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**Supporting document 2 – Assessment of galacto-oligosaccharides against three beneficial physiological effects**

A1178 – AOAC 2017.16 as a new method of analysis for total dietary fibre

# Executive summary

Application A1178 seeks to amend the *Australian New Zealand Food Standards Code* (the Code) to permit the addition of an alternate method of analysis of dietary fibre, AOAC Method 2017.16 (McCleary 2019). As described in Supporting Document 1 (SD1), galacto-oligosaccharides (GOS) are a component measured by AOAC Method 2017.16 and contribute to the method’s analytical value for total fibre. The assessment described in this Supporting Document considers whether GOS meet the three beneficial physiological effects listed in the Code’s definition of dietary fibre provided in Standard 1.1.2 – Definitions used throughout the Code.

We assessed evidence from human trials that investigated whether GOS promote at least one of the beneficial physiological effects prescribed in the Code’s definition of dietary fibre. The beneficial physiological effects are:

* 1. *laxation;*
  2. *reduction in blood cholesterol;*
  3. *modulation of blood glucose.*

Current evidence shows that a higher dietary fibre intake, from foods naturally rich in intact dietary fibre, lowers the risk of many non-communicable diseases. Inverse dose-response relationships between fibre intake and all-cause mortality, colorectal cancer, type 2 diabetes mellitus, and incidence of coronary heart disease have been demonstrated (Reynolds et al. 2019). These desirable health outcomes are mediated, in part, through the effect of dietary fibre on cardiometabolic risk factors and intermediary physiological outcomes; three of which are sufficiently well-established to be incorporated as health criteria that define dietary fibre in the Code. Consuming foods naturally high in dietary fibre is an important part of dietary guidelines and nutrition education. Many consumers perceive fibre as being ‘good for health’. It is common for processed foods to be manufactured with added dietary fibre, whether extracted or synthetically produced. It may be possible for some extracted or synthetically produced dietary fibres to exert physiological effects; however, the evidence supporting fibre’s role in preventing non-communicable disease is based primarily on intake of fibre from whole grains, fruits, and vegetables (Reynolds et al. 2019) but not added fibre. Thus, the benefits of synthetic analogues of dietary fibres tend to be assessed according to their effects on physiological or biochemical outcomes linked with non-communicable disease risk; in this case the three outcomes listed in the Code: *laxation; reduction in blood cholesterol; and, modulation of blood glucose.*

The current assessment evaluates parallel or crossover controlled trials in humans, where the intervention group consumed additional GOS in isolation to other non-digestible carbohydrates, and that reported an outcome related to the three beneficial physiological effects listed in the Code. The eligible studies were mainly randomised trials with a placebo control. We conducted a meta-analysis if the outcome was assessed by at least two studies. A narrative review was completed, for other outcomes.

The results of our meta-analyses indicate that GOS intake does not alter total or LDL cholesterol, fasting glucose, or stool weight (summary effect sizes are provided in Table 1). The results from the trials also show that GOS do not affect other outcomes related to blood lipids, glycaemic control, or laxation, including: HDL-cholesterol; fasting triglycerides; glycosylated haemoglobin; postprandial glycaemic response; fasting insulin; HOMA-IR; five additional insulin-related outcomes; relative stool weight; bowel movement or stool frequency; and, intestinal transit time (summary effects of some outcomes are provided in Table 5, Appendix Section 2.1). Adverse effects were noted in two studies that tested the lowest GOS intake level of all included studies and had excluded volunteers with gastrointestinal tract conditions. One participant withdrew due to gastrointestinal upset (Pedersen et al. 2016) and another due to tolerance problems (Vulevic et al. 2008), however, the severity of symptoms and possible cause were not reported.

**Table 1: Galacto-oligosaccharide intake has no overall mean effect on outcomes related to laxation, blood cholesterol, and blood glucose.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Outcome** | **Units** | **Mean difference (95% CI)1** | ***P*2** | **Number of included studies and pair-wise comparisons** |
| Stool weight | gram | 6.34 (-6.21, 18.9) | 0.32 | 3, 6 |
| Total cholesterol | mmol/L | -0.13 (-0.34, 0.08) | 0.21 | 4, 4 |
| LDL-cholesterol | mmol/L | -0.11 (-0.29, 0.08) | 0.25 | 3, 3 |
| Fasting glucose3 | mmol/L | -0.09 (-0.19, 0.00) | 0.06 | 4, 4 |

CI, confidence interval; L, litre; LDL, low density lipoprotein. 1Mean difference is calculated as intervention minus placebo. 2*P-value* pertains to overall mean difference (i.e. effect size). 3Fasting status confirmed by three of four studies and assumed for one study (Vulevic et al. 2013).

Most of this assessment’s evidence is derived from seven controlled studies, involving a total of 223 participants. These studies collectively assessed a wide range of intakes of added GOS, up to very high intakes of 15 g per day. All seven trials had links with industry, whether through declared interests or funding; however, the small number of trials and lack of studies without industry associations precluded any subgroup analysis to examine potential bias in the findings. The body of evidence about the physiological effects of GOS only includes results from clinical trials which used synthetic analogues, not the natural forms, and shows that GOS does not affect the three beneficial physiological effects listed in the Code. Therefore, insofar as naturally occurring GOS is concerned, the physiological effects are inferred on the basis of structural similarities to synthetic analogues and extend from an indirect body of evidence (i.e. synthetic analogues).The chemical composition of GOS mixtures, which can include large proportions of non-GOS components, is highly variable and this is likely to contribute to variability in physiological effects reported in human trials.

Overall, the mean effect estimates show that GOS do not exert a beneficial effect on stool weight, total or LDL-cholesterol, or fasting blood glucose. All mean effect sizes for the relevant physiological outcomes were small (e.g. a decrease of ~0.1 mmol/L for the blood markers) and 95% confidence intervals spanned the null (i.e. zero effect). Although the mean effect sizes are small, the lower limits of the confidence intervals include a possible desirable outcome, meaning a beneficial effect cannot be excluded entirely. However, the likelihood of this is low because the results of future research would need to differ considerably from the current body of evidence to shift the mean effect size enough to show a clinically meaningful benefit. In general, as evidence accrues, summary estimates of effect tend to be attenuated rather than increased (Strazzullo et al. 2009). A summary of the strengths and limitations of our assessment is provided in Table 6 (see Appendix Section 2.1).

We conclude, based on the current body of evidence, that GOS intake does not exert clinically meaningful or beneficial effects on laxation, blood cholesterol, or blood glucose. This is consistent with international assessments by the US Food and Drug Administration and the UK Food Standards Agency. The assessment of the evidence provides little reason to indicate that the consumption of GOS will have desirable effects on health, as judged by the three physiological outcomes that define dietary fibre in the Code. Thus, these carbohydrates do not meet any of the three beneficial physiological effects listed in the Code’s definition of dietary fibre.

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# Scope of the assessment: rationale and limitations

The assessment described in this Supporting Document answers the risk assessment question: “to what extent does GOS meet the Code’s definition of dietary fibre for beneficial physiological effects for any population group except infants and young children?” These beneficial physiological effects are:

1. *laxation;*
2. *reduction in blood cholesterol;*
3. *modulation of blood glucose;*

We included publications that reported an outcome related to the Code’s three beneficial physiological effects. We categorised them into primary and secondary outcomes, according to their relevance to each of the three beneficial physiological effects listed in the Code’s definition of dietary fibre (i.e. “laxation”, “reduction in blood cholesterol”, and “modulation of blood glucose”). If the outcome was assessed in at least two studies, we conducted a meta-analysis. The primary outcomes assessed using meta-analyses include: absolute stool weight; total and LDL-cholesterol; and, fasting blood glucose. The primary outcomes assessed by narrative review include: glycosylated haemoglobin; postprandial glycaemic response; and, relative stool weight. Secondary outcomes assessed using meta-analyses include: HDL-cholesterol; fasting triglycerides; fasting insulin; and, HOMA-IR. Secondary outcomes assessed by narrative review include: bowel movement or stool frequency; intestinal transit time; and, five additional insulin-related outcomes.

The body of evidence about the physiological effects of GOS only includes results from clinical trials which used synthetic analogues, not the natural forms, and shows that GOS does not affect the three beneficial physiological effects listed in the Code. Therefore, insofar as naturally occurring GOS is concerned, the physiological effects are inferred on the basis of structural similarities to synthetic analogues and extend from an indirect body of evidence (i.e. synthetic analogues).Further, GOS were consumed in powdered form, mixed into beverages or foods; therefore, the current assessment has not evaluated the effects of synthetic analogues of GOS when incorporated into the food matrix of processed foods.

The Supporting Document 1 explains that sugars with a degree of polymerisation of two or less (i.e. mono- and di-saccharides) will not be detected as dietary fibre by the AOAC 2017.16 Method. Table 7 illustrates that the studies used GOS mixtures where the GOS component contained varying levels of disaccharides (Appendix Section 2.5). For example, Ito et al. (1990) stated that Oligomate-50 contains 16% disaccharides (either galactosyl glucose or galactosyl galactose; w/w dry matter) which contributes almost one-third of the total GOS (52%) in the Oligomate-50 mixture (w/w dry matter). This disaccharide component (16%) excludes the lactose (10%) that is also present in Oligomate-50. Torres et al. (2010) presented the chemical composition of other commercial GOS products which contain saccharides of different degrees of polymerisation, in different proportions of the total amount of GOS. Total GOS content varied between 48% and 100% (w/w dry matter) in the six products reviewed by Torres et al. (2010). The non-GOS components of mixtures represent differing proportions of disaccharides (lactose) and monosaccharides (glucose or galactose). The GOS’ degrees of polymerisation also varied; saccharides with a degree of polymerisation of two contributed from 0% to 29% (w/w dry matter) to the commercial product. Thus, there are some considerations to bear in mind. First, the impact of different degrees of polymerisation on physiological outcomes is unknown. The current evidence is unable to distinguish the nature of a relationship, if any, between GOS mixtures’ degrees of polymerisation and physiological outcomes. Second, if GOS mixtures reduce their mono- or di-saccharide component in future, it is unknown what physiological effect the new mixture would exert. Third, the reported fibre content will differ between the commercially reported information and the measured fibre content when using the AOAC 2017.16 Method. In addition, some studies used a GOS mixture containing mono- and di-saccharides, but did not match this sugar or energy content in the placebo that was provided to the control group. Therefore, it is not always possible to attribute any observed effect to GOS.

We included parallel or crossover controlled trials in humans where the intervention group consumed additional GOS in isolation to other non-digestible carbohydrates. The resulting studies were mainly randomised trials with a placebo control, although we had not intentionally limited the inclusion criteria to this, due to an expected low number of relevant studies. Studies with an inclusion criteria specific to infants, young children, or people with gastrointestinal conditions (such as inflammatory bowel disease, irritable bowel syndrome, short bowel syndrome, or ulcerative colitis) were excluded from this review. The reasons for excluding these participant groups were: 1, GOS intake contributes to undesirable gut symptoms in people with irritable bowel syndrome (Tuck et al. 2018) which affects 8.1% (95% CI: 7.0, 8.3) of the North America/Europe/Australia/New Zealand population (Sperber et al. 2017); 2, lowering blood cholesterol and fasting glucose is not generally of critical importance in infants and young children; and, 3, the reporting of the fibre content of infant formula is not required by the Code.

# 1.2 Laxation

## 1.2.1 Assessment: background

There are several hypotheses to explain the effect of dietary fibre on laxation. Cummings (2001) outlines four probable mechanisms of action: (i) plant cell walls resist breakdown and retain water, and may have the physical effect of increasing intestinal bulk which stimulates colonic movement; (ii) the degradation of fibre by microflora leads to increased microbial growth and excretion of microbial products in faeces which increases stool weight; (iii) increasing bulk in the large intestine may shorten the transit time, in turn reducing the water absorption by the colon and increasing the amount of moisture in the stool; and (iv) gas (H2, CH4, and CO2) created by fermentation and trapped within gut contents increases intestinal bulk. It is hypothesised that, through one or more of these potential mechanisms, fibre promotes normal laxation by increasing stool weight (Cummings 2001) and reducing the transit time in the intestinal tract (Slavin 2013).

Adequate stool or faecal weight for a laxative effect may be assessed using a benchmark of a greater than 1 g increase in faecal wet weight per gram of test fibre consumed (hereon in, >1 g/g). FSANZ used this benchmark in the assessment of two previous applications: A1142 - [Addition of Prescribed Method of Analysis for Resistant Starch](https://www.foodstandards.gov.au/code/applications/Pages/A1142Method-of-Analysis-for-Resistant-Starch.aspx); and, A491 - [Resistant maltodextrin as dietary fibre](https://www.foodstandards.gov.au/code/applications/Pages/applicationa491resistantmaltodextrinasdietaryfibre/index.aspx). A1142 used a benchmark of >1 g/g due to a precedent set by A491 (FSANZ 2018). A491 states this benchmark was used due to it being established in A277, noting “however this benchmark has not been further consolidated as a formal requirement”. . . “nor have any benchmarks been established in the scientific literature” (FSANZ 2004, p. 37). A277 - [Inulin as a dietary fibre](https://www.foodstandards.gov.au/code/applications/Pages/applicationa277inulinasadietaryfibre/index.aspx) does not refer to a benchmark *per se*. It states that, according to a consensus paper by Van Loo et al. (1999), fructo-oligosaccharide (FOS) intake is associated with “a mild increase in faecal output comparable to soluble dietary fibres and resistant starch (1-2 g faecal weight increase/g FOS ingested at intakes 15-40 g/day)” (FSANZ 2001, p. 4). The faecal outputs of soluble dietary fibres and resistant starch on which this statement is based, is not outlined or cited. Other favourable physiological properties of fructans related to bowel habit and gut flora are also noted.

Cummings reviewed about 100 studies published between 1932 and 1984, that investigated the effect of dietary fibre intake on faecal output. The fibre sources were categorised into eight groups: wheat (mainly bran); fruits and vegetables; gums and mucilages; cellulose; oats; corn; legumes; and, pectin (Cummings 2001). Cummings concluded that all these sources of fibre increased faecal output. However, stool weight per gram of fibre consumed varied considerably. Legumes, which the SD1 outlines are high in naturally occurring GOS, gave an increase of 2.2 ± 0.3 g (mean ± standard error of the mean, SE) in stool weight per gram of fibre, based on 17 studies. The least effective fibre was pectin which gave an increase in stool weight of 1.2 ± 0.3 g/g of fibre, based on 11 studies. Whereas fruits and vegetables, and wheat (mostly bran), had a four-fold greater impact (an increase of 4.7 ± 0.7 g/g based on 28 studies, and 5.4 ± 0.7 g/g based on 41 studies, respectively). A benchmark threshold of >1 g/g therefore aligns with the least effective source of fibre (pectin) reported by Cummings (2001). This benchmark reflects a laxative effect that is up to five times lower than the laxative effect of other fibre sources.

The use of stool weight to evaluate the effect of fibre on laxation has been referred to by other agencies such as the National Health and Medical Research Council (NHMRC 2006) and the Scientific Advisory Committee on Nutrition (SACN 2008). In setting Nutrient Reference Values, the NHMRC acknowledge that establishing dietary fibre requirements is difficult. They state that in the absence of biochemical markers, clinical endpoints were considered, however, the potential clinically-related endpoints are ill defined. The NHMRC used “adequate gastrointestinal function and adequate laxation rather than reduction of risk for chronic disease” to estimate dietary fibre requirements that prevent deficiency states (i.e. the Adequate Intake values; NHMRC 2006 p. 41). Change in stool weight is discussed by the NHMRC but does not appear to have been used in establishing the dietary fibre Nutrient Reference Values. The NHMRC state that an increased stool weight alone does not guarantee improved laxation. The NHMRC did not set an Estimated Average Requirement and instead established an Adequate Intake derived from median intakes in populations where laxation problems are not common. The Adequate Intake is 30 g and 25 g per day for men and women (not pregnant or lactating) aged 19 years or older. The NHMRC noted that a dietary fibre intake higher than the Adequate Intake may reduce the risk of obesity and chronic disease. The NHMRC outline the association between dietary fibre intake and the risk of obesity, cardiovascular disease, diabetes, and some cancers. The Suggested Dietary Target proposes a dietary fibre intake to reduce the risk of coronary heart disease: 38 g and 28 g per day for men and women, respectively.

## 1.2.2 Assessment: results

Three studies are reviewed here, to evaluate the relationship between added GOS intake, and absolute and relative change in stool weight. Relative change in stool weight is the absolute change relative to the intake level of added GOS. We compared the studies’ relative change findings against a benchmark of greater than 1 g and 5 g increase in faecal wet weight per gram of added GOS (hereon in, >1 g/g and >5 g/g). This aligns with the least and most effective source of fibre on stool weight (pectin and wheat, mostly bran, respectively; Cummings 2001). Each study had a small sample size (n=12 for both crossover studies conducted by Ito et al. 1990, and van Dokkum et al. 1999; n=40 for the parallel controlled trial by Alles et al. 1999). Randomisation was not stated for one crossover trial (Ito et al. 1990) and one parallel controlled trial (Alles et al. 1999); we contacted the corresponding authors for clarification but received no response. Each studies’ results are summarised in Table 2. We report the methodology of our review and the included studies in Appendices Sections 2.2 and 2.6.

**Table 2: Galacto-oligosaccharide intake is not associated with stool weight.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **First author (year)** | **GOS intake level (g/day)** | **Stool weight** | | | | ***P*7** |
| **Baseline (mean ± SE, g/day)1** | **Follow-up (mean ± SD or SE2, g/day)3** | **Mean difference4 ± SE5 (g/day)** | **Mean difference ± SE relative to GOS6 (g/day per GOS g)** |
| Ito (1990) | 0.0 | n/a | 151 ± 63.6 | n/a | n/a | n/a |
| 2.5 | n/a | 134 ± 49.6 | -16.8 ± 13.2 | -6.7 ± 5.3 | NSD |
| 5.0 | n/a | 151 ± 77.1 | 0.0 ± 16.1 | 0.0 ± 3.2 | NSD |
| 10.0 | n/a | 163 ± 71.4 | 11.3 ± 15.2 | 1.1 ± 1.5 | NSD |
| van Dokkum (1999) | 0.0 | n/a | 129 ± 42.5 | n/a | n/a | n/a |
| 15.0 | n/a | 149 ± 51.5 | 19.5 ± 10.8 | 1.3 ± 0.7 | NSD |
| Alles (1999) | 0.0 | 147 ± 11 | 139 ± 14 | n/a | n/a | n/a |
| 8.58 | 113 ± 12 | 127 ± 14 | 22.3 ± 22.2 | 2.6 ± 2.6 | NSD |
| 14.48 | 146 ± 22 | 142 ± 18 | 3.5 ± 21.8 | 0.2 ± 1.5 | NSD |

GOS, galacto-oligosaccharide; g, gram; n/a, not applicable or not available; SE, standard error of the mean; SD, standard deviation; NSD, not significantly different (*P*>0.05).

1 Baseline stool weight is provided only for the parallel controlled trial by Alles et al. (1999). This represents stool weight at the end of a run-in diet period, common to all conditions.

2 SD for Ito et al. (1990) and van Dokkum et al. (1999). SE for Alles et al. (1999).

3 Represents stool weight at end of the intervention period for the crossover studies (Ito et al. 1990 and van Dokkum et al. 1999) and parallel controlled trial (Alles et al. 1999). van Dokkum et al. (1999) reports stool weight as g/48 hour and we converted the mean and SD to g/day by dividing both by 2.

4 Calculated mean difference = mean (intervention follow-up) – mean (placebo follow-up) for the crossover studies (Ito et al. 1990 and van Dokkum et al. 1999). For the parallel controlled trial, the difference provided by Alles et al. (1999) represents group x time (i.e. adjusted for stool weight at the end of a run-in diet period).

5 All SEs were calculated. Appendix Section 2.6 outlines the data and formulae used to calculate the SE. Note that the SE for the parallel controlled trial was calculated from the 95% confidence interval of the difference which was provided by Alles et al. (1999): (-21.2, 65.9) for the placebo versus 8.5 g GOS / day condition; and, (-39.3, 46.3) for the placebo versus 14.4 g GOS / day condition.

6 The mean difference relative to GOS intake level and its standard error was calculated. Appendix Section 2.6 outlines the data and formulae used.

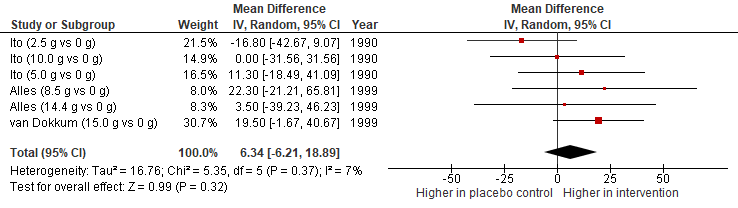
7 Reflects statistical (non-)significance of the difference in stool weight between placebo and intervention group at follow-up (Ito el al. 1990 and van Dokkum et al. 1999) or statistical (non-)significance of the difference between placebo and intervention group, over time (Alles et al. 1999). Exact *P* values are not provided for Ito el al. (1990), van Dokkum et al. (1999), and Alles et al. (1999).

8 Alles et al. (1999) state that non-placebo participants were provided an intake level of GOS aimed to be 7.5 g/day or 15.0 g/day. They later state that the low and high intake diet provided a mean of 8.5 g/day and 14.4 g/day, respectively.

The control and assessment of dietary intake is relevant to the validity of all three physiological outcomes. Morel et al. (2015) found that appetite and food intake decreased with α-GOS intake. Further, Ito et al. (1990) report that their decision to use 10 g/day as their maximum GOS intake was based on preliminary, unpublished trials that found a single dose of 15 g/day led to extreme fullness. With respect to laxation, if food intake decreases, stool weight or frequency may decrease. Of the three studies assessed, two included high intakes (15.0 g/day and 14.4 g/day were used by van Dokkum et al. 1999 and Alles et al. 1999, respectively). Participants’ diets were either controlled and reported to be not statistically different between conditions (Alles et al. 1999), controlled and not measured (van Dokkum et al. 1999), or minimally controlled and not measured (Ito et al. 1990). Although van Dokkum et al. (1999) did not assess diet, meals and snacks were provided to control the diet, and body weight, which can be used as a proxy for diet stability, did not change over time. There is no evidence to suggest that high intake levels of GOS with the potential effect of decreasing food intake, has affected the validity of the current studies’ stool weight and frequency results.

The differences in relative stool weight between intervention and control groups differ within and between the three studies. The largest difference was a lower relative stool weight, -6.7 ± 5.3 g (mean ± SE) indicating GOS intake may promote constipation. When using a >5 g/g benchmark as a proxy for a laxative effect, the mean differences in relative stool weight indicate a worsened laxation (one from six comparisons), and no effect on laxation (five from six comparisons). When using a >1 g/g benchmark, the mean differences in relative stool weight indicate a promotion of laxation (three from six comparisons), worsened laxation (one from six comparisons), and no effect (two from six comparisons). In one comparison, converted data from van Dokkum et al. (1999) found that a 15.0 g/day intake did meet a benchmark of >1 g/g. Based on the findings of Ito et al. (1990), a benchmark was not met when GOS were provided at a low intake level (2.5 and 5.0 g/day; in fact, the lowest intake level produced an entirely different direction of effect, with a 6.7 g/g lower relative stool weight, indicating GOS intake may promote constipation) and was met at a moderate intake level (10.0 g/day). The reverse was true for Alles et al. (1999); results demonstrate the moderate intake (8.5 g GOS/day) met a benchmark of >1 g/g, but the high intake (14.4 g/day) did not. The two studies that tested more than one intake level (Ito et al. 1990 and Alles et al. 1999) provide no evidence of a causal relationship between GOS intake and increased stool weight; the size and direction of effect vary substantially at different intake levels. Next, wide variances undermine the accuracy of the mean differences and reduce the representativeness of these samples’ results to that of the true population. The standard errors for all mean differences (ranging from 0.7 g to 5.3 g) are much larger than pectin’s, which this assessment’s low benchmark (>1 g/g) is based on: the standard error of the mean difference for pectin is 0.3 g (Cummings 2001). A >1 g/g benchmark is reached in half the comparisons and only when using the point estimate of the mean difference between the two groups, but never when the variance is considered. To illustrate, van Dokkum et al. (1999) used the highest intake level of the three studies: 15.0 g GOS per day. At this intake level, the point estimate of the mean difference between the two groups is 1.3 g/day per gram of GOS, however, the variance (SE is 0.7 g/day per GOS g) demonstrates that the true mean difference could be substantially lower; as low as 0.6 g/day per GOS g, which is lower than a benchmark of >1 g/g. The variances of Ito et al. (1990) and Alles et al. (1999) are at least twice the size, which diminishes the precision of their point estimates to a much larger extent than the aforementioned results of van Dokkum et al. (1999). Future research using larger sample sizes should reduce the variance.

The size and direction of effect of GOS intake in absolute stool weight also varies substantially at different intake levels, as we saw for relative stool weight. No statistically significant effects in absolute stool weight are reported by all three studies (Ito et al. 1990, van Dokkum et al. 1999, and Alles et al. 1999; Table 2). In comparison to 0 g, an intake between 2.5 and 15.0 g/day has an overall mean (95% confidence interval, CI) effect in stool weight of 6.34 g (-6.21, 18.89, *P*=0.32; Figure 1). The confidence intervals of the weighted pooled estimate and all intervention effect estimates cross the line of no effect. There is no evidence of a difference in stool weight between intervention and control groups. Heterogeneity was very low, meaning that any variability between interventions’ effects is largely due to random variation (chance), not clinical or methodological diversity (I2=7%).



**Figure 1: Galacto-oligosaccharide intake has no overall effect in stool weight.**

On the presumption that any relationship between GOS intake and stool weight is dose-dependent, we expect lower and more realistic total GOS intake levels between 0 and 5 g/day to produce smaller effect sizes. A meta-regression or sub-group analysis to determine the impact of GOS intake level or study quality in stool weight was not conducted due to the small number of studies and intake levels. In lieu of this, we refer to our narrative review, detailed in Appendix Section 2.2.2. In summary, our review concludes that greater weight should be given to the data from Alles et al. (1999) as their study design produced a stronger level of evidence. Three conditions were used to test the effect of three intake levels of GOS (0.0, 8.5 and 14.4 g/day) in a parallel, placebo-controlled study. The duration of the intervention period was three weeks, participants’ diets were well controlled, and a three week ‘run-in’ period was incorporated prior to the intervention period. Alles et al. (1999) demonstrated mixed results when assessing stool weight expressed in absolute and relative terms. As already stated, both conditions did not met a benchmark of >5 g/g, and one but not both conditions met a benchmark threshold of >1 g/g and only when using the point estimate of the mean difference (2.6 g) but not when considering the variance (SE is 2.6 g) which is reason to interpret the mean difference with caution. The variance is large (2.6 g) with respect to the size of the point estimate (also 2.6 g) and in comparison to that of pectin (0.3 g). It is almost nine-fold larger than that of pectin (SE of 2.6 g versus 0.3 g; Cummings 2001), which this assessment’s low benchmark (>1 g/g) is based on. Further, since the mean differences in relative stool weight vary so substantially (2.6 g versus 0.2 g; more than a ten-fold difference) and decrease with increasing intake, the results of Alles et al. (1999) do not support a positive dose-response relationship. This reduces the likelihood of a causal relationship of GOS intake on stool weight.

Additional quantitative measures of laxation include self-reported bowel movement or stool frequency, and intestinal transit time, which were measured in seven studies. No effect on self-reported bowel movement or stool frequency was reported by five of six studies (Ito et al. 1990, Alles et al. 1999, Davis et al. 2010, Piirainen et al. 2008, and Whisner et al. 2013). The sixth study’s results suggested defecation frequency was higher during the GOS study period than the control period, however, the relationship was not statistically analysed (Teuri & Korpela 1998). No effect on intestinal transit time, using radio-opaque pellets swallowed, was reported by one of one study (van Dokkum et al. 1999). Not included in the above seven studies is Canfora et al. (2017) who reported no change in stool frequency. This, however, appears to be incorporated in their reporting of adverse events rather than an outcome of interest. Further appraisal of these studies’ methodology and results was not considered necessary, as described in Appendix Section 2.2.1.

The US Food and Drug Administration reviewed evidence of the effects of isolated and synthetically produced non-digestible carbohydrate intake (FDA 2016). Their summaries of three studies (Ito et al. 1990, Walton et al. 2012, and Davis et al. 2010) report that GOS intake did not have a laxative effect, as assessed by the number of bowel movements and stool frequency. At the request of the UK Food Standards Agency, the Scientific Advisory Committee on Nutrition evaluated the effect of potential dietary fibre components on health outcomes (SACN 2008). This incorporated an assessment of the effect of oligosaccharide intake on colonic function, as determined by faecal output. Their review included one study testing the effect of GOS (Alles et al. 1999). They concluded that oligosaccharide intake had very little effect on faceal weight, meaning oligosaccharides could not be considered a dietary fibre when using faceal weight as a criterion.

In summary, the evidence demonstrates:

* The body of evidence as a whole does not demonstrate that GOS intake promotes laxation. Absolute and relative stool weight were evaluated using three well designed studies with small sample sizes and industry support. A wide range of intake levels was evaluated, including very high intakes which provide ample opportunity for a causal effect to be seen if one exists.
* No overall effect of GOS intake on absolute stool weight based on individual studies and pooled data.
* No overall effect of GOS intake on relative stool weight.
  + None of the point estimates of the mean differences from six comparisons of three studies, meet a threshold of >5 g/g.
  + Only half the point estimates, of the mean differences from six comparisons of three studies, meet a threshold of >1 g/g. The relative stool weights from all six comparisons do not meet the threshold when considering their variances, all of which include weights below this threshold.
  + The mean difference in relative stool weight varies considerably in direction and size across the six comparisons and does not consistently meet a >1 g/g threshold. This suggests an absence of a positive dose-response relationship. Further, the largest difference was a lower relative stool weight, -6.7 g ± 5.3 g (mean ± SE).
  + The mean differences in relative stool weight, averaged with equal weighting across the six comparisons, is -1.5 g/g. This may indicate GOS intake may have a small effect towards constipation.
* Limitations with the use of stool weight and a >1 g/g benchmark as intermediary outcomes for ‘healthy’ laxation. Different types of fibre have different magnitudes of effect on stool weight. One benchmark that we used, >1 g/g, is lower than that of the least effective source of fibre, pectin, which is associated with a 1.2 ± 0.3 g (mean ± SE) increase in stool weight per gram of fibre (Cummings 2001). At best, this benchmark represents the minimum threshold required to demonstrate a laxative effect. Further, we have not assessed the evidence to determine the nature of the relationship between fibre and stool weight across a range of intake levels. This could improve the threshold and units of a benchmark for relative stool weight. Last, an increased stool weight does not guarantee improved laxation, as fluid ingestion also has an effect.
* No effect of GOS intake on bowel movement or stool frequency, nor on intestinal transit time.

We conclude that, when taken as a whole, the body of evidence does not convincingly demonstrate that GOS intake promotes laxation. Based on current evidence, added GOS intake has no effect on laxation.

