*Special Article*

# Short-chain fatty acids as anti-inflammatory agents in overweight and obesity: a systematic review and meta-analysis

Shaun Eslick, Cherry Thompson, Bronwyn Berthon, and Lisa Wood

***Context:*** *Short-chain fatty acids (SCFAs) derived from microbial fermentation of prebiotic soluble fibers are noted for their anti-inflammatory benefits against obese systemic inflammation.* ***Objective:*** *A systematic review and meta-analysis were undertaken to investigate the effect of SCFAs and prebiotic interventions on systemic inflammation in obesity.* ***Data Sources:*** *Relevant studies from 1947 to August 2019 were collected from the Cumulative Index to Nursing and Allied Health Literature, Embase, Medline, and Cochrane databases. Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were followed.* ***Study Selection:*** *Of 61 included studies, 29 were of humans and 32 of animals.* ***Data Extraction:*** *Methodological quality of studies was assessed using the critical appraisal checklist of the Academy of Nutrition and Dietetics. Data pertaining to population, intervention type and duration, and markers of systemic inflammation were extracted from included studies.* ***Results:*** *Of 29 included human studies, 3 of 4 SCFA interventions and 11 of 25 prebiotic interventions resulted in a significant decrease in $\geq$1 biomarker of systemic inflammation. Of 32 included animal studies, 10 of 11 SCFA interventions and 18 of 21 prebiotic interventions resulted in a significant reduction of $\geq$1 biomarker of systemic inflammation. Meta-analysis revealed that prebiotics in humans reduced levels of plasma high-sensitivity C-reactive protein (standard mean difference [SMD], −0.83; 95%CI: −1.56 to −0.11; $I^2$: 86%; P = 0.02) and plasma lipopolysaccharide (SMD, −1.20; 95%CI: −1.89 to −0.51; $I^2$: 87%; P = 0.0006), and reduced TNF−α levels in animals (SMD, −0.63; 95%CI: −1.19 to −0.07; P = 0.03). Heterogeneity among supplement types, duration, and dose across studies was significant.* ***Conclusion:*** *Evidence from this review and meta-analysis supports the use of SCFAs and prebiotics as novel aids in treatment of obese systemic inflammation.* ***Systematic Review Registration:*** *PROSPERO registration no. CRD42020148529.*

## INTRODUCTION

Obesity is a global pandemic: prevalence more than doubled from 1980 to 2014.[1] In 2016, >1.9 billion adults were overweight, with 650 million of those people classed as obese, equating to 13% of the world's population.[1] The loss of life expectancy for an obese person is significant compared with that of a person of healthy

Affiliation: *S. Eslick*, *C. Thompson*, *B. Berthon*, and *L. Wood* are with the Level 2, Hunter Medical Research Institute, University of Newcastle, Kookaburra Circuit, New Lambton Heights, New South Wales, Australia

Correspondence: S. Eslick, Centre for Asthma and Respiratory Diseases, Level 2, Hunter Medical Research Institute, Kookaburra Circuit, New Lambton Heights, NSW 2305, Australia. E-mail: Shaun.eslick@uon.edu.au .

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weight: at only 25 years of age, remaining life expectancy is expected to decrease by approximately 12 years.[2]

In obesity, translocation of lipopolysaccharide (LPS) from colonic bacteria has been characterized as a hallmark trigger of low-grade systemic inflammation. LPS is a main component of outer-membrane gram-negative bacteria, with increases in LPS linked to obesity and high-fat diets as well as diets low in fiber and high in sugars.[3,4] A common feature of obesity is gut dysbiosis, where imbalances in the composition and function of intestinal microbes occur as a result of high-fat diets.[3] In turn, as a result of adversely altered gut composition, increased intestinal permeability occurs due to fewer and disorganized tight-junction proteins, ultimately elevating LPS levels.[5,6] LPS drives the overproduction of pro-inflammatory cytokines and reactive oxygen species, resulting in systemic inflammation.[5,7]

Systemic inflammation also occurs in obesity due to the expansion of adipocytes, which induces substantial tissue remodeling and angiogenesis, requiring regulated local pro-inflammatory responses.[8] Because of rapid growth, local hypoxia occurs, resulting in cell death, thereby triggering an accumulation of pro-inflammatory mediators that spill over into the bloodstream.[9] Additionally, prolonged lipogenesis renders adipocytes unable to package excess lipids, resulting in a spillover of free fatty acids into circulation, activating a number of inflammatory responses.[10] These dysfunctional processes are responsible for chronic low-grade systemic inflammation that is observed in obesity. Systemic inflammation manifests as an elevation in levels of proinflammatory mediators such as C-reactive protein (CRP), interleukins (ILs; eg, IL-1$\beta$, IL-6), and tumor necrosis factor $\alpha$ (TNF-$\alpha$).[9] Systemic inflammation contributes to the development of various chronic diseases such as type 2 diabetes (T2DM), liver disease, arthritis, and some types of cancer, through multiple organs and immune systems.[8]

Dietary fiber has health benefits due to both its anti-inflammatory properties and its ability to positively alter the composition of the gut microbiota in obesity.[11,12] One factor contributing to the anti-inflammatory properties of dietary fiber is the production of short-chain fatty acids (SCFAs). SCFAs are metabolically active end products of dietary fiber fermentation by intestinal microbial fermentation.[13] Indigestible fermentable fibers such as resistant starch, oligosaccharides, and inulin are the primary prebiotic substrates from which SCFAs are produced, with acetate, butyrate, and propionate composing >95% of SCFA content.[14,15] Numerous factors can affect SCFA production, such as the type of dietary fiber available for fermentation as well as the bacteria present in the available microbiome. Fiber types such as cellulose have poor fermentation, whereas soluble fibers such as oligosaccharides and resistant starch are potent SCFA substrates.[16] They also promote the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*, which are potent SCFA producers.[16,17]

In vitro, SCFAs have been found to exhibit anti-inflammatory effects by altering cytokine production and chemotaxis in immune cells such as neutrophils, monocytes, and macrophages.[18] Furthermore, SCFAs have been proposed to inhibit LPS-induced inflammation, a primary trigger of systemic inflammation in obesity.[19] SCFAs' anti-inflammatory effects have been highlighted through a number of mechanisms. SCFAs have been proposed to activate free fatty acid receptors 2 and 3 (FFARs), whereby activation initiates downstream signaling cascades that inhibit inflammation.[20] FFAR2 is primarily expressed on immune cells such as neutrophils and monocytes, as is FFAR3, albeit at lower levels.[20] FFAR3 is highly expressed on adipocytes in adipose tissue and therefore has been proposed to have implications in obesity.[20] SCFAs also have been suggested to inhibit histone deacetylase (HDAC) activity: inhibition of HDACs leads to a reduction in pro-inflammatory gene transcription, ultimately decreasing pro-inflammatory cytokine production.[20] Hence, SCFAs produced via fermentation of prebiotics may be useful for alleviating systemic inflammation in obesity.

A limited number of studies have examined the effects of SCFA interventions in humans. Several studies have examined mechanisms for SCFA action in animal studies, because immune cells present in mice feature similar receptors such as FFARs and HDACs found on human immune cells.[20] Therefore, in this review, we evaluated the effect of SCFAs and prebiotics on inflammation in overweight/obesity in both humans and animals.

## METHODS

The electronic databases Cumulative Index to Nursing and Allied Health Literature, Embase, Medline, and Cochrane were searched for articles from 1947 to August 2019, using keyword search terms and medical subject headings. A search strategy included the following key search terms: overweight, obese, obesity, body weight, weight gain, adipose tissue, energy intake, short-chain fatty acids, volatile fatty acids, butyrate, acetate, propionate, soluble fiber, dietary fiber, inflammation, inflammatory mediators, interleukins, TNF-$\alpha$, and C-reactive protein.

### Article inclusion and exclusion criteria

The Participants, Intervention, Comparators, Outcomes and Study Design (PICOS) criteria are displayed in

*Table 1* **PICOS criteria for inclusion of studies**

| Parameter | Criteria |
|---|---|
| Population | Overweight/obese humans or obese or high-fat-diet–treated animals |
| Intervention | Short-chain fatty acids or prebiotics, delivered orally, via diet, or via enema |
| Comparison | Control group (ie, maltodextrin or starch), unexposed group (no short-chain fatty acid or no prebiotic) |
| Outcome | Markers of systemic inflammation (eg, interleukins such as IL-6 and IL-1$\beta$, C-reactive protein, tumor necrosis factor-$\alpha$, and lipopolysaccharide) |
| Study design | Randomized controlled trial, quasi-experimental studies, cohort studies, case-control studies, before and after studies, and cross sectional studies |

Table 1. In this review, studies were included if they investigated the effect of SCFAs or prebiotics delivered orally, intravenously, or via enema on any biomarker of systemic inflammation in an obese or overweight context in either humans or animals of any age or sex. In humans, the target population was assessed as overweight if body mass index (BMI) was 25–29.9 $kg/m^2$ and obese if BMI was $>30\,kg/m^2$. In animals, the target population were defined as obese animals or animals fed a high-fat diet. Randomized controlled trials (RCTs), quasi-experimental studies, cohort studies, case-control studies, before-and-after (pre-post) studies, and cross-sectional studies were included. Exclusion criteria for this review were narrative or systematic reviews, case studies, conference abstracts, interventions that included synbiotics, or studies that were not in English.

### Critical appraisal and data extraction

Studies retrieved after database searches were independently reviewed by 2 reviewers (S.E., C.T.) first on the basis of title, followed by abstract and full text. A disagreement on the inclusion of any study was settled by a third independent reviewer (B.B.). During screening, reasons for exclusion were noted. After full-text screening, included studies underwent appraisal for methodological quality by 2 reviewers (S.E., C.T.) using a standardized critical appraisal checklist designed by the Academy of Nutrition and Dietetics.[21] This checklist consists of 10 validity questions to assess methodological strength. Two reviewers (S.E., C.T.) independently rated each study as negative, neutral, or positive. Studies that did not meet $\geq$6 validity questions were rated negative; those that did not meet criteria of questions 2, 3, 6, or 7 but met most other questions were rated neutral; and those that met the criteria for questions 2, 3, 6, and 7 and most other questions were rated positive. Last, the level of evidence for each article was decided according to study design, per the evidence hierarchy of the National Health and Medical Research Council of Australia.[22]

### Data extraction

The following data were extracted from included studies: country, population, publication year, study design, sample size, details of intervention, duration of intervention, and outcomes of interest: systemic inflammatory biomarkers or measures of SCFAs.

### Meta-analyses

For this review, meta-analysis was conducted using RevMan, version 5.3 (Nordic Cochrane Centre). Heterogeneity of studies was determined using the $X2$ test (significant heterogeneity determined at $P < 0.1$) and the $I^2$ factor (30%–60% = moderate, 50%–90% = substantial, and 75%–100% = considerable heterogeneity).[23] When substantial heterogeneity was found, subgroup analysis was performed to address possible contributing factors. Studies in this review were considered heterogeneous as a result of type and dosage of SCFAs or prebiotic supplementation and study population, such as disease status and country of origin; therefore, the random-effects meta-analysis model was used in meta-analyses. The inverse-variance statistical method was used, with standardized mean difference (SMD) reflecting effect size and consequent 95%CIs calculated. Interventions were included on the basis of guidelines of the *Cochrane Handbook for Systematic Reviews of Interventions*.[23] Where studies reported multiple treatments or doses (ie, varying doses of a prebiotic), groups were combined into a single pair-wise comparison.

Crossover studies were not included in meta-analyses if there was inadequate detail to reject the possibility of carry-over effects, if data were not reported in an appropriate form (ie, individual participant data or within-patient differences) for paired analyses to be approximated, or if results from paired analyses were not reported. Additionally, some studies and/or data from studies could not be included in the meta-analysis because of to the statistical analysis (ie, analysis of covariance models) used or the method of data reporting.

## RESULTS

A total of 6323 articles were found, from which 1740 duplicates were removed (Figure 1). Thus, 4583 titles were examined and 1028 articles were retrieved for abstract review. After abstract review, 704 articles were

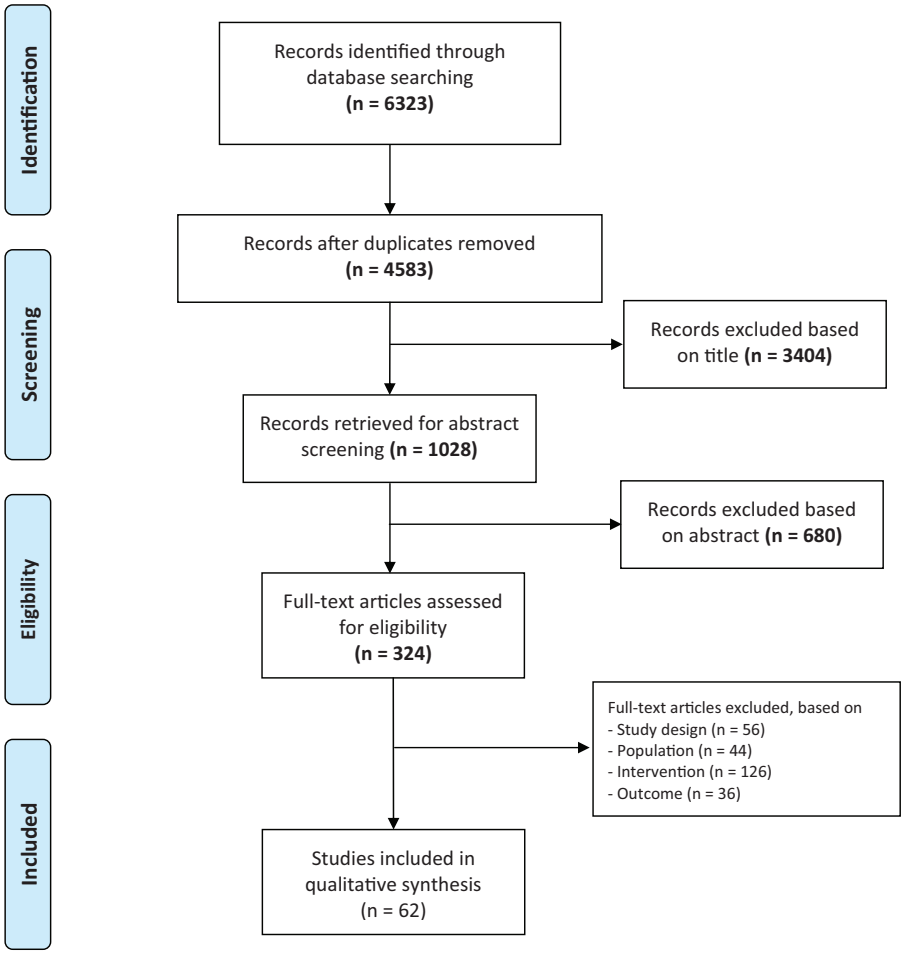


*Figure 1* Flowchart of articles for inclusion in systematic review for the effects of short-chain fatty acids and prebiotics on systemic inflammation in overweight or obese humans and animals

excluded, leaving 324 articles to be retrieved for full-text review. Data extraction and quality assessment were undertaken for 61 articles that met review criteria. Of these, 51 studies (84%) included in this review had positive methodological quality. Methodological vigor of these studies was strengthened by their use of random allocations to intervention, incorporation of controls or treatment sequence (crossover trials), blinding, and comparability of study groups. Ten studies (16%) were rated as having neutral quality, primarily as a result of inadequate detail pertaining to intervention protocol or design and/or methods of outcome measures. Nil studies were rated as negative.

## Description of included human studies

Of the 61 studies included, 29 studies (48%) were of humans. Of the 29 human studies, 27 (93%) were RCTs (n = 10 crossover trials), 1 was an intervention study, and 1 was a secondary analysis of an RCT. Included studies were published from 2008 to 2019. Ten studies (34%) were conducted in Europe,[13,24–32] 9 (31%) in North America,[33–41] 8 (28%) in Asia,[42–49] and 2 (7%) in South America.[50,51] A total of 28 studies (97%) were undertaken in adults; the remaining 1 study (3%) was carried out with children. All the studies included overweight or obese individuals and 12 studies (41%) included participants who had T2DM, or were prediabetic, or had insulin resistance. Two studies (7%) included participants with metabolic syndrome, 1 study (4%) included participants with both metabolic syndrome and T2DM, 1 study (4%) included participants with nonalcoholic fatty liver disease, 1 study (4%) included persons with nonalcoholic steatohepatitis, and 1 study (4%) included bariatric patients. The remaining studies included participants who were overweight/obese and had no other comorbidities reported.

Of the experimental studies in humans; 4 (14%) used SCFAs (ie, sodium acetate or sodium butyrate) (Table 2),[13,24,33,42] whereas 25 (86%) used a form of prebiotic fiber (ie, resistant starch, polysaccharide, or oligosaccharide) (Table 3).[25–32,34–41,43–51] The intervention period ranged from 1 hour to 36 weeks.

*Table 2* **Oral, intravenous, and colonic SCFA interventions in overweight and obese humans**

| Reference; country | Type of study/ evidence level[a] | Quality[b] | No. of participants (M/F) | Age range, y | Population characteristics | Intervention | Control | Duration | Result |
|---|---|---|---|---|---|---|---|---|---|
| van der Beek et al[24]; Netherlands 2016 | RCT-crossover/II | + | 6/0 | 20–50 | BMI,[c] 25–35 with no other comorbidities | Distal/proximal colon sodium acetate infusion (100/180 mM) 2×3 d IV catheter | 0.9% NaCl, 120 mL | 3 d arm with;7 d w/o | ↔ Plasma TNF-α, IL-8, and IL-6<br>↔ Plasma acetate, butyrate, or propionate |
| Canfora et al[13]; Netherlands 2017 | RCT-crossover/II | + | 12/0 | 20–50 | BMI 25–35 with no other comorbidities | SCFA mixtures HA, HB, and HP , 200 mL; enema (200 mM) | Placebo: 40 mm sodium chloride dissolved in 200 mL sterile water | 4×1 d arm with; 5 d w/o | ↓ Plasma IL-1$\beta$ (acetate vs propionate), ↔ plasma TNF-α, IL-8, IL-6<br>↑ Fasting plasma acetate (HA and HP vs placebo),<br>↑ Fasting plasma butyrate (HA, HB, and HP vs placebo),<br>↔ Fasting plasma propionate |
| Freeland et al[33]; Canada 2009 | RCT-crossover/II | Ø | 0/6 | Mean ± SD, 44 ± 9.8 | Hyper-insulemic obese (mean BMI, 31) | 20 mmol sodium acetate IV, 60 mmol sodium acetate enema | Normal saline, 300 mL enema or 100 mL intravenously | 4×1 h per occasion | ↓ Plasma TNF-α (acetate vs saline)<br>↔ Plasma acetate |
| Roshanravan et al[42]; Iran 2017 | RCT/II | + | 22/37 | 30–55 | Obese T2DM; BMI range, 27–35 | 600 mg/d sodium butyrate (6×100 mg oral tablets), 10 g/d inulin, inulin, and sodium butyrate | 10 g/d starch powder | 45 d | ↓ mRNA TNF-α gene expression (all groups vs control), ↓ plasma - (all groups vs control) |

Abbreviations: BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; HA, high in acetate; HB, high in butyrate; HP, high in propionate; IL, interleukin; IV, intravenous; mRNA, messenger RNA; RCT, randomized controlled trial; SCFA, short-chain fatty acid; T2DM, type 2 diabetes mellitus; TNF-α, tumor necrosis factor α; w/o, washout; +, positive study quality; Ø, neutral study quality; ↓, decrease; ↑, increase; ↔, no change.

[a] Evidence level according to the National Health and Medical Research Council.

[b] Methodological quality of study decided using the American Dietetic Association critical appraisal checklist.

[c] Calculated as weight (kg)/height ($m^2$).

*Table 3* **Oral prebiotic interventions in overweight and obese humans**

| Reference; country | Type of study/ evidence level[a] | Quality[b] | No. of participants (M/F) | Age range, y | Population (BMI) | Intervention | Control | Duration | Result |
|---|---|---|---|---|---|---|---|---|---|
| Aliasgharzadeh et al[43]; Iran 2015 | RCT/II | + | 0/55 | 30–65 | T2DM (>25) | Resistant dextrin, 10 g/d | MDX, 10 g/d | 8 wk | ↓Fasting plasma LPS, TNF-α, and IL-6, ↔ fasting plasma hs-CRP |
| Bodinham et al[25]; United Kingdom 2014 | RCT-crossover/II | + | 12/5 | Mean ± SD, 55 ± 9.9 | T2DM (Mean BMI[c], 30.6) | RS (type 2, Hi-Maize), 40 g/d | Amoica supplement, 27 g/d | 12 wk; 12 wk w/o | ↓ Fasting plasma TNF-α, ↔ fasting plasma IL-6, ↓ fasting plasma butyrate and propionate |
| Bomhof et al[34]; Canada 2018 | RCT/II | + | 8/6 | ≥18 | NASH (White population, >25; Asian population, >23) | OF, (8 g/d for 12 wk then 16 g/d for 24 wk) | MDX, (8 g/d for 12 wk then 16 g/d for 24 wk) | 36 wk | ↔ TNF-α, IL-6, and LPS |
| Canfora et al[26]; Netherlands 2017 | RCT/II | + | 23/21 | 45–70 | Prediabetic (28–40) | GOS, 15 g/d | MDX, 16.95 g/d | 12 wk | ↔ Fasting plasma TNF-α, IL-8, and IL-6, ↔ fecal or fasting plasma SCFAs |
| Chambers et al[27]; United Kingdom 2019 | RCT-crossover/II | + | 3/9 | 49–65 | Overweight/obese (25–40) | INU-propionate ester or INU, 20 g/d | Cellulose, 20 g/d | 6 wk | ↔ Fasting serum CRP, IL-6, IL-8, IL-10, and IL-12, ↑ fecal propionate (INU-propionate ester vs inulin + cellulose), ↔ fecal butyrate or acetate, ↔ serum acetate, butyrate or propionate |
| Dall'Alba et al[50]; Brazil 2013 | RCT/II | + | 27/17 | >30 | T2DM and metabolic syndrome | SF, 10 g/d (Benefiber) | N/A | 6 wk | ↔ Serum hs-CRP |
| Dehghan et al[46]; Iran 2014a | RCT/II | Ø | 0/49 | 20–65 | T2DM (>25) | INU, 10 g/d | MDX, 10 g/d | 8 wk | ↓ Serum hs-CRP, LPS, and TNF-α, ↔ serum IL-10 |
| Dehghan et al[45]; Iran 2014 b | RCT/II | Ø | 0/52 | 20–65 | T2DM (>25) | OF-enriched INU, 10 g/d | MDX, 10 g/d | 8 wk | ↓ Serum LPS, TNF-α, and hs-CRP, ↔ serum IL-10 and IL-6 |
| Dehghan et al[44]; Iran 2016 | RCT/II | + | 0/46 | 30–65 | T2DM (range, 25–34.99) | OF-enriched INU, 10 g/d | MDX, 10 g/d | 8 wk | ↓ Serum IL-12, ↑ serum IL-4 |
| Dewulf et al[28]; United Kingdom 2013 | Intervention study/III | + | 0/30 | 18–65 | Obese (> 30) | INU/OF (50/50 mix), 16 g/d | MDX, 16 g/d | 12 wk | ↔ Plasma CRP and LPS |
| Fernandes et al[51]; Brazil 2016 | RCT/II | Ø | 1/5 | 18–65 | Bariatric patients (<40) vs healthy (range, 18.5-24.9) | FOS, 6 g/d | MDX, 6 g/d | 15 d | ↔ Plasma IL-1B, IL-6, TNF-α, and CRP |
| Javadi et al[47]; Iran 2018 | RCT/II | + | 16/3 | 20–60 | NAFLD (25.5–35.5) | INU, 10 g/d + probiotic placebo | MDX, 10 g/d + probiotic placebo | 12 wk | ↓ Serum TNF-α and hs-CRP, ↔ IL-6 |
| Johnston et al[30]; United Kingdom 2010 | RCT/II | + | 12/8 | 21–70 | Insulin resistant, overweight/obese | RS (type 2, Hi-Maize), 40 g/d | 27 g/d rapidly digestible starch | 12 wk | ↔ Plasma hs-CRP and IL-6 |
| Kamalpour et al[48]; Iran 2018 | RCT/II | + | 12/25 | ≥30 | T2DM (25–35) | Psyllium, 7 g/d | Cellulose, 7 g/d | 2 wk | ↓ Plasma TNF-α (vs baseline), ↔ plasma TNF-α (vs control) |
| Karimi et al[49]; Malaysia 2015 | RCT/II | + | 0/56 | 30–65 | T2DM (>25) | RS (type 2, Hi-Maize), 10 g/d | MDX, 10 g/d | 8 wk | ↓ Plasma hs-CRP |
| King et al[35]; United States 2007 | RCT- crossover/II | Ø | 3/14 | 21–49 | Overweight /obese (≥27) | High-fiber DASH diet (30 g/d) or psyllium (30 g/d) | N/A | 3 wk | ↔ Serum hs-CRP |
| King et al[36]; United States | RCT/II | Ø | 44/118 | 40–65 | Overweight | Psyllium, 7 or 14 g/d | N/A | 12 wk | ↔ Serum CRP or IL-6 |

(continued)

*Table 3* **Continued**

| Reference; country | Type of study/ evidence level[a] | Quality[b] | No. of participants (M/F) | Age range, y | Population (BMI) | Intervention | Control | Duration | Result |
|---|---|---|---|---|---|---|---|---|---|
| 2008 | | | | | /obese (>25) | | | | |
| Maki et al[37]; United States 2012 | RCT –crossover/II | Ø | 11/22 | 18–69 | Obese and insulin resistant | RS (type 2), 15 or 30 g/d | Amoica, 15 or 30 g/d | 3×4 wk; 3 wk w/o | ↑ Plasma acetate in women (30 g/d vs 15 g/d and control), ↔ serum hs-CRP |
| Morel et al[29]; France 2015a | RCT/II | Ø | 44/44 | 18–60 | Overweight (25–28) | α-GOS, 6, 12, or 18 g/d | Dried glucose syrup, 6, 12, 18 g/d | 2 wk | ↓ Plasma LPS (all groups vs control) |
| Morel et al[29]; France 2015 b | RCT/II | Ø | 44/44 | 18–60 | Overweight (25–28) | α-GOS, 12 g/d | Dried glucose syrup, 12 g/d | 2 wk | ↓ Plasma CRP and LPS (all groups vs control) |
| Nicoluci et al[38]; Canada 2017 | RCT/II | + | 24/18 | 7–12 children | >85th BMI percentile | OF-INU, 8 g/d | MDX, 8 g/d | 16 wk | ↔ Serum IL-6 and LPSIL-10, CRP, IL-1β, TNF-α, IL-4s and IL-33 |
| Parnell et al[39]; Canada 2017 | Secondary Analysis of RCT/III | + | 48 | N/a | Overweight/ obese | OF, 21 g/d | MDX, 21 g/d | 12 wk | ↔ Plasma IL-6, LPS, TNF-α, and MCP-1 |
| Reimer et al[40]; Canada 2013 | RCT/II | + | 25/31 | 20–65 | Overweight/ obese (range, 24–30) | PS, 15 g/d fiber product "PGX" | Rice flour, 15 g/d | 14 wk | ↓ Serum IL-1β and IL-6 (PGX vs baseline), ↔ serum TNF-α, ↓ fecal butyrate (PGX vs baseline) |
| Reverri et al[41]; United States 2015 | RCT-crossover/II | + | 6/6 | >18 | Metabolic syndrome | SF matched meal (2 g IF and 8 g SF) | Antioxidant matched meal (0 g fiber) | 3×1 d arm; 1 wk w/o | ↑Plasma IL-6 postprandial (all groups), ↔ plasma IL-1β and IL-6 |
| Rizkalla et al[31]; France 2012 | RCT- crossover/II | + | 8/5 | 30–60 | Overweight/ obese (27–38) | SF, 6.9 g | Low-calorie conventional diet (1200 kcal) | 2×4 wk arm; 8 wk w/o | ↔ Fasting plasma TNF-α or IL-6 |
| Vulevic et al[32]; United Kingdom 2013 | RCT crossover/II | Ø | 16/29 | 18–65 | Predisposed to metabolic syndrome >3 risk factors (>25) | GOS, 5.5 g/d | MDX 5.5 g/d | 10 wk arm; 4 wk w/o | ↔ Plasma TNF-α, IL-6, and IL-1β (vs control), ↓ plasma CRP (vs control) |

Abbreviations: BMI, body mass index; CRP, C-reactive protein; DASH, Dietary Approaches to Stop Hypertension; FOS, fructo-oligosaccharide; GOS, galacto-oligosaccharide; IF, insoluble fiber; INU, inulin; kcal, kilocalorie; MDX, maltodextrin; NAFLD, nonalcoholic fatty liver disease; RS, resistant starch; N/A, not applicable; NASH, nonalcoholic steatohepatitis; OF, oligofructose; PS, polysaccharide; RCT, randomized controlled trial; SF, soluble fiber; T2DM, type 2 diabetes mellitus; w/o, washout; +, positive study quality; Ø, neutral study quality; ↓, decrease; ↑, increase; ↔, no change.

