The effect of short-chain fatty acids on glycemic control in humans: A systematic review and meta-analysis

Running title: Short-chain fatty acids and glycemia in humans

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ACRONYMS AND ABBREVIATIONS: AE, adverse event; CI, confidence intervals; CHO, carbohydrate; FBG, Fasting blood glucose; FFAR, Free fatty acid receptor; FI, Fasting insulin; GLP-1, Glucagon-like protein 1; HbA1c; Glycated hemoglobin; HV, healthy volunteers; iAUC, incremental area under the curve; IGT, Impaired glucose tolerance; IR, insulin resistance; PICOS; population, Intervention, Comparison, Outcomes and Study; PBG, postprandial blood glucose; PI, postprandial insulin; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PYY, peptide YY; QUICKI, Quantitative Insulin-Sensitivity Check Index; RCT, Randomized controlled trial; SCFA, short-chain fatty acid; SD, Standard deviation; SMD, Standard mean difference; T2D, type 2 diabetes; XO, Crossover

ABSTRACT

Background

Non-communicable disease development is related to impairments in glycaemic and insulinemic response, which can be modulated by fiber intake. Fiber's beneficial effect upon metabolic health can be partially attributed to the production of short-chain fatty acids (SCFAs) via microbial fermentation of fiber in the gastrointestinal tract.

Objective

We aimed to determine the effect of the SCFAs, acetate, propionate, and butyrate on glycemic control in humans.

Methods

CENTRAL, Embase, PubMed, Scopus and Web of Science databases were searched from inception to the 07/12/2021. Papers were included if they reported a randomized, controlled trial measuring glucose and/or insulin compared to a placebo in adults. Studies were categorized by the type of SCFA and intervention duration. Random effects meta-analyses were performed for glucose and insulin for those subject categories with ≥ 3 studies, or a narrative review was performed.

Results

We identified 43 eligible papers, with 46 studies within those records (n=913), 44 studies were included in the meta-analysis. Vinegar intake decreased acute glucose response, standard mean difference (SMD) and (95% CI) -0.53 (-0.92, -0.14) (n=67) in individuals with impaired glucose tolerance or type 2 diabetes and in healthy (SMD) -0.27 (-0.54, 0.00) (n=186). The meta-analyses for acute acetate as well as acute and chronic propionate studies had no significant effect.

Conclusions

Vinegar decreased glucose response acutely in healthy and non-healthy. Acetate, propionate, butyrate, and mixed SCFAs had no effect on blood glucose and insulin in humans. Significant heterogeneity, risk of bias, and publication bias were identified in several study categories, including acute vinegar glucose response. As evidence was very uncertain, caution is urged when interpreting these results.

Further high-quality research is required to determine the effect of SCFAs on glycemic control. **PROSPERO registration number** CRD42021231115.

KEY WORDS: short-chain fatty acids, acetate, propionate, butyrate, glycemic control, systematic review, meta-analysis, insulin

INTRODUCTION

Non-communicable diseases such as type 2 diabetes (T2D) and cardiovascular disease accounted for 44% of global deaths in 2019 (1). T2D diagnoses have quadrupled globally, from 108 million to 422 million, in the last 40 years (2). Elevations in blood glucose and insulin play a significant role in non-communicable disease development, specifically of T2D (3–6). Improving glycemic control can reduce the risk of complications associated with T2D (7).

Diet is a primary risk factor for the development of non-communicable diseases. Western diets are often nutrient deficient, energy-dense and low in fiber (8) and populations following Western dietary patterns have high incidences of chronic disease (9,10). Fiber intake plays a determining role in non-communicable disease risk and is a strong indicator of all-cause chronic disease mortality risk (11). Previous human nutrition studies have shown that dietary fibers have a beneficial effect on glycemia (12).

Dietary fiber passes through the upper gastrointestinal tract undigested and can act as a substrate for bacterial fermentation throughout the gut (13). After undergoing fermentation in the gastrointestinal tract, 10g of fiber yields approximately 100 mmol/L of short-chain fatty acids (SCFAs). Acetate, butyrate, and propionate are produced in the largest quantities at a molar ratio of 3:1:1 respectively (14). SCFAs activate G-protein coupled receptors, known as free fatty-acid receptors (FFAR) -2 and -3 which are expressed in the gut and in metabolically active tissues such as liver, adipocytes, myocytes and pancreas (15). *In vitro* and animal studies have shown SCFAs influence glucose metabolism in glucose-disposal tissues such as hepatocytes (16), adipocytes (17), and myocytes (18). These SCFAs have been shown to directly stimulate the release of anorectic hormones such as glucagon like peptide-1 (GLP-1) and peptide YY (PYY) in colonic enteroendocrine cells (19–21). However, increasing fiber intake at population level has proven challenging. Hence, providing a similar metabolic benefit via alternative methods is the aim of much current research.

Overall, evidence suggests that SCFAs may influence human glucose homeostasis. Compiling the studies exploring the impact of SCFAs on glycemic control may help to elucidate the therapeutic

potential of SCFAs within the systemic circulation and gut, when administered at concentrations at or above that produced when the recommended fiber intake (30g/d) is consumed. Here, we aim to investigate the effects of SCFA administration on glycaemic control.

METHODS

A systematic review of peer-reviewed literature published since inception was performed. The systematic review was conducted in adherence to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (22). The formal screening of papers begun the 15th of November 2020 and the registration of the protocol for this review to PROSPERO was submitted on the 14th of January 2021 with the reference CRD42021231115. The PRISMA checklist for this review can be found in the **Supplemental Material.**

Eligibility Criteria

The PICOS (patients, intervention, comparator, outcome, study design) criteria were used to establish study eligibility (**Table 1**).

Search Strategy

The online databases PubMed (Medline), Cochrane CENTRAL, EMBASE, Web of Science and Scopus were used to identify records published from inception to December 7th, 2021. The search algorithm used for each database is described in **Supplemental Table 1**. In addition, a manual search of reference lists of reviews on the topic was performed, to identify additional relevant articles. When necessary, the authors were contacted to obtain data of interest. Studies were excluded if authors did not respond.

Study Selection

The study selection was performed using the online software Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia. Available at www.covidence.org). All articles identified by the search strategy were screened by title and abstract by 2 reviewers independently (S.A and J.E.P). Post-screening, full texts deemed to be potentially relevant were assessed for eligibility against the defined inclusion and exclusion criteria independently (S.A and J.E.P) (**Table 1**). During

study screening and assessment, any discrepancies in eligibility of papers were resolved by consulting a third party (A.C-M). Excluded studies and reasons for exclusion can be found in **Supplemental Table 2**.

Data extraction and quantification

Data was extracted by four reviewers independently (S.A, A.C-M, D.H. and J.E.P). Articles deemed eligible for inclusion were assigned to subject categories according to the nature of the study intervention (acute or chronic), type of SCFA (acetate, butyrate, propionate, mixed or vinegar (acetic acid)). Study characteristics were extracted for each category including first authors' last name, publication year, study design, length of intervention (acute (<24h) or chronic), sample size, participants demographic characteristics (gender, age, BMI, any health conditions), SCFA concentration, route of administration (oral, intravenous, gastrointestinal), measurement period, energy and macronutrient matching, and outcomes analyzed. Some identified records contained multiple studies, which were extracted individually. Whilst all comparisons within the same record were captured in the summary of studies tables, not all comparisons were meta-analyzed. Selection of the comparisons against the control was based on the highest dose, or based on the format used in real-life (e.g. liquid vinegar over pill). **Table 2** summarizes the eligible study characteristics.

Data synthesis and statistical analysis

Descriptive data were reported as mean ± standard deviation (SD) unless otherwise stated. Glycemic control measurements included blood glucose and insulin (raw or change from baseline) area under the curve (AUC) or incremental area under the curve (iAUC), fasting blood glucose or insulin, glycated hemoglobin (Hb1Ac) (%), or insulin sensitivity indexes (e.g., homeostatic model assessment-insulin resistance). For the subject categories which included <3 studies, a narrative review was conducted in accordance with the Cochrane handbook for systematic review of interventions (23). For categories which had ≥3 studies, a meta-analysis was performed. For the meta-analysis, raw data or changes from baseline iAUC were extracted, and variance was transformed to SD. When data was available as individual time points, means and variance were extracted using the

online tool WebPlotDigitizer 4.4 (Available in: https://apps.automeris.io/). Then, the iAUC of the mean and variance was calculated by the trapezoidal rule (24).

Meta-analysis

A meta-analysis to estimate the pooled effect of the SCFAs on the different glycemic outcomes, was performed for each subject category that included ≥3 studies reporting the same glycemic outcome. These were: acute acetate, acute vinegar, and acute and chronic propionate administration. For acute studies, meta-analyses of postprandial glucose (PBG) and insulin iAUC were performed. For chronic studies, meta-analyses of PBG and insulin iAUC, fasting glucose and insulin, and glycated hemoglobin (HbA1c) were performed. For these outcomes for each subject category, the weighted effect estimates as standardized mean difference (SMD) and corresponding 95% CIs were calculated as using a Sidik-Jonkman random-effects model to allow a wide 95% CI to reflect uncertainty in the estimation of between-study heterogeneity. For crossover studies, the SMD was calculated by assuming a parallel design for a more conservative analysis. Heterogeneity was assessed using the I^2 statistic and visual inspection of the Galbraith plot (Supplemental Online Material). I^2 values range from 0% to 100%, with values of 25% to 49%, 50% to 74% and ≥75% classified as low, moderate, and high, respectively (25). Confidence intervals were determined using the 'heterogi' command in Stata. Publication bias was assessed via funnel plots for each meta-analysis (Supplemental Online Material). To assess influential studies, a sensitivity (leave-one-out) analysis was performed for all categories (Supplemental Online Material). Statistical significance was determined at a p-value ≤0.05. All reported P values are 2-sided. All statistical analyses were performed with Stata 17.0.

Risk-of-bias assessment

Studies were assessed for risk of bias by 3 independent reviewers (S.A, J.E.P, D.H) following the revised Cochrane risk-of-bias tool for randomized trials (26). The 6 methodological features assessed were randomization, assignment of intervention, adherence to intervention, missing outcome data, measuring the outcome and selection of the reported result. Studies were classified as "high risk" if they contained methodological flaws that may have influenced the results, "low risk" if the flaw was

not deemed to have affected the results and "some concerns" if not enough information was provided to pass a judgement. Disagreements in the classification were resolved by consulting a third party (A.C-M).

Certainty of evidence - Grading of Recommendations, Assessment, Development and Evaluation (GRADE)

The summary of findings table was constructed using GRADEpro software (http://gradepro.org, accessed 10th December 2021). Certainty of evidence was assessed using Grading of Recommendations, Assessment, Development and Evaluation (GRADE) recommendations. Certainty of evidence was graded as high, moderate, low, and very low (27).

Assessment of confounders of glycemic control

Studies that controlled for factors influencing glycemic response may produce more accurate results. Acute confounders of glycemic control are physical activity, length of fasting period and fiberor alcohol intake. Chronic confounders include changes in body weight and body fat percentage over the course of the study. Included studies were assessed for how they controlled for elements known to confound glycemic control (e.g. body weight or body fat change, physical activity or alcohol intake prior to the intervention or an overnight fast) and the results were summarized in **Supplemental Table 3**.

RESULTS

Description of studies

The systematic literature search produced a total of 5932 references following the database and manual search (**Figure 1**). Specifically, 3514 publications were identified from PubMed, 289 from Cochrane CENTRAL, 1018 from EMBASE, 325 from Web of Science, 786 from Scopus and 1 from the manual search. Duplicates (n=1862) were removed. After screening, 4014 records were excluded. A total of 56 full-text articles were assessed for eligibility against the PICOS criteria, 14 of which were excluded. This yielded 43 records to be included in this review. Some records had more than 2

intervention arms/test interventions or more than 2 studies within the same record. This was the case for acetate studies such as Scheppach *et al*., 1988 (28) (2 studies within the same record), and vinegar studies such as Brighenti *et al.,* 1995 (29) (2 interventions vs. same control), Johnston *et al*., 2005 (30) (2 arms with different food matrices), Johnston *et al*., 2010 (31) (3 studies within the same record), Liatis *et al*., 2010 (32) (2 arms with different glycemic indexes), Darzi *et al*., 2014 (33) (2 studies within the same record) and Feise *et al*., 2020 (34) (3 interventions vs. same control). Nevertheless, not all comparisons were meta-analyzed. The comparisons chosen for meta-analysis are described in the footnote of each forest plot. Therefore, from the 43 identified records, there were 52 studies within those records, 44 of which, were included in the meta-analysis. Five investigated acetate (all acute interventions), two investigated butyrate (both reporting the same chronic study), 14 investigated propionate (8 acute and 6 chronic interventions), 31 studies investigated vinegar (25 acute and 6 chronic interventions), and five investigated mixed SCFAs.

Tables 2-6 describe the study design and participant characteristics of all eligible studies. In the interest of clarity, SCFA interventions will be referred in the text in the simple form of acetate, butyrate, or propionate. However, some studies have used different compounds of the SCFAs, e.g., sodium propionate.

Risk of bias in included studies

The risk of bias for the included studies is described in **Figure 2**. Out of the studies, 47% were determined to have a high risk of bias, 44% a moderate risk of bias and 9% a low risk of bias. The domains of greatest concern were risk of bias arising from deviations from intended interventions (D2.2), risk of bias in measurement of the outcome (D4) and in selection of reported result (D5).

Effects of interventions

Acetate

The characteristics of the eligible acetate studies are summarized in **Table 2**.

Acute interventions

Seven studies were meta-analyzed for blood glucose (28,35–37) and 6 for insulin (28,35–37). Forest

plots of the pooled effect of acetate interventions on postprandial blood glucose and insulin are shown in **Figure 3 and 4**.

A random-effects model showed that acute acetate interventions had no effect on postprandial blood glucose (SMD = 0.09; 95% CI: -0.26, 0.44; n = 44) and non-significant interstudy heterogeneity (I^2= 23.1%, p=0.59; 95% CI: 0, 71) (**Figure 3**). For insulin, acute acetate interventions had no significant effect on postprandial blood insulin iAUC (SMD = 0.35; 95% CI: -0.07, 0.77; n=35) and moderate interstudy heterogeneity (I^2= 30.7%, p=0.53; 95% CI: 0, 75) (**Figure 4**).

Homogeneity via Galbraith plot, publication bias via funnel plot and sensitivity analysis via leave-one out plot were assessed and reported in **Supplemental Figures 1-2.**

Vinegar

The characteristics of the vinegar studies eligible are summarized in **Table 3**.

Acute interventions

During the literature search, 15 studies within 11 references were identified that investigated the effects of acute vinegar administration on glycemic control. Of these 15 studies were meta-analyzed (38,29,39,33,30,40,41,31,42,34,43,41) for post-prandial blood glucose response in healthy individuals and seven in metabolically compromised individuals (31,32,43–45). Five of the healthy volunteer studies (30,40,42,43) and six of the non-healthy volunteer studies (32,43–45) were also meta-analyzed for post-prandial insulin response.

Forest plots of the pooled effect of vinegar interventions on postprandial blood glucose and insulin are shown in **Figure 5 - 8**. For blood glucose, a random-effects model showed that acute vinegar interventions had a significant effect on postprandial blood glucose in healthy subjects (SMD = -0.27; 95% CI: -0.54, 0.00, n = 186) (**Figure 5**). Interstudy heterogeneity was significant (I^2 = 66.2%, p = 0.001; 95% CI: 48, 82). Acute interventions with vinegar had a significant effect on postprandial blood glucose in subjects with impairments in glucose tolerance (SMD = -0.53; 95% CI: -0.92, -0.14, n = 67) (**Figure 6**). Interstudy heterogeneity was not significant (I^2 = 53.0%, p=0.11; 95% CI: 0, 75).

Acute interventions with vinegar had no significant effect on postprandial insulin (PI) in healthy subjects (SMD = -0.29; 95% CI: -0.66, 0.08; n = 55) (**Figure 7**). The studies had non-significant heterogeneity (I^2 = 44.6%, p = 0.21; 95% CI: 0, 74). Acute interventions with vinegar had no significant effect on PI in subjects with impaired glucose tolerance (IGT) or T2D (SMD = -0.16; 95% CI: -0.75, 0.44, n = 58) (**Figure 8**). Substantial heterogeneity was seen between the studies included in this meta-analysis (I^2=77.0%, p = 0.001; 95% CI: 39, 88).

Homogeneity via Galbraith plot, publication bias via funnel plot and sensitivity analysis via leave-one out plot were assessed and reported in **Supplemental figures 3-7.**

Chronic interventions

During the literature search, 7 chronic intervention studies using vinegar were identified, 6 were included in the meta-analysis (46–51) investigating fasting blood glucose. Chronic interventions with vinegar had no significant effect on fasting glucose (SMD = -1.60; 95% CI: -4.30, 1.09; n = 143) (**Figure 9**). Interstudy heterogeneity was significant (I^2= 99.0%, p=0.001; 95% CI: 92,97).

Three studies were identified which investigated the effect of chronic vinegar on fasting insulin response (46–48). Chronic interventions with vinegar had a significant effect on fasting blood insulin (SMD = 0.06; 95% CI: -0.50, 0.62; n = 89) (**Figure 10**). Interstudy heterogeneity was substantial (I^2= 72.2%, p=0.03; 95% CI: 6, 92).

Homogeneity via Galbraith plot, publication bias via funnel plot and sensitivity analysis via leave-one out plot were assessed and reported in **Supplemental Figure 8.**

Two eligible studies (49,50) investigated the effect of chronic vinegar supplementation on HbA1c in individuals with T2D. Patients were supplemented with 15 mL and 20 mL of vinegar for a month or 10 weeks, respectively (49,50). Both authors reported a significant decrease in HbA1c of 7% and 9%, whereas in the placebo group HbA1c decreased by 1% and increased by 2%, respectively (49,50).

Three studies investigated the degree of insulin resistance, using Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), following chronic vinegar intake (46–48). Two of these were

investigated a healthy cohort, showing that while a 4-week supplementation of 21g of vinegar did not result in a significant change compared to the control group (48), an 8-week supplementation led to a significant decrease of 8% in HOMA-IR compared to the control (47). One study supplemented with vinegar people with T2D for 8 weeks and reported that HOMA-IR, Quantitative Insulin-Sensitivity Check Index (QUICKI) and HOMA-beta was not significantly different compared to the control group (46).

Butyrate

The characteristics of the eligible butyrate studies are summarized in **Table 4**.

Chronic interventions

Two interventions reported glycemic outcomes following a chronic intervention with butyrate (52,53). These two records described the same study, so a meta-analysis was not possible. In this study, 60 participants with type 2 diabetes were randomized in a parallel design to 4 groups (n=15 in each) in which they had to consume 6 (100 mg) oral capsules and 10g of powder a day for 45 days. Two of the interventions were assessed in this review. These were sodium butyrate capsules and starch powder (intervention a), and starch capsules and starch powder (control). One publication reported no significant difference in fasting blood glucose, postprandial blood glucose at 2h, fasting insulin, HbA1c and HOMA-IR post-intervention compared to the control (52). The other reported QUICKI, which was not significantly different from control following the 45-day intervention (p=0.137) (53). GLP-1 secretion significantly increased following the butyrate intervention, compared to the control by 22.57 pg/ml (p=0.008) when adjusted for baseline value, BMI and blood pressure (52).

Propionate

The characteristics of the eligible propionate studies are summarized in **Table 5**.

Acute interventions

Eight studies were found to investigate the effects of acute propionate administration on glycemic control. Of these, all were meta-analyzed (36,54–60) for glucose and seven were analyzed for insulin (36,54,56–60).

Forest plots of the pooled effect of propionate acute interventions on glycemic outcomes are shown in **Figure 11 - 12**. For postprandial blood glucose (**Figure 11**) and insulin (**Figure 12**), random-effects model of the acute interventions with propionate had no significant effect (SMD = 0.07; 95% CI: -0.32, 0.47; n = 123 and SMD = 0.24; 95% CI: -0.30, 0.78; n = 117). Interstudy heterogeneity was non-significant for both outcomes (I^2= 75.8%, p = 0.14; 95% CI: 0, 72 and I^2= 86.7%, p =0.12; 95% CI: 0,75, respectively).

Homogeneity via Galbraith plot, publication bias via funnel plot and sensitivity analysis via leave-one out plot were assessed and reported in **Supplemental figures 9-10.**

Chronic interventions

Five studies were found to investigate the effects of chronic propionate administration on glycemic control, all of which were meta-analyzed for postprandial blood glucose (55,61–64). Four studies were identified for PI (61–64). Four studies measured fasting glucose and insulin (61–64).

Forest plots are shown in **Figures 13-16**. Chronic interventions with propionate had no significant effect postprandial blood glucose (**Figure 13**) or insulin (**Figure 14**) iAUC (SMD = -0.08; 95% CI: -0.43, 0.27; n = 73 and SMD = -0.06; 95% CI: -0.39, 0.27; n = 67). Interstudy heterogeneity was non-significant for PBG and PI (I^2= 15.3%, p=0.79; 95% CI: 0, 79 and I^2=0.3%, p=0.97; 95% CI: 0, 85). Chronic interventions with propionate had no significant effect on fasting blood glucose (**Figure 15**) or insulin (**Figure 16**) (SMD = -0.14; 95% CI: -0.47, 0.19; n = 67 and SMD = -0.22; 95% CI: -0.65, 0.21; n = 67). Interstudy heterogeneity non-significant glucose and insulin (I^2= 1.3%, p=0.93; 95% CI: 0, 85 I^2= 37.4%, p=0.26; 95% CI: 0, 88).

Homogeneity via Galbraith plot, publication bias via funnel plot and sensitivity analysis via leave-one out plot were assessed and reported in **Supplemental Figures 11-14.**

Mixed SCFAs

The characteristics of the eligible studies using mixed SCFAs are summarized in **Table 6.**

Acute administration

Five studies (36,65–68) were found to investigate the effects of acute mixed SCFA administration on glycemic control, all of which were given via the ileum or rectum. Wolever and colleagues rectally infused different ratios of SCFA mixtures: acetate 180 mmol/L and propionate 60 mmol/L or acetate 90 mmol/L and propionate 30 mmol/L, and an isotonic saline solution into 6 healthy individuals (65). Neither solution induced a change in blood glucose concentrations. However, the high acetate mixture led to a decrease in free fatty acids and an increase in total cholesterol and triglyceride concentrations.

The same research team then rectally administered a combination of acetate (180 mmol/L) and propionate (60 mmol/L) to the same population, compared to acetate, propionate, and saline solutions alone (66). The results showed that blood glucose increased by +0.16 mmol/L ($p \leq 0.05$), and that insulin decreased by –17 pmol/L ($p \leq 0.05$) with the mixed SCFAs administration compared to acetate alone, independently on changes in glucagon, free fatty acids, and total cholesterol.

Another study in healthy lean males, who received a 18h ileal perfusion of SCFAs (acetate 60 mmol/L, propionate 25 mmol/L, butyrate 15 mmol/L) alone (67). This was followed by saline solution at 12h. The study showed that mixed SCFA administration did not have an effect on insulin sensitivity, basal hepatic glucose production, or concentrations of triacylglycerol, total cholesterol and insulin between the three conditions (67).

Canfora and team used a rectal infusion of 200 mmol/L of high-acetate, -propionate, -butyrate or placebo solutions in healthy participants, followed by a 75 g glucose load (68). There was no differential effect on blood glucose or insulin levels between the 3 SCFA mixtures.

Certainty of evidence

The certainty of evidence ranged from low to very low for all glycemic outcomes in all SCFA investigated. GRADE assessments of each outcome can be found in **Tables 7-11**. Certainty of evidence was downgraded due to high risk of bias, imprecision due to small sample size and wide confidence intervals and differences in study methodology, dose size and study population.

Furthermore, for many of the studies glycemic response was not the primary outcome reducing the likelihood that researchers would be able to detect an effect.

Confounders of glycemic control

The studies were assessed for controlling for known confounders of glycemic control (body weight and fat change, standard evening meal, whether fiber, strenuous exercise and alcohol were avoided, and whether an overnight fast was completed prior to study visit) which are were summarized in **Supplemental Table 3**.

In acute studies, one acetate (28) and three vinegar (31,33,41) studies instructed participants to consume a standard evening meal. Participants in four vinegar studies (31,33,34,41) and two propionate studies (54,57), avoided strenuous physical activity. One acetate study (28), three vinegar studies (33,34,41) and three propionate studies (54,57,59) discouraged participants from consuming alcohol. Three acetate studies (28,35,36), one vinegar study (54), two propionate studies (36,54) and two mixed vinegar studies (36,67) prescribed a low fiber diet before the study visit. All acute studies, excluding one which provided no information (29), required participants to fast for more than 6 hours before the study visit.

In chronic studies, 50% of studies accounted for changes in body weight and body fat (52,53,55,61–64,69). Three chronic propionate studies instructed participants to consume a standard evening meal (62,63,69). Four propionate studies instructed participants to avoid strenuous physical activity (61–63,69). One vinegar study (50) and four propionate studies (61–63,69) instructed participants to abstain from alcohol before the study visit. All studies requested participants to fast for > 6 hours before the visit.

Adverse Events

The Adverse Events (AEs) reported for each intervention arm for each study category were assessed **(Supplemental Table 4)**. Adverse event data was not disclosed in all publications.

In acute interventions, no AEs were reported for acetate nor vinegar (35,41,44,45). Two studies reported AEs for propionate interventions; one case of nausea was reported (57) and one documented no AEs (54). Two studies reported AEs for mixed SCFA administration, and one had up to 6 incidences of belching, in both intervention and control groups (65), the other reported no AEs (68).

In chronic interventions with propionate, there were 3 incidences of flatulence when consuming inulin-propionate ester bread (61). Another study with propionate reported 6 cases of nausea, 2 cases of constipation, 1 case of flatulence and 4 cases of vomiting were reported in the intervention group, although nausea, constipation and flatulence was also reported in the control group (64). One study with propionate recorded AEs but did not have any to report (63). Three vinegar studies reported AEs (47,48,50), but only one reported one case of nausea, stomach ache and headache (48).

Compliance

Study adherence to the intervention for each study category was assessed (**Supplemental Table 5)**. Withdrawals were defined as participants that dropped out after randomization. One acute study (35), reported a 40% withdrawal rate, researchers failed to clip the catheter to the colonic mucosa, which meant SCFA could not be administered. Studies acutely supplementing propionate had 100% adherence, whereas chronic interventions had an average of 12% withdrawal, one study reported a participant withdrawal due to nausea (64). Studies using chronic butyrate had a 1.7% withdrawal due to lost to follow up (52,53).

DISCUSSION

A total of 43 publications with 46 studies and 913 participants were incorporated into our analysis, which showed that acute vinegar administration had a favorable effect on blood glucose in subjects with T2D or IGT and healthy participants. Acute and/or chronic administrations of acetate, vinegar, propionate, butyrate and mixed SCFAs had no effect on glycemic measures including FBG, FI, PBG and PI. A summary of the results of the meta-analyses can be found in **Table 12.**

Acute SCFA administration

The effect of acute SCFA administration upon glycemic response has been explored using acetate, vinegar, propionate and mixed SCFA. Doses varied highly from 12 – 200 mmol/L and duration ranged from 60 to 1080 minutes. Our findings, which suggest that acute vinegar influences PBG, correspond with a recent meta-analysis (70), in which all participants were pooled and vinegar was shown to reduce glucose and insulin concentrations. This could bode positively for future treatments to halt the progression of glycemic deterioration.

Previous studies have suggested that vinegar, propionate and acetate delay gastric emptying, slowing the rate of glucose absorption from a meal (54,56,71,72). Moreover, vinegar and propionate could inhibit digestive starch enzymes (55,73), although digestion could be modulated by phenolic compounds rather than by the presence of acetic acid in vinegar (74). Increased fecal bulk was also reported after propionate administration (55), suggesting that undigested starch could be reaching the colon, thereby reducing glycemic load. Furthermore, acetate and propionate administration promoted gluconeogenesis in rodent studies (75,76), but gastric administration of propionate was not shown to have any effect (36). One study (66), reported an increase in PBG and a reduction in PI after administration of a propionate-acetate mixture, compared to acetate alone, suggesting that propionate has gluconeogenic potential (77).When acetate is administered via the distal colon (35), it is able to bypass oxidation by the liver and enter the systemic circulation via rectal venous plexus (68). Reductions in circulating free fatty acids have been observed after propionate and acetate administration, which could suggest acetate found in the peripheral circulation may influence fat oxidation (28,35,36,59,60,65,68). However, when acetate was administered via constant gastric infusion in rats in another study, researchers reported increases in lipogenesis and insulin resistance (78).

In the few acute studies which recorded AEs, nausea and belching was reported. The lack of AE reporting makes it difficult to determine the tolerability and safety of SCFA administration. Reported

withdrawals were due to methodological issues, non-compliance, unpleasant taste of intervention and participant availability. Although there were AEs, results suggest SCFAs could be tolerated by participants. Unfortunately, this cannot be confirmed as most studies did not report compliance or withdrawals.

Acute vinegar supplementation was shown to significantly improve PBG in all participants. However, the GRADE certainty of evidence for all acute outcomes, except for mixed SCFA was very low. Caution should be taken when interpreting these results as there is still little to no certainty that acute SCFA administration has any effect on PBG and PI. The low certainty of evidence and lack of significant results in this systematic review stems from variability in the route of administration, dosage, participant health and sample size demonstrated by the high heterogeneity of some outcomes (PBG in HV and PI in unhealthy volunteers after vinegar supplementation) and wide I^2 95% CIs. Furthermore, publication bias was detected in several outcomes, (excluding PBG for vinegar (HV), propionate and mixed SCFA and PI for propionate). Sensitivity analyses also indicated that three studies (29,31,38), were driving the outcome for acute PBG of vinegar (HV) and one study (40), had a significant influence on the outcome of acute PI in HV. These factors influenced the precision and directness of the outcomes which downgraded the quality of evidence.

Chronic SCFA administration

The effect chronic SCFA supplementation in the form of vinegar, butyrate and propionate has been investigated. Doses were from 12 - 200 mmol/L and study durations ranged between 2 to 70 days. All interventions were orally administered, and some studies used alimentary vehicles, such as bread, smoothies, cheese, and dietary fibers. In this review and meta-analysis, chronic supplementation of SCFAs had no significant effect on PBG, PI, FBG or FI.

Two studies administered more than 15 ml of vinegar per day and reported a significant reduction in fasting blood glucose (46,50). Reducing the rate of gastric emptying, via vinegar administration, could have an effect on fasting glucose and insulin concentration in the long term (65,71,72). However, this is yet to be extensively studied. Some studies attributed changes in fasting glycemia to reduced oxidative stress which can be associated with both acetic acid and phenolic compounds present in

vinegar (79,80), but only one study reported an increase in 2,20-Diphenyl-1-picrylhydrazyl, a free-radical (46). Oxidative stress is associated with reductions in insulin sensitivity and glycemic deterioration (81).

Chronic sodium butyrate supplementation showed no effect on glycemic measures compared to a starch placebo. Furthermore, a study using ^{13}C-labelled butyrate administered in the colon demonstrated that only 2% of butyrate is found systemically (82). At this concentration it is unlikely that butyrate would exert an effect on metabolically active tissues such as adipocytes and skeletal muscle to alter glucose tolerance.

Chambers and colleagues found that fasting insulin, Matsuda index (a measure of insulin sensitivity) and HOMA-IR improved when propionate was supplemented compared to the cellulose group, but were not significantly different to the inulin control (62). Perhaps reinforcing previous findings suggesting that incubation with propionate inhibits apoptosis and stimulate insulin release in pancreatic beta-cells *in vitro* (69). As inulin can be used for bacterial fermentation it is not possible to distinguish whether the effect seen was due to increased propionate or overall SCFA production.

An increase in 2-h postprandial GLP-1 was detected following the butyrate intervention (52). Butyrate and other SCFA act as signaling molecules in the colon, binding to FFAR2 and FFAR3 expressed in the enteroendocrine L-cells and triggering the release of gut hormones such as GLP-1 (83). Chronic propionate administration to adipocytes expressing FFAR2 has been shown to inhibit lipolysis and reduce circulating non-esterified fatty acid concentrations (92, 95). Maintaining low circulating concentrations of non-esterified fatty acids may prevent β-cell dysfunction and peripheral insulin resistance over time (85).

Adverse events reported included nausea, flatulence, constipation, vomiting and belching. Reported withdrawals were due to non-compliance, lost to follow up, personal reasons, AEs both unrelated and related (vinegar, propionate) to the intervention, consent withdrawal and unpleasant taste of intervention (vinegar). In some cases, withdrawal reasons were not reported, thus whether they are related to the intervention is not known. These results could suggest that these interventions, are not feasible or tolerable for chronic supplementation in a free-living population.

The GRADE certainty of evidence for all chronic outcomes was very low. Caution should be taken when interpreting these results as there is still little to no certainty that chronic SCFA administration has any effect on PBG, PI, FBG or FI. High heterogeneity was detected in chronic FBG and PBG response to vinegar supplementation and wide 95% CIs were seen for all heterogeneity results excluding chronic vinegar supplementation and postprandial glucose response, indicative of lower certainty of heterogeneity value. Furthermore, publication bias was detected in all chronic outcomes. These factors influenced the precision, consistency and directness of the outcomes which downgraded the quality of evidence.

Future research on SCFAs and glycemic control

Further high-quality double-blind RCTs using standardized study methodology (sites of administration, types of interventions), powered to detect changes in glycemic response are required. Future studies should employ gold-standard methodology (e.g., euglycemic hyperinsulinemic clamps), to elucidate the impact of SCFA on insulin sensitivity. Studies should also account for and report glycemic confounders such physical activity and body weight change. Researchers should ensure test foods are blinded and palatable to avoid triggering delayed gastric emptying and confounding results. Further research should attempt to elucidate the effect of SCFA interconversion in the colon by gut microbiota on host glucose metabolism. Furthermore, tracer and dose response studies could help to determine optimal concentrations of SCFA and the role of SCFA in different metabolic states. Lastly, improving understanding of FFAR desensitization during chronic administration of SCFAs, may provide insight into how chronic SCFA supplementation might play a therapeutic role in future.

Strengths and limitations

One strength of this systematic review is that studies were separated according to categories to increase homogeneity. By separating individuals based on health status, we were able to demonstrate that vinegar supplementation may influence glycemia in metabolically compromised individuals, which to our knowledge, was previously unreported. One limitation of this review is that the paired

nature of data from crossover studies was not accounted for so these studies may be underweighted. Our restrictive inclusion criteria identified a small and heterogeneous pool of studies for each subject category. Some records did not report uniform glycemic measures e.g., area under curve, for acute interventions and could not be included in the meta-analysis, which highlighted the need to standardize glycemic outcome reporting to aid future meta-analysis. In this review, we have analyzed the PBG and PI via the iAUC, potentially masking any significant differences at specific time points (e.g., first phase insulin response), which could be informative of metabolic disease progression for subjects with or at risk of T2D.

CONCLUSION

The present systematic review and meta-analysis found that acetate, butyrate and propionate have no effect on glycemic control. Acetic acid, in form of vinegar acutely reduced blood glucose in adults with T2D and IGT and healthy adults. This evidence comes from a very limited and heterogeneous number of studies for all categories with a moderate to high risk of bias and a low to very low certainty of evidence. Future high-quality research should be focused on investigating the effect of both acute and chronic interventions of SCFAs with glycemic control measures as the primary outcome in subjects of all health statuses. Such studies should be controlled for confounders that can affect glycemia to ensure that high-quality evidence is produced.

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AUTHOR'S RESPONSABILITIES

The authors' contributions were as follows: A.C-M and J.E.P wrote the introduction; S.AA and J.E.P designed the research; J.E.P and A.C-M conducted the database search; S.AA and J.E.P. screened the articles; A.C-M resolved conflicts during screening; A.C-M, J.E.P, S.AA, D.H extracted the data; A.C-M conducted the meta-analysis and A.C-M and J.E.P wrote the methods sections; all authors interpreted the findings and wrote the discussion. A.C-M has primary responsibility for final content. All authors read and approved the final manuscript.

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Table 1. PICOS Criteria for study eligibility		
	Inclusion	**Exclusion**
Participants	Humans who are healthy, overweight, or obese, have metabolic syndrome or type 2 diabetes.	Other type of diseased humans and animals. Humans undergoing clamps such as hypoglycemic or hyperinsulinemic euglycemic clamp which do not represent a real physiological setting.
Intervention	Acetate, propionate, butyrate alone or mixed and vinegar administration. Both acute (for 24h) and chronic (over 24h) administrations	SCFA conjugated with drugs or hormones.
Comparator	Placebo	Against diseased humans, between different doses of SCFA.
Outcome	Quantifiable measures of glycemic control as main or secondary outcome such as fasting glucose/insulin, postprandial glucose/insulin (i.e. area under the curve), Hb1Ac, insulin sensitivity indexes (i.e. HOMA-IR, clamps)	Studies which do not include a quantifiable measure of the outcomes of interest.
Study design	Study designs which generate empirical data from interventional studies which are randomized controlled trials (RCTs). Only results analyzed statistically will be included.	Reviews, conference abstracts, dissertation abstracts, lectures, information pieces, study registers and *corrigendums* were not included. Studies were limited to English language published from 1980 onwards.

Table 2. Summary of the acetate studies design, participant and intervention characteristics and outcome analyzed

		Participant's characteristics				Intervention characteristics				
Reference	**Study design**	**Health status**	**Sample size (M/F)**	**Age (y)**	**BMI (kg/m^2)**	**Amount given (rate)**	**Route of administration**	**Duration**	**Control**	**Glycemic outcome analyzed**
Acute										
Scheppach 1988 (study 1) (28)	RCT, XO	HV	5	33	22.4	195 mmol (15mmol/15 min) +50g CHO	Oral (drink)	360min	Chloride	PBG PI
Laurent 1995 (36)	RCT, XO	HV	6 (3/3)	22	21.2	12 mmol/h	Intragastric (infusion)	300min	Saline	PBG PI
Freeland 2010 (31)	RCT, XO	Hyper-insulinemic	6 (0/6)	44	31	20 mmol/L (12.5ml/ min)	Intravenous (infusion)	60min	Saline IV	PBG PI
						60 mmol/L (37.5ml/ min)	Rectal (infusion)	60min	Saline R	PBG PI
Van der Beek 2016 (35)	RCT, XO	HV	6 (6/0)	35	31	100-180 mmol/L	colonic (infusion)	300min/d x3d	Saline	PBG PI

CHO, carbohydrate; d, day; FBG, fasting blood glucose; FI, fasting insulin; HV, healthy volunteers; M, matched for energy and macronutrients; min, minutes; NI, no information; PBG, postprandial blood glucose; PI, postprandial insulin; RCT, randomized controlled trial; T2D, Type 2 diabetes; XO, cross-over

Table 3. Summary of the oral intake of vinegar studies design, participant and intervention characteristics and outcome analyzed

Reference	Study design	Health status	Sample size (Male/ Female)	Age (y)	BMI (kg/m^2)	Amount	Type of vinegar	Acetic acid (%)	Duration	Co-ingested with CHO (g)	Control	Outcomes analyzed
Acute												
Brighenti 1995 (29)	RCT, XO	HV	5 (4/1)	37	98	20 ml	White (acetic acid from vinegar)	5	95 min	50	White Bread	PBG
Johnston 2004 (43)	RCT, XO	HV	8	NI	NI	20 g	Apple cider	5	60 min	87	Placebo drink (not specified)	PBG PI
Johnston 2004a (43)	RCT, XO	T2D	11	NI	NI	20 g	Apple cider	5	60 min	87	Placebo drink (not specified)	PBG PI
Johnston 2004b (43)	RCT, XO	IR	10	NI	NI	20 g	Apple cider	5	60 min	87	Placebo drink (not specified)	PBG PI
Johnston 2005a (30)	RCT, XO	HV	11 (1/10)	27.9	22.7	20 g	Apple cider	5	60 min	87	Sweetened water	PBG PI
Johnston 2005b (30)	RCT, XO	HV	11 (1/10)	27.9	22.7	20 g	Apple cider	5	60 min	52	Sweetened water	PBG PI
Leeman 2005 (42)	RCT, XO	HV	13 (3/10)	19–32	22.5	28 g	White	6	120 min	50	Boiled potatoes	PBG PI
Ostman 2005 (40)	RCT, XO	HV	12 (10/2)	22.9	21.5	28 g	White	6	120 min	50	White bread	PBG PI
Hlebowicz 2008 (39)	RCT, XO	HV	13 (6/7)	25	22.8	28 g	White wine	5	120 min	50	White bread	PBG