# 1.3 Reduction in blood cholesterol

## 1.3.1 Assessment: results

Five studies are reviewed here to evaluate the relationship between added GOS intake, and outcomes related to blood cholesterol. Participants’ diets were either controlled (van Dokkum et al. 1999), measured and reported to be unchanged (Vulevic et al. 2013, Pedersen et al. 2016, and Canfora et al. 2017), or not measured (Vulevic et al. 2008). In the intervention conditions, participants consumed a GOS intake of 2.6 g/day (Vulevic et al. 2008, Vulevic et al. 2013, and Pedersen et al. 2016) or 15 g/day (van Dokkum et al. 1999, and Canfora et al. 2017). The intervention conditions’ duration ranged from three weeks (van Dokkum et al. 1999) to 10 weeks (Vulevic et al. 2008) and 12 weeks (Vulevic et al. 2013, Pedersen et al. 2016, and Canfora et al. 2017). This duration provides an adequate opportunity for blood lipid concentrations to respond to the treatment. We report the methodology of our review and the included studies in Appendices Sections 2.3 and 2.6. We note that in one study a participant withdrew after three weeks due to “tolerance problems” which is not further described (Vulevic et al. 2008). Pedersen et al. (2016) also reported one participant withdrew due to gastrointestinal upset, however, the severity of symptoms and possible cause are also not specified.

Selected outcomes, based on their relevance to the beneficial physiological effect listed in the Code’s definition of dietary fibre (i.e. “reduction in blood cholesterol”) and assessed by at least two studies, are presented in Table 3. Thus, we are primarily interested in assessing whether GOS intake reduces total and LDL-cholesterol concentration. We also assessed related benefits: increases to HDL-cholesterol concentrations; and, reductions to triglyceride concentrations which, while not a blood cholesterol, is a blood lipid marker relevant to dyslipidaemia and cardiovascular disease. A secondary reason to focus on total and LDL-cholesterol reduction is that, in Australia, dyslipidaemia is more commonly diagnosed on the basis of high total or LDL-cholesterol, rather than high triglycerides, low HDL-cholesterol, or lipid-modifying medication use with normal lipid levels. The most recent national, measured data was collected in 2011-12; 63% of Australians aged 18 and over had dyslipidaemia, one in three (33%) had high fasting total cholesterol (≥5.5 mmol/L), and one in three (33%) had high fasting LDL-cholesterol (≥3.5 mmol/L; ABS 2013). No improvements in the four blood lipid measures (total, LDL-, and HDL-cholesterol, and triglycerides) were found by four of five studies (van Dokkum et al. 1999, Vulevic et al. 2008, Pedersen et al. 2016, or Canfora et al. 2017). The remaining study by Vulevic et al. (2013) found half the blood lipid outcomes were unchanged and half improved (a small, statistically significant reduction in total cholesterol and triglyceride concentration; see Table 3). Vulevic et al. (2013) also found a fifth outcome, total cholesterol:HDL-cholesterol, was lower (improved) in the GOS condition at the end of the 12 week study period (data not shown in Table 3; see Appendix Section 2.3.2). Participants in the latter study were aged 18 to 65 years, had a body mass index >25 kg/m2, and had ≥ three metabolic syndrome risk factors. These risk factors included: fasting glucose >5.6 mmol/L; high blood pressure (not defined by authors); dyslipidaemia (HDL-cholesterol <1 mmol/L, triglyceride >1.3 mmol/L); and, waist circumference (>94 cm in men, >80 cm in women). Participants also met extensive exclusion criteria including but not limited to: myocardial infarction/stroke or cancer in the previous 12 months; diagnosed diabetes, fasting glucose >7 mmol/L, or other endocrine disorders; chronic coronary disorders, cholestatic liver, or pancreatitis; receiving medication for hyperlipidaemia, hypertension, inflammation, or hypercoagulation; trying or intending to complete a weight loss regime; and, smoking or a history of alcohol or drug abuse. Further criteria are listed in Appendix Section 2.3.2. The lipid outcomes of Vulevic et al. (2013) are mixed and, when taken in context of the other four studies, may indicate that the beneficial effect of GOS intake on total cholesterol and triglycerides could be limited to those with dyslipidaemia. However, we question the *P* values of Vulevic et al. (2013) for the difference in total cholesterol and triglyceride levels between groups at follow-up (i.e. end of the 12-week condition). We sought clarification from the corresponding author on some of these statistically different results, but did not receive a response. First, the size of the mean differences and standard deviations relative to means, suggests that a significant difference is unlikely. Second, we have assumed that all plasma samples were taken from fasted participants at all time points. This, however, is not clearly stated; fasting status is recommended for triglycerides in order to detect potentially small changes, because triglyceride levels can be influenced by a high fat meal intake prior to, and on the day of, blood collection (Nordestgaard et al. 2016). Third, the total cholesterol results conflict with a similarly designed study. In contrast, the earlier and similarly designed study by Vulevic et al. (2008) demonstrated no difference in total cholesterol between the intervention and control groups over time (*P*>0.05; triglycerides were not measured). The main difference between these two studies are the samples’ characteristics. In comparison to Vulevic et al. (2013), the earlier study’s sample: was not recruited on the basis of metabolic syndrome risk factors; were assessed as in “good health”; had a ~1.3 mmol/L lower total cholesterol (mean ± SD: ~5.0 ± ~0.9 mmol/L); were older (mean ± SD: 69.3 ± 4.0 years; range: 64–79 years); had a lower body mass index (range: 22–31 kg/m2); and, had almost the same male:female (16:28). This supports the hypothesis that the results of Vulevic et al. (2013) may not be widely generalisable.

**Table 3: The association between galacto-oligosaccharide intake and blood total, LDL- and HDL-cholesterol, and triglycerides.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First author (year)** | **GOS intake level (g/day)** | **Total cholesterol (mmol/L)** | | | | **LDL-cholesterol (mmol/L)** | | | | **HDL-cholesterol (mmol/L)** | | | | **Triglycerides (mmol/L)1** | | | |
| **Baseline (mean ± SD or SE)2** | **Follow-up (mean ± SD or SE2)3** | **Mean difference4 ± SE5** | ***P*6** | **Baseline (mean ± SD or SE)2** | **Follow-up (mean ± SD or SE2)3** | **Mean difference4 ± SE5** | ***P*6** | **Baseline (mean ± SD or SE)2** | **Follow-up (mean ± SD or SE2)3** | **Mean difference4 ± SE5** | ***P*6** | **Baseline (mean ± SD or SE)2** | **Follow-up (mean ± SD or SE2)3** | **Mean difference4 ± SE5** | ***P*6** |
| van Dokkum (1999) | 0.0 | n/a | 4.56 ± 0.62 | n/a | n/a | n/a | 2.82 ± 0.51 | n/a | n/a | n/a | 1.14 ± 0.22 | n/a | n/a | n/a | 1.40 ± 0.68 | n/a | n/a |
| 15.0 | n/a | 4.58 ± 0.78 | 0.02 ± 0.14 | NSD | n/a | 2.87 ± 0.67 | 0.05 ± 0.12 | NSD | n/a | 1.11 ± 0.20 | -0.03 ± 0.04 | NSD | n/a | 1.46 ± 0.66 | 0.06 ± 0.12 | NSD |
| Vulevic (2008) | 0.0 | 4.94 ± 0.88 | 4.97 ± 0.977 | n/a | n/a | n/a | n/a | n/a | n/a | 1.29 ± 0.32 | 1.29 ± 0.317 | n/a | n/a | n/a | n/a | n/a | n/a |
| 2.6 | 5.02 ± 0.93 | 5.07 ± 0.997 | 0.02 ± 0.137 | NSD | n/a | n/a | n/a | n/a | 1.31 ± 0.27 | 1.28 ± 0.277 | -0.03 ± 0.047 | NSD | n/a | n/a | n/a | n/a |
| Vulevic (2013) | 0.0 | 6.2 ± 1.3 | 6.2 ± 1.2 | n/a | n/a | 4.2 ± 1.1 | 4.3 ± 1.0 | n/a | n/a | 1.4 ± 0.4 | 1.4 ± 0.4 | n/a | n/a | 1.6 ± 0.7 | 1.6 ± 0.7 | n/a | n/a |
| 2.6 | 6.3 ± 1.3 | 5.9 ± 1.1 | -0.4 ± 0.177 | < 0.0018,9 | 4.2 ± 1.1 | 4.1 ± 1.0 | -0.2 ± 0.147 | NSD | 1.4 ± 0.3 | 1.4 ± 0.4 | 0.0 ± 0.057 | NSD | 1.6 ± 0.8 | 1.5 ± 0.6 | -0.1 ± 0.107 | < 0.0058,10 |
| Pedersen (2016) | 0.0 | 3.44 ± 0.26 | 3.65 ± 0.21 | n/a | n/a | 2.02 ± 0.20 | 2.11 ± 0.17 | n/a | n/a | 1.00 ± 0.07 | 1.09 ± 0.07 | n/a | n/a | 0.91 ± 0.11 | 0.97 ± 0.09 | n/a | n/a |
| 2.6 | 3.40 ± 0.20 | 3.33 ± 0.16 | -0.28 ± 0.20 | 0.068 | 1.91 ± 0.18 | 1.77 ± 0.16 | -0.23 ± 0.16 | 0.051 | 1.03 ± 0.08 | 1.09 ± 0.09 | -0.03 ± 0.07 | 0.798 | 1.03 ± 0.10 | 1.02 ± 0.13 | -0.07 ± 0.10 | 0.534 |
| Canfora (2017) | 0.0 | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | 1.17 ± 0.45 | 1.31 ± 0.51 | n/a | n/a |
| 15.0 | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | 1.28 ± 0.42 | 1.50 ± 0.59 | 0.08 ± 0.10 | 0.54 |

GOS, galacto-oligosaccharide; g, gram; L, litre; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; n/a, not applicable or not available; SD, standard deviation; SE, standard error of the mean; NSD, not significantly different (*P*>0.058).

1 Fasting status (triglycerides): fasting (van Dokkum et al. 1999, Canfora et al. 2017, and Pedersen et al. 2016), and assumed to be fasting (Vulevic et al. 2013).

2 SD for van Dokkum et al. (1999), Vulevic et al. (2008), Vulevic et al. (2013), and Canfora et al. (2017). SE for Pedersen et al. (2016).

3 Represents blood lipid values at end of the intervention period for the crossover studies (van Dokkum et al. 1999, Vulevic et al. 2008, and Vulevic et al. 2013) and parallel randomised controlled trial (Pedersen et al. 2016, and Canfora et al. 2017).

4 Calculated mean difference = mean (intervention follow-up) – mean (placebo follow-up) for one crossover study (van Dokkum et al. 1999). Calculated mean difference = (mean (intervention follow-up) – mean (intervention baseline)) – (mean (placebo follow-up) – mean (placebo baseline)) for two crossover studies (Vulevic et al. 2008 and Vulevic et al. 2013) and two parallel randomised controlled trials (Pedersen et al. 2016 and Canfora et al. 2017).

5 All SEs were calculated. Appendix Section 2.6 outlines the data and formulae used to calculate the SE.

6 Reflects statistical (non-)significance of the difference between placebo and intervention group at follow-up (van Dokkum et al. 1999, Vulevic et al. 2008, and Vulevic et al. 2013) or statistical (non-)significance of the difference in changes (from baseline to follow-up) between groups, using a 2-way repeated-measures analysis of variance (Canfora et al. 2017) or with baseline values as covariate (ANCOVA; Pedersen et al. 2016). Exact *P* values are not provided for van Dokkum et al. (1999), Vulevic et al. (2008), and Vulevic et al. (2013).

7 These values represent the follow-up time points at week 10 and 12 for Vulevic et al. (2008) and Vulevic et al. (2013), respectively.

8 ForVulevic et al. (2013), *P* values were corrected for multiple testing and significance was set at *P*<0.005 after Bonferroni adjustment.

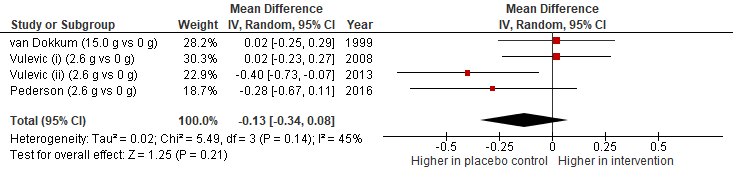
9 Two *P* values are provided by Vulevic et al. (2013): *P*<0.0001 (tabulated); and, *P*<0.001 (in text).

10 Two *P* values are provided by Vulevic et al. (2013): *P*<0.005 (tabulated); and, *P*<0.0005 (in text).

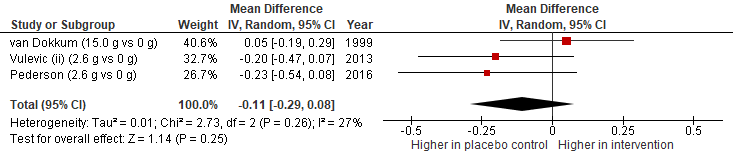
Meta-analyses were conducted for each lipid outcome. There is no evidence of a difference in our primary outcomes (total and LDL-cholesterol concentration) and secondary outcomes (HDL-cholesterol and triglyceride) between intervention and control groups. In comparison to 0 g/day, an intake of 2.6 or 15.0 g/day of added, synthetically produced GOS has an overall mean (95% CI) effect on:

* Total cholesterol of -0.13 mmol/L (-0.34, 0.08, *P*=0.21; Figure 2)
* LDL-cholesterol of -0.11 mmol/L (-0.29, 0.08, *P*=0.25; Figure 3)
* HDL-cholesterol of -0.02 mmol/L (-0.07, 0.02, *P*=0.31; Figure 4)
* Fasting triglyceride of -0.01 mmol/L (-0.12, 0.09, *P*=0.80; Figure 5)

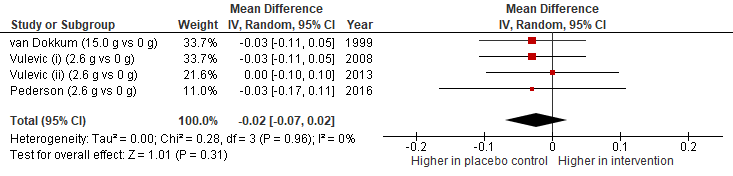
The confidence intervals of all weighted pooled estimates and all intervention effect estimates, except one (Vulevic et al. 2013 for total cholesterol), cross the line of no effect. Heterogeneity between interventions varied by outcome: none for HDL-cholesterol and triglyceride (I2=0%); low for LDL-cholesterol (I2=27%); and, moderate for total cholesterol (I2=45%). For LDL- and total cholesterol, the differences in observed intervention effects cannot be explained by random variation alone. Chance accounts for the variability between interventions’ effects for HDL-cholesterol and triglyceride.



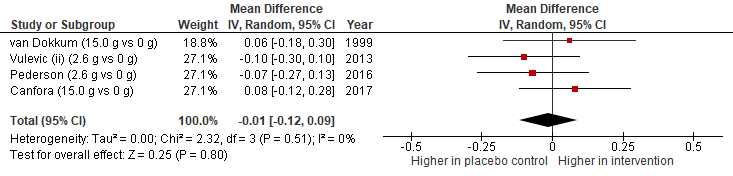
**Figure 2: Galacto-oligosaccharide intake has no overall effect on total cholesterol.**



**Figure 3: Galacto-oligosaccharide intake has no overall effect on LDL-cholesterol.**



**Figure 4: Galacto-oligosaccharide intake has no overall effect on HDL-cholesterol.**

**Figure 5: Galacto-oligosaccharide intake has no overall effect on fasting triglyceride1.**

1Fasting status: fasting (van Dokkum et al. 1999, Canfora et al. 2017, and Pedersen et al. 2016), and assumed to be fasting (Vulevic et al. 2013).

Blood cholesterol related outcomes were evaluated using five well designed studies with small sample sizes and industry support. We conclude that, when taken as a whole, the body of evidence does not convincingly demonstrate that GOS intake promotes a reduction in blood cholesterol (total or LDL-cholesterol) or a beneficial effect on other lipids. Based on current evidence, added GOS intake has no effect on blood cholesterol (total or LDL-cholesterol) or other lipids.

The US Food and Drug Administration (FDA 2016) reviewed two studies with respect to blood cholesterol (Vulevic et al. 2008 and Vulevic et al. 2013) with summaries aligning with ours.

# 1.4 Modulation of blood glucose

## 1.4.1 Assessment: results

Four studies are reviewed here to evaluate the relationship between added GOS intake and outcomes related to blood glucose. Participants’ diets were reported to be controlled (van Dokkum et al. 1999) or measured and unchanged (Canfora et al. 2017, Vulevic et al. 2013, and Pedersen et al. 2016). In the intervention conditions, participants’ intake of GOS was 2.6 g/day for 12 weeks (Vulevic et al. 2013, and Pedersen et al. 2016), 15 g/day for 12 weeks (Canfora et al. 2017), or 15 g/day for three weeks (van Dokkum et al. 1999). The intervention conditions’ duration provides sufficient opportunity for blood glucose- and insulin-related outcomes to respond to the treatment, including glycosylated haemoglobin which was reported by one 12 week study (Pedersen et al. 2016). None of the glucose or insulin related outcomes differed between groups at baseline, in three from four studies (Canfora et al. 2017, Vulevic et al. 2013, and Pedersen et al. 2016). The remaining study by van Dokkum et al. (1999) does not report baseline concentrations, however, state that these participants had “normal health” as assessed using clinical laboratory tests and vital signs. Three out of four studies’ participants had either a minimum or mean body mass index of >25 kg/m2 and required participants to have a marker of abnormal glucose metabolism, such as type 2 diabetes mellitus (well-controlled; total diabetes medication use: n=23 from 29; metformin use: n=20 from 29), at least three metabolic syndrome risk factors, or prediabetes (Canfora et al. 2017, Vulevic et al. 2013, and Pedersen et al. 2016). In contrast, the remaining study used a more homogenous sample of young adult males (mean age: 23 years), with a mean body mass index of approximately 23 kg/m2, of “normal health”, and with lower blood glucose and insulin levels than the other studies’ samples (Table 4; van Dokkum et al. 1999). We note that one participant withdrew due to gastrointestinal upset (Pedersen et al. 2016), however, the severity of symptoms and cause are not specified. We report the methodology of our review and the included studies in Appendices Sections 2.4 and 2.6.

We are primarily interested in assessing whether GOS intake modulates blood glucose, glycosylated haemoglobin, and postprandial glycaemic response, due to their relevance to the beneficial physiological effect listed in the Code’s definition of dietary fibre (i.e. “modulation of blood glucose”). Baseline and follow-up data were reported by: four studies for blood glucose (mainly fasting; see Table 4); one study for glycosylated haemoglobin (Pedersen et al. 2016; see Table 4); and, one study each for the postprandial glycaemic response to an insulin-modified intravenous glucose tolerance test (Pedersen et al. 2016; see Appendix Section 2.4.2) or oral glucose tolerance test (van Dokkum et al. 1999; see Appendix Section 2.4.2). No statistically significant differences between the intervention and control conditions were reported for any of these primary outcomes, at the individual study level. One study reported an increased fasting glucose in the intervention condition only (*P*<0.05) and small increases in glycosylated haemoglobin in both the intervention and control group (*P*>0.05) for within-group change over the 12 week trial (Pedersen et al. 2016). We conducted a meta-analysis for blood glucose (discussed below), because this outcome was assessed by at least two studies.

We also assessed modulations to related, secondary outcomes including: insulin (reported by four studies; see Table 4); homeostatic model assessment of insulin resistance (HOMA-IR, reported by two studies; see Table 4); postprandial insulin response to an insulin-modified intravenous glucose tolerance test (Pedersen et al. 2016) or oral glucose tolerance test (van Dokkum et al. 1999); peripheral insulin sensitivity and adipose tissue insulin sensitivity (Canfora et al. 2017); and, HOMA for insulin sensitivity and HOMA for β-cell function (Pedersen et al. 2016). As insulin and HOMA-IR were assessed by at least two studies, these results are included in Table 4 and are further assessed by meta-analyses (discussed below). The results of other secondary outcomes are detailed in Appendix Section 2.4.2. No statistically significant differences between the intervention and control conditions were reported for any of the secondary outcomes. One exception was for insulin, where one out of four studies did report a difference (Vulevic et al. 2013; see Table 4). It is not stated, however, if Vulevic et al. (2013) measured insulin in a fasting state at all time points, unlike the other three studies that reported fasting insulin with no statistically significant differences (van Dokkum et al. 1999, Pedersen et al. 2016, and Canfora et al. 2017; see Table 4). One study reported an increased postprandial insulin (incremental AUC) response to an insulin-modified intravenous glucose tolerance test in the intervention condition only, over the 12 week trial (*P*<0.05; Pedersen et al. 2016).

**Table 4:** **The association between galacto-oligosaccharide intake and blood glucose and insulin outcomes.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First author (year)** | **GOS intake level (g/day)** | **Glucose (mmol/L)1** | | | | **HbA1c (%)** | | | | **Insulin (mU/L or pmol/L)1,11** | | | | **HOMA-IR2** | | | |
| **Baseline (mean ± SD or SE3)** | **Follow-up (mean ± SD or SE3)4** | **Mean difference5 ± SE6** | ***P*7** | **Baseline (mean ± SD or SE3)** | **Follow-up (mean ± SD or SE3)4** | **Mean difference5 ± SE6** | ***P*7** | **Baseline (mean ± SD or SE3)** | **Follow-up (mean ± SD or SE3)4** | **Mean difference5 ± SE6** | ***P*7** | **Baseline (mean ± SD3)** | **Follow-up (mean ± SD3)4** | **Mean difference5 ± SE6** | ***P*7** |
| van Dokkum (1999) | 0.0 | n/a | 4.8 ± 0.212 | n/a | n/a | n/a | n/a | n/a | n/a | n/a | 10.6 ± 4.312 | n/a | n/a | n/a | n/a | n/a | n/a |
| 15.0 | n/a | 4.7 ± 0.212 | -0.1 ± 0.05 | NSD | n/a | n/a | n/a | n/a | n/a | 9.4 ± 3.612 | -1.2 ± 1.0411 | NSD | n/a | n/a | n/a | n/a |
| Vulevic (2013) | 0.0 | 5.2 ± 0.9 | 5.6 ± 0.8 | n/a | n/a | n/a | n/a | n/a | n/a | 64.8 ± 30.6 | 70.1 ± 36.8 | n/a | n/a | n/a | n/a | n/a | n/a |
| 2.6 | 5.4 ± 0.6 | 5.6 ± 0.7 | -0.2 ± 0.1 | NSD | n/a | n/a | n/a | n/a | 67.3 ± 30.9 | 58.1 ± 29.7 | -14.5 ± 6.1 | <0.0058 | n/a | n/a | n/a | n/a |
| Pedersen (2016) | 0.0 | 6.2 ± 0.3 | 6.5 ± 0.3 | n/a | n/a | 6.4 ± 0.2 | 6.6 ± 0.2 | n/a | n/a | 94.6 ± 15.3 | 83.0 ± 13.0 | n/a | n/a | 1.9 ± 1.3 | 1.8 ± 1.0 | n/a | n/a |
| 2.6 | 6.1 ± 0.4 | 6.810 ± 0.4 | 0.4 ± 0.4 | 0.227 | 6.8 ± 0.3 | 7.0 ± 0.3 | 0.0 ± 0.3 | n/a | 83.5 ± 14.7 | 94.0 ± 18.7 | 22.1 ± 20.0 | 0.543 | 1.6 ± 1.0 | 1.8 ± 1.3 | 0.3 ± 0.4 | 0.199 |
| Canfora (2017) | 0.0 | 5.8 ±  0.4 | 5.8 ±  0.5 | n/a | n/a | 5.6 ± 0.4 | n/a | n/a | n/a | 19.1 ± 17.39 | 18.3 ± 10.1 | n/a | n/a | 5.1 ± 2.412 | 4.7 ± 2.812 | n/a | n/a |
| 15.0 | 6.0 ± 0.5 | 6.0 ± 0.5 | 0.0 ± 0.1 | 0.79 | 5.6 ± 0.3 | n/a | n/a | n/a | 20.7 ± 8.89 | 18.9 ± 8.3 | -1.0 ± 3.311 | 0.71 | 5.2 ± 2.612 | 5.3 ± 3.412 | 0.5 ± 0.8 | 0.598 |

GOS, galacto-oligosaccharide; g, gram; L, litre; n/a, not applicable or not available; SD, standard deviation; SE, standard error of the mean; NSD, not significantly different (*P*>0.058); HbA1c, glycosylated haemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance.

1 Fasting status (glucose and insulin): fasting (van Dokkum et al. 1999, Canfora et al. 2017, and Pedersen et al. 2016), and assumed to be fasting (Vulevic et al. 2013).

2 Units of measurement are not stated for Canfora et al. (2017) and are ‘%’ for Pedersen et al. (2016).

3 SD for van Dokkum et al. 1999, Vulevic et al. 2013, and Canfora et al. 2017, for all outcomes. Pedersen et al. (2016) uses SE for glucose, insulin, and HbA1c outcomes. Pedersen et al. (2016) provides medians and interquartile ranges (IQR) for HOMA-IR, from which we estimated means and SDs (means and SDs are presented in Table 4) as described in Appendix Section 2.6. The medians (IQR) published by Pedersen et al. (2016) for HOMA-IR are: 1.88 (1.15–2.77) for placebo baseline; 1.58 (1.27–2.56) for placebo follow-up; 1.60 (1.03–2.18) for intervention baseline; and, 1.7 (1.08–2.68) for intervention follow-up.

4 Represents values at end of the intervention period for all outcomes and studies.

5 Calculated mean difference = mean (intervention follow-up) – mean (placebo follow-up) for one crossover study (van Dokkum et al. 1999). Calculated mean difference = (mean (intervention follow-up) – mean (intervention baseline)) – (mean (placebo follow-up) – mean (placebo baseline)) for one crossover study (Vulevic et al. 2013) and two parallel randomised controlled trials (Canfora et al. 2017, and Pedersen et al. 2016).

6 All SEs were calculated. Appendix Section 2.6 outlines the data and formulae used to calculate the SE.

7 Reflects statistical (non-)significance of the difference between placebo and intervention group at follow-up (van Dokkum et al. 1999, and Vulevic et al. 2013) or statistical (non-)significance of the difference in changes (from baseline to follow-up) between groups, using a 2-way repeated-measures analysis of variance (Canfora et al. 2017) or with baseline values as covariate (ANCOVA; Pedersen et al. 2016). Exact *P* values are not provided for van Dokkum et al. (1999), and Vulevic et al. (2013).

8 Note that *P* values were corrected for multiple testing and significance was set at *P*<0.005 after Bonferroni adjustment.

9 These pre-intervention values, extracted from Table 3 (Canfora et al. 2017), differ to the baseline values presented in Table 1 of Canfora et al. (2017) (19.1 ± 7.2 and 20.7 ± 6.7 for the placebo and intervention, respectively). It is likely that data presented in Table 1 of Canfora et al. (2017) represent results from the screening phase.

10 Significant within-group change (*P*<0.05).

11 Units of measurement for insulin concentrations are mU/L (conventional units; van Dokkum et al. 1999, and Canfora et al. 2017) and pmol/L (Système International units; Vulevic et al. 2013, and Pedersen et al. 2016). The difference in insulin concentrations (between groups at follow-up for van Dokkum et al. 1999 or between groups over time for Canfora et al. 2017) can be converted to ‘pmol/L’ units using the conversion factor: 1 µIU/mL = 6.00 pmol/L (Knopp et al. 2019). The subsequent differences (mean ± SE) are: -7.2 ± 6.2 pmol/L and -6.0 ± 20.0 pmol/L for van Dokkum et al. (1999) and Canfora et al. (2017), respectively. The latter ‘pmol/L’ values have been used in our meta-analysis.

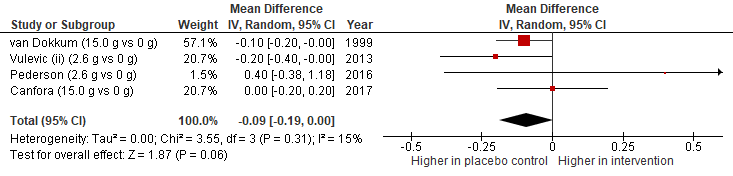
12 Data have been estimated by extracting numerical data from published images (figures) using an online tool, ‘WebPlotDigitizer’. The baseline HOMA-IR values reported by Canfora et al. (2017) in Table 1 differ to the data extracted from Figure 2C. For the placebo condition, the baseline data was 5.10 ± 2.7 (Table 1) and 5.1 ± 2.4 (Figure 2C). For the intervention condition, the baseline data was 5.34 ± 2.7 (Table 1) and 5.2 ± 2.6 (Figure 2C). The difference may be due to either: (1) the tabulated ‘baseline’ data may represent data from the screening phase, whereas the data used for the figure may represent data from a separate pre-intervention data collection phase; and/or, (2) error associated with using the online tool to extract data from the figure. For consistency, the ‘difference’ in HOMA-IR (mean ± SE) between groups over time has been calculated using baseline and follow-up data extracted from Figure 2C.

Meta-analyses were conducted for outcomes assessed by two or more studies: the primary blood glucose outcome, and the secondary outcomes of blood insulin and HOMA-IR. In comparison to 0 g/day, an intake of 2.6 or 15.0 g/day of added, synthetically produced GOS has an overall mean (95% CI) effect on:

* Fasting glucose of -0.09 mmol/L (-0.19, 0.00, *P*=0.06; Figure 6)
* Fasting insulin of -8.92 pmol/L (-18.0, 0.15, *P*=0.05; Figure 7)
* HOMA-IR of 0.34 (-0.36, 1.04, *P*=0.34; Figure 8).

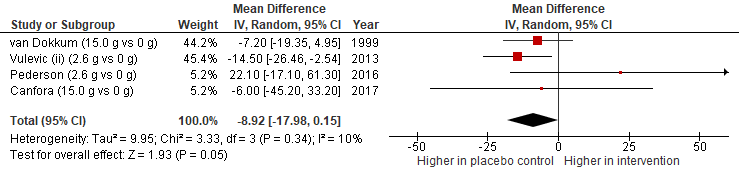
There is no evidence of a clinically meaningful difference in blood glucose or insulin concentration, or HOMA-IR between intervention and control groups. The difference in fasting glucose level is negligibly small; 0.09 mmol/L lower in the intervention condition compared to the placebo. This mean difference almost reached statistical significance (*P*=0.06) and the upper confidence interval was above the line of no effect. One of the seven secondary outcomes, insulin, also had a small difference of -8.92 pmol/L (equivalent to -1.49 mU/L), was close to statistical significance (*P*=0.05) and included the line of no effect. The confidence intervals of all weighted pooled estimates and all intervention effect estimates except one (Vulevic et al. 2013 for insulin) includes the line of no effect. Heterogeneity between interventions was none for HOMA-IR (I2=0%), and low for glucose (I2=15%) and insulin (I2=10%). The differences in observed intervention effects on insulin is more likely due to random variation rather than clinical or methodological diversity, or both, among the studies.

All meta-analyses include intake levels that are much higher than would be expected from the intake of naturally occurring, added, or total GOS. For example, the analysis for glucose has a 78% weighting towards the studies using a 15 g/day intake level (Figure 6). On the presumption that any relationship between GOS intake and these outcomes are dose-dependent, we expect lower and more realistic total GOS intake levels between 0 and 5 g/day to produce much smaller effect sizes. However, based on a visual inspection of Figures 6 to 8, there does not appear to be a dose-response relationship, which reduces the likelihood of a causal effect. The summary effect for glucose and insulin (Figures 6 and 7) are heavily influenced by the results of van Dokkum et al. (1999), which have a disproportionate weighting (57% and 44% for glucose and insulin, respectively) compared to the sample size entered into the meta-analysis for this study (13% for both outcomes). The total sample size entered into the meta-analyses for both blood glucose and insulin (Figures 6 and 7) is n=187. van Dokkum et al. (1999) accounts for 13% (n=24) of the total sample size entered, yet carries much more weight in the meta-analyses: 57% and 44% for blood glucose and insulin, respectively (Figures 6 and 7). This discrepancy is likely due to the narrower variance of van Dokkum et al. (1999; Table 4). The lower variance could be attributed to a more homogenous sample, as well as having lower blood glucose and insulin levels and no inclusion criteria related to abnormal glucose metabolism (see Section 1.4.1 p. 17) that may otherwise create greater within-study heterogeneity in glucose and insulin responses to an experimental condition. There is a possibility, therefore, that the effect of GOS may have a different effect on blood glucose-related outcomes depending on the sample characteristics; the extent or duration of abnormal glucose metabolism may potentially affect the size and/or variability of changes to blood glucose parameters. More studies and a subgroup analysis are required in order to assess this. Last, one of the studies (Vulevic et al. 2013) may not have used fasting status for blood glucose or insulin. We note this study had a 21% and 45% weighting in the meta-analyses for blood glucose or insulin, respectively (Figures 6 and 7).



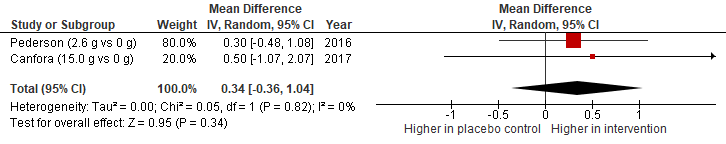
**Figure 6: Galacto-oligosaccharide intake has no overall effect on fasting glucose1.**

1Fasting status: fasting (van Dokkum et al. 1999, Canfora et al. 2017, and Pedersen et al. 2016), and assumed to be fasting (Vulevic et al. 2013).



**Figure 7: Galacto-oligosaccharide intake has no overall effect on fasting insulin1.**

1Fasting status: fasting (van Dokkum et al. 1999, Canfora et al. 2017, and Pedersen et al. 2016), and assumed to be fasting (Vulevic et al. 2013). Mean difference (95% CI) equates to -1.49 (-3.00, 0.025) mU/L, using the conversion factor: 1 µIU/mL = 6.00 pmol/L (Knopp et al. 2019).



**Figure 8: Galacto-oligosaccharide intake has no overall effect on HOMA-IR1.**

1Homestatic model assessment of insulin resistance.

Blood glucose related outcomes were evaluated using four well designed studies with small sample sizes and industry support. A wide range of intake levels was evaluated, including very high intakes which provide ample opportunity for a causal effect to be seen if one exists.

We conclude, when taken as a whole, the body of evidence does not convincingly demonstrate that GOS intake promotes a clinically meaningful modulation (reduction) in fasting blood glucose, glycosylated haemoglobin, or postprandial glycaemic response, nor a beneficial effect on secondary outcomes including fasting blood insulin and HOMA-IR and five insulin-related outcomes. Based on current evidence, added GOS intake has no clinically meaningful effect on fasting blood glucose (a primary outcome), or fasting blood insulin and HOMA-IR (secondary outcomes).

The US Food and Drug Administration (FDA 2016) reviewed one study with respect to blood glucose (Vulevic et al. 2013) with a summary aligning with ours.

# References

ABS (2013) Australian Health Survey: biomedical results for chronic diseases, 2011–12. ABS. cat. no. 4364.0.55.005. Canberra: Australian Bureau of Statistics.

Alles MS, Meyboom HS, Harryvan JL, Van Laere KM, Nagengast FM, Huatvast JG (1999) Effect of transgalactooligosaccharides on the composition of the human intestinal microflora and on putative risk markers for colon cancer. Am J Clin Nutr 69(5):980-91.

Borenstein M, Hedges LV, Higgins JPT, Rothstein HR (2009) Effect sizes based on means. Ch 4 In: Introduction to meta-analysis. 1st ed, John Wiley & Sons Ltd, West Sussex, pp. 21-32.

Bouhnik Y, Flourie B, D’Agay-Abensour L, Pochart P, ve Gramet G, le Durand M & Rambaud J-C (1997) Administration of transgalacto-oligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. J Nutr 127:444-8.

Canfora EE, van der Beek CM, Hermes GDA, Goossens GH, Jocken JWE, Holst JJ, van Eijk HM, Venema K, Smidt H, Zoetendal EG, Dejong CHC, Lenaerts K & Blaak EE (2017) Supplementation of diet with galacto-oligosaccharides increases bifidobacteria, but not insulin sensitivity, in obese prediabetic individuals. Gastoenterol 153:87-97.

Cummings JH (2001) The effect of dietary fibre on fecal weight and composition. Chapter 4.4 In: Spiller GA (ed) CRC Handbook of dietary fiber in human nutrition. 3rd ed, CRC Press, Florida, pp. 183-252.

Davis LMG, Martinez I, Walter J & Hutkins R (2010) A dose dependent impact of prebiotic galactooligosaccharides on the intestinal microbiota of healthy adults. Int J Food Micro 144:285-92.

Demonty I, Ras RT, van der Knaap HCM, Duchateau GSMJE, Meijer L, Zock PL, Geleijnse JM, Trautwein EA (2009) Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake. J Nutr 139(2):271–84.

FDA (2016) Science review of isolated and synthetic non-digestible carbohydrates. United States Department of Health and Human Services, Food and Drug Administration.

FSANZ (2001) A277 - Inulin and fructooligosaccharides as dietary fibre: full assessment report and regulatory impact assessment. Canberra: FSANZ. https://www.foodstandards.gov.au/code/applications/documents/A277\_FAR.doc

FSANZ (2004) A491 - Resistant maltodextrin as dietary fibre: final assessment report. Canberra: FSANZ. https://www.foodstandards.gov.au/code/applications/documents/A491%20Maltodextrin%20FAR%20Final.doc

FSANZ (2010) Consideration of the effect of a 1:1 isomer mix of conjugated linoleic acid on HDL- and LDL-cholesterol levels (A1005): supporting document 1. FSANZ, Canberra. https://www.foodstandards.gov.au/code/applications/Documents/A1005%20Tonalin%20CLA%20SD1%20Lipids.pdf

FSANZ (2016) Systematic review of the evidence for a relationship between β-glucan from oats or barley and peak postprandial blood glucose concentration.

https://www.foodstandards.gov.au/consumer/labelling/nutrition/Documents/Beta-glucan%20glucose%20review.pdf

FSANZ (2018) Assessment of resistant starch as dietary fibre; and suitability of AOAC 2002.02 as a regulatory method of analysis (A1142): supporting document 1. FSANZ, Canberra. https://www.foodstandards.gov.au/code/applications/Documents/A1142%20SD1.docx

Higgins JPT, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327:557–60.

Ito M, Deguchi Y, Miyamori A, Matsumoto K, Kikuchi H, Matsumoto K, Kobayashi Y, Yajima T & Kan T (1990) Effects of administration of galactooligosaccharides on the human faecal microflora, stool weight and abdominal sensation. Microb Ecol Health Dis. 3:285-92.

Knopp JL, Holder-Pearson L & Chase JG (2019) Insulin units and conversion factors: a story of truth, boots, and faster half-truths. J Diabetes Sci Technol 13:597-600.

McCleary BV (2019). Total Dietary Fiber (CODEX Definition) in Foods and Food Ingredients by a Rapid Enzymatic‐Gravimetric Method and Liquid Chromatography: Collaborative Study, First Action 2017.16. J AOAC Int 102(1):196-207.

Morel FB, Dai Q, Ni J, Thomas D, Parnet P & Fanҫa-Berthon P (2015). α-galacto-oligosaccharides dose-dependently reduce appetite and decrease inflammation in overweight adults. J Nutr 145:2052-9.

NHMRC, Australian Government Department of Health and Ageing, New Zealand Ministry of Health (2006). Nutrient reference values for Australia and New Zealand. NHMRC, Canberra.

Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, Watts GF, Sypniewska G, Wiklund O, Borén J, Chapman MJ, Cobbaert C, Descamps OS, von Eckardstein A, Kamstrup PR, Pulkki K, Kronenberg F, Remaley AT, Rifai N, Ros E & Langlois M (2016). Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points—a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. Eur Heart J 37:1944-58.

Pedersen C, Gallagher E, Horton F, Ellis RJ, Ijaz UZ, Wu H, Jaiyeola E, Diribe O, Duparc T, Cani PD, Gibson GR, Hinton P, Wright J, La Ragione R & Robertson MD (2016). Host-microbiome interactions in human type 2 diabetes following prebiotic fibre (galacto-oligosaccharide) intake. Brit J Nutr 116:1869-77.

Piirainen L, Kekkonen RA, Kajander K, Ahlroos T, Tynkkynen S, Nevala R & Korpela R (2008). In school-aged children a combination of galacto-oligosaccharides and *Lactobacillus* GG increases bifidobacteria more than *Lactobacillus* GG on its own. Ann Nutr Metab 52:204–8.

Reynolds A, Mann J, Cummings J, Winter N, Mete E & Te Morenga L (2019). Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. The Lancet 393:434-45.

Sakata and Shinbo (2003). Fecal weight and its daily fluctuation in young women. Part 2. Related parameters. Jpn J Public Health 50:890-6.

Scientific Advisory Committee on Nutrition (SACN) (2008). Summary of narrative synthesis of the health effects of potential dietary fibre components. Government of the United Kingdom: Food Standards Agency / Medical Research Council Human Nutrition Research Group.

Slavin J (2013). Fibre and prebiotics: mechanisms and health benefits. Nutrients 5(4):1417-35.

Sperber AD, Dumitrascu D, Fukudo S, Gerson C, Ghoshal UC, Gwee KA, Hungin APS, Kang J, Minhu C, Schmulson M, Bolotin A, Friger M, Freud T & Whitehead W (2017). The global prevalence of IBS in adults reains elusive due to the heterogeneity of studies: a Rome Foundation working team literature review. Gut 66:1075-82.

Strazzullo P, D’Elia L, Kandala N & Cappuccio FP (2009). Salt intake, stroke, and cardiovascular disease: meta-analysis of prospective studies. BMJ 339:b4567.

Teuri U & Korpela R (1998). Galacto-oligosaccharides relieve constipation in elderly people. Ann Nutr Metab 42(6):319-27.

The Nordic Cochrane Centre TCC (2014). Review Manager (RevMan) Version 5.3.

Torres DPM, Gonҫalves MF, Teixeira JA & Rodrigues LR (2010). Galacto-oligosaccharides: production, properties, applications, and significance as prebiotics. Compr Rev Food Sci F 9:438-54.

Tuck C, Taylor K, Gibson P, Barrett J & Muir J (2018). Increasing symptoms in irritable bowel symptoms with ingestion of galacto-oligosaccharides are mitigated by α-galactosidase treatment. Am J Gastroenterol 113(1):124-34.

van Dokkum W, Wezendonk B, Srikumar TS & van den Heuvel EGHM (1999). Effect of nondigestible oligosaccharides on large-bowel functions, blood lipid concentrations and glucose absorption in young healthy male subjects. Eur J Clin Nutr 53:1-7.

Van Loo J, Cummings J, Delzenne N, Englyst H, Franck A, Hopkins M, Kok N, Macfarlane G, Newton D, Quigley M, Roberfroid M, van Vliet T & van den Heuvel E (1999). Functional food properties of non-digestible oligosaccharides: a consensus report for the ENDO project (DGXII AIRII-CT94-1095). Brit J Nutr 81:121-32.

Vulevic J, Drakoularakou A, Yaqoob P, Tzortzis G & Gibson GR (2008). Modulation of the fecal microflora profile and immune function by a novel trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. Am J Clin Nutr 88:1438-46.

Vulevic J, Juric A, Tzortzis G & Gibson GR (2013). A mixture of trans-galactooligosaccharides reduces markers of metabolic syndrome and modulates the fecal microbiota and immune function of overweight adults. J Nutr 143:324-31.

Walton GE, van den Heuvel EGHM, Kosters MHW, Rastall RA, Tuohy KM & Gibson GR (2012). A randomised crossover study investigating the effects of galacto-oligosaccharides on the faecal microbiota in men and women over 50 years of age. Brit J Nutr 107:1466-75.

Wan X, Wang W, Liu J & Tong T (2014). Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol 14,135.

Whisner CM, Martin BR, Schoterman MHC, Nakatsu CH, McCabe LD, McCabe GP, Wastney ME, van den Heuvel EGHM & Weaver CM (2013). Galacto-oligosaccharides increase calcium absorption and gut bifidobacteria in young girls: a double-blind cross-over trial. Brit J Nutr 110:1292-1303.

# Appendix

## 2.1 Summary of meta-analyses’ results, and our assessment’s strengths and limitations

**Table 5: Galacto-oligosaccharide intake has no overall mean effect on outcomes related to laxation, blood cholesterol, and blood glucose.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Outcome** | **Units** | **Mean difference (95% CI)1** | ***P*2** | **I2** | **Number of included studies pair-wise comparisons, and individuals3** |
| *Outcomes of primary interest to the definition of dietary fibre (Standard 1.1.2)* | | | | | |
| Stool weight | gram | 6.34 (-6.21, 18.9) | 0.32 | 7 | 3, 6, 113 (64) |
| Total cholesterol | mmol/L | -0.13 (-0.34, 0.08) | 0.21 | 45 | 4, 4, 225 (127) |
| LDL-cholesterol | mmol/L | -0.11 (-0.29, 0.08) | 0.25 | 27 | 3, 3, 143 (86) |
| Fasting glucose4 | mmol/L | -0.09 (-0.19, 0.00) | 0.06 | 15 | 4, 4, 187 (130) |
| *Outcomes of secondary interest to the definition of dietary fibre (Standard 1.1.2)* | | | | | |
| HDL-cholesterol | mmol/L | -0.02 (-0.07, 0.02) | 0.31 | 0 | 4, 4, 225 (127) |
| Fasting triglycerides4 | mmol/L | -0.01 (-0.12, 0.09) | 0.80 | 0 | 4, 4, 187 (130) |
| Fasting insulin4 | pmol/L | -8.92 (-18.0, 0.15)5 | 0.05 | 10 | 4, 4, 187 (130) |
| HOMA-IR |  | 0.34 (-0.36, 1.04) | 0.34 | 0 | 2, 2, 73 (73) |

CI, confidence interval; L, litre; LDL, low density lipoprotein; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance. 1Mean difference is calculated as intervention minus placebo. 2*P-value* pertains to overall mean difference (i.e. effect size). 3The number of individuals reflects the sample size entered into the meta-analysis, with the value in brackets representing the number of unique individuals (the sum of sample sizes from each study) included in the meta-analysis. Differences between values are due to sample sizes being treated differently depending on the study design (that is, crossover versus parallel controlled trials, with participants from crossover studies acting as their own control and, thus, increasing the non-bracketed value entered in to the meta-analysis). 4Fasting status confirmed by three of four studies and assumed for one study (Vulevic et al. 2013). 5Equates to -1.49 (-3.00, 0.025) mU/L, using the conversion factor: 1 µIU/mL = 6.00 pmol/L (Knopp et al. 2019).

**Table 6: Strengths and limitations of our assessment.**

|  |  |
| --- | --- |
| **Strengths** | **Limitations** |
| The inclusion of all data from all identified studies in humans that measured any outcome of direct or indirect relevance to the three physiological effects. | Publication bias could not be ascertained because of the small number of trials. In general, publication bias tends to favour studies reporting ‘positive’ results and leads to an overestimation of the true mean effect size. |
| Effect sizes were estimated using meta-analyses. Pooling data increases our ability to detect a real effect if one exists, improves our estimation of the size and direction of effect together with a 95% level of confidence, and decreases the risk of reviewers’ bias in interpreting differing results from individual studies. | All the studies included in the meta-analyses had links to commercial interests. The small number of studies prevented subgroup analysis to assess possible bias of industry links on overall results (see Table 7, Appendix Section 2.5). |
| Outcomes are assessed across a range of intake levels, from 2.5 g to 15.0 g added GOS per day (in addition to the intake of naturally occurring amounts in the habitual diet, common to all participants). Many of these intake levels are likely to be higher than actual consumption. However, this may change if a potential approval of AOAC Method 2017.16 and a reduction in cost to produce synthetic analogues encourages manufacturers to increase their addition of GOS to foods for commercial interests. | The current intake of added GOS is likely to be substantially lower than the intake levels tested in the intervention arms. As such, the size of the effects shown in Tables 1 and 5, small as they are, are likely to be even smaller in practice. |
| The studies included in the meta-analyses were crossover or parallel controlled trials and included a placebo control. Five of seven studies were randomised. Randomisation was not stated for one crossover and one parallel controlled trial (authors were contacted for clarification but did not respond). | Commercial GOS mixtures are variable in their structure which may contribute to physiological effects differently. |
|  | A formal risk of bias checklist was not completed. |
|  | Data was not extracted in duplicate. |

GOS, galacto-oligosaccharides.

## 2.2.1 Laxation: review methodology and results

We identified original research publications using the search terms, galacto-oligosaccharides and, stool or faecal weight, and a hand search of relevant reviews’ reference lists. The applicant did not identify any further studies. Four studies (Ito et al. 1990, Bouhnik et al. 1997, van Dokkum et al. 1999, and Alles et al. 1999) report outcomes expressed as absolute change in stool weight, from which we calculated the relative change. The study by Bouhnik et al. (1997) was excluded because it lacked a control group. Data extraction, conversion, and analyses are explained in Table 2 footnotes and Appendix Section 2.6.

We conducted a review of studies reporting on the effect of GOS intake on additional quantitative laxative outcomes: bowel movement or stool frequency; or, intestinal transit time. For efficiency, we planned to first categorise results as positive, negative, neutral, or mixed. If a positive or mixed effect emerged in the majority of studies, we planned secondly to conduct a rigorous appraisal of the studies’ methodology and results. We identified eight studies reporting self-reported stool frequency and intestinal transit time (Ito et al. 1990, van Dokkum et al. 1999, Alles et al. 1999, Canfora et al. 2017, Davis et al. 2010, Piirainen et al. 2008, Teuri & Korpela 1998, and Whisner et al. 2013). Although Canfora et al. (2017) state that participants did not report “side effects . . . such as changes in stool frequency or gastrointestinal complaints”, we excluded this study as stool frequency is not listed as an outcome and the method of assessment was not stated. We assume that this result reflects participants’ voluntary reporting on adverse outcomes ad hoc and, therefore, this study was excluded from the assessment of laxative effect. The first step involving categorisation of study outcomes demonstrated a consistent, neutral (i.e. no) effect of GOS intake on laxative outcomes and, therefore, the second step of study appraisal was not undertaken. Stool softening or consistency, ease or straining during defecation, gastrointestinal comfort, flatulence, or similar qualitative or subjective outcomes have not been used as measures of laxation in the current assessment as they could be attributed to factors other than laxation.

## 2.2.2 Laxation: included studies’ methodology and results

Laxation was assessed using bowel movement or stool frequency, and intestinal transit time (see Appendix Section 2.2.1), and absolute and relative stool weight (see below and Section 1.2.2). The ‘benchmark’ criteria we used for relative stool weight is a greater than 1 g and 5 g increase in faecal wet weight per gram of GOS consumed (see Section 1.2.1). A detailed review of the three studies that reported faecal output from a collection period of at least 24 hours, permitting an assessment of absolute and relative stool weight, is provided below and in Table 7 (Appendix Section 2.5).

Ito et al. (1990) recruited “healthy” males, who did not receive medication within two weeks before or during the study period, aged 26 to 48 years (n=12) to explore the laxative effect of GOS intake in a Latin square, single-blinded (participants), crossover study. Four 7-day conditions were tested: placebo and three intake levels of GOS. The washout period varied depending on treatment order and was a minimum of seven days. Randomisation for, or method of allocation to, treatment order is not mentioned; we contacted the corresponding author to seek clarification, but received no response. Participants’ diet was not controlled, except for three restrictions: no extra lactose; no milk; and, no fermentation products. Dietary intake (including fluid intake) was not assessed. During the four 7-day conditions, participants were provided an oral dose of Oligomate-50, consumed once per day after lunch. Four intake levels of Oligomate-50 were used (0.0, 4.8, 9.6 or 19.2 g/day) which provided 0.0, 2.5, 5.0, or 10.0 g/day of GOS, respectively (for consumption instructions, structure and form, see Table 7, Appendix Section 2.5). The authors report that bowel habits were normal at the start of the study. Stool weight reflects the mean daily wet weight at intervention follow-up only, using stools collected on days 5 to 7 of each experimental condition. Stool weight was not statistically different at any intake level: 151 ± 63.6; 134 ± 49.6; 151 ± 77.1; and, 163 ± 71.4 g/day (mean ± SD; for the GOS intakes, 0, 2.5, 5.0, or 10.0 g/day, respectively, relative to the placebo condition). The authors did not provide exact *P* values for these comparisons. We converted their findings to the mean differences in absolute stool weight (see Table 2) and relative stool weight (-6.72, 0.00, and 1.13 g/day per gram of GOS for the 2.5, 5.0, or 10.0 g GOS/day condition, respectively). The largest difference was a lower relative stool weight, -6.7 g ± 5.3 g (mean ± SE) indicating GOS intake may promote constipation. When compared to a proxy for a laxative effect, a benchmark of greater than 5 g increase in faecal wet weight per gram of test fibre consumed, the mean differences in relative stool weight indicate a worsened laxation (one from three comparisons), and no effect on laxation (two from three). When using a >1 g/g benchmark, the mean differences in relative stool weight indicate a worsened laxation (one from three comparisons), no effect on laxation (one from three), and a promotion of laxation when using the point estimate of the mean difference but not when the variance is considered (one from three). Wide variances of relative stool weight are discussed in Section 1.2.2. In this sample of healthy males, an inconsistent effect on laxation was demonstrated, when consuming GOS at three intake levels (2.5, 5.0 or 10.0 g/day) for seven days.

van Dokkum et al. (1999) recruited “healthy” males (n=12, mean ± SD age=23 ± 3 years, mean ± SD weight=79.8 ± 9.2 kg, with normal bowel habits) in a Latin square, randomised, double-blinded, diet-controlled study. From the reported mean weight and height, we calculated the sample’s mean body mass index as approximately 23 kg/m2. Four 3-week conditions were tested: inulin; fructo-oligosaccharide; GOS; and, a placebo. No washout period was included. Participants were randomised to one of the treatment orders. We report only on the results of GOS and placebo conditions. Participants’ diet was carefully controlled. Meals and snacks were provided to participants. Dinner meals were consumed from a metabolic ward during the first two weeks. In the last one week, participants stayed at, and ate all foods from, the metabolic ward. The basal diet reflected a normal Dutch food pattern (based on a national food survey), did not contain non-digestible oligosaccharides, and aimed to maintain a constant body weight. The basal diet, without added GOS, provided 24.1 g/day dietary fibre. We note this is lower than the Adequate Intake and Suggested Dietary Target values recommended by the NHMRC (30 g and 38 g per day, respectively) for males aged 19 years and over. The average fluid intake was 2050 mL/day. Diet adherence was not reported but mean body weight did not statistically differ over time (*P*≥0.05) with only marginally changes: 79.8 kg (baseline); 79.1 kg (week 3); and, 78.1 kg (week 12). During the 3-week GOS condition, participants consumed 15 g of GOS daily (for consumption instructions, structure and form, see Table 7, Appendix Section 2.5). Stool wet weight, representing the weight over 48 hours at the end of the 3-week condition, did not differ between the GOS and placebo condition at follow-up (*P*≥0.05). Stool wet weight at the end of 3-week GOS and placebo condition, respectively, was: 297 ± 103 g/48 h (mean ± SD) which we converted to 149 ± 51.5 g/day; and, 258 ± 85 g/48 h (mean ± SD) which we converted to 129 ± 42.5 g/day. The relative stool weight at follow-up was 1.3 ± 0.7 g/day per gram of GOS consumed, that is, a higher stool weight in the intervention condition relative to the placebo. This exceeds the >1 g/g but not the > 5 g/g benchmark. When the variance (SE is 0.7 g/day per GOS g) is taken into account, however, the >1 g/g benchmark may not be met (the relative stool weight may be as low as 0.6 g/day per GOS g). In this sample of healthy young males, there was a possibility of a laxative effect when consuming GOS at a high intake level (15 g/day) for three weeks.

Alles et al. (1999) conducted a parallel, single-blind (participants), controlled trial. Randomisation is not mentioned; we contacted the corresponding author to seek clarification, but received no response. Adults aged 18-75 years with a stable body weight (n=41 but 1 withdrew in first week and data represents n=40; 18 females and 22 males) were recruited. The exclusion criteria encompassed: antibiotic or laxative use, or surgery, during the last 12 months; complaints of diarrhoea, obstipation, or abdominal pain; medication use for gastrointestinal function; gastrointestinal or gallbladder disorders; a serum triacylglycerol of ≥2.5 mmol/L and a serum cholesterol of ≥7.0 mmol/L; and, abnormal hemocytometric values and urinary values for protein, glucose, and pH. A breath-lactose test was conducted to exclude hydrogen responders to lactose. Participants completed two consecutive 3-week blocks: a 3-week run-in diet (common to all participants) followed by a three 3-week experimental condition. Participants were divided in to one of three conditions which varied by the GOS intake level: placebo (0 g/day, n=13); low intake (intended to be 7.5 g/day, n=13); and, high intake (intended to be 15.0 g/day, n=14). The mean baseline body mass index and age was approximately 23 kg/m2 and 39 years; neither significantly differed between groups. Participants’ diet was carefully controlled: ~90% of participants’ energy intake was from supplied foods; and, ~10% was from ‘free choice’ foods chosen by the participant from a list of non-fibre containing foods (they were encouraged not to change their selection throughout the study). Participants’ habitual energy intake was estimated and they received a study diet that met their individual energy needs. The 21 3-week ‘menus’ included conventional foods, were of a similar nutrient composition other than the oligosaccharides, and are described as being rich in animal protein and low in fibre. During the study, energy intakes were adjusted in response to body weight change (which was recorded three times per week) to maintain a stable weight. Weekday lunches were consumed at the Department. Other weekday food was packaged and provided daily. Weekend food was provided on Fridays. Participants’ intake of ‘free choice’ foods and any deviations from their usual diet was documented. Energy intake from the ‘free choice’ foods did not significantly differ between conditions (accounting for 11.2% of total energy with a range of 7.7 to 14.4%), nor did the intake of energy (mean ± SEM ranged from 10.5 ± 0.4 MJ to 11.0 ± 0.5 MJ), fibre (mean ± SEM ranged from 1.74 ± 0.02 g/MJ to 1.77 ± 0.02 g/MJ), and percentage of energy from macronutrients from the diet overall. Fluid intake is not reported. Compliance for GOS intake was “near 100%” based on inspection of juice bottles and intake of the lunch meal in the Department. Alles et al. (1999) stated that, during the three 3-week intervention period, non-placebo participants were provided an intake level of GOS aimed to be 7.5 g/day or 15.0 g/day. They later stated that the low and high intake diet provided a mean of 8.5 g/day and 14.4 g/day, respectively (for consumption instructions, structure and form, see Table 7, Appendix Section 2.5). Change in stool weight, reflecting stool collected during the last weekend of the run-in and intervention period, was not significantly different between low intake and placebo or high intake and placebo (*P*≥0.05). The difference in the change of stool weight over time between the low intake condition and placebo was 22.3 g/day (-21.2, 65.9; mean, 95% CI), that is, a relative increase in stool weight in the low intake condition. Although the stool weight decreased within the high intake condition by 4 g/day, the difference in the change of stool weight over time between the high intake condition and placebo was 3.52 g/day (-39.3, 46.3; mean, 95% CI). We converted these values (22.3 g/day and 3.52 g/day) for comparison against the benchmarks of >1 g/g and >5 g/g. Our calculations are based on thechange in stool weight by time and group, relative to the amount of GOS consumed (8.5 g/day and 14.4 g/day) rather than the amount aimed for (7.5 g/day or 15.0 g/day). An increased stool weight of 2.6 g/day (SE: 2.6) and 0.2 g/day (SE: 1.5) per gram of GOS consumed was demonstrated in the low and high intake condition, respectively. That is, an increased stool weight, 2.6 g/day, in the low intake condition relative to the placebo that exceeds a >1 g/g but not a >5 g/g benchmark. When the variance (SE is 2.6 g/day per GOS g) is taken into account, however, the >1 g/g benchmark may not be met (the relative stool weight may be as low as 0.0 g/day per GOS g). A meaningful relative increase in the stool weight was not, however, demonstrated in the high intake condition (0.2 g/day)[[1]](#footnote-2) for either the >1 g/g or >5 g/g benchmark. Thus, in this sample of adults with no notable risk factors, Alles et al. (1999) provided inconsistent findings, suggesting there is a possibility of a laxative effect of GOS’ when consumed at a moderate (8.5 g/day) but not high (14.4 g/day) intake level for three weeks.

## 2.3.1 Blood cholesterol: review methodology and results

We identified original research publications using search terms related to galacto-oligosaccharides and cholesterol. The applicant identified one further study (Pedersen et al. 2016). Five studies are relevant (van Dokkum et al. 1999, Vulevic et al. 2008, Vulevic et al. 2013, Pedersen et al. 2016, and Canfora et al. 2017). Data extraction, conversion, and analyses are explained in Table 3 footnotes and Appendix Section 2.6.

## 2.3.2 Blood cholesterol: included studies’ methodology and results

A detailed review of the five studies is provided below and in Table 7 (Appendix Section 2.5).

The study design of van Dokkum et al. (1999) has been summarised in Appendix Section 2.2.2. With regards to blood cholesterol, participants’ baseline blood lipid concentrations were not reported although authors state they had “normal health” as assessed using a medical history, clinical laboratory tests, and vital signs. Fasting blood lipid concentrations represent the mean from blood sampled on two consecutive days at the end of each treatment period. The measures included: total serum cholesterol (mmol/L); HDL-, HDL2-, HDL3- and LDL-cholesterol (mmol/L); phospholipids (mmol/L); triacylglycerol (mmol/L); and, apolipoprotein A-1 and B (g/L). None of the blood lipid concentrations were statistically significantly different between the GOS condition and placebo at the end of the 21-day treatment period (*P*>0.05). In this sample of healthy adult males, these findings suggest GOS intake has no effect on blood cholesterol or other blood lipid outcomes when consumed at a high intake level (15 g/day) over three weeks.

Vulevic et al. (2008) conducted a double-blind, randomised, controlled, crossover study. Participants were elderly (age: 69.3 ± 4.0 years) males (n=16) and females (n=28) with a body mass index range of 22-31 kg/m2. The data represented n=41 as three participants did not complete the study. We note that one participant withdrew after three weeks, due to experiencing “tolerance problems”; we presume this occurred during the intervention condition, but it is not stated nor are the severity of symptoms specified. Exclusion criteria were applied to prevent volunteers with gastrointestinal tract dysfunction from participating, such as medication use, or history or evidence of disorder of the gastrointestinal tract. Exclusion criteria also included: antibiotic use three months before the study; use of pre-, pro-, or sym-biotic supplements, either regularly within two weeks before the study, or in another study six months before this study; use of immunosuppressive or anti-inflammatory drugs or drugs affecting intestinal mobility; or, a body mass index of <20 kg/m2 (women) or <22 kg/m2 (men). Other than being in “good health” the authors did not mention whether participants were screened on the basis of blood lipid concentration. Two 10-week conditions were tested, a placebo and GOS condition, with a 4-week washout period between conditions. Participants’ diet was not assessed. Although body weight was measured and compliance with the study protocol was recorded, neither were reported. During the 10-week GOS condition, participants consumed 2.6 g of GOS daily (for consumption instructions, structure and form, see Table 7, Appendix Section 2.5). Plasma lipid measures included total cholesterol and HDL-cholesterol. Blood lipids were measured at three time points during each condition: day 0, week 5, and week 10. Participants’ baseline total and HDL-cholesterol levels were not significantly different between groups (*P*≥0.05): the mean total cholesterol for the two conditions was 4.9 and 5.0 mmol/L and the mean HDL-cholesterol for both conditions, 1.3 mmol/L. Total and HDL- cholesterol did not significantly differ between the two conditions at baseline, week 5 or week 10, and did not change over time (*P*≥0.05). In this sample of healthy elderly adults, mostly without dyslipidaemia, these findings suggest GOS have no effect on blood cholesterol when consumed at a low intake level (2.6 g/day) for 10 weeks.

Vulevic et al. (2013) conducted a double-blind, randomised, placebo-controlled, crossover study. Participants were overweight (>25 kg/m2) males (n=16, age 42.8 ± 12.1 years) and females (n=29, age 46.4 ± 11.8 years) with ≥3 risk factors associated with metabolic syndrome. Extensive inclusion and exclusion criteria were used beyond this. Some criteria is listed in Section 1.3.1. Additional criteria excluded participants: with renal or bowel disease or gastrointestinal disorders; taking drugs that affect intestinal motility or absorption, antibiotics in the one month prior to study commencement, dietary antioxidants or phytochemicals, prebiotic or probiotic supplements; who were pregnant or lactating; and, were anaemic (haemoglobin: men >140 g/L, women >115 g/L). Two 12-week conditions were tested, a placebo (maltodextrin) and GOS condition, with a 4-week washout period between conditions. During the 12-week GOS condition, participants consumed 2.6 g of GOS daily (for consumption instructions, structure and form, see Table 7, Appendix Section 2.5). The authors reported that participants’ body weight, energy intake and percentage contribution of macronutrients to total energy was stable throughout the study. Outcomes included total cholesterol, triglycerides, LDL- and HDL-cholesterol, and total cholesterol:HDL-cholesterol, measured at three time points during each condition: week 1, 6, and 12. Participants’ baseline lipids indicated some dyslipidaemia: 93% had total cholesterol concentrations >5.0 mmol/L; 76% had HDL-cholesterol <1.03 mmol/L in men and <1.29 mmol/L in women; and, 40% had plasma triglyceride concentrations >1.7 mmol/L. None of the five plasma lipid outcomes differed between the two conditions at baseline. At week 12, three out of five outcomes were lower in the GOS condition (exact *P* values are not stated for any): total cholesterol (*P*<0.001[[2]](#footnote-3)); triglycerides (*P*<0.005[[3]](#footnote-4)); and, total cholesterol:HDL-cholesterol (*P*<0.0001). ForVulevic et al. (2013), *P* values were corrected for multiple testing and significance was set at *P*<0.005 after Bonferroni adjustment. Given the small size of the differences and differing *P* values, and other reasons provided in Section 1.3.1, we sought clarification from the corresponding author on some of these statistically different results but did not receive a response. In a sample comprising of adults with three or more metabolic risk factors, Vulevic et al. (2013) provided inconsistent findings when GOS are consumed at a low intake (2.6 g/day) for 12 weeks, however, the reporting and generalisability of the findings are uncertain (see Section 1.3.1).

Pedersen et al. (2016) conducted a randomised, double-blind, placebo-controlled, parallel study. Participants (n=32) were males with well-controlled type 2 diabetes mellitus, aged 42-65 years. The exclusion criteria were: use of antibiotics in the prior three months; use of anti-inflammatory medications (except a low-dose aspirin), diuretics, and proton-pump inhibitors; and, inflammatory bowel disease, Crohn’s disease, coeliac disease, and irritable bowel disease. Three withdrew and/or were excluded from data analysis due to gastrointestinal upset (n=1) and antibiotic treatment (n=2), therefore, the data represented n=29. Two 12-week conditions were tested: a placebo (maltodextrin) and GOS condition. During the 12-week GOS condition, participants consumed 2.6 g of GOS daily (for consumption instructions, structure and form, see Table 7, Appendix Section 2.5). Participants were asked not to change their lifestyle during the study. Participants were asked to exclude probiotic products and prebiotic supplements (beyond the study supplement) for the two weeks prior to, and during, the study. Diet was assessed at baseline and week 12 via a seven day diary; dietary fibre intake was ~22 g/day. At baseline, there were no significant differences between groups for blood lipids, glucose tolerance, body composition, or diet. No significant differences in the change between groups were found except for the percentage of dietary energy from protein which increased by 1.1% in the placebo condition (*P*=0.004). Body weight (kg), body mass index (kg/m2), body fat (%; measured by bioimpedance), and waist circumference (cm), did not change between groups over the 12 weeks (*P*=0.335, *P*=0.333, *P*=0.514, and *P*=0.451, respectively). Compliance to the consumption of maltodextrin or GOS mixture powders was reported to be 96%, as assessed via the return of sachets. Diet did not differ between conditions over time, with exception for the percentage of energy attributed to protein (*P*=0.004). Plasma lipid measures included concentrations of triglycerides, and total, LDL- and HDL-cholesterol. None were significantly different between the two conditions over time (triglycerides, *P*=0.534; HDL-cholesterol, *P*=0.798). The between-group difference in change over time approached significance for total and LDL-cholesterol (*P*=0.068 and *P*=0.051, respectively). In this sample of adults with well-controlled type 2 diabetes mellitus, no effect on any blood cholesterol outcome was found with a low GOS intake of 2.6 g/day over 12 weeks.

Canfora et al. (2017) conducted a randomised (stratified for age and sex), double-blinded, placebo-controlled, parallel trial. Participants (n=44, aged 45-70 years) were overweight or obese (body mass index mean: ~33 kg/m2, and range: 28–40 kg/m2) and weight stable (in the three months prior to study commencement), with impaired fasting glucose (defined as a fasting plasma glucose ≥ 5.6 mmol/L) or impaired glucose tolerance (defined as a plasma glucose between 7.8 and 11 mmol/L at two hours post-oral glucose tolerance test containing 75 g glucose). Criteria excluded volunteers: with diabetes, cardiovascular disease, gastroenterologic diseases, abdominal surgery, or liver or kidney malfunction; taking lipid- or glucose-lowering medication, *β*-blockers, antioxidants, or chronic corticosteroids; consuming antibiotics, prebiotics, or probiotics during or in the three months prior to the study; following a hypocaloric diet; or, with a life expectancy less than five years. Two dropped out of the study (final n=44). Two 12-week conditions were tested: an isocaloric placebo (maltodextrin, n=23); and, a GOS condition (n=21). During the 12-week GOS condition, participants consumed 15 g of GOS daily (for consumption instructions, structure and form, see Table 7, Appendix Section 2.5). Participants were instructed to maintain their current diet and physical activity. Diet (energy, macronutrient and fibre intake via a three-day weighed food record), body composition (via dual-energy X-ray absorptiometry and body weight), and physical activity were not significantly different between groups over time, at week 12 (*P* ranged from 0.114 to 0.912). Compliance to the consumption of maltodextrin or GOS mixture powders was reported to be 98%, as assessed via the return of sachets and participants’ recording of intake. Plasma triacylglycerol was the only outcome assessed of relevance to blood cholesterol. It was not used in the study’s screening criteria and it was not stated whether the baseline concentrations statistically differed between conditions. After the 12 week period, and after a 12 hour fast, no significant differences between groups over time were detected for fasting plasma triacylglycerol (presented in Table 3; *P*=0.54) and steady-state plasma triacylglycerol (during the hyperinsulinemic-euglycemic clamp method; data not extracted in Table 3; *P*=0.71) levels. In this sample of adults with impaired fasting glucose or impaired glucose tolerance, no effect on plasma triacylglycerol concentration was found with a high GOS intake of 15 g/day over 12 weeks.

## 2.4.1 Blood glucose: review methodology and results

Our search identified published research using search terms related to galacto-oligosaccharides and, blood glucose or insulin. The applicant identified one further study (Pedersen et al. 2016). Four studies are relevant (van Dokkum et al. 1999, Vulevic et al. 2013, Pedersen et al. 2016, and Canfora et al. 2017). Data extraction, conversion, and analyses are explained in Table 4 footnotes and Appendix Section 2.6.

## 2.4.2 Blood glucose: included studies’ methodology and results

A detailed review of the four studies is provided below and in Table 7 (Appendix Section 2.5).

The study designed by van Dokkum et al. (1999) has been summarised in Appendix Section 2.2.2. With regards to blood glucose, participants’ baseline blood glucose and insulin concentrations were not reported although authors report that participants had “normal health” as assessed using a medical history, clinical laboratory tests, and vital signs. In the third week of the 3-week treatment period and after an overnight fast, participants’ glucose and insulin response to an oral glucose tolerance test was measured using five time points (0, 30, 60, 90, and 120 minutes). The comparison was a glucose test solution (50 g of glucose) with 5 g of GOS or no non-digestible oligosaccharides (control). Neither the mean blood glucose or insulin response (including the fasted value at the time point, 0 minutes) were statistically significantly (*P*>0.05) different between the GOS and control condition at the end of the 21-day treatment period. In this sample of healthy adult males, no effect on blood glucose or insulin was found with a high GOS intake of 15 g/day over three weeks.

The study conducted by Vulevic et al. (2013) has been described in Appendix Section 2.3.2. Participants were included in the study if they were aged 18 to 65 years, had a body mass index >25 kg/m2, and had three or more metabolic risk factors, one of which could be a fasting glucose of >5.6 mmol/L (see Section 1.3.1 for other factors’ thresholds). Extensive inclusion and exclusion criteria are stated, such as, excluding volunteers with: diagnosed diabetes, with a fasting glucose >7 mmol/L, with other endocrine disorders, or trying or intending to complete a weight loss regime (and Sections 1.3.1 and 2.3.2 for other criteria). Participants’ baseline values indicated some impaired glucose metabolism: 78% had plasma insulin concentrations >40 pmol/L; and, 27% had plasma glucose concentrations >5.6 mmol/L. Plasma glucose concentration did not significantly differ between the two conditions at baseline, week 6 or week 12. Plasma insulin concentration was not statistically different between conditions at baseline (*P* value not stated) or week 6 (*P*=0.0084) and was lower in the GOS condition at week 12 (*P*<0.005[[4]](#footnote-5); exact *P* value not stated). In this sample of adults with metabolic risk factors, no effect on blood glucose was found with a low GOS intake of 2.6 g/day over 12 weeks.

The design of the study conducted by Pedersen et al. (2016) has been summarised in Appendix Section 2.3.2. None of the glucose tolerance related outcomes were significantly different between the two conditions at baseline (all *P*>0.05). None of the outcomes were significantly different between the two conditions over the 12 weeks: fasting glucose (*P*=0.227); fasting insulin (*P*=0.543); glycosylated haemoglobin (HbA1c; *P* value not provided when using ‘%’ units, and are *P*=0.946 when reported using the units, ‘mmol/mol’); HOMA-IR (*P*=0.199); HOMA for insulin sensitivity (*P*=0.215); HOMA for *β*-cell function (*P*=0.362); and, the postprandial glucose (*P*=0.485 for total AUC and *P*=0.221 for incremental AUC over 180 minutes) and postprandial insulin (*P*=0.112 for total AUC and *P*=0.171 for incremental AUC over 180 minutes) response to an insulin-modified intravenous glucose tolerance test. Fasting glucose and postprandial insulin (incremental AUC) response to an insulin-modified intravenous glucose tolerance test are the only two outcomes with significant within-group change (*P*<0.05); this occurred in the intervention condition only. Metformin use was considered to be a confounding factor due to its effect on intestinal bacterial populations but a subgroup analysis was not possible because 13 of 14 participants in the GOS condition were using metformin. In this sample of males with well-controlled type 2 diabetes mellitus, no effect on blood glucose was found with a low GOS intake of 2.6 g/day over 12 weeks.

The study design of Canfora et al. (2017) has been described in Appendix Section 2.3.2. Participants had impaired fasting glucose or impaired glucose tolerance but volunteers diagnosed with diabetes or taking glucose-lowering medication were excluded. With regards to blood glucose, baseline values did not differ between conditions for glucose (*P=*0.095), insulin (*P*=0.721[[5]](#footnote-6)), mean homeostatic model assessment of insulin resistance (HOMA-IR; *P*=0.829), glycated haemoglobin (HbA1c; *P*=0.509), and a 2 hour plasma glucose response to an oral glucose tolerance test (OGTT; *P*=0.259). After a 12 hour fast, participants’ peripheral insulin sensitivity was measured by the one-step hyperinsulinemic-euglycemic clamp method and assessed by the M value. The M value (mg/kg/min) represents the mean glucose infusion rate over the last 30 minutes of euglycemia. Blood plasma was sampled during fasting (time: -5 min) and euglycemic (time: 90 and 120 min) periods of the hyperinsulinemic-euglycemic clamp, for additional outcomes. Adipose tissue insulin sensitivity was measured by insulin-stimulated suppression of circulating free fatty acids during steady-state clamp. After the 12-week period, no significant differences between groups over time were detected for peripheral insulin sensitivity (as assessed by the M value, *P*=0.467), HOMA-IR (*P*=0.598), and adipose tissue insulin sensitivity (as assessed by free fatty acid suppression; *P*=0.808). No significant differences between groups over time were found for fasting plasma glucose (*P*=0.79) and fasting insulin (*P*=0.71). Follow-up values for glycated haemoglobin (HbA1c) and glycaemic response to the OGTT were not reported; it is likely that these were assessed for screening purposes only. In this sample of adults with impaired fasting glucose or impaired glucose tolerance, no effect on blood glucose was found with a high GOS intake of 15 g/day over 12 weeks.

## 2.5 Industry involvement and characteristics of galacto-oligosaccharides used in selected studies

**Table 7: Industry involvement and instructions, composition, and form of galacto-oligosaccharides used in studies included in meta-analyses and narrative review1.**

| **Physiological outcome measured** | **First author (year)** | **Study design** | **Intervention & Comparator** | **GOS** | **Industry involvement** |
| --- | --- | --- | --- | --- | --- |
| Laxation | Ito (1990) | Crossover.  Randomisation is not stated.  n=12  Four 7-day conditions: placebo and three intake levels of GOS.  Washout: variable length, minimum 7 days.  Study location: not stated but likely Japan. | I: Oral dose of 115 mL apple juice containing Oligomate-50, consumed once per day after lunch.  Three intake levels of Oligomate-50 (4.8, 9.6 or 19.2 g/day) containing 2.5, 5.0 or 10.0 g/day GOS.  C: Oral dose of 115 mL apple juice consumed once per day after lunch. | Oligomate-50.  Composition: 52% GOS (Gal-(Gal)n-Glc (n=0-4); 16% disaccharides, 24% trisaccharides, 10% tetrasaccharides, 2% penta- and hexa-saccharides); 38% monosaccharide; and, 10% lactose. (We note that lactose, a disaccharide, does not seem to be included in the previously listed 16% disaccharide content that forms part of GOS.)  Galactosyllactoses were produced from lactose by the enzymatic action of β-D-galactosidase (produced by *Aspergillus oryzae* and *Streptococcus thermophillus*).  Adherence to apple juice consumption was not reported. | Authors are employees of Yakult Central Institute for Microbiological Research, Japan.  The study protocol was approved by the Yakult Central Institute Ethics Committee. |
| Laxation, blood cholesterol, blood glucose | van Dokkum (1999) | Crossover.  Randomised.  n=12  Four 3-week conditions: inulin, FOS, GOS, and placebo.  Washout: nil.  Study location: The Netherlands. | I: GOS condition:  Oral dose of 100 mL orange juice containing 15 g/day GOS, consumed in three even intakes (5 g) at breakfast, lunch and dinner.  C: Oral dose of 100 mL orange juice. | GOS supplement composition: oligo-fructose (0% of dry matter); oligo-galactose (85%); glucose/fructose/sucrose (3.3%); galactose (0.3%); lactose (10.7%); and, ash (1%).  Adherence to orange juice was not reported. | TNO Nutrition and Food Research Institute, The Netherlands.  GOS supplements were provided by Borculo Whey Products, Borculo, The Netherlands. |
| Laxation | Alles (1999) | Parallel, controlled.  Randomisation not stated.  n=40 (placebo n=13, low-GOS n=13, and high-GOS n=14).  Participants divided into three groups. ‘Run-in diet’ for 3-weeks, followed by one of three 3-week conditions: placebo and two intake levels of GOS.  Washout: n/a.  Study location: The Netherlands. | I: Oral dose of 150 g fruit juice containing Elix’or (providing a total GOS intake level of 8.5 g/day or 14.4 g/day), consumed in three equal intakes per day with each meal.  C: Oral dose of 150 g fruit juice, containing lactose and glucose only, consumed three times per day with each meal. | Elix’or; a low- and high-GOS mixture was composed of a GOS syrup. The high-GOS mixture consisted of “75% dry matter, of which 62% was GOS with a DP of 2 (32% nondigestible disaccharides), 3 (35%), 4 (23%), 5 (8%), or >5 (2%). The remaining dry matter consisted of 20% lactose and 18% monosaccharides (mainly glucose).”  The low-GOS mixture, contained less GOS syrup, and more glucose and lactose to equalise the amount of non-oligosaccharide components.  The placebo mixture contained glucose and lactose in amounts equal to that in the GOS mixtures.  Adherence: “one of the 3 daily servings was consumed with a hot meal at our department. The other 2 servings were consumed outside the department; however, compliance was near 100% based on inspection of the juice bottles.” | Elix’or was provided by Borculo Whey Products (Borculo, Netherlands).  Research was supported by the Netherlands Ministry of Agriculture, the Dutch Dairy Foundation on Nutrition and Health, AVEBE (Netherlands), Nutreco (Netherlands), and ORAFTI (Belgium). |
| Blood cholesterol | Vulevic (2008) | Crossover.  Randomised.  Data represents n=41.  Two 10-week conditions: placebo and GOS.  Washout: 4 weeks.  Study location: United Kingdom. | I: B-GOS (Bi2muno; 5.5 g/day) provided in powder form providing 2.64 g GOS/day.  C: maltodextrin (5.5 g/day) provided in powder form.  I+C: participants instructed to reconstitute sachets by mixing with water immediately prior to consumption. Instructed to consume it at the same time of day, once per day. | GOS content of B-GOS (Bi2muno) = 48% (wt:wt). DP of B-GOS (% of GOS content): DP=2 (52%); 3 (26%); 4 (14%); and, 5 (8%). Saccharide linkages of B-GOS (% of GOS content): β1→3 (26%); β1→4 (23%); and, β1→6 (51%).  Adherence to study protocol was measured but not reported. | One author was an employee of Clasado Ltd (Milton Keynes, United Kingdom).  Research was supported by a grant from Clasado Ltd.  Clasado Ltd supplied the GOS mixture. |
| Blood cholesterol, blood glucose | Vulevic (2013) | Refer to Vulevic (2008) except study periods were for 12 weeks (not 10).  n=45  Study location: United Kingdom. | Refer to Vulevic (2008) | Refer to Vulevic (2008) | Two authors were employees of Clasado Research Services Ltd. A third author was not an employee at the time of the study, but became an employee by the time of publication.  Research was supported by a grant from Clasado Ltd.  Clasado Ltd supplied the GOS mixture. |
| Blood cholesterol, blood glucose | Canfora (2017) | Parallel, controlled.  Randomised.  Data represents n=44.  Two 12-week conditions: placebo or GOS.  Washout: n/a.  Study location: The Netherlands. | I: Vivinal (7.04 g) provided in powder form providing 5 g GOS, consumed three times per day with meals (total 15 g GOS/day).  C: Maltodextrin (5.65 g; isocaloric to I dose i.e. 270 kJ/day) provided in powder form, consumed three times per day with meals.  I+C: participants instructed to consume powder (provided in sachets) with a 200 mL low-fat yoghurt drink. Yoghurt did not contain any probiotic strains or supplemented GOS. | Vivinal (FrieslandCampina Domo, Amersfoort, The Netherlands) contains 69% GOS, 23% lactose, 5% monosaccharides (glucose and galactose), and 3% moisture.  Adherence to powder intake via diary and return of sachets: 98% (98% of sachets were empty on return). | One author was affiliated with the Beneficial Microbes Consultancy (Wageningen, The Netherlands). |
| Blood cholesterol, blood glucose | Pedersen (2016) | Parallel, controlled study.  Randomised.  Data represents n=29.  Two 12-week conditions: placebo or GOS.  Washout: n/a.  Study location: United Kingdom. | Refer to Vulevic (2008), however, for I+C, unlike Vulevic (2008), the instructions provided to participants for consumption are unclear but authors state the powders “were readily mixed into beverages or food”. | Refer to Vulevic (2008).  Adherence to powder intake via return of sachets: 96%. | Authors acknowledge Clasado Ltd for providing the supplements. |

I, intervention; C, comparator; GOS, galacto-oligosaccharide; FOS, fructo-oligosaccharide; NDO, non-digestible oligosaccharide; g, gram; d, day; n/a, not applicable; and, DP, degree of polymerisation.

1 Excludes four (Davis et al. 2010, Piirainen et al. 2008, Whisner et al. 2013, and Teuri & Korpela 1998) out of seven studies which report results only for bowel movement or stool frequency. These studies did not undergo rigorous appraisal or form the basis for conclusions regarding a laxative effect (see Sections 1.2.2 and 2.2.1). The remaining three from seven studies reported outcomes included in the meta-analyses or narrative review.

## 2.6 Review: data extraction, conversion, and analyses

*Data extraction*

Study characteristics and data were extracted by one assessor. If data were present only in graphs, the relevant statistics were extracted using the online program [WebPlotDigitizer](http://arohatgi.info/WebPlotDigitizer/index.html) Version 3.12.

*Data conversion*

Case-by-case conversion of data for standardisation is explained in the footnotes to Tables 2, 3, and 4. The raw mean differences (*D*) and their standard errors (*SED*) were calculated in Microsoft® Excel® 2016 using the following formulae.

**Table 8: Formulae used for data conversion.**

|  |  |  |
| --- | --- | --- |
| **First author (year)** | **Raw mean difference (*D*)** | **Standard error of *D (SED)*** |
| Ito (1990) | 13 | 2, 3, 4 |
| Van Dokkum (1999) | 13 | 2, 3, 4 |
| Alles (1999) | n/a | 5 |
| Vulevic (2008) | 64 | 7, 8, 9, 10, 11 |
| Vulevic (2013) | 64 | 7, 8, 9, 10, 11 |
| Pedersen (2016)1 | 12, 144 | 13, 7, 8, 9, 10, 11 |
| Pedersen (2016)2 | 64 | 15, 16, 17 |
| Canfora (2017) | 64 | 7, 8, 9, 10, 11 |

n/a, not applicable as this was directly reported.

1 For one outcome, HOMA-IR, only.

2 For all outcomes except HOMA-IR.

3 Difference between groups at follow-up.

4 Change scores; difference between groups over time, from baseline to follow-up.

Two crossover or Latin square studies (Ito et al. 1990, and van Dokkum et al. 1999) reported the mean and standard deviation at follow-up only. The mean difference (*D*) was calculated as:

(1)

For these crossover or Latin square studies (Ito et al. 1990 and van Dokkum et al. 1999), the standard error of the difference was calculated using equations appropriate for studies using matched groups (Borenstein et al. 2009):

The standard error of *D* is,

(2)

where the variance of *D* (*VD*) is,

(3)

where the standard deviation of the mean difference (*Sdiff*) is,

(4)

where *S1* and *S2* are the sample standard deviations at the follow-up time points of the two conditions (intervention and placebo, respectively), *r* is the correlation coefficient, and *n* is the number of pairs.

A correlation coefficient (*r*) of 0.7 was imputed as the correlation between intervention and placebo follow-up scores for the laxation outcomes (stool weight) presented in Table 2 for Ito et al. (1990) and van Dokkum et al. (1999). This value was chosen based on weak evidence from Sakata and Shinbo (2003). van Dokkum et al. (1999) also report lipid outcomes (total, LDL-, and HDL-cholesterol, and triglycerides, presented in Table 3) and glucose-related outcomes (glucose, and insulin, presented in Table 4). For these outcomes *r*=0.8 (as described by Demonty et al. 2009, and FSANZ 2010) and *r*=0.6 (based on unpublished capillary blood glucose data, as described by FSANZ 2016 p. 5) was imputed, respectively.

Alles et al. (1999), a parallel study, reported the difference and, therefore, additional calculations were not required for the mean difference (*D*). The standard error of the difference, obtained from the reported 95% confidence interval of the difference, was calculated:

The standard error of *D* is,

(5)

To convert the absolute difference in stool weight (mean ± SE; g/day) to a value relative to the GOS consumed (g/day per GOS gram), the mean difference and its standard error were both divided by the GOS intake level (g/day) used in the intervention condition. This approach was applied to all three studies: Ito et al. (1990); van Dokkum et al. (1999); and, Alles et al. (1999).

Two crossover studies (Vulevic et al. 2008, and Vulevic et al. 2013) reported the mean and standard deviation at both baseline and follow-up. The mean difference was calculated as:

(6)

For these crossover studies (Vulevic et al. 2008, and Vulevic et al. 2013), the standard error of the difference between groups over time (*SED*) was calculated as:

The standard error of *D* is,

(7)

where and are the sample standard errors of the change over time (from baseline to follow-up) for the intervention and placebo conditions, respectively, and are calculated as:

(8)

and,

(9)

where and are the sample standard deviations of the change over time (from baseline to follow-up) for the intervention and placebo conditions, respectively, and are calculated as:

(10)

(11)

where a correlation coefficient (*r*) of 0.8 was used as the correlation between repeated measures of all lipid outcomes (total, LDL-, and HDL-cholesterol, and triglycerides presented in Table 3; Demonty et al. 2009, FSANZ 2010) and *r*=0.6 for two glucose-related outcomes (glucose, and insulin, presented in Table 4; based on unpublished capillary blood glucose data, as described by FSANZ 2016 p. 5) of the studies by Vulevic et al. (2008) and Vulevic et al. (2013).

HOMA-IR was reported by Pedersen et al. (2016) as medians with interquartile ranges for both groups at baseline and follow-up. Means and standard deviations were estimated from the published medians and interquartile ranges. Due to a small sample size and a suggestion of a skewed distribution, the following formulae recommended by Wan et al. (2014) was used:

(12)



(13)



where,

*a* = the minimum value

*q1* = the first quartile

*m* = the median

*q3* = the third quartile

*b* = the maximum value

*n* = the sample size

Where was selected from Table 2 of Wan et al. (2014) based on Q, where *n = 4Q + 1,* and *n*=15 (placebo) and *n*=14 (intervention).

The estimated mean difference over time between groups for HOMA-IR of Pedersen et al. (2016) was then calculated using:

(14)

For HOMA-IR, reported by Pedersen et al. (2016), the estimated standard deviations derived from formula 13 were used to estimate the standard error of the difference between groups over time (*SED*) using formulae 7 to 11 above, with a correlation coefficient (*r*) of 0.6, based on unpublished capillary blood glucose data (as described by FSANZ 2016 p. 5) for the correlation between repeated measures.

Two parallel studies report the mean at both baseline and follow-up (Canfora et al. 2017 and all outcomes except HOMA-IR from Pedersen et al. 2016) and we calculated the mean difference over time between groups using formula 6 above.

For one of these parallel studies (Pedersen et al. 2016), which reported the SE at both baseline and follow-up for each condition for all outcomes except HOMA-IR (described above), the standard error of the difference between groups over time (for the former seven outcomes) was calculated as:

The standard error of *D* is,

(15)

where is,

(16)

where is,

(17)

where *SE1* and *SE2* are the sample standard errors of the change over time (from baseline to follow-up) for the intervention and placebo conditions, respectively, and *r* is the correlation coefficient.

A correlation coefficient (*r*) of 0.8 was used as the correlation between repeated measures of all lipid outcomes (total, LDL-, and HDL-cholesterol, and triglycerides presented in Table 3; Demonty et al. 2009; FSANZ 2010) and *r*=0.6 for three glucose-related outcomes (glucose, insulin, and HbA1C presented in Table 4; based on unpublished capillary blood glucose data, as described by FSANZ 2016 p. 5).

Another parallel study (Canfora et al. 2017) reported the standard deviation at both baseline and follow-up for triglyceride and glucose-related outcomes (glucose, insulin, and HOMA-IR). We calculated the standard error of the difference between groups over time using formulae 7 to 11, using a correlation coefficient (*r*) between repeated measures of 0.8 and 0.6 for the triglyceride and glucose-related outcomes, respectively.

*Statistical analyses*

Meta-analyses were conducted in Review Manager 5.3, developed by The Cochrane Collaboration (The Nordic Cochrane Centre 2014). Meta-analysis was performed using a random effects model and generic inverse-variance method. I2 was used to assess heterogeneity between interventions. It describes the “percentage of total variation across studies that is not due to chance” and 0%, 25%, 50% and 75% could be interpreted as indicating no, low, medium, and high heterogeneity respectively (Higgins et al. 2003). No meta-regression or sub-group analyses were conducted due to the small number of included studies, intake levels, and populations to compare.

The mean difference and its standard error of each comparison, together with the sample size for the intervention and control groups, was entered directly into Review Manager 5.3. Two of three studies measuring stool weight included two or three intervention arms (Alles et al. 1999 and Ito et al. 1990, respectively). To prevent double counting of the control group, we planned to include just one intervention arm; selecting the arm that tested an intake level closest to what is consumed in a normal diet (i.e. typically the arm with the lowest intake level). The mean differences are, however, very different in size and direction across the different intervention groups (e.g. see Table 2) and omitting some arms may increase the risk of biasing the outcome of the analysis. For this reason, we included all pair-wise comparisons and divided the shared control groups’ sample size evenly among the comparisons. For example, for the crossover study by Ito et al. (1990; n=12 with three pair-wise comparisons), we entered n=4 (control=12 divided by 3). Likewise, for Alles et al. (1999; parallel trial with two comparisons of independent groups), the shared control (n=13) was entered as n=7 in the meta-analysis.

1. SACN (2008) reports a 1.9 g and 0.3 g difference in stool weight per gram of test fibre for the low and high intake GOS condition, respectively, compared to the placebo. Our respective values are a 2.6 g and 0.2 g difference in stool weight per gram of test fibre. [↑](#footnote-ref-2)
2. Two *P* values are provided by Vulevic et al. (2013): *P*<0.0001 (tabulated); and, *P*<0.001 (in text). [↑](#footnote-ref-3)
3. Two *P* values are provided by Vulevic et al. (2013): *P*<0.005 (tabulated); and, *P*<0.0005 (in text). [↑](#footnote-ref-4)
4. Note that *P* values were corrected for multiple testing and significance was set at *P*<0.005 after Bonferroni adjustment. [↑](#footnote-ref-5)
5. Table 4 (see Section 1.4.1) presents the pre-intervention plasma insulin data extracted from Table 3 of Canfora et al. (2017). This differs to the baseline values presented in Table 1 by Canfora et al. (2017), which likely reflect data from the screening phase. The *P* value of 0.721 pertains to the latter values from Table 1 (mean ± SD: 19.1 ± 7.2 mU/L and 20.7 ± 6.7 mU/L for the placebo and intervention, respectively). [↑](#footnote-ref-6)