[a]Evidence level indicated as per the National Health and Medical Research Council.

[b]Methodological quality of study decided with the use of American Dietetic Association critical appraisal checklist.

[c]Calculated as weight (kg)/height ($m^2$)

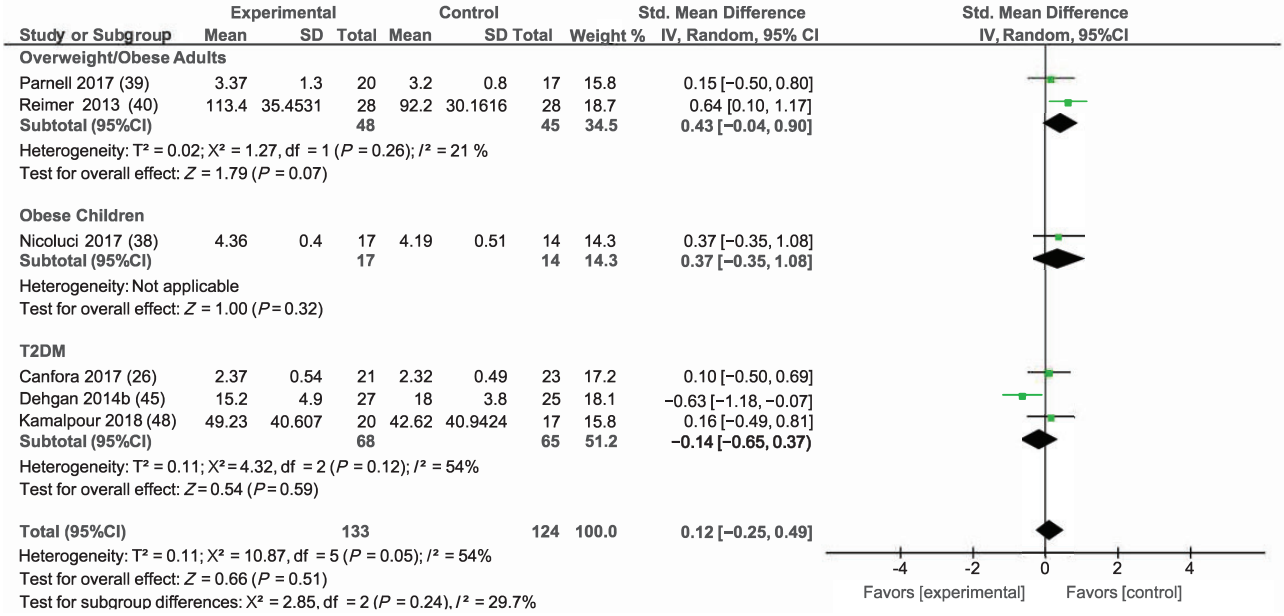


*Figure 2* **Forest plot of randomized controlled trials examining the effect of prebiotic supplementation on circulating tumor necrosis factor α (TNF-α), subgrouped by adults, children, and disease status. Pooled effect estimates (diamond) for TNF-α are shown. Values are expressed as standardized mean differences with 95%CIs determined by a generic IV random-effects model. Heterogeneity measured by $I^2$ at a significance of $P < 0.10$.** df, degrees of freedom; IV, inverse variance; Std., standardized; T2DM, type 2 diabetes mellitus

## Description of included animal studies

Of the 61 studies included in the review, 32 (52%) were studies of animal models, published from 2005 to 2019. Of these, 12 animal studies (38%) were conducted in Europe,[6,52–62] 14 (44%) in Asia,[63–76] 3 (9%) in North America,[77–79] 2 (6%) in South America,[80,81] and 1 (3%) in Africa.[82] Animal models consisted of rats or mice that were obese or fed a high-fat diet, wherein mice were fed a high-fat diet for a period during or before treatment. Eight studies (25%) used diabetic mice; 1 study (3%) observed pregnant, obese mice; and 1 study (3%) used mice with steatosis induced by a high-fat diet.

Of the included animal studies, 11 (34%) studied the delivery of SCFAs and 21 (66%) supplemented animals with a form of prebiotic fiber. Intervention periods ranged from 3 weeks to 25 weeks.

## Effects of SCFA on systemic inflammation in humans

Characteristics of human experimental studies investigating SCFAs are presented in Table 2. van der Beek et al[24] examined the effect of sodium acetate infusions in distal (100 mm/l) and proximal (180 mmol/l) segments of the colon on systemic inflammation. The 100 mmol/l distal administration led to a nonsignificant reduction in plasma TNF-α levels ($P = 0.067$) vs placebo.[24] The 180 mmol/l distal administration also led to a nonsignificant increase in plasma acetate levels ($P = 0.069$) vs placebo. Proximal infusions did not have any significant affects.[24] Canfora et al[13] investigated the effect of SCFA mixtures high in butyrate, acetate, or propionate, delivered via enema, and their effect on systemic inflammation. The high-acetate mixture significantly reduced fasting plasma IL-1β levels compared with the high-propionate infusion; however, the change was not different than that observed after administration of the placebo infusion.[13] No significant changes were observed in other systemic inflammatory markers (namely, TNF-α, IL-6, and IL-8).[13] Freeland and Wolever[33] infused sodium acetate intravenously and via enema, which results in a significant decrease in plasma levels of TNF-α in both groups compared with the control (saline). Roshanravan et al[42] administered 600 mg/d sodium butyrate, 10 g/d inulin, or both orally for 45 days. A significant decrease in plasma hs-CRP and TNF-α messenger RNA gene expression was observed as a result of all interventions, compared with the control.[42] A significant decrease in inflammation was detected in 3 of the 4 included SCFA studies (75%). Meta-analysis could not be performed with data from these studies, due to small sample sizes and heterogeneity in study designs.

## Effects of prebiotics on systemic inflammation in human studies

Characteristics of the 25 included prebiotic studies (41%) conducted with humans are described in Table 3. Of these studies, 11 (44%) reported a significant change in ≥1 pro-inflammatory (eg, TNF-α, IL-6, IL-1β, CRP)

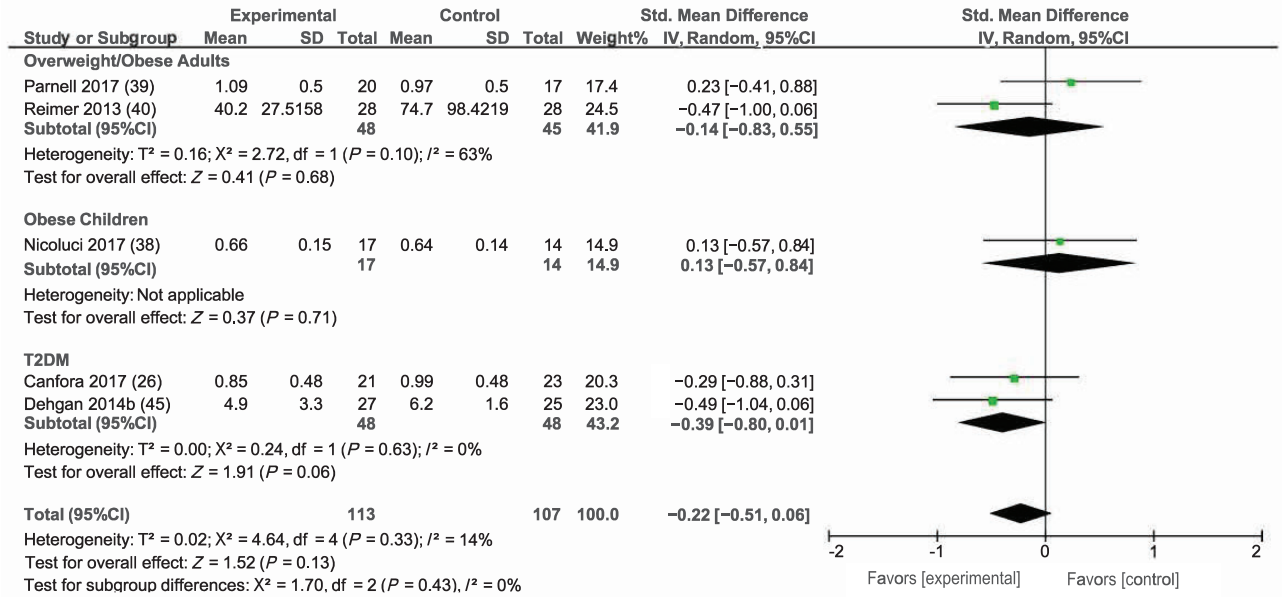


*Figure 3* **Forest plot of randomized controlled trials examining the effect of prebiotic supplementation on circulating interleukin-6 (IL-6), subgrouped by adults, children, and disease status. Pooled effect estimates (diamond) for IL-6 are shown. Values are expressed as standardized mean differences with 95%CIs determined by a generic IV random-effects model. Heterogeneity measured by $I^2$ at a significance of $P < 0.10$.** df, degrees of freedom; IV, inverse variance; Std., standardized; T2DM, type 2 diabetes mellitus

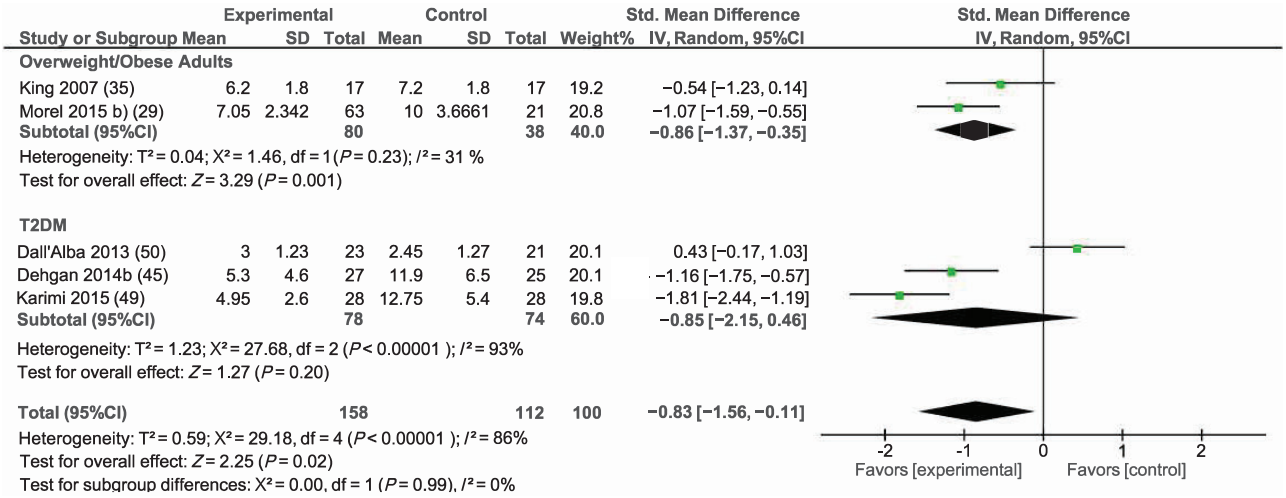


*Figure 4* **Forest plot of randomized controlled trials examining the effect of prebiotic supplementation on circulating high-sensitivity C-reactive protein (hs-CRP), subgrouped by adults, children, and disease status. Pooled effect estimates (diamond) for hs-CRP are shown. Values are expressed as standardized mean differences with 95%CIs determined by a generic IV random-effects model. Heterogeneity measured by $I^2$ at a significance of $P < 0.10$.** df, degrees of freedom; IV, inverse variance; Std., standardized; T2DM, type 2 diabetes mellitus

or anti-inflammatory (eg, IL-4, IL-10) biomarker of systemic inflammation. A total of 13 studies (52%) reported no significant change in systemic inflammatory markers, whereas in 1 study (4%) with a high soluble-fiber meal, authors reported a postprandial increase in plasma IL-6 levels.[41]

Of prebiotic interventions in overweight and obese humans, 14 interventions (56%) included a oligosaccharide prebiotic (ie, fructo-oligosaccharide, galacto-oligosaccharide, or oligofructose).[26–29,31,32,34,38,39,44–47,51] Six (43%) of these studies resulted in a significant change in ≥1 pro-inflammatory or anti-inflammatory biomarker of systemic inflammation.[29,32,44–47] No significant change was reported in the remaining 8 studies (57%).[26–28,31,34,38,39,51] Of the remaining studies, 6 (26%) investigated polysaccharide interventions,[35,36,40,41,48,50] and 5 (22%) looked at resistant-starch interventions.[25,30,37,43,49]

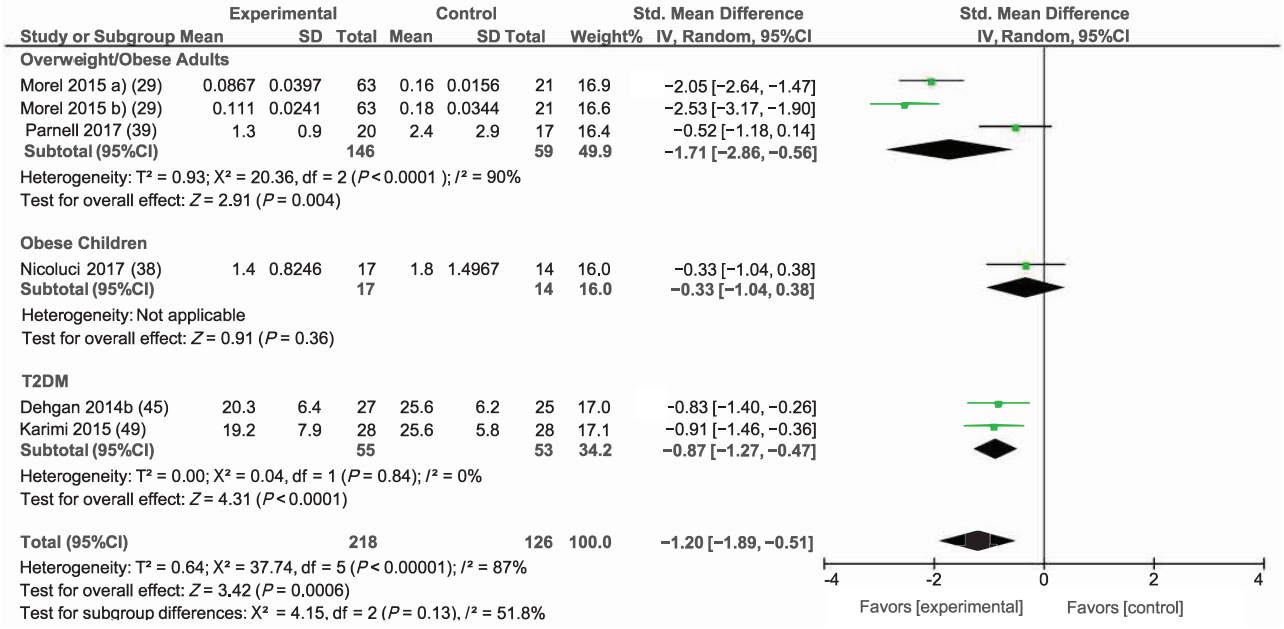


*Figure 5* **Forest plot of randomized controlled trials examining the effect of prebiotic supplementation on circulating lipopolysaccharide (LPS), subgrouped by adults, children, and disease status. Pooled effect estimates (diamond) for LPS are shown. Values are expressed as standardized mean differences with 95%CIs determined by a generic IV random-effects model. Heterogeneity measured by $I^2$ at a significance of $P < 0.10$.** IV, inverse variance; df, degrees of freedom; Std., standardized; T2DM, type 2 diabetes mellitus

Two of the 6 studies (33%) investigating polysaccharide supplementation resulted in a significant decrease in ≥1 marker of systemic inflammation compared with baseline or a control.[40,48] The remaining 4 studies (67%) reported no anti-inflammatory effects after polysaccharide supplementation.[50] In the 5 studies (17%) in which resistant-starch interventions were examined, 3 (60%) reported a significant decrease in ≥1 marker of systemic inflammation.[43] Johnston et al[30] and Maki et al[37] did not report any significant changes in insulin-resistant participants after resistant-starch supplementation.

SCFAs as an outcome of a prebiotic intervention were measured in 5 studies, 2 of which reported an increase in plasma levels of an SCFA after intervention. Supplementation of high-amylose maize starch significantly increased plasma acetate levels in women compared with the control group, and, compared with inulin and cellulose, an inulin-propionate ester significantly increased fecal propionate in overweight/obese participants.[27,37] In 1 study, a decrease in plasma levels of SCFAs after a resistant-starch intervention was reported, 1 study reported a decrease in fecal SCFAs after a polysaccharide intervention, and 1 study observed no effect of oligosaccharides on fecal or plasma SCFAs.[25,26,40]

Meta-analysis was undertaken to observe the effect of prebiotic interventions on TNF-α (n = 6), IL-6 (n = 5), hs-CRP (n = 5), and LPS (n = 6). Prebiotics overall did not have a significant effect on TNF-α (SMD, 0.12; 95%CI: −0.25 to 0.49; $I^2 = 54\%$; $P = 0.51$) (Figure 2).[26,38–40,45,48] Prebiotic interventions did not significantly change plasma IL-6 levels overall or in any subgroup (SMD, −0.22; 95%CI: −0.51 to 0.06; $I^2 = 14\%$; $P = 0.13$) (Figure 3).[26,38–40,45] Results from the meta-analysis showed that prebiotic supplementation significantly decreased hs-CRP levels compared with those of control subjects, and heterogeneity was also significant between studies (SMD, −0.83; 95%CI: −1.56 to −0.11; $I^2 = 86\%$; $P = 0.02$) (Figure 4).[29,35,45,49,50] Last, results of the meta-analysis also indicated that prebiotic supplementation significantly reduced LPS levels in humans compared with a control and, similarly, heterogeneity was significant between studies (SMD, −1.20; 95%CI: −1.89 to −0.51; $I^2 = 87\%$; $P = 0.0006$) (Figure 5).[29,38,39,45,49]

## Geographic location and the effects of SCFAs and prebiotics on systemic inflammation

Of human studies included in this review, a total of 10 (34%) were conducted in Europe, 9 (31%) in North America, 8 (28%) in Asia, and 2 studies (7%) were conducted in South America.

When subgrouped by continent, 4 studies (40%) conducted in Europe, 2 studies (22%) from in North America, and 8 studies (100%) conducted in Asia reported a significant decrease in ≥ 1 inflammatory biomarker. No studies (0%) from South America reported a significant decrease in ≥ 1 inflammatory biomarker,

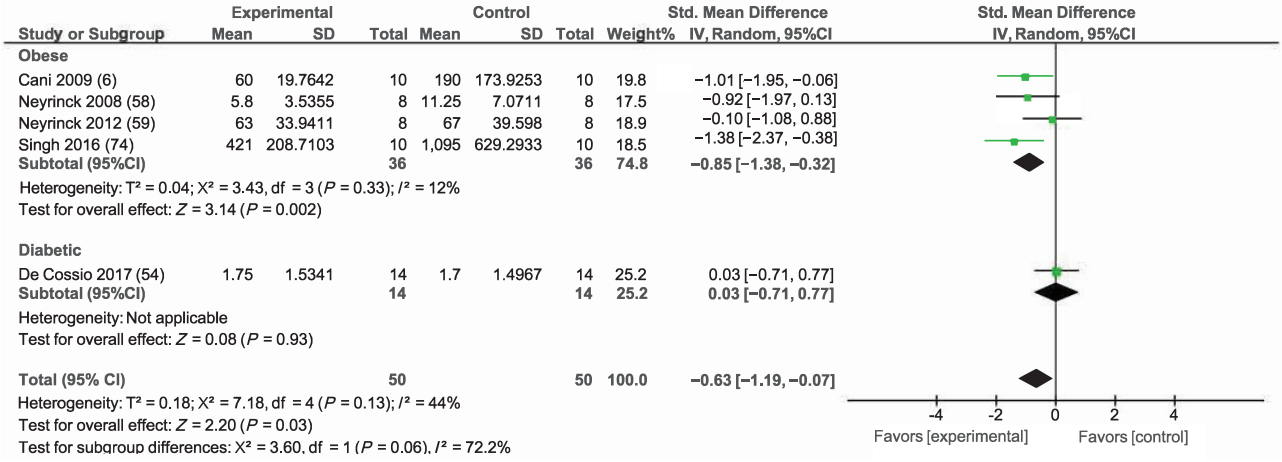


*Figure 6* **Forest plot of randomized controlled trials examining the effect of prebiotic supplementation on circulating tumor necrosis factor α (TNF-α) in obese animals. Pooled effect estimates (diamond) for TNF-α are shown. Values are expressed as standardized mean differences with 95%CIs determined by a generic IV random-effects model. Heterogeneity measured by $I^2$ at a significance of $P < 0.10$.** df, degrees of freedom; IV, inverse variance; Std., standardized

and 1 study from North America reported a significant increase in ≥ 1 inflammatory biomarker.