Mettler 2009 (38)	RCT, XO	HV	27 (9/18)	26	22.5	28 g	NI	NI	120 min	75	Vanilla milk rice + cinnamon	PBG
Johnston 2010 -study 1 (31)	RCT, XO	HV	10 (4/6)	35	27.5	2–20 g	Apple cider	5	120 min	0	0g acetic acid drink	PBG
Johnston 2010-study 2a (31)	RCT, XO	HV	9 (2/7)	50	33.7	20 g	Raspberry	5	120 min	75	Placebo (not specified)	PBG
Johnston 2010-study 3 (31)	RCT, XO	HV	10 (2/8)	38	26.3	20 g	Apple cider	5	120 min	75	Placebo (not specified)	PBG
Johnston 2010 -study 4 (31)	RCT, XO	T2D	9 (4/5)	69	31.4	20 g	Apple cider	5	120 min	75	Placebo (not specified)	PBG
Liatis 2010a (32)	RCT, XO	T2D	8 (3/5)	57.4	29.8	20 g	Wine (High glycemic index 86/100)	6	120 min	51	High glycemic meal	PBG PI
Liatis 2010b (32)	RCT, XO	T2D	8 (4/4)	61.4	30.1	20 g	Wine (Low glycemic index 38/100)	6	120 min	52	Low glycemic meal	PBG PI
Darzi 2014 - study 1 (33)	RCT, XO	HV	16 (3/13)	22.2	22.1	30 g	White wine (palatable)	6	180 min	94.5	75g sugar free squash	PBG
Darzi 2014 - study 2 (33)	RCT, XO	HV	14 (6/8)	27.5	22.7	30 g	White wine	6	180 min	60	Water	PBG
Mitrou 2015a (45)	RCT, XO	T2D	11 (4/7)	53.0	25.0	30 ml	NI	6	300 min	75	Water	PBG PI muscle glucose uptake
Mitrou 2015b (44)	RCT, XO	IGT	8 (4/4)	46.0	30.0	30 ml	Wine	6	300 min	75	Water	PBG PI Muscle glucose

												uptake
Feise 2020a (34)	XO	HV	12 (5/7)	22.6	21.2	25 g	Liquid vinegar	5	60 min	64	Water	PBG
Feise 2020b (34)	XO	HV	12 (5/7)	22.6	21.2	25 g	Vinegar pills	5	60 min	64	Water	PBG
Feise 2020c (34)	XO	HV	12 (5/7)	22.6	21.2	25 g	Crushed vinegar pills	5	60 min	64	Water	PBG
Zhao 2020 (41)	RCT, XO	HV	15 (0/15)	23.6	20.3	30 g	Black rice	5	200 min	35	White rice, dried apple	PBG
Chronic												
White 2007 (51)	XO	T2D	11 (4/7)	40-72	29.1	30 ml	Apple cider	0.002	2 d	Usual diet	Water	FBG
Hosseini 2011 (49)	parallel	T2D	30 (15/15)	30-60	NI	15 ml	Vinegar	NI	4 wks.	Usual diet	Water	FBG HbA1c
Derakhshande h-Rishehri 2014 (48)	RCT, parallel	HV	72 (32/40)	31.6	25.3; 22.8	21.66 g	Honey	NI	4 wks.	Usual diet	Normal diet	FBG FI HOMA-IR
Ali 2019 (50)	RCT, parallel	T2D	55 (26/29)	30-60	NI	20 ml	Dates	NI	10 wks.	Usual diet	Honey in water	FBG HbA1c
Gheflati 2019 (46)	RCT, parallel	T2D	62 (20/42)	49.5; 52.1	29.0; 28.9	20 ml	Apple	5	8 wks.	Usual diet	Normal diet	FBG FI HOMA-IR QUICKI
Jasbi 2019 (47)	RCT, parallel	HV	45 (41/4)	29.6; 30.1	27.8; 28.5	60 ml	Red wine	6	8 wks.	Usual diet	Apple cider vinegar tablet	FBG FI HOMA-IR

CHO, carbohydrate; d, days; FBG, fasting blood glucose; FI, fasting insulin; HbA1c, glycated hemoglobin; HV, healthy volunteers; min, minutes; NI, no information; PBG, postprandial blood glucose; PI, postprandial insulin; QUICKI, Quantitative insulin sensitivity index; RCT, randomized controlled trial; T2D, Type 2 diabetes; XO, cross-over.

Table 4. Summary of the butyrate studies design, participant and intervention characteristics and outcome analyzed

		Participant's characteristics				Intervention characteristics				
Chronic										
Reference	**Study design**	**Health status**	**Sample size (Male/ Female)**	**Age (y, mean)**	**BMI (kg/m^2, mean)**	**Amount given**	**Route of administration**	**Duration**	**Control**	**Outcome analyzed**
Roshanravan 2017 (52)	RCT, Parallel	T2D	30 (10/20)	49	30.3	600 mg Butyrate+ 10g butyrate powder	Oral (capsule)	45 d	Starch	FBG FI PBG PI HOMA-IR HbA1c
Roshanravan 2018 (53)	RCT, Parallel	T2D	30 (10/20)	49	30.3	600 mg Butyrate+ 10g starch powder	Oral (Capsule)	45 d	Starch	QUICKI

d, days; FBG, fasting blood glucose; FI, fasting insulin; HbA1c, glycated hemoglobin; PBG, postprandial blood glucose; PI, postprandial insulin; QUICKI, Quantitative insulin sensitivity index; RCT, randomized controlled trial; T2D, Type 2 diabetes; XO, cross-over

Table 5. Summary of the propionate studies design, participant and intervention characteristics and outcome analyzed

		Participant's characteristics				Intervention characteristics				
Reference	**Study design**	**Health status**	**Sample size (Male/ Female)**	**Age (y)**	**BMI (kg/m^2)**	**Amount given**	**Route of administration**	**Time-course**	**Control**	**Glycemic outcome analyzed**
Acute)										
Todesco 1991 (55)	RCT, XO	HV	6 (3/3)	32	22.5	3.3. g	oral (bread)	180min	Bread	PBG
Laurent 1995 (36)	RCT	HV	6 (3/3)	22	21.2	4 mmol/h	Intragastric (infusion)	300min	Saline	PBG PI
Darwiche 2001 (56)	RCT, XO	HV	9 (5/4)	32	23.6	1.85 g	oral (bread)	125min	Bread	PBG PI
Darzi 2012 (54)	RCT, XO	HV	20 (9/11)	25	23.1	43.8 g	oral (Sourdough bread)	180min	Control bread	PBG PI
Byrne 2016 (57)	RCT, XO	HV	20 (20/0)	52	25.2	10 g	oral (powder)	360min	Inulin	PBG PI
Chambers 2018 (59)	RCT, XO	HV	18 (9/9)	50	30.5	6.8 g	oral (tablet)	180 min	NaCl 4164 mg	PBG PI
Tirosh 2019 (60)	RCT, XO	HV	14 (9/5)	41	23.7	1.0g	oral (Calcium propionate)	240 min	Placebo (not specified)	PBG PI
Adler 2021 (58)	RCT, XO	HV	27 (12/15)	30	26.7	1.5 g	oral (calcium propionate)	240 min	Calcium carbonate	PBG
Chronic										

Venter 1990 (64)	Paired comparison	HV	20 (0/20)	20-21	18-22.5	7.5 g/d	oral (capsule)	7 wks.	calcium phosphate	PBG PI
Todesco 1991 (55)	RCT, XO	HV	6 (3/3)	32	22.5	9.9 g/d	oral (Propionate bread)	1 wk.	Propionate free white bread	PBG
Chambers 2015 (63)	RCT, Parallel	HV	49 (19/30)	54	32.5	10 g/d	GIT (powder)	24 wks.	Inulin	FI HbA1c HOMA-IR
Pingitore 2017 (69)	RCT, Parallel	HV	49 (19/30)	53	32.5	10 g/d	GIT (sachet)	24 wks.	Inulin	PBG PI
Chambers 2019 (62)	RCT, XO	HV	12 (3/6)	60	29.8	20 g/d	GIT (powder)	42 d	Cellulose	FBG FI HOMA-IR
Byrne 2019 (61)	RCT, XO	HV	21 (9/12)	18-65	60	10 g/d	GIT (Bread roll and smoothie)	1 wk.	Bread	PBG PI

d, days; FBG, fasting blood glucose; FI, fasting insulin; GIT, gastrointestinal tract; HbA1c, glycated hemoglobin; HV, Healthy volunteers, min, minutes; NI, no info; PBG, postprandial blood glucose; PI, postprandial insulin; QUICKI, Quantitative insulin sensitivity index; RCT, randomized controlled trial; T2D, Type 2 diabetes; XO, cross-over

Table 6. Summary of the mixed SCFAs studies design, participant and intervention characteristics and glycemic outcome analyzed

		Participant's characteristics				Intervention characteristics				
Reference	**Study design**	**Health status**	**Sample size (M/F)**	**Age (y)**	**BMI (kg/m^2)**	**Amount given (acetate, propionate, butyrate)**	**Route of administration**	**Time-course**	**Control**	**Glycemic outcomes analyzed**
Mixed SCFAs (acute)										
Wolever 1988 (65)	RCT, XO	HV	6 (3/3)	33.0	NI	Acetate 90 mmol/L + 30 mmol/L Propionate or Acetate 180 mmol/L + 60 mmol/L Propionate	Rectal	120min	Saline	PBG PI
Wolever 1991 (66)	RCT, XO	HV	6	29.0	24.1	Acetate (180 mmol/L) + propionate (60 mmol/L)	Rectal	120min	Saline	PBG PI
Laurent 1995 (36)	RCT, XO	HV	6 (3/3)	22	21.2	Acetate 12 mmol/h Propionate 4 mmol/h	Intragastric (infusion)	300min	Saline	PBG PI
Alamowitch 1996 (67)	RCT, XO	HV	6	26.0	20.9	90 mmol/L (Acetate: propionate: butyrate; 60,25,15 mmol/L)	Ileal	18h	Saline	basal hepatic glucose production insulin sensitivity
Canfora 2017 (68)	RCT, XO	HV	12 (12/0)	36.0	25-35	200 mmol/L (High-acetate: 24,8,8 mmol/L) (High-butyrate: 8,8,24 mmol/L) (High-propionate: 8,24,8 mmol/L)*	Rectal	300min	Saline	PBG PI Carbohydrate oxidation

*Second enema given 3h after first infusion and given with 75g of oral glucose load to represent postprandial state.

h, hours; HV, healthy volunteers; min, minutes; NI, no information; PBG, postprandial blood glucose; PI, postprandial insulin; RCT, randomized controlled trial; T2D, Type 2 diabetes; XO, cross-over

Table 7: GRADE Summary of results for acetate compared to placebo or usual treatment for HV and patients with IGT and T2D

Outcomes	**Anticipated absolute effects*** (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with Placebo or usual treatment	**Risk with Acetate**				
Acute postprandial glucose	-	SMD = **0.09** (-0.26, 0.44)	-	44 (7 RCTs)	⨁◯◯◯ Very low[a,b,c,d]	The evidence suggests that acetate results in little to no difference in postprandial glucose.
Acute postprandial insulin	-	SMD = **0.35** (-0.07, 0.77)	-	35 (6 RCTs)	⨁◯◯◯ Very low[a,c,d,e,f]	Acetate may result in little to no difference in postprandial insulin.

a. Risk of bias due to lack of blinding and selective outcome reporting.
b. Study populations differed between studies (HV, T2D, hyperinsulinemia, overweight/obese). Dosage of acetate varied highly between 1 – 360 mmol.
c. Not generalizable due to small sample size. Not applicable due to administration methods for acetate.
d. 95% CI are very wide
e. Study populations differed between studies (HV, hyperinsulinemia, overweight/obese). Dosage of acetate varied highly between 36 – 360 mmol. None of the studies had PI as their primary outcome.
f. Possible publication bias detected by funnel plot

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI, 95% confidence interval; HV, healthy volunteers; IGT, impaired glucose tolerance; IR, insulin resistant; SMD, standardized mean difference; T2D, type 2 diabetes.

Table 8: GRADE Summary of results for vinegar compared to placebo or usual treatment for HVs and patients with IGT and T2D

Outcomes	**Anticipated absolute effects*** (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo or usual treatment	**Risk with vinegar**				
Acute postprandial glucose (HV)	-	SMD = **-0.27** (-0.54, 0.00)	-	186 (15 RCTs)	⨁◯◯◯ Very low[a,b,c]	Vinegar may reduce/have little to no effect on postprandial glucose in HV, but the evidence is very uncertain.
Acute postprandial glucose (T2D, IR, IGT)	-	SMD = **-0.53** (-0.92, 0.14)	-	67 (7 RCTs)	⨁◯◯◯ Very Low[d,e,f]	Vinegar may reduce/have little to no effect on postprandial glucose in individuals with T2D, IR or IGT but the evidence is very uncertain.
Chronic fasting blood glucose (all studies)	-	SMD = **-1.60** (-4.30, 1.09)	-	143 (6 RCTs)	⨁◯◯◯ Very low[f,g,h,i]	Vinegar may reduce/have little to no effect on fasting blood glucose, but the evidence is very uncertain.
Acute postprandial insulin (HV)	-	SMD = **-0.29** (-0.66, 0.08)	-	55 (5 RCTs)	⨁◯◯◯ Very low[f e,f,h]	Vinegar may reduce/have little to no effect on postprandial insulin in HV, but the evidence is very uncertain.
Acute postprandial insulin (T2D, IR, IGT)	-	SMD = **-0.16** (-0.75, 0.44)	-	58 (6 RCTs)	⨁◯◯◯ Very low[f,j,k]	Vinegar may reduce/have little to no effect on postprandial insulin in individuals with T2D, IR or IGT but the evidence is very uncertain.
Chronic fasting insulin (all studies)	-	SMD = **0.06** (-0.50, 0.62)	-	89 (3 RCTs)	⨁◯◯◯ Very low[f,l,m,n,]	Vinegar may increase/have little to no effect on fasting insulin, but the evidence is very uncertain.

a. Risk of bias arose from lack of allocation concealment, through lack of blinding and in measurement of outcomes.
b. Different types of vinegar were used, although similar concentrations of acetic acid were documented.
c. Serious inconsistency (severe heterogeneity I^2 = 65.1%, p = 0.001). 95% CI is wide.
d. Risk of bias arose from lack of allocation concealment, due to lack of blinding, measurement of outcome, and selective outcome reporting.
e. Inconsistency due to very wide 95% CI
f. Possible publication bias detected by funnel plot
g. Serious inconsistency (severe heterogeneity I^2 = 99.0%, p = 0.001). Very wide 95% CI
h. Risk of bias arose from lack of allocation concealment, lack of blinding, and in the measurement of the outcome
i. Studies were not generalizable as mostly conducted on metabolically unhealthy individuals. Some study comparators contained small amounts of vinegar/acetic acid.
j. Serious inconsistency (severe heterogeneity I^2 = 77.0%, p = 0.001). 95% CI is very wide (i)
k. Risk of bias due to lack of allocation concealment, lack of blinding, incomplete accounting of patients and outcome events and selective outcome reporting
l. Risk of bias arose from lack of allocation concealment and due to lack of blinding.

