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Review Article EP151157.RA

GUT MICROBIOTA, PREBIOTICS, PROBIOTICS, AND SYNBIOTICS IN MANAGEMENT OF OBESITY AND PREDIABETES: REVIEW OF RANDOMIZED CONTROLLED TRIALS.

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Abbreviations:

A1c = glycohemoglobin A1c'**; GMB =** gut (large bowel) microbiota**; DM2 =** diabetes mellitus type 2**; HOMA-IR =** homeostatic model assessment - insulin resistance; **LPS =** lipopolysaccharide**; RTC =** Randomized controlled trial.

INTRODUCTION

Nutrition affects health and disease. Homo sapiens have evolved ~100,000 years ago in an environment with sporadic food availability favoring evolutionary selection of "thrifty" genes promoting preservation and storage of nutrients (1). Leap to the present and nutrient abundance instigates human obesity of epidemic proportion. The consequences of obesity, diabetes mellitus type 2 (DM2), non-alcoholic fatty liver disease (NAFLD) or steato-hepatitis (NASH), cardiovascular disease (CVD), and cancer, are main causes of morbidity and mortality in developed countries (2,3). Humans have coevolved with microbacteria, the first form of life to appear on Earth ~3.5 billion years ago (4). Bacteria in the gut are called gut microbiota (called GMB or microbiota for the purpose of this review). "Microbiota" comes from Greek "*mikros*" meaning small and "*bios"* meaning life. Emerging evidence suggest an essential role of microbiota in human health and disease including digestion, energy and glucose metabolism, as well as immunomodulation and brain function (4-9). Diet-related interventions causing beneficial changes of GMB could also include prebiotics, probiotics and synbiotics (combining prebiotics and probiotics). There are many challenges in studying the usefulness of pre-, pro- and synbiotics for the human health. Multiple hypotheses still

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need to be tested and controversies resolved. For example, it is not clear whether potential health benefits result from interaction of '–biotics' with the microbiota and if the effect sizes seen in studies are clinically relevant. This review focuses on published randomized controlled trials (RCTs) of microbiota, pre-, pro- and synbiotics for metabolic conditions (obesity, prediabetes, and DM2).

METHODS

Randomized controlled trials were sought and retrieved from electronic databases and reference lists. The search engines included MEDLINE (http://www.ncbi.nlm.nih.gov/pubmed), Science Direct (www.sciencedirect.com) and Cochrane library (http://www.cochranelibrary.com). The terms used were: overweight, obesity, prediabetes, diabetes mellitus type 2, metabolic syndrome, gut microbiota, microbiome, prebiotic, oligosaccharides, fructo-oligosaccharide, galacto-oligosaccharide, inulin, lactulose, oligofructose, probiotic, synbiotic, yogurt, yoghurt, milk, dairy, glucose, insulin sensitivity, insulin resistance, HOMA, weight loss, low calorie diet, endotoxin, glucose metabolism, dysglycemia, cholesterol, hyperlipidemia and dyslipidemia. The terms used in search engines were employed to help with article identification. The terms were AND, OR, quotation marks, asterisks, and parenthesis. The articles published in English until October 30, 2015 were retrieved and reviewed. Relevant articles from the reference lists were retrieved and reviewed as well. The method was consistent with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (10).

MICROBIOTA COMPOSITION AND FUNCTION IN METABOLIC DISEASE

DOI:10.4158/EP151157.RA

Gut microbiota colonize the body at birth with the newborn swallowing microbacteria from the birth canal, and evolve with aging (4,5,9). Bacteria make up most of the gut microorganisms and up to 60% of the dry fecal mass (4). The GMB is comprised of ~100 trillion bacteria, 10-fold the number of cells in the human body. The collective genome of these bacteria (microbiome) is 150-fold larger than the human genome (4,5,9). These bacteria are from ~500 species with 99% belonging to 30 – 40 species from the four main families (phyla), i.e. *Firmicutes* (64%), *Bacteroidetes* (23%), *Proteobacteria* (8%), and *Actinobacteria* (3%) (4).

The human body offers ecosystem and nourishment to microbacteria in the lumen and bowel mucosal surfaces. The human host affects GMB survival by dietary composition (4-9) and the use of prebiotics, probiotics, and antibiotics (4,11,12). Reciprocally, GMB abundance, composition, and function enable nutrient absorption, processing of vitamins, drugs, and hormones, detoxification of carcinogens and possibly influences longevity (4-9,13). Interruption of healthy symbiotic relationship results in gut *dysbiosis* that is proposed as a contributing factor to obesity and its consequences, DM2, NAFLD, CVD and cancer. Dysbiosis is multifactorial in origin, one factor being the obesogenic diet (4-9). Dysbiotic bacteria maintain a vicious cycle by increasing efficiency of energy harvesting from the diet (5,9). GMB dysbiosis triggers increased shedding of lipopolysaccharide endotoxin (LPS), a molecule from the outer membrane of Gram(-) bacteria. It is suggested that LPS disrupts gut mucosal immunity and the mucosal barrier, creating a “leaky-gut” and activating inflammatory pathways and systemic immunity (5-9). Subclinical inflammation from “dys-nutrition” and dysbiosis could perpetuate the vicious cycle with the double-hit of steatosis (ectopic fat

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accumulation) with inflammation (i.e. “-itis”) resulting in steato-hepatitis (e.g. NASH), steato-pancreatitis (e.g, DM2), and steato-arteritis (e.g. CVD), among other conditions (9). In addition, NAFLD is linked to other obesity-related conditions including polycystic ovary syndrome (PCOS) and obstructive sleep apnea (OSA) (14) and pre-/probiotics show promise as therapeutic agents for NAFLD (7).

The co-risks of obesity and DM2 are genetics and lifestyle (1-3). While genetic predisposition is usually considered “non-modifiable,” the microbiome (microbiota genes) could potentially be modified by lifestyle and nutrition including pre- and probiotics, thus offering a novel approach to the management of metabolic disorders.

PREBIOTICS USE FOR METABOLIC DISEASE

Definition

Prebiotics are defined as food expected to cause beneficial changes in gut microbiota (11,12). These changes could confer health benefits to the human host. The term prebiotic was first defined by Marcel Roberfroid in 1995: "A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" (11). It can be argued that Elie Metchnikoff was a pioneer of this concept in 1907 suggesting that “…the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" (15). Prebiotics are complex carbohydrates, e.g., dietary fiber. The most studied prebiotics are fructans and arabinoxylan. Fructans are polymers of fructose molecules, composed of the linear

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chains of fructose units linked by the β-glycosidic bonds and typically terminating in a glucose unit (16). There are short-chain (oligofructose) and long-chain (polyfructose, i.e., inulin and levan) fructans, typically functioning in the roots as the energy pools for many plants instead of starch. An arabinoxylan is a hemicellulose, a copolymer of two pentose sugars, arabinose and xylose. It is located in the cell walls of plants and mainly serves a structural role (16). Fructans are found in soluble and arabinoxylans among both soluble and insoluble dietary fibers (16).

Function

Prebiotics are fermented by GMB into short-chain fatty acids (SCFAs: acetate, propionate, and butyrate), L-lactate, CO_2, hydrogen, methane, and other metabolites regulating downstream metabolic processes in multiple ways (9,17,18). The prebiotics reduce constipation, foster weight gain or loss, improve levels of glucose and lipids, and appear to exert an anticarcinogenic effect among other actions (9,17,18).

Research data (Table 1)

Preclinical studies using multiple in vitro and in vivo models produced convincing evidence of prebiotics affecting energy and glucose homeostasis (4-9,11,13,17,18). The mechanisms of prebiotics and GMD interactions need further clarification but include changing GMB relative abundance (e.g., decreased *Firmicutes* and increased *Bacteroidetes*), altering levels of satietogenic gut peptides, decreasing systemic inflammation, and improving glucose tolerance (9,11,18). Review of the preclinical

DOI:10.4158/EP151157.RA

studies is beyond the scope of this manuscript. Numerous original papers and reviews of the preclinical data have been published (4-9,11,13,17,18).

Randomized controlled trials in subjects with obesity and prediabetes (19-24) as well as patients with DM2 (25-27) (Table 1) showed inconsistent results with predominantly neutral effect on all evaluated metabolic parameters including body weight, BMI, fasting and postprandial glucose and insulin, glycohemoglobin A1c (A1c) and HOMA-IR (19-27). Similarly, variable results were produced for lipid profile with some studies showing reduced total cholesterol (24,26), low density lipoprotein (LDL) (20,26), and triglycerides (TG) (24,26) but with the majority of the studies showing no effect on lipid profile (19-24, 26) (Table 1). Subclinical inflammation markers appear to be reduced (24-27), sometimes substantially. For example, markers were decreased as follows: hsCRP -35.6% (26), TNF-α -20-23% (26,27), lipopolysaccharide (LPS) -22-28% (26,27), marker of oxidative stress malondialdehyde -37.2% (25). Conversely, antioxidant defense (total antioxidant capacity) was increased +18.8% (25). In one trial including 16 healthy adults, a single evening meal of brown beans compared with white wheat bread (WWB, reference product) produced significant and clinically relevant improvement of glycemic and inflammatory markers (28). The effects shown at a subsequent standardized breakfast included lowered blood glucose (-15%, $p<0.01$) and insulin (-16%, $p<0.05$), increased satiety hormones (PYY +51% and GLP-2 +8.4%, $p<0.05$ for both), decreased hunger hormone (ghrelin -14%, $p<0.05$), hunger sensations (-15%, $p = 0.05$), and suppressed inflammatory markers (IL-6 -35% and IL-18 -8.3%, $p<0.05$ for both). An increase in breath H_2 (+141%, $p<0.01$), propionate (+16%, $p<0.05$),

DOI:10.4158/EP151157.RA

and isobutyrate (+18%, P<0.001) were significant after brown beans compared to after WWB, indicating involvement of colonic fermentation (28).

The results of various studies were confirmed by a recent meta-analysis that included 13 trials, representing 513 adults with BMI ≥25 kg/m² (29). Overall, prebiotic supplementation reduced plasma total cholesterol (standardized mean difference [SMD] -0.25; 95% CI -0.48, -0.02), LDL (SMD -0.22; 95% CI -0.44, -0.00), and triglycerides (SMD -0.72; 95% CI -1.20, -0.23) and increased HDL (SMD +0.49; 95% CI +0.01, +0.97) in diabetic trials. Synbiotic supplementation reduced plasma fasting insulin (SMD -0.39; 95% CI -0.75, -0.02) and triglycerides (SMD -0.43; 95% CI -0.70, -0.15). The authors concluded that the data supported prebiotics and synbiotics supplementation as an adjuvant therapy in obesity-related comorbidities, such as dyslipidemia and insulin resistance (29). However, the effect size was small to moderate considering that SMD values of 0.2, 0.5 and 0.8 represent small, moderate and large effect sizes.

Availability

The raw natural food particularly rich in prebiotics (percent content by weight) include chicory root (~65%), Jerusalem artichoke (~32%), barley (22%), garlic (~18%), onion (~10%), globe artichoke (~7%), rye bran or grain (~7%), wheat bran (~5%), and asparagus (4%), and cooked food are chocolate (9%) and white bread (~3%) (30). There are no published guidelines for daily prebiotics intake. Four to 10 g daily is suggested to be beneficial (11,12). Some examples of common food to achieve 6 g serving of prebiotics are as follows (amount [calories]): raw Jerusalem artichoke 19 g (15 kcal), raw garlic 34.3 g (45 kcal), raw leek 51.3 g (32 kcal), raw onion 70 g (20 kcal),

DOI:10.4158/EP151157.RA

cooked onion 120 g (55 kcal), raw wheat bran 120 g (250 kcal), whole cooked wheat flour 125 g (410 kcal), raw banana 600 g (525 kcal) (30). Dietary increase of prebiotics can be associated with increased bloating and bowel movements due to increased fermentation and SCFA production (31). These symptoms may or may not resolve after prebiotic-induced changes in microbiota (31).

PROBIOTICS AND SYNBIOTICS USE FOR METABOLIC DISEASE

Definition

Probiotics are defined as microorganisms expected to be beneficial for humans. The concept is attributed to Nobel laureate Elie Metchnikoff who suggested in 1907 "…to replace the harmful microbes by useful microbes" (15). The World Health Organization's definition of probiotics is "…live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (12). The probiotic candidate must be a taxonomically defined (genus, species, and strain level) and safety and health benefits supported by reproducible human studies (12). Synbiotics refer to synergistic blend of prebiotics and probiotics.

Function

Probiotics and synbiotics have multiple potential functions. Probiotics are suggested to play an important role in immunomodulation and in regulating cytokines (4,6,7,9,32). This is particularly meaningful since obesity is considered as a state of subclinical low-grade inflammation with significant expression and/or production of cyto-

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and chemokines (4-9,32). The majority of known probiotics is species of human microbiota and expected to have the same functions as symbiotic microbiota (4-9,17,18).

Research data (Table 1)

Preclinical studies in cell lines and animal models reported possible probiotic usefulness for weight loss, insulin resistance and hyperlipidemia management (32-35). An investigation of metabolism-related mechanisms of probiotic action showed ability to hydrolyze bile salts, to reduce fat accumulation and systemic inflammation, to decrease plasma leptin, and down-regulate peroxisome proliferator-activated receptor-γ (PPAR-γ) in the liver (32-35). These data need further confirmation.

The majority of RCTs of probiotics were small (less than 100 participants), of short duration (12 weeks or less) and used yogurt or capsules containing probiotics (Table 1). The results showed non-significant or small, clinically irrelevant changes in body weight, blood pressure, A1c and other biomarkers including waist circumference, visceral fat, basal metabolic rate, lipid profile, HOMA-IR, insulin sensitivity index, and inflammation (CRP, IL-6, TNF-α) (Table 1) (36-47). Similarly, results were inconsistent from trials using synbiotics or other supplement combinations (48-55).

The meta-analysis of 368 articles of probiotic use for treatment of obesity had chosen only 4 randomized trials that provided direct comparison of therapeutic efficacy of probiotics and placebo (56). The meta-analysis of these data showed no significant effect of probiotics on body weight (kg) and BMI (kg/m^2): body weight, n = 196; SMD -1.77; 95% CI, -4.84, 1.29; P = .26; BMI, n = 154; SMD 0.77; 95% CI, -0.24, 1.78; P = .14. The authors concluded that probiotics have limited efficacy in terms of decreasing

DOI:10.4158/EP151157.RA

body weight and BMI and were not effective for weight loss (56). In contrast, a meta-analysis of 614 patients (from 11 RCTs) with DM2 pooled results showed a decrease in A1c (%) and fasting glucose (mg/dl): SMD -0.32; 95% CI -0.57, -0.07, P = 0.01 and SMD -9.36; 95% CI -16.56, -1.98, P =0.01 for A1c and glucose, respectively (57). There was, however, no effect on fasting insulin and HOMA-IR (57). In a meta-analysis of probiotics use for lipid lowering and other CVD risks (15 studies with 788 subjects), statistically significant pooled effects were found on reduction of BMI, waist circumference, total cholesterol, LDL, and inflammatory markers (58). However, the mean reductions were small, averaging 0.3 kg/m^2 for BMI, 1.82 cm for waist circumference, and ~10% for total cholesterol and LDL. Subgroup analysis revealed statistically significant effects of probiotics on total cholesterol and LDL when the medium was fermented milk or yogurt rather than a capsule form, consumption was at least 8 weeks in duration, and the probiotics consisted of multiple strains rather than a single strain. Among single strains, Lactobacillus gasseri was predominantly associated with weight loss (29) and Lactobacillus Acidophilus with reduction in LDL (58). There were inconsistencies and contradictions in observations depending on variability of a strain or strains of probiotics, dosage, duration of treatment and patient population. Overall, it can be concluded that current results demonstrated small, if any, changes in body weight (< 3%) and metabolic parameters suggesting low clinical relevance and lack of evidence for using probiotics for weight loss or metabolic benefits.

Availability

DOI:10.4158/EP151157.RA

Fermented food provides natural source of live probiotic cultures. The freeze-dried bacteria are available in tablets, capsules, powders, and sachets. Probiotics in fermented food include fermented milk (e.g. yogurt, buttermilk, kefir), fermented (pickled) vegetables (e.g. sauerkraut, cabbage kimchee, pickled ginger), fermented bean paste (e.g. miso, tempeh, natto) and other fermented foods and beverages (59). Fermented milk and fermented plants have similar probiotic bacteria containing L. acidophilus, L. paracasei, L. rhamnosus and L. plantarum among other species. The National Yogurt Association gives a Live & Active Cultures seal to yogurt products, which contain 10^8 Colony Forming Units (CFU) per gram at the time of manufacturing (59). Fermented plants appear attractive for obesity management as they contain probiotics and prebiotics and have low energy density. There are no randomized trials for pickled vegetables and a trial for fermented soy is unconvincing (60).

PREBIOTICS, PROBIOTIC, SYNBIOTICS AND MORBIDITY OR MORTALITY RISK

There are no randomized trials to answer the ultimate question of whether prebiotics, probiotics or synbiotics decrease morbidity or mortality risk. Prospective cