8.2. Risk of obesity

Body weight and BMI (or BMI standardised by age and sex and expressed as BMI z-scores for studies conducted in children) were eligible endpoints in RCTs with an intervention period of at least 6 weeks. Body weight and BMI were not assessed as endpoints in studies conducted under neutral energy balance because these studies were designed to maintain body weight constant (i.e. target energy intakes were adjusted to that end, even weekly in some studies). Percent body fat (%BF) and waist circumference (WC) were eligible endpoints in studies conducted ad libitum and in studies conducted under neutral energy balance. This is because both endpoints could theoretically change together with body weight or independently of it through changes in body composition and body fat redistribution. Measurements of %BF using bioelectrical impedance analysis (BIA) or skinfold thickness were not eligible for intervention studies because these techniques are generally not appropriate to assess small changes in body fat when used alone, particularly in obese subjects and/or when significant changes in body water compartments occur.

8.2.1. Total sugars

sQ1.1. Total sugars and risk of obesity

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of obesity, incidence of abdominal obesity	0	0
LoE2. Standalone (surrogate)	Body weight/BMI, waist circumference	0	**3**
LoE3. Complementary	Body fat, abdominal fat	0	2

8.2.1.1. Observational studies

Three prospective cohorts of children investigated the association between the intake of total sugars and BMI (SCES, (Gopinath et al., 2013); NGHS, (Lee et al., 2015); KoCAS, (Hur et al., 2015)), of which two also assessed WC (SCES, NGHS) and two %BF (SCES, KoCAS). The studies used either the nutrient residuals model or the standard multivariable model (in continuous analysis) to adjust for TEI, and thus kept TEI constant. The evidence table, including the effect estimates and confidence intervals, is in **Annex J**.

LoE2. Standalone (surrogate): Body weight/BMI, waist circumference. PCs. The SCES cohort (RoB tier 2) reports non-significant associations (negative in females, positive in males) between total sugars intake at baseline and change in BMI or WC over the 5-year follow-up. In the NGHS cohort (RoB tier 1), a non-significant (positive) association was found between 1-year changes in total sugars intake and concurrent changes in BMI z-scores and WC in the most adjusted models. Associations between absolute intake of total sugars at baseline and BMI z-scores at the end of the 4-year follow-up were positive and non-significant in the KoCAS (RoB tier 3).

Preliminary UA. The Panel notes the limited number of studies available, that the direction of the relationship is inconsistent across studies, and that none shows significant associations between the intake of total sugars and BMI (or BMI z-scores) or WC. The heterogeneity of these studies with respect to the exposure–endpoint relationships investigated (baseline intake vs. changes in the endpoint, changes in intake vs. changes in the endpoint, baseline intake vs. endpoint at the end of follow-up) precludes the calculation of pooled mean estimates across studies, as evidence is sparse by type of relationship.

The Panel considers that the available BoE does not suggest a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of obesity. **No comprehensive UA is performed**.

LoE3. Complementary: Body fat, abdominal fat. PCs. The Panel notes that the BoE is limited to two PCs (SCES, KoCAS), which are inconsistent regarding the direction of the association between total sugars intake and %BF (negative in SCES, significant in males only, RoB tier 2; positive in KoCAS, RoB tier 3).

The Panel considers that the available BoE does not suggest a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and %BF.

8.2.1.2. Overall conclusion on sQ1.1

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of obesity. Total sugars were not investigated under other dietary conditions (e.g. not keeping TEI constant in the analysis).

8.2.2. Added and free sugars

sQ2.1. Added and free sugars and risk of obesity

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of obesity, incidence of abdominal obesity	0	0
LoE2. Standalone (surrogate)	Body weight/BMI, waist circumference	**11 (+2)**	**8**
LoE3. Complementary	Body fat, abdominal fat	5	4

8.2.2.1. Intervention studies

LoE 2. Standalone (surrogate): Body weight/BMI, waist circumference. RCTs. Changes in body weight were investigated in 11 studies of which six manipulated sugars from beverages and five from a combination of solid foods and beverages. Seven RCTs were conducted in overweight/obese individuals and two were in children and adolescents. Between-arm differences in added sugar intakes ranged from 6 to 24 E%. Of these, two studies investigated changes in WC. WC was also measured in two studies conducted under neutral energy balance. The results of the individual studies are in **Appendix F**.

Preliminary UA

At the end of the intervention, body weight was higher in the high sugars arm relative to the low sugars arm in all the 11 studies considered. The effect was statistically significant in three studies. Six RCTs were at low RoB (tier 1) and five at moderate RoB (tier 2). The mean pooled effect (95% CI) is 1.15 kg (0.53, 1.77; I^2 = 29%) **(Appendix G, Figure G.1a)**. The results on BMI followed the same pattern in the six studies which assessed this endpoint, as expected in studies conducted mainly in adults **(Appendix G, Figure G.1b)**. The mean pooled effect (95% CI) is 0.38 kg/m^2 (0.10, 0.66).

In the four studies which investigated changes in WC, between-arm differences in added sugars intake ranged from 6 to 22 E% **(Appendix G, Figure G.1c)**. Within each dietary condition (i.e. ad libitum, under neutral energy balance), the two studies available showed changes in WC in opposite directions. One RCT was at low RoB (tier 1) and three RCTs were at moderate RoB (tier 2). The mean pooled effect (95% CI) is 0.25 cm (−0.47, 0.97; I^2 = 50%). Changes in WC were consistent with changes in body weight within each study conducted ad libitum, and consistent with changes in %BF within each study conducted under neutral energy balance.

The Panel considers that the available BoE suggests a positive relationship between the intake of added and free sugars and risk of obesity.

Comprehensive UA

Selection of the endpoint. Owing to the low number of studies having WC as an endpoint and the lower reliability of this measurement as compared to body weight, the Panel selected body weight as the key endpoint for the comprehensive UA in relation to sQ2.1 (**Table 13**).

Dose-response relationship. In the linear dose-response meta-regression analysis conducted by EFSA (**Annex L**), the intake of added or free sugars expressed as E% could not significantly explain the variability in the between-arm differences in body weight changes (the fit of the model measured by the Akaike information criteria (AIC), equal to 36.1, was not dissimilar from that of the model with no explanatory variables, AIC equal to 36.5). Thus, evidence does not support a linear dose-response relationship between the intake of added or free sugars as E% ad libitum and body weight change (estimated regression coefficient 0.0479, 95%CI: −0.0623; 0.1582, p = 0.3941). Consequently, the impact of other variables as possible modifiers of the effect was not explored. A non-linear dose-response was not investigated based on the graphical exploration of the data. Dose-response was not investigated in individual studies.

LoE3. Complementary: Body fat, abdominal fat. RCTs. Five studies assessed changes in %BF, of which two were at neutral energy balance and three ad libitum. Between-arm differences in added sugars intake ranged from 10 to 23 E% **(Appendix G, Figure G.1d)**. In all studies except one, %BF was higher in the high sugars arm relative to the low sugars arm at the end of the intervention relative to baseline. The mean pooled effect (95% CI) is 0.22% (−0.05, 0.50; I^2 = 0%). Changes in %BF were generally consistent with changes in body weight within each study conducted ad libitum, and consistent with changes in WC within each study conducted under neutral energy balance.

Consistency across LoEs. The Panel notes that changes in body weight were generally consistent with changes in WC and % BF within each study, but few RCTs investigated these endpoints.

Table 13: sQ2.1. RCTs. Comprehensive analysis of the uncertainties in the BoE and in the methods.

What is the level of certainty in a positive and causal relationship between intake of **added and free** sugars *ad libitum* and the risk of obesity at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE2. Standalone (surrogate). Endpoint:** body weight **11 RCTs, 1,328 participants.** Pooled mean effect estimate (95% CI) = 1.15 kg (0.53, 1.77) assuming a within-subject correlation coefficient of 0.82. The correlation coefficient for this endpoint is expected to be > 0.82. **(Appendix G, Figure G.1a)**.	**Initial certainty: High (> 75–100% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	6 studies in tier 1; 5 studies tier 2 (**Appendix I, Table I.1**) Between low and moderate Key questions: • Randomisation: low • Exposure assessment: generally low • Outcome assessment: mixed low and probably high Probably high for allocation concealment and blinding	Serious
Unexplained inconsistency	Low statistical heterogeneity (I^2 = 29% for the pooled mean effect). Mean effect estimates are similar across studies and 95%CI largely overlap.	Not serious
Indirectness	Surrogate endpoint	Serious
Imprecision	Low. It could be even lower because the correlation coefficient for this endpoint is expected to be > 0.82 **(Appendix G, Figure G.1a)**.	Not serious
Publication bias	Funnel plot suggests low risk of publication bias (**Appendix H, Figure H.1**). Public (n = 3), private (n = 3) and mixed (n = 4) funding (NR for one study).	Undetected
Upgrading factors	None identified	None
Final certainty	Started high, downgraded one level for indirectness. RoB was not considered sufficiently serious to downgrade because it was between low and moderate, and generally low for 2 out of the 3 key questions.	**Moderate (> 50–75% probability)**

Conclusion sQ2.1. RCTs. The level of certainty in a positive and causal relationship between the intake of added and free sugars and risk of obesity is **moderate** (rationale in **Table 13**). The studies were conducted ad libitum. Between-arm differences in added and free sugars intake were between 6 and 24 E%. Most RCTs were in overweight/obese adult subjects, and two were in children and adolescents.

8.2.2.2. Observational studies

Eight PCs investigated the association between added sugars (QUALITY, (Wang et al., 2014); NGHS, (Lee et al., 2015)), free sugars (DONALD, (Herbst et al., 2011); KoCAS, (Hur et al., 2015)), added and free sugars (Mr and Ms OS, (Liu et al., 2018) or sucrose (PHHP, (Parker et al., 1997); EPIC-Norfolk, (Kuhnle et al., 2015); NSHDS, (Winkvist et al., 2017)) and body weight, BMI or BMI z-scores. Of these, three also investigated WC (QUALITY, NGHS, EPIC-Norfolk), and three either BF, abdominal fat or both (DONALD, QUALITY, Mr and Ms OS). Evidence tables are in **Annex J**.

LoE2. Standalone (surrogate): Body weight/BMI, waist circumference. PCs. The four studies on added or free sugars were conducted in children (DONALD, QUALITY, NGHS, KoCAS), whereas the study on added and free sugars was in the older adults (Mr and Ms OS) and the three studies on sucrose were in adults (PHHP, EPIC-Norfolk, NSHDS).

Sugars intake was analysed as continuous variable in all the studies. Six PCs used either the nutrient residuals model (DONALD, EPIC-Norfolk) or the standard multivariable model (QUALITY, KoCAS, PHHP, NGHS) to adjust for TEI, and thus kept TEI constant. Two studies used the multivariable energy density model not including TEI as covariate (NSHDS, Mr and Ms OS).

Six studies investigated the association between added sugars, free sugars or sucrose intake at baseline and either the change in endpoint over follow-up (PHHP, QUALITY, Mr and Ms OS) or the endpoint at the end of follow-up (EPIC-Norkfolk, DONALD, KoCAS), while two studies investigated the association between change in added sugars or sucrose intake and change in endpoints over follow-up (NSHDS, NGHS).

Preliminary UA

Negative (DONALD, KoCAS, EPIC-Norfolk) or null (QUALITY, PHHP) associations between the intake of added sugars, free sugars or sucrose at baseline and measures of body weight are reported in all studies except one (Mr and Ms OS). Plot can be found in **Appendix K, Figure K.1a** (EPIC-Norfolk and PHHP could not be included). The EPIC-Norfolk study reported a positive association when sucrose in spot urine samples was used as a marker of sucrose intake. The direction of the associations observed with WC were consistent with those for body weight measurements within each study (QUALITY, NGHS, EPIC-Norfolk). Positive (NGHS) and negative (NSHDS) associations between changes in the intake of added sugars or sucrose and measures of body weight were reported. The Panel notes that in NSHDS and Mr and Ms OS, multivariable nutrient density models were applied without adjustment for TEI (NSHDS, Mr and Ms OS).

Two PCs were in RoB tier 1 (NGHS, QUALITY), five in tier 2 (DONALD, EPIC-Norfolk, PHHP, NSHDS, Mr and Ms OS) and one in tier 3 (KoCAS) for these endpoints. Confounding was a critical domain for all, except for those in tier 1, and attrition was a critical domain in all except Mr and Ms OS. The heat map for the RoB assessment is in **Appendix L, Table L.1a**.

The Panel notes that the available studies are heterogeneous in relation to the analytical strategies applied to investigate the relationship between added sugars, free sugars or sucrose and measures of BW and WC, i.e. baseline intake vs. change in intake analyses, and models used to account for TEI. Also, the heterogeneity of the studies with respect to the exposure–endpoint relationships investigated precludes the calculation of pooled mean effect estimates across studies, as evidence is sparse by type of relationship. Such relationships were mostly negative or null, regardless of the RoB tier, particularly in PCs using adequate statistical models to account for TEI. Therefore, the Panel considers that the available BoE does not suggest a positive relationship between the intake of added and free sugars in isocaloric exchange with other macronutrients and risk of obesity. **No comprehensive UA is performed**.

LoE3. Complementary: Body fat, abdominal fat. PCs. Of the above-mentioned studies, four had either %BF (DONALD and KoCAS; RoB tier 3), BF in kg (QUALITY, RoB tier 1), abdominal fat (kg) or a combination of these (Mr and Ms OS, RoB tier 2), as endpoints. The results for BF and abdominal fat were generally consistent with those for body weight/BMI and WC, respectively, within each study, except in KoCAS. Studies on BF (%) are plotted in **Appendix K, Figure K.1b**.

The Panel notes the heterogeneity of these studies with respect to the exposure–endpoint relationships investigated, that no clear pattern is observed with respect to the direction of the association and that changes in %BF were consistent with measures of body weight except in KoCAS (RoB tier 3).

The Panel considers that the available BoE does not suggest a positive relationship between the intake of added and free sugars in isocaloric exchange with other macronutrients and body fat.

Conclusion sQ2.1. PCs. The Panel considers that the available BoE from PCs does not suggest a positive relationship between the intake of added and free sugars in isocaloric exchange with other macronutrients and risk of obesity.

8.2.2.3. Overall conclusion on sQ2.1

There is evidence from RCTs for a positive and causal relationship between the intake of added and free sugars ad libitum and risk of obesity (**moderate** level of certainty). The available BoE from PCs cannot be used to modify the level of certainty in this conclusion.

8.2.3. Fructose

sQ3.1. Fructose and risk of obesity

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of obesity, incidence of abdominal obesity	0	0
LoE2. Standalone (surrogate)	Body weight/BMI, waist circumference	**2**	**2**
LoE3. Complementary	Body fat, abdominal fat	1	1

8.2.3.1. Intervention studies

LoE2. Standalone (surrogate). Body weight/BMI, waist circumference. RCTs. Two RCTs (Stanhope et al., 2009; Angelopoulos et al., 2015) assessed the effects of fructose and glucose in beverages at doses of 9 and 25E% in the respective studies. The studies were conducted ad libitum in overweight and obese males and females and lasted 10 and 8 weeks, respectively.

Preliminary UA. The consumption of fructose and glucose as beverages increased body weight significantly (all study arms combined) regardless of the type of sugar administered during the intervention with no differences between fructose and glucose in any of the two RCTs, which were at moderate RoB (tier 2). The pooled mean effect estimate is 0.02 kg (95% CI = −2.26, 2.29). The results of the individual studies are in **Appendix F**. Similar results were obtained for WC and BMI (Stanhope et al., 2009; Angelopoulos et al., 2015).

The Panel notes the limited number of studies available and that effect of fructose vs. glucose on body weight and WC was null. The Panel considers that the BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with glucose and risk of obesity. **No comprehensive UA is performed**.

LoE3. Complementary: Body fat, abdominal fat. RCTs. Results for %BF were consistent with those for body weight in the only study which reported on this outcome (Stanhope et al., 2009).

The Panel considers that the available BoE does not suggest a positive relationship between the intake fructose in isocaloric exchange with glucose and %BF.

Conclusion sQ3.1. RCTs. The Panel considers that the available BoE from RCTs does not suggest a positive relationship between the intake of fructose in isocaloric exchange with glucose and risk of obesity.

8.2.3.2. Observational studies

The relationship between the intake of fructose and changes in WC during follow-up was investigated in two prospective cohorts (SCES, (Gopinath et al., 2013); TLGS, (Bahadoran et al., 2017)), one of which (SCES) also assessed changes in BMI and %BF. These studies used either the nutrient residuals model (SCES) or the multivariable nutrient density model (TLGS) to account for TEI in the analyses, and thus aimed at investigating the relationship between fructose and the endpoints while keeping TEI constant. Evidence tables are in **Annex J**.

LoE2. Standalone (surrogate): Body weight/BMI, waist circumference. PCs. In the SCES cohort of children (RoB tier 2), separate analyses are given for males and females. For males, results refer to fructose at baseline by tertiles of intake, whereas for females, results refer to changes in fructose intake over the follow-up as continuous variable. Reasons for the different analysis by sex are unclear. The relationship between fructose intake and changes in BMI and WC over the 5-year follow-up was positive but non-significant in both sexes. In the TLGS cohort of adult males and females (RoB tier 2), the relationship between fructose intake at baseline and change in WC over the mean follow-up of 6.7 years was positive and statistically significant. The only variable considered for adjustment in the model was age.

Preliminary UA. The Panel notes that only two PCs are available and that, although both report a positive association between the intake of fructose and WC (significant in one), both studies are at moderate RoB (tier 2) for that endpoints. Critical domains were confounding and exposure (TLGS), and selective reporting (other sources of bias) and attrition (SCES).

The Panel considers that the available BoE from PCs does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and risk of obesity. **No comprehensive UA is performed**.

LoE3. Complementary: Body fat, abdominal fat. PCs. Only the SCES cohort (RoB tier 2) investigated the relationship between fructose intake (at baseline for males, as changes in intake over follow-up for females) and changes in %BF over the 5-year follow-up (positive, non-significant in both sexes).

The Panel considers that the available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and %BF.

Conclusion sQ3.1. PCs. The Panel considers that the available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and risk of obesity.

8.2.3.3. Overall conclusion on sQ3.1

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of fructose in isocaloric exchange with glucose or other macronutrients and risk of obesity. Fructose was not investigated under other dietary conditions (e.g. not keeping TEI constant).

8.2.4. Sugar-sweetened beverages

sQ4.1. SSBs and risk of obesity

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of obesity, incidence of abdominal obesity	0	**10**
LoE2. Standalone (surrogate)	Body weight/BMI, waist circumference	**6(+2)**	**21**
LoE3. Complementary	Body fat, abdominal fat	4	6

8.2.4.1. Intervention studies

LoE2. Standalone (surrogate): Body weight/BMI, waist circumference. RCTs. Among the RCTs which investigated the effect of high vs. low sugars intake ad libitum on body weight (discussed in Section 8.2.2.1), six assessed the consumption of SSBs vs. a sugar-free alternative. The between-group target difference in sugars intake from beverages was between 6 and 20E%. Studies lasted between 12 and 72 weeks and most (n = 5) were conducted in overweight/obese individuals (**Appendix F**).

Preliminary UA

At the end of the intervention, body weight was higher in the SSBs group relative to the sugar-free alternative in all studies. The effect was statistically significant in two studies. Three studies were at low RoB (tier 1) and three at moderate RoB (tier 2). The mean pooled effect (95% CI) is 0.82 kg (0.36, 1.29; I^2 = 0%) **(Appendix G, Figure G.1a)**.

Results for BMI in the four studies reporting on this outcome were in the same direction. Mean pooled effect (95% CI) is 0.29 kg/m^2 (0.06, 0.51, I^2 = 0%) **(Appendix G, Figure G.1b)**. Results for WC were as for added sugars (Section 8.2.2.1) because all four studies reporting on this outcome were conducted with beverages **(Appendix G, Figure G.1c)**.

The Panel considers that the available BoE suggest a positive relationship between the intake of SSBs as compared to a sugar-free alternative and risk of obesity.

Comprehensive UA

Selection of the endpoint. Owing to the low number of studies having WC as an endpoint and the lower reliability of this measurement as compared to body weight, the Panel selected body weight as the key endpoint for the comprehensive UA in relation to sQ4.1 for RCTs (**Table 14**).

Dose-response relationship. Dose-response relationships were not investigated in individual studies or by meta-regression analysis across studies, and there was no indication of a dose-response relationship by visual examination of the forest plot.

LoE3. Complementary: Body fat, abdominal fat. RCTs. Four studies assessed changes in % BF, of which two at neutral energy balance and two ad libitum **(Appendix G, Figure G.1d)**. In all studies except one, %BF was higher with high vs. low consumption of SSBs at the end of the intervention relative to baseline. Changes in %BF were generally consistent with changes in body weight within each study conducted ad libitum, and consistent with changes in WC within each study conducted under neutral energy balance.

Consistency across LoEs. The Panel notes that changes in body weight were generally consistent with changes in WC and % BF, but few RCTs investigated these endpoints.

Table 14: Q4.1. RCTs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty in a positive and causal relationship between intake of **SSBs** *ad libitum* and the risk of obesity at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE2. Standalone (surrogate). Endpoint: body weight** **6 RCTs, 1,036 participants.** Pooled mean effect estimate (95%CI) = 0.82 kg (0.36, 1.29) assuming a within-subject correlation coefficient of 0.82. The correlation coefficient for this endpoint is expected to be > 0.82. **(Appendix G, Figure G.1a).**	**Initial certainty: High (> 75–100% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	3 studies in tier 1; 3 studies tier 2 (**Appendix I, Table I.1**) Between low and moderate Key questions: • Randomisation: low • Exposure assessment: generally low • Outcome assessment: mixed low and probably high Probably high for allocation concealment and blinding	Serious
Unexplained inconsistency	Low statistical heterogeneity (I^2 = 0% for the pooled mean effect). Mean effect estimates are similar across studies and 95%CI largely overlap.	Not serious
Indirectness	Surrogate endpoint.	Serious
Imprecision	Low. It could be even lower because the correlation coefficient for this endpoint is expected to be > 0.82 **(Appendix G, Figure G1.a).**	Not serious
Publication bias	Funnel plot suggests low risk of publication bias (**Appendix H, Figure H.1**). Public (n = 2), private (n = 2) and mixed (n = 2) funding.	Undetected
Upgrading factors	None identified	None
Final certainty	Started high, downgraded one level for indirectness. RoB was not considered sufficiently serious to downgrade because it was between low and moderate, and generally low for 2 out of the 3 key questions.	**Moderate (> 50–75% probability)**

Conclusion sQ4.1. RCTs. The level of certainty in a positive and causal relationship between the intake of SSBs and risk of obesity is **moderate** (rationale in **Table 14**). The studies were conducted ad libitum using sugar-free alternatives as control. Between-arm differences in sugars intake from beverages were between 6 and 20 E%. Most RCTs were in overweight/obese subjects, and two were in children and adolescents.

8.2.4.2. Observational studies

LoE1. Standalone (main): Incidence of obesity, incidence of abdominal obesity. PCs

Incidence of obesity

Six PCs investigated the relationship between the intake of SSBs and incidence of overweight and/or obesity in non-overweight/obese individuals. Of these, four were in infants, toddlers and young children (DDHP (Lim et al., 2009); Amsterdam (Weijs et al., 2011); Generation R (Leermakers et al., 2015); ELEMENT (Cantoral et al., 2015)) and one in young adolescents of both sexes (PHI, (Ludwig et al., 2001)), whereas one was in adult black females (BWHS, (Boggs et al., 2013)). One study also investigated the association between the intake of ASBs and incidence of obesity (PHI). The evidence table is in **Annex J**.

Among the three PCs that analysed the exposure by categories of intake, BWHS did not adjust for TEI and ELEMENT adjusted for non-SSBs energy, and thus did not keep TEI constant. The exception was the Generation R, which standardised the exposure using the nutrient residuals model and included TEI as covariate. The remaining PCs performed continuous analyses using the standard multivariable model (DDHP, PHI) or the multivariable nutrient density model not including TEI as covariate (Amsterdam). All PCs adjust for baseline BMI except the three studies conducted in infants, which use either infant body weight (Amsterdam, Generation R) or maternal obesity at 12 months post-partum (ELEMENT) as a proxy.

Five PCs report a positive association between the intake of SSBs at baseline (BWHS, RoB tier 1; PHI and DDHP, RoB tier 2; Amsterdam, RoB tier 3) or the cumulative intake between 1 and 5 years of age (ELEMENT, RoB tier 3) and incidence of overweight and/or obesity (significant in 3 out of 5), whereas in one PC (Generation R, RoB tier 2), the association was positive in females and negative in males **(Appendix K, Figure K.2a)**. In the PHI, a significant positive association was reported for changes in intake of SSSDs over follow-up and incidence of obesity, whereas the association was negative for ASBs. The heat map for RoB assessment is in **Appendix L, Table L.2**.

Incidence of abdominal obesity

The relationship between the intake of SSBs and incidence of abdominal obesity was investigated in five PCs, one in infants (ELEMENT, (Cantoral et al., 2015)), one in children and adolescents (TLGS, (Mirmiran et al., 2015)) and three in adults of both sexes (Girona, (Funtikova et al., 2015); KoGES, (Kang and Kim, 2017); CARDIA, (Duffey et al., 2010)). Evidence table is in **Annex J**.

Four PCs analyse the intake of SSBs as categorial variable using the standard multivariable model and including either TEI (Girona, TLGS, KoGES) or non-SSBs energy (ELEMENT) as covariate, whereas one analysed the exposure as a continuous variable adjusting for non-SSBs energy (CARDIA). All studies adjust for either WC, BMI, body weight at baseline or maternal obesity at 12 months post-partum as a proxy (ELEMENT).

All PCs report a positive relationship (significant in 4 out of 5) between the intake of SSBs at baseline or the cumulative intake of SSBs over 4 years and incidence of abdominal obesity at the end of follow-up **(Appendix K, Figure K.2b)**. Two PCs were in RoB tier 1 (CARDIA, Girona), one in tier 2 (KoGES) and two in tier 3 (ELEMENT, TLGS). Heat map for RoB assessment is in **Appendix L, Table L.3**.

Preliminary UA

The Panel notes that all PCs report positive associations between the intake of SSBs and incidence of obesity and/or abdominal obesity (n = 10). The association was statistically significant in six out of the seven PCs which did not keep TEI constant in the analysis, and in one out of the three PCs which kept TEI constant in the analysis. Five PCs were in RoB tier 1, two in tier 2 and three in tier 3. Critical domains were confounding, exposure assessment and attrition.

The Panel considers that the available BoE from PCs suggests a positive relationship between the consumption of SSBs and risk of obesity, particularly when TEI is not kept constant in the analysis.

Comprehensive UA

Selection of the endpoint. The Panel notes that the overlap between the PCs that investigated incidence of obesity and incidence of abdominal obesity is limited to one study (ELEMENT). The Panel also notes that incidence of (whole body) obesity and abdominal obesity are closely related measures at a population level and show a similar relationship with disease risk. Therefore, the Panel considers that the evidence on both endpoints can be combined and addressed in the comprehensive UA. Pooled mean effect estimates, however, were not calculated because, out of the 10 PCs available, three PCs did not report the number of cases across categories of intake (Girona, TLGS, Generation R), one did not report the exposure as used for data analysis (CARDIA) and one assessed cumulative exposure over 4 years (ELEMENT) (**Appendix K, Figure K.3**).

Dose-response relationship. Linear dose-response relationships across categories of SSBs intake were explored in six PCs. Significant positive linear dose-response relationships were reported in three PCs (ELEMENT, TLGS, GIRONA). In the BWHS cohort the relationship was borderline significant, whereas no evidence for a dose-response relationship was reported in the Generation R and KoGES cohorts. The Panel notes that two out of the three PCs reporting a significant positive linear dose-response were at high RoB (tier 3). Dose-response relationships were not investigated by meta-regression analysis because the data required (e.g. number of cases, exposure) were not available for most PCs.

LoE2. Standalone (surrogate): Body weight/BMI, waist circumference. PCs. A total of 21 PCs investigated the relationship between the intake of SSBs and measures of body weight or BMI, five of which also report on measures of WC, whereas one cohort reports only on WC (EPIC-Diogenes). Evidence tables are in **Annex J**.

Ten PCs investigated the relationship between the intake of SSBs at baseline and measures of body weight or BMI, four of which were in adults and six in children and/or adolescents. Of these, eight analysed the exposure as continuous variable using the standard multivariable model (n = 6) or the

nutrient residuals model (n = 1), thus keeping TEI constant. One PC (CoSCIS) did not adjust for TEI **(Appendix K, Figure K.4a)**. The two PCs which analysed the exposure as categorical variable (not included in the forest plot) used the multivariable nutrient density model not including TEI as covariate (MIT-GDS) or the standard multivariable model (Framingham-3Gen), and thus did not keep TEI constant in the analysis.

Seven PCs (DCH, (Olsen et al., 2016); MONICA, (Olsen et al., 2016); AGAHLS, (Stoof et al., 2013); DONALD, (Libuda et al., 2008); HSS-DK, (Zheng et al., 2015); MIT-GDS, (Phillips et al., 2004); GUTS, (Berkey et al., 2004)) report positive associations (statistically significant in DCH and MIT-GDS) between the intake of SSBs and measures of body weight or BMI, whereas three report non-significant negative associations (Inter99, (Olsen et al., 2016); CoSCIS, (Jensen et al., 2013); Framingham-3Gen, (Ma et al., 2016b)). In the PCs which provide models with and without TEI as covariate (n = 7, **Appendix K**, **Figure K.4a**), the introduction of this factor in the model did not substantially change the estimates of the association.

Thirteen PCs investigated the relationship between change in SSBs intake and measures of body weight or BMI **(Appendix K, Figure K.4b)**. Seven were in children and/or adolescents (GUTS, (Berkey et al., 2004); GUTS II, (Field et al., 2014); NGHS, (Striegel-Moore et al., 2006); ALSPAC, (Bigornia et al., 2015); MOVE, (Carlson et al., 2012); DONALD, (Libuda et al., 2008); WAPCS, (Ambrosini et al., 2013)) and six in adults (MTC, (Stern et al., 2017); HPFS, NHS and NHS II (Pan et al., 2013); SUN, (Barrio-Lopez et al., 2013); WHI; (Auerbach et al., 2018)). Eleven PCs analysed change in SSBs intake as a continuous variable. Of these, four used the standard multivariable model (GUTS, NGHS), the nutrient residuals model (WHI) or the multivariable nutrient density model (DONALD) and thus kept TEI constant in the analysis, whereas seven did not adjust for TEI. The two PCs analysing change in SSBs intake as categorical variable used either the standard multivariable model (SUN) or did not adjust for TEI (WAPCS), and thus did not keep TEI constant.

All 13 PCs report positive relationships between changes in intake of SSBs and measures of body weight or BMI, and these were statistically significant in eight studies (WAPCS only in females), seven of which did not keep TEI constant and six of which adjusted for measures of BMI at baseline. Among the five PCs in which the relationship was not significant, three kept TEI constant and one adjusted for measures of BMI at baseline.

A total of nine PCs also addressed the relationship between the intake of ASBs and measures of body weight or BMI. Only in two studies such relationship was positive (GUTS, GUTSII), whereas the remaining seven PCs report either null or negative associations. In six out of these seven PCs, the relationship between intake of SSBs and measures of body weight or BMI was positive and statistically significant (HPFS, NHS, NHSII, HSS-DK, NGHS, MTC).

In the three PCs which investigated the intake of SSBs at baseline in relation to measures of WC (DCH and Inter 99 (Olsen et al., 2016); EPIC-DiOGenes (Romaguera et al., 2011)), the direction of the association was inconsistent **(Appendix K, Figure K.4c)**. TEI was kept constant in all studies and one PC adjusted for BMI. Conversely, the three PCs which assessed changes in SSBs intake (MTC, (Stern et al., 2017); ALSPAC, (Johnson et al., 2007); WAPCS, (Ambrosini et al., 2013)) report significant positive associations (WAPCS only in males) between the exposure and measures of WC **(Appendix K, Figure K.4d)**. None of these kept TEI constant and two adjusted for BMI. Measures of WC were generally consistent with measures of BMI within each study.

Of the 21 PCs considered in this LoE, nine were in RoB tier 1, six in tier 2 and seven in tier 3 for measures of body weight/BMI. The WAPCS was in RoB tier 1 for BMI and in RoB tier 2 for WC. The heat map for the RoB assessment is in **Appendix L**, **Table L.4a**.

The Panel notes that the analytical strategy undertaken to investigate the association between the intake of SSBs and measures of body weight, BMI and WC differs among the PCs available. Most PCs report positive (and significant) associations between the intake of SSBs at baseline or changes in SSBs consumption and the endpoints particularly when TEI was not kept constant in the analysis, and thus allowing for the contribution of SSBs to excess energy intake. In contrast, the relationship is non-significant, null or even negative when TEI is kept constant (i.e. when SSBs are investigated in isocaloric exchange with other dietary sources of energy).

The Panel considers that the available BoE suggests a positive relationship between the intake of SSBs and measures of body weight, BMI and WC when TEI is not kept constant in the analysis.

LoE3. Complementary: Body fat, abdominal fat. PCs. Only four of the above-mentioned PCs investigated measures of BF in relation to baseline intake of SSBs and the results were mixed. The relationship was negative (non-significant) in CoSCIS, DONALD (males) and AGAHLS (females), positive (non-significant) in females (MIT-GDS and DONALD) and positive and significant in the

AGAHLS cohort for males. Measures of BF were consistent with measures of BMI in the four cohorts (DONALD, CoSCIS and MIT-GDS, RoB tier 2; AGAHLS, RoB tier 3) which measured both endpoints, except for females in the AGAHLS and for males in DONALD (**Appendix K**, **Figure K.4a**). Conversely, the three PCs which assessed changes in SSBs consumption in relation to measures of BF report a positive association, which was statistically significant in two PCs (MOVE, RoB tier 3; ALSPAC, RoB tier 1). Measures of BF were consistent with measures of BMI in the three cohorts (**Appendix K**, **Figure K.4b**). In a separate publication reporting on the ALSPAC cohort (Johnson et al., 2007), there was a negative (non-significant) association between the intake of SSBs at baseline and body fat at end of follow-up.

Abdominal fat was only investigated in one PC (AGAHLS, **Appendix K**, **Figure K.4c**), and only in relation to baseline intake of SSBs, the results of which are mixed (positive and significant relationship for males, negative and non-significant relationship for females).

The Panel notes the limited data available on the association between the consumption of SSBs and measures of BF. The Panel also notes that measures of BF were generally consistent with measures of BMI in the few studies which assessed both endpoints.

Consistency across LoEs. The Panel notes that a large BoE suggests a positive relationship between the intake of SSBs and measures of body weight, BMI and WC when TEI is not kept constant in the analysis. Measures of BF were generally consistent with measures of BMI in the few studies which assessed both endpoints.

Table 15: sQ4.1. PCs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty in a positive and causal relationship between intake of **SSBs** and the risk of obesity at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE1. Standalone (main). Endpoints:** incidence of obesity and incidence of abdominal obesity **10 PCs, 32,282 participants.** Pooled mean effect estimates could not be calculated because the minimum dataset needed to calculate RRs per unit of intake was not available for about half of the PCs (**Appendix K**, **Figure K.3**)	**Initial certainty: Moderate (> 50–75% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	Three PCs in tier 1; 4 PCs in tier 2, 4 PCs in tier 3 (**Appendix L**, **Tables L.2 and L.3**) Generally moderate Key questions: • Confounding: mixed probably low and probably high • Exposure assessment: most probably high • Outcome assessment: most probably low Most probably high for attrition	Serious
Unexplained inconsistency	All PCs (n = 10) report positive relationships between the intake of SSBs and incidence of obesity and/or abdominal obesity.	Not serious
Indirectness	Direct endpoint	Not serious
Imprecision	Low in most studies	Not serious
Publication bias	Few studies available. RRs per unit of change in the exposure cannot be estimated for about half of the PCs. Risk of publication bias cannot be assessed. Public (n = 7) and mixed (n = 3) funding.	Undetected (cannot be assessed)
Upgrading factors	Consistency: a large BoE suggests a positive relationship between the intake of SSBs not keeping TEI constant in the analysis and measures of body weight, BMI and WC, whereas the relationship was null or negative for ASB in most of the PCs which also assessed this exposure (LoE2). Measures of BF where generally consistent with measures of BMI in the few studies which assessed both endpoints (LoE3).	Yes (consistency across LoEs)
Final certainty	Started moderate, decreased one level for RoB, increased one level for consistency across LoE	**Moderate (> 50–75% probability)**

Conclusion sQ4.1. PCs. The level of certainty in a positive and causal relationship between the intake of SSBs and risk of obesity is **moderate** (rationale in **Table 15**). The relationship was observed

not keeping TEI constant in the analysis, and thus allowing for the contribution of SSBs to excess energy intake.

8.2.4.3. Overall conclusion on sQ4.1

There is evidence from RCTs for a positive and causal relationship between the intake of SSBs ad libitum and risk of obesity (**moderate** certainty). The Panel considers that the available BoE from PCs (**moderate** certainty) can be used to upgrade this level of certainty to **high** (> 75–100% probability), considering that the main uncertainty in the BoE from RCTs was indirectness (downgrading factor).

8.2.5. Fruit juices

sQ5.1. FJs and risk of obesity

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of obesity, incidence of abdominal obesity	0	**2**
LoE2. Standalone (surrogate)	Body weight/BMI, waist circumference	0	**10**
LoE3. Complementary	Body fat, abdominal fat	0	3

8.2.5.1. Observational studies

LoE1. Standalone (main): Incidence of obesity, incidence of abdominal obesity. PCs. Among the 5 PCs which assessed SSBs in relation to the incidence of abdominal obesity, two (CARDIA, (Duffey et al., 2010); Girona, (Funtikova et al., 2015)) also investigated FJs. No PCs on FJs had incidence of obesity as endpoint. The evidence table is in **Annex J**.

Preliminary UA

Both cohorts report non-significant negative associations between the intake of FJs and incidence of abdominal obesity after adjustment for relevant covariates, including baseline BMI or WC, respectively (**Appendix K, Figure K.2b**). As for SSBs, FJs was analysed as categorial variable using the standard multivariable model to adjust for TEI (Girona) or as continuous variable adjusting for non-FJs energy intake (CARDIA). In both cases, TEI is not kept constant.

The Panel notes that the two studies available are at low RoB (tier 1) and report a non-significant negative relationship between the intake of FJs and incidence of abdominal obesity.

The Panel considers that the available BoE does not suggest a positive relationship between the intake of FJs and risk of obesity. **No comprehensive UA is performed on this LoE**.

LoE2. Standalone (surrogate): Body weight/BMI, waist circumference. **PCs**. Ten PCs investigated the association between the intake of FJs and body weight or BMI-related endpoints. Five cohorts included adults, three of which only females (WHI, (Auerbach et al., 2018); NHS and NHS II, (Pan et al., 2013)), one only males (HPFS, (Pan et al., 2013)) and one males and females combined (EPIC-DiOGenes, (Romaguera et al., 2011)). The remaining PCs were in children and/or adolescents, (GUTS, (Field et al., 2003); NGHS, (Striegel-Moore et al., 2006); MOVE, (Carlson et al., 2012); Project Viva, (Sonneville et al., 2015); DONALD, (Libuda et al., 2008)). All were US cohorts, except two (DONALD, Germany; EPIC-Diogenes, five European countries). Evidence tables are in **Annex J**.

Preliminary UA

Eight PCs (all except Project Viva and EPIC-DiOGenes) investigated changes in the exposure vs. concurrent changes in the endpoints as continuous variables. Of these, three adjusted for TEI using the standard multivariable model (GUTS, NGHS) or the nutrient residuals model (WHI), and thus kept TEI constant, whereas five did not adjust for TEI (HPFS, NHS, NHS II, MOVE) or adjusted for energy intake from other sources using an energy partition model (DONALD), not keeping TEI constant. Only the five PCs in adults and two PCs in children (GUTS, DONALD) adjusted for baseline BMI-related endpoints.

The four PCs in adults report statistically significant positive associations between changes in the intake of FJs and changes in body weight (HPFS, NHS, NHS II, WHI; RoB tier 1) **(Appendix K, Figure K.5)**. In two PCs in children, the association between changes in FJs intake and changes in BMI z-scores (MOVE) or BMI (NGHS) was not statistically significant (negative in MOVE and positive in NGHS; RoB tier 2). The Panel notes that these PCs did not adjust for baseline measures of BMI. In the

remaining two PCs in children, the association was positive and statistically significant for females (GUTS, RoB tier 2; DONALD, RoB tier 1). For males, the association was positive in GUTS and negative in DONALD (both non-significant). In GUTS and WHI, which introduced TEI stepwise in the multivariable models, adjustment for TEI did not substantially change the estimates of the association.

Three PCs (Project viva, DONALD, EPIC-DiOGenes) assessed FJs at baseline in relation to BMI z-scores or WC regressed to BMI. In the Project viva (RoB tier 3), which analysed categories of exposure using the standard multivariable model vs. BMI z-scores at the end of follow-up, the relationship was positive and statistically significant in the least adjusted model and after adjustment for BMI z-scores at baseline, but became non-significant when TEI was included in the model as covariate. Non-significant (negative in females, positive in males) associations were reported in DONALD (RoB tier 1) between baseline intake of FJs and change in BMI z-scores over follow-up. Similarly, a non-significant negative association was reported between the intake of FJs at baseline and annual changes in WC regressed to BMI in the EPIC-DiOGenes (RoB tier 3). These three PCs were at probably high RoB for confounding owing to the lack of adjustment for diet quality and physical activity.

The heat map for the RoB assessment can be found in **Appendix L**, **Table L.5**.

The Panel notes that seven out the eight PCs reported positive associations between changes in the intake of FJ and concurrent changes in body weight or BMI z-scores. The relationship was statistically significant in the four studies conducted in adults (3 cohorts in females, one cohort in males) and in two of the four studies conducted in children in females only. Conversely, non-significant positive and negative associations were reported in three PCs which addressed intakes of FJs at baseline and changes in BMI z-scores or WC regressed to BMI.

The Panel considers that the available BoE suggests a positive relationship between the intake of FJs and risk of obesity.

Comprehensive UA

Selection of the exposure and selection of the endpoint. The Panel decided to conduct the comprehensive UA on changes in FJs intake vs. concurrent changes in body weight (adults) and BMI z-scores (children) because of the higher number of studies available (vs FJs intake at baseline, vs. measures of WC) and owing to the consistency of the results across studies.

The Panel notes that the PCs investigated different exposure–endpoint relationships which were very heterogeneous both in terms of unit of change in exposure and definition of the endpoint. This precludes the calculation of pooled mean effect estimates across studies **(Appendix K**, **Figure K.5)**.

Dose-response relationship. Dose-response relationships across categories of intake were not investigated in any study. Dose-response relationships were not investigated by meta-regression analyses owing to the heterogeneity of the exposure–endpoints investigated.

LoE3. Complementary: Body fat, abdominal fat. **PCs**. Three PCs (all in children) investigated the association between the intake of FJs and BF. Two analysed intakes of FJs at baseline vs. body fat (kg) at the end of follow-up (ALSPAC, (Johnson et al., 2007); RoB tier 1) or vs. change in body fat (%) over follow-up (DONALD, (Libuda et al., 2008)) and two analysed changes in FJs intake vs. changes in body fat (%) over follow-up (DONALD, RoB tier 2; MOVE, (Carlson et al., 2012), RoB tier 3). All studies report negative (non-significant) relationships between the intake of FJs and the endpoints except the DONALD cohort for females only, where the relationship between changes in FJs intake and change in % body fat was positive (non-significant).

The Panel notes the limited data available on the relationship between the consumption of FJs and measures of BF. The Panel also notes that measures of body fat where generally consistent with measures of BMI in the only two studies which assessed both endpoints.

Consistency across LoEs. The Panel notes that changes in measures of body weight and BMI were consistent with measures of body fat (**LoE3**) but inconsistent with incidence of abdominal obesity in the few PCs which assessed these endpoints (**LoE1**).

Table 16: sQ5.1. PCs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty in a positive and causal relationship between intake of **FJs** and the risk of obesity at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE2. Standalone (surrogate). Endpoints: changes in body weight and BMI z-scores** **8 PCs, 191,881 participants.** Pooled mean effect estimates across studies cannot be calculated because of the heterogeneity of the exposure–endpoint relationships investigated **(Appendix K, Figure K.5)**. Most PCs found positive relationships between the intake of FJs and changes in the endpoints except for two children cohorts (MOVE, both sexes combined; DONALD, males only).	**Initial certainty: Low (> 15–50% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	Five PCs in tier 1; 3 PCs in tier 2 (**Appendix L**, **Table L.5**) Between low and moderate Key questions: • Confounding: mixed probably low and probably high • Exposure assessment: probably low • Outcome assessment: probably low Confounding was a critical domain in studies conducted in children, mostly because the lack of control for physical activity and the quality of the diet	Serious
Unexplained inconsistency	Inconsistency in the results of the two PCs in children (MOVE, DONALD) could be explained by differences in age, the type of analysis performed (e.g. by sex), sample size or by a combination of these factors.	Not serious
Indirectness	Surrogate endpoint	Serious
Imprecision	Low in most studies	Not serious
Publication bias	Few studies available, also heterogeneous. It cannot be assessed. Public (n = 6), mixed (n = 1) and unclear (n = 1) funding	Undetected (cannot be assessed)
Upgrading factors	None identified	None
Final certainty	Started low, downgraded for indirectness (one level). RoB was not considered sufficiently serious to downgrade because it was between low and moderate, and probably low for 2 out of the 3 key questions.	**Very low (0–15% probability)**

Conclusion sQ5.1. PCs. The level of certainty in a positive and causal relationship between the intake of FJs and risk of obesity is **very low** (rationale in **Table 16**).

8.2.5.2. Overall conclusion on sQ5.1

There is evidence from PCs for a positive and causal relationship between the intake of FJs and risk of obesity (**very low** level of certainty).

8.3. Risk of NAFLD/NASH

Standalone LoEs for the risk of NAFLD/NASH include studies reporting on the incidence of NAFLD/NASH (main LoE) and studies reporting changes in liver fat (surrogate LoE). The Panel decided to consider changes in skeletal muscle fat and visceral adipose tissue (VAT) in a complementary LoE because these two variables are reported in studies which investigate the effect of sugars on liver fat.

Ectopic fat deposition was an eligible endpoint in RCTs conducted ad libitum and in studies conducted in isocaloric conditions lasting at least 2 weeks if assessed by computed tomography (CT), magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS) or in biopsies.

For plotting, standardised mean differences were calculated for liver fat and VAT, owing to the different units of measurement in which these endpoints were reported in the RCTs and the lack of conversion factors. Data on skeletal muscle fat are not plotted due to lack of comparability across studies (i.e. biopsies were obtained from different muscles depending on the study).

8.3.1. Total sugars

sQ1.2. Total sugars and risk of NAFLD/NASH

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of NAFLD/NASH	0	**1**
LoE2. Standalone (surrogate)	Liver fat	0	0
LoE3. Complementary	Changes in skeletal muscle fat and visceral adipose tissue	0	0
LoE4. Complementary	Risk of obesity	sQ1.1	sQ1.1

8.3.1.1. Observational studies

LoE1. Standalone (main): Incidence of NAFLD/NASH. PCs. One PC investigated the relationship between the intake of total sugars and incidence of NAFLD/NASH. Evidence table is in **Annex J**.

Preliminary UA

In the ALSPAC cohort (Anderson et al., 2015), energy-adjusted total sugars intake (nutrient residuals model) at 3, 7 and 10 years of age was positively but not significantly associated with the risk of NAFLD at 17–18 years of age or with liver stiffness as a surrogate marker for NASH, either in the crude model or after adjustment for relevant confounders. Results were similar in sensitivity analyses restricting the sample to plausible reporters of dietary intake or to participants with a complete data set for all variables. The only dietary variable consistently and significantly positively correlated with these endpoints was total energy intake, and the association appeared to be mediated by total body fat at the time of the endpoint assessment. The study was at low RoB (tier 1) for both endpoints.

The Panel considers that the available BoE does not suggest a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of NAFLD/NASH. **No comprehensive UA is performed**.

8.3.1.2. Overall conclusion on sQ1.2

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of NAFLD/NASH. Total sugars were not investigated under other dietary conditions (e.g. not keeping TEI constant in the analysis).

8.3.2. Added and free sugars

sQ2.2. Added and free sugars and risk of NAFLD/NASH

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of NAFLD/NASH	0	0
LoE2. Standalone (surrogate)	Liver fat	**4**	0
LoE3. Complementary	Skeletal muscle fat and visceral adipose tissue	2/3	0
LoE4. Complementary	Risk of obesity	sQ2.1	sQ2.1

8.3.2.1. Intervention studies

The effect of high vs. low added sugar intakes on liver fat was assessed in four intervention studies (5 study groups), three of which (4 study groups) also investigated VAT and two of which also report on skeletal muscle fat (Maersk et al., 2012; Lowndes et al., 2014b) **(Appendix F)**.

LoE2. Standalone (surrogate): Liver fat. RCTs

Preliminary UA

Liver fat accrual was higher in the high sugar arm relative to the low sugar arm in all the studies which investigated this endpoint, three of which recruited exclusively overweight/obese individuals **(Appendix G, Figure G.2a)**. Between-arm differences in added and free sugar intakes ranged from

18 to 22 E%, and study duration between 10 and 24 weeks. Three studies used beverages and one foods and beverages. The increase in liver fat was similar among overweight subjects with and without NAFLD (Umpleby et al., 2017). The pooled standardised mean effect estimate (95%CI) was 0.66 (0.45, 0.86). The mean difference in body weight change between the high and the low sugar arms ranged from 0.85 to 2.3 kg regardless of whether the study aimed at neutral energy balance (i.e. and thus investigated added or free sugars in isocaloric exchange with other macronutrients, n = 2) or was conducted ad libitum (n = 2). In one study (Maersk et al., 2012) changes in liver fat were already adjusted for changes in body weight, suggesting an effect of added and free sugars on liver fat beyond any effect on body weight. Studies were at low to moderate RoB (1 in tier 1; 3 in tier 2).

The Panel considers that the available BoE from RCTs suggests a positive relationship between the intake of added and free sugars ad libitum and in isocaloric exchange with other macronutrients and risk of NALFLD/NASH.

Comprehensive UA

Selection of the endpoint. The only endpoint in this standalone LoEs is liver fat.

Dose-response relationship. No dose-response relationship between the intake of added sugars and liver fat was reported in one study which tested three sugar doses (8, 18 and 30E%) (Lowndes et al., 2014b). Dose-response was not investigated by meta-regression analysis owing to the low number of studies available. Visual inspection of the forest plot **(Appendix G, Figure G.2a)** does not suggest a dose-response relationship. The sugars dose range investigated (between-arm difference) is narrow (18–22E%).

LoE3. Complementary: Skeletal muscle fat and visceral adipose tissue. RCTs. Changes in Skm followed the same trend as liver fat in the two studies which assessed this variable (Maersk et al., 2012; Lowndes et al., 2014b). Changes in VAT followed the same trend as liver fat in overweight subjects without NAFLD, but no differences in VAT were observed between the high and the low sugar arms in subjects with NAFLD (Umpleby et al., 2017) **(Appendix G, Figure G.2b)**.

LoE4 (sQ2.1). **Complementary: risk of obesity. RCTs**. There is evidence from RCTs for a positive and causal relationship between the intake of added and free sugars ad libitum and an increased risk of obesity (**moderate** level of certainty).

Consistency across LoEs. The Panel notes that changes in skeletal muscle fat and VAT were consistent with changes in LF except for changes in VAT in subjects with NAFLD, but few RCTs investigated these endpoints. Consistent with an increased risk of obesity.

Table 17: sQ2.2. RCTs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty that the intake of **added and free sugars** is positively and causally associated with the risk of NAFLD/NASH at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE2. Standalone (surrogate). Endpoint: liver fat** **4 RCTs, 87 participants**. Pooled standardised mean effect estimate (95% CI) = 0.66 (0.45, 0.86) assuming a within-subject correlation coefficient of 0.82. The correlation coefficient for this endpoint is expected to be < 0.82. **(Appendix G, Figure G.2a)**.	**Initial certainty: High (> 75–100% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	1 study in tier 1; 3 studies tier 2 (**Appendix I, Figure I.2**) Generally moderate. Key questions: • Randomisation: low • Exposure assessment: generally low • Outcome assessment: generally low Probably high for allocation concealment, blinding and attrition	Serious
Unexplained inconsistency	Substantial statistical heterogeneity (I^2 = 67% for the pooled standardised mean effect). However, the number of studies is small, mean effect estimates are similar across studies and 95% CI largely overlap	Not serious

Indirectness	Surrogate endpoint for risk of NAFLD. Indirectness is bigger for risk of NASH.	Serious
Imprecision	Low. It could be higher because the expected correlation coefficient for this endpoint is < 0.82, but still low **(Appendix G, Figure G.2a)**.	Not serious
Publication bias	The few (n = 4) studies available are small (n = 7–13 subjects per arm) possibly due to the nature of the endpoint measured and all show significant effects, as illustrated in the funnel plot **(Appendix H, Figure H.2)**. It is unclear whether this is due to publication bias. Public (n = 1), private (n = 1) and mixed (n = 2) funding.	Undetected (it cannot be assessed)
Upgrading factors	None identified	None
Final certainty	Started high, downgraded one level for risk of bias and one level for indirectness	**Low (> 15–50% probability)**

Conclusion sQ2.2. RCTs. The level of certainty in a positive and causal relationship between the intake of added and free sugars and risk of NAFLD/NASH is **low** (rationale in **Table 17**). RCTs were in adults, mostly overweight/obese. Between-arm differences in added and free sugars were between 18 and 22E%, consumed *ad libitum* or in isocaloric exchange with other macronutrients.

8.3.2.2. Observational studies

There are no eligible PCs for standalone LoEs in relation to this sQ and there is no supportive evidence from complementary LoEs (sQ2.1, Section 8.3.1.2).

8.3.2.3. Overall conclusion on sQ.2.2

There is evidence from RCTs for a positive and causal relationship between the intake of added and free sugars *ad libitum* or in isocaloric exchange with other macronutrients and risk of NAFLD/NASH (**low** level of certainty). The available BoE from PCs cannot be used to modify the level of certainty in this conclusion.

8.3.3. Fructose

sQ3.2. Fructose and risk of NAFLD/NASH

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of NAFLD/NASH	0	0
LoE2. Standalone (surrogate)	Liver fat	**3**	0
LoE3. Complementary	Skeletal muscle fat and visceral adipose tissue	2/2	0
LoE4. Complementary	Risk of obesity	sQ3.1	sQ3.1

8.3.3.1. Intervention studies

LoE2. Standalone (surrogate): Liver fat. RCTs. Three RCTs (4 study groups) assessed the effects of fructose vs. glucose provided as beverages at doses from 22 to 25 E% on liver fat. The interventions lasted between 2 and 4 weeks (**Appendix F**).

Preliminary UA

The three studies showed lower liver fat accrual with fructose vs. glucose when fructose and glucose were consumed either ad libitum (Jin et al., 2014) or in positive energy balance (Silbernagel et al., 2011; Johnston et al., 2013). The opposite was observed in the study by (Johnston et al., 2013) under neutral energy balance. The effect was not statistically significant in any of the studies, which were at low to moderate RoB (2 in tier 1; 1 in tier 2) **(Appendix G, Figure G.3a)**. The pooled mean effect (standardised effect estimate) is −0.4 (95% CI = −0.20, 0.12). The Panel notes that the BoE is limited to three RCTs conducted under three different dietary conditions.

The Panel considers that the available BoE from RCTs does not suggest a positive relationship between fructose in isocaloric exchange with glucose and risk of NAFLD/NASH. **No comprehensive UA is performed**.

LoE3. Complementary: Skeletal muscle fat and visceral adipose tissue. RCTs. Similar results to liver fat were obtained for skeletal muscle fat (Silbernagel et al., 2011; Johnston et al., 2013). In relation

to VAT (2 studies), one (Stanhope et al., 2009) showed an increase in VAT with fructose relative to glucose in men only (sensitivity analysis by sex, **Appendix F**), whereas the second (Silbernagel et al., 2011) showed no difference between these two sugars **(Appendix G, Figure G.3b)**.

In the study by Johnston et al. (2013), conducted in males with abdominal obesity, both glucose and fructose (providing 25E% as beverages) increased liver fat and skeletal muscle fat when subjects were on positive energy balance, but not when these sugars were consumed under neutral energy balance. In the study by Silbernagel et al. (2011), no changes in liver fat or skeletal muscle fat were observed with either fructose or glucose on positive energy balance. The Panel notes that the BoE is limited to two RCTs, which show conflicting results.

The Panel considers that the available BoE from RCTs does not suggest a positive relationship between fructose in isocaloric exchange with glucose and ectopic fat deposition.

LoE 4 (sQ3.1). Complementary: Risk of obesity. RCTs. The available BoE from RCTs does not suggest a positive relationship between the intake of fructose in isocaloric exchange with glucose and risk of obesity.

Conclusion sQ3.2. RCTs. The Panel considers that the available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with glucose and risk of NAFLD/NASH.

8.3.3.2. Observational studies

There are no eligible PCs for standalone LoEs in relation to this sQ3.2. and there is no supportive evidence from complementary LoEs (sQ3.1, Section 8.3.3.2).

8.3.3.3. Overall conclusion on sQ3.2

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of fructose in isocaloric exchange with glucose or other macronutrients and risk of NAFLD/NASH.

8.3.4. Sugar-sweetened beverages

sQ4.2. SSBs and risk of NAFLD/NASH

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of NAFLD/NASH	0	0
LoE2. Standalone (surrogate)	Liver fat	**3**	0
LoE3. Complementary	Skeletal muscle fat/visceral adipose tissue	2/2	0/1
LoE4. Complementary	Risk of obesity	sQ4.1	sQ4.1

8.3.4.1. Intervention studies

LoE2. Standalone (surrogate): Liver fat. RCTs. Three out of the four RCTs which investigated the effect of high vs. low sugars intake on liver fat (Section 8.3.2.1) were conducted with beverages **(Appendix G, Figure G.2a)**. The between-arm target difference in sugars intake from beverages was between 18 and 22E% and study duration between 10 and 24 weeks.

Preliminary UA

Liver fat was significantly higher in the high vs. the low sugar arms in the three RCTs. One study was at low RoB (tier 1) and two at moderate RoB (tier 2). The pooled standardised mean effect estimate (95% CI) for these studies was 0.65 (0.31, 0.99, I^2 = 85%).

The Panel considers that the available BoE suggests a positive relationship between the intake of SSBs and risk of NAFLD/NASH.

Comprehensive UA

Selection of the endpoint. The only endpoint in this standalone LoE is liver fat.

Dose-response relationship. No dose-response relationship between the intake of sugars in beverages and liver fat was reported in one study using sucrose and HFCS in beverages at doses of 8, 18 and 30E% (Lowndes et al., 2014b). Dose-response was not investigated by meta-regression analysis owing to the low number of studies available. Visual inspection of the forest plot

(Appendix G, Figure G.2a) does not suggest a dose-response relationship, but the number of studies is small and the dose range investigated is narrow (18–22E%).

LoE3. Complementary: Skeletal muscle fat/visceral adipose tissue. RCTs. The two RCTs which investigated the effect of high vs. low sugars intake on skeletal muscle and two out of the three which reported on VAT (Section 8.3.2.1) were conducted with beverages **(Appendix G, Figure G.2b)**. In these studies, skeletal muscle fat and VAT were significantly higher in the high vs. the low sugar arm.

LoE4 (sQ4.1). **Complementary: Risk of obesity. RCTs**. There is evidence for a positive and causal relationship between the intake of SSBs and risk of obesity (**moderate** certainty).

Consistency across LoE. The Panel notes that changes in skeletal muscle fat and VAT were consistent with changes in LF except for changes in VAT in subjects with NAFLD, but few RCTs investigated these endpoints. Consistent with an increased risk of obesity.

Table 18: sQ4.2. RCTs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty that the intake of **SSBs** is positively and causally associated with the risk of NAFLD/NASH at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE2. Standalone (surrogate). Endpoint: liver fat** **3 RCTs, 70 participants**. Pooled standardised mean effect estimate (95% CI) = 0.65 (0.31, 0.99) assuming a within-subject correlation coefficient of 0.82. The correlation coefficient for this endpoint is expected to be < 0.82 **(Appendix G, Figure G.2a)**.	**Initial certainty: High (> 75–100% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	1 study in tier 1; 2 studies tier 2 (**Appendix I, Figure I.2**) Generally moderate. Key questions: • Randomisation: low • Exposure assessment: low • Outcome assessment: generally low Probably high for allocation concealment, blinding and attrition	Serious
Unexplained inconsistency	Substantial statistical heterogeneity (I^2 = 83% for the pooled standardised mean effect). However, the number of studies is small, mean effect estimates are similar across studies and 95% CI largely overlap.	Not serious
Indirectness	Surrogate endpoint for risk of NAFLD. Indirectness is bigger for risk of NASH	Serious
Imprecision	Low. It could be higher because the expected correlation coefficient for this endpoint is < 0.82, but still low.	Not serious
Publication bias	The few (n = 3) studies available are small (n = 7–13 subjects per arm) possibly due to the nature of the endpoint measured and all show significant effects, as illustrated in the funnel plot **(Appendix H, Figure H.2)**. It is unclear whether this is due to publication bias. Private (n = 1) and mixed (n = 2) funding.	Undetected (cannot be assessed)
Upgrading factors	None identified	None
Final certainty	Started high, downgraded one level for RoB and one level for indirectness	**Low (> 15–50% probability)**

Conclusion sQ4.2. RCTs. The level of certainty in a positive and causal relationship between the intake of SSBs and risk of NAFLD/NASH is **low** (rationale in **Table 18**). Most RCTs were conducted in overweight/obese subjects. Beverages were consumed ad libitum or under neutral energy balance and between arm differences in sugars from beverages were between 18 and 20E%.

8.3.4.2. Observational studies

No PCs were eligible for standalone LoEs in relation to sQ4.2.

LoE3. Complementary: Skeletal muscle fat/visceral adipose tissue. PCs. One PC (Framingham-3Gen, (Ma et al., 2016b)) investigated the relationship between the intake of SSBs at baseline and changes in VAT and VAT:SAAT ratio over the 6-year follow-up in adult males and females.

SSBs were analysed as categorical variable using the standard multivariable model for energy adjustment, thus not keeping TEI constant. The evidence table is in **Annex J**.

A significant positive linear dose-response relationship between the intake of SSBs and changes in VAT and the VAT:SAAT ratio was reported after adjusting for confounders, including changes in body weight, whereas no relationship was found with the intake of ASBs. The study was a low RoB (tier 1), the critical domain being the exposure assessment.

Although this study suggests a positive relationship between the consumption of SSBs not keeping TEI constant and ectopic fat deposition in VAT, the Panel notes that only one PC is available on this endpoint.

LoE4 (sQ4.1). **Complementary: Risk of obesity. PCs**. There is evidence for a positive and causal relationship between the intake of SSBs and risk of obesity (**moderate** certainty).

Conclusion sQ4.2. PCs. Although there is some evidence from PCs in complementary LoE that SSBs could increase the risk of obesity (**moderate** certainty, **LoE4 (sQ4.1)**) and ectopic fat deposition in VAT (**LoE3**), no PCs were eligible for standalone LoEs in relation to this sQ. Thus, the Panel considers that the available BoE does not suggest a positive relationship between the consumption of SSBs and risk of NAFLD/NASH.

8.3.4.3. Overall conclusion on sQ4.2

There is evidence from RCTs for a positive and causal relationship between the intake of SSBs ad libitum or under neutral energy balance and risk of NAFLD/NASH (**low** level of certainty). The available BoE from PCs cannot be used to modify the level of certainty in this conclusion.

8.3.5. Fruit juices

sQ5.2. FJs and risk of NAFLD/NASH			
LoE1. Standalone (main)	Incidence of NAFLD/NASH	0	0
LoE2. Standalone (surrogate)	Liver fat	0	0
LoE3. Complementary	Skeletal muscle fat and visceral adipose tissue	0	0
LoE4. Complementary	Risk of obesity (sQ5.1)	sQ5.1	sQ5.1

8.3.5.1. Observational studies

No PCs were eligible for standalone LoEs in relation to sQ5.2.

LoE4 (sQ5.1). Complementary: Risk of obesity. PCs. There is evidence for a positive relationship between the intake of FJs and risk of obesity (**very low** certainty).

Conclusion sQ5.2. **PCs**. The Panel considers that the available BoE does not suggest a positive relationship between the intake of FJs and risk of NAFLD/NASH.

8.3.5.2. Overall conclusion on sQ5.2

Since no studies were available for standalone LoEs in relation to this sQ, the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of FJs and risk of NAFLD/NASH.

8.4. Risk of type 2 diabetes mellitus

8.4.1. Total sugars

sQ1.3. Total sugars and risk of type 2 diabetes mellitus (T2DM)			
LoE	**Endpoints**	**RCTs (n)**	**PCs (n)**
LoE1. Standalone (main)	Incidence of T2DM	0	4*
LoE2. Standalone (surrogate)	Measures of glucose tolerance	0	1
LoE3. Complementary	Indices of insulin sensitivity/beta-cell function	0	0
LoE4. Complementary	Measures of insulin sensitivity	0	0
LoE5. Complementary	Risk of obesity	sQ1.1	sQ1.1

*: Of which one was a PCC.

8.4.1.1. Observational studies

LoE1. Standalone (main): Incidence of T2DM. PCs. Three PCs (FMCHES, (Montonen et al., 2007); WHS, (Janket et al., 2003); WHI, (Tasevska et al., 2018)) and one PCC (EPIC-Interact, (Sluijs et al., 2013)) investigated the relationship between total sugars and incidence of T2DM. The evidence table is in **Annex J**. Three studies analysed total sugars as categorical variable (EPIC-Interact, FMCHES, WHS) and one as continuous variable (WHI). Mean/median intakes of total sugars were 24.8 E% in the WHI and ranged between 65 g/day and 134–137 g/day in the EPIC-Interact and WHS, and between 92 and 171 g/day in the FMCHES (all energy-adjusted values) across categories of intake.

The multivariable nutrient density model (WHI) or the nutrient residuals model with (EPIC-Interact, FMCHES) or without (WHS) further adjustment for TEI were used to investigate total sugars while keeping TEI constant. In the WHI, energy partition models were also built to assess the full effect of total sugars intake on T2DM risk (i.e. the energy and non-energy contribution of the nutrient while keeping energy intake from other nutrients constant).

Preliminary UA

Three studies (EPIC-Interact, WHI, WHS) report significant negative associations between total sugars intake and incidence of T2DM in energy substitution models **(Appendix K, Figure K.6)**. The associations were attenuated in all cohorts after adjustments for relevant covariates, including baseline BMI and/or TEI, and remained statistically significant in the WHI only. Similar results were obtained using energy partition models in the WHI cohort (results not plotted). In contrast, the FMCHES reports a non-significant positive association between the intake of total sugars and incidence of T2DM, with a relative risk of 1.42 (95% CI = 0.90, 2.24) for the highest vs. the lowest quartile of energy-adjusted total sugars intake. The relationship was observed at higher levels of total sugars intake as compared to the other PCs.

Similar results were found in the four studies described above when cases of T2DM diagnosed in the first 2–4 years of follow-up and/or cases of hypertension, dyslipidaemia and/or CVD at baseline were excluded in sensitivity analyses to address reverse causality.

Two studies were at low RoB (tier 1; FMCHES, WHS) and two were at moderate RoB (tier 2; EPIC-Interact, WHI), critical domains being outcome assessment (n = 3), attrition (n = 2) and confounding (n = 1). The heat map for the RoB assessment is in **Appendix L, Table L.6**.

The Panel notes that three out the four studies available report a negative relationship between the intake of total sugars in isocaloric exchange with other macronutrients and incidence of T2DM. In one study, negative relationships were also reported when the full effect (the energy and non-energy components) of total sugars was assessed (energy partition models).

The Panel considers that the available BoE from PCs does not suggest a positive relationship between the intake of total sugars and incidence of T2DM. **No comprehensive UA is performed on this LoE**.

LoE2. Standalone (surrogate): Measures of glucose tolerance. PCs. Only one PC investigated the relationship between the intake of total sugars and measures of glucose tolerance (Feskens et al., 1995). The evidence table is in **Annex J**.

Preliminary UA

In a 20-year follow-up of a random sample from the Seven Countries cohort (Feskens et al., 1995) including 338 males from the Netherlands and Finland, a non-significant negative relationship was reported between the intake of total sugars at baseline and blood glucose concentrations at 2 h during an OGTT at the end of follow-up. A non-significant positive association was observed when change in total sugar intake over follow-up was used as the exposure variable. The multivariable nutrient density model was used to adjust for TEI. The study was at moderate RoB (tier 2), critical domains being confounding and attrition.

The Panel considers that the available BoE from PCs does not suggest a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and adverse effects on measures of glucose tolerance. **No comprehensive UA is performed**.

LoE5 (sQ1.1). Complementary: Risk of obesity. PCs. The available BoE does not suggest a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of obesity.

8.4.1.2. Overall conclusion on sQ1.3

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of total sugars (as net intake or in isocaloric exchange with other macronutrients) and risk of T2DM.

8.4.2. Added and free sugars

sQ2.3. Added and free sugars and risk of Type 2 diabetes mellitus

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of T2DM	0	**4***
LoE2. Standalone (surrogate)	Measures of glucose tolerance	**17**	**2**
LoE3. Complementary	Indices of insulin sensitivity/beta-cell function	5	2
LoE4. Complementary	Measures of insulin sensitivity	7	0
LoE5. Complementary	Risk of obesity	sQ2.1	sQ2.1

*: Of which one was a PCC.

8.4.2.1. Intervention studies

LoE2. Standalone (surrogate): Measures of glucose tolerance. RCTs. Ten RCTs assessed the effect of high vs. low intakes of added sugars on blood glucose at 120′ during an OGTT, eight of which were conducted in isocaloric exchange with starch under neutral energy balance and two were ad libitum **(Appendix G, Figure G.4a)**. The same studies except Huttunen et al. (1976) also measured insulin at 120′ **(Appendix G, Figure G.4b)**. Between-arm differences in added sugar intakes ranged from 10 to 54 E%, and study duration between 1 and 56 weeks. Six RCTs were in healthy subjects, two were in overweight/obese individuals and two included individuals with hyperinsulinaemia **(Appendix F)**.

Seventeen studies (19 groups) assessed the effect of high vs. low added and free sugars intake (8–43E%) on fasting glucose, of which nine were conducted in isocaloric exchange with starch under neutral energy balance and eight were ad libitum **(Appendix G, Figure G.4c)**. Most of these also measured fasting insulin **(Appendix G, Figure G.4d)**. Study duration ranged from 4 to 36 weeks. Eight RCTs were in overweight/obese individuals and two RCTs included subjects with hyperinsulinaemia.

Preliminary UA

Results for blood glucose and insulin at 120′ during an OGTT were mixed and apparently unrelated to the difference in added sugars intake between the study arms **(Appendix G, Figures G.4a and G.4b)**. An additional study (Lewis et al., 2013) not included in the forest plots (values for glucose and insulin at 120′ not shown in the publication) reported no significant differences in the iAUC for glucose ad insulin during the OGTT between the high and the low sugar arms (18 E% difference). The only two studies showing a significant effect of added sugars on glucose at 120′ were restricted to subjects with hyperinsulinaemia (Israel et al., 1983) or included a group of subjects with hyperinsulinaemia (Hallfrisch et al., 1983a). The only RCTs showing a significant effect of added sugars on insulin at 120′ was restricted to overweight/obese individuals. These RCTs used either fructose (Hallfrisch et al., 1983a) or sucrose (Israel et al., 1983; Lewis et al., 2013) in isocaloric exchange with starch. In the study by Israel et al. (1983), conducted in men and women with hyperinsulinaemia, glucose and insulin responses during the OGTT significantly increased with increasing doses of sucrose (2E%, 15E % and 30E% in isocaloric exchange with starch) in a dose-response manner (Appendix F). The Panel notes that these individuals were at high risk for developing T2DM. Five RCTs were in RoB tier 1 and five in tier 2. Critical domains were randomisation, allocation concealment and blinding. The Panel notes that these individuals were at high risk for developing T2DM. Five RCTs were in RoB tier 1 and five in tier 2. Critical domains were randomisation, allocation concealment and blinding.

Fasting glucose was higher in the high sugar arm relative to the low sugar arm in 11 of the 17 studies, whereas the effect of the intervention was null in three studies and negative in the remaining three studies **(Appendix G, Figure G.4c)**. The mean pooled effect (95% CI) is 1.94 mg/dL (0.23, 3.66; I^2 = 87%). The mean pooled effect (95% CI) for studies in isocaloric exchange with other macronutrients (starch in most studies) at neutral energy balance is 3.01 mg/dL (0.41, 5.60; I^2 = 89%), and for studies conducted ad libitum is 0.48 mg/dL (−1.48, 2.44; I^2 = 79%).

Similar results were obtained for fasting insulin **(Appendix G, Figure G.4d)**. The mean pooled effect (95% CI) is 16.21 ρmol/L (3.91, 28.50; I^2 = 93%). The mean pooled effect (95% CI) for studies in isocaloric exchange with starch at neutral energy balance is 19.99 ρmol/L (0.67, 39.31; I^2 = 93%), and 7.58 ρmol/L (1.04, 14.12; I^2 = 34%) for studies ad libitum.

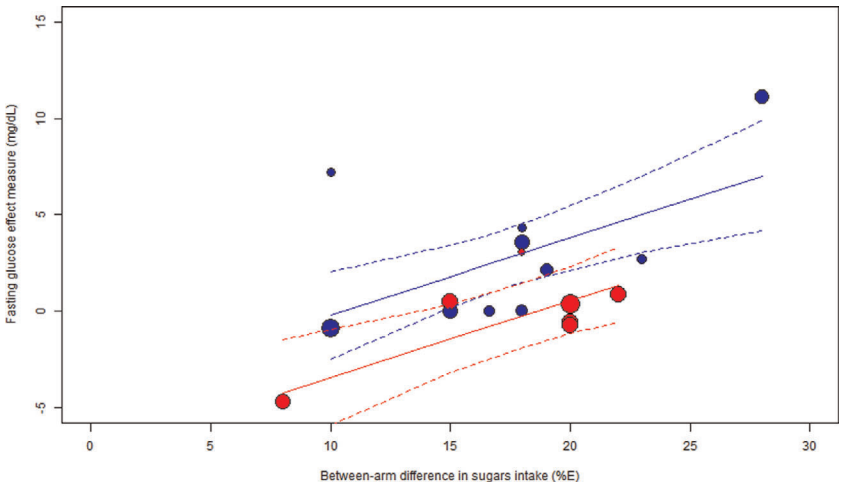
The Panel considers that the available BoE suggest a positive relationship between the intake of added and free sugars and risk of T2DM.

Comprehensive UA

Selection of the endpoint. Within this LoE2, which includes two surrogate endpoints for the risk of T2DM (fasting glucose and glucose at 120' during an OGTT), the Panel decided to perform a comprehensive UA on fasting blood glucose owing to: (a) the higher number of studies available, particularly in ad libitum conditions; (b) the consistency of the results across studies; and c) to the higher reliability of the measurement, as the type of sugar used in the OGTT challenge (sucrose vs. glucose) and the amount of sugar given (fixed vs. relative amounts depending on body weight) varied across studies **(see Appendix F)**.

Dose-response relationship. A linear dose-response relationship was observed between the intake of sucrose at doses 2, 15 and 30 E% in isocaloric exchange with starch and fasting glucose and insulin levels in the RCT by Israel et al. (1983) conducted in men and women with hyperinsulinaemia.

A meta-regression linear dose-response analysis was performed to investigate the association between the difference in sugars intake between arms (dose range 6–43%) and the corresponding difference in fasting glucose. A total of 19 observations from 18 RCTs were eligible for the analysis. Potential effect-modifiers were identified using a graphical display of the stratified dose-response curves. These include main characteristics of the exposure (i.e. sugars source and type, dietary conditions) and methodological aspects related to study design and duration, run-in and RoB. The only adjusting factor retained in the final model was RoB, owing to the best fit performance (AIC = 75) and the statistical significance of the parameters. Residual heterogeneity remains high (Cochran Q-test = 43.26) and statistically significant ($p < 0.0001$) for the best fitting model, suggesting that other factors not identified in the BoE, or for which it was not possible to adjust due to the low number of studies, might play a role in explaining differences across studies. Several diagnostics, the Hat indicator, the Cook distance and the influence analysis (One-At-a-Time leave out analysis), identified one study (Moser et al., 1986), conducted on the subgroup of young women taking contraceptives, as highly influential because of the high sugars dose and the particularly small size of the effect. Since the results of the study-subgroup were counter-conservative (i.e. very low responses at high doses), and their impact was to flatten the dose-response, it was decided to exclude the observation from the dose-response analysis. Despite not being influential and showing a pattern fitting well the model, also the other sub-group (women not taking contraceptives) from the same study was dropped from the analysis because randomisation was performed for the two sub-groups combined. Therefore, the final dose-response model was set up on 17 observations from 17 RCTs (**Figure 12**). The difference in sugars intake between arms in the final model was between 6 and 30 E%. The model indicates an expected increase of around 4 mg/dL (95% CI: 1.7–6.3, $p < 0.01$) of blood fasting glucose levels per each increase of 10E% intake from sugar. Adjusting for RoB leads to higher absolute fasting glucose mean expected levels for the same dose of sugars intake when considering RCTs at low RoB (tier 1; intercept = −4.2mg/dL, 95% CI = −8.4, 0.03) as compared to RCTs at moderate RoB (tier 2; intercept = −7.4, 95% CI = −13.91, −0.95). Between-arm differences in sugars intake (E%) and RoB only accounted for 25.6% of the variability across studies, thus leaving most of the heterogeneity unexplained. In this context, the Panel considers that this analysis can be used to conclude on the direction of the linear dose-response relationship, but not to make a quantitative prediction of the effect of added or free sugars on fasting glucose levels. A meta-regressive non-linear dose-response relationship was also investigated using a cubic spline function with three knots. Non-linearity was supported by the model. The shape of the non-linear dose-response was monotonically positive. However, the AIC showed a slightly better fit for the linear model, which was retained.



Blue = RoB Tier 1; Red = RoB Tier 2.

Figure 12: Meta-regressive dose-response linear model between the intake of added and free sugars (E%) and fasting glucose

A series of linear and non-linear dose-response models were explored for assessing the relationship between the difference in sugars intake between arms and the corresponding difference in fasting insulin changes during the intervention. All the models were highly sensitive to one study and to other methodological choices (i.e. hypothesised level of the correlation between observations at beginning and end of the intervention). Therefore, none of them was considered sufficiently robust to be used for drawing conclusions on the shape and strength of the dose-response relationship.

The full report of the dose-response analyses can be found in **Annex L**.

LoE3. Complementary: Indices of insulin sensitivity/beta-cell function. RCTs. Among the above-mentioned studies reporting on fasting glucose and insulin and/or glucose and insulin during an OGTT, five (Raben et al., 2002; Maersk et al., 2012; Campos et al., 2015; Lowndes et al., 2015; Umpleby et al., 2017) also report on indices of insulin sensitivity/insulin resistance (HOMA-IR, n = 5; ISI indices during an OGTT, n = 2) and/or indices of beta-cell function (HOMA-β, n = 1) **(Appendix F)**.

No significant differences were observed in any of these indices between the high and the low sugar arms in any study. The Panel notes that changes in glucose and insulin (fasting conditions or during an OGTT) were also not significantly different between the high and low sugar arms in these studies. Three studies were in RoB tier 1 and two were in tier 2. Critical domains were allocation concealment and blinding.

LoE4. Complementary: Measures of insulin sensitivity. RCTs. A total of seven RCTs investigated the effect of high vs. low added sugars intake on measures of insulin sensitivity **(Appendix F)**. In five studies, an euglycaemic hyperinsulinaemic clamp was performed to assess insulin sensitivity in steady-state conditions (Black et al., 2006; Le et al., 2009; Aeberli et al., 2013; Lewis et al., 2013; Schwarz et al., 2015), whereas two studies were conducted in non-steady state conditions using an IVITT (Beck-Nielsen et al., 1978) or a stable labelled intravenous glucose tolerance test (SLIVGTT, (Sunehag et al., 2008)). The testing conditions (e.g. one vs. two or three-step clamps, insulin infusion rates), the endpoint variables used to assess insulin sensitivity, the dietary conditions (i.e. isocaloric with neutral or positive energy balance, hypercaloric, ad lib*itum) and* the type of sugar assessed (e.g. sucrose, fructose) varied from study to study. All RCTs were in young or middle age adults (4 in males and 3 in males and females) and had a duration between 1 and 6 weeks.

Higher intakes of sucrose in mixed diets (25 E% vs. 10 E% and 15 E% vs. 5 E%) had no effect on whole-body insulin sensitivity (glucose disposal) or hepatic insulin sensitivity (suppression of endogenous glucose production) in steady-state conditions (euglycaemic hyperinsulinaemic clamp) and neutral energy balance (Black et al., 2006; Lewis et al., 2013), whereas sucrose (32 E%) decreased whole-body insulin sensitivity in non-steady state conditions (IVITT) and positive energy balance as compared to fat (Beck-Nielsen et al., 1978).

Fructose given as beverages significantly decreased hepatic insulin sensitivity (euglycaemic hyperinsulinaemic clamp) at intakes of 20 E% in isocaloric exchange with starch on neutral energy

balance in non-obese males (Schwarz et al., 2015), at intakes of 35E% in hypercaloric conditions in subjects with and without family history of type 2 diabetes (Le et al., 2009) and at intakes of 16 E% when consumed ad libitum as compared to sucrose or glucose given in the same amounts or to fructose given at 8E% in normal weight males (Aeberli et al., 2013). In these studies, whole body glucose disposal was generally not affected. No significant differences were observed in whole body insulin sensitivity (SLIVGTT) or indices of insulin secretion between high (24E%) and low (6E%) intakes of fructose in mixed diets on neutral energy balance in the only study performed in adolescents (Sunehag et al., 2008).

Five studies were at low RoB (tier 1: Black et al. (2006); Sunehag et al. (2008); Le et al. (2009); Aeberli et al. (2013); Lewis et al. (2013)) and two were at moderate RoB (tier 2: Beck-Nielsen et al. (1978); Schwarz et al. (2015)). Critical domains were allocation concealment and blinding.

The Panel considers that the available BoE suggests an adverse effect of fructose given as beverages for short periods of time (1–6 weeks) on hepatic insulin sensitivity in isocaloric exchange with other carbohydrates (glucose, starch) regardless the dietary conditions in which fructose is consumed. This effect is generally not observed on measures of whole-body insulin sensitivity or with comparable amounts of sucrose. The Panel notes that, whereas the effect is observed at intakes of 16 E% and above (lowest dose tested), the available RCTs do not allow identifying a level of fructose intake, either alone or in combination with glucose, at which the risk is not increased.

LoE5 (sQ2.1). **Complementary: Risk of obesity. RCTs**. There is evidence from RCTs for a positive and causal relationship between the intake of added and free sugars ad libitum and an increased risk of obesity (**moderate** level of certainty).

Consistency across LoEs. The Panel notes that changes in fasting glucose were consistent with changes in fasting insulin but less consistent with other measures of glucose tolerance and with measures of insulin sensitivity/resistance in the few and heterogeneous RCTs available on these endpoints. Consistent with an increased risk of obesity.

Table 19: sQ2.3. RCTs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty in a positive and causal relationship between intake of **added and free** sugars and the risk of T2DM at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE2. Standalone (surrogate). Endpoint: fasting glucose 17 RCTs, 935 participants.** Pooled mean effect estimate (95% CI) = 1.94 mg/dL (0.23, 3.66); assuming a within-subject correlation coefficient of 0.82. Considering that blood glucose levels are under homeostatic control in non-diabetic subjects, the correlation coefficient for this endpoint is expected to be > 0.82. **(Appendix G, Figure G.4c)**.	**Initial certainty: High (> 75–100% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	11 studies in tier 1; 6 studies in tier 2 (**Appendix I, Figure I.3**) Generally low Key questions: • Randomisation: low • Exposure assessment: generally low • Outcome assessment: generally low Probably high for allocation concealment and blinding	Not serious
Unexplained inconsistency	High heterogeneity (I^2 = 87%) for the pooled mean effect estimate. Point estimates vary widely, and 95% CI show minimal overlap. Residual heterogeneity in dose-response analysis remained high and statistically significant. Between-arm difference in sugars intake (E%) plus RoB only accounted for 34.4% of the variability across studies.	Very serious
Indirectness	Surrogate endpoint	Serious
Imprecision	Low. It could be even lower because the correlation coefficient for this endpoint is expected to be > 0.82	Not serious

Publication bias	Funnel plot shows a slight association between the magnitude of the effect and the SE, and Egger's test was significant (p = 0.004), suggesting a small risk of publication bias (**Appendix H**, **Figure H.3**). However, there is some indication for true heterogeneity in small studies. Public (n = 3), private (n = 6), mixed (n = 4) and NR (n = 4) funding.	Undetected
Upgrading factors	Dose-response: The dose-response meta-regression analysis conducted by EFSA showed that an increase of at least 11E% from sugar is needed to predict a positive effect on fasting glucose. Any further increase of 10E% from sugar leads to an increase of 4 mg/dL in fasting glucose (linear dose-response).	Yes (dose-response)
Final certainty	Downgraded two levels for unexplained inconsistency and one level for indirectness. Upgraded one level for dose-response.	**Low (> 15–50% probability)**

Conclusion sQ2.3. RCTs. The level of certainty in a positive and causal relationship between the intake of added and free sugars and risk of T2DM is **low** (rationale in **Table 19**). RCTs included only adults. About half of the RCTs were in overweight/obese subjects and two were limited to (or included a group of) hyperinsulinaemic individuals. Added and free sugars were consumed ad libitum or in isocaloric exchange with other macronutrients and between-arm differences in added and free sugars intake were between 8 and 43 E%.

8.4.2.2. Observational studies

LoE1. Standalone (main): Incidence of T2DM. PCs. The relationship between sucrose and incidence of T2DM was investigated in four PCs (EPIC-Norfolk, (Ahmadi-Abhari et al., 2014); FMCHES, (Montonen et al., 2007); MDCS, (Sonestedt et al., 2012); WHS, (Janket et al., 2003)). The MDCS cohort also reports on added sugars from all sources. Three PCs analysed sucrose as categorical variable (FMCHES, MDCS, WHS) and one both as categorical and continuous variable (EPIC-Norfolk). The multivariable nutrient density model (EPIC-Norfolk, MDCS) or the nutrient residuals model with (FMCHES) and without (WHS) further adjustment for TEI were used to investigate sucrose while keeping TEI constant. In the EPIC-Norfolk cohort, energy partition models were also built to assess the full effect of sucrose on T2DM risk (i.e. keeping energy intake from other nutrients constant). The evidence table is in **Annex J**.

Preliminary UA

Three PCs report either a non-significant negative (EPIC-Norfolk, WHS) or no (MDCS) association between sucrose intake while keeping TEI constant and incidence of T2DM **(Appendix K**, **Figure K.7)**. Similar results were obtained using energy partition models in the EPIC-Norfolk cohort (results not plotted). In contrast, the FMCHES cohort reports a non-significant positive association between the intake of sucrose and incidence of T2DM, with a relative risk of 1.22 (95% CI = 0.77, 1.92) for the highest vs. the lowest quartile of energy-adjusted sucrose intake (most adjusted model), with no apparent dose-response relationship. Similar results were found in EPIC-Norfolk, WHS and FMCHES when cases of T2DM diagnosed in the first 2–4 years of follow-up and/or cases of hypertension, dyslipidaemia and/or CVD at baseline were excluded in sensitivity analyses to address reverse causality. In the MDCS cohort, a significant negative relationship between the intake of added sugars and incidence of T2DM became non-significant when BMI was included in the model as covariate (**Annex J)**.

Three PCs were at low RoB (Tier 1; Epic-Norfolk, FMCHES, WHS) and one at moderate RoB (Tier 2; MDCS). The heat map for the RoB assessment is in **Appendix L**, **Table L.7**.

The Panel notes that these studies were inconsistent in the direction of the association and that in three out of the four PCs the relationship was null or negative. The Panel considers that the available BoE does not suggest a positive relationship between the intake of added or free sugars in isocaloric exchange with other macronutrients and incidence of T2DM. **No comprehensive UA is performed on this LoE**.

LoE2. Standalone (surrogate): Changes in glucose tolerance. PCs. Two PCs assessed the relationship between the intake of added sugars (QUALITY, (Wang et al., 2014)), or sucrose (CARDIA, (Folsom et al., 1996)), and changes in glucose tolerance. The QUALITY study investigated the relationship between the baseline intake of added sugars from solids and from liquids and changes in fasting glucose and insulin over a follow-up of 2 years in children 8–10 years of age. Results for added

sugars from all sources are not reported. The CARDIA cohort of young adults investigated the relationship between changes in sucrose intake and concurrent changes in fasting insulin over the 7-year follow-up.

In the QUALITY cohort, added sugars from solids and from liquids were analysed as continuous variables using the standard multivariable model to adjust for TEI. In the CARDIA cohort, sucrose was analysed as a continuous variable using repeated measures analysis, without adjustment for TEI. The evidence table is in **Annex J**.

Preliminary UA

Baseline intake of added sugars from solid foods was not associated with changes in fasting glucose or fasting insulin over follow-up in the QUALITY cohort. A significant positive relationship was found, however, between the intake of added sugars from liquid sources at baseline and changes in fasting glucose and insulin over follow-up. For each 10 g/day increase in added sugars from liquids, mean fasting glucose increased by 0.039 mmol/L (95% CI: 0.015, 0.063, $p < 0.01$) and mean fasting insulin by 2.261 ρmol/L (95% CI: 0.676, 3.845, $p < 0.01$).

In the CARDIA cohort, changes in sucrose were not associated with changes in fasting insulin over the follow-up, with the exception of white females, where a significantly inverse association was found; for each 6E% from sucrose there was a fasting insulin decrease of 0.7 μU/mL (spread values not reported) over the follow-up.

Both studies were at moderate RoB (Tier 2), critical domains being attrition (QUALITY only) and other sources of bias (selective reporting). Confounding was a critical domain in the CARDIA only (**Annex K**).

The Panel notes that added sugars from all sources were not investigated in the QUALITY cohort. The Panel considers that the available BoE does not suggest a positive relationship between the intake of added or free sugars in isocaloric exchange with other macronutrients and measures of glucose tolerance. **No comprehensive UA is performed on this LoE**.

LoE3. Complementary: Changes in indices of insulin sensitivity/beta-cell function. PCs. In the QUALITY cohort (Wang et al., 2014), baseline intake of added sugars from solid foods was not associated with changes in the HOMA-IR[15] index or the Matsuda-IS index[16] over follow-up. A significant positive relationship was found, however, between the intake of added sugars from liquid sources at baseline and changes in the HOMA-IR index and the Matsuda-ISI. For each 10 g/day increase in added sugars from liquids at baseline, mean HOMA-IR was +0.091 (95% CI: 0.034, 0.149, $p < 0.01$) and mean Matsuda-IS index was −0.356 (95% CI: −0.628, −0.084, $p < 0.01$), suggesting an increase in hepatic and whole-body insulin resistance (RoB tier 2). Conversely, in the DONALD cohort of adolescents followed up for 12.6 years (Goletzke et al., 2013b), baseline intake of free sugars from all sources or from liquid sources only was not associated with HOMA-IR or HOMA-β at the end of follow-up (RoB tier 1).

The Panel notes from the limited number of studies available that the direction of the relationship is inconsistent across studies for added and free sugars from liquids, and that free sugars from all sources were not associated with adverse effects on indices of insulin sensitivity/resistance or beta-cell function. The Panel considers that the available BoE does not suggest a positive relationship between the intake of added or free sugars in isocaloric exchange with other macronutrients and indices of insulin sensitivity/resistance or beta-cell function.

LoE5 (sQ2.1) Complementary: Risk of obesity. PCs. The available BoE does not suggest a positive relationship between the intake of added or free sugars in isocaloric exchange with other macronutrients and risk of obesity.

Conclusion sQ2.3. PCs. The available BoE does not suggest a positive relationship between the intake of added or free sugars in isocaloric exchange with other macronutrients and risk of T2DM.

8.4.2.3. Overall conclusion on sQ2.3

There is evidence from RCTs for a positive and causal relationship between the intake of added and free sugars and risk of T2DM (**low** certainty). The available BoE from PCs cannot be used to modify the level of certainty in this conclusion.

[15] Fasting plasma glucose (mmol/L) x fasting plasma insulin (pmol/L)/22.5.
[16] 10,000/square root [(fasting plasma glucose x fasting plasma insulin) x (mean OGTT glucose 3 mean OGTTinsulin)].

8.4.3. Fructose

sQ3.3. Fructose and risk of Type 2 diabetes mellitus

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of T2DM	0	**3***
LoE2. Standalone (surrogate)	Changes in glucose tolerance	**10**	0
LoE3. Complementary	Changes in indices of insulin sensitivity/beta-cell function	5	1
LoE4. Complementary	Changes in insulin sensitivity	6	0
LoE5. Complementary	Risk of obesity	sQ3.1	sQ3.1

*: Of which one was a PCC.

8.4.3.1. Intervention studies

LoE2. Standalone (surrogate): Changes in glucose tolerance. RCTs. The effect of fructose vs. glucose on fasting glucose was investigated in eight RCTs (of which seven also measured fasting insulin) under different dietary conditions (neutral energy balance, positive energy balance, ad libitum) and in different population groups (with NGT or IGT, with NAFLD, overweight/obese, with BMI $< 35 kg/m^2$, healthy subjects) at doses between 9 and 42.5 E% **(Appendix G, Figures G.5a and G.5b)**. Two additional studies (Hallfrisch et al., 1983a; Swanson et al., 1992) assessed the effect of different doses of fructose in isocaloric exchange with starch on fasting glucose, one of which (Hallfrisch et al., 1983a) also reported on fasting insulin **(Appendix G, Figures G.4c and G.4d)**. Finally, the effect of fructose vs. glucose on glucose and insulin at 120' during an OGTT was investigated at doses of 15E% in mixed diets under neutral energy balance (Koh et al., 1988) and at doses of 25E% given as beverages ad libitum (Stanhope et al., 2009) (**Appendix F**).

Preliminary UA

The results of RCTs comparing fructose in isocaloric exchange with glucose were mixed. Overall fasting glucose was lower in three studies (4 arms) and higher in five studies with fructose than with glucose. The pooled mean effect estimate (95% CI) was −2.67 mg/dL (−6.46, 1.11). Results for fasting insulin followed a similar pattern (pooled mean effect estimate and 95% CI = −0.77 ρmol/L and −20.07, 18.53) except in the study by Jin et al. (2014) in adolescents with NAFLD, where fructose intake (20E%) significantly increased fasting insulin and decreased fasting glucose as compared to glucose when consumed ad libitum in beverages.

The study by Hallfrisch et al. (1983a) showed no effect of fructose in solid foods at 15 E% as compared to starch on fasting glucose and no difference between hyper- and normo-insulinaemic subjects. Fasting insulin, however, was significantly higher with fructose vs. starch though only in hyperinsulinaemic individuals. No significant differences in fasting glucose were noted between fructose at similar levels of intake (16.6 E%) and starch in the study by Swanson et al. (1992) conducted in healthy subjects.

No effect of fructose vs. glucose was reported on glucose or insulin at 120' during an OGTT at doses of 15 and 25 E% in the two studies that assessed this endpoint (Koh et al., 1988; Stanhope et al., 2009).

The Panel considers that the available BoE does not suggest an adverse effect of fructose on measures of glucose tolerance when consumed in isocaloric exchange with other carbohydrates (glucose, starch). **No comprehensive UA is performed**.

LoE3. Complementary: Changes in indices of insulin sensitivity/beta-cell function. RCTs. A total of five RCTs investigated the effects of fructose vs. glucose from beverages at doses from 9 to 25 E% on indices of insulin sensitivity/resistance (**Appendix F**). Changes in the HOMA-IR did not differ significantly between the fructose and glucose arms in the five studies which assessed this endpoint (Stanhope et al., 2009; Silbernagel et al., 2011; Jin et al., 2014; Mark et al., 2014; Lowndes et al., 2015). The Matsuda ISI, calculated from glucose and insulin values during an OGTT, significantly decreased in both arms with no differences between fructose and glucose in positive energy balance (Silbernagel et al., 2011), but decreased significantly more in the fructose arm when both sugars in beverages were provided ad libitum (Stanhope et al., 2009). In the latter RCTs, the increase in body weight was similar in the glucose and fructose arms, whereas the increase in total fat and VAT was significantly higher in the fructose vs. the glucose arm.

The Panel considers that the available BoE does not suggest an adverse effect of fructose on indices of insulin sensitivity/resistance when consumed in isocaloric exchange with glucose under controlled energy conditions.

LoE4. Complementary: Changes in insulin sensitivity. **RCTs**. Three studies investigated the effect of fructose vs. glucose given as beverages on measures of insulin sensitivity, two in steady-state conditions using the euglycaemic hyperinsulinaemic clamp (Aeberli et al., 2013; Johnston et al., 2013) and one in non-steady state conditions using an IVITT (Beck-Nielsen et al., 1980) (Appendix F). The effect of fructose was also investigated in studies providing different amounts of fructose *ad libitum* (Aeberli et al., 2013), in isocaloric exchange with starch (Sunehag et al., 2008; Schwarz et al., 2015), in hypercaloric conditions (Le et al., 2009) and in isocaloric exchange with sucrose (Aeberli et al., 2013). The results of these studies are discussed in Section 8.4.2.1 under LoE 4 for added and (free) sugars.

The Panel considers that the available BoE suggests an adverse effect of fructose given as beverages for short periods of time (1–6 weeks) on hepatic insulin sensitivity in isocaloric exchange with other carbohydrates (glucose, starch) regardless the dietary conditions in which fructose is consumed. This effect is generally not observed on measures of whole-body insulin sensitivity. The Panel notes that, whereas the effect is observed at intakes of 16 E% and above, the available RCTs do not allow identifying a level of fructose intake at which the risk is not increased.

LoE5. Complementary: risk of obesity. RCTs. The available BoE from RCTs does not suggest a positive relationship between the intake of fructose in isocaloric exchange with glucose and risk of obesity.

Conclusion sQ3.3. RCTs. Whereas there is some evidence for an adverse effect of fructose on hepatic insulin sensitivity when consumed in isocaloric exchange with other carbohydrates (glucose, starch), which could eventually lead to hyperinsulinaemia and in the long term to the development of T2DM, the RCTs available do not suggest an adverse effect of fructose on measures of glucose tolerance. Therefore, the Panel considers that the available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other carbohydrates (glucose, starch) and risk of T2DM.

8.4.3.2. Observational studies

LoE1. Standalone (main): Incidence of T2DM. **PCs**. The relationship between the intake of free fructose and free glucose (as mono-saccharides) and incidence of T2DM was investigated in three cohorts, one of females (WHS, (Janket et al., 2003)) and two of males and females combined (EPIC-Norfolk, (Ahmadi-Abhari et al., 2014)); FMCHES, (Montonen et al., 2007)). The Epic-Norfolk was a PCC. Free fructose and free glucose were analysed as categorical variables in all the studies. The dose ranges covered were similar across the PCs, with intakes of free fructose being slightly higher than those of free glucose in all the studies. The multivariable nutrient density model (Epic-Norfolk) or the nutrient residuals model with (FMCHES) and without (WHS) further adjustment for TEI were used to investigate free fructose and glucose while keeping TEI constant. The evidence table is in **Annex J**.

Preliminary UA

The results of these studies were mixed. In the most adjusted models including TEI and baseline BMI, the incidence of T2DM significantly increased across categories of free fructose intake (from lowest to highest) in the FMCHES cohort and significantly decreased in the EPIC-Norfolk cohort. No association between free fructose intake and incidence of T2DM was observed in the WHS **(Appendix K, Figure K.8)**. Similar results were obtained for free glucose, although the negative relationship reported in the EPIC-Norfolk cohort was not statistically significant for this exposure **(Appendix K, Figure K.9)**. The three PCs were at low RoB (tier 1).

In the EPIC-Norfolk cohort, free fructose and free glucose were also analysed using the nutrient residuals and the standard multivariable models for energy adjustment, obtaining similar results. Using the multivariable nutrient density model and modelling specific substitution patterns, replacement of free fructose with other carbohydrates did not affect the risk of T2DM, whereas replacement of saturated fatty acids and protein with an isocaloric amount of fructose significantly decreased the risk of T2DM. This was also the case when the energy partition model was used, where higher intakes of free fructose and free glucose were negatively associated with T2DM risk while keeping energy intake from other macronutrients constant.

The Panel notes the low number of PCs available and the inconsistency of the results across studies. The Panel considers that the available BoE from PCs does not suggest a positive relationship

between the intake of fructose in isocaloric exchange with glucose or other macronutrients and incidence of T2DM.

LoE3. Complementary: Changes in indices of insulin sensitivity/beta-cell function. PCs. The relationship between the intake of fructose and indices of insulin resistance was investigated only in the TLGS cohort of males and females in Iran (Bahadoran et al., 2017). Fructose intake at baseline (E%, continuous analysis) was positively associated with an increase in fasting insulin and HOMA-IR over follow-up. This study was at high RoB (tier 3). The only covariate included in the model for data analysis was age.

The Panel considers that the available BoE from PCs does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and adverse effects on indices of insulin resistance.

Conclusion sQ3.3. PCs. The available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with glucose or other macronutrients and risk of T2DM.

8.4.3.3. Overall conclusion on sQ3.3

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of fructose in isocaloric exchange with other carbohydrates (glucose, starch) or other macronutrients and risk of T2DM.

8.4.4. Sugar-sweetened beverages

sQ4.3. SSBs and risk of Type 2 diabetes mellitus

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of T2DM	0	**14***
LoE2. Standalone (surrogate)	Measures of glucose tolerance	**7**	**1**
LoE3. Complementary	Indices of insulin sensitivity/beta-cell function	3	2
LoE4. Complementary	Measures of insulin sensitivity	3	0
LoE5. Complementary	Risk of obesity	sQ4.1	sQ4.1

*: Of which one was a PCC.

8.4.4.1. Intervention studies

LoE2. Standalone (surrogate): Measures of glucose tolerance. RCTs. Out of the 17 RCTs which investigated the effect of high vs. low added and free sugars intake on fasting glucose (see Section 8.4.2.1), seven were conducted with beverages **(Appendix G, Figure G.4c2)**. Pooled mean effect estimates (95% CI) for sugars from different sources were 0.82 mg/dL (−1.46, 3.10) for beverages (n = 7, dose range = 8–22E%), 0.67 mg/dL (−0.77, 2.12) for mixtures of food and beverages (n = 7, 8 study groups, dose range = 10–23E%) and 6.63 mg/dL (0.52, 12.75) for solid foods (n = 3, 4 study groups, dose range = 15–43E%). The Panel notes that, although the pooled effect estimates vary across food sources, the 95% CI overlap. The Panel also notes that the sugar doses investigated were different across food sources, and that the study by Moser et al. (1986) using 43 E% in solid foods was dropped from the dose-response meta-regression analysis (leverage point).

In the dose-response meta-regression analysis conducted by EFSA (technical report in **Annex L**), the sugar source was not found to be a significant modifying factor of the dose-response relationship, although the BoE had obvious limitations to test this hypothesis owing to the low number of studies which used solid foods only. The Panel also notes that the conclusions on complementary LoEs 3 and 4 for added and free sugars were mainly driven by studies conducted with beverages.

Based on the available BoE from RCTs, the Panel has the same level of certainty on a positive and causal relationship between the intake of SSBs and risk of T2DM as for added and free sugars (**low** certainty).

Conclusion sQ4.3. RCTs. The level of certainty in a positive and causal relationship between the intake of SSBs and risk of T2DM is **low**.

8.4.4.2. Observational studies

LoE1. Standalone (main): Incidence of T2DM. PCs. The relationship between the intake of SSBs and incidence of T2DM was investigated in 14 studies, of which 13 were PCs and one was a PCC

study (EPIC-InterAct; InterAct consortium, 2013). These include three PCs in which the endpoint was high fasting glucose (> 100 or 110 mg/dL, depending on the study) or the use of hypoglycaemic medications (CARDIA, KoGES, TLGS) and one PC which investigated incidence of pre-diabetes and incidence of T2DM as a composite endpoint (Framingham Offspring).

Three PCs included only females (BWHS, (Palmer et al., 2008); NHS II, (Schulze et al., 2004); WHI, (Huang et al., 2017)); two included only males (HPFS (de Koning et al., 2011); Toyama (Sakurai et al., 2014)); in three PCs, males and females were analysed separately (KoGES, (Kang and Kim, 2017); JPHC (Eshak et al., 2013); ARIC (Paynter et al., 2006)) and the remaining studies were on males and females combined (FMCHES, (Montonen et al., 2007); CARDIA, (Duffey et al., 2010); EPIC-InterAct (InterAct consortium, 2013); Framingham Offspring, (Ma et al., 2016a); MDCS, (Ericson et al., 2018); TLGS, (Mirmiran et al., 2015)). All the studies were in adults, except for the TLGS (children and adolescents 6–18 years of age). Six of these studies (Framingham Offspring, HPFS, NHS II, Toyama, WHI, EPIC-InterAct) also investigated the association between the intake of ASBs and incidence of T2DM.

All studies analyse the intake of SSBs as categorial variable using the standard multivariable model to adjust for energy except CARDIA, which analyses the exposure as a continuous variable adjusting for non-SSBs energy. In both cases, the analysis allows for TEI to change as a function of SSBs consumption. The EPIC-InterAct also analyses the exposure as a continuous variable adjusting for TEI. All studies include BMI (or body weight in CARDIA) as covariate in the most adjusted models. The evidence table is in **Annex J**.

Preliminary UA

A positive relationship between the consumption of SSBs and incidence of T2DM was observed in 13 out of the 14 studies considered (ARIC, BWHS, FMCHES, KoGES, MDCS, TLGS, Toyama; statistically significant in EPIC-InterAct, Framingham Offspring, HPFS, NHS II, WHI and JPHC in females only), whereas the relationship was null in the CARDIA and in the JPHC for males. The forest plot for the 13 studies in adults can be found in **Appendix K**, **Figure K.10**. The TLGS cohort in children and adolescents is not included (number of cases was not reported).

The association between the consumption of SSBs and incidence of T2DM was attenuated when BMI was included in the model as an additional variable after adjusting for relevant covariates in four (BWHS, EPIC-InterAct, MDCS, NHSII) out of the eight studies which tested this hypothesis (exceptions were Framingham-Offspring, Toyama, HPFS and TLGS), suggesting that the relationship may be in part mediated by BMI.

Out of the six studies which addressed the relationship between ASBs and incidence of T2DM, the association was weaker than for SSBs in five (Framingham Offspring, HPFS, NHS II, WHI, EPIC-InterAct) and non-significant in four (EPIC-InterAct, Framingham Offspring, HPFS, NHS II), whereas one PC reported a stronger and statistically significant association as compared to SSBs (Toyama). The Panel notes that the relationship between ASBs and incidence of T2DM in these studies is inconsistent and generally weaker than for SSBs.

Five studies were in RoB tier 1 (ARIC, BWHS, Framingham Offspring, HPFS, Toyama), six were in tier 2 (CARDIA, EPIC-InterAct, FMCHES, JPHC, NSH II, TLGS) and three were in tier 3 (KoGES, MDCS, WHI). The heat map can be found in **Appendix L**, **Table L.8**.

The Panel considers that the available BoE suggests a positive relationship between the consumption of SSBs and risk of T2DM.

Comprehensive UA

Selection of the endpoint. The only eligible endpoint in this LoE1 is incidence of T2DM. As anticipated in the protocol for this scientific opinion, the definition of T2DM and the methods used for the identification of cases varied from study to study. True incidence of T2DM may have been underestimated in some studies (e.g. when cases were identified through drug reimbursement records only) and overestimated in others (e.g. when high fasting glucose below the diagnostic threshold for diabetes and diagnosis or treatment of diabetes were combined in composite endpoints).

Dose-response relationship. A significant linear dose-response relationship across categories of SSBs intake was originally reported in eight (BWHS, FMCHES, EPIC-InterAct, Framingham Offspring, HPFS, NHS II, JPHC in women only, WHI) of the 13 studies which performed a categorical analysis. Upon request for additional data from the study authors of EPIC-InterAct, individual country-specific cohort risk estimates were included in the dose-response analysis.

In the dose-response meta-analysis conducted by EFSA, parametric dose-response models were estimated based on summarised data. Both linear and non-linear (restricted cubic splines) dose-response relationships were investigated. Random-effects models were fitted on risk ratios from most adjusted multivariable models via restricted maximum likelihood using a one-stage and a two-stage approach (to estimate individual studies pooled effects across exposure categories). The reference dose chosen was zero mL/day. The between-study heterogeneity was investigated with Cochran's Q test and the I^2 statistic; to explore possible sources of heterogeneity, adjusted study-specific RRs per 250 mL/day increase in intake were stratified by age, sex, study location, categorisation of exposure, follow-up time and tier of reliability. Sensitivity analyses were run to address the uncertainty in the exposure characterisation, in the choice of splines knots and in the internal validity of the individual studies. Publication bias was assessed using Egger's test and funnel plot on the same study-specific RRs used in the subgroup analyses.

Fifty-five non-referent RRs from 19 study-specific analyses were included (I^2 = 51%; p = 0.001) in the dose-response analysis. The TLGS (number of cases not reported), BWHS (model diagnostics) and CARDIA (RR already provided per unit increase) cohorts were excluded. The predicted pooled relative risk of T2DM was 1.13 (95% CI: 1.07, 1.20) for an increase in SSBs intake of 250 mL/day in the linear model (p for linear trend < 0.0001) and 1.13 (95% CI: 1.07, 1.20) at 250 mL/day in the non-linear model (RCS with three knots at fixed percentiles, 10%, 50% and 90%, of the distribution; p for non-linearity = 0.816) (**Figure 13**). The subgroup analyses did not identify clear sources of heterogeneity: there was a suggestion that the risk was higher in subjects younger than 55 years old; in Asian populations; in cohorts with longer follow-up; in RoB tier 2 studies. A sensitivity analysis excluding RoB tier 3 studies confirmed no evidence of departure from linearity (p = 0.295) and showed higher RRs estimates (1.15 (95% CI: 1.06, 1.24); 1.19 (95% CI: 1.09, 1.29)), narrower exposure range and improved fitting. The funnel plot and related Egger's regression suggested the possibility of a 'small-study effect' (larger effects in PCs where RRs are more imprecise). This can be interpreted as publication bias (e.g. study results not published or not located) or can be explained by actual heterogeneity (e.g. differences in the underlying risk across populations), outcome reporting or poor quality of small studies. In this case, the Panel considers that the 'small-study effect' can be explained by true heterogeneity. The PC driving the asymmetry of the funnel plot was a cohort of Finnish males and females (FMCHES) with very low incidence of T2DM. The technical report and all related references are in **Annex J**.

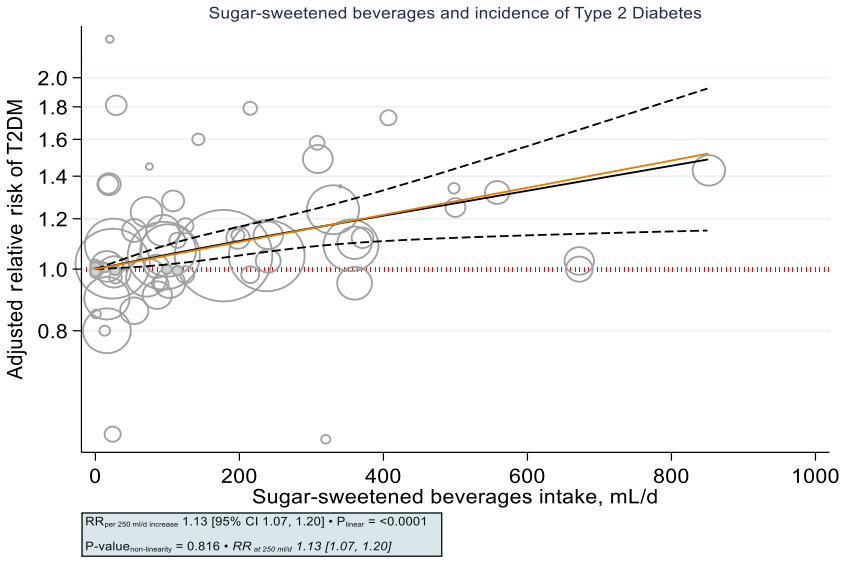


Figure 13: Dose-response meta-analysis on the relationship between the intake of sugar-sweetened beverages and incidence of type 2 diabetes mellitus (T2DM)

LoE2. Standalone (surrogate): Measures of glucose tolerance. PCs. One PC (WAPCS, (Ambrosini et al., 2013)), investigated the relationship between changes in SSBs intake and concurrent changes in fasting glucose and fasting insulin over the 3-year follow-up. Change in SSBs intake was analysed as a categorical variable and TEI was not adjusted for (WAPCS). The evidence table is in **Annex J**.

Non-significant negative associations were reported for changes in fasting glucose and fasting insulin in the highest vs. lowest tertile of increase in SSBs intake in males and females after adjusting for BMI and major dietary patterns.

The study was at low RoB (tier 1), the critical domain being attrition (**Annex K**).

The Panel notes the limited evidence available from PCs. The Panel considers that the available BoE does not suggest a positive relationship between intake of SSBs and measures of glucose tolerance.

LoE3. Complementary: Indices of insulin sensitivity/resistance or beta-cell function. **PCs**. The Framingham-Offspring (Ma et al., 2016a) investigated the relationship between the cumulative intake of SSBs and HOMA-IR at end of follow-up, while the WAPCS (Ambrosini et al., 2013) investigated changes SSBs intake and concurrent changes in HOMA-IR over the follow-up (**Annex J**). The Framingham-Offspring reports a positive and significant relationship between SSBs intake and insulin resistance, whereas the WAPCS reports a negative non-significant association for changes in HOMA-IR across tertiles of increase in SSBs intake over the follow-up. In the Framingham-Offspring, no relationship was observed between the intake of ASBs and HOMA-IR. Both PCs were at low RoB (tier 1), the critical domains being attrition (WAPCS) and confounding (Framingham-Offspring) (**Annex K**).

The Panel notes from the limited number of studies available that the direction of the relationship is inconsistent across studies. The Panel considers that the available BoE does not suggest a positive relationship between the intake of SSBs and indices of insulin resistance.

LoE5 (sQ4.1). **Complementary: Risk of obesity. PCs**. There is evidence for a positive and causal relationship between the intake of SSBs and risk of obesity (**moderate** certainty).

Consistency across LoE. The Panel notes an increased incidence of T2DM is consistent with an increased risk of obesity. However, few PCs assessed endpoints for other LoEs specific to this sQ (e.g. measures of glucose tolerance, indices of insulin sensitivity/resistance or beta-cell function).

Table 20: sQ4.3. PCs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty in a positive and causal relationship between intake of **SSBs** and the risk of T2DM at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE1. Standalone (main). Endpoint:** incidence of T2DM **13 PCs and 1 PCC, 338,007 participants.** 19 study-specific analyses from 11 PCs were included in the dose-response analysis. (**Appendix K**, **Figure K.10**)	**Initial certainty: Moderate (> 50–75% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	Five PCs in tier 1; 6 PCs in tier 2, 3 PCs in tier 3 (**Appendix L**, **Table L.8**) Generally moderate Key questions: • Confounding: most probably low • Exposure assessment: mixed probably low and probably high • Outcome assessment: mixed low and probably high Mixed probably low and probably high for attrition	Serious
Unexplained inconsistency	Moderate heterogeneity (I^2 = 51%) for the pooled mean effect estimate of study-specific RRs per unit increase of intake. RRs are similar across large studies; small studies show higher effects, but confidence intervals overlap. No clear sources of heterogeneity identified.	Not serious
Indirectness	Direct endpoint in most studies	Not serious
Imprecision	Low	Not serious
Publication bias	Funnel plot showed asymmetry and Egger's test was significant (p = 0.021), suggesting a possible small-study effect **(Annex M)**. However, the number of studies available is small, and there is some indication for true heterogeneity of small (vs large) studies. Public (n = 13) and mixed (n = 1) funding.	Undetected

Upgrading factors	Dose-response: A significant linear dose-response relationship across categories of SSBs intake was reported in eight of the 13 PCs which performed a categorical analysis. The dose-response meta-analysis conducted by EFSA showed a significant linear positive dose relationship (linear pooled mean effect estimate (95% CI) = 1.13 (1.07, 1.20) for 250 mL/d increase with no support for non-linearity (p = 0.816). In sensitivity analysis, exclusion of PCs at high RoB (tier 3) had a negligible impact on the dose-response relationship (**Annex M**).	Yes (dose-response)
Final certainty	Started moderate, upgraded one level for dose-response. Not downgraded for RoB because PCs at high RoB (tier 3) had a negligible impact on the dose-response relationship.	**High (> 75–100% probability)**

Conclusion sQ4.3. PCs. The level of certainty in a positive and causal relationship between the intake of SSBs and risk T2DM is **high** (rationale in **Table 20**). The relationship was mostly observed for SSBs not keeping TEI constant.

8.4.4.3. Overall conclusion on sQ4.3

There is evidence from PCs for a positive and causal relationship between the intake of SSBs and risk of T2DM (**high** certainty). Evidence from RCTs (**low** certainty) supports the relationship.

8.4.5. Fruit juices

sQ5.3. FJs and risk of Type 2 diabetes mellitus

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of T2DM	0	**9***
LoE2. Standalone (surrogate)	Measures of glucose tolerance	0	0
LoE3. Complementary	Indices of insulin sensitivity/beta-cell function	0	0
LoE4. Complementary	Measures of insulin sensitivity	0	0
LoE5. Complementary	Risk of obesity	sQ5.1	sQ5.1

*: Of which one was a PCC.

8.4.5.1. Observational studies

LoE1. Standalone (main): Incidence of T2DM. PCs. The relationship between the intake of FJs and incidence of T2DM was investigated in nine studies, of which eight were PCs and one was a PCC (EPIC-InterAct; InterAct consortium, 2013). In the CARDIA cohort the endpoint was high fasting glucose (> 110 mg/dL) or the use of hypoglycaemic medications.

Four PCs included only females (BWHS (Palmer et al., 2008); NHS and NHS II (Muraki et al., 2013); WHI (Auerbach et al., 2017)); one included only males (HPFS, (Muraki et al., 2013)); in one, males and females were analysed separately (JPHC, (Eshak et al., 2013)); and the remaining were on males and females combined (CARDIA, (Duffey et al., 2010); EPIC-InterAct, (InterAct consortium, 2013); SUN, (Fresan et al., 2017)). All the studies were in adults.

All studies analysed the intake of FJs as categorial variable using the standard multivariable model to adjust for energy except WHI, which used the residuals (energy-adjusted) model, the CARDIA, which analysed the exposure as a continuous variable adjusting for non-SSBs energy, and the BWHS, which did not adjust for TEI. In all cases except for the WHI, the analysis allows for TEI to change as a function of FJs consumption. All studies except the BWHS include BMI (or body weight in CARDIA) as covariate in the most adjusted models. EPIC-InterAct, NHS, NHSII and HPFS also report results for FJs analysed as a continuous variable, and thus in isocaloric exchange with other food sources. The evidence table is in **Annex J**.

Preliminary UA

A positive relationship between the consumption of FJs and incidence of T2DM was observed in six studies (EPIC-InterAct, BWHS, JPHC and statistically significant in HPFS, NHS and NHS II), whereas it was null in one (CARDIA) and negative (non-significant) in two (SUN and WHI). The forest plot can be found in **Appendix K**, **Figure K.11**. The Panel notes that, in the WHI cohort, TEI was kept constant in the analysis. Results in the EPIC-InterAct, NHS, NHSII and HPFS cohorts were similar when FJs were

analysed as a continuous variable using the standard multivariable model to adjust for TEI, and thus in isocaloric exchange with other food sources.

Three PCs are in RoB tier 1 (BWHS, HPFS, WHI), five in tier 2 (CARDIA, EPIC-InterAct, NHS, NSH II, SUN) and one in tier 3 (JPHC). The heat map can be found in **Appendix L**, **Table L.9**.

The Panel considers that the available BoE suggests a positive relationship between the consumption of FJs and risk of T2DM.

Comprehensive UA

Selection of the endpoint. The only eligible endpoint in this LoE is incidence of T2DM.

Dose-response relationship. A significant linear dose-response relationship across categories of FJs intake was reported in three (HPFS, NHS, NHS II) of the eight PCs which performed a categorical analysis. Upon request for additional data from the study authors of EPIC-InterAct, individual country-specific cohort risk estimates were included in the dose-response analysis.

In the dose-response meta-analysis conducted by EFSA, parametric dose-response models were estimated based on summarised data. Both linear and non-linear (restricted cubic splines) dose-response relationships were investigated. The methodological approach applied was the same as for the dose-response meta-analyses of SSBs intake and incidence of T2DM (**Annex M**).

Forty-two non-referent RRs from 13 study-specific analyses were included in the dose-response meta-analysis (I^2 = 3%; p = 0.414). The BWHS (RRs not adjusted for BMI and EI), CARDIA (RR already provided per unit increase), SUN and WHI (model diagnostics) cohorts were excluded. The predicted pooled relative risk of T2DM was 1.16 (95% CI: 1.09, 1.24) for an increase in FJs intake of 250 mL/day in the linear model (p for linear trend < 0.0001) and 1.19 (95% CI: 1.11, 1.28) at 250 mL/day in the non-linear model (RCS with three knots at fixed percentiles, 10%, 50% and 90%, of the distribution; p for non-linearity = 0.372) (**Figure 14**). The subgroup analyses did not identify clear sources of heterogeneity, also given the overall heterogeneity quantified as 3%. A sensitivity analysis excluding RoB tier 3 studies confirmed no evidence of departure from linearity (p = 0.704) and showed similar RRs estimates (1.17 (95% CI: 1.09, 1.25); 1.18 (95% CI: 1.10, 1.27)) and improved fitting. The funnel plot and related Egger regression did not support a possible small-study effect.

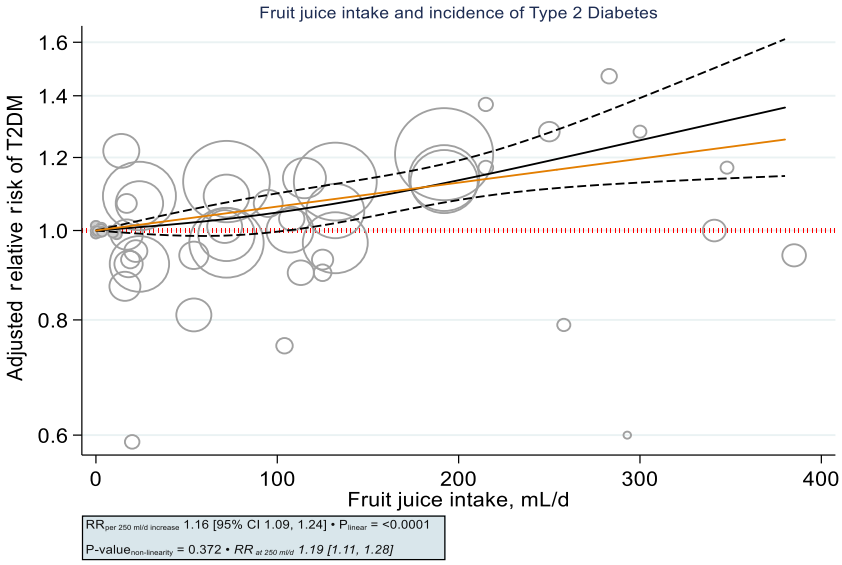


Figure 14: Dose-response meta-analysis on the relationship between the intake of fruit juices and incidence of Type 2 diabetes mellitus (T2DM)

LoE5. Complementary: Risk of obesity. PCs. There is evidence for a positive and causal relationship between the intake of FJs and risk of obesity (**very low** level of certainty).

Consistency across LoEs. The Panel notes and an increased incidence of T2DM is consistent with an increased risk of obesity. However, no PCs are available from other standalone or complementary LoEs which are specific to this sQ.

Table 21: sQ5.3. PCs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty in a positive and causal relationship between intake of **FJs** and risk of T2DM at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE1. Standalone (main). Endpoint: incidence of T2DM 8 PCs and 1 PCC, 419,152 participants.** 13 study-specific analyses from 5 PCs were included in the dose-response analysis.	**Initial certainty: Moderate (> 50–75% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	Three PCs in tier 1; 5 PCs in tier 2, 1 in tier 3 (**Appendix L**, **Table L.9**) Generally moderate Key questions: • Confounding: probably low • Exposure assessment: mixed probably low and probably high • Outcome assessment: most probably high Mixed low and probably high for attrition	Serious
Unexplained inconsistency	No heterogeneity detected (I^2 = 3%) for the pooled mean effect estimate of study-specific RRs per unit increase of intake. RRs are similar across studies and confidence intervals overlap.	Not serious
Indirectness	Direct endpoint in most studies.	Not serious
Imprecision	Low	Not serious
Publication bias	No evidence of asymmetry in funnel plot and Egger test was not significant (p = 0.703). Limited number of studies (**Annex M**). Public funding (n = 9).	Undetected
Upgrading factors	Dose-response: A significant linear dose-response relationship across categories of FJs intake was reported in 3 (HPFS, NHS, NHS II) of the 8 PCs which performed a categorical analysis. The dose-response meta-analysis conducted by EFSA showed a significant linear positive relationship (linear pooled mean effect estimate (95% CI) = 1.16 (1.09, 1.24; I^2 = 3%) for 250 mL/d increase with weak support for non-linearity (p = 0.372) (**Annex M**).	Yes (dose-response)
Final certainty	Started moderate, downgraded one level for RoB, upgraded one level for dose-response.	**Moderate (> 50–75% probability)**

Conclusion sQ5.3. PCs. The level of certainty in a positive and causal relationship between the intake of FJs and risk T2DM is **moderate** (rationale in **Table 21**). The relationship was observed for FJs both keeping and not keeping TEI constant in the analysis.

8.4.5.2. Overall conclusion on sQ5.3

There is evidence from PCs for a positive and causal relationship between the intake of FJs and risk of T2DM (**moderate** level of certainty).

8.5. Risk of dyslipidaemia

8.5.1. Total sugars

sQ1.4. Total sugars and risk of dyslipidaemia

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of high total-c, LDL-c, TG or low HDL-c	0	0
LoE2. Standalone (surrogate)	Changes in total-c, LDL-c, TG, HDL-c or derived indices	0	**2**
LoE3. Complementary	Risk of obesity	sQ1.1	sQ1.1
LoE4. Complementary	Risk of Type 2 diabetes mellitus	sQ1.3	sQ1.3

8.5.1.1. Observational studies

LoE2. Standalone: Total-c, LDL-c, TG, HDL-c or derived indices. PCs. Two PCs investigated the relationship between total sugars intake and blood lipid levels, one in the older adults (BMES, (Goletzke et al., 2013a)) and one in toddlers (ALSPAC, (Cowin and Emmett, 2001)) of both sexes. Total sugars were analysed as continuous variable using either the nutrient residuals (energy adjusted) model (ALSPAC) or the nutrient density (energy adjusted) model (BMES), and thus in isocaloric exchange with other macronutrients. The evidence table is in **Annex J**.

Preliminary UA

The BMES found no association between changes in total sugars intake and concurrent changes in TG and HDL-c over the 5-year follow-up. In the ALSPAC, a non-significant positive correlation was found between energy-adjusted total sugar intakes at baseline and blood lipid levels (total cholesterol, HDL-c and LDL-c) at the end of the 13-month follow-up. In a backward stepwise regression analysis that excluded the least significant variables until all were $p < 0.1$, total sugars intake was retained in the model only for the T-c:HDL-c ratio and only for females, showing a positive association ($p = 0.052$).

Both PCs were at moderate RoB (tier 2), with critical domains being confounding and attrition (**Annex K**).

The Panel notes that the two PCs available were heterogeneous regarding the population studied and the exposure–endpoint combinations assessed (total sugars intake at baseline vs. blood lipid levels at the end of follow-up; changes in total sugars intake vs. concurrent changes in blood lipids) and that total sugars intake was largely unrelated to blood lipid levels in both studies after adjusting for relevant covariates, including dietary fat.

The Panel considers that the available BoE does not suggest a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and adverse effects on blood lipids. **No comprehensive UA is performed**.

Complementary LoE3: Risk of obesity and LoE4: Risk of T2DM. **PCs**. The available BoE does not suggest a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of obesity (sQ1.1, Section 8.2.1.1) or risk of T2DM (sQ1.3, Section 8.4.1.1).

Conclusion sQ1.4. PCs. The available BoE does not suggest a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of dyslipidaemia.

8.5.1.2. Overall conclusion on sQ1.4

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of dyslipidaemia. Total sugars were not investigated under other dietary conditions (e.g. not keeping TEI constant).

8.5.2. Added and free sugars

sQ2.4. Added and free sugars and risk of dyslipidaemia

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of high total-c, LDL-c, TG or low HDL-c	0	0
LoE2. Standalone (surrogate)	Changes in total-c, LDL-c, TG, HDL-c or derived indices	**24**	**3**
LoE3. Complementary	Risk of obesity	sQ2.1	sQ2.1
LoE4. Complementary	Risk of Type 2 diabetes mellitus	sQ2.3	sQ2.3

8.5.2.1. Intervention studies

LoE2. Standalone (surrogate): Changes in total-c, LDL-c, TG, HDL-c or derived indices. RCTs. Twenty-four RCTs (29 study groups) investigated the effect of high vs. low sugar intakes on changes in total cholesterol **(Appendix G, Figure G.6a1)**, of which 17 (21 study groups) also assessed changes in LDL-cholesterol **(Appendix G, Figure G.6b1)**, 20 (24 study groups) report on changes in HDL-cholesterol **(Appendix G, Figure G.6c1)** and 23 (29 study groups) on fasting triglycerides (TG) **(Appendix G, Figure G.6d1)**. Differences in sugar intakes in the high vs. the low sugar arms ranged from 6 to 43 E% and study duration from 4 to 72 weeks. Six RCTs were conducted

with solid foods, seven with beverages and 11 with mixtures of solid foods and beverages (**Appendix F**). All the studies were in adults: six were in healthy subjects and the remaining in selected population subgroups (e.g. overweight/obese, BMI < 35 kg/m^2, individuals with gallstones, hypertriglyceridaemia, hyperinsulinaemia, etc.).

Added and free sugars were provided under neutral energy balance in isocaloric exchange with other macronutrients (mostly starch) (13 studies) or ad libitum (11 studies). In 10 studies conducted under neutral energy balance, the macronutrient composition of the background diet was known and controlled by the investigators. Of these, eight RCTs also controlled for the polyunsaturated/saturated (P/S) fatty acid ratio (**Appendix F**).

Preliminary UA

Total-c and fasting TG were higher in the high vs. the low sugar arm in 20 and 19 out of the 29 study groups, respectively. Pooled mean effect estimates (95%CI) are 8.71 mg/dL (2.86, 14.56; I^2 = 87%) for total-c **(Appendix G, Figure G.6a1)** and 14.59 mg/dL (7.16, 22.02; I^2 = 81%) for fasting TG **(Appendix G, Figure G.6d1)**. LDL-c was also higher in the high vs. the low sugar arm in 16 out of the 21 study groups. The pooled mean effect estimate (95%CI) is 4.50 mg/dL (−0.88, 9.87; I^2 = 90%) **(Appendix G, Figure G.6b1)**. Conversely, HDL-c was minimally affected by the intervention (pooled mean effect estimate (95% CI) = 0.83 mg/dL (−0.25, 1.91; I^2 =77%) **(Appendix G, Figure G.6c1)**. Heterogeneity across studies was high and statistically significant.

The effect of high vs. low sugars intake was of bigger magnitude and statistically significant for all blood lipid variables when the analysis was restricted to studies conducted under neutral energy balance in isocaloric exchange with starch, of which most controlled for the macronutrient composition of the diet and the P/S ratio. Pooled mean effect estimates (95%CI) are 13.40 mg/dL (6.63, 20.16, I^2 = 75%) for total-c **(Appendix G, Figure G.6a1)**, 7.88 mg/dL (1.82, 13.94; I^2 = 75%) for LDL-c, 1.98 mg/dL (0.96, 2.99; I^2 = 32%) for HDL-c and 17.24 mg/dL (7.67, 26.81; I^2 = 79%) for fasting TG **(Appendix G, Figures G.6b1, G.6c1 and G.6d1)**.

In studies conducted ad libitum, the effect of high vs. low sugars intake on fasting TG was consistent with that observed in studies under neutral energy balance, although not statistically significant (pooled effect estimate and 95% CI = 10.32 mg/dL, −2.04 to 22.68; I^2 = 85%) (**Appendix G, Figure G.6d1**), whereas the effect on total-c, LDL-c and HDL-c was negligible (**Appendix G, Figures G.6a1, G.6b1 and G.6c1**).

Twelve RCTs were at low RoB (tier 1) and 12 at moderate RoB (tier 2). The heat map is in **Appendix I, Figure I.4**.

The Panel considers that the available BoE suggests a positive relationship between the intake of added and free sugars and risk of dyslipidaemia.

Comprehensive UA

Selection of the endpoint. The Panel decided to conduct the comprehensive UA on fasting TG for the following reasons: (a) the effect of the intervention on fasting TG was higher than on any other blood lipid fraction; (b) dietary lipids, which can affect total-c and LDL-c, were not controlled for in studies ad libitum; (c) TG are more likely to be affected by dietary sugars (particularly fructose) than any other blood lipid fraction (see Section 3.6.1.3).

Dose-response relationship. A dose-response relationship between the intake of sucrose (doses 2, 15 and 30E%) in isocaloric exchange with starch and fasting TGs was observed in the RCT by Israel et al. (1983) conducted in individuals with hyperinsulinaemia (men only). A dose-response relationship between the intake of fructose (doses 0, 7.5 and 15 E%) in isocaloric exchange with starch and fasting TGs was also reported in the RCT by Hallfrisch et al. (1983a)* conducted in men with hyperinsulinaemia.

A meta-regression linear dose-response analysis was performed by EFSA to investigate the association between the difference in sugars intake and the difference in fasting TG between study arms. A total of 29 observations were eligible for the analysis. Potential effect-modifiers were identified using graphical displays of the stratified dose-response curves. These variables included main characteristics of the exposure (i.e. sugars source and type, dietary conditions), methodological aspects related to study design (parallel or cross-over, with and without wash-out) and RoB. The final model was chosen considering goodness of fit, significance of the parameters, explained heterogeneity and robustness in response to the inclusion/exclusion of individual studies. Although various models with adjustment factors were able to improve the model fit, the estimates of the related parameters were not statistically significant and the explained heterogeneity was lower than in the final model

(24%). Therefore, no adjusting factors have been retained in the final dose-response model. Residual heterogeneity remained high (Cochran Q-test = 66.39) and statistically significant ($p < 0.0001$), indicating that other factors not identified in the BoE, or for which it was not possible to adjust due to the low number of studies available, play a role in explaining differences across studies.

Several diagnostics, the Hat indicator, the Cook distance and the influence analysis (One-at-a-Time leave out analysis), identified one study (Moser et al., 1986), conducted on two subgroups of young women taking/not taking contraceptives, as highly influential because of the high sugars dose and the particularly small size of the effect. Since the results of the study were counterconservative (i.e. very low responses at high sugar doses), and their impact was to flatten the dose-response, it was decided to exclude the two observations from the dose-response analysis. The final model was set up on 27 observations with sugars E% intake ranging between 6% and 30%). It indicates an expected increase in fasting TG of around 17 mg/dL (95% CI: 8.9, 25.8, $p < 0.01$) per each increase of 10E% intake from sugar with a negative estimate of the intercept (−16.70 mg/dL, 95% CI: −32.88, −0.53, $p = 0.04$). A meta-regressive non-linear dose-response relationship was also investigated using a restricted cubic spline (RCS) with three knots. The linear model was retained as the parameter entailing the quadratic component of the model was not statistically significant (**Figure 15**). In the final linear model, between-arm differences in sugars intake (E%) only accounted for around 20% of the variability across studies thus leaving most of the heterogeneity unexplained. In this context, the Panel considers that this analysis can be used to conclude on the shape and direction of the dose-response relationship, but not to make a quantitative prediction of the effect of added or free sugars on fasting levels of triglycerides. The Panel notes that RCTs showing the highest absolute difference in fasting triglycerides between arms for the same difference in sugars intake were conducted in subjects with obesity, hypertriglyceridaemia or hyperinsulinaemia. These are represented by points outside the upper bound of the 95% CI in **Figure 15**. The technical report can be found in **Annex L**.

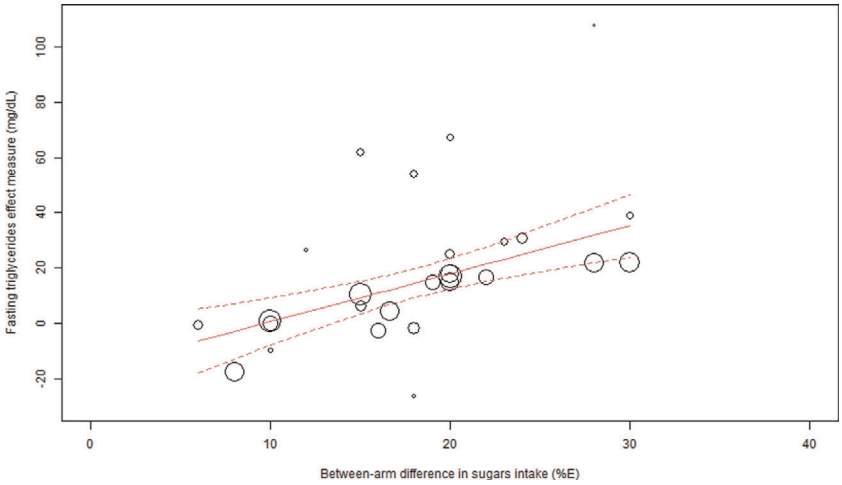


Figure 15: Meta-regressive dose-response linear model between the intake of added and free sugars (E%) and fasting triglycerides

Complementary LoE3: Risk of obesity and LoE4: Risk of T2DM. RCTs. There is evidence from RCTs for a positive and causal relationship between the intake of added and free sugars ad libitum and risk of obesity (moderate certainty, sQ2.1, Section 8.2.2.1) and for a positive and causal relationship between the intake of added and free sugars *ad libitum* or in isocaloric exchange with other macronutrients and risk of T2DM (low certainty, sQ2.3, Section 8.4.2.1).

Consistency across LoE. The effect on total TG was consistent with the effect on total-c and LDL-c, particularly in RCTs conducted under neutral energy balance in isocaloric exchange with starch, where the macronutrient composition and P/S ratio were controlled for, whereas HDL-c was minimally affected (LoE2). It is also consistent with an increased risk of obesity (LoE3) and T2DM (LoE4).

Table 22: sQ2.4. RCTs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty that the intake of **added and free sugars** is positively and causally associated with the risk of dyslipidaemia at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?

BoE (standalone)	**LoE2. Standalone (surrogate). Endpoint:** fasting TG **23 RCTs (29 study groups), 1,086 participants**. Pooled mean effect estimate (95% CI) = 14.59 mg/dL (7.16, 22.02) for all studies combined, assuming a within-subject correlation coefficient of 0.82. The correlation coefficient for this endpoint is expected to be lower. **(Appendix G, Figure G.6d1)**.	**Initial certainty: High (> 75–100% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	12 studies in tier 1; 11 studies tier 2 (**Appendix I, Figure I.4**) Between low and moderate. Key questions: • Randomisation: low • Exposure assessment: low • Outcome assessment: low Probably high for allocation concealment and blinding	Serious
Unexplained inconsistency	High heterogeneity. I^2 = 81% (p < 0.01) for the pooled mean effect. Point estimates vary widely, and 95% CI show minimal overlap. Residual heterogeneity in dose-response analysis is high (Cochran Q-test=66.39) and statistically significant. Between-arm difference in sugars intake (E%) only accounted for 24% of the variability across studies.	Very serious
Indirectness	Surrogate endpoint	Serious
Imprecision	Low. It could be higher because the expected correlation coefficient for this endpoint is < 0.82, but still low **(Appendix G, Figure G.6d1)**.	Not serious
Publication bias	Funnel plot shows a slight association between the magnitude of the effect and the SE, and Egger's test was significant (p = 0.004), suggesting a risk of publication bias (**Appendix H, Figure H.4**). However, there is some indication for true heterogeneity in small studies. Public (n = 5), private (n = 5), mixed (n = 5) and NR (n = 8) funding.	Undetected
Upgrading factors	Dose-response: two RCTs reported linear dose-response relationships for fructose (doses between 0 and 15E%) and sucrose (doses between 2 and 30E%) in men with hyperinsulinaemia. In the meta-regression dose-response analysis, a between-arm difference in added sugars intake of at least 9.6E% is needed to predict a positive effect on fasting TG. Any further increase of 10E% in the between-arm difference in added sugars intake leads to an increase in fasting TG of 17mg/dL (linear dose-response). Consistency: The effect on TG is consistent with the effect on total-c and LDL-c, particularly in RCTs conducted under neutral energy balance in isocaloric exchange with starch, where the macronutrient composition and P/S ratio were controlled for. It is also consistent with a positive and causal relationship between the intake of added and free sugars ad libitum and risk of obesity (LoE3; **moderate** certainty) and with a positive and causal relationship between the intake of added and free sugars ad libitum or in isocaloric exchange with other macronutrients risk of T2DM (LoE4; **low** certainty).	Yes (dose-response and consistency)
Final certainty	Started high, downgraded two levels for heterogeneity and one level for indirectness, upgraded one level for dose-response and one level for consistency. RoB was not considered sufficiently serious to downgrade because it was between low and moderate but low for the three key questions.	**Moderate (> 50–75% probability)**

Conclusions sQ2.4. RCTs. The level of certainty in a positive and causal relationship between the intake of added and free sugars and risk of dyslipidaemia is **moderate** (rationale in **Table 22**). The effect is particularly observed under neutral energy balance in isocaloric exchange with starch while controlling for the macronutrient composition and P/S ratio of the diet. RCTs included only adults. About half of the RCTs were in overweight/obese subjects and three included a group of hyperinsulinaemic individuals. Between-arm differences in added and free sugars intake were between 6 and 43E%.

8.5.2.2. Observational studies

LoE2. Standalone (surrogate): Changes in total-c, LDL-c, TG, HDL-c or derived indices. PCs. Three PCs studies on the relationship between the intake of added sugars (NGHS, (Lee et al., 2014)) or sucrose (CARDIA, (Archer et al., 1998); NSHDS, (Winkvist et al., 2017)) and blood lipids were available. Two report on changes in HDL-c and one on changes in total cholesterol and fasting TG. All PCs analysed the exposure as a continuous variable and used the nutrient density model for energy adjustment, but only the NGHS included TEI in the models as a covariate. Evidence table is in **Annex J**.

Preliminary UA

In the NGHS cohort of black and Caucasian female adolescents, HDL-c was significantly higher by 0.26 mg/dL per year (95% CI: 0.04, 0.48; p = 0.02) in the group consuming < 10E% as added sugars vs. the group consuming > 10E% over the 10-year follow-up (RoB tier 1). This was mostly due to an increase in HDL-c in the first group, whereas HDL-c concentrations in the second group were virtually unchanged. Similar results were obtained for sucrose in the CARDIA cohort of young black and white males and females. A negative association was observed between the intake of sucrose and HDL-c concentrations in both ethnicities and sexes over the 7-year follow-up. The relationship was statistically significant in all groups except black males. Per each 10E% increase in sucrose intake, mean reductions in HDL-c ranged between 0.3 and 0.04 mmol/L (SE between 0.01 and 0.02) (RoB tier 2). Sucrose intake was not significantly associated with changes in total cholesterol (positive) or fasting TG (negative) in the large NSHDS cohort of middle age Swedish males and females followed-up for 10 years (RoB tier 2).

Critical domains across studies in the RoB assessment were confounding and attrition (**Annex K**).

The Panel notes the small number of PCs available and the different blood lipid fractions assessed. Whereas added sugars and sucrose were negatively associated with HDL-c in the NGHS and CARDIA cohorts, both studies were at probably high risk of bias for confounding. The Panel considers the available BoE from PCs does not suggest a positive relationship between the intake of added and free sugars and adverse effects on blood lipids. **No comprehensive UA is performed**.

Complementary LoE3: Risk of obesity and LoE4: Risk of T2DM. PCs. The available BoE does not suggest a positive relationship between the intake of added or free sugars in isocaloric exchange with other macronutrients and risk of obesity (sQ2.1, Section 8.2.2.2) or risk of T2DM (sQ1.3, Section 8.4.2.2).

sQ2.4. PCs. The available BoE does not suggest a positive relationship between the intake of added and free sugars in isocaloric exchange with other macronutrients and risk of dyslipidaemia.

8.5.2.3. Overall conclusion on sQ2.4

There is evidence from RCTs for a positive and causal relationship between the intake of added and free sugars and risk of dyslipidaemia (**moderate** level of certainty). The available BoE from PCs cannot be used to modify the level of certainty in this conclusion.

8.5.3. Fructose

sQ3.4. Fructose and risk of dyslipidaemia			
LoE1. Standalone (main)	Incidence of high total-c, LDL-c, TG or low HDL-c (cut-offs)	0	0
LoE2. Standalone (surrogate)	Changes in total-c, LDL-c, TG, HDL-c or derived indices	**10**	**1**
LoE3. Complementary	Risk of obesity	sQ3.1	sQ3.1
LoE4. Complementary	Risk of Type 2 diabetes mellitus	sQ3.3	sQ3.3

8.5.3.1. Intervention studies

LoE2. Standalone (surrogate): Changes in total-c, LDL-c, TG, HDL-c or derived indices RCTs. A total of seven RCTs (9 study groups) assessed the effect of fructose vs. glucose on fasting TG under different dietary conditions (under neutral or positive energy balance, ad libitum), of which six also reported on total-c, LDL-c and HDL-c **(Appendix G, Figures G.7a–G.7d)**. Doses of fructose and glucose ranged from 9 to 25 E% and study duration between 4 and 10 weeks. All RCTs were in adults selected based on BMI (overweight obese, BMI < 32 or 35 kg/m^2), glucose tolerance status (NGT, IGT) or liver fat (NAFLD).

Three additional RCTs investigated the effect of doses of fructose between 15 and 20E% in isocaloric exchange with starch under neutral energy balance **(Appendix G, Figures G.6a–G.6d)**. Study duration was between 4 and 5 weeks. One study (Swanson et al., 1992) was in healthy males and females, whereas two RCTs were in males and included one group with normoinsulinaemia and one group with hyperinsulinaemia (Hallfrisch et al., 1983a; Reiser et al., 1989a).

Preliminary UA

The results of the RCTs assessing the effect of fructose vs. glucose were mixed (**Appendix F**). Pooled effect estimates (95%CI) were 1.5mg/dL (−2.97, 6.10) for total-c **(Appendix G, Figure G.7a)**, −0.03 mg/dL (−1.64, 1.59) for LDL-c **(Appendix G, Figure G.7b)**, −0.29 mg/dL (−1.25, 0.68) for HDL-c **(Appendix G, Figure G.7c)** and 4.25 mg/dL (−7.68, 16.17) for fasting TG **(Appendix G, Figure G.7d)**. The only RCT which showed a consistent significant effect of fructose vs. glucose across the blood lipid profile was conducted at doses of 22 E% with beverages in positive energy balance (Silbernagel et al., 2011). RoB was low for five studies (tier 1) and moderate for two (tier 2). Overall, these studies do not suggest a positive relationship between fructose in isocaloric exchange with glucose and adverse effects on blood lipids.

Conversely, fructose consistently increased total-c, LDL-c, HDL-c and fasting TG when consumed in isocaloric exchange with starch under neutral energy balance in the three RCTs which investigated this relationship (Reiser et al., 1989a)*(Hallfrisch et al., 1983a; Swanson et al., 1992)*. The effect on fasting TG was particularly marked in men with hyperinsulinaemia (Reiser et al., 1989a)*(Hallfrisch et al., 1983a)*; **Appendix G, Figure G.6d**), which are at higher risk for developing T2DM. A positive dose-response relationship between the intake of fructose (at doses of 0, 7.5 and 15 E%) in isocaloric exchange with starch and fasting TGs was reported by (Hallfrisch et al., 1983a)* in this population subgroup.

The Panel notes that RCTs investigating the effect of fructose in isocaloric exchange with starch were part of the BoE used to reach conclusions on a positive and causal relationship between the intake of added (and free sugars) and risk of dyslipidaemia and considers that the same conclusions apply, since the type of sugar used in the studies (fructose, mixtures of fructose and glucose) was not a significant modifying factor (see Section 8.5.2.1). The Panel also considers that the available BoE from RCTs does not suggest a positive relationship between the intake of fructose in isocaloric exchange with glucose and risk of dyslipidaemia. **No comprehensive UA is performed**.

Complementary LoE3: Risk of obesity and LoE4: Risk of T2DM. RCTs. The available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with glucose and risk of obesity (sQ3.1, Section 8.2.3.1) or T2DM (sQ3.3, Section 8.4.3.1).

Conclusion sQ3.4. RCTs. The available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with glucose and risk of dyslipidaemia. The Panel considers, however, that the conclusions for a positive and causal relationship between the intake of added and free sugars and risk of dyslipidaemia also apply to fructose in isocaloric exchange with starch (**moderate** certainty).

8.5.3.2. Observational studies

LoE2. Standalone (surrogate): Total-c, LDL-c, TG, HDL-c or derived indices. PCs. Only one PC investigated the relationship between fructose intake and changes in blood lipids (fasting TG and HDL-c). In the TLGS cohort of males and females (Bahadoran et al., 2017) each 1E% from fructose was associated with non-significant mean increase in fasting TG of 0.310 mg/dL (95% CI: −0.521, 1.145) and with a significant mean decrease in HDL-c of −0.297 mg/dL (95% CI: −0.410, −0.184). This study, however, was at high RoB (tier 3) and at definitively high RoB for confounding (i.e. the only variable included in the model was age).

The Panel considers that the available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and adverse effects on blood lipids.

Complementary LoE3: Risk of obesity and LoE4: Risk of T2DM. PCs. The available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and risk of obesity (sQ3.1, Section 8.2.3.2) or risk of T2DM (sQ3.3, Section 8.4.3.2).

Conclusion sQ3.4. PCs. The Panel considers that the available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and risk of dyslipidaemia.

8.5.3.3. Overall conclusion on sQ3.4

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of fructose in isocaloric exchange with glucose or other macronutrients and risk of dyslipidaemia. The Panel considers, however, that the conclusions for a positive and causal relationship between the intake of added and free sugars and risk of dyslipidaemia also apply to fructose in isocaloric exchange with starch (**moderate** certainty).

8.5.4. Sugar-sweetened beverages

sQ4.4. SSBs and risk of dyslipidaemia

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of high total-c, LDL-c, TG or low HDL-c (cut-offs)	0	**5**
LoE2. Standalone (surrogate)	Changes in total-c, LDL-c, TG, HDL-c or derived indices	**7**	**4**
LoE3. Complementary	Risk of obesity (sQ4.1)	sQ4.1	sQ4.1
LoE4. Complementary	Risk of Type 2 diabetes mellitus (sQ4.3)	sQ4.3	sQ4.3

8.5.4.1. Intervention studies

LoE2. Standalone (surrogate): Changes in total-c, LDL-c, TG, HDL-c or derived indices. RCTs. Of the 24 RCTs which investigated the effect of high vs. low added and free sugars intake on changes in total cholesterol (see Section 8.5.2.1), seven were conducted with beverages. The same studies also investigated changes in LDL-cholesterol, HDL-cholesterol and fasting TG, except for Campos et al., 2015, which did not report on LDL-cholesterol. The between-group target difference in sugars intake from beverages was between 8 and 22 E% and study duration from 4 to 36 weeks. Two studies were under neutral energy balance and the other five were conducted ad libitum. Six RCTs were in adults selected based on BMI (overweight, obese and BMI < 35 kg/m^2) and one in healthy subjects (**Appendix F**).

Preliminary UA

The results of RCTs comparing a high sugar dose from SSBs to a lower one, or to a sugar-free alternative, were mixed for all blood lipids. At the end of the intervention, total cholesterol was higher in the high sugar arm relative to the low sugar arm in two studies, lower in three and null in the other two. The pooled mean effect estimate (95%CI) for these studies was −0.30 mg/dL (−14.02, 13.41; I^2 = 90%) (**Appendix G**, **Figure G.6a2**). The results on LDL-c, HDL-c and fasting TG followed a similar pattern. The pooled mean effect estimates (95%CI) are −2.50 mg/dL (−13.52, 8.52; I^2 = 87%) (**Appendix G**, **Figure G.6b2**), 0.16 mg/dL (−1.69, 2.01; I^2 = 78%) (**Appendix G**, **Figure G.6c2**) and 6.10 mg/dL (−12.43, 24.64; I^2 = 88%) (**Appendix G**, **Figure G.6d2**), respectively. There was high heterogeneity across the studies. Three RCTs were at low RoB (tier 1) and four at moderate RoB (tier 2) (**Appendix I, Table I.4**).

The Panel considers that the available BoE from RCTs does not suggest a positive relationship between consumption of SSBs and adverse effects on blood lipids. The Panel notes, however, that most studies were conducted ad libitum and thus did not control for the lipid profile of the diet. This is consistent with the fact that the strongest relationship between the intake of added and free sugars and adverse effects on blood lipids was observed in RCTs conducted at neutral energy balance in isocaloric exchange with starch while controlling for the macronutrient composition and P/S ratio of the diet (see Section 8.5.2.1). **No comprehensive UA is performed**.

Complementary LoE3: Risk of obesity and LoE4: Risk of T2DM. RCTs. There is evidence from RCTs for a positive and causal relationship between the intake of SSBs and risk of obesity (moderate certainty, sQ4.1, Section 8.2.4.1) and T2DM (low certainty, sQ4.3, Section 8.4.4.1).

Conclusions sQ4.4. RCTs. While there is evidence for a positive and causal relationship between consumption of SSBs and risk of obesity and T2DM, the available BoE does not suggest a positive relationship between the intake of SSBs and risk of dyslipidaemia. The Panel notes, however, that most RCTs were conducted ad libitum and thus did not control for the lipid profile of the diet.

8.5.4.2. Observational studies

LoE1. Standalone (main): Incidence of high total-c, LDL-c, TG or low HDL-c (cut-offs). **PCs**. Five PCs, four of which were in adults (KoGES, (Kang and Kim, 2017); CARDIA, (Duffey et al., 2010); Framingham-3Gen and Framingham Offspring, (Haslam et al., 2020)) and one in children and adolescents (TLGS), investigated the relationship between the intake of SSBs and incidence of high triglycerides and low HDL-cholesterol. The CARDIA, Framingham-3Gen and Framingham Offspring cohorts also investigated the relationship with incidence of high LDL-cholesterol (≥ 4.1 mmol/L). Cut-off values for high triglycerides were ≥ 1.7 mmol/L except for Framingham-3Gen and Framingham Offspring (≥ 2.0 mmol/L). Cut-off values for low HDL-cholesterol were < 1.04 mmol/L for men and < 1.3 mmol/L for women in all cohorts. The use of cholesterol-lowering medication was also considered part of the incidence case criteria in the CARDIA cohort and for subjects age > 18 years in the TLGS cohort. Evidence table can be found in **Annex J**.

The TLGS, KoGES, Framingham-3Gen and Framingham Offspring cohorts analysed SSBs as a categorical variable using the standard multivariable model for energy adjustment and the CARDIA cohort analysed the exposure as a continuous variable adjusting for non-SSBs energy intake. In both cases, TEI was not kept constant.

Preliminary UA

All PCs report positive relationships between the intake of SSBs and incidence of high TG. The positive relationship was statistically significant in the Framingham Offspring cohort. The KoGES, CARDIA, Framingham-3Gen and Framingham Offspring cohorts report a positive relationship between the intake of SSBs and incidence of low HDL-c, significant only in the CARDIA cohort. Contrariwise, in the TLGS cohort the association was negative (non-significant). In the CARDIA, Framingham-3Gen and Framingham Offspring cohorts, the relationship between the intake of SSBs and incidence of high LDL-c was positive, but statistically significant only in CARDIA.

One study was at low RoB (tier 1; Framingham Offspring), three at moderate RoB (tier 2; CARDIA, TLGS and Framingham-3Gen) and one at high RoB (tier 3; KoGES), critical domains being confounding, exposure and attrition (**Appendix L**, **Table L.10**).

The Panel notes that most PCs available report positive and non-significant relationships between the intake of SSBs and incidence of high-TG, low-HDL-c and high-LDL-c. The direction of the relationship was negative (non-significant) for low-HDL-c in the TLGS cohort of children and adolescents. The Panel considers that the available BoE supports a positive relationship between the consumption of SSBs and risk of dyslipidaemia.

Comprehensive UA

Selection of the endpoint. The Panel decided to conduct the comprehensive UA on the incidence of high fasting TG because of the higher number of studies, the consistency of the relationship, and because TG are more likely to be affected by dietary sugars (particularly fructose) than any other blood lipid fraction (see Section 3.6.1.3) **(Appendix K**, **Figure K.12)**. Pooled mean effect estimates, however, were not calculated because, out of the five PCs available, one did not report the number of cases across categories of intake (TLGS), one did not report the exposure as used for data analysis (CARDIA) and one assessed cumulative mean intakes up to diagnosis for cases and over the entire follow-up for non-cases (Framingham Offspring).

Dose-response relationship. Linear dose-response relationships across categories of SSBs intake were explored in four PCs. Significant positive linear dose-response relationships were reported only in one PC (Framingham Offspring). Dose-response relationships were not investigated by meta-regression analysis because the data required (e.g. number of cases, exposure) were not available for most PCs.

LoE2. Standalone (surrogate): Changes in total-c, LDL-c, TG, HDL-c or derived indices. PCs

Two cohorts of children (Daily-D (Van Rompay et al., 2015); WAPCS (Ambrosini et al., 2013)) and two cohorts of adults (Framingham-3Gen and Framingham Offspring, (Haslam et al., 2020)) investigated the relationship between intake of SSBs and changes in blood lipids over the follow-up. The Daily-D cohort investigated the relationship between SSBs intake at baseline, as well changes in SSBs intake and changes in TG and HDL-cholesterol over the one-year follow-up. The WAPCS cohort investigated changes in SSBs intake and concurrent changes in TG, HDL-c and LDL-c over the 3-year follow-up. The Framingham-3Gen and Framingham Offspring cohorts assessed average intakes of SSBs over a 4-year period and concurrent changes in TG, HDL-c and LDL-c. The evidence table is in **Annex J**.

The Daily-D, Framingham-3Gen and Framingham Offspring cohorts analysed SSBs as a categorical variable using the standard multivariable model for energy adjustment. Although the WAPCS cohort had not adjusted for energy intake in the multivariable models for which results were presented, associations were reported to be unchanged after additional adjustment for TEI in separate models (data not shown).

The four PCs reported positive relationships between the intake of SSBs and changes in fasting TG over follow-up, which remained statistically significant in the Framingham-3Gen and Framingham Offspring cohorts after adjusting for relevant confounders. Similarly, the relationship between SSBs intake and changes in HDL-c was negative in all PCs and statistically significant in all but for females in the WAPCS. The results for LDL-c were mixed in the three PCs which assessed this endpoint (Framingham-3Gen, Framingham Offspring, WAPCS).

The WAPCS, Framingham-3Gen and Framingham Offspring cohorts were at low RoB (tier 1) and the Daily-D cohort at moderate RoB (tier 2), critical domains being exposure and attrition (**Annex K**).

The Panel notes the consistency of the results across PCs regarding the positive and negative relationships between the intake of SSBs and changes in fasting TG and HDL-c, respectively, and that most studies were at low RoB (tier 1). The Panel considers that the available BoE from PCs suggests a positive relationship between the intake SSBs and adverse effects on blood lipids.

Complementary LoE3: Risk of obesity and LoE4: Risk of T2DM (sQ4.1). PCs. There is evidence from PCs for a positive and causal relationship between the intake of SSBs ad libitum and risk of obesity (moderate certainty, sQ4.1, Section 8.2.4.2) and T2DM (moderate certainty, sQ4.3, Section 8.4.4.2).

Consistency across LoE. An increased incidence of high-TG with higher intakes of SSBs is consistent with an increased incidence of low HDL-c, with changes in TG and HDL-c as continuous variables in the same direction, respectively, and with an increased risk of obesity and T2DM. This lipid profile (high TG, low HDL-c) is characteristic of the metabolic syndrome, a risk factor for the development of T2DM, possibly mediated by insulin resistance. Changes in LDL-c were less consistent.

Table 23: sQ4.4. PCs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty that the intake of SSBs is positively and causally associated with the risk of dyslipidaemia at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE1. Standalone (main). Endpoint:** incidence of high TG **5 PCs, 12,660 participants**. Pooled mean effect estimates were not calculated because the data required were not available from the individual PCs.	**Initial certainty: Moderate (> 50–75% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	1 PC in tier 1; 3 PCs in tier 2, 1 PC in tier 3 **(Appendix L, Table L.10)** Generally moderate. Key questions: • Exposure assessment: between low and probably high • Outcome assessment: low • Confounding: between low and probably high Probably high for attrition	Serious

Unexplained inconsistency	All PCs report positive relationships between the intake of SSBs and incidence of high TG.	Not serious
Indirectness	Direct endpoint	Not serious
Imprecision	High in most studies	Serious
Publication bias	Few studies available, also heterogeneous. It cannot be assessed. Public (n = 4) and mixed (n = 1) funding.	Undetected (cannot be assessed)
Upgrading factors	Consistency: An increased incidence of high TG with higher intakes of SSBs is consistent with an increased incidence of low HDL-c, with changes in TG and HDL-c as continuous variables in the same direction, respectively, and with an increased risk of obesity and T2DM. This lipid profile (high TG, low HDL-c) is characteristic of the metabolic syndrome, a risk factor for the development of T2DM, possibly mediated by insulin resistance. Changes in LDL-c were less consistent.	Yes (consistency)
Final certainty	Started moderate, downgraded for RoB (one level) and imprecision (one level), upgraded for consistency (one level).	**Low (> 15–50% probability)**

Conclusions sQ4.4. PCs. The level of certainty in a positive and causal relationship between the intake of SSBs and risk of dyslipidaemia is **low** (rationale in **Table 23**).

8.5.4.3. Overall conclusion on sQ4.4

There is evidence from PCs for a positive and causal relationship between the intake of SSBs and risk of dyslipidaemia (**low** level of certainty). The available BoE from RCTs cannot be used to modify the level of certainty in this conclusion.

8.5.5. Fruit juices

sQ5.4. FJs and risk of dyslipidaemia			
LoE1. Standalone (main)	Incidence of high total-c, LDL-c, TG or low HDL-c (cut-offs)	0	**1**
LoE2. Standalone (surrogate)	Changes in total-c, LDL-c, TG, HDL-c or derived indices	0	0
LoE3. Complementary	Risk of obesity (sQ5.1)	sQ5.1	sQ5.1
LoE4. Complementary	Risk of Type 2 diabetes mellitus (sQ5.3)	sQ5.3	sQ5.3

8.5.5.1. Intervention studies

No RCTs were eligible for sQ5.4.

8.5.5.2. Observational studies

LoE1. Standalone (main): Incidence of high total-c, LDL-c, TG or low HDL-c (cut-offs). PCs

Only one PC investigated the relationship between FJs intake and incidence of high triglycerides, high LDL-cholesterol and low HDL-cholesterol (CARDIA, (Duffey et al., 2010)). The evidence table is in **Annex J**.

Preliminary UA

No significant relationships were observed between the intake of FJs at baseline and incidence of high TG (negative), high LDL-c (positive) or low HDL-c (null) at the end of the 20-year follow-up. The study was at moderate RoB (tier 2), critical domains being confounding and attrition (**Annex K**).

The Panel considers that the available BoE from PCs does not suggest a positive relationship between the intake of FJs and incidence of high TG, high LDL-c or low HDL-c. **No comprehensive UA is performed**.

Complementary LoE3: Risk of obesity and LoE 4: T2DM. PCs. There is evidence from PCs for a positive and causal relationship between the intake of FJs and risk of obesity (very low certainty sQ5.1, Section 8.2.5.1) and T2DM (moderate certainty, sQ5.3, Section 8.4.5.1).

Conclusions sQ5.4. PCs. While there is evidence for a positive and causal relationship between consumption of FJs and risk of obesity and T2DM, the available BoE does not suggest a positive relationship between the intake of FJs and risk of dyslipidaemia.

8.5.5.3. Overall conclusion on sQ5.4

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of FJs and risk of dyslipidaemia.

8.6. Risk of hypertension

8.6.1. Total sugars

sQ1.5. Total sugars and risk of hypertension

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of hypertension	0	0
LoE2. Standalone (surrogate)	Changes in SBP and/or DBP	0	**1**
LoE3. Complementary	Incidence of hyperuricaemia/changes in uric acid	0	0
LoE4. Complementary	Risk of obesity	sQ1.1	sQ1.1
LoE5. Complementary	Risk of Type 2 diabetes mellitus	sQ1.3	sQ1.3

8.6.1.1. Intervention studies

No RCTs were eligible for sQ1.5.

8.6.1.2. Observational studies

LoE2. Standalone (surrogate): Changes in SBP and/or DBP. PCs.

One PC (SCES, (Gopinath et al., 2012)) investigated the relationship between total sugars intake and BP in adolescents of both sexes. The evidence table is in **Annex J**.

Preliminary UA

The SCES cohort reports a positive association between changes in total sugar intake and concurrent changes in BP over the 5-year follow-up (statistically significant in females only), both in the crude model and after adjusting for relevant covariates, which included TEI and baseline BP. The study was at low RoB (tier 1), with attrition being the only critical domain. The Panel notes, however, that only one PC with about 500 participants is available.

The Panel considers that the available BoE does not suggest a positive association between the intake of total sugars in isocaloric exchange with other macronutrients and an increased risk of obesity.

LoE4 (sQ1.1). Complementary: Risk of obesity. PCs. The available evidence does not suggest a positive association between the intake of total sugars in isocaloric exchange with other macronutrients and an increased risk of obesity.

LoE5 (sQ1.3). Complementary: Risk of T2DM. PCs. The available evidence does not suggest a positive association between the intake of total sugars in isocaloric exchange with other macronutrients and an increased risk of type 2 diabetes mellitus.

sQ1.5. PCs. The Panel considers the available BoE does not suggest a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of hypertension.

8.6.1.3. Overall conclusion on sQ1.5

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of hypertension. Total sugars were not investigated under other dietary conditions (e.g. not keeping TEI constant).

8.6.2. Added and free sugars

sQ2.5. Added and free sugars and risk of hypertension

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of hypertension	0	0
LoE2. Standalone (surrogate)	Changes in SBP and/or DBP	**10**	**2**
LoE3. Complementary	Incidence of hyperuricaemia/ uric acid	0/7	0
LoE4. Complementary	Risk of obesity	sQ2.1	sQ2.1
LoE5. Complementary	Risk of Type 2 diabetes mellitus	sQ2.3	sQ2.3

8.6.2.1. Intervention studies

LoE2. Standalone (surrogate): Changes in SBP and/or DBP. RCTs. The effect of high vs. low added sugar intakes on changes in blood pressure was investigated in 10 intervention studies (11 study groups), four of which had the sugar source as beverages, two as solid foods and the remaining four as combinations of beverages and solid foods. Between-arm differences in added sugar intakes ranged from 10 to 28 E%, and study duration between 6 and 36 weeks (**Appendix F**). Five RCTs were ad libitum and five were conducted under neutral energy balance, most in isocaloric exchange with starch. Two RCTs selected subjects based on serum insulin concentrations (were on, or included one group of, hyperinsulinaemic individuals) and the remaining on the basis of BMI cut-offs (five were in overweight/obese individuals, one in non-obese and two in subjects with BMI < 35 kg/m^2).

Preliminary UA

Seven RCTs found SBP to be higher in the high vs. the low sugar arm, whereas three studies (four study groups) showed the opposite (**Appendix G**, **Figure G.8a1**). The pooled mean effect estimate (95% CI) for SBP is 1.47 mmHg (−0.75, 3.68, I^2 = 83%). The pooled mean effect estimate (95% CI) for studies under neutral energy balance in isocaloric exchange with starch is 0.47 mmHg (−2.60, 3.55, I^2 = 82%) and for RCTs conducted ad libitum is 2.77 mmHg (−0.72, 6.26, I^2 = 85%). A similar pattern was observed for DBP (**Appendix G**, **Figure G.8b1**), with the pooled mean effect estimate (95% CI) being 1.48 mmHg (−0.05, 3.00, I^2 = 73%). Three RCTs were at low RoB (tier 1) and seven at moderate RoB (tier 2).

The Panel considers that the available BoE suggests a positive relationship between the intake of added and free sugars and risk of hypertension.

Comprehensive UA

Selection of the endpoint. The Panel decided to conduct the comprehensive UA on SBP because SBP, rather than DBP, is used for CVD risk stratification owing to its higher predictive value (Graham et al., 2007).

Dose-response relationship. It was not investigated in individual RCTs. No meta-regression analysis could be performed owing to the small number of RCTs available. Visual inspection of the forest plots does not suggest a dose-response relationship.

LoE3. Complementary: Incidence of hyperuricaemia/uric acid. RCTs. A total of seven RCTs (8 study groups) investigated the effect of high vs. low sugar intake on uric acid, four of which also report on blood pressure (Israel et al., 1983; Maersk et al., 2012; Lowndes et al., 2014b; Campos et al., 2015) (Appendix F). Between-arm differences in added sugar intakes that ranged from 16 to 30E%. Except for Lowndes et al. (2014b) and Campos et al. (2015), which found no differences between the two sugar arms, uric acid levels were higher in the high sugar arm relative to low sugar arm. The pooled mean effect estimate (95% CI) is 0.39 mg/dL (0.14, 0.64, I^2 = 59%) (**Appendix G**, **Figure G.10a**). Pooled mean effect estimates (95%CI) are similar for studies conducted in isocaloric exchange with starch at neutral energy balance (0.35 mg/dL (0.03, 0.68), I^2 = 69%) and for studies conducted ad libitum (0.47 mg/dL (0.03, 0.91), I^2 = 41%). Mean differences in body weight change between the high and low sugar arms ranged between −4.1 and 2.3 kg when these were reported and were apparently unrelated to changes in uric acid (**Appendix G**, **Figure G.10a**).

The Panel considers that the available BoE suggests a positive relationship between the intake of added sugars at doses between 16 to 30E% and uric acid levels, both when consumed ad libitum and in isocaloric exchange with starch. The effect appears to be independent of changes in body weight.

Complementary LoE4: Risk of obesity and LoE5: Risk of T2DM. RCTs. The is evidence from RCTs for a positive and causal relationship between the intake of added and free sugars and risk of obesity (moderate certainty, sQ2.1, Section 8.2.2.1) and T2DM (low certainty, sQ2.3, Section 8.4.2.1).

Consistency across LoE. Changes in SBP are consistent with changes in DBP, with changes in uric acid and consistent with an increased risk of obesity and T2DM.

Table 24: sQ2.5. RCTs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty that the intake of added and free sugars is positively and causally associated with the risk of hypertension at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE2. Standalone (surrogate). Endpoint:** SBP **10 RCTs (11 study groups), 568 participants**. Pooled mean effect estimate (95%CI) = 1.47 mmHg (−0.75, 3.68) assuming a within-subject correlation coefficient of 0.82. The correlation coefficient for this endpoint is expected to be close to that value. (**Appendix G**, **Figure G.8a1**)	**Initial certainty: High (> 75–100% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	3 studies in tier 1; 7 studies tier 2 (**Appendix I**, **Figure I.5**) Generally moderate. Key questions: • Randomisation: generally low • Exposure assessment: generally low • Outcome assessment: between low and probably high Probably high for allocation concealment and blinding	Serious
Unexplained inconsistency	High heterogeneity. I^2 = 83% for the pooled mean effect. Point estimates vary widely, and 95% CI show minimal overlap.	Very serious
Indirectness	Surrogate endpoint	Serious
Imprecision	High. The 95%CI includes 0 and thus the possibility of a beneficial (rather than adverse) effect. **(Appendix G**, **Figure G.8a1)**	Serious
Publication bias	Funnel plot does not suggest a high risk of publication bias and the Egger's test was not significant (p = 0.209) **(Appendix H**, **Figure H.5)** Private (n = 5), mixed (n = 2) and NR (n = 3) funding.	Undetected
Upgrading factors	Consistency: Changes in SBP are consistent with changes in DBP, with changes in uric acid and consistent with an increased risk of obesity and T2DM.	Yes (consistency)
Final certainty	Started high, downgraded one level for RoB, one level for heterogeneity, one level for indirectness and one level for imprecision; upgraded one level for consistency.	**Very low (0–15% probability)**

Conclusions sQ2.5. **RCTs**. The level of certainty in a positive and causal relationship between the intake of added and free sugars and risk of hypertension is **very low** (rationale in **Table 24**). RCTs included only adults. About half of the RCTs were in overweight/obese subjects and two were in (or included a group of) hyperinsulinaemic individuals. Added and free sugars were consumed ad libitum or in isocaloric exchange with starch and between-arm differences in added and free sugars intake ranged between 10 and 28 E%.

8.6.2.2. Observational studies

LoE2. Standalone (surrogate): Changes in SBP and/or DBP. **PCs**. Two prospective cohorts investigated the relationship between change in intake of added sugars (SCES, (Gopinath et al., 2012) or sucrose (NSHDS, (Winkvist et al., 2017)) over follow-up and concurrent changes in blood pressure. The exposure was analysed as a continuous variable using either the nutrient residuals model (SCES) or the nutrient density model (NSHDS) for analysis, and thus aimed at maintaining TEI constant. The Panel notes, however, that TEI was not included as additional factor in the model in the NSHDS cohort. The evidence table is in **Annex J**.

Preliminary UA

In the SCES cohort of adolescent males and females, a positive relationship between changes in added sugars intake and changes in SBP and DBP was observed in females. The relationship was statistically significant only for changes in DBP. Each standard deviation (27.63 g/day) increase in added sugar intake during the 5-year follow-up was concurrently related to an increase in DBP of 1.31 mmHg (SE: 0.57, $p < 0.02$). Non-significant relationships between changes in added sugars intake and SBP (negative) or DBP (positive) were reported for males.

In the NSHDS cohort, female and male adults had a mean baseline consumption of sucrose of 6.5 and 6.6E%, respectively. Each 1E% increase in sucrose intake over follow-up was related to a decrease in SBP of 0.66 mmHg (SE: 0.38, $p = 0.08$) in females and with an increase of 0.38 mmHg (SE: 0.32, $p = 0.22$) in males during the 10-year follow-up. The study did not report results for DBP.

These studies were at RoB tier 1 (SCES) and tier 3 (NSHDS), critical domains being confounding, outcome assessment and attrition (**Annex K**).

The Panel notes the paucity of data available from PCs. The Panel also notes that in the PC at low RoB, changes in SBP were inconsistent between sexes and inconsistent with changes in DBP in males.

The Panel considers that the available BoE does not suggest a positive relationship between the intake of added sugars in isocaloric exchange with other macronutrients and BP.

Complementary LoE4: Risk of obesity and LoE5: Risk of T2DM. PCs. The available BoE does not suggest a positive relationship between the intake of added or free sugars in isocaloric exchange with other macronutrients and risk of obesity (sQ2.1, Section 8.2.2.2) or T2DM (sQ2.3, Section 8.4.2.2).

sQ2.5. PCs. The available BoE does not suggest a positive relationship between the intake of added or free sugars in isocaloric exchange with other macronutrients and risk of hypertension.

8.6.2.3. Overall conclusion on sQ2.5

There is evidence from RCTs for a positive and causal relationship between the intake of added and free sugars ad libitum and isocaloric exchange with starch and risk of hypertension (**very low** certainty). The available BoE from PCs cannot be used to modify the level of certainty in this conclusion.

8.6.3. Fructose

sQ3.5. Fructose and risk of hypertension

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of hypertension	0	**3**
LoE2. Standalone (surrogate)	Changes in SBP and/or DBP	**5**	**2**
LoE3. Complementary	Incidence of hyperuricaemia/uric acid	0/5	0
LoE4. Complementary	Risk of obesity	sQ3.1	sQ3.1
LoE5. Complementary	Risk of Type 2 diabetes mellitus	sQ3.3	sQ3.3

8.6.3.1. Intervention studies

LoE2. Standalone (surrogate): Changes in SBP and/or DBP. RCTs. Four RCTs investigated the effects of fructose in isocaloric exchange with glucose at doses between 9 and 25 E% on blood pressure. The results of the individual studies can be found in **Appendix F**.

Preliminary UA

All RCTs except Angelopoulos et al. (2015) show a decrease in SBP and DBP with fructose relative to glucose, with a pooled mean effect estimate (95% CI) of −1.61 (−4.61, 1.38, $I^2 = 57\%$) and −2.09 mmHg (−4.30, 0.13, $I^2 = 65\%$), respectively **(Appendix G, Figures G.9a,b)**.

All these studies were at moderate RoB (tier 2), the critical domains being randomisation, allocation concealment, blinding and endpoint assessment (**Appendix I**, **Figure I.6**).

One cross-over design study investigated the effect of varying levels of fructose (0, 7.5 and 15 E%) in isocaloric exchange with starch for 5 weeks (Hallfrisch et al., 1983a)*. SBP was significantly lower with diets providing 7.5 and 15 E% from fructose than with the diet providing 0 E% from fructose ($p < 0.015$).

The Panel considers that the available evidence from RCTs does not suggest a positive relationship between the intake of fructose in isocaloric exchange with glucose or starch and SBP or DBP. **No comprehensive UA is performed**.

LoE3. Complementary: Incidence of hyperuricaemia/uric acid. RCTs. The same four studies that reported on the effect of fructose vs. glucose on changes in BP also report on changes in fasting uric acid levels. Uric acid levels were higher in four out of the five study groups when fructose was consumed, the effect being statistically significant only in the study by Stanhope et al. (2009) conducted ad libitum (results in Cox et al. (2012)). The exception were subjects with IGT in the study by Koh et al. (1988), which showed lower uric acid levels with fructose compared to glucose. The pooled mean effect estimate (95% CI) is 0.12 (−0.16, 0.40, I^2 = 74%). Mean differences in body weight change between the fructose and glucose arms ranged between −1.5 and 0.1 kg when these were reported, suggesting that the effect is independent of changes in body weight (**Appendix G**, **Figure G.11**).

In another study by Reiser et al. (1989a), fructose intake at 20 E% in isocaloric exchange with starch significantly increased uric acid levels in normo- and hyperinsulinaemic individuals. The mean effect (95%CI) was 0.54 mg/dL (0.19, 0.89).

The Panel considers that there is some evidence from RCTs for a positive relationship between the intake of fructose in isocaloric exchange with other carbohydrates (i.e. glucose, starch) at doses between 9 and 25E% and uric acid levels. The effect appears to be independent of changes in body weight.

Complementary LoE4: Risk of obesity and LoE5: risk of T2DM. RCTs. The available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with glucose and risk of obesity (sQ3.1, Section 8.2.3.1) or T2DM (low certainty, sQ3.3, Section 8.4.3.1).

Conclusions sQ3.5. RCTs. The Panel considers that the available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with glucose and risk of hypertension.

8.6.3.2. Observational studies

LoE1. Standalone (main): Incidence of hypertension. PCs. Three large independent PCs of male (HPFS) and female (NHS and NHS-II) health professionals in the USA reported in the same publication (Forman et al., 2009) investigated the relationship between fructose (E%, quintiles of intake) and incidence of hypertension. Models were adjusted for both baseline BMI and TEI. TEI was kept constant in the analyses. Evidence table is in **Annex J**.

Preliminary UA

No significant relationship was found between fructose and incidence of hypertension across quintiles of intake in any cohort (most adjusted models). Median intakes ranged from about 6 E% to about 14 E% across quintiles of fructose. Duration of follow-up ranged from 14 to 20 years (**Appendix K**, **Figure K.14**). The three PCs were at low RoB (tier 1) for this endpoint and no critical domains were identified.

The Panel considers that the available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and incidence of hypertension. **No comprehensive UA is performed on this LoE**.

LoE2. Standalone (surrogate): Changes in SBP and/or DBP. PCs. Two PCs (SCES, (Gopinath et al., 2012); TLGS, (Bahadoran et al., 2017)) investigated associations between fructose intake and changes in SBP and DBP. The evidence table is in **Annex J**.

Preliminary UA

The SCES cohort reported a statistically significant association between fructose intake and BP in female adolescents, but no association was found among males (RoB tier 1). In females, each standard deviation increase in fructose intake over the 5-year follow-up (1 SD = 14.19 g/day) was concurrently related to an increase of 1.80 mmHg (SE = 0.82; p = 0.03) in SBP and of 1.67 mmHg (SE = 0.61; p = 0.01) in DBP. In the TLGS cohort of Iranian adults with a mean baseline fructose consumption of 6.4 E%, each 1 E% of fructose intake at baseline was related to an increase of 0.217 mmHg (95% CI: 0.063 to 0.371) in SBP and 0.267 mmHg (95% CI: 0.157, 0.376) in DBP during a mean follow-up of 6.7 year. The only adjustment made in the linear regression was age (RoB tier 3).

The Panel notes that the available BoE is limited to two PCs, one of which is at high RoB.

The Panel considers that the available BoE from PCs does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and blood pressure.

No comprehensive UA is performed on this LoE.

Complementary LoE4: Risk of obesity and LoE5: risk of T2DM. PCs. The available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and risk of obesity (sQ3.1, Section 8.2.3.2) or T2DM (sQ3.3, Section 8.4.3.2).

Conclusions sQ3.5. PCs. The Panel considers that the available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and risk of hypertension.

8.6.3.3. Overall conclusion on sQ3.5

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of fructose in isocaloric exchange with glucose or other macronutrients and risk of hypertension.

8.6.4. Sugar-sweetened beverages

sQ4.5. SSBs and risk of hypertension			
LoE1. Standalone (main)	Incidence of hypertension	0	**7**
LoE2. Standalone (surrogate)	SBP and/or DBP	**4**	**1**
LoE3. Complementary	Incidence of hyperuricaemia/uric acid	0/3	1/0
LoE4. Complementary	Risk of obesity (sQ4.1)	sQ4.1	sQ4.1
LoE5. Complementary	Risk of Type 2 diabetes mellitus (sQ4.3)	sQ4.3	sQ4.3

8.6.4.1. Intervention studies

LoE2. Standalone (surrogate): Changes in SBP and/or DBP. RCTs. Four of the 10 intervention studies that investigated the effect of high vs. low added sugar intakes on changes in BP (see Section 8.6.2.1) were on beverages.

For SBP (**Appendix G**, **Figure G.8a2**), the variable used for the comprehensive UA, pooled mean effect estimates (95%CI) for sugars from different sources were 3.05 mmHg (−0.96, 7.06, I^2 = 91%) for beverages (n = 4, dose range 18–22E%), 2.04 mmHg (−1.98, 6.07, I^2 = 77%) for mixtures of food and beverages (n = 4, dose range = 10–23E%) and −1.14 mmHg (−4.58, 2.30, I^2 = 63%) for solid foods (n = 2, 3 study groups, dose range = 15–28E%). A similar pattern was observed for DBP (**Appendix G**, **Figure G.8b2**), with the pooled mean effect estimate (95% CI) for beverages being 2.25 mmHg (−0.70, 5.21, I^2 = 75%).

LoE3. Complementary: Incidence of hyperuricaemia/uric acid. RCTs. Out of the seven RCTs that investigated the effect of high vs. low sugar intake on uric acid levels (see Section 8.6.2.1), three were conducted with beverages (**Appendix F**). Between-arm differences in energy derived from SSBs ranged from 18 to 22E%. Uric acid levels were significantly higher in the high vs. the low sugar arm in one study conducted ad libitum, whereas no difference was observed in another RCTs conducted *ad libitum*. In the study conducted at neutral energy balance, uric acid levels were lower in the high vs. the low sugar arms. The pooled mean effect estimate (95% CI) is 0.10 mg/dL (−0.42, 0.63, I^2 = 63%) (**Appendix G**, **Figure G.10b**). One of the studies was at low RoB (tier 1) and two were at moderate RoB (tier 2).

The Panel notes the low number of RCTs available on the effect of SSBs on uric acid levels and the inconsistency of the results across studies. The Panel considers that the available BoE does not suggest a positive relationship between the intake of SSBs and uric acid levels.

Complementary LoE4: Risk of obesity and LoE5: Risk of T2DM. RCTs. There is evidence from RCTs for a positive and causal relationship between the intake of SSBs and risk of obesity (moderate certainty, sQ4.1, Section 8.2.4.1) and T2DM (low certainty, sQ4.3, Section 8.4.4.1).

Based on the available BoE from RCTs, the Panel has the same level of certainty on a positive and causal relationship between the intake of SSBs and risk of hypertension as for added and free sugars (**very low** certainty).

Conclusion sQ4.5. RCTs. The level of certainty in a positive and causal relationship between the intake of SSBs and risk of hypertension is **very low**.

8.6.4.2. Observational studies

LoE1. Standalone (main): Incidence of hypertension. PCs. Seven PCs, six in adults and one in children and adolescents (TLGS), investigated the relationship between intake of SSBs and incidence of hypertension. In five PCs (KoGES, (Kwak et al., 2018); HPFS, NHSII and NHS, (Cohen et al., 2012); SUN, (Sayon-Orea et al., 2015)) hypertension was defined as SBP $\geq$ 140 mmHg and/or DBP $\geq$ 90 mmHg or use of antihypertensive medication, whereas lower thresholds of $\geq$ 130 mmHg and $\geq$ 85 mmHg, respectively, were used in TLGS (Mirmiran et al., 2015) and the CARDIA (Duffey et al., 2010) cohort of young adults.

Six cohorts analysed SSBs as a categorical variable using the standard multivariable model for energy adjustment and one cohort (CARDIA) analysed the exposure as a continuous variable adjusting for non-SSBs energy intake. In both cases, the analysis allows for TEI to change as a function of SSBs consumption. Three cohorts (NHS, NHSII, HPFS) also investigated the relationship between ASBs and incidence of hypertension. Evidence table is in **Annex J**.

Preliminary UA

All cohorts report a positive association between the intake of SSBs and incidence of hypertension and the associations were significant in four of the seven cohorts (KoGES, NHS, NHSII, SUN). The forest plot for the six PCs in adults can be found in **Appendix K**, **Figure K.14**. The TLGS cohort in children and adolescents is not included (number of cases was not reported).

The three cohorts that analysed consumption of ASBs showed similar, or even stronger (HPFS), associations with hypertension as for SSBs. The associations were positive and statistically significant in all three cohorts. Data from these cohorts were collected and analysed using the same methodology.

Five PCs were at low RoB (tier 1), one at moderate RoB (tier 2) and one at high RoB (tier 3) (**Appendix L**, **Table L.11**).

The Panel considers that the available BoE suggests a positive relationship between the consumption of SSBs and risk of hypertension.

Comprehensive UA

Selection of the endpoint. The only eligible endpoint in this LoE is incidence of hypertension. The definition of hypertension and the methods used for the identification of cases were similar for all cohorts, except for the CARDIA and TLGS cohorts which used lower SBP and DPB thresholds for defining hypertension.

Dose-response relationship. A significant linear dose-response relationship across categories of SSBs intake was reported in five (KoGES, NHS, NHSII, SUN, TLGS) of the six PCs which performed a categorical analysis.

In the dose-response meta-analysis conducted by EFSA, parametric dose-response models were estimated based on summarised data. Both linear and non-linear (restricted cubic splines) dose-response relationships were investigated. The methodological approach applied was the same as for the dose-response meta-analyses of SSBs intake and incidence T2DM (**Annex M**).

Fourteen non-referent RRs from five study-specific analyses were included in the dose-response meta-analysis (I^2 = 70.5%; p = 0.009). The TLGS (number of incident cases not reported) and CARDIA (RR already provided per unit increase) cohorts were excluded. The predicted pooled relative risk of HTN was 1.06 (95% CI: 1.04, 1.08) for an increase in SSBs intake of 250 mL/day in the linear model (p for linear trend < 0.0001) and 1.07 (95% CI: 1.04, 1.11) at 250 mL/day in the non-linear model (RCS with three knots at fixed percentiles, 10%, 50% and 90%, of the distribution; p for non-linearity = 0.237) (**Figure 16**). The subgroup analyses did not identify clear sources of heterogeneity, also given the limited number of studies across strata. The funnel plot and related Egger regression were not carried out as the number of studies was very limited.

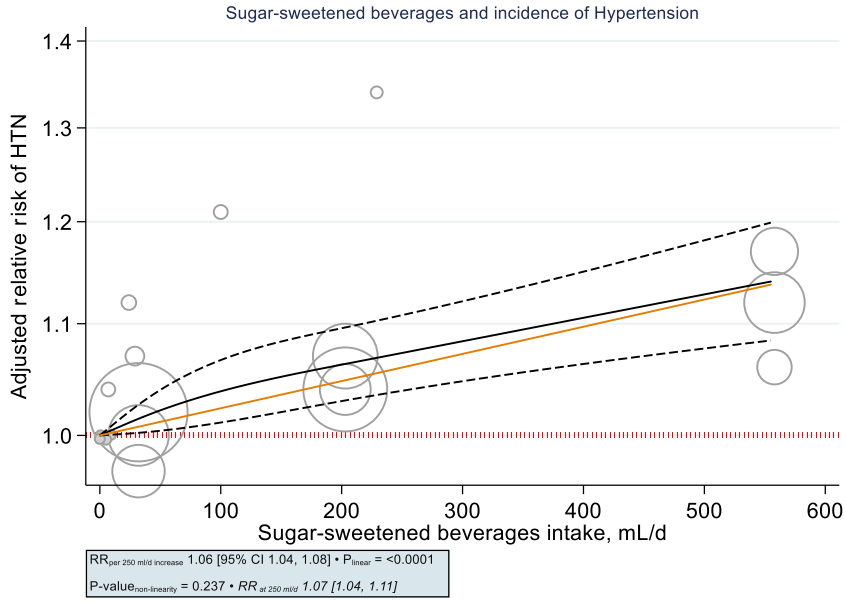


Figure 16: Dose-response meta-analysis on the relationship between the intake of sugar-sweetened beverages and incidence of hypertension (HTN)

LoE2. **Standalone (surrogate): Changes in SBP and/or DBP**. **PCs**. One PC (WAPCS, (Ambrosini et al., 2013)), investigated the relationship between changes in SSBs intake and concurrent changes in BP over the 3-year follow-up. Evidence table is in **Annex J**.

Non-significant positive (for SBP) and negative (for DBP) associations were reported for changes in BP across tertiles of increase in SSBs intake in males and females after adjusting for BMI and major dietary patterns. The authors state that these relationships were unchanged after additional adjustment for TEI in separate models (data not shown). The study was at low RoB (tier 1). The critical domain was attrition.

The Panel notes the limited evidence available from PCs. The Panel considers that the available BoE does not suggest a positive relationship between intake of SSBs and changes in BP.

LoE3. Complementary: Incidence of hyperuricaemia/uric acid. PCs. One PC (ARIC, (Bomback et al., 2010)) investigated the relationship between intake of SSBs and incidence of hyperuricaemia. SSBs were analysed as a categorical variable without adjustment for energy intake. Evidence table is in **Annex J**.

There was a positive (non-significant) association between consumption of SSBs and incidence of hyperuricaemia. In comparison to the referent category consuming less than one serving or 355 mL per day), those consuming more than one serving per day had an OR for incident hyperuricaemia of 1.17 (95% CI: 0.95, 1.43, $p = 0.1$). A negative (non-significant) relationship with incident hyperuricaemia (OR 0.97, 95% CI: 0.83, 1.14) was found for ASBs. The study was at low RoB (tier 1).

The Panel notes the paucity of data available and considers that the available BoE does not suggest a positive relationship between intake of SSBs and incidence of hyperuricaemia.

Table 25: sQ4.5. PCs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty in a positive and causal relationship between intake of SSBs and the risk of hypertension at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE1. Standalone (main). Endpoint: incidence of hypertension** **7 PCs, 246,572 participants.** Five study-specific analyses from five PCs were included in the dose-response analysis.	**Initial certainty: Moderate (> 50–75% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	Five PCs in tier 1; 1 PC in tier 2; 1 PC in tier 3 (**Appendix L**, **Table L.11**). Generally low Key questions: • Confounding: most probably low • Exposure assessment: most probably low • Outcome assessment: most probably low Mixed probably low and probably high for attrition The study at RoB tier 3 (TLGS) was not included in the dose-response analysis (number of cases not reported).	Not serious
Unexplained inconsistency	All PCs (n = 7) report positive relationships between the intake of SSBs and incidence of hypertension. Substantial heterogeneity (I^2 = 70.5%) for the pooled mean effect estimate of study-specific RRs per unit increase of intake. RRs are similar across large studies; small studies show higher effects, but confidence intervals overlap. No clear sources of heterogeneity identified beyond sample size.	Not serious
Indirectness	Direct endpoint	Not serious
Imprecision	Low	Not serious
Publication bias	Limited number of studies, it cannot be assessed. Public (n = 6) and mixed funding (n = 1).	Undetected (cannot be assessed)
Upgrading factors	Dose-response: A significant linear dose-response relationship across categories of SSBs intake was reported in 5 of the 6 PCs which performed a categorical analysis. The dose-response meta-analysis conducted by EFSA showed a significant linear positive dose relationship (linear pooled mean effect estimate (95%CI) = 1.06 (1.04, 1.08) for 250 mL/d increase with no support for non-linearity (p = 0.237).	Yes (dose-response)
Final certainty	Started moderate, upgraded one level for dose-response.	**High (> 75–100% probability)**

Complementary LoE4: Risk of obesity and LoE5: risk of T2DM. PCs. There is evidence from PCs for a positive and causal relationship between the intake of SSBs and risk of obesity (moderate certainty, sQ4.1, Section 8.2.4.2) and T2DM (moderate certainty, sQ4.3, Section 8.4.4.2).

Consistency across LoEs. The Panel notes that an increased incidence of hypertension is consistent with an increased risk of obesity and T2DM, but very few PCs assessed endpoints for other LoEs specific to this sQ (e.g. changes in BP, incidence of hyperuricaemia).

Conclusion sQ4.5. PCs. The level of certainty in a positive and causal relationship between the intake of SSBs and risk of hypertension is **high** (rationale in **Table 25**). The relationship was observed for SSBs not keeping TEI constant in the analysis.

8.6.4.3. Overall conclusion on sQ4.5

There is evidence from PCs for a positive and causal relationship between the intake of SSBs and risk of hypertension (**high** certainty). Evidence from RCTs (**very low** certainty) supports the relationship.

8.6.5. Fruit juices

sQ5.5. FJs and risk of hypertension			
LoE1. Standalone (main)	Incidence of hypertension	0	**2**
LoE2. Standalone (surrogate)	Changes in SBP and/or DBP	0	0
LoE3. Complementary	Incidence of hyperuricaemia/uric acid	0	0
LoE4. Complementary	Risk of obesity (sQ5.1)	sQ5.1	sQ5.1
LoE5. Complementary	Risk of Type 2 diabetes mellitus (sQ5.3)	sQ5.3	sQ5.3

8.6.5.1. Observational studies

LoE1. Standalone (main): Incidence of hypertension. PCs. Two PCs (CARDIA, (Duffey et al., 2010); WHI, (Auerbach et al., 2017)) investigated the relationship between FJs intake and incidence of hypertension. The CARDIA cohort analysed the exposure as a continuous variable adjusting for non-SSBs energy intake, thus not keeping TEI constant. Conversely, the WHI cohort analysed the exposure as a categorical variable using the nutrient residual (energy adjusted) model and thus kept TEI constant. In the WHI cohort, participants were considered to have incident hypertension if they initiated medication for treatment and in the CARDIA cohort either use of antihypertensive medication or BP $\geq$ 130 mmHg/$\geq$ 85 mmHg. Evidence table is in **Annex J**.

Preliminary UA

Both PCs found that the association between FJs intake and incidence of hypertension was null. The Panel notes that, in the WHI cohort, TEI was kept constant in the analysis. Both cohorts were at low RoB (tier 1).

The Panel notes the small number of studies available. The Panel considers that the available BoE does not suggest a positive relationship between intake of FJs and incidence of hypertension. **No comprehensive UA is performed**.

Complementary LoE4: Risk of obesity and LoE5: risk of T2DM. PCs. There is evidence from PCs for a positive and causal relationship between the intake of FJs and risk of obesity (very low, sQ5.1, Section 8.2.5.1) and T2DM (moderate, sQ5.3, Section 8.4.5.1).

8.6.5.2. Overall conclusion on sQ5.5

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of FJs and risk of hypertension.

8.7. Risk of cardiovascular diseases

8.7.1. Total sugars

sQ1.6. Total sugars and risk of cardiovascular diseases (CVDs)			
LoE	**Endpoints**	**RCTs (n)**	**PCs (n)**
LoE1. Standalone (main)	Incidence and mortality: CVD (composite endpoint), CHD or stroke	0	**8**
LoE2. Complementary	Risk of obesity	sQ1.1	sQ1.1
LoE3. Complementary	Risk of Type 2 diabetes mellitus	sQ1.3	sQ1.3
LoE4. Complementary	Risk of dyslipidaemia	sQ1.4	sQ1.4
LoE5. Complementary	Risk of hypertension	sQ1.5	sQ1.5
LoE6. Complementary	Incidence of hyperuricaemia/uric acid	LoE3 for sQ1.5	LoE3 for sQ1.5

8.7.1.1. Observational studies

LoE1. Standalone (main): Incidence and mortality: CVD (composite endpoint), CHD or stroke. PCs. Two publications report on the relationship between the intake of total sugars and incidence of CVDs using data from one PC (WHI) or several PCs (EPIC-Multicentre). The WHI cohort of post-menopausal women (Tasevska et al., 2018) provides results for incidence of CVD, CHD and stroke, whereas the EPIC-Multicentre study (Sieri et al., 2020) reports on incidence of CHD. For three centres included in that study (EPIC-Utrecht, EPIC-Morgen, EPICOR), results on incidence of CHD and stroke are reported in separate publications (EPIC-Utrecht: (Beulens et al., 2007), EPIC-Morgen: (Burger et al., 2011), EPICOR: (Sieri et al., 2010, 2013)). Results on incidence of CHD for these centres have not been considered in the final data set because of the overlap with the EPIC-Multicentre. The EPIC-Utrecht also reports on CVD incidence (Beulens et al., 2007).

In addition, three PCs provide results on the relationship between the intake of total sugars and mortality from CVDs, two on CVD mortality as a composite endpoint (NIH-AARP, (Tasevska et al., 2014b); Takayama, (Nagata et al., 2019)) and one on CHD mortality (SCHS, (Rebello et al., 2014)).

The cohorts involved Asian populations (Takayama, SCHS), US populations (WHI, NIH-AARP) and European populations (EPIC cohorts).

In these PCs, total sugars were analysed either as a continuous (WHI) variable, as categorical variable (all other cohorts) or both, using either the nutrient residuals (energy-adjusted) model or the nutrient density (energy-adjusted) model for energy adjustment, and thus, total sugars were investigated in isocaloric exchange with other macronutrients. The WHI also analysed the data applying energy partition models to investigate the full effect of total sugars intake on CVD risk (i.e. the energy and non-energy contribution of the nutrient while keeping energy intake from other nutrients constant). All PCs included BMI in most-adjusted models. The evidence table is in **Annex J**.

Preliminary UA

CVD (incidence and mortality). Results on the relationship between total sugars intake and CVD (composite endpoint) were mixed in the four PCs reporting on this endpoint. The relationship was positive and non-significant in the NIH-AARP (mortality) for males and females, positive and significant for males and null for females in the Takayama (mortality), null for the EPIC-Utrecht cohort of females and negative (non-significant) in the WHI cohort (incidence). These data are plotted in **Appendix K**, **Figure K.15a**.

CHD (incidence and mortality). A positive and significant relationship between total sugars intake and CHD (incidence) was observed in the EPIC-Multicentre study. Conversely, negative relationships were reported in the WHI (incidence) and the SCHS (mortality) cohorts. The negative relationship was statistically significant for males in the SCHS (**Appendix K**, **Figure K.15b**).

Stroke (incidence). The results on incidence of stroke in the three EPIC centres reporting on this endpoint were mixed. The relationship was positive and non-significant in EPICOR for males and females combined, null for males and negative, non-significant for females in EPIC-Morgen and null for the female-only cohort of EPIC-Utrecht. A negative (non-significant) association between the intake of total sugars and incidence of stroke was reported in the WHI cohort (**Appendix K**, **Figure K.15b**).

Six out of the eight PCs were at low risk of bias (tier 1; EPIC-Multicentre, EPIC-Utrecht, EPIC-Morgen, EPICOR, NIH-AARP, SCHS) and two at moderate RoB (tier 2; WHI and Takayama) for all the endpoints assessed in each study. Critical domains were exposure and outcome assessment (Takayama) and outcome assessment and attrition (WHI) (**Appendix L**, **Table L.12**).

Complementary LoE2: risk of obesity, LoE3: risk of T2DM, LoE4: risk of dyslipidaemia and LoE5: risk of hypertension. PCs. The available BoE does not support a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of obesity (sQ1.1, Section 8.2.1.1), T2DM (sQ1.3, Section 8.4.1.1), dyslipidaemia (sQ1.4, Section 8.5.1.1) or hypertension (sQ1.5, Section 8.6.1.2).

The Panel notes that most PCs report null or negative relationships between the intake of total sugars and incidence of stroke, and that these PCs were mostly at low RoB. The Panel also notes that, for CHD and CVD (composite endpoint), the results were mixed across cohorts.

For CHD, the Panel considers that the EPIC-Multicentre study is most relevant to the present assessment because it consists of a pooled analysis of data from 23 centres representing eight European countries, including males and females 35–70 years of age. RR (95%CI) for the highest vs. the lowest quartile of total sugars intake (energy-adjusted intakes using the residual method = ≤ 77.2 g/day and > 129.3 g/day, respectively) was 1.24 (1.09, 1.40). The RR per each 50 g/day

increase in total sugars was 1.09 (1.02, 1.17). When pooled effect estimates were calculated by country (continuous analysis), heterogeneity was found to be low (I^2 = 29.6%) and results varied across countries, with five countries reporting a positive association, two reporting a negative association and one where the relationship was null. The Panel notes that this study was at low RoB (tier 1). The Panel also notes, however, that these results are inconsistent with data from two other cohorts included in the assessment (WHI, SCHS) which show a negative relationship between the intake of total sugars and CHD, and are not supported by PCs on the relationship between total sugars and CVD risk or risk factors for CVDs (namely obesity, T2DM, dyslipidaemia and hypertension).

The Panel therefore considers that the available evidence does not suggest a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and incidence of CHD. **No comprehensive UA is performed**.

Conclusion sQ1.6. PCs. The Panel considers the available BoE does not suggest a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of CVDs.

8.7.1.2. Overall conclusion on sQ1.6

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of CVDs. Total sugars were not investigated under other dietary conditions (e.g. not keeping TEI constant).

8.7.2. Added and free sugars

sQ2.6. Added and free sugars and risk of cardiovascular diseases

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence and mortality: CVD (composite endpoint) or as CHD or stroke	0	**3**
LoE2. Complementary	Risk of obesity	sQ2.1	sQ2.1
LoE3. Complementary	Risk of Type 2 diabetes mellitus	sQ2.3	sQ2.3
LoE4. Complementary	Risk of dyslipidaemia	sQ2.4	sQ2.4
LoE5. Complementary	Risk of hypertension	sQ2.5	sQ2.5
LoE6. Complementary	Incidence of hyperuricaemia/uric acid	LoE3 for sQ2.5	LoE3 for sQ2.5

8.7.2.1. Intervention studies

No RCTs were eligible for standalone LoEs in relation to sQ2.6.

Complementary LoE2: risk of obesity, LoE3: risk of T2DM, LoE4: risk of dyslipidaemia and LoE5: Risk of hypertension. RCTs. There is evidence for a positive and causal relationship between the intake of added and free sugars and risk of obesity (**moderate**, sQ2.1, Section 8.2.2.1), T2DM (**low**, sQ2.3, Section 8.4.2.1), dyslipidaemia (**moderate**, sQ2.4, Section 8.5.2.1) and hypertension (**very low**, sQ2.5, Section 8.6.2.1).

Complementary LoE6 (LoE3 for sQ2.5): Incidence of hyperuricaemia/uric acid. RCTs. There is evidence for a positive relationship between the intake of added sugars at doses between 16 to 30E% and uric acid levels, both when consumed ad libitum and in isocaloric exchange with starch. The effect appears to be independent of changes in body weight.

Conclusion sQ2.6. RCTs. Although there is some evidence for a positive and causal relationship between the intake of added and free sugars and adverse effects on established risk factors for cardiovascular diseases (i.e. body weight, glucose metabolism, blood lipids, blood pressure and uric acid), no RCTs on cardiovascular disease endpoints are available. In the absence of data from standalone LoEs, the available BoE from RCTs **cannot be used to conclude** on a positive relationship between the intake of added or free sugars and risk of cardiovascular diseases (see Section 8.1.3).

8.7.2.2. Observational studies

LoE1. Standalone (main): Incidence and mortality: CVD (composite endpoint), CHD or stroke. PCs. Three PCs investigated CVD (composite endpoint) in relation to the intake of added or free sugars (Mr and Ms Os, (Liu et al., 2018)), sucrose (MDCS, (Sonestedt et al., 2015)) and added sugars or sucrose (NIH-AARP, (Tasevska et al., 2014b) expressed as E% or in g/1,000 kcal across quintiles of intake. Of these, one (MDCS) reports on CVD incidence and two (Mr and Ms Os, NIH-AARP) on CVD mortality. The MDCS cohort also investigated sucrose in relation to the incidence of CHD and ischaemic stroke. The evidence table is in **Annex J**.

The three PCs analysed the exposure as categorical variable and used the energy density (energy adjusted) model or the residual model to account for TEI, and thus investigated sugars in isocaloric exchange with other macronutrients.

Preliminary UA

CVD (incidence and mortality). Negative and non-significant associations between the intake of added sugars, free sugars or sucrose and incidence of fatal CVD were reported in Mr and Ms Os and NIH-AARP cohorts. This was also the case for major sources of added sugars, including beverages, in the Mr and Ms Os cohort. Most adjusted models included TEI, dietary factors, BMI and other risk factors for CVD. In the MDCS cohort (Sonestedt et al., 2015), a positive but non-significant association was found between sucrose intake and incidence of CVD ($HR_{Q5\ vs.\ Q1}$: 1.08; 95% CI: 0.96, 1.21; P-trend = 0.18).

CHD, ischaemic stroke (incidence). When investigating the association with CHD or stroke separately (Warfa et al., 2016) in the MDCS cohort, sucrose intake was positively and significantly associated with the incidence of CHD ($HR_{Q5\ vs.\ Q1}$: 1.37; 95% CI: 1.13, 1.66; P-trend = 0.008). A non-linear dose-response relationship between sucrose intake and risk of coronary events was modelled using a restricted cubic spline with four knots and the median sucrose intake (8.2 E%) as reference. This analysis indicated that the coronary event risk associated with sucrose intake increased above the median intake, with statistically significant levels above 13 E% from sucrose. Conversely, the relationship between sucrose intake and incidence of ischaemic stroke was negative and non-significant (HR_{Q5} vs. Q1: 0.94; 95% CI: 0.77, 1.14; P-trend = 0.66).

The three PCs were a low RoB (tier 1) for all the exposures and endpoints assessed (**Annex K**).

The Panel notes that, whereas negative and non-significant associations are reported between the intake of added and free sugars (and sucrose as a proxy) and CVD mortality (Mr and Ms Os, NIH-AARP), a positive relationship was observed between the intake of sucrose and incidence of CVD mostly driven by a positive and significant relationship with the incidence of CHD (MDCS). However, the Panel also notes that only one PC is available for that exposure and endpoint. The Panel considers that the available BoE does not suggest a positive relationship between the intake of added or free sugars and risk of CVD. **No comprehensive UA is performed**.

Complementary LoE2: risk of obesity, LoE3: risk of T2DM, LoE4: risk of dyslipidaemia and LoE5: Risk of hypertension. PCs. The available BoE does not support a positive relationship between the intake of added and free sugars in isocaloric exchange with other macronutrients and risk of obesity (sQ2.1, Section 8.2.2.2), T2DM (sQ2.3, Section 8.4.2.2), dyslipidaemia (sQ2.4, Section 8.5.2.2) or hypertension (sQ2.5, Section 8.6.2.2).

Conclusions sQ2.6. PCs. The Panel considers that the available BoE does not support a positive relationship between the intake of added and free sugars in isocaloric exchange with other macronutrients and risk of CVD.

8.7.2.3. Overall conclusions on sQ2.6

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of added or free sugars in isocaloric exchange with other macronutrients and risk of CVD.

8.7.3. Fructose

sQ3.6. Fructose and risk of cardiovascular diseases

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence and mortality: CVD (composite endpoint) or as CHD or stroke	0	**3**

sQ3.6. Fructose and risk of cardiovascular diseases			
LoE	**Endpoints**	**RCTs (n)**	**PCs (n)**
LoE2. Complementary	Risk of obesity	sQ3.1	sQ3.1
LoE3. Complementary	Risk of Type 2 diabetes mellitus	sQ3.3	sQ3.3
LoE4. Complementary	Risk of dyslipidaemia	sQ3.4	sQ3.4
LoE5. Complementary	Risk of hypertension	sQ3.5	sQ3.5
LoE6. Complementary	Incidence of hyperuricaemia/uric acid	LoE3 for sQ3.5	LoE3 for sQ3.5

8.7.3.1. Intervention studies

No RCTs were eligible for standalone LoEs in relation to sQ3.6.

Complementary LoE4: risk of obesity, LoE5: risk of T2DM, LoE6: risk of dyslipidaemia and LoE7: Risk of hypertension. RCTs. The available BoE does not support a positive relationship between the intake of fructose in isocaloric exchange with glucose and risk of obesity (sQ3.1, Section 8.2.3.1), T2DM (sQ3.3, Section 8.4.3.1), dyslipidaemia (sQ3.4, Section 8.5.3.1) or hypertension (sQ3.5, Section 8.6.3.1).

LoE8 (LoE3 for sQ3.5). Complementary: Risk of incidence of hyperuricaemia/uric acid. RCTs. There is some evidence from RCTs for a positive relationship between the intake of fructose in isocaloric exchange with other carbohydrates (i.e. glucose, starch) at doses between 9 and 25E% and uric acid levels. The effect appears to be independent of changes in body weight.

Conclusion sQ3.6. RCTs. The Panel considers that the available BoE does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other carbohydrates (glucose, starch) and risk of cardiovascular diseases.

8.7.3.2. Observational studies

LoE1. Standalone (main): Incidence and mortality: CVD (composite endpoint), CHD or stroke. PCs. Three PCs investigated CVD (composite endpoint) in relation to the intake of fructose expressed as E% or in g/1,000 kcal across categories of intake. Of these, one (TLGS; (Bahadoran et al., 2017)) reports on CVD incidence and two (NIH-AARP, (Tasevska et al., 2014b); Takayama; (Nagata et al., 2019)) on CVD mortality. The evidence table is in **Annex J**.

The three PCs analysed the exposure as categorical variable and used the energy density (energy adjusted) model to account to TEI, and thus investigated fructose in isocaloric exchange with other macronutrients. TLGS also analysed fructose as a continuous variable.

Preliminary UA

CVD (incidence and mortality). The three PCs report positive relationships between the intake of fructose and risk of CVD (incidence or mortality). The relationship was statistically significant in the TLGS cohort (incidence, males and females combined) and in the NIH-AARP and Takayama cohorts (mortality) for males only (**Appendix K, Figure K.16a**). In the NIH-AARP, fructose from solid foods was negatively associated with the incidence of fatal CVD, whereas the relationship was positive for fructose from beverages. These relationships were statistically significant for both males and females. The TLGS cohort also reported results for added and naturally occurring fructose separately. Similarly to the relationship with total fructose, a statistically significant positive association was observed for added fructose ($HR_{T3\ vs.\ T1}$ = 1.80, 95%CI: 1.04, 3.12), while the relationship with naturally occurring fructose was positive but non-significant ($HR_{T3\ vs.\ T1}$ = 1.19, 95%CI: 0.69, 2.05). The cohorts widely differed in the number of participants (2,369 in TLGS; 29,079 in Takayama; 353,751 in NIH-AARP), the length of follow-up (6.7 years in TLGS vs. 13 and 14 years in the NIH-AARP and Takayama, respectively) and the range of fructose intake (median intakes in the highest categories for the Takayama cohort corresponded to the lowest categories of intake for the NIH-AARP and TLGS cohorts). The strongest association was reported for the smaller study (TLGS) with the shortest follow-up, in which the number of cases was small (**Appendix K, Figure K.16a**).

These PCs were at low (RoB tier 1; NIH-AARP), moderate (RoB tier 2; Takayama) and high (RoB tier 3; TLGS) risk of bias. Critical domains were confounding, exposure and outcome assessment. The heat map is in **Appendix L, Table L.13**.

The Panel considers that the available evidence suggests a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and risk of CVD.

Comprehensive UA

Selection of the endpoint. The only endpoint in this LoE for which data are available is CVD (composite endpoint). The pooled mean effect estimate of study-specific HRs for the highest vs. the lowest categories of intake is 1.11 (1.01, 1.21; I^2 = 31.7%) (**Appendix K, Figure K.16b**).

Dose-response relationship. Significant linear positive dose-response relationships were reported in two (TLGS, Takayama males only) out of the three PCs available. Dose-response relationships were not investigated across the BoE owing to the limited number of PCs available.

Complementary LoE2: risk of obesity, LoE3: risk of T2DM, LoE4: risk of dyslipidaemia and LoE5: risk of hypertension. PCs. The available BoE does not support a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and risk of obesity (sQ3.1, Section 8.2.3.2), T2DM (sQ3.3, Section 8.4.3.2), dyslipidaemia (sQ3.4, Section 8.5.3.2) or hypertension (sQ3.5, Section 8.6.3.2).

Consistency across LoE. An increased risk of CVD with increasing intakes of fructose in isocaloric exchange with other macronutrients is not supported by the results of PCs on the relationship between fructose intake and risk factors for CVDs (namely obesity, T2DM, dyslipidaemia and hypertension).

Table 26: sQ3.6. PCs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty in a positive and causal relationship between intake of fructose and the risk of CVDs at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE1. Standalone (main). Endpoint: CVD (composite endpoint)** **3 PCs, 385,199 participants**. Pooled mean effect estimate (HR and 95%CI) on five estimates from three PCs = 1.11 (1.01, 1.21), I^2 = 31.7% **(Appendix K, Figure K16.b)**	**Initial certainty: Moderate (> 50–75% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	1 PCs in tier 1; 1 PC in tier 2; 1 PC in tier 3 (**Appendix L, Table L.13**). Generally moderate Key questions: • Confounding: most probably low • Exposure assessment: most probably high • Outcome assessment: most probably high	Serious
Unexplained inconsistency	All 3 PCs report positive relationships between the intake of fructose and CVD (incidence or mortality). Heterogeneity for the pooled mean effect estimate of study-specific HRs for the highest vs. the lowest categories of intake was low (I^2 = 31.7%).	Not serious
Indirectness	Direct endpoint	Not serious
Imprecision	Low	Not serious
Publication bias	Limited number of studies, it cannot be assessed. Public funding (n = 3).	Undetected (cannot be assessed)
Upgrading factors	None	No
Final certainty	Started moderate, downgraded one level for RoB.	**Low (> 15–50% probability)**

Conclusion sQ3.6. PCs. The level of certainty in a positive and causal relationship between the intake of fructose and risk of cardiovascular diseases is **low** (rationale in **Table 26**).

8.7.3.3. Overall conclusion on sQ3.6

There is evidence from PCs for a positive and causal relationship between the intake of fructose in isocaloric exchange with other macronutrients and risk of cardiovascular diseases (**low** certainty). The available BoE from RCTs cannot be used to modify the level of certainty in this conclusion.

8.7.4. Sugar-sweetened beverages

sQ4.6. SSBs and risk of cardiovascular diseases

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence and mortality: CVD (composite endpoint) or as CHD or stroke	0	**9**
LoE2. Complementary	Risk of obesity	sQ4.1	sQ4.1
LoE3. Complementary	Risk of Type 2 diabetes mellitus	sQ4.3	sQ4.3
LoE4. Complementary	Risk of dyslipidaemia	sQ4.4	sQ4.4
LoE5. Complementary	Risk of hypertension	sQ4.5	sQ4.5
LoE6. Complementary	Incidence of hyperuricaemia/uric acid	LoE3 for sQ4.5	LoE3 for sQ4.5

8.7.4.1. Intervention studies

No RCTs were eligible for standalone LoEs in relation to sQ4.6.

Complementary LoE2: risk of obesity, LoE3: risk of T2DM, LoE4: risk of dyslipidaemia and LoE5: Risk of hypertension. RCTs. There is evidence for a positive and causal relationship between the intake of SSBs and risk of obesity (**moderate**, sQ4.1, Section 8.2.4.1), T2DM (**low**, sQ4.3, Section 8.4.4.1) and hypertension (**very low**, sQ4.5, Section 8.6.4.1), whereas the available BoE from RCTs does not support a positive relationship with the risk of dyslipidaemia (sQ4.4, Section 8.5.4.1).

Complementary LoE6 (LoE3 for sQ4.5): Incidence of hyperuricaemia/uric acid. RCTs. The available BoE does not suggest a positive relationship between the intake of SSBs and uric acid levels.

Conclusion sQ4.6. RCTs. Although there is some evidence for a positive and causal relationship between the intake of SSBs and adverse effects on risk factors for cardiovascular diseases (i.e. body weight, glucose metabolism and blood pressure), no RCTs cardiovascular disease endpoints are available. In the absence of data from standalone LoEs, the available BoE from RCTs **cannot be used to conclude** on a positive relationship between the intake of SSBs and risk of cardiovascular diseases (see Section 8.1.3).

8.7.4.2. Observational studies

LoE1. Standalone (main): Incidence and mortality: CVD (composite endpoint), CHD or stroke. PCs. Five PCs report on the relationship between SSBs consumption and CVD (composite endpoint) incidence (MDCS, (Sonestedt et al., 2015); CTS, (Pacheco et al., 2020)) or mortality (EPIC-Multicentre, (Mullee et al., 2019); NHS and HPFS, (Malik et al., 2019)), of which MDCS, CTS and EPIC-Multicentre also have CHD and stroke as separate endpoints and NHS, HPFS also report on incidence of stroke in separate publications (Bernstein et al., 2012). The EPIC-Multicentre includes data from seven European countries. The HPP (Keller et al., 2020), a pooled analysis of seven individual studies and REGARDS (Collin et al., 2019) report on CHD incidence and mortality, respectively, whereas the JPHC (Eshak et al., 2012) has incidence of CHD and stroke as endpoints. The Framingham-Offspring (Pase et al., 2017) reports on stroke incidence. The EPIC-Multicentre also provides results on the relationship between the intake of ASBs and all the endpoints assessed in relation to SSBs, whereas the NHS and HPFS only assess ASBs in relation to stroke incidence (Bernstein et al., 2012).

Most studies analyse the exposure as a categorical variable using the standard multivariate model for energy adjustment, and thus do not keep TEI constant. Exceptions are the MDCS, which used the nutrient residuals (energy-adjusted model) and the REGARDS, which used the energy density model with no further adjustment for energy. All studies include BMI as covariate in the adjustment strategy. The HPP, REGARDS, NHS and HPFS also provide a continuous analysis using the standard multivariate (energy-adjusted) model or nutrient density model (REGARDS), thus keeping TEI constant. Evidence tables are in **Annex J**.

Preliminary UA

CVD (incidence and mortality). Four (CTS, EPIC-Multicentre, NHS, HPFS) of the five PCs which investigate the relationship between SSBs and CVD (composite endpoint) report a positive association, which was statistically significant in the CTS and NHS cohorts. The exception is the MDCS cohort, in which TEI was kept constant in the analysis (**Appendix K**, **Figure K.17a1**). The pooled mean effect estimate (95%CI) of study-specific HR for the highest vs. the lowest categories of intake is 1.15 (1.03, 1.29), I^2 = 66.1% (**Appendix K**, **Figure K.17a2**).

In the EPIC-Multicentre, the relationship between the intake of ASBs and CVD mortality was stronger than for SSBs and statistically significant. The HR (95%CI) for the highest vs. the lowest categories of intake were 1.52 (1.30, 1.78) and 1.11 (0.95, 1.30), respectively. In the NHS and HPFS, the relationship between the intake of ASBs and CVD mortality was similar to that for SSBs, and statistically significant in the NHS. The HR (95%CI) for the highest vs. the lowest categories of ASBs intake was 1.43 (1.10, 1.87; P_{trend} = 0.02) and 1.21 (0.86, 1.70; P_{trend} = 0.23), in the NHS and HPFS, respectively.

CHD (incidence and mortality). Among the six studies reporting on this endpoint, three show a positive (non-significant) relationships between the intake of SSBs and CHD (HPP, REGARDS, CTS) and in three the relationship is close to the null (MDCS, JPHC, EPIC-Multicentre) (**Appendix K**, **Figure K.17b1**). The pooled mean effect estimate (95%CI) of study-specific HR for the highest vs. the lowest categories of intake is 1.08 (1.00, 1.18), I^2 = 0% (**Appendix K**, **Figure K.17b2**).

In the EPIC-Multicentre, the relationship between the intake of ASBs and CHD fmortality was positive and statistically significant. The HR (95%CI) for the highest vs. the lowest categories of intake for SSBs and ASBs were 1.04 (0.87, 1.23; p per trend = 0.84) and 1.41 (1.11, 1.79; p per trend = 0.003), respectively.

Stroke (incidence and mortality). A positive relationship between the intake of SSBs and stroke is reported in four PCs (CTS, JPHC in females, NHS, HPFS, EPIC-Multicentre; statistically significant in CTS), whereas in one PC the relationship was close to null (MDCS) and it was negative in another two (Framingham-Offspring and JPHC; statistically significant only in males in JPHC) (**Appendix K**, **Figure K.17c1**). The pooled mean effect estimate (95%CI) of study-specific HR for the highest vs. the lowest categories of intake is 1.07 (0.96, 1.19), I^2 = 45.9% (**Appendix K**, **Figure K.17c1**). The Framingham-Offspring also reports on ischaemic stroke and observes a similar association as for total stroke. The HPFS and NHS also report on ischaemic and haemorrhagic stroke separately. The association with haemorrhagic stroke is negative in both studies, whereas the association with ischaemic stroke is positive in the NHS and null in the HPFS. When SSBs intake was analysed as a continuous variable, the positive association with incidence of total stroke and ischaemic stroke was statistically significant in the NHS and positive (non-significant) for haemorrhagic stroke in the HPFS.

The relationship between ASBs and stroke was similar to that of SSBs in three PCs which reported on this exposure (positive and non-significant; EPIC-Multicentre, NHS and HPFS). In the Framingham-Offspring, which reports a negative relationship between the intake of SSBs and incidence of stroke, the association was positive for ASBs [HR (95%CI) $_{C3\ vs.\ C1}$: 1.97 (1.10, 3.55) for 'recent intake'; HR (95%CI) $_{C3\ vs.\ C1}$: 1.79 (0.91, 3.52) for 'cumulative intake']. The relationship between the intake of ASBs and incidence of ischaemic and haemorrhagic stroke was positive in both the HPFS and NHS, and statistically significant for ischaemic stroke in the NHS ($HR_{Qc3\ vs.\ non\text{-}c}$ 1.55 (95% CI: 1.20, 2.00); P per trend < 0.0001).

Five out of the nine PCs were at low RoB (tier 1; HPFS, JPHC, MDCS, NHS, Framingham-Offspring), two at moderate RoB (tier 2; CTS, HPP) and two at high RoB (tier 3; EPIC-Multicentre, REGARDS) for all the endpoints assessed in each study (**Appendix L**, **Table L.14**). Critical domains were exposure and outcome assessment and confounding for PCs in RoB tier 3.

In sensitivity analyses excluding studies at high RoB (tier 3, EPIC-Multicentre, REGARDS) the pooled mean effect estimates of study-specific HRs (95%CI) for the highest vs. the lowest categories of intake for CVD (composite endpoint), CHD and stroke were 1.17 (1.01, 1.35), 1.07 (0.98, 1.18) and 1.04 (0.92, 1.18), respectively.

The Panel considers that the available BoE suggests a positive relationship between the intake of SSBs and risk of CVDs.

Comprehensive UA

Selection of the endpoint. The Panel decided to conduct the comprehensive UA on CVD (composite endpoint) owing to the consistency of the results across cohorts, the higher precision of the pooled mean effect estimates as compared to either CHD or stroke and the fact that these two endpoints are the major components of the CVD composite endpoint.

Dose-response relationship. A positive linear dose-response relationship was observed in three (CTS, HPFS, NHS) out of the five PCs in categorical analyses.

In the dose-response meta-analysis conducted by EFSA, parametric dose-response models were estimated based on summarised data. Both linear and non-linear (restricted cubic splines) dose-response relationships were investigated. The methodological approach applied was the same as for the dose-response meta-analyses of SSBs intake and incidence of T2DM (see Section 8.6.4.2 and **Annex M**).

Fifteen RRs from four study-specific analyses were included in the dose-response meta-analysis (I^2 = 0%; p = 552). The MDCS cohort was excluded (model diagnostics). The predicted pooled relative risk of CVD (composite endpoint) was 1.06 (95% CI: 1.04, 1.09) for an increase in SSBs intake of 250 mL/day in the linear model (p for linear trend < 0.0001), and 1.07 (95% CI: 1.03, 1.11) at 250 mL/day in the non-linear model (RCS with three knots at fixed percentiles, 10%, 50% and 90%, of the distribution; p for non-linearity = 0.800) (**Figure 17**). The subgroup analyses did not identify clear sources of heterogeneity, also given the limited number of studies across strata. The funnel plot and related Egger regression were not carried out as the number of studies was very limited.

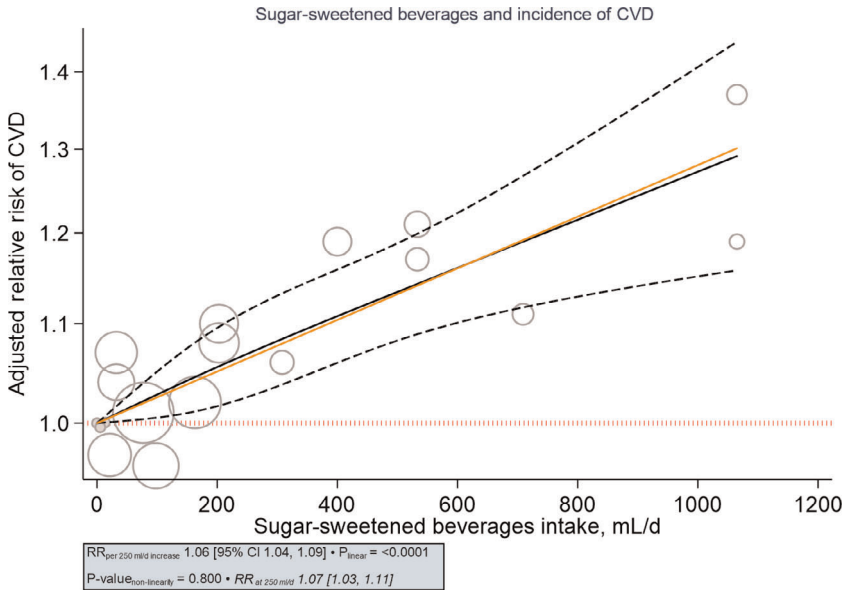


Figure 17: Dose-response meta-analysis on the relationship between the intake of sugar-sweetened beverages and risk of cardiovascular disease (CVD) – composite endpoint

Complementary LoE2: risk of obesity, LoE3: risk of T2DM, LoE4: risk of dyslipidaemia and LoE5: Risk of hypertension. PCs. There is evidence for a positive and causal relationship between the intake of SSBs and risk of obesity (**moderate**, sQ4.1, Section 8.2.4.2), T2DM (**high**, sQ4.3, Section 8.4.4.2), dyslipidaemia (**low**, sQ4.4, Section 8.5.4.2) and hypertension (**high**, sQ4.5, Section 8.6.4.2).

Consistency across LoE. The positive relationship between the intake of SSBs and risk of CVD (composite endpoint) is supported by the positive association between the intake of SSBs and risk of CHD and stroke, and by PCs on risk factors for CVDs, namely obesity, T2DM, dyslipidaemia and hypertension.

Table 27: sQ4.6. PCs. Comprehensive analysis of the uncertainties in the BoE and in the methods

What is the level of certainty in a positive and causal relationship between intake of SSBs and the risk of CVDs at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?		
BoE (standalone)	**LoE1. Standalone (main). Endpoint: CVD (composite endpoint)** **5 PCs, 575,966 participants.** Four study-specific analyses from four PCs were included in the dose-response analysis	**Initial certainty: Moderate (> 50–75% probability)**
Domain	**Rationale**	**Evaluation**
Risk of bias	3 PCs in tier 1; 1 PC in tier 2; 1 PC in tier 3 (**Appendix L**, **Table L.14**).	Serious

	Generally moderate Key questions: • Confounding: most probably low • Exposure assessment: most probably high • Outcome assessment: most probably high	
Unexplained inconsistency	Four out of the five PCs report positive relationships between the intake of SSBs and CVD (incidence or mortality). The exception is the MDCS, where TEI was kept constant in the analysis. Heterogeneity is low (I^2 = 0%) for the pooled mean effect estimate of study-specific RRs per unit increase of intake. RRs are similar across studies. No clear sources of heterogeneity identified.	Not serious
Indirectness	Direct endpoint	Not serious
Imprecision	Low	Not serious
Publication bias	Limited number of studies, it cannot be assessed. Public funding (n = 5).	Undetected (cannot be assessed)
Upgrading factors	**Dose-response relationship.** A significant linear dose-response relationship across categories of SSBs intake was reported in 3 of the 5 PCs which performed a categorical analysis. The dose-response meta-analysis conducted by EFSA showed a significant linear positive dose relationship (linear pooled mean effect estimate (95%CI) = 1.06 (1.04, 1.09) for 250 mL/d increase with no support for non-linearity (p = 0.800). In sensitivity analysis, exclusion of the PC at high RoB (tier 3) had a negligible impact on the dose-response relationship (**Annex M**). **Consistency across LoE.** The positive relationship between the intake of SSBs and risk of CVD (composite endpoint) is supported by the positive association between the intake of SSBs and risk of CHD and stroke, and by PCs on risk factors for CVDs, namely obesity, T2DM, dyslipidaemia and hypertension.	Yes (dose-response and consistency across LoE)
Final certainty	Started moderate, downgraded for RoB (one level); upgraded for consistency (one level) and dose-response (one level).	**High (> 75–100% probability)**

Conclusion sQ4.6. PCs. The level of certainty in a positive and causal relationship between the intake of SSBs and risk of CVDs is **high** (rationale in **Table 27**). The relationship was observed for SSBs not keeping TEI constant.

8.7.4.3. Overall conclusion sQ4.6

There is evidence from PCs for a positive and causal relationship between the intake of SSBs and risk of CVDs (**high** level of certainty).

8.7.5. Fruit juices

sQ5.6. FJs and risk of cardiovascular diseases

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence and mortality: CVD (composite endpoint) or as CHD or stroke	0	**3**
LoE2. Complementary	Risk of obesity	sQ5.1	sQ5.1
LoE3. Complementary	Risk of Type 2 diabetes mellitus	sQ5.3	sQ5.3
LoE4. Complementary	Risk of dyslipidaemia	sQ5.4	sQ5.4
LoE5. Complementary	Risk of hypertension	sQ5.5	sQ5.5
LoE6. Complementary	Incidence of hyperuricaemia/uric acid	LoE3 for sQ5.5	LoE3 for sQ5.5

8.7.5.1. Observational studies

LoE1. Standalone (main): Incidence and mortality: CVD (composite endpoint), CHD or stroke. PCs. The MDCS (Sonestedt et al., 2015) reports on incidence of CVD, CHD and ischaemic stroke in relation to the intake of FJs. The NHS and HPFS report on the relationship between the

intake of FJs and incidence of ischaemic stroke (Joshipura et al., 1999). In the MDCS cohort, FJs was analysed as a categorical variable using the nutrient residuals model to adjust for energy intake, and thus was assessed keeping TEI constant across tertiles of intake vs. non-consumers (reference category). In the NHS and HPFS, FJs was analysed both as a categorical and continuous variable, using the multivariable model to adjust for TEI, thus keeping TEI constant. The evidence table is in **Annex J**.

Preliminary UA

The intake of FJs was unrelated to the incidence of CVD, CHD or ischaemic stroke in the MDCS cohort. In the NHS and HPFS, the intake of FJs was inversely related to the incidence of ischaemic stroke, significant in the NHS only.

The MDCS and HPFS were at low RoB (tier 1). The NHS was at moderate RoB (tier 2), with the critical domain being outcome and attrition (**Annex K**).

The Panel considers that the available BoE does not support a positive relationship between the intake of FJs and risk of CVDs. **No comprehensive UA is performed**.

Conclusion sQ5.6. PCs. The available BoE does not support a positive relationship between the intake of FJs and risk of CVDs.

8.7.5.2. Overall conclusion on sQ5.6

Since no studies were available for standalone LoEs in relation to this sQ, the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of FJs and risk of CVDs.

8.8. Risk of gout

8.8.1. Total sugars

sQ1.7. Total sugars and risk of gout

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of gout	0	0
LoE2. Complementary	Incidence of hyperuricaemia/uric acid	LoE3 for sQ1.5	LoE3 for sQ1.5
LoE3. Complementary	Risk of obesity	sQ1.1	sQ1.1

8.8.1.1. Observational studies

No PCs were eligible for standalone LoEs in relation to sQ1.7.

LoE3 (sQ1.1). **Complementary: Risk of obesity. PCs**. The available evidence does not suggest a positive association between the intake of total sugars in isocaloric exchange with other macronutrients and risk of obesity.

Conclusion sQ1.7. PCs The available evidence does not suggest a positive association between the intake of total sugars in isocaloric exchange with other macronutrients and risk of gout.

8.8.1.2. Overall conclusion on sQ1.7

Since no studies were available for standalone LoEs in relation to this sQ, the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of total sugars and risk of gout.

8.8.2. Added and free sugars

sQ2.7. Added and free sugars and risk of gout

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of gout	0	0
LoE2. Complementary	Incidence of hyperuricaemia/uric acid	LoE3 for sQ2.5	LoE3 for sQ2.5
LoE3. Complementary	Risk of obesity	sQ2.1	sQ2.1

8.8.2.1. Intervention studies

No RCTs were eligible for standalone LoEs in relation to sQ2.7.

LoE2 (LoE3 for sQ2.5). **Complementary: Incidence of hyperuricaemia/uric acid. RCTs**. There is evidence from RCTs for a positive relationship between the intake of added sugars and uric acid levels, both when consumed ad libitum and in isocaloric exchange with starch. The effect appears to be independent of changes in body weight.

LoE3 (sQ2.1). **Complementary: Risk of obesity. RCTs**. There is evidence from RCTs for a positive and causal relationship between the intake of added and free sugars ad libitum and risk of obesity (**moderate** level of certainty).

Conclusion sQ2.7. RCTs. Whereas there is evidence from RCTs for a positive relationship between the intake of added and free sugars and both uric acid levels and risk of obesity, which are established risk factors for gout, no RCTs on incidence of gout are available. In the absence of data from standalone LoEs, the available BoE from RCTs **cannot be used to conclude** on a positive relationship between the intake of added or free sugars and risk of gout (see Section 8.1.3).

8.8.2.2. Observational studies

No PCs were eligible for standalone LoEs in relation to sQ2.7.

LoE3 (sQ2.1). **Complementary: Risk of obesity. PCs**. The available evidence from PCs does not suggest a positive relationship between the intake of added or free sugars in isocaloric exchange with other macronutrients and risk of obesity.

Conclusion sQ2.7. PCs. The available evidence from PCs does not suggest a positive relationship between the intake of added or free sugars in isocaloric exchange with other macronutrients and risk of gout.

8.8.2.3. Overall conclusions on sQ2.7

Since no studies were available for standalone LoEs in relation to this sQ, the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of added or free sugars and risk of gout.

8.8.3. Fructose

sQ3.7. Fructose and risk of gout

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of gout	0	**2**
LoE2. Complementary	Incidence of hyperuricaemia/uric acid	LoE3 for sQ3.5	LoE3 for sQ3.5
LoE3. Complementary	Risk of obesity	sQ3.1	sQ3.1

8.8.3.1. Intervention studies

No RCTs were eligible for standalone LoEs in relation to sQ3.7.

LoE2 (LoE3 for sQ3.5). **Complementary: Incidence of hyperuricaemia/uric acid. RCTs**. There is some evidence from RCTs for a positive relationship between the intake of fructose in isocaloric exchange with other carbohydrates (i.e. glucose, starch) and uric acid levels. The effect appears to be independent of changes in body weight.

LoE3 (sQ1.3). **Complementary: Risk of obesity. RCTs**. The available evidence from RCTs does not suggest a positive relationship between the intake of fructose in isocaloric exchange with glucose and risk of obesity.

Conclusion sQ3.7. RCTs. Whereas there is evidence from RCTs for a positive relationship between the intake of fructose in isocaloric exchange with other carbohydrates (i.e. glucose, starch) and uric acid levels, an established risk factor for gout, no RCTs on incidence of gout are available. Therefore, the Panel considers that the available BoE from RCTs **cannot be used to conclude** on positive relationship between the intake of fructose in isocaloric exchange with other carbohydrates and risk of gout.

8.8.3.2. Observational studies

LoE1. Standalone (main): Incidence of gout. PCs. Two PCs investigated the relationship between the consumption of total fructose and free fructose in isocaloric exchange with other macronutrients and the incidence of gout. Both studies, one in males (HPFS (Choi and Curhan, 2008)) and one in females (NHS (Choi et al., 2010)), were conducted in middle-aged health professionals living in the USA, used the same semiquantitative FFQ to assess the exposure and the same criteria to ascertain the endpoint, and considered similar confounders in multivariable models. Total and free fructose were analysed as categorical and continuous variables using the energy density (energy-adjusted) model. In addition, two energy partition models were built: one assessed total and free fructose in isocaloric exchange with fat and the second in isocaloric exchange with other carbohydrates. The evidence table is in **Annex J**.

Preliminary UA

A positive linear dose-response relationship between the consumption of total fructose and free-fructose and incidence of gout was observed in both sexes (**Annex J; Appendix K, Figures K.18a and K.18b**). Both in males and females, RRs were higher in models considering fructose in isocaloric exchange with other carbohydrates than in those considering fructose in isocaloric exchange with fat. The relationship was stronger for free fructose than for total fructose. In females, the multivariable RR for each 5 E% increment in energy intake from free fructose at baseline, compared with equivalent energy intake from other types of carbohydrates, was 1.86 (95% CI = 1.44, 2.40) and the corresponding RR for total fructose was 1.47 (95% CI = 1.20, 1.80). In males, the multivariable RR for each 5 E% increment in energy intake from free fructose, as compared with equivalent energy intake from other types of carbohydrates, was 2.10 (95% CI = 1.53–2.77), and the corresponding RR for total fructose was 1.52 (95% CI = 1.23–1.88).

In the systematic review on fructose intake and risk of gout by Jamnik et al. (2016), only these two PCs were eligible for this exposure. The pooled RR estimate (95%CI) for the highest quintile of fructose intake compared to the lowest (reference) quintile in most adjusted models considering fructose in isocaloric exchange with other carbohydrates was 1.62 (1.28, 2.03), I^2 = 0%.

HPFS was at low RoB (tier 1) and NHS at moderate RoB (tier 2), critical domains being attrition (NHS only) and outcome assessment (**Annex K**).

The Panel notes the consistency of results between sexes, the large sample size and number of cases (HPFS, n = 46,393, cases = 755; NHS, n = 78,906, cases = 778) over a long follow-up (12 and 22 years, respectively), and that the study was between low and moderate RoB.

The Panel considers that the available BoE suggests a positive relationship between the intake of fructose in isocaloric exchange with other carbohydrates and incidence of gout.

Comprehensive UA

The Panel considers that it would be inappropriate to proceed with a comprehensive UA because several downgrading factors cannot be assessed with less than three independent studies. The initial level of certainty assigned to the relationship is **very low** (0–15% probability) to reflect the limited BoE available (see Section 8.1.3).

The Panel notes the large sample size of the study, the long duration of follow-up, the magnitude of the effect, the low RoB and the biological plausibility of the relationship. There are indeed several mechanisms by which fructose could increase uric acid levels (see Section 3.6.1.4) and evidence from RCTs that it does in isocaloric exchange with glucose and starch (see Section 8.6.3.1). Considering the above, the Panel considers that the level of certainty in the relationship is **moderate** (> 50–75% probability).

LoE3 (sQ3.1). **Complementary: Risk of obesity. PCs**. The available evidence does not suggest a positive relationship between the intake of fructose in isocaloric exchange with other macronutrients and an increased risk of obesity.

Conclusions sQ3.7. PCs. The level of certainty in a positive and causal relationship between the intake of fructose in isocaloric exchange with other carbohydrates and risk of gout is **moderate** (>50–75% probability).

8.8.3.3. Overall conclusions for sQ3.7

There is evidence from PCs for a positive and causal relationship between the intake of fructose in isocaloric exchange with other carbohydrates and risk of gout (**moderate** certainty).

8.8.4. Sugar-sweetened beverages

sQ4.7. SSBs and risk of gout			
LoE	**Endpoints**	**RCTs (n)**	**PCs (n)**
LoE1. Standalone (main)	Incidence of gout	0	**2**
LoE2. Complementary	Incidence of hyperuricaemia/uric acid	LoE3 for sQ4.5	LoE3 for sQ4.5
LoE3. Complementary	Risk of obesity (sQ4.1)	sQ4.1	sQ4.1

8.8.4.1. Intervention studies

No RCTs were eligible for standalone LoEs in relation to sQ4.7.

LoE2 (LoE3 for sQ3.5). Complementary: Incidence of hyperuricaemia/uric acid. RCTs. The available BoE does not suggest a positive relationship between the intake of SSBs and uric acid levels.

LoE3 (sQ1.3). Complementary: Risk of obesity. RCTs. There is evidence for a positive and causal relationship between the intake of SSBs and risk of obesity (**moderate** certainty).

Conclusion sQ3.7. RCTs. Whereas there is evidence from RCTs for a positive relationship between the intake of SSBs and risk of obesity, an established risk factor for gout, no RCTs investigating the relationship between the intake of SSBs and incidence of gout are available. Therefore, the Panel considers that the available BoE from RCTs cannot be used to conclude on a positive relationship between the intake of SSBs and risk of gout.

8.8.4.2. Observational studies

LoE1. Standalone (main): Incidence of gout. PCs. The same two PCs which investigated the relationship between the intake of fructose and incidence of gout (see Section 8.8.3.2) also explored the relationship between the intake of SSBs (as source of fructose intake) and the intake of ASBs in relation to that endpoint (HPFS, (Choi and Curhan, 2008); NHS, (Choi et al., 2010)).

SSBs were analysed as categorical variable using standard multivariable model for energy adjustment, and thus, TEI was not kept constant in the analysis. The evidence table is in **Annex J**.

Preliminary UA

A positive linear dose-response relationship between the consumption of SSBs and incidence of gout was observed in both sexes across categories of intake (**Appendix K**, **Figure K.19**), whereas no association was found between the intake of ASBs and incidence of gout. In the systematic review on fructose intake and risk of gout by Ayoub-Charette et al. (2019), only these two PCs were eligible for this exposure. The pooled RR estimate (95%CI) for the highest (> 2 servings per day) category of SSBs intake compared to the lowest (reference, < 1 serving per month; serving size = 355mL) in most adjusted models was 2.08 (95%CI = 1.28, 2.03), I^2 = 0%.

As for fructose, HPFS was at low RoB (tier 1) and NHS at moderate RoB (tier 2), critical domains being attrition (NHS only) and outcome assessment (**Annex K**).

The Panel notes the consistency of results between sexes, the large sample size and number of cases over a long follow-up, and that the study was between low and moderate RoB. The Panel considers that the available BoE suggests a positive relationship between the intake of SSBs and incidence of gout.

Comprehensive UA

As for fructose, the Panel considers that it would be inappropriate to proceed with a comprehensive UA because several downgrading factors cannot be assessed with less than three independent studies. The initial level of certainty assigned to the relationship is **very low** (0–15% probability) to reflect the limited BoE available (see Section 8.1.3).

LoE2. Complementary (LoE3 for sQ4.5): Incidence of hyperuricaemia/uric acid. PCs. The available BoE does not suggest a positive relationship between intake of SSBs and incidence of hyperuricaemia.

LoE3 (sQ3.1). Complementary: Risk of obesity. PCs. There is evidence for a positive and causal relationship between the intake of SSBs and risk of obesity (**moderate** certainty, Section 8.2.4.2).

The Panel notes the large sample size of the study, the long duration of follow-up, the large magnitude of the effect, the low RoB and the biological plausibility of the relationship. SSBs were an important contributor to fructose and free fructose intake in the study, there are several mechanisms by which fructose could increase uric acid levels (see Section 3.6.1.4) and evidence from RCTs that it does in isocaloric exchange with glucose and starch (see Section 8.6.3.1), and evidence from PCs and RCTs on a positive and causal relationship between the intake of SSBs and increased risk of obesity, a risk factor for gout. Therefore, the Panel considers that the level of certainty in the relationship is **moderate** (> 50–75% probability). The relationship is observed for SSBs consumed not keeping TEI constant in the analysis.

Conclusions sQ4.7. PCs. The level of certainty in a positive and causal relationship between the intake of SSBs and risk of gout is **moderate**.

8.8.4.3. Overall conclusions for sQ4.7

There is evidence from PCs for a positive and causal relationship between the intake of SSBs and risk of gout (**moderate** certainty). Evidence from RCTs on a positive and causal relationship between the intake of SSBs ad libitum and risk of obesity, a risk factor for gout, has already been considered by the Panel when assigning this level of certainty to the relationship.

8.8.5. Fruit juices

sQ5.7. FJs and risk of gout

LoE	Endpoints	RCTs (n)	PCs (n)
LoE1. Standalone (main)	Incidence of gout	0	**2**
LoE2. Complementary	Incidence of hyperuricaemia/uric acid (LoE 3 for sQ5.5)	LoE3 for sQ5.5	LoE3 for sQ5.5
LoE3. Complementary	Risk of obesity (sQ5.1)	sQ5.1	sQ5.1

8.8.5.1. Observational studies

LoE1. Standalone (main): Incidence of gout. PCs. The same two PCs which investigated the relationship between the intake of fructose (see Section 8.8.3.2) and SSBs (see Section 8.8.4.2) and incidence of gout also explored the relationship between the intake of FJs (as source of fructose intake) and that endpoint (HPFS, (Choi and Curhan, 2008); NHS, (Choi et al., 2010)).

In the HPFS, the intake of total FJs was used for analysis. Data are also reported for orange or apple juice. In the NHS, the intake of orange juice and the intake of other FJs are reported and analysed separately. For this opinion, the Panel decided to extract orange juice as the exposure of interest because it was the major contributor among juices to free fructose intake (17% vs. 2.9% for apple juice and 2.65% for other juices).

In both PCs, FJs was analysed as categorical variable using standard multivariable model for energy adjustment, and thus, TEI was not kept constant in the analysis. The evidence table is in **Annex J**.

Preliminary UA

A positive linear dose-response relationship between the consumption of FJs and incidence of gout was observed in both sexes across categories of intake (**Appendix K**, **Figure K.20**). The RR estimate (95%CI) for the highest (> 2 servings per day) category of FJs intake compared to the lowest (reference, < 1 serving per month; serving size = 177mL) in most adjusted models was 1.81 (95%CI = 1.12, 2.93) for males and 2.42 (95%CI = 1.27, 4.63) for females.

As for fructose and SSBs, HPFS was at low RoB (tier 1) and NHS at moderate RoB (tier 2), critical domains being attrition (NHS only) and outcome assessment (**Annex K**).

The Panel notes the consistency of results between sexes, the large sample size and number of cases over a long follow-up, the large magnitude of the effect and that the study was between low and moderate RoB. The Panel considers that the available BoE suggests a positive relationship between the intake of FJs and incidence of gout.

Comprehensive UA

As for fructose and SSBs, the Panel considers that it would be inappropriate to proceed with a comprehensive UA because several downgrading factors cannot be assessed with less than three independent studies. The initial level of certainty assigned to the relationship is **very low** (0–15% probability) to reflect the limited BoE available (see Section 8.1.3). The relationship is observed for FJs not keeping TEI constant in the analysis.

LoE3 (sQ3.1). **Complementary: Risk of obesity. PCs**. There is evidence for a positive and causal relationship between the intake of FJs and risk of obesity (**very low** certainty).

The Panel notes the large sample size of the study, the long duration of follow-up, the larger magnitude of the effect as compared to SSBs (similar RR for half of the amount), the low RoB and the biological plausibility of the relationship. FJs were an important contributor to fructose and free fructose intake in the study, there are several mechanisms by which fructose could increase uric acid levels (see Section 3.6.1.4 and evidence from RCTs that it does in isocaloric exchange with glucose and starch (see Section 8.6.3.1), and limited evidence from PCs for a positive and causal relationship between the intake of FJs (not keeping TEI constant) and increased risk of obesity, a risk factor for gout. Therefore, the Panel considers that the level of certainty in the relationship is **moderate** (> 50–75% probability). The relationship is observed for FJs not keeping TEI constant.

Conclusions sQ3.7. PCs. The level of certainty in a positive and causal relationship between the intake of FJs and risk of gout is **moderate** (> 50–75% probability).

8.8.5.2. Overall conclusions for sQ5.7

There is evidence from PCs for a positive and causal relationship between the intake of 100%FJs and risk of gout (**moderate** certainty).

8.9. Overall conclusions on hazard identification: metabolic diseases

Conclusions on the level of certainty for a positive and causal relationship for each exposure and disease endpoint by study design, as well as the overall conclusions for both study designs combined, are summarised in **Table 28**.

Table 28: Summary conclusions on the level of certainty in the body of evidence for hazard identification[1]

Exposure, study design, dietary conditions	**Disease**						
Total sugars	**Obesity**	**NAFLD**	**T2DM**	**Dyslipidaemia**	**HTN**	**CVD**	**Gout**
RCTs.	No data	No data	No data	No data	No data	No data	No data
PCs. Mainly keeping TEI constant in the analysis	No support	No support	No support	No support	No support	No support	No data[2]
Overall conclusion	**No conclusion**[3]	**No conclusion**[3]	**No conclusion**[3]	**No conclusion**[3]	**No conclusion**[3]	**No conclusion**[3]	**No conclusion**[3]
Added and free sugars	**Obesity**	**NAFLD**	**T2DM**	**Dyslipidaemia**	**HTN**	**CVD**	**Gout**
RCTs. Ad libitum or in isocaloric exchange with other macronutrients (mainly starch)	Moderate *(Ad libitum)*	Low	Low	Moderate *(mostly in isocaloric exchange with starch)*	Very low	No data[2]	No data[2]
PCs. Mainly keeping TEI constant in the analysis	No support	No support	No support	No support	No support	No support	No data[2]
Overall conclusion	**Moderate** *(Ad libitum)*	**Low**	**Low**	**Moderate** *(mostly in isocaloric exchange with starch)*	**Very low**	**No conclusion**[3]	**No conclusion**[3]
Fructose	**Obesity**	**NAFLD**	**T2DM**	**Dyslipidaemia**	**HTN**	**CVD**	**Gout**
RCTs. Isocaloric exchange with glucose	No support	No support	No support	No support	No support	No data[2]	No data[2]
PCs. Keeping TEI constant in the analysis	No support	No data[2]	No support	No support	No support	Low	Moderate
Overall conclusion	**No conclusion**[3]	**No conclusion**[3]	**No conclusion**[3]	**No conclusion**[3]	**No conclusion**[3]	**Low**	**Moderate**
SSBs	**Obesity**	**NAFLD**	**T2DM**	**Dyslipidaemia**	**HTN**	**CVD**	**Gout**
RCTs. Ad libitum or at neutral energy balance	Moderate *(Ad libitum)*	Low	Low	No support *(Ad libitum)*	Very low	No data[2]	No data[2]
PCs. Mainly not keeping TEI constant in the analysis	Moderate	No data[2]	High	Low	High	High	Moderate
Overall conclusion	**High**	**Low**	**High**	**Low**	**High**	**High**	**Moderate**
FJs	**Obesity**	**NAFLD**	**T2DM**	**Dyslipidaemia**	**HTN**	**CVD**	**Gout**
RCTs.	No data	No data	No data	No data	No data	No data[2]	No data

Exposure, study design, dietary conditions	Disease						
Total sugars	**Obesity**	**NAFLD**	**T2DM**	**Dyslipidaemia**	**HTN**	**CVD**	**Gout**
PCs. Mainly not keeping TEI constant in the analysis	Very low	No data[2]	Moderate	No support	No support	No support	Moderate
Overall conclusion	**Very low**	**No conclusion**[3]	**Moderate**	**No conclusion**[3]	**No conclusion**[3]	**No conclusion**[3]	**Moderate**

CVD = cardiovascular disease; HTN = hypertension; NAFLD = non-alcoholic fatty liver disease; PCs = prospective cohorts; RCTs = randomised controlled trials; T2DM = type 2 diabetes mellitus; TEI = total energy intake.

1: Levels of certainty on a positive and causal relationship are associated with the following probability ranges: high (75–100% probability), moderate (50–75%), low (15–50% probability), very low (0–15% probability).

2: No data on standalone LoEs.

3: Since no standalone LoEs passed the screening step (preliminary uncertainty analysis), the available body of evidence cannot be used to conclude on a positive and causal relationship between the exposure and the disease risk.

8.9.1. Total sugars

Total sugars intake corresponds to all mono- and disaccharides supplied by the diet. In European populations, core food groups (i.e. fresh fruits and vegetables, milk and dairy and cereal products) represent a large proportion of total sugars intake while non-core food groups such as beverages (SSBs, fruit juices), fine bakery wares and sugars and confectionery are other major contributors (see Section 4.3). The contribution of such food groups to mean total sugars intake varies across population groups and among countries (e.g. between 30% and 60% for core food groups and between 10% and 30% for beverages in most population groups except infants and toddlers), so that very different dietary patterns may lead to similar total sugars intake.

Given the complex nature of this exposure, no RCT addressed the effect of total sugars intake on health outcomes. The BoE is limited to PCs on the intake of total sugars from all relevant dietary sources, which vary widely in their nutritional profile and role in the diet.

The eligible PCs investigated the associations between total sugars intake and the risk of obesity, NAFLD, T2DM, dyslipidaemia, hypertension and CVD. TEI was generally considered a potential confounder, thus models fully accounting for TEI were applied (see Section 5). Hence, the BoE addresses the potential role of total sugars in disease risk independent of their contribution to energy intake, i.e. the inherent properties of sugars as compared to other macronutrients.

The Panel notes that one large European cohort study (EPIC-Multicentre, (Sieri et al., 2020)) reports a positive and significant linear dose-response relationship between the intake of total sugars in isocaloric exchange with other macronutrients and incidence of CHD. The results of this study, however, were at odds with the results obtained in other cohorts outside Europe and not supported by PCs on total sugars and risk factors for CHD, namely obesity, T2DM, dyslipidaemia and hypertension. Overall, the Panel considers that the available BoE from PCs does not support a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and any of the chronic metabolic diseases assessed in this opinion.

The Panel notes that total sugars intake reflects very heterogeneous food sources and dietary patterns. The Panel considers that the relative contribution of different food groups to total sugars intake may be more relevant in relation to chronic disease risk than the intake of total sugars *per se*.

8.9.2. Added and free sugars

Added sugars intake corresponds to all mono- and disaccharides added to foods as ingredients during processing or preparation at home, and sugars eaten separately or added to foods at the table; free sugars include added sugars plus sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates. The Panel notes that the BoE considered in this opinion does not allow comparison of health effects based on the separate classification of dietary sugars as added or free (see Sections 8.1.1 and 8.1.2).

Food groups contributing the most to the intake of added and free sugars in European countries were 'sugars and confectionery', followed by beverages (SSBs, fruit and vegetable juices) and fine bakery wares in most population groups, with high variability across countries. The main difference between the intake of added and free sugars was accounted for by juices (mostly fruit juices). In infants, children and adolescents, sweetened milk and dairy products were also major contributors to mean intakes of added and free sugars. Different from total sugars, added and free sugars mainly originate from non-core food groups, except for sweetened milk and dairy products in young consumers.

In the present assessment, mean intakes obtained using the EFSA food composition and consumption databases may be accurate for free sugars, but possibly overestimated for added sugars because all sweetening ingredients were considered to be added sugars, and thus, the difference between added and free sugars is limited to sugars from fruit and vegetable juices, and to sugars from fruit and vegetable juice concentrates, honey and syrups only when used as such by the consumer. Mean intakes estimates for both added and free sugars calculated by EFSA using the EFSA food composition database were, however, generally lower than those estimated at national level using national food composition data for the same dietary surveys.

Evidence for a positive and causal relationship between the intake of added and free sugars and risk of chronic metabolic diseases arises from RCTs that were used to investigate the effect of 'high' vs. 'low' sugars intake on surrogate disease endpoints, i.e. body weight, liver fat, measures of glucose tolerance, blood lipids and blood pressure. Because the evidence from RCTs was limited to data on

surrogate endpoints, the conclusions of the Panel assume that a sustained adverse effect on the surrogate measures over time would eventually lead to an increased risk of disease.

Evidence from PCs on disease endpoints could not be used to address this uncertainty as there was no support from PCs for a positive and causal relationship between the intake of added or free sugars and risk of chronic metabolic diseases. The BoE from PCs mostly investigated whether the consumption of added (and/or free) sugars could affect the risk of these diseases independent from a contribution to excess energy intake (i.e. intake standardised to energy for the analyses). In addition, few PCs report on the intake of added and/or free sugars from all sources. A major uncertainty in the BoE in relation to observational studies lies on the different definitions and food composition databases used to assess the intake of added and free sugars. For example, when the exact food product consumed is not specified (as the case may be when FFQs are used for the dietary assessment), or the ingredient used for sweetening purposes (e.g. sucrose, fructose, syrups, honey, fruit juice concentrates, other) is not specified, then the amount of added and free sugars originating from the different foods cannot not be accurately assigned.

Overall, the Panel concludes that the level of certainty for a positive and causal relationship between the intake of added and free sugars and risk of chronic metabolic diseases is moderate for obesity and dyslipidaemia (> 50–75% probability), low for NAFLD/NASH and T2DM (> 15–50% probability) and very low for hypertension (0–15% probability).

Although RCTs conducted in isocaloric conditions provide some evidence that the mechanism by which added and free sugars could increase liver fat, fasting glucose, fasting triglycerides and SBP may not only be mediated by energy, the Panel notes the difficulty of fully controlling for energy intake in nutrition intervention studies. Across RCTs, mean changes in body weight were of a similar order of magnitude whether the interventions aimed at modifying sugars intake were conducted ad libitum or under neutral energy balance. Data were insufficient to adequately explore the modifying effect of body weight changes in these relationships. Regarding the risk of dyslipidaemia, the relationship was more apparent in studies conducted at neutral energy balance while controlling for the macronutrient and lipid profiles of the diet than in studies ad libitum. This suggests that the (uncontrolled) impact of modifying sugars intakes ad libitum on the macronutrient and lipid profile of the background diet may have attenuated the relationship in free living conditions.

The BoE includes RCTs on mixtures of fructose and glucose in solid foods, beverages and foods and beverages combined, as well as a few studies conducted with fructose in isocaloric exchange with starch. RCTs with SSBs (and on mixtures of glucose and fructose in beverages) were a substantial part of the BoE available for added and free sugars in relation to all endpoints investigated, except blood lipids. In subgroup analysis, the effect of added and free sugars in foods and/or mixtures of foods and beverages was as strong or stronger than the effect of added and free sugars in beverages for the majority of the endpoints assessed (e.g. body weight and other measures of body fatness; fasting glucose and other measures of glucose tolerance; measures of insulin sensitivity, blood lipids, uric acid). However, these RCTs also differ in other characteristics (e.g. sugars dose, study population, duration of the intervention), so that the available data were insufficient to explore whether the source of added and free sugars could be a modifying factor of the relationship between their intake and the endpoints investigated.

Regarding the external validity of the BoE, the Panel notes that:

1) Most RCTs were conducted in adult subjects from either the general population or specific risk groups (e.g. overweight/obese, hyperinsulinaemic) including males, females or individuals both sexes combined. RCTs in children were scarce and mainly investigated the relationship between added or free sugars and measures of body weight and body fat. Data from RCTs were insufficient to explore whether age, sex or risk factors for disease could be modifying factors of the relationship between the intake of added and free sugars and the endpoints investigated.
2) Most PCs were conducted in adult subjects from the general population or convenience samples thereof (e.g. health practitioners) living in Europe, the US or Asian countries. As for RCTs, PCs in children were scarce and mainly investigated the relationship between added and/or free sugars and measures of body weight and body fat. PCs conducted in Europe were available for most of the exposure–disease relationships assessed and the results were in line with those reported in other geographical areas.

Overall, the Panel notes that the BoE has adequate external validity because it covers the target population for the assessment (i.e. the general population and subgroups thereof, including children

and individuals at risk of disease but not on pharmacological treatment for a disease, as specified in Section 5.3 of the protocol). The Panel also notes that, although age, sex and other individual factors could impact the strength of the relationships, the mechanisms by which dietary sugars could increase the risk of metabolic diseases are expected to be the same across population groups (see Section 8.9.5). Therefore, the Panel considers that the conclusions on hazard identification apply to the general European population and subgroups thereof.

Major sources of uncertainty in the BoE and in the methods used for data analysis are as follows:

1) RCTs explored the relationship between the intake of added or free sugars and surrogate but not direct disease endpoints.
2) In RCTs, between-arm differences in added or free sugars intake only refer to the dietary fraction that was manipulated by the intervention, and not necessarily to the intake of added and free sugars from all sources. This requires the assumption that the effect observed for a given change in added or free sugars intake is independent of the background intake (i.e. that moving from 10 E% to 20 E% intake from added and free sugars from all sources would have the same impact on the endpoints as moving from 20% to 30 E% intake), and that the intervention equally affects the consumption of added and free sugars from the background diet in the two study arms that are being compared.
3) Dose-response relationships across the BoE from RCTs between the intake of added and free sugars and surrogate disease endpoints could not be explored for liver fat owing to the limited number of studies available and the narrow range of sugars doses investigated, whereas no apparent dose-response relationships were observed for SBP (visual inspection of data, not formally assessed) or body weight (formally assessed). In addition, the residual heterogeneity in the positive linear dose-response relationships identified between the intake of added and free sugars and fasting glucose and fasting triglycerides was high, so that they could only be used to conclude on the direction of the linear dose-response relationship, but not to make a quantitative prediction of the effect of added and free sugars on fasting glucose or triglyceride levels.
4) Data from RCTs were insufficient to explore whether the source of added and/or free sugars could be a modifying factor of the relationship between the intake of added and free sugars and the endpoints investigated.
5) In PCs, sources of uncertainty in the BoE include the use of self-reported methods to assess the intake of added and free sugars, limitations in the food composition databases used to classify sugars as added or free, the use of sucrose as a surrogate for added and free sugars and the unclear impact that different adjustment strategies to account for possible mediators and confounders (e.g. TEI, BMI, diet quality) could have on the results.

8.9.3. Fructose

Glucose and fructose as monosaccharides are found naturally in fruits, berries, juices and some vegetables and honey. Sucrose (glucose-fructose disaccharide) is naturally present in sugar cane and sugar beet, in honey and in many vegetables, berries and fruits. Sucrose and isoglucose (a source of glucose and fructose monosaccharides) are also used as sweetening agents. Pure fructose is seldom used as sweetening agent in Europe. Intakes of fructose and its sources in European populations could not be calculated in this assessment because data on the content of single mono- and disaccharides in foods in the EFSA Nutrient Composition Database are scarce and not adequate to provide estimates of intake for individual sugar types.

Eligible PCs investigated the relationship between fructose intake from all sources and disease risk, i.e. namely risk of obesity, T2DM, dyslipidaemia, HTN, CVD and gout. The available BoE supports a positive and causal relationship between the intake of fructose in isocaloric exchange with other macronutrients and risk of gout (fructose and free fructose) and risk of CVDs (fructose from all sources), respectively. No support was found for a positive relationship with other chronic metabolic diseases. The Panel notes that fructose and glucose intakes in mixed diets are highly correlated because they share the same dietary sources, and that it is difficult to disentangle the contribution of these specific sugar types to disease risk in PCs. The relationship between the intake of glucose (and free glucose) and risk of gout or CVDs was not investigated in these PCs. In addition, contributors to fructose intake widely vary in their nutritional profile and role in the diet, and disentangling the effect of fructose *per se* from that of the food sources from which it is obtained (or from associated dietary patterns thereof) in observational studies is difficult.

Eligible RCTs investigated the effect of added fructose as monosaccharide in isocaloric exchange with added glucose as monosaccharide on surrogate disease endpoints, i.e. namely body weight, liver fat, measures of glucose tolerance, blood lipids and blood pressure. The effects of fructose and glucose on these endpoints did not appear to be different from each other. The Panel notes that there is some evidence from RCTs for a specific effect of fructose on hepatic insulin resistance and uric acid levels. The Panel also notes that the latter is a risk factor for hypertension, CVDs and gout, and that mechanisms underlying such specific effect of fructose are well-established (see Section 3.6.1.4).

Overall, the Panel concludes that the level of certainty for a positive relationship between the intake of fructose and risk of chronic metabolic disease is moderate for gout (> 50–75% probability) and low for CVDs (> 15–50% probability).

Regarding the external validity of the BoE, the Panel notes that:

1) The relationships between the intake of fructose and the risk of gout and CVDs have not been investigated in European populations, and the BoE for each relationship is limited to two and three cohorts, respectively.
2) The BoE does not include studies (RCTs or PCs) in children.

In this context, the Panel notes that it is unclear whether the conclusions on the relationship between the intake of fructose from all sources and the risk of CVDs (investigated in cohorts from US, Japan and Iran) and gout (investigated in US cohorts only) could be extrapolated to European populations because several factors could affect both the direction and the strength of the association (e.g. differences in the intake of fructose as E%, in the dietary sources of fructose and/or in the associated dietary patterns; differences in the incidence of CVDs and gout).

Major sources of uncertainty in the BoE and in the methods used for data analysis are as follows:

1) RCTs explored the relationship between the intake of fructose and surrogate (but not direct) disease endpoints.
2) In RCTs comparing the effects of fructose vs. glucose, the sugar dose (as free fructose or free glucose) only refers to the dietary fraction that was manipulated with the intervention, and not necessarily to the intake of fructose and glucose from all sources.
3) In RCTs comparing the effect of different doses of fructose as monosaccharide in isocaloric exchange with starch, between-arm differences in fructose intake only refer to the dietary fraction that was manipulated with the intervention, and not to the intake of fructose from all sources. As for added and free sugars, this leads to the assumption that the effect observed for a given change in fructose intake is independent of the background intake, and that the intervention equally affects the consumption of fructose from the background diet in the two study arms that are being compared.
4) Fructose and glucose intakes (as monosaccharides or bound as sucrose) in mixed diets are highly correlated because they share the same dietary sources, and it is difficult to disentangle the contribution of these specific sugar types to disease risk in PCs.

The Panel notes the uncertainties related to the external validity of the findings in relation to the risk of CVD and gout and the difficulties to disentangle the contribution of glucose and fructose to disease risk in PCs. The Panel also notes, however, that fructose is a component of added and free sugars in mixed diets and considers that the conclusions for added and free sugars also apply to fructose in that context.

8.9.4. Sources of added and free sugars

Intakes of added and free sugars from all sources in European countries were higher in consumers of SSBs (sugar-sweetened soft drinks and sugar-sweetened fruit drinks) than in consumers of any other food group in virtually all countries and population groups. The maximum contribution of SSBs to mean intakes of added and free sugars in consumers of these beverages ranged between 40% and 60% approx. depending on the population group, with high variation across countries. A notable exception is the intake of free sugars in toddlers, which was higher in consumers of fruit juices than in consumers of any other food group. Fruit juices contributed up to 48% to the intake of free sugars in this population group (see Section 4.3).

Conclusions from RCTs on SSBs are like those for added and free sugars. RCTs on SSBs (and on mixtures of glucose and fructose in beverages) were a substantial part of the BoE available for added and free sugars in relation to all endpoints except blood lipids. In that case, the effect of added and

free sugars was observed primarily in RCTs at neutral energy balance while controlling for the macronutrient and lipid profiles of the diet as mentioned above, whereas the few RCTs available on SSBs were conducted ad libitum.

Conversely, the overall evidence from PCs on SSBs supports a positive and causal relationship between the exposure and the risk of chronic metabolic diseases, whereas this was not the case for added and free sugars from all sources. Different from added and free sugars, SSBs were analysed not keeping TEI constant. Positive and causal relationships were identified in PCs between the intake of SSBs and incidence of obesity, T2DM, dyslipidaemia, hypertension, CVDs and gout. In addition, positive linear dose-response relationships were identified across the body of evidence between the intake of SSBs and incidence of T2DM, hypertension and CVD, with no evidence of non-linearity and no major sources of heterogeneity identified among those it was possible to explore (age, sex, study location, follow-up time, categorisation of exposure, tier of reliability).

A source of uncertainty is whether these relationships could be attributed, at least in part, to the sugars fraction of the beverages. The relationship between ASBs consumption and incidence of obesity, T2DM and risk of gout was null, negative or inconsistent in the studies included that also report on this exposure, suggesting that the positive relationship observed for SSBs in relation to these endpoints could be attributed, at least in part, to the sugars fraction of the beverage. Conversely, the relationship between the consumption of ASBs and incidence of hypertension and CVDs was similar to or stronger than for SSBs in these studies, suggesting that factors other than the sugar content of these beverages may play a role (e.g. associated dietary patterns and lifestyle factors), although reverse causality (i.e. individuals at higher risk of disease switching to ASBs) cannot be excluded. The Panel wishes to reiterate that such data do not allow drawing conclusions about the relationship between the intake of ASBs and risk of chronic disease because the systematic review was not set for that purpose, ASBs being out of the scope for this assessment.

Overall, the Panel concludes that the level of certainty for a positive and causal relationship between the intake of SSBs and risk of chronic metabolic disease is considered to be high for obesity, T2DM, HTN and CVD (> 75–100% probability), moderate for gout (> 50–75% probability) and low for NAFLD/NASH and dyslipidaemia (> 15–50% probability).

The number of PCs available for FJs, a major source of free sugars, was lower than for SSBs, as were the levels of intake. Only one RCT investigating different levels of intake of free sugars from FJs was identified, thus considered insufficient to draw conclusions. Overall, the Panel concludes that the level of certainty for a positive and causal relationship between the intake of FJs and risk of chronic metabolic disease is considered to be moderate for T2DM and gout (> 50–75% probability), and very low for obesity (0–15% probability), based on data from PCs. As for SSBs, FJs were analysed in most studies not keeping TEI constant.

As for added and free sugars, most RCTs on SSBs were conducted in adult subjects from either the general population, including males, females or individuals of both sexes combined, or specific risk groups. RCTs in children were scarce and mainly investigated the relationship between SSBs and measures of body weight and body fat. Most PCs on SSBs and FJs were conducted in adult subjects from the general population or convenience samples thereof (e.g. health practitioners) living in Europe, the US or Asian countries. PCs in children mainly investigated the relationship between the intake of these beverages and measures of body weight and body fat, and the results were consistent with those in adults. PCs conducted in Europe were available for most of the exposure–disease relationships assessed (as for fructose, a notable exception are PCs investigating the incidence of gout) and the results were in line with those reported in other geographical areas. Therefore, the Panel considers that, except for the risk of gout, the BoE has good external validity and that the conclusions on hazard identification apply to the general European population and subgroups thereof.

Major sources of uncertainty in the BoE and in the methods used for data analysis are as follows:

1) The available data from RCTs were insufficient to explore whether the source of added and free sugars could be a modifying factor of the relationship between their intake and the endpoints investigated.
2) No RCTs investigating different levels of intake of free sugars from FJs could be identified.
3) The BoE from PCs does not allow exploring whether the source of dietary sugars could be a modifying factor of the relationship between their intake and the endpoints investigated. This is because most PCs exploring the relationship between different sources of dietary sugars and disease risk did not quantify sugar intakes from those sources. In that context, it was possible to estimate sugar intakes from SSBs and FJs because the variability in the

sugar content per unit of volume was relatively low at the time intake estimates were assessed in the PCs available (i.e. a mean content of 10 g of sugars per 100 mL of the beverage is assumed). However, this was not possible for sources of sugars reported as combined categories including foods or food groups with very different sugar content, and for which the relative contribution of each food or food group to the combined category was unknown (e.g. 'sweets and cakes', 'sweet beverages including milkshakes, coffee and tea', 'cereal products', 'fruit and vegetable products', 'dairy products', etc.).

4) Differences in the classification of SSBs and fruit juices across PCs, in the methods used to assess their intake, and the fact that several PCs rely on one exposure assessment at the beginning of long follow-ups, through which subjects could have changed their habits in relation to the consumption of these beverages, are sources on uncertainty.
5) Adjusting for the rest of the diet when investigating the contribution of a single food source (SSBs, FJs) to disease risk is challenging, whereas the implications of different analytical strategies (e.g. adjustment for the energy contribution or the intake of other food sources, of specific nutrients, of specific foods; adjustment for total diet scores) on the results are unclear.
6) The relationship between the consumption of ASBs and incidence of hypertension and CVDs was similar to or stronger than for SSBs in the PCs included in the assessment, which questions the role of the sugar fraction in SSBs on the development of these metabolic diseases.

8.9.5. Mode of action

Exploring the relationship between the intake of dietary sugars, an energy-containing macronutrient and risk of chronic metabolic diseases is challenging. A notable limitation in the body of evidence (BoE) is that the energy and non-energy contribution (i.e. the molecule-specific effect) of dietary sugars from one or more sources to metabolic disease risk could not be systematically addressed across studies and endpoints. On the one hand, the characterisation of the specific (non-energy related) effects of sugars was hampered by the limitations of individual studies (e.g. incomplete control for energy in RCTs, inadequate control for energy in PCs), and by the disparity of available studies in terms of the choice and characterisation of the exposure of interest, the measurement of health endpoints and the analytical strategies used for data analysis and control for mediators/confounders. On the other hand, energy-related effects of dietary sugars from one or more sources could derive from excess energy intake likely owing to their hedonic properties, as suggested by the effect of sugars on body weight in RCTs conducted ad libitum and possibly to a lower satiating effect when consumed as liquids, as suggested by PCs not keeping TEI constant in the analysis (e.g. mostly on liquid sources of sugars). However, this was not addressed in the majority of eligible PCs on dietary (total/added/free) sugars from all sources, which mostly aimed at keeping TEI constant in the analysis.

Excess energy intake leading to positive energy balance and body weight gain is one mechanism by which the intake of dietary sugars can contribute to the risk of chronic metabolic diseases (Section 3.6.1.1). There is evidence for a positive and causal relationship between the intake of added and free sugars and their liquid sources, body weight gain and risk of obesity, both from RCTs conducted ad libitum and from PCs not keeping TEI constant in the analysis. Obesity is a well-established risk factor for several chronic metabolic diseases.

The available evidence also indicates a specific effect of dietary sugars on liver fat, glucose tolerance and blood triglycerides. High intakes of dietary sugars have been shown to induce *de novo* lipogenesis in the liver and the gut, increase the secretion of TG-rich lipoprotein particles (TRL) in the circulation and decrease their clearance. In addition, high *de novo* lipogenesis can lead to ectopic fat deposition (e.g. in the liver), increase hepatic insulin resistance and impair glucose tolerance in the long term (see Sections 3.6.1.2 and 3.6.1.3). Taking together studies conducted at neutral energy balance in isocaloric exchange with starch and studies conducted ad libitum, positive linear dose-response relationships were identified between the intake of added and free sugars (mostly as mixtures of glucose and fructose) and fasting glucose and triglyceride levels in RCTs, with no evidence for non-linearity. The dietary conditions in which the studies were conducted were not identified as a major source of heterogeneity. However, unexplained heterogeneity remained high and data were insufficient to adequately explore the modifying effect of body weight changes in these relationships.

Since starch is absorbed as glucose in the bloodstream, the fructose component could have been responsible for the specific metabolic effects of added and free sugars when consumed in isocaloric

exchange with starch. Fructose has been shown to increase hepatic insulin resistance more than equivalent amounts of glucose or sucrose. In addition, there are specific mechanisms by which fructose can increase uric acid levels, a risk factor for the development of hypertension and gout. High fructose intakes lead to an increase in hepatic fructose uptake and phosphorylation to fructose-1-P, while degradation of fructose-1-P to trioses phosphate is slightly delayed. This results in a transient depletion of intrahepatic ATP stores, leading to the formation of AMP and to the degradation of purines. Fructose may also impair renal uric acid clearance and fractional excretion (see Section 3.6.1.4).

Based on the available evidence, the Panel considers that excess energy intake leading to positive energy balance and body weight gain is the main mechanism by which the intake of dietary sugars may contribute to the development of chronic metabolic diseases in free living conditions. The Panel also considers that mechanisms which are specific to sugars as found in mixed diets (i.e. *de novo* lipogenesis leading to ectopic fat deposition, increased hepatic insulin resistance and impaired glucose tolerance in the long term; increase in uric acid levels) may also play a role, particularly in positive energy balance.

8.10. Metabolic diseases: data gaps and research needs

The Panel notes that the amount of evidence available across different exposures and endpoints is very variable. Main data gaps identified in the BoE relate to the characterisation of dietary sugars in the whole diet (as total, added and free sugars; as sugar types), the quantification of sugar intakes from different sources (not only beverages) and the relationship between all these variables and chronic disease endpoints.

To that end, the use of accurate food composition databases based on food analyses, repeated measures of the exposure through the studies to assess habitual intakes the development and validation of reliable methods and (bio)markers of intake are of paramount importance.

In the context of a safety assessment, PCs allow to assess the relationship between the intake of dietary sugars and their sources and chronic disease risk in free-living conditions across wide ranges of intake, provided that possible mediators and confounders are reliably measured and accounted for. Particular attention should be paid to the analytical strategies used to account for both energy intake and BMI (or measures thereof), which could be both mediators and confounders of the relationship. The contribution of RCTs investigating the effect of dietary sugars and their sources on surrogate disease endpoints are important to establish the causality to the relationships identified in epidemiological studies, as well as to investigate the mechanisms underlying such relationships.

9. Hazard identification: pregnancy endpoints

9.1. Body of evidence

9.1.1. Intervention studies

No intervention studies were identified in relation to pregnancy-related endpoints.

9.1.2. Observational studies

Among the seven PCs eligible for this review, three investigated the relationship between the intake of dietary sugars in women in child-bearing age and incidence of gestational diabetes mellitus (GDM) (ALSWH cohort, (Looman et al., 2018); SUN cohort, (Donazar-Ezcurra et al., 2018); NHS II, (Chen et al., 2009a)) among the women who became pregnant during the follow-up of the study. These studies did not assess the intake of dietary sugars or their sources during pregnancy. The remaining four PCs investigated the relationship between the intake of dietary sugars during pregnancy and birthweight-related endpoints (Camden cohort, (Lenders et al., 1997); HSS-USA cohort (Crume et al., 2016); MoBa cohort, (Grundt et al., 2017); GeliS cohort (Günther et al., 2019)) in women recruited in the first trimester of pregnancy. The exposures of interest investigated in these studies were total sugars, SSBs and fruit juice.

Evidence tables of the observational studies on pregnancy-related endpoints can be found in **Annex J**.

9.2. Principles applied to assess the body of evidence: evidence integration and uncertainty analysis

The principles applied to assess the body of evidence are as described for metabolic diseases (Section 8.1.3), including the elements considered for preliminary and comprehensive UAs.

Table 29 summarises the subquestions for hazard identification in relation to pregnancy endpoints, the LoEs and the number of studies included by study design and exposure. Total sugars, SSBs and FJs were investigated in relation to the risk of GDM (**sQA**), whereas total sugars and SSBs were assessed in relation to the risk of adverse birth-weight-related endpoints (**sQB**).

In relation to the risk of GDM, incidence of GDM was the only eligible endpoint, and thus, there is only one standalone (main) LoE. Obesity pre-pregnancy and weight gain during pregnancy could both increase the risk of GDM. The available studies in the BoE which investigated incidence of GDM did not assess the intake of dietary sugars during pregnancy, and studies on the relationship between the intake of dietary sugars and weight gain during pregnancy have not been systematically searched for in this assessment. However, the Panel considers that the conclusions regarding the risk of obesity as assessed in the section of metabolic diseases (Section 8.2) for the general population also apply to women in child-bearing age pre-pregnancy, and thus, risk of obesity will be considered as a complementary LoE. In addition, GDM increases the risk of T2DM, and factors increasing the risk of T2DM in women of child-bearing age could also increase the risk of GDM. For this reason, risk of T2DM as assessed in the section of metabolic diseases (Section 8.4) for the general population will also be considered as a complementary LoE. These complementary LoEs, on their own, cannot answer the sQ on risk of GDM (see Section 8.1.3).

In relation to the risk of adverse birth-weight related endpoints, a standalone (main) LoE includes incidence of low birthweight (LBW), small for gestational age (SGA), high birthweight (HBW) and large for gestational age (LGA) as eligible endpoints, whereas a standalone (surrogate) LoE includes birthweight.

Table 29: Subquestions for hazard identification, lines of evidence and number of studies included by exposure and study design

sQ1. Is the intake of **total sugars** positively and causally associated with adverse pregnancy endpoints at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?			
LoE	**Endpoints**	**RCTs (n)**	**PCs (n)**
sQ1.A Risk of GDM			
LoE1. Standalone (main)	Incidence of GDM	0	1
LoE2. Complementary	Risk of obesity (sQ1.1)	sQ1.1	sQ1.1
LoE3. Complementary	Risk of Type 2 diabetes mellitus (sQ1.3)	sQ1.3	sQ1.3
sQ1.B Risk of adverse birthweight-related endpoints			
LoE1. Standalone (main)	Incidence of LBW, SGA, HBW, LGA	0	1
LoE2. Standalone (surrogate)	Birthweight	0	1
sQ2. Is the intake of **SSBs** positively and causally associated with adverse pregnancy endpoints at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?			
LoE	**Endpoints**	**RCTs (n)**	**PCs (n)**
sQ2.A Risk of GDM			
LoE1. Standalone (main)	Incidence of GDM	0	2
LoE2. Complementary	Risk of obesity (sQ4.1)	sQ4.1	sQ4.1
LoE3. Complementary	Risk of Type 2 diabetes mellitus (sQ4.3)	sQ4.3	sQ4.3
sQ2.B Risk of adverse birthweight-related endpoints			
LoE1. Standalone (main)	Incidence of LBW, SGA, HBW, LGA	0	2
LoE2. Standalone (surrogate)	Birthweight	0	2

sQ3. Is the intake of **FJs** positively and causally associated with adverse pregnancy endpoints at the levels of intake and in the population subgroups investigated in the studies eligible for this assessment?

LoE	Endpoints	RCTs (n)	PCs (n)
sQ4.A Risk of GDM			
LoE1. Standalone (main)	Incidence of GDM	0	2
LoE2. Complementary	Risk of obesity (sQ5.1)	sQ5.1	sQ5.1
LoE3. Complementary	Risk of Type 2 diabetes mellitus (sQ5.3)	sQ5.3	sQ5.3

9.3. Incidence of gestational diabetes mellitus

9.3.1. Total sugars

9.3.1.1. Intervention studies

No RCTs were available for sQ1.A

9.3.1.2. Observational studies

LoE1. Standalone (main): Incidence of GDM. PCs. One PC investigated the relationship between the intake of total sugars at baseline and incidence of GDM in the subset of women who became pregnant during follow-up. Total sugars intake during pregnancy was not assessed.

In the ALSWH cohort (Looman et al., 2018), 3,607 women between 25 and 30 years of age with complete data and no diagnosis of diabetes at baseline (type 1, type 2 or GDM) reported at least one pregnancy (total of 6,263 pregnancies) during a 12-year follow-up. Total sugars intake was analysed by categories of intake and adjusted for TEI using the nutrient residuals model, so TEI was kept constant in the analysis.

Preliminary UA. The incidence of GDM significantly decreased across increasing quartiles of total sugars intake when the model was adjusted for relevant covariates and TEI. With the additional adjustment for E% from fat and protein, the negative relationship became non-significant ($RR_{Q4\ vs.\ Q1}$: 0.83; 95% CI: 0.56, 1.23; p per trend = 0.32). Further adjustment for pre-pregnancy BMI had no impact on the relationship. This PC was at high RoB (tier 3). Critical domains were confounding, outcome assessment and attrition.

The Panel considers that the available BoE does not support a positive relationship between the intake of total sugars and incidence of GDM. **No comprehensive UA is performed**.

Complementary LoE2: Risk of obesity and LoE3: Risk of T2DM. **PCs**. The available BoE does not suggest a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of obesity (sQ1.1, Section 8.2.1.1) or risk of T2DM (sQ1.3, Section 8.4.1.1).

Conclusion sQ1.A. PCs. The available BoE does not support a positive relationship between the intake of total sugars in isocaloric exchange with other macronutrients and risk of GDM.

9.3.1.3. Overall conclusion on sQ1.A

Since no standalone LoE passed the screening step (preliminary UA), the Panel considers that the available BoE cannot be used to conclude on a positive and causal relationship between the intake of total sugars and risk of GDM.

9.3.2. Sugar-sweetened beverages

9.3.2.1. Intervention studies

No RCTs were available for standalone LoEs in relation to sQ2.A.

Complementary LoE2: Risk of obesity and LoE3: Risk of T2DM. RCTs. There is evidence from RCTs for a positive and causal relationship between the intake of SSBs ad libitum and risk of obesity (moderate certainty, sQ4.1, Section 8.2.4.1) and T2DM (low certainty, sQ4.3, Section 8.4.4.1).

Conclusion sQ2.A. RCTs. Whereas there is evidence from RCTs for a positive relationship between the intake of SSBs and risk of obesity and T2DM, no RCTs investigating the relationship between the intake of SSBs and incidence of GDM are available. Therefore, the Panel considers that the available BoE from RCTs does not suggest a positive relationship between the intake of SSBs and risk of GDM.

9.3.2.2. Observational studies

LoE1. Standalone (main): Incidence of GDM. PCs. Two PCs (SUN, (Donazar-Ezcurra et al., 2018); NHSII, (Chen et al., 2009a)) report on the relationship between the intake of SSBs and incidence of GDM in the subset of women who became pregnant during follow-up. Data on SSBs were collected at baseline in both cohorts, and at 6 and 10 years of follow-up in the SUN cohort. None of the PCs assessed intake of SSBs during pregnancy.

Either the standard multivariable model was used for categorical analyses (SUN) or TEI was not included in the models (NHS II), so that TEI was not kept constant in the analyses. Both PCs include BMI in the most adjusted models. The evidence table can be found in **Annex J**.

Preliminary UA

In the SUN cohort, a significant positive dose-response relationship was observed between the intake of SSBs and incidence of GDM in a population of 3,396 women reporting a live birth during the 10.3 years of follow-up. In the model adjusted for relevant covariates, incidence of GDM significantly increased across categories of SSBs intake ($OR_{C4\ vs.\ C1}$ = 2.06, 95%CI = 1.28, 3.34) in a dose-response manner (p for trend=0.006). Additional adjustment for TEI did not substantially modify the results. The increased risk of GDM was already significant at intakes between 1 and 3 servings/month and < 1 serving/week (1 serving = 200 mL). When repeated measurements of SSBs intake were considered in the analysis (at baseline, 6 and 10 years of follow-up), the increase in incidence of GDM was only significant for the highest category of intake (> 2 servings/week) and the RR was reduced ($OR_{C4\ vs.\ C1}$ = 1.70, 95%CI = 1.02, 2.81; p for trend = 0.017). This PC was at low RoB (tier 1).

In the NHS II cohort (Chen et al., 2009a), a significant positive dose-response relationship was reported between the intake of SSBs and incidence of GDM in a population of 13,475 women reporting a live birth during the 10 years of follow-up. In the model adjusted for relevant covariates, including BMI, physical activity and family history of diabetes, each serving/day (334 mL/day) was associated with a RR of 1.23 (95%CI = 1.05, 1.43) of developing GDM. Additional adjustment for Western dietary pattern scores attenuated the association (RR = 1.16; 95%CI = 0.99, 1.36), suggesting that the relationship may be in part mediated and/or confounded by dietary habits associated with the consumption of SSBs. Models were not adjusted for TEI. This PC was at moderate RoB (tier 2), critical domains being outcome assessment and attrition.

The Panel considers that the available BoE suggests a positive relationship between the intake of SSBs and risk of GDM.

Comprehensive UA

The BoE on the relationship between the intake of SSBs and risk of GDM is limited to two PCs. The Panel considers that it would be inappropriate to proceed with a comprehensive UA because several downgrading factors cannot be assessed with less than three independent studies. The initial level of certainty assigned to the relationship is **very low** (0–15% probability) to reflect the limited BoE available (see Section 8.1.3).

Complementary LoE2: Risk of obesity and LoE3: Risk of T2DM. PCs. There is evidence from PCs for a positive and causal relationship between the intake of SSBs ad libitum and risk of obesity (moderate certainty, sQ4.1, Section 8.2.4.2) and T2DM (moderate certainty, sQ4.3, Section 8.4.4.2).

The Panel notes that the BoE consists of two independent cohorts of women adequately powered with an appropriate follow-up and at low to moderate RoB. However, the Panel also notes that the relationship was strongest in the smallest study and apparent at levels of intake as low as 200 mL/week, corresponding to 20 g of sugars per week. Taking into account that the relationship between the intake of SSBs and risk of GDM is consistent with evidence from PCs and RCTs for an increased risk of obesity and T2DM in the general population, which includes women in childbearing age, the Panel considers that the level of certainty in the relationship is **low** (> 15–50% probability).

Conclusion sQ2.A. PCs. The level of certainty in a positive and causal relationship between the intake of SSBs and risk of GDM is **low**. The relationship was observed not keeping TEI constant in the analysis.

9.3.2.3. Overall conclusion on sQ2.A

There is evidence from PCs for a positive and causal relationship between the intake of SSBs and risk of GDM (**low** level of certainty).