m. Study populations differed between the three studies. Different types of vinegar were used.
n. Serious inconsistency (severe heterogeneity $I^2 = 70.6\%$, $p = 0.02$

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI, 95% confidence interval; HV, healthy volunteers; IGT, impaired glucose tolerance; IR, insulin resistant; SMD, standardized mean difference; T2D, type 2 diabetes

Table 9: GRADE Summary of results for propionate compared to placebo or usual treatment for HVs and patients with IGT and T2D

Outcomes	**Anticipated absolute effects*** (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo or usual treatment	**Risk with Propionate**				
Acute postprandial blood glucose	-	SMD = **0.07** (-0.32, 0.47)	-	123 (8 RCTs)	⨁◯◯◯ Very low[a,b,c]	Propionate may increase/have little to no effect on postprandial glucose (acute) but the evidence is very uncertain.
Chronic postprandial blood glucose	-	SMD = **-0.08** (-0.43, 0.27)	-	73 (5 RCTs)	⨁⨁◯◯ Low[c,d,e]	The evidence is very uncertain about the effect of propionate on postprandial glucose (chronic).
Chronic fasting glucose	-	SMD = **-0.14** (-0.47, 0.19)	-	67 (4 RCTs)	⨁◯◯◯ Very low[d,e,f]	The evidence suggests that propionate results in little to no difference in fasting glucose (chronic).
Acute postprandial insulin	-	SMD = **0.24** (-0.30, 0.78)	-	117 (7 RCTs)	⨁◯◯◯ Very low[a,b,c]	The evidence is very uncertain about the effect of propionate on postprandial insulin (acute).
Chronic postprandial insulin	-	SMD = **- 0.06** (-0.39, 0.27)	-	167 (4 RCTs)	⨁◯◯◯ Very low[a,b,e,f]	The evidence suggests that propionate results in little to no difference in postprandial insulin (chronic).
Chronic fasting insulin	-	SMD = **-0.22** (-0.65, 0.21)	-	67 (4 RCTs)	⨁◯◯◯ Very low[c,d,e]	The evidence is very uncertain about the effect of propionate on fasting Insulin (chronic).

a. The risk of bias mainly arises from lack of allocation concealment and incomplete accounting of patients and outcome events.
b. Inconsistency may stem from differing interventions (including sodium propionate, calcium propionate and inulin propionate-ester) and dosage (ranging from <1g - 9.9g of intervention). PBG was the main outcome in 50% of studies.
c. Very wide 95% CI.
d. The risk of bias mainly arose from due to lack of blinding.
e. possible publication bias detected by funnel plot
f. Wide 95% CI

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.
*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI). CI, 95%confidence interval; HV, healthy individuals; IGT, impaired glucose tolerance; IR, insulin resistant; SMD, standardized mean difference; T2D, type 2 diabetes

Table 10: GRADE Summary of results for mixed SCFAs compared to placebo or usual treatment for HV and patients with IGT and T2D

Outcomes	**Anticipated absolute effects*** (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with Placebo or usual treatment	**Risk with Mixed SCFA**				
Acute postprandial blood glucose	not pooled	not pooled	-	42 (4 RCTs)	⨁⨁◯◯ Low[a,b]	The evidence suggests that mixed SCFA results in little to no difference in acute postprandial blood glucose. The majority of studies found no significant difference in postprandial blood glucose. One study saw a small, significant increase in blood glucose.
Acute postprandial insulin	not pooled	not pooled	-	36 (3 RCTs)	⨁⨁◯◯ Low[a,b]	The evidence suggests that mixed SCFA results in little to no difference in acute postprandial insulin. One study saw a small, significant decrease in insulin secretion after mixed SCFA administration.

a. Difficult to generalize results due to the small sample sizes. Rectal and gastric infusions mean that interventions are not very applicable, replicable, or tolerable, reducing transferability.
b. Studies tend to have small sample sizes, increasing imprecision.

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

***The risk in the intervention group** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI, 95% confidence interval; HV, healthy volunteers; IGT, impaired glucose tolerance; IR, insulin resistant; SMD, standardized mean difference; T2D, type 2 diabetes

Table 11: GRADE Summary of results for butyrate compared to placebo or usual treatment for patients with T2D

Outcomes	**Anticipated absolute effects*** (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with Placebo or usual treatment	**Risk with Butyrate**				
Fasting blood glucose	-	**MD = -1.20** (-2.91 to 0.51)	-	30 (1 RCT)	⨁◯◯◯ Very low[a,b]	The evidence is very uncertain about the effect of butyrate on fasting blood glucose as there is only one study on chronic butyrate which fit our criteria
Fasting insulin	-	**MD = 0.9** (0.57, 1.31)	-	30 (1 RCT)	⨁◯◯◯ Very low[a,b]	The evidence is very uncertain about the effect of butyrate on fasting insulin as there was only one study investigating butyrate which fit our criteria.

a. Risk of bias was high for study due to lack of blinding, incomplete accounting of patients and outcome events, and selective outcome reporting.
b. Publication bias is suspected as there is only one study investigating butyrate and the only study demonstrates butyrate to have a significant effect.

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI, 95% Confidence interval; HV, healthy volunteer; IGT, impaired glucose tolerance; IR, insulin resistant; MD, mean difference; T2D, type 2 diabetes

Table 12. Summary of the results from the meta-analysis for all subject categories.

		Acute	Chronic
Acetate	**Glucose**	0.09 (-0.26, 0.44), n = 44	No studies found.
	Insulin	0.35 (-0.07, 0.77), n = 35	No studies found.
Propionate	**Glucose**	0.07 (-0.32, 0.47), n = 123	PBG -0.08 (-0.43, 0.27), n = 73 FBG -0.14 (-0.47, 0.19), n = 67
	Insulin	0.24 (-0.30, 0.78), n = 117	PI -0.06 (-0.39, 0.27), n = 67 FI -0.22 (-0.65, 0.21), n = 67
Butyrate	**Glucose**	No studies found.	No meta-analysis possible. See narrative review.
	Insulin	No studies found.	No meta-analysis possible. See narrative review.
Mixed SCFAs	**Glucose**	No meta-analysis possible. See narrative review.	No studies found.
	Insulin	No meta-analysis possible. See narrative review.	No studies found.
Vinegar	**Glucose**	-0.27 (-0.54, 0.00), n = 186, healthy -0.53 (-0.92, -0.14), n = 67, T2D, IGT	-1.60 (-4.30, 1.09), n = 143
	Insulin	-0.29 (-0.66, 0.08), n = 55, healthy -0.16 (-0.75, 0.44), n = 58, T2D, IGT	0.06 (-0.50, 0.62), n = 89

Results are shown in SMD (standard mean difference) for acute studies and Hedges' g for chronic studies (95 % CI), p-value ≤0.05 was significant; n, sample size of the number of participants pooled from all studies included in meta-analysis. Results are shown for all type of participants (healthy and non-healthy) and for postprandial outcomes, unless otherwise stated. FBG, fasting blood glucose; FI, fasting insulin; PBG, postprandial blood glucose; PI, postprandial insulin; SCFA, short chain fatty acids.

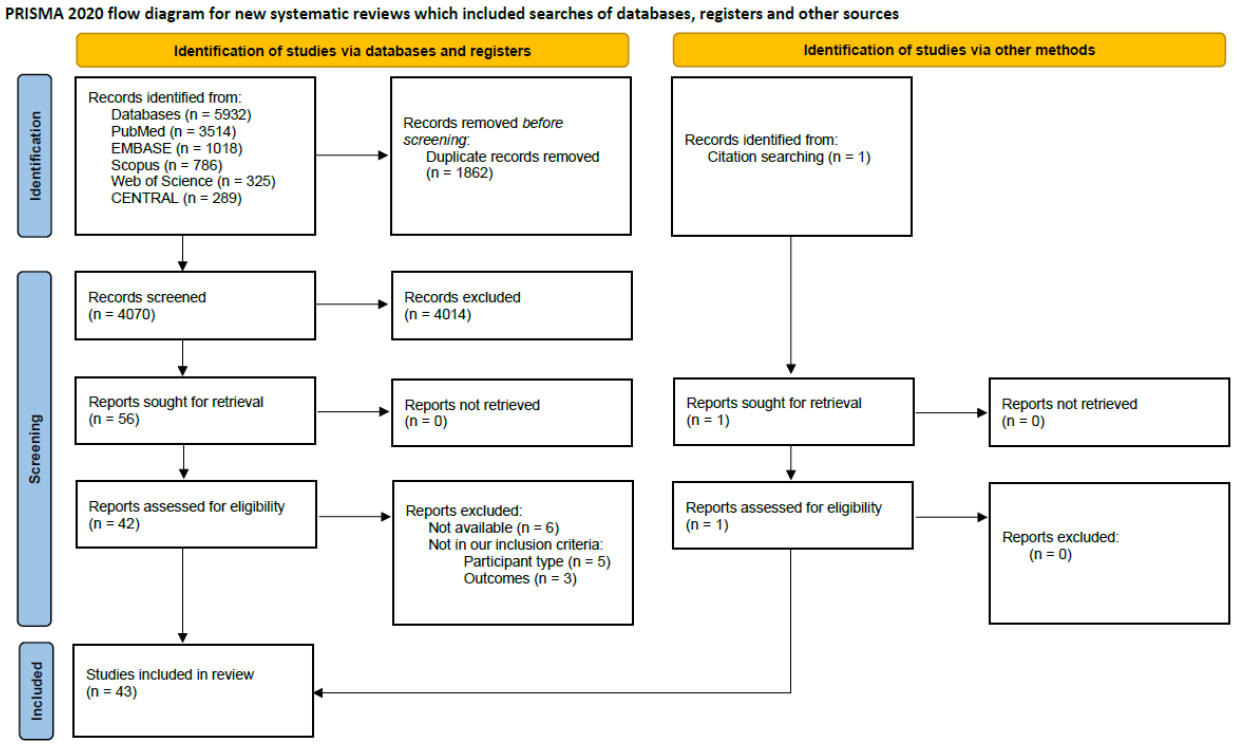

Figure 1. PRISMA Flow Diagram of references identified and evaluated. PRISMA, preferred reporting items for systematic reviews and meta-analyses.

Study name	D1	D2.1	D2.2	D3	D4	D5	Overall
ACETATE							
Freeland 2010 (37)	-	+	x	+	+	+	x
Johnston 2010 (31)	+	+	+	+	-	+	-
Laurent 1995 (36)	-	+	+	+	+	-	-
Scheppach 1988 (28)	-	-	x	x	-	-	x
Van der Beek 2016 (35)	+	+	+	+	+	+	+
BUTYRATE							
Roshanravan 2017 (52)	+	-	+	+	-	-	x
Roshanravan 2018 (53)	+	-	+	+	-	-	x
PROPIONATE							
Adler 2021 (58)	+	+	+	-	+	-	-
Byrne 2016 (57)	+	+	+	+	+	-	-
Byrne 2019 (61)	+	+	+	+	+	+	+
Chambers 2015 (63)	+	+	-	-	+	+	-
Chambers 2018 (59)	-	+	+	+	+	+	-
Chambers 2019 (62)	+	-	x	+	+	+	x
Darwiche 2001 (56)	+	+	+	+	x	-	x
Darzi 2012 (54)	+	-	+	+	-	+	-
Laurent 1995 (36)	-	+	+	+	+	-	-
Pingitore 2017 (69)	+	+	-	-	+	+	-
Tirosh 2019 (60)	-	+	+	+	+	-	-
Todesco 1991 (55)	-	+	+	+	x	-	x
Venter 1990 (64)	+	+	-	+	+	-	-
MIXED							
Alamowitch 1996 (67)	+	+	+	+	+	+	+
Canfora 2017 (68)	+	+	+	+	+	+	+
Laurent 1995 (36)	-	+	+	+	+	-	-
Wolever 1988 (65)	-	-	+	+	+	+	-
Wolever 1991 (66)	-	+	-	+	-	+	x
VINEGAR							
Ali 2019 (50)	+	-	+	+	-	+	-
Brighenti 1995 (29)	-	-	-	+	-	+	x
Darzi 2014 (33)	+	+	+	+	x	+	x
Derakhshandeh-Rishehri 2014 (48)	-	x	x	-	+	+	x
Feise 2020 (34)	-	-	+	+	-	+	x
Gheflati 2019 (46)	-	+	+	+	-	+	-
Hlebowicz 2008 (39)	-	+	+	+	x	+	x
Hosseini 2011 (49)	-	-	x	+	x	+	x
Jasbi 2019 (47)	-	+	-	+	-	+	x
Johnston 2010 (31)	+	+	+	+	-	+	-
Johnston 2004 (43)	-	-	-	+	-	-	x
Johnston 2005 (30)	-	-	-	+	-	+	x
Leeman 2005 (42)	-	+	-	+	+	+	-
Liatis 2010 (32)	-	+	-	-	+	+	x
Mettler 2009 (38)	+	-	+	+	-	-	x
Mitrou 2015a (45)	-	+	+	+	+	+	-
Mitrou 2015b (44)	-	+	-	+	+	+	-
Ostman 2005 (40)	-	+	+	+	+	+	-
White 2007 (51)	-	+	-	+	x	+	x
Zhao 2020 (41)	+	+	+	+	x	+	x

Judgement	
+	Low
-	Some concerns
x	High
Risk of Bias…	
D1	…arising from randomization process
D2.1	…due to deviations from the intended interventions (asssignment to the intervention)
D2.2	…due to deviations from the intended interventions (effect of adhering to intervention)
D3	…due to missing outcome data
D4	…in measurement of outcome
D5	…in selection of reported result

Figure 2, Risk of bias summary for all studies by length-intervention category (acute or chronic) and intervention (acetate, propionate, butyrate, vinegar, mixed SCFAs). SCFA; short chain fatty acids.

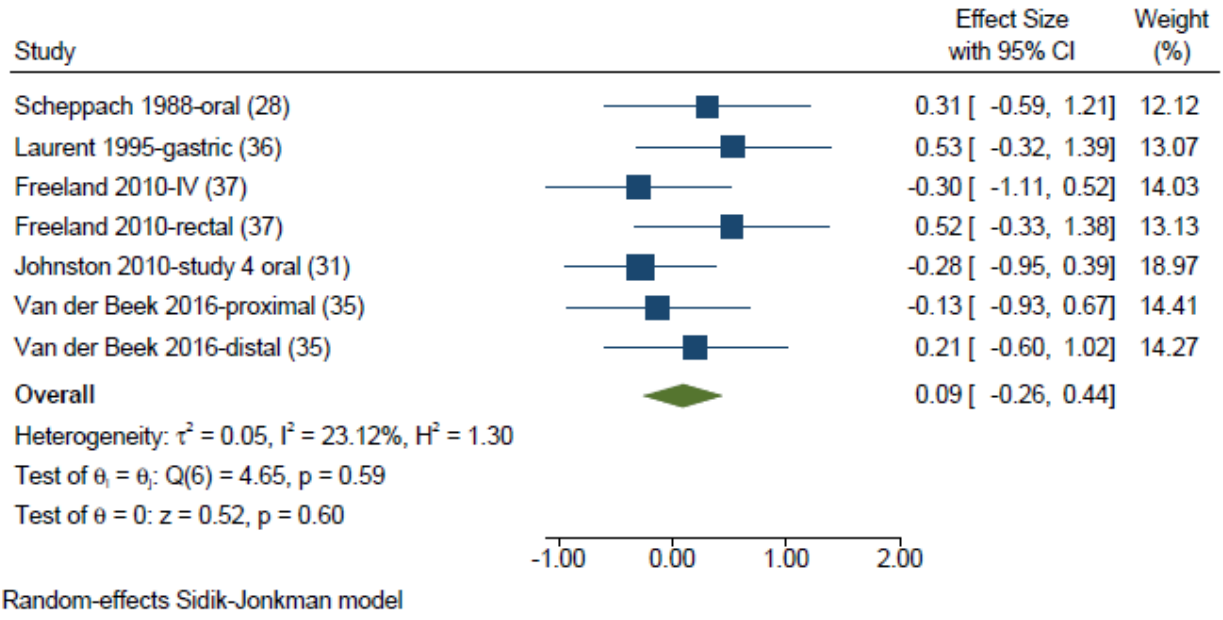

Figure 3. Forest plots for randomized controlled trials of acute acetate on postprandial blood glucose. Acute interventions with acetate had a main effect of 0.09 (-0.26, 0.44) (p=0.60) on post-intervention postprandial blood glucose iAUC (n = 44). Johnston 2010 study 4-oral, in T2D. Random-effects model was used to calculate standardized mean differences (squares), 95% CI (horizontal lines), and summary effect (SMD) (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. Interpretation of SMDs (or 'effect sizes') is <0.40 = small effect size, 0.40 to 0.70 = moderate effect size, >0.70 = large effect size. GIT, gastrointestinal tract; SMD, standard mean difference.

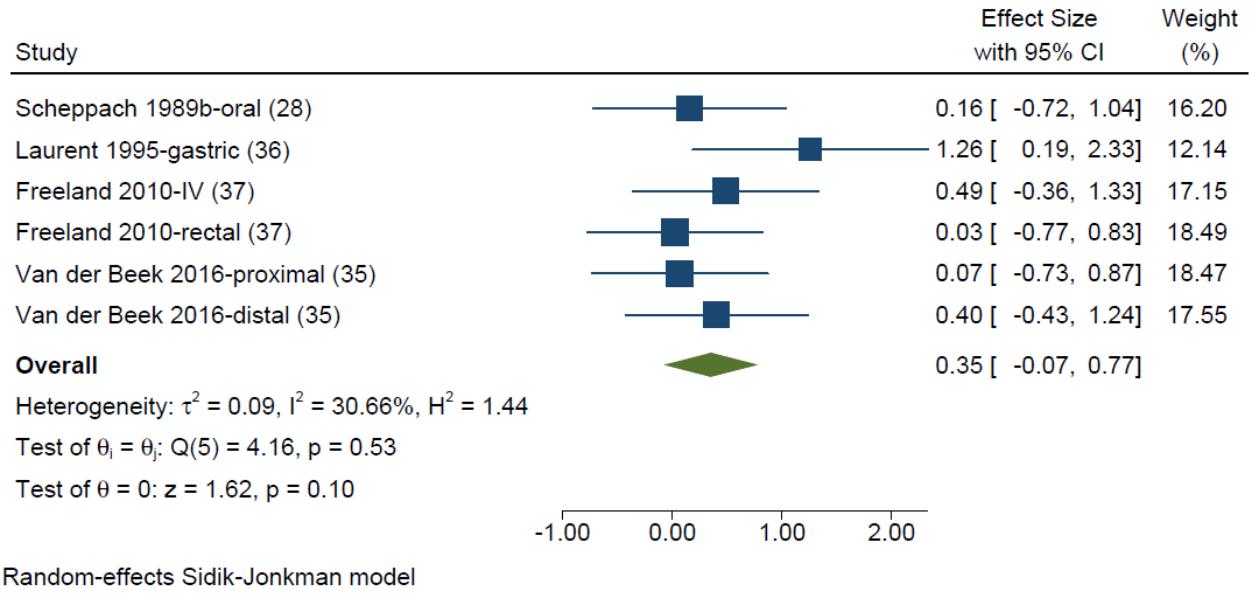

Figure 4. Forest plots for randomized controlled trials of acute acetate on postprandial blood insulin. Acute interventions with acetate had a main effect of 0.35 (-0.07, 0.77) (p=0.10) on post-intervention postprandial blood insulin iAUC (n = 35). Random-effects model was used to calculate standardized mean differences (squares), 95% Cl (horizontal lines), and summary effect (SMD) (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. Interpretation of SMDs (or 'effect sizes') is <0.40 = small effect size, 0.40 to 0.70 = moderate effect size, >0.70 = large effect size. GIT, gastrointestinal tract; SMD, standard mean difference.

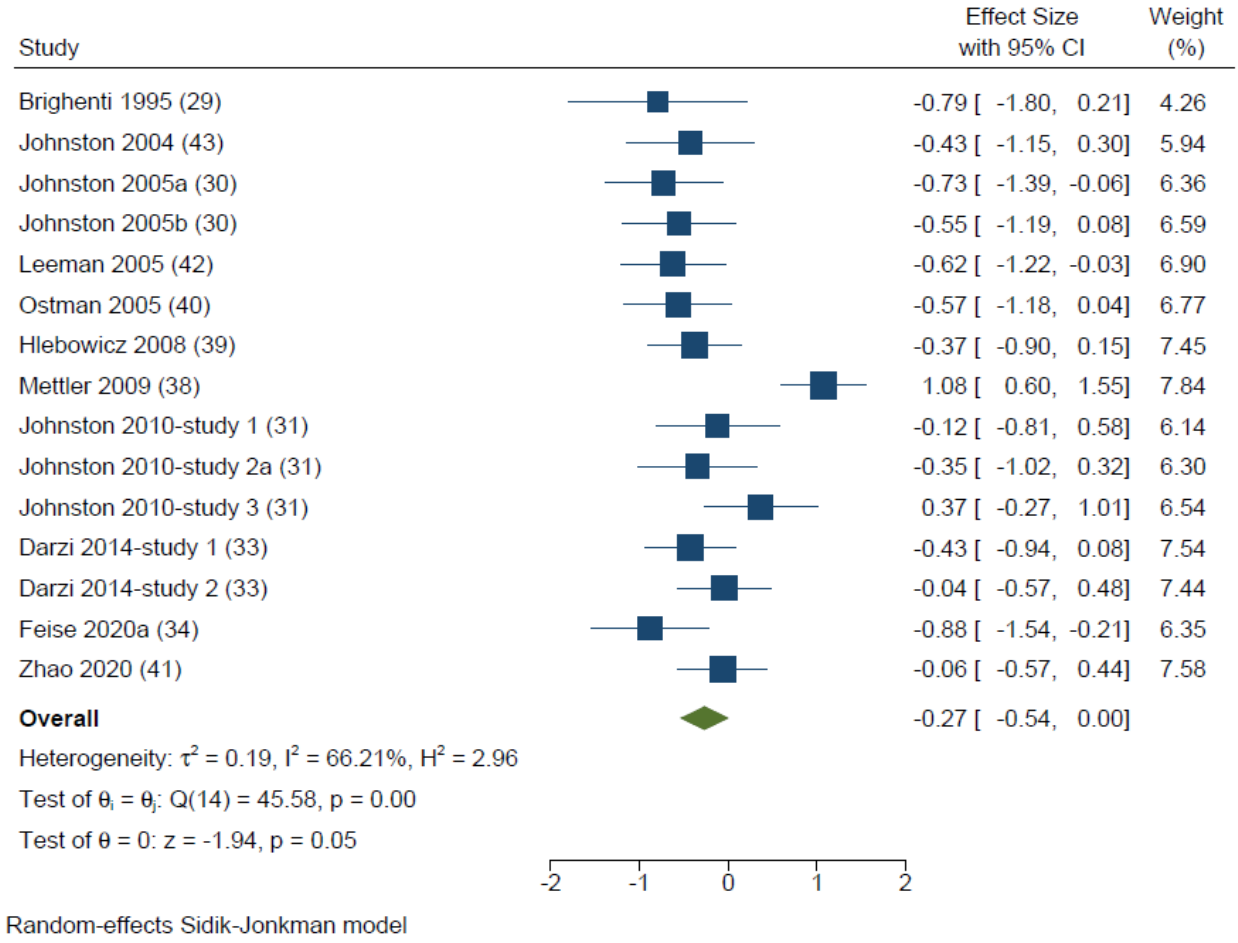

Figure 5. Forest plots for randomized controlled trials of acute vinegar intake on postprandial blood glucose in healthy volunteers. Acute interventions with vinegar had a main effect of -0.27 (-0.54, 0.00) (p=0.05)-on post-intervention postprandial blood glucose iAUC (n = 186) in healthy subjects. Random-effects model was used to calculate standardized mean differences (squares), 95% Cl (horizontal lines), and summary effect (SMD) (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. Interpretation of SMDs (or 'effect sizes') is <0.40 = small effect size, 0.40 to 0.70 = moderate effect size, >0.70 = large effect size. Brighenti 1995, acetic acid within vinegar (co-ingested with 50g of CHO). Johnston 2005, (a) bagel and juice meal, (b) chicken teriyaki. Johnston 2010, (study 1) 1g of acetic acid as vinegar consumed prior to the test meal (bagel + juice), (Study 2a) 1g of acetic acid as vinegar consumed with the test meal, , (Study 3) 1g of acetic acid as vinegar ingested immediately prior to a 75-gram dextrose load in 10 healthy adults. Darzi 2014, (Study 1) vinegar within unpalatable drink alongside a mixed breakfast in comparison to a non-vinegar control; consisting of 25 g vinegar+25 g sugar-free squash+100 g water in one drink that was consumed first, followed by 50 g sugar-free squash+100 g water in a second drink (Study 2) vinegar drink intake following a milkshake preload compared to a non-vinegar control; consisting of 30 g of vinegar (containing 6% acetic acid) + 150 g water. Feise 2020, (a) 25 g liquid vinegar (1.25 g acetic acid),. SMD, standard mean difference.

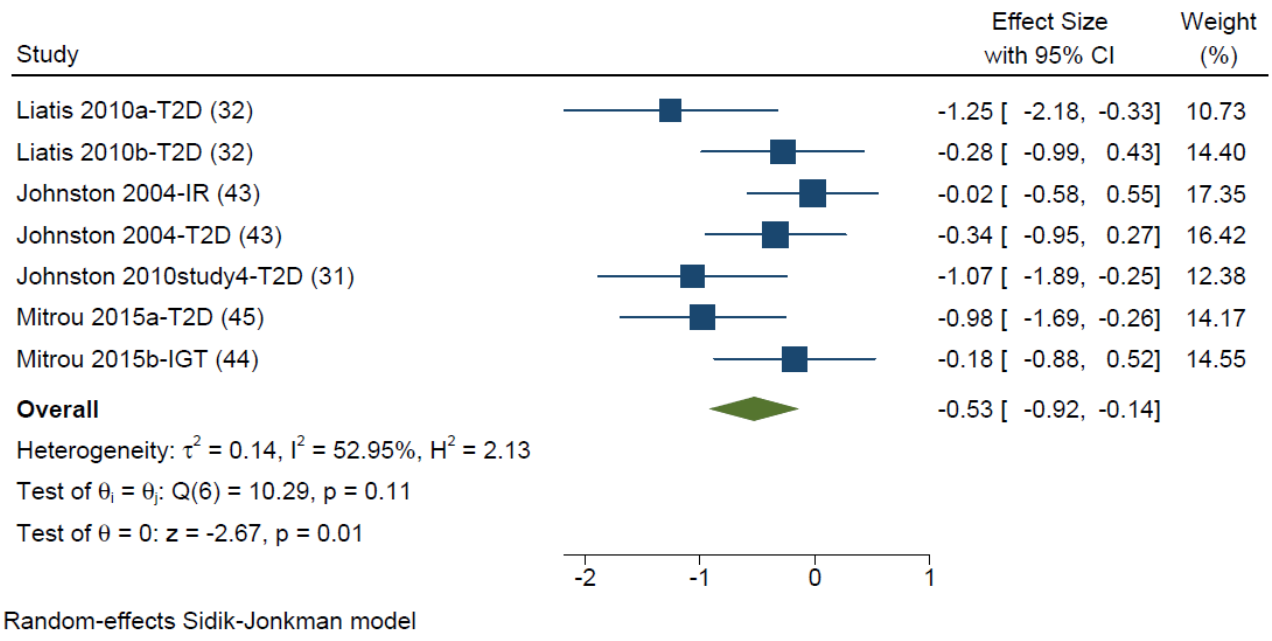

Figure 6. Forest plots for randomized controlled trials of acute vinegar intake on postprandial blood glucose in non-healthy adults. Acute interventions with vinegar had a main effect of -0.53 (-0.92, -0.14) (p=0.01) on post-intervention postprandial blood glucose iAUC (n = 67) in non-healthy subjects. Random-effects model was used to calculate standardized mean differences (squares), 95% Cl (horizontal lines), and summary effect (SMD) (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. Interpretation of SMDs (or 'effect sizes') is <0.40 = small effect size, 0.40 to 0.70 = moderate effect size, >0.70 = large effect size. Liatis 2010, (a) High-GI meal, (b) Low-GI meal. Mitrou 2015, (a) Journal of Diabetes Research, (b) European Journal of Clinical Nutrition. T2D, type 2 diabetes; IGT, impaired glucose tolerance; IR, insulin resistant; SMD, standard mean difference.

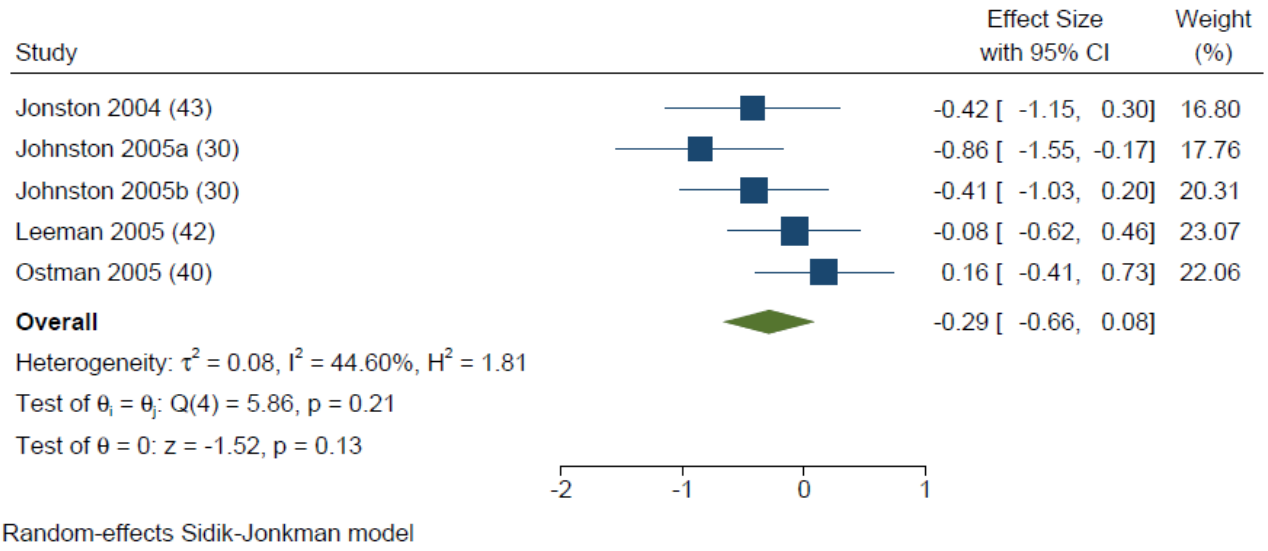

Figure 7. Forest plots for randomized controlled trials of acute vinegar intake on postprandial blood insulin in healthy volunteers. Acute interventions with vinegar had a main effect of -0.29 (-0.66, 0.08) (p=0.13) on post-intervention postprandial insulin iAUC (n = 55) in healthy subjects. Random-effects model was used to calculate standardized mean differences (squares), 95% Cl (horizontal lines), and summary effect (SMD) (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. Interpretation of SMDs (or 'effect sizes') is <0.40 = small effect size, 0.40 to 0.70 = moderate effect size, >0.70 = large effect size. Johnston 2005, (a) bagel and juice meal, (b) chicken teriyaki. SMD, standard mean difference.

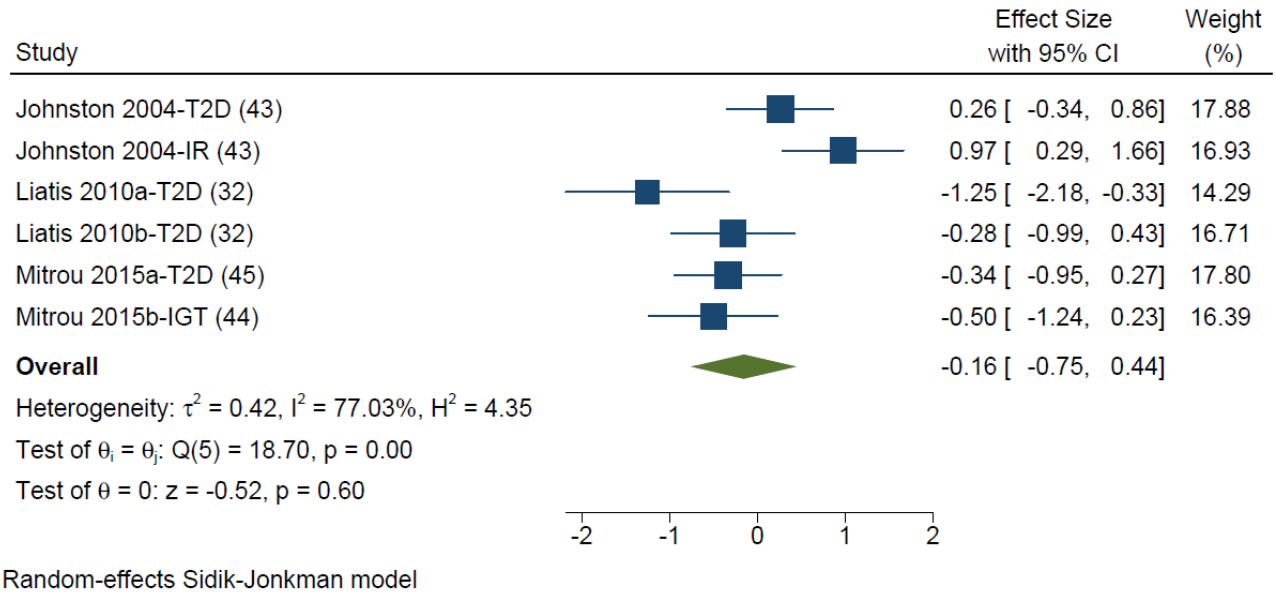

Figure 8. Forest plots for randomized controlled trials of acute vinegar intake on postprandial blood insulin in non-healthy adults. Acute interventions with vinegar had a main effect of -0.16 (-0.75, 0.44) (p=0.60) on post-intervention postprandial blood glucose iAUC (n = 58) in non-healthy adults. Random-effects model was used to calculate standardized mean differences (squares), 95% Cl (horizontal lines), and summary effect (SMD) (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. Interpretation of SMDs (or 'effect sizes') is <0.40 = small effect size, 0.40 to 0.70 = moderate effect size, >0.70 = large effect size. Liatis 2010, (a) High-GI meal, (b) Low-GI meal. Mitrou 2015, (a) Journal of Diabetes Research, (b) European Journal of Clinical Nutrition. T2D, type 2 diabetes; IGT, impaired glucose tolerance; IR, insulin resistant; SMD, standard mean difference.

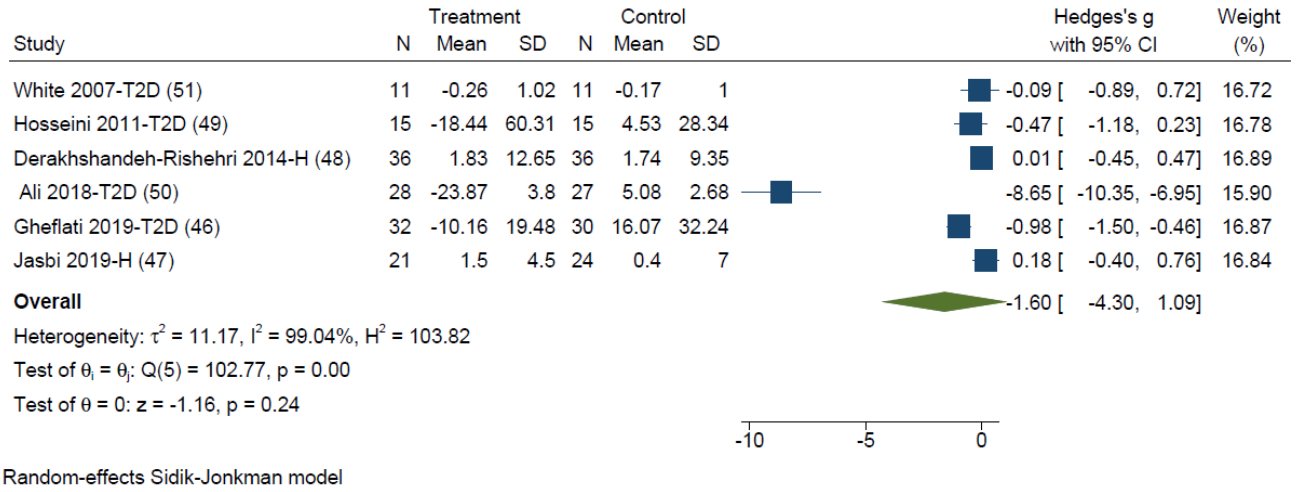

Figure 9. Forest plots for randomized controlled trials of chronic vinegar intake on postprandial blood glucose. Chronic interventions with vinegar had a main effect of -1.60 (-4.30, 1.09) (p=0.24) on post-intervention postprandial blood glucose iAUC (n = 143). Random-effects model was used to calculate Hedge's g (squares), 95% Cl (horizontal lines), and summary effect (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. T2D, type 2 diabetes; IGT, impaired glucose tolerance; IR, insulin resistant.

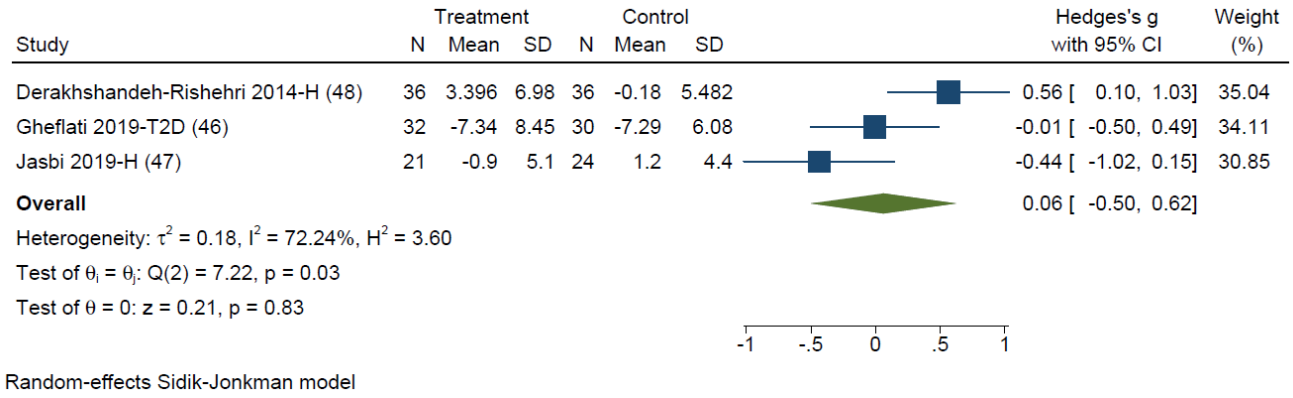

Figure 10. Forest plots for randomized controlled trials of chronic vinegar intake on postprandial blood insulin. Chronic interventions with vinegar had a main effect of 0.06 (-0.50, 0.62) (p=0.83) on post-intervention postprandial blood insulin iAUC (n = 89) Random-effects model was used to calculate Hedge's g (squares), 95% Cl (horizontal lines), and summary effect (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. Interpretation of the 'effect sizes' is <0.40 = small effect size, 0.40 to 0.70 = moderate effect size, >0.70 = large effect size. T2D, type 2 diabetes; IGT, impaired glucose tolerance; IR, insulin resistant; SMD, standard mean difference.

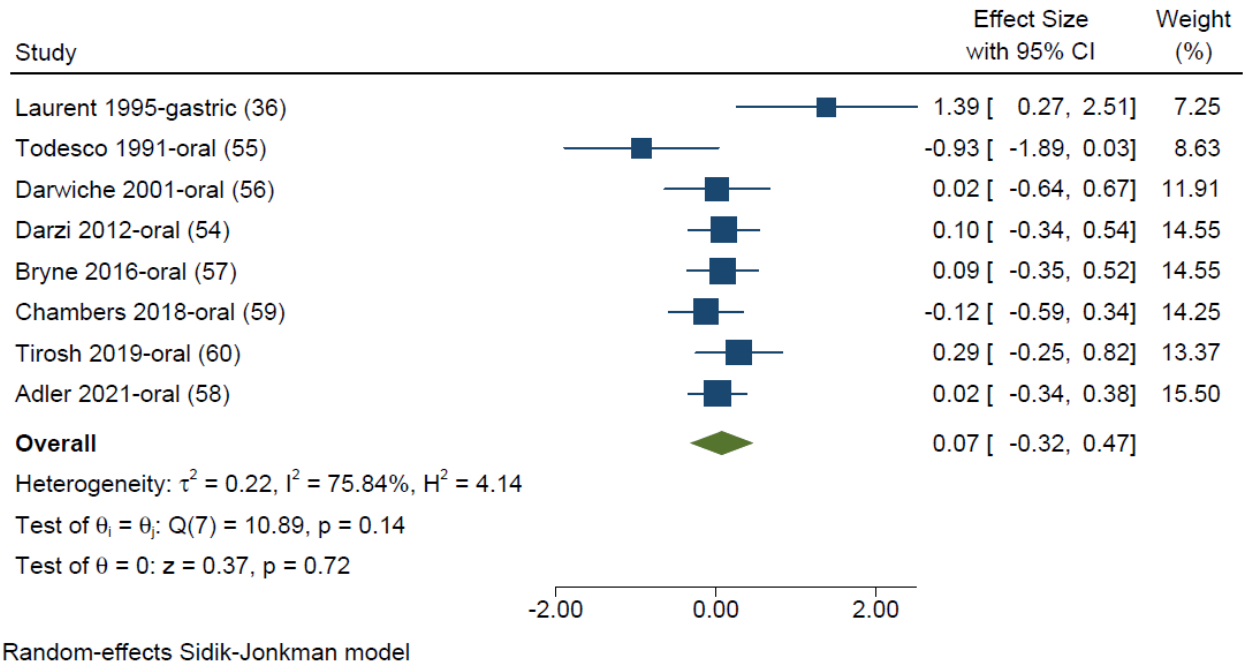

Figure 11. Forest plots for randomized controlled trials of acute propionate on postprandial blood glucose. Acute interventions with propionate had a main effect of 0.07 (-0.32, 0.47) (p=0.72) on post-intervention postprandial blood glucose iAUC (n = 123). Random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effect (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. Interpretation of the 'effect sizes' is <0.40 = small effect size, 0.40 to 0.70 = moderate effect size, >0.70 = large effect size. GIT, gastrointestinal tract; SMD, standard mean difference.

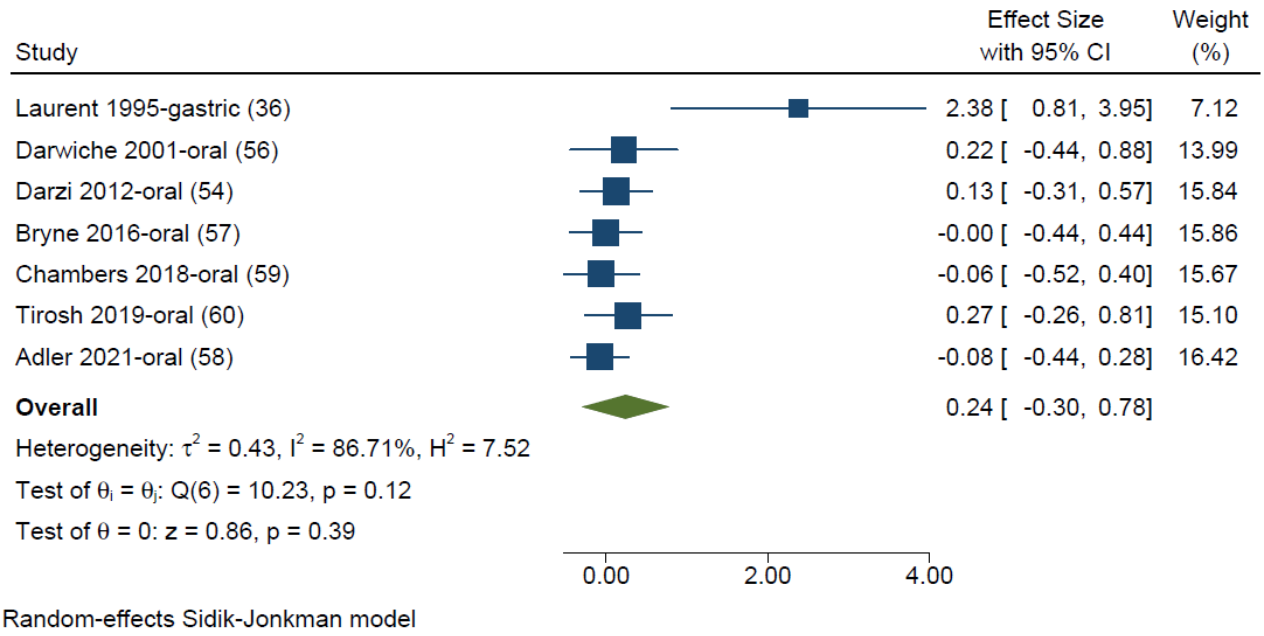

Figure 12. Forest plots for randomized controlled trials of acute propionate on postprandial blood insulin. Chronic interventions with propionate had a main effect of 0.24 (-0.30, 0.78) (p=0.39) on post-intervention intervention postprandial blood insulin iAUC (n = 117). Random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effect (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. Interpretation of the 'effect sizes' is <0.40 = small effect size, 0.40 to 0.70 = moderate effect size, >0.70 = large effect size. GIT, gastrointestinal tract; SMD, standard mean difference.

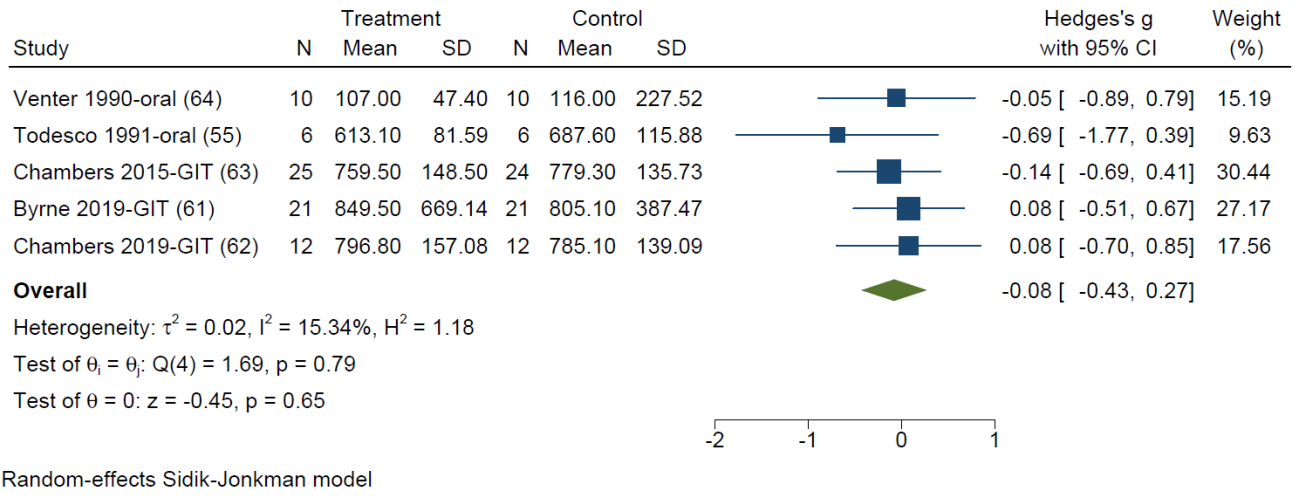

Figure 13. Forest plots for randomized controlled trials of chronic propionate on postprandial blood glucose. Chronic interventions with propionate had a main effect of -0.08 (-0.43, 0.27) (p=0.65) on post-intervention postprandial blood glucose iAUC (n = 73). Chambers et al., 2015 and Pingitore et al., 2017 reported the same study so only Chambers et al., 2015 was reported in the meta-analysis. Random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effect (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. Interpretation of the 'effect sizes' is <0.40 = small effect size, 0.40 to 0.70 = moderate effect size, >0.70 = large effect size. GIT, gastrointestinal tract; SMD, standard mean difference.

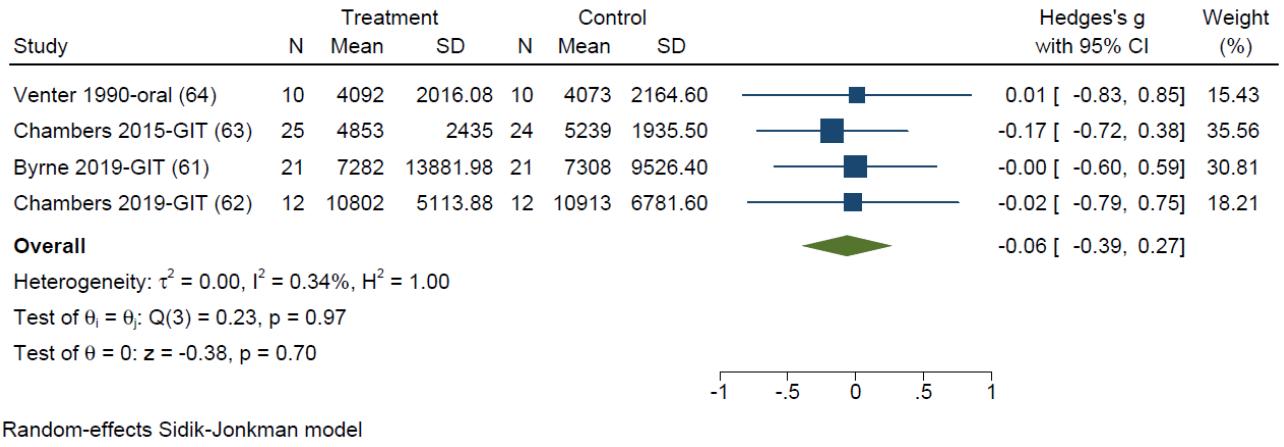

Figure 14. Forest plots for randomized controlled trials of chronic propionate on postprandial blood insulin. Chronic interventions with propionate had a main effect of -0.06 (-0.39, 0.27) (p=0.70) on post-intervention intervention postprandial blood insulin iAUC (n = 67). Random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effect (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. Interpretation of the 'effect sizes' is <0.40 = small effect size, 0.40 to 0.70 = moderate effect size, >0.70 = large effect size. GIT, gastrointestinal tract; SMD, standard mean difference.

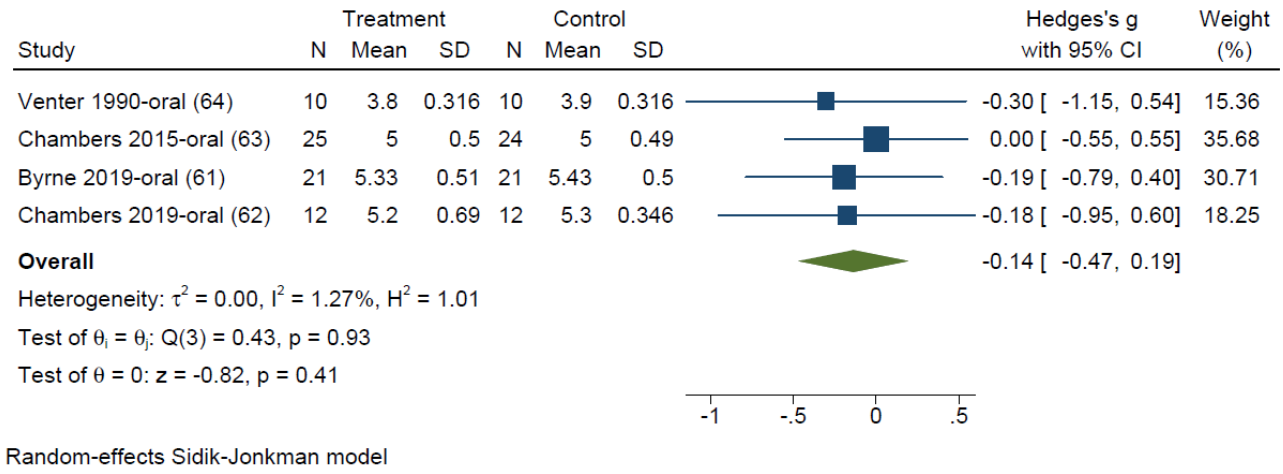

Figure 15. Forest plots for randomized controlled trials of chronic propionate on fasting blood glucose. Chronic interventions with propionate had a main effect of -0.14 (-0.47, 0.19) (p=0.41) on post-intervention fasting blood glucose (n = 67). Random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effect (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. Interpretation of the 'effect sizes' is <0.40 = small effect size, 0.40 to 0.70 = moderate effect size, >0.70 = large effect size. GIT, gastrointestinal tract; SMD, standard mean difference.

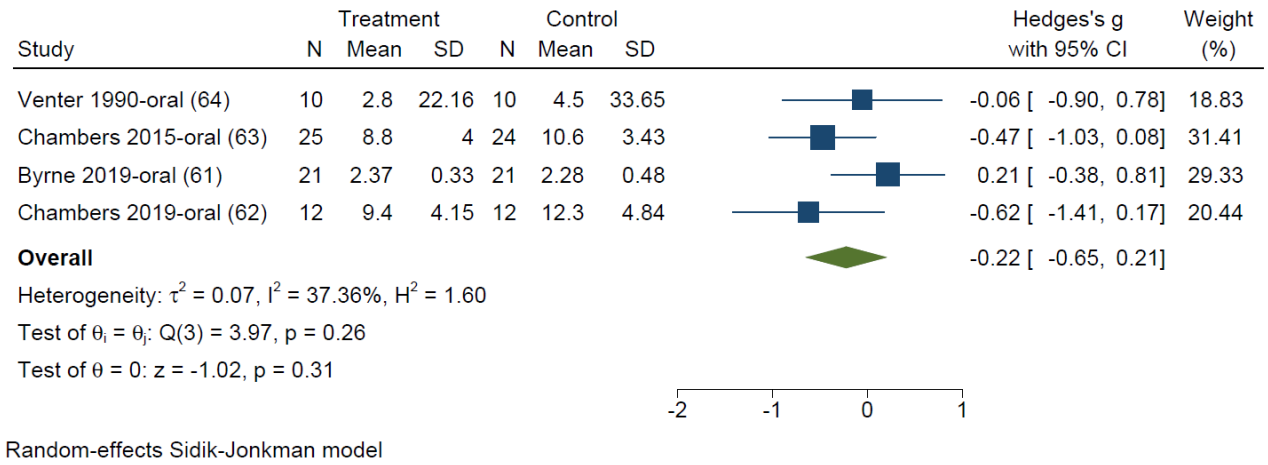

Figure 16. Forest plots for randomized controlled trials of chronic propionate on fasting blood insulin. Chronic interventions with propionate had a main effect of -0.22 (-0.65, 0.21) (p=0.31) on post-intervention intervention fasting blood insulin (n = 67). Random-effects model was used to calculate standardized mean differences (squares), 95% CIs (horizontal lines), and summary effect (diamond). The study weight (expressed as %) indicates the relative contribution of individual studies to the overall pooled effect size. Between studies heterogeneity was calculated using the I^2 statistic. P-value≤0.05 was considered statistically significant. Interpretation of the 'effect sizes' is <0.40 = small effect size, 0.40 to 0.70 = moderate effect size, >0.70 = large effect size. GIT, gastrointestinal tract; SMD, standard mean difference.