131 cases (women and men aged 45–83 years)	Pancreatic	≥ 2 drinks/d *v.* 0 (no) drink/d	2·3	1·35, 3·92	Age, sex, education, smoking, BMI, and energy, and alcohol consumption.
2006 Mayne[11]	Case–control studies	United States	Not provided	255 cases and 687 controls (men and women, 30–79 years)	Gastric-Cardia	≥ 365 drinks (/year *v.* 0–11 drinks (lowest quartile) / year	0·74	0·46, 1·16	Study center, sex, age, race, income, education, reflux symptoms, caloric intake, meat intake, and vegetable intake.
				In women	Gastric-Cardia		0·46	0·12, 1·74	
				In men	Gastric-Cardia		0·85	0·50, 1·38	
				352 cases and 687* controls (men and women, 30–79 years)	Gastric-nonCardia		0·65	0·43, 0·98	
				In women	Gastric-nonCardia		0·63	0·27, 1·46	
				In men	Gastric-nonCardia		0·62	0·38, 1·01	
				206 cases and 687* controls (men and women, 30–79 years)	Oesophageal-SCC		0·85	0·48, 1·52	

Table 1 *Continued*

Study (reference)	Type of study	Country	Years enrolled	Population (gender, age)	Type of GI cancer	Definition of ASSD intake (highest *v.* lowest category)	OR/RR/HR	95 % CI	Adjusted variables
				In women	Oesophageal-SCC		0·36	0·08, 1·71	
				In men	Oesophageal-SCC		0·99	0·52, 1·90	
				282 cases and 687* controls (men and women, 30–79 years)	Oesophageal-AC		0·47	0·29, 0·76	
				In women	Oesophageal-AC		0·40	0·1, 1·55	
				In men	Oesophageal-AC		0·46	0·27, 0·79	
2007 Gallus[12]	Case–control study	Italy	1991–2004	1225 cases and 7028 controls (men and women, average 62 and 58 years; cases and controls)	Colorectal (Colon)	> 2drinks /d *v.* none	0·89	0·65, 1·21	Age, sex, study centre, education, tobacco smoking, alcohol drinking, BMI, total energy intake, intake of hot beverages, parity, and menopausal status.
				In women	Colorectal (Colon)		0·92	0·84, 1·01	
				In men	Colorectal (Colon)		1·00	0·91, 1·1	
				728 cases and 7028* controls (men and women, average 62 and 58 years; cases and controls)	Colorectal (Rectum)		0·8	0·54, 1·19	
				In women	Colorectal (rectum)		0·92	0·81, 1·03	
				In men	Colorectal (rectum)		0·98	0·87, 1·10	
				304 cases and 7028* controls (men and women, median age 60 years	Oesophageal		1·24	0·54, 2·81	
				In women	Oesophageal		0·80	0·48, 1·31	
				In men	Oesophageal		1·07	0·88, 1·32	
2008 Bao[23]	Prospective cohort study	United States	1995–2003	487 922 persons and 1258 cases (men and women, 50–71 years)	Pancreatic	Highest quintiles of diet soft drinks/d *v.* never.	1·11	0·86, 1·44	Age, sex, BMI, smoking, and physical activity
2008 Ibiebele[13]	Case–control study	Australia	2001–2005	294 cases and 1484 controls (men and women, 18–79 years)	Oesophageal (AC)	≥ 4 times/d *v.* never.	1·06	0·72, 1·56	Age, sex, BMI, heartburn and acid reflux, smoking, alcohol, education, total energy intake, and vegetable intake.
				238 cases and 1484* controls (men and women, 18–79 years)	Oesophageal (SCC)		0·64	0·45, 0·92	
				325 cases and 1484* controls (men and women, 18–79 years)	Oesophageal (AEG)		0·71	0·51, 0·99	