## Effects of SCFAs on systemic inflammation in animal models

Of the 61 studies included in this review, 11 (18%) examined the effects of an SCFA intervention on systemic inflammatory markers in overweight/obese mice (Table 4).[52,63–69,80–82] Of these 11 studies, 10 (91%) reported a significant decrease in a marker of systemic inflammation (ie, cytokine production or gene expression of TNF-α, IL-6, or IL-1β).[52,64–69,80–82] Beh et al[63] supplemented diet-induced obese mice with synthetic acetic acid or a vinegar source of acetic acid and reported no change in inflammatory markers. In 8 of these 11 animal studies (73%), sodium butyrate was used in interventions that spanned 6–16 weeks, and all reported a significant decrease in ≥1 marker of systemic inflammation as a result.[52,65–68,80]

Two studies (18%) reported analysis of SCFAs in feces as an outcome of an SCFA intervention. Lu et al[67] observed a significant increase in fecal butyrate and propionate levels as a result of sodium butyrate and sodium propionate supplementation, respectively, compared with the control group. Zhai et al[69] reported a significant increase in fecal propionate and butyrate levels after a sodium butyrate intervention.

Meta-analysis was unable to be completed for the effect of SCFAs on systemic inflammation in animals, due to the heterogeneity in study design and reported outcomes.

## Effects of prebiotics on systemic inflammation in animal studies

In this review, 21 articles (34%) reported on investigation of prebiotic effects on systemic inflammation in obese mice (Table 5).[6,53–62,70–79] Of these 21 studies, 18 (86%) reported a significant change in ≥1 pro-inflammatory (eg, TNF-α, IL-6, IL-1β, CRP) or anti-inflammatory (eg, IL-4, IL-10) biomarker of systemic inflammation. Of the remaining studies, 2 (10%) reported an increase in pro-inflammatory markers, and 1 study (5%) reported no significant changes.

In 10 studies (48%), supplementation of oligosaccharides was explored in animals; in 9 of the 10 (90%), a significant decrease was reported in ≥1 marker of systemic inflammation.[6,53,59,62,70,73–75,79] A study by de Cossio et al[54] reported a significant increase in levels of the anti-inflammatory IL-10 and pro-inflammatory IL-6 after oligofructose supplementation for 8 weeks in obese T2DM mice.

Polysaccharides were investigated in 3 of the included animal studies (14%).[71,72,78] All 3 studies found significant decreases in inflammatory responses after polysaccharide supplementation, compared with control animals that were fed only a high-fat diet.[71,72,78] Two of the included animal studies investigated the effects of resistant starch as a prebiotic intervention. Polakof et al[60] observed significant decreases in liver mRNA, TNFrsf1a, NFκBia, and IL-18r1 as a result of a diet supplemented with 40% resistant starch, whereas Barouei et al[77] reported no changes in inflammatory markers after a 6-week resistant-starch intervention.

In 6 studies (37%), supplementation with other soluble fibers were explored in animals. In 5 of the 6

*Table 4* **Oral SCFA interventions in obese mice**

| Reference; country | No. **Quality**[a] | Population | Intervention | Control | Duration | Result |
|---|---|---|---|---|---|---|
| Aguilar et al[80]; Brazil 2018 | 36 + | Obese mice | HFD + sodium butyrate (1% of diet) | HFD | 10 wk | NF-κB downregulation via ↓ mRNA p65 and IκBα |
| Beh et al[63]; Malaysia 2017 | 36 + | Obese mice | 2 mL/kg body weight of synthetic acetic acid (4%) or Nipa vinegar (4% acetic acid) | Normal diet (standard chow mixture) | 10 wk | ↔ Liver NF-κB mRNA |
| Bounihi et al[82]; Algeria 2017 | 72 + | Obese mice | Acetic acid (7 mL/kg/d), pomegranate vinegar (3.5, 7, 14 mL/kg/d), prickly pear vinegar (3.5, 7, 14 mL/kg/d) or apple vinegar (3.5, 7, 14 mL/kg/d) | Distilled water | 18 wk | ↓ Plasma CRP and TNF-α (all groups vs obese control) |
| Fang et al[64]; China 2019 | 16 + | HFD-treated mice | Sodium butyrate (0.1 mM) | HFD | 12 wk | ↓ Serum TNF-α and IL-6 |
| Lee et al[65]; South Korea 2017 | 18 + | Obese mice | HFD + sodium butyrate 5% wt/wt | HFD | 16 wk | ↔ Serum TNF-α and IL-6, ↓ serum IL-1β, ↓ peripheral blood cells mRNA TNF-α, IL-6, IL-1β, MCP-1 |
| Li et al[66]; China 2013 | 40 + | Pregnant, obese mice | HFD + 5% sodium butyrate | HFD | 14 wk | ↓ Serum TNF-α and IL-1β |
| Lu et al[67]; China 2016 | 40 + | Obese mice | HFD-A or HFD-B or HFD-P or HFD-SCFA | HFD | 12 wk | ↓ Plasma IL-1β (HFD-A vs HFD), ↓ plasma IL-6 (HFD-A, HFD-B, HFD-P vs HFD), ↓ plasma MCP-1 (HFD-P vs HFD), ↓ plasma TNF-α (HFD-B vs HFD), ↔ plasma IL-10, ↑ fecal propionate (HFD-P vs HFD), ↑ fecal butyrate (HFD-B vs HFD) |
| Mattace Raso et al[52]; Italy 2013 | 18 + | HFD-induced steatosis | HFD + 20 mg/kg) sodium butyrate or HFD + 42.5 mg/kg *N*-butyramide | HFD | 6 wk | ↓ Liver mRNA TNF-α, IL-6, and IL-1β (HFD + sodium butyrate, HFD + *N*-butyramide vs HFD), ↓ liver NF-κB activation (HFD + sodium butyrate, HFD + *N*-butyramide vs HFD) |
| Vinolo et al[81]; Brazil 2012 | 18 + | HFD-treated mice | HFD + tributyrin (2 g/kg/BW) | HFD | 10 wk | ↔ Epididymal white adipose tissue IL-6 ↓ Epididymal white adipose tissue mRNA TNF-α, MCP-1, and IL-1β (vs HFD) |
| Wang et al[68]; China 2015 | 16 + | Obese diabetic mice (db/db) | Sodium butyrate intraperitoneal injection (1 mg/kg) | Saline intraperitoneal injection (1 g/kg) | 6 wk | ↓ Epididymal adipose tissue mRNA IL-1β, IL-6, and TNF-α ↓ Subcutaneous adipose tissue mRNA IL-1β, IL-6, and TNF-α |
| Zhai et al[69]; China 2019 | 10 + | HFD-treated mice | HFD + sodium butyrate (1% wt/wt) | HFD | 8 wk | ↓ Liver mRNA expression IL-1β and IL-6 ↑ Fecal propionate and butyrate |

*Abbreviations:* BW, body weight; CRP, C-reactive protein; HFD, high-fat diet; HFD-A, high-fat diet plus 5% wt/wt sodium acetate; HFD-B, high-fat diet plus 5% wt/wt sodium butyrate; HFD-P, high-fat diet plus 5% wt/wt sodium propionate; HFD-SCFA, 5% wt/wt mixture high-fat diet plus IL, interleukin; MCP-1, monocyte chemoattractant protein 1; mRNA, messenger RNA; NF-κB, nuclear factor κ B; SCFA, short-chain fatty acid; TNF-α, tumor necrosis factor α; wt, weight; +, positive study quality; Ø, neutral study quality; ↓, decrease; ↑, increase; ↔, no change.

[a]Methodological quality of study decided using the American Dietetic Association critical appraisal checklist.

*Table 5* **Prebiotic interventions in obese mice**

| Reference; country | No. | **Quality**[a] | Population | Intervention | Control | Duration | Result |
|---|---|---|---|---|---|---|---|
| Ahmadi et al[78]; United States 2019 | 32 | + | Obese T2DM mice | HFD + INU, sago PS or acorn PS | HFD | 5 wk | ↓ mRNA TNF-α (sago vs control), mRNA IL-6 (acorn vs control), mRNA MCP-1 (INU and sago vs control) |
| Barouei et al[77]; United States 2017 | 20 | + | Obese mice | RS (type 2) (4.5 kcal/g) + HFD | HFD | 6 wk | ↑ Epididymal adipose tissue IL-6<br>↔ Epididymal adipose tissue TNF-α and MCP-1<br>↔ mRNA TNF-α, IL-10, and MCP-1 |
| Cani et al[53]; Belgium 2007 | 32 | + | Diabetic mice | OF (10%) + HFD | HFD | 14 wk | ↓ Plasma IL-6, IL-1β, and IL-1α, ↓ adipose tissue mRNA TNF-α |
| Cani et al[6]; Belgium 2009 | 20 | + | Obese mice | OF 9:1 (ratio of weight of control diet to weight of fibers) | Control diet | 5 wk | ↓ Plasma TNF-α, MCP-1, IL-1β, IL-10, IL-15, and IL-18, ↓ mRNA TNF-α |
| De Cossio et al[54]; France 2017 | 28 | + | Obese T2DM mice | OF (0.6 g/d) in rap water | Tap water | 8 wk | ↑ Plasma IL-10, ↑ plasma IL-6, ↔ plasma IL-1β, and TNF-α |
| Everard et al[62]; Belgium 2011 | 20 | + | Obese mice | OF (0.3 g/d) | Standardized control diet | 5 wk | ↓ Colon IL-1 mRNA |
| Galisteo et al[56]; Spain 2005 | 28 | + | Obese T2DM rats | SF 5 g/d | Standardized control diet | 25 wk | ↓ Plasma TNF-α |
| Galisteo et al[55]; Spain 2010 | 50 | + | Obese mice | SF (3.5 g/d) | Obese control with standard diet | 10 wk | ↓ Adipose tissue TNF-α |
| Jakobsdottir et al[57]; Sweden 2013 | 28 | + | Obese mice | Pectin 80 g/kg (acetic acid), guar gum 91 g/kg (propionic acid) or mixture (butyrate) | Fiber-free control diet | 6 wk | ↓ Portal serum MCP-1 (guar gum at wk 2 + 4 vs Fiber-free, pectin, and mixture), ↔ portal vein serum IL-1β, IL-1α, IL-6, IL-10, IL-18, and TNF-α, ↑ serum acetate and propionate (interventions vs control at wk 4 + 6), ↑ serum butyrate (guar gum and mixture vs control and pectin at wk 6) |
| Jangra et al[70]; India 2019 | 12 | Ø | HFD-treated mice | HFD + INU | HFD | 18 wk | ↓ mRNA gene expression IL-6 and TNF-α |
| Kanagasabapathy et al[71]; Malaysia 2012 | 30 | + | Diabetic mice | Glucan-rich PS (GE) (60, 120, 240 mg/kg BW) | HFD or HFD + metformin | 16 wk | ↓NF-κB (all GE groups vs HFD group)<br>↓ Adipose tissue gene expression IL-6, TNF-α, CRP, and MCP-1 (all GE groups vs HFD group) |
| Li et al[73]; China 2019 | 30 | + | Obese diabetic mice (db/db) | INU (5% wt/wt) | Saline | 6 wk | ↓ Plasma LPS, IL-6, IL-17A, IL-10, and TNF-α |
| Murakami et al[72]; Japan 2015 | 14 | + | HFD-treated mice | HFD + 10% epilactose | HFD | 8 wk | ↓ Epididymal adipose tissue gene expression TNF-α, MCP-1, ↑ caecum acetate, propionate, *n*-butyrate |
| Neyrinck et al[58] Belgium 2008 | 24 | + | Obese mice | SF + HFD | HFD | 3 wk | ↓ Plasma IL-6, ↔ plasma IL-1β, TNF-α, MCP-1, and IL-1α, ↔ visceral adipose tissue mRNA IL-1 β, IL-6, and TNF-α |
| Neyrinck et al[59]; Belgium 2012 | 16 | + | Obese mice | HFD + 7.5% arabinoxylan oligosaccharides | HFD | 8 wk | ↓ Plasma IL-6, ↔ plasma IL-1β, IL-13, TNF-α, IL-1α, IL-10, and MCP-1 |
| Polakof et al[60]; France 2013 | 20 | + | HFD-treated rats | HFD + 41.6% RS | HFD | 9 wk | ↓ Liver mRNA Tnfrsf1a, Nfκbia, and IL-18r1 |
| Sanchez et al[61]; Spain 2011 | 0/40 | Ø | Obese rats | 10% high-methoxylated apple pectin, 5% soluble cocoa fiber or 10% β-glucan–enriched diet | Standard diet | 7 wk | ↔ Plasma TNF-α |

(continued)

*Table 5* **Continued**

| Reference; country | No. **Quality**[a] | Population | Intervention | Control | Duration | Result |
|---|---|---|---|---|---|---|
| Singh et al[74]; India 2016 | 20/0+ | HFD-treated mice | HFD + IOS (1 g/kg) | HFD | 12 wk | ↓ Serum TNF-$\alpha$ and IL-1$\beta$, ↓ liver cytokines TNF-$\alpha$, ↓ liver mRNA gene expression IL-6, TNF-$\alpha$, NF-$\kappa$B, and TLR-4 |
| Singh et al[75]; India 2017 | 10/0+ | HFD-treated mice | HFD + IOS (1 g/kg) | HFD | 12 wk | ↓ Liver cytokines TNF-$\alpha$ and IL-1$\beta$, ↔ liver mRNA gene expression TNF-$\alpha$, TLR-4, Nf-$\kappa$B, and IL-6 |
| Tingting et al[76]; China 2016 | 16/0+ | Obese mice | HFD + pectin (5% wt/wt) | HFD | 6 wk | ↓ Ileal mRNA gene expression IL-6, TNF-$\alpha$, and TLR4, ↑ ileal mRNA gene expression IL-10 |
| Zou et al[79]; United States 2018 | 26 + | HFD-treated mice | HFD + 200 g INU/4057 kcal | HFD | 4 wk | ↑ Colon IL-22 |

*Abbreviations:* BW, body weight; CRP, C-reactive protein; HFD, high-fat diet; IL, interleukin; INU, inulin; IOS, isomalto-oligosaccharide; MCP-1, monocyte chemoattractant protein 1; mRNA, messenger RNA; NF-$\kappa$B, nuclear factor kappa B; T2DM, type 2 diabetes mellitus; OF, oligofructose; PS, polysaccharide; RS, resistant starch; SF, soluble fiber; wt, weight; TNF-$\alpha$, tumor necrosis factor $\alpha$; +, positive study quality; Ø, neutral study quality; ↓, decrease; ↑, increase; ↔, no change.

[a]Methodological quality of study decided using the American Dietetic Association critical appraisal checklist.

(83%), a significant decrease was reported in ≥1 marker of systemic inflammation, compared with control groups, and 1 study reported no significant changes.[55–58,61,76]

Two of these included studies assessed SCFAs as an outcome of a prebiotic intervention. Jakobsdottir et al[57] explored serum SCFA levels after prebiotic supplementation in obese mice. They reported that all interventions increased serum acetate and propionate, compared with a fiber-free control diet, whereas guar gum and mixture interventions significantly increased SCFA levels compared with pectin and fiber-free diets. Additionally, Murakami et al[72] found in their study that epilactose supplementation in mice fed a high-fat diet significantly increased caecum acetate, *n*-butyrate, and propionate levels, compared with those of a high-fat-only group.

Meta-analysis was performed on the effects of a prebiotic interventions on the systemic inflammatory marker TNF-α (n = 6). Analysis of results (Figure 6)[6,54,58,59,74] from 5 studies studying TNF-α as an outcome revealed a significant decrease in TNF-α as a result of a prebiotic intervention (SMD, −0.63; 95%CI: −1.19 to −0.07; $I^2$ = 44%; $P$ = 0.03). Meta-analysis could not be performed on other markers of inflammation, due to heterogeneity in study design and reported outcomes.

## DISCUSSION

In this review, we evaluated evidence for the effect of SCFAs and prebiotics on systemic inflammation in overweight or obese humans or animals. In 14 of 29 human studies (48%), a significant decrease was reported in levels of ≥1 marker of systemic inflammation. Of included animal studies, 28 (88%) reported a significant beneficial change of ≥1 marker of systemic inflammation (ie, decrease in pro-inflammatory or increase in anti-inflammatory marker). The meta-analysis indicated that prebiotic intervention in overweight/obese humans was associated with a significant decrease in circulating levels of hs-CRP and LPS, and that TNF-α levels were significantly lowered in animals as a result of a prebiotic intervention.

SCFAs can be directly administered via several methods. In humans, SCFAs may be given orally (in tablet form), intravenously, via infusion, or by enema. In 3 of the 4 studies using an SCFA intervention in obese humans, a decrease in a marker of systemic inflammation was reported. An oral tablet of butyrate was used in 1 study, which reported a significant decrease in hs-CRP level, whereas a crossover intervention showed reductions in plasma TNF-α levels after intravenous and enema acetate interventions.[33,42] Canfora et al[13]

administered SCFAs via enema, and plasma IL-1$\beta$ levels were significantly reduced after acetate administration, compared with propionate. These SCFA interventions suggest there is a role for SCFAs in alleviating inflammation in obesity. However, these studies have done little to explore the mechanisms behind the anti-inflammatory effects behind SCFAs. Mechanisms of action such as activation of FFARs and inhibition of HDACs have been explored in animals; however, little work has been undertaken in humans to explore these mechanisms. Work in humans should particularly look to investigate the role of SCFAs on these mechanisms. With heterogeneity between types and delivery of SCFA administration in these interventions, more studies examining these factors are warranted to determine SCFAs as a novel aid in reducing obese systemic inflammation.

SCFAs can also be produced from prebiotic substrates, particularly from soluble fibers via fermentation by gut microbiota.[83] In obesity, SCFAs have been proposed to reduce systemic inflammation in obesity by directly increasing the lipid-buffering capacity of adipose tissue, reducing spillover of free fatty acids into circulation, and, ultimately, reducing production of pro-inflammatory cytokines. Prebiotic interventions in humans resulted in a significant decrease in $\geq 1$ marker of systemic inflammation in 11 of 25 studies; mixed results were observed across all types of prebiotics, suggesting that no specific type of prebiotic was favorable compared with another. Variables such as prebiotic dose and/or study duration in these studies may have also influenced the heterogeneity of results. The meta-analysis revealed that a significant decrease in hs-CRP levels occurred in overweight/obese humans as a result of prebiotic interventions. Similarly, in their meta-analysis, McLoughlin et al[84] examined the effect of prebiotic supplementation on CRP in the general population, reporting a significant decrease (SMD, −0.60; 95%CI: −0.98 to −0.23; $I^2 = 64\%$; $P = 0.002$), where considerable heterogeneity was also observed. In contrast, a systematic review of prebiotic interventions by Kellow et al[7] showed 3 of 4 studies reported a significant decrease in CRP levels in overweight and obese adults with T2DM when compared with controls. However, when pooled analysis (n = 181) was performed, CRP levels were not significantly reduced (SMD, −0.85; 95 %CI: −2.11, 0.42; $P = 0.19$).[7] Differences in meta-analysis results perhaps lie in methodology, such that our review and the review of Mcloughlin et al[84] excluded crossover studies from meta-analysis, whereas the review of Kellow et al[7] did not. Disparity between studies, relating to supplement types and dosage, intervention duration, biomarker measurement, and study population may have also factored into differences between results. Analysis performed by McLoughlin et al[84] indicated that prebiotics did not significantly change levels of TNF-α and IL-6 in the general population. Similarly, our meta-analysis showed that, overall, TNF-α and IL-6 were not significantly changed as a result of prebiotic intervention in an obese population. Our review highlights current evidence, specifically for the impact of prebiotics on systemic inflammation in obesity. Although evidence appears supportive for the use of prebiotics as a novel aid against systemic inflammation, more work elucidating the optimal dose and best form of prebiotic in reducing systemic inflammation in obesity is warranted.

It is believed that SCFA production from prebiotics can promote gastrointestinal barrier integrity, thereby preventing access of pathogenic intestinal bacteria and LPS into circulation.[85] Meta-analysis revealed that prebiotic supplementation significantly reduced LPS levels in humans. Levels of LPS are often higher in obesity, particularly in obese individuals with T2DM. A systematic review by Gomes et al[86] indicated that individuals with T2DM had 66.4% higher levels of plasma LPS than persons without diabetes. SCFAs have been proposed to inhibit LPS-induced inflammation, directly or indirectly, via activation of FFAR2 and FFAR3.[87,88] LPS is a hallmark trigger of systemic inflammation in obesity; therefore, decreasing levels of LPS in obesity is crucial in decreasing systemic inflammation. The minimum dose of prebiotic found to significantly reduce plasma LPS levels was 6 g of α-galacto-oligosaccharide.[29] Although several studies have provided evidence for prebiotics' ability to decrease LPS levels in obesity, more studies exploring the best form of prebiotic and the optimal dose of prebiotic required to attenuate LPS in the obese context are needed.

A number of determinants such as mode of birth delivery, genetics, diet, medical history, and social history can influence gut microbiota composition.[89] Moreover, recent studies have shown that significant variations exist in gut microbiota composition in healthy individuals from different countries and of different races.[90,91] Subgroup analysis within this review showed that in all studies (n = 8) conducted in Asia, a significant reduction in $\geq 1$ biomarker of systemic inflammation in overweight and obese individuals was reported. Results of studies from other continents were not as conclusive, with only 40% of studies conducted in Europe, 22% of studies in North American, and 0% of studies in South America reporting significant reduction of $\geq 1$ biomarker of systemic inflammation. These results may suggest that SCFA and prebiotic interventions are more effective in Asian populations than in populations from other continents. However, it is important to note that 7 of 8 human studies conducted in

Asia included in this review were from Iran; therefore, this sample population may not be truly reflective of populations from other Asian countries. Furthermore, significant differences between types and dose of SCFA or prebiotic treatments may also reflect differences seen among populations from different geographic locations. Currently, there is a paucity of research examining the difference in effects of SCFAs and prebiotics in individuals from different races and geographic locations. Studies should consider differences present in gut microbiota composition between individuals from differing geographic locations because these differences may affect the effectiveness of SCFA and prebiotic interventions. Understanding these differences may be important in determining whether specific SCFA and prebiotic treatments are needed to achieve reductions in systemic inflammation among differing populations

Overall, the majority of animal studies (88%) included in this review reported a decrease in $\geq$1 marker of systemic inflammation in response to an SCFA intervention. To our knowledge, this is the first systematic review evaluating the effect of SCFAs or prebiotic interventions in animal studies. Interestingly, sodium butyrate was used in 8 animal studies (73%), and all 8 reported a significant decrease in inflammation post-intervention. Butyrate is considered the most potent HDAC inhibitor, with inhibition shown to suppress TNF-$\alpha$ production and suppress NF-$\kappa$B activity as well as promote the production of anti-inflammatory cytokines.[92,93] Meta-analysis could not be undertaken for SCFA supplementation in animal studies, due to the heterogeneity in outcome measures as well as intervention types carried out. The animal studies that investigated the impact of prebiotics on systemic inflammation in obese mice also showed an overall beneficial effect, with 86% of studies reporting a reduction in $\geq$1 marker of systemic inflammation. Meta-analysis revealed that prebiotics significantly reduced TNF-$\alpha$ in obese mice. Hence, this review highlights a large number of studies that support an anti-inflammatory role of SCFA in animals. Because few SCFA studies have been undertaken in humans, these studies should inform future directions for clinical SCFA interventions. It is important to highlight, however, that animal models often use large doses of fiber, which cannot be replicated in human interventions, potentially explaining differences observed between animals and human studies.[94]

In this review, 8 studies in humans and 4 studies in animals measured SCFAs as an outcome of SCFA or prebiotic intervention. Conflicting results were observed between human studies reporting SCFA measurements. Of the 3 studies that examined plasma SCFAs after SCFA interventions in humans, only 1 reported a significant increase in plasma levels of SCFAs.[13] Of 5 studies examining SCFAs as an outcome following prebiotic interventions, 1 study reported no significant changes in plasma or fecal SCFA levels; 1 study reported an increase in fecal SCFA levels but no changes in plasma SCFAs; 1 study reported a decrease in plasma SCFA levels; 1 study reported an increase in plasma levels of SCFAs; and 1 study reported a decrease in fecal levels of SCFAs.[25–27,37,40] Of the 4 animal studies, 2 studies reported assessment of SCFA after prebiotic intervention, 1 study measured SCFAs in plasma, and 1 study measured SCFAs in the caecum and 2 studies measured SCFA in feces post-SCFA intervention. All 4 animal studies reported a significant increase in SCFA levels.[57,67,69,72]

The complexity of exposures is impossible to replicate in animal models and it is likely that inherent differences explain why consistent SCFA increases were seen in animal models, compared with those in humans.[89] Because of heterogeneity of the available research, more studies need to be undertaken in humans, analyzing SCFA levels to clarify the best types of prebiotic and SCFA supplementation to increase SCFA production, as well as determining the amount and type of prebiotics or SCFAs required to induce an anti-inflammatory response. Importantly, researchers should consider analyzing SCFAs in plasma, because plasma SCFA levels may be more reflective of SCFA production: approximately 95% of SCFAs are absorbed by colonocytes within the gastrointestinal tract.[83] SCFA absorption by colonocytes is rapid and efficient; thus, only 5%–10% of SCFA may be excreted within feces. Therefore, analyzing SCFA levels in feces alone may not be an accurate measure of SCFA production.[83]

It is important to highlight the limitations of the research that formed the basis of this systematic review. First, heterogeneity between studies with respect to dose and type of SCFA and prebiotic intervention, study duration, and systemic inflammatory outcomes restricted the number of studies available for meta-analysis. Furthermore, as a result of this limitation, the magnitude of changes to cytokines due to interventions could not be deduced. Second, several studies did not consider background dietary intake during the intervention period, which may have confounded the results, either due to consumption of dietary soluble fiber or consumption of other nutrients (ie, vitamins and antioxidants) that can alter systemic inflammation. Only 18 of the included human studies (62%) assessed dietary intake or used a standardized diet, which is an important consideration in future research. Fourth, another limitation was the exclusion of studies not in English, potentially introducing selection bias that may have affected the results. Fifth, although the anti-inflammatory effects of prebiotics can be largely attributable to SCFA

production, other actions of prebiotics are likely to be important for reducing systemic inflammation in obesity. Prebiotic-stimulated synthesis of beneficial bacteria by gut microbiota such as *Lactobacillus* and the bifidobacteria may indirectly affect inflammation via the maintenance and repair of epithelial barriers, which may reduce the affect of pro-inflammatory triggers such as LPS.[95] Altered gut bacteria may also influence the differentiation and action of immune cells and their production of cytokines.[96] This highlights the importance of measuring SCFA levels, particularly in plasma where SCFA action occurs, to determine the relative contribution of SCFA to the anti-inflammatory effects of fiber.

Despite these limitations, this appears to be is the first study in which SCFAs and prebiotics were evaluated in obese humans and animals, providing a comprehensive overview of current evidence for SCFAs and their anti-inflammatory potential. Most (92%) of human studies included in this review were classified as level II evidence according to the National Health and Medical Research Council hierarchy of evidence, which strengthens the conclusions we have drawn.

## CONCLUSION

In summary, this systematic review highlights the anti-inflammatory potential of SCFAs and prebiotics in attenuating systemic inflammation in overweight/obesity in animals and humans. Overall, the evidence for an effect of SCFAs and prebiotics on systemic inflammation is supportive. Because of heterogeneity among studies, the optimal dose, type, and duration of intervention was unable to be determined and these details should be addressed in future work. Furthermore, studies reporting SCFA levels as an outcome of SCFA or prebiotic interventions are required to help determine the optimal delivery method for SCFA, as well as to quantify what increases of SCFA levels are required for clinically significant anti-inflammatory effects. Exploring these questions would be paramount in confirming SCFAs and prebiotics as a novel approach for the treatment of systemic inflammation in overweight and obesity and to reduce the risk of chronic disease.


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