Table 1 *Continued*

Study (reference)	Type of study	Country	Years enrolled	Population (gender, age)	Type of GI cancer	Definition of ASSD intake (highest *v.* lowest category)	OR/RR/HR	95 % CI	Adjusted variables
2009 Bosetti[14]	Case–control study	Italy	1997–2007	230 cases and 547 controls (men and women, 22–80 years)	Gastric	Ever *v.* never users	0·8	0·45, 1·43	Age, sex, study center, year of interview, education, BMI, tobacco smoking, history of diabetes, consumption of hot beverages, and total energy intake.
				In women	Gastric		0·93	0·39, 2·18	
				In men	Gastric		0·76	0·32, 1·79	
			1991–2007	326 cases and 652 controls (men and women, 34–80 years)	Pancreatic	Ever *v.* never users	0·62	0·37, 1·04	
				In women	Pancreatic		0·62	0·29, 1·34	
				In men	Pancreatic		0·66	0·31, 1·38	
2009 Chan[15]	Case–control study	United states	1995–1999	532 cases and 1701 controls (men and women, 21–85 years)	Pancreatic	≥ 2 drinks/d *v.* none	1·5	1·2, 2·1	Age, sex, energy intake, race, education, smoking, BMI, physical activity, history of diabetes, and total fat consumption.
				In women	Pancreatic		1·40	0·90, 2·30	
				In men	Pancreatic		1·80	1·10, 2·80	
2010 Gallus[16]	Case–control study	Italy	1991–2008	326 cases and 652 controls (men and women, 63 (Median) years)	Pancreatic	≥ 15 drinks/week *v.* none	1·02	0·72, 1·44	Age, sex, center, year of interview, education, BMI, smoking, alcohol intake, energy intake, diabetes, and family history of pancreatic cancer.
2014 Mahfouz[17]	Case–control study	Egypt	2011	150 cases and 300 controls (men and women, age not reported)	Colorectal	Yes *v.* no	4·60	1·9, 11·01	Age, sex, residence, education, and occupation
2016 Navarrete-Munoz[24]	Prospective cohort study	Europe	1992–2009	477 199 persons and 865 cases (men and women, average age 51 years)	Pancreatic	> 246·3 g (Quintile 5) of drinks /d *v.* none	1·07	0·67, 1·73	Age, Sex, BMI, Waist circumference, smoking, alcohol, physical activity, study center, education, juice, and nectar.
				In women	Pancreatic		1·09	1·03, 1·15	
				In men	Pancreatic		0·91	0·80, 1·04	
2016 Stepien[18]	Nested case–control study	Europe	2006	121 cases and 241 controls (men and women, mean age 57 years)	Liver	> 6 cans /week *v.* none	1·18	1·04, 1·34	Age, sex, study center, non-alcoholic energy intake, BMI, sex-specific physical activity, education, alcohol intake, alcohol intake pattern, smoking intensity, duration and history, and diabetes status.
	Prospective cohort study	Europe	1992–2010	477 206 persons and 191 cases (men and women, mean age 57 years)	Liver	> 6 cans/week *v.* none	1·83	1·11, 3·02	Age, sex, study center, non-alcoholic energy intake, BMI, sex-specific physical activity, education level, alcohol intake at recruitment, alcohol intake pattern, smoking intensity, duration and history, and diabetes status.

Table 1 *Continued*

Study (reference)	Type of study	Country	Years enrolled	Population (gender, age)	Type of GI cancer	Definition of ASSD intake (highest *v.* lowest category)	OR/RR/HR	95 % CI	Adjusted variables
2017 Hodge[25]	Prospective cohort study	Australia	1990–2007	35 593 persons, 165 cases (men and women, 40–69years)	Gastric	≥ 1 drink frequency/d *v.* never	1·03	0·53, 1·98	Sex, country of birth, smoking, alcohol intake, physical activity, and Mediterranean diet.
				35 593* persons,1055 cases (men and women, 40–69years)	Colorectal		0·79	0·6, 1·06	
2017 Li[19]	Pooled analysis of case–control studies	United states	2002–2005	472 cases and 492 controls (men and women, 18–79 years)	Oesophageal (Barrett)	Highest quartile in g/d *v.* lowest	1·51	0·98, 2·33	Age, sex, race, total energy intake, fruit and vegetable intake, BMI, and frequency of gastro-esophageal reflux.
2017 Li[20]	Pooled analysis of case–control study	United states	1993–95 and 1992–97	500 cases and 2027 controls (men and women, 30–79 years)	Oesophageal	Highest quintile of standard serving/d *v.* lowest	1·22	0·87, 1·70	Age, sex, race, study indicator, BMI, fruits and vegetables intake, cigarette smoking, GERD frequency, and total energy intake.
				529 cases and 2027* controls (men and women, 30–79 years)	Gastric	Highest quintile of standard serving/d *v.* lowest	1·21	0·86, 1·69	
2019 Bassett[26]	Prospective cohort study	Australia	1990–2015	35 109 persons, 125 cases (men and women, 27–76 years)	Gastric	> 1 drinks/d *v.* never/< 1/month	1·23	1·02, 1·48	Age, sex, country of birth, BMI. Mediterranean diet, alcohol, smoking, and physical activity.
2019 Chazelas[27]	Prospective cohort study	France	2009–2017	101 257 persons, 166 cases (males and females, mean age of 42·2 years	Colorectal	Highest quartile of drinks/d *v.* lowest	0·8	0·44, 1·46	Age, BMI, physical activity level, smoking, history of cancer, diabetes, hypertension, major cardiovascular event and dyslipidemia, menopausal status, education, alcohol intake, oral contraception, hormonal therapy for menopause, carbohydrate intake, total lipid intake, sodium intake, and sugar sweetened drinks.
2019 Luo*[28]	Prospective cohort study	United states	1980–2012	137 608 persons, 160 cases (males and females; 30–55, 40–75 years	Liver	Highest quartile of drinks/d *v.* lowest	1·26	0·79, 2·01	Age, gender, BMI, race, physical activity, smoking, alcohol, aspirin use, and total calorie intake.
2019 Malik[29]	Prospective cohort study	United states	1986–2014	173 229 persons, 160 cases (males and females; 30–55, 40–75 years	Colorectal	≥ 2 drinks/d *v.* < 1 drink /month	1·01	0·77, 1·31	Age, BMI, smoking, postmenopausal hormone use (Nurses-Health-Study), physical activity, family history of cancer, diabetes, myocardial infarction, hypertension and

Table 1 *Continued*

Study (reference)	Type of study	Country	Years enrolled	Population (gender, age)	Type of GI cancer	Definition of ASSD intake (highest *v.* lowest category)	OR/RR/HR	95 % CI	Adjusted variables
2019 Mullee[30]	Prospective cohort study	Europe	1992–2018	451 743 persons, 160 cases (males and females, mean age 50·8 years	Colorectal	≥ 1 glass of drinks/d *v.* < 1 glass of drink /month	1·22	0·91, 1·64	hypercholesterolemia, multivitamin use, ethnicity, aspirin use, total energy intake, red and processed meat intake, whole grains, fruits, and vegetables intake. BMI, physical activity, alcohol, smoking, education, red and processed meat intake, fruits and vegetable intake, contraceptive use, and menopausal status.

AC, adenocarcinoma; SCC, squamous cell carcinoma; AEG, adenocarcinomas of oesophaggastric junction; GERD, gastroesophageal reflux disease; RR, relative ratio.
*Reported artificially sweetened drinks as diet drinks.

cancer, lymphoma and leukaemia in rats with a dose–response relationship[7,8]. Moreover, these were conducted at a dose level similar to the recommended daily intake levels in humans[7,8]. Soffritti *et al.* also found a neoplastic induction by aspartame regarding carcinogenesis in the liver and lung in mice[35], which is linked to the production of formaldehyde from the methanol constituent of aspartame. Liver and other body tissues metabolise methanol into formaldehyde[36–38]. Formaldehyde is genotoxic and damages the DNA due to the formation of formaldehyde adducts that increases the risk of chromosomal mutations due to DNA-protein cross-links formation[5]. Therefore, lifespan exposure to ASSD could increase the risk of liver cancer in human.

Effects of acidulants, colouring (4-methylimidazole) and flavouring agents in artificially sweetened soft drinks

Soft drinks in addition to artificial sweeteners contain acidulants and colouring and flavouring agents[6]. A colourant known as caramel (4-methylimidazole) is classified into Group 2B (possibly carcinogenic) by the international agency for research on cancer[39]. Caramel is used as a colouring agent in the production of both the artificially sweetened and sugar-sweetened soft drinks in similar permissible level[40]. Some artificial flavouring agents in ASSD are also chemically synthesised. However, their effects to promote neoplastic induction remain unclear.

Inflammation by artificial ingredients and proinflammatory markers

Systemic inflammation from the artificial ingredients in ASSD and proinflammatory markers such as C-reactive protein have also been implicated. C-reactive protein is a non-specific acute phase protein primarily synthesised by the liver and used as a systemic inflammatory marker have been suggested to promote carcinogenesis[41]. For example, C-reactive protein was found to increase the risk of breast cancer in a meta-analysis of cohort studies (RR, 1·26; 95 % CI, 1·07, 1·49)[41].

Also, altered GI track microbiota by ASSD might be related to inflammation. In both animal and human studies, Suez *et al.*[42] demonstrated that consumption of artificially sweetened products of saccharin, aspartame and sucralose triggers glucose intolerance due to the altered composition and functions of GI track microbiota. The altered microbiota decrease bacterial heterogeneity and the relative ratio of Bacteroidetes and Firmicutes[42,43]. More importantly, lipopolysaccharide, a part of outer membrane of Gram-negative bacteria, is suggested to initiate obesity-related inflammation and insulin resistance. In the liver or adipose tissues, lipopolysaccharide triggers the innate immune response that increases proinflammatory cytokine expression[44].

Additionally, weight gain and elevated glycaemic index might be associated with promotion of inflammation, which could lead to the development of cancer. Fowler *et al.*[45] in a cohort study on 'obesity epidemic' observed

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Table 2 Methodological quality of studies included in the final analysis based on the Newcastle-Ottawa scale* for assessing the quality of case–control studies and cohort studies (*n* 22)

	Selection				Comparability	Exposure			
Case–control studies (*n* 11)	Adequate definition of cases	Representativeness of cases	Selection of controls	Definition of controls	Control for important factor or additional factor	Ascertainment of exposure (blinding)	Same method of ascertainment for participants	Nonresponse rate	Total
2006 Lagergren[10]	1	1	0	1	1	0	1	0	5
2006 Mayne[11]	1	1	1	0	2	0	1	0	6
2006 Gallus[12]	1	1	1	1	2		1	1	9
2008 Ibiebele[13]	1	1	1	1	2	0	1	1	8
2009 Bosetti[14]	1	1	1	1	2	0	1	0	7
2009 Chan[14]	1	1	1	1	2	0	1	0	7
2010 Gallus[16]	1	1	1	1	1	1	1	1	8
2014 Mahfouz[17]	1	1	0	1	0	0	1	1	5
2016 Stepien[18]	1	1	1	1	2	1	1	1	9
2017 Li[19]	1	1	1	1	2	1	1	1	9
2017 Li[20]	1	1	1	1	2	1	1	1	9
								Average score = 7	

	Selection				Comparability	Outcome			
Cohort studies (*n* 11)	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome of interest was not present at start of study	Control for important factor or additional factor	Assessment of outcome	Follow-up long enough for out-comes to occur	Adequacy of follow-up of cohorts	Total
2004 Khan[21]	1	1	1	1	2	1	1	0	8
2006 Larson[22]	1	1	0	1	2	1	0	0	6
2008 Bao[23]	1	1	0	1	1	1	0	1	6
2016 Navarrete-Munoz[24]	1	1	0	1	2	1	1	1	8
2016 Stepien[18]	1	1	1	1	2	1	1	1	9
2017 Hodge[25]	0	1	1	1	2	1	1	1	8
2019 Bassett[26]	1	1	1	1	2	1	1	1	9
2019 Chazelas[27]	1	1	1	1	2	1	0	1	8
2019 Luo[28]	1	1	1	1	2	1	1	1	9
2019 Malik[29]	1	1	1	1	2	1	1	1	9
2019 Mullee[30]	1	1	1	1	2	1	1	1	9
								Average score = 8	

*Each study can be awarded a maximum of one star for each numbered item within the selection and exposure categories, while a maximum of two stars can be given for the comparability category. 2016 Stepien *et al.*'s study consists of both a case–controls study and a cohort study.

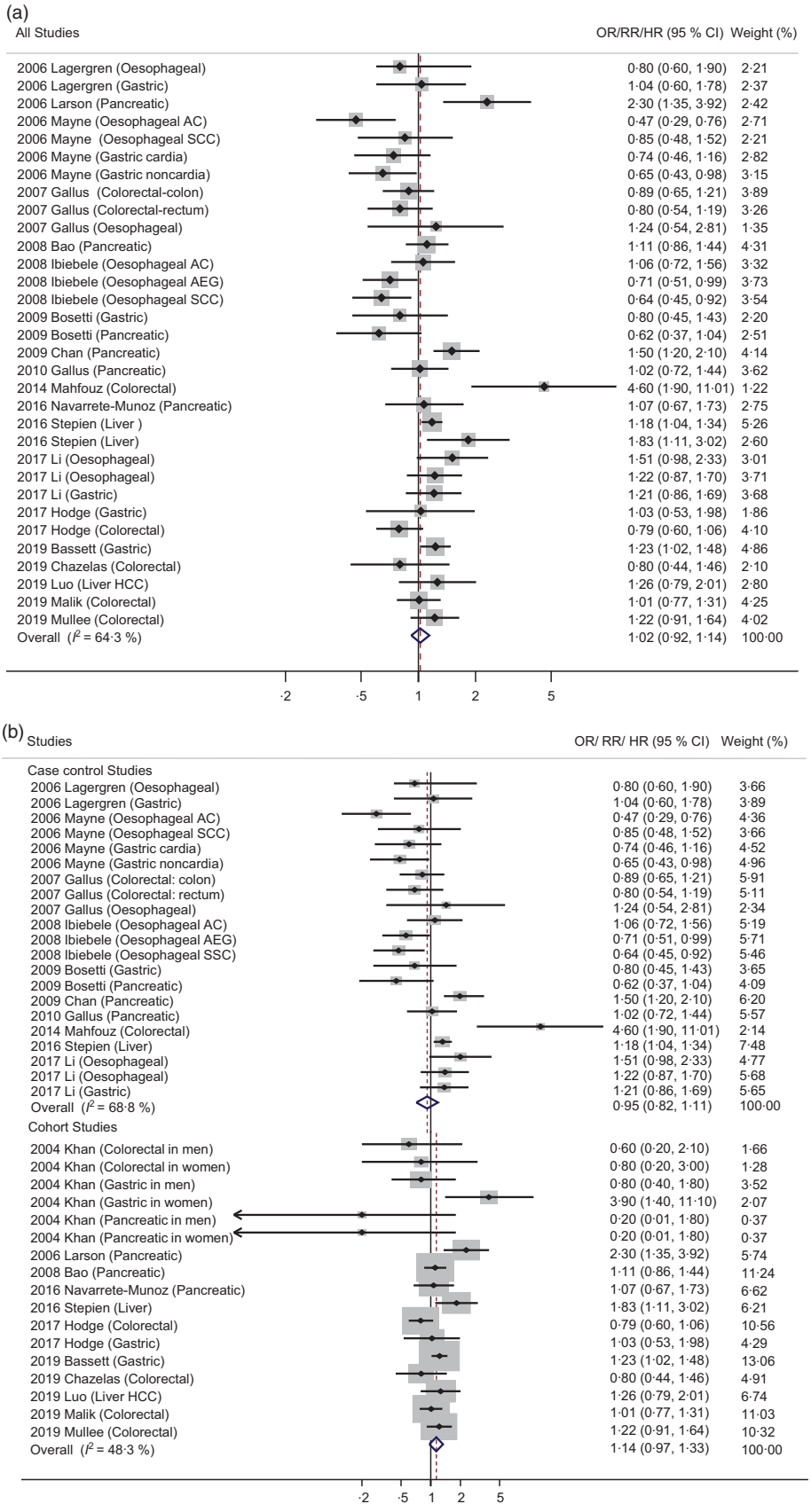

Fig. 2 (colour online) Consumption of artificially sweetened soft drinks and risk of gastrointestinal (GI) cancer in a random-effects meta-analysis of observational epidemiological studies. (a) All studies; (b) subgroup meta-analysis by type of study design; (c) subgroup meta-analysis by type of GI cancer. OR, OR; RR, relative risk; CI, CI; AC, adenocarcinoma; SCC, squamous cell carcinoma; AEG, adenocarcinomas of oesophagogastric junction, and HCC, hepatocellular carcinoma. *During data analysis using Stata SE version 16.1 statistical software, the lower limit of the 95 % CIs of 0·0 that were observed in both men and women by Khan *et al.* (2004) study was rejected by the STATA software. We, therefore, chose 0·01 (the closest value to 0·0 that could be accepted by the software for the analysis to proceed)

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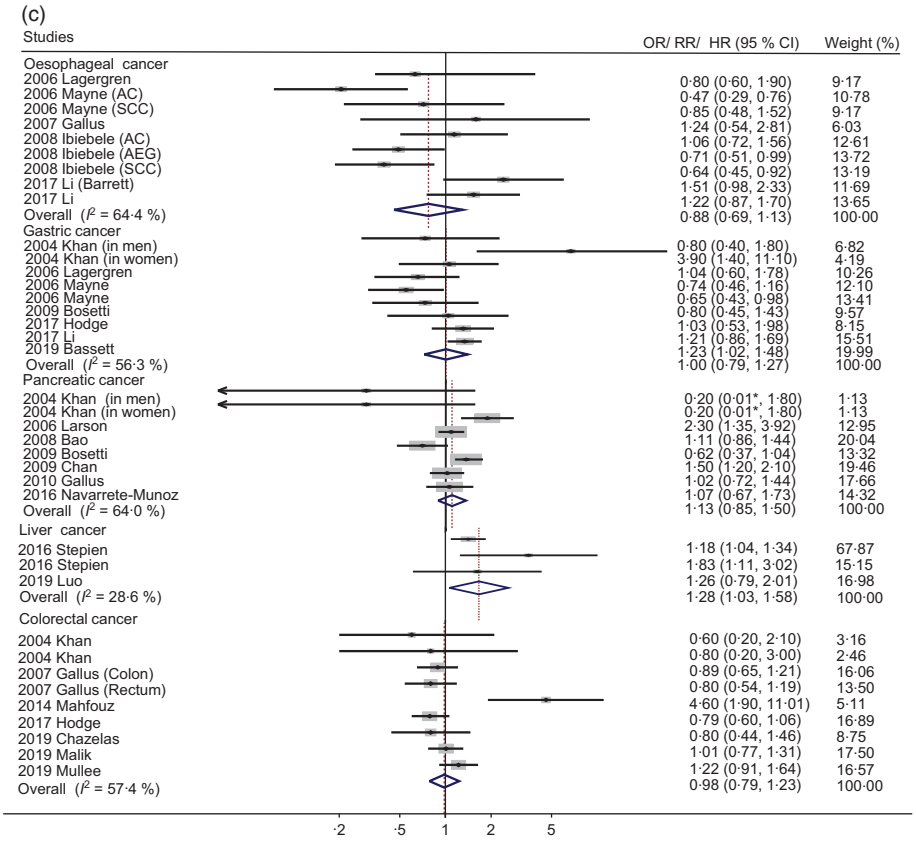

Fig. 2 (colour online) (Continued).

that participants who consumed ASSD showed a significant weight gain and obesity than those who did not consume ASSD. Similar findings were reported in two adolescent cohorts that examined effects of ASSD intake on BMI and fat percentage[46]. These findings suggest that artificial sweeteners stimulate food intake and weaken the validity of sweet taste by desensitising the natural ability of sweet taste to evoke physiological responses[2,43,47]. This could induce higher glycaemic index, hyperinsulinaemia and systemic inflammation that promote tumorigenesis[48]. However, since ASSDa are generally viewed as healthier substitutes due to absence of sugar, individuals with underlying health disorders like obesity, diabetes and CVD might have experienced high consumption of ASSD in the past.

Possible explanations for no significant association between artificially sweetened soft drinks consumption and gastrointestinal cancer risk

We do not have clear explanations for no significant association between the consumption of ASSD and the risk of GI cancer. However, there are some possible ones for it. First, findings from preclinical studies such as laboratory studies or experimental animal studies are not always directly applied to humans. Laboratory studies and experimental animal studies are usually conducted in the limited and controlled settings and environments, and observational epidemiological studies are conducted in the different environmental settings. In addition,lifestyle factors could affect the disease outcomes in humans. Second, the lack of an apparent association in this meta-analysis might be due to confounders, which might affect the disease outcomes. For example, some studies did not adjust for the intake of fruits, fruit juice and vegetables, which contain various antioxidants that could attenuate the harmful effects of ASSD[49]. Third, the fundamental metabolic differences between humans and animals could also lead to different health status outcomes from a specific exposure such as ASSD. Besides the metabolic differences between laboratory animals and human, their anatomical, physiological and biochemical differences in particular in their GI tracts could influence the absorption and bioavailability values[50] of ASSD and their ingredients. Fourth, the accuracy of the dietary survey to estimate the consumption of ASSD could also affect detecting the exact influence of ASSD on the development of GI cancer. Last, most of the observational studies included in the current meta-analysis used data from a single measurement of dietary intake at baseline. Therefore, such data might not reflect long-term dietary intake behaviour.

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Table 3 Association between artificially sweetened soft drinks and risk of gastrointestinal cancers in subgroup meta-analyses using a random-effects model

Factors	Number of studies	Summary OR or RR or HR	95 % CI	Heterogeneity, I^2 (%)
All studies[10–30]	32	1·02	0·92, 1·14	64·3
Type of cancer				
Oesophageal cancer[10–13,19]	9	0·88	0·69, 1·13	64·4
Gastric cancer[10,11,14,20,21,25,26]	9	1·00	0·79, 1·27	56·3
Pancreatic cancer[14–16,21–24]	8	1·13	0·85, 1·50	64·0
Liver cancer[18,28]	3	1·28	1·03, 1·58	28·6
Colorectal cancer[12,17,21,25,27,29,30]	9	0·98	0·79, 1·23	57·4
Gender				
Female[11,12,14,15,21,24]	14	0·96	0·84–1·10	59·1
Male[11,12,14,15,21,24]	14	0·94	0·84- 1·05	44·9
Region				
Africa[17]	1	4·60	1·91, 11·07	NA
America[11,15,19,20,23,28,29]	11	1·02	0·84, 1·24	66·4
Australia[13,25,26]	6	0·89	0·69, 1·14	70·6
Asia[21]	6	0·87	0·39, 1·93	53·5
Europe[10,12,14,16,18,22,24,27,30]	14	1·06	0·91, 1·23	49·5
Methodological quality				
High quality[11–16,18–21,24–30]	29	0·98	0·88, 1·10	58·7
Low quality[10,17,22,23]	5	1·46	0·91, 2·33	76·5
Subgroup meta-analyses of case–control studies				
Type of cancer				
All types of GI cancer[10–20]	21	0·95	0·82, 1·11	68·8
Oesophageal cancer[10–13,19]	9	0·88	0·69, 1·13	64·4
Gastric cancer[10,14,20,21]	5	0·88	0·68, 1·14	38·1
Pancreatic cancer[14–16]	3	1·02	0·64, 1·63	78·8
Liver cancer[18]	1	1·18	1·04,1·34	NA
Colorectal cancer[16,21]	3	1·29	0·64, 2·58	84·9
Gender				
Female[11,12,14,15]	10	0·91	0·85, 0·98	0·5
Male[11,12,14,15]	10	0·94	0·82, 1·09	55·5
Region				
Africa[17]	1	4·60	1·91, 11·07	NA
America[11,15,19,20]	8	0·97	0·73, 1·29	75·9
Australia[13]	3	0·78	0·58, 1·03	48·5
Asia	No study	–		–
Europe[10,12,14,16,18]	9	0·96	0·82, 1·12	33·6
Methodological quality				
High quality[11–16,18–20]	18	0·92	0·79, 1·07	67·4
Low quality[10,17]	3	1·47	0·60, 3·56	82·1
Subgroup meta-analyses of cohort studies				
Type of cancer				
All types of GI cancer[18,21–30]	17	1·14	0·97, 1·33	48·3
Oesophageal cancer	No study	–		–
Gastric cancer[25,26]	4	1·26	0·82, 1·93	52·4
Pancreatic cancer[21–24]	4	1·17	0·56, 2·43	69·0
Liver cancer[18,28]	2	1·50	1·04, 2·16	12·4
Colorectal cancer[21,25,27,29,30]	6	0·96	0·81, 1·14	9·5
Gender				
Female[21,24]	4	1·25	0·55, 2·81	60·8
Male[21,24]	4	0·90	0·79, 1·02	0·0
Region				
Africa	No study	NA		NA
America[23,28,29]	3	1·09	0·91, 1·29	0·0
Australia[25,26]	3	1·01	0·72, 1·41	69·4
Asia[21]	6	0·87	0·39, 1·93	53·5
Europe[18,22,24,27,30]	5	1·35	0·98, 1·85	58·0
Methodological quality				
High quality[18–21,24–30]	15	1·09	0·92, 1·29	41·3
Low quality[22,23]	2	1·54	0·76, 3·13	82·8

Abbreviation: NA, not applicable.

Strengths

This is the first most comprehensive meta-analysis of observational epidemiological studies such as case–control studies and cohort studies on this topic. Although a meta-analysis of observational epidemiological studies regarding this topic has been published in 2019, it only included pancreatic cancer and involved sweetened beverages including both sugar-sweetened soft drinks and ASSD[51]. Moreover, the authors only investigated the combined effects of both types of beverages.

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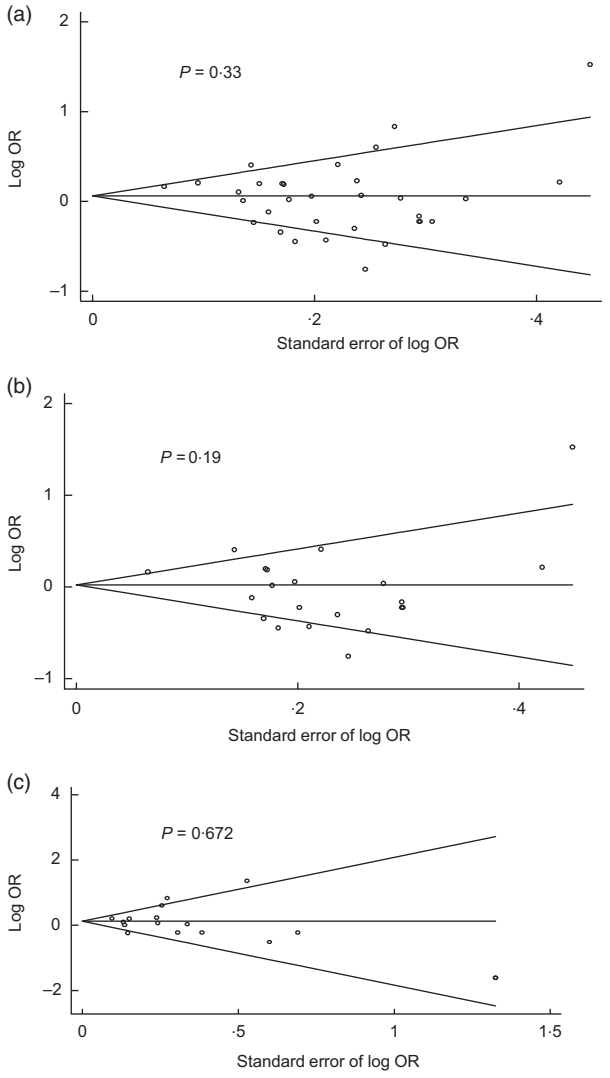

Fig. 3 Assessment of publication bias by Begg's funnel plots with 95 % confidence limits on consumption of artificially sweetened soft drinks and risk of gastrointestinal cancer in a random-effects meta-analysis of observational epidemiological studies. (a) All studies (*n* 32); (b) case–control studies (21); (c) cohort studies (*n* 17)

Limitations

There are several limitations in our study. We included only observational epidemiological studies such as case–control and cohort studies in the current meta-analysis. In terms of evidence-based medicine, randomised controlled trials, which give us a higher level of evidence than observational studies, are warranted to confirm the association between the consumption of ASSD and the risk of GI cancer. However, no randomised controlled trials on this topic have been published so far, and it is not easy to conduct randomised controlled trials on this topic because of ethical concerns. Additionally, although we found that the consumption of ASSD increased the risk of liver cancer, the number of the included studies is too small to confirm the association between them. We included only three studies with a case–control study and two cohort studies for this association.

Conclusions

In this meta-analysis of observational epidemiological studies, we found that the consumption of ASSD is not associated with the risk of GI cancer. Further large prospective cohort studies are warranted to confirm its effect on the risk of liver cancer.

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Supplementary material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S136898002100104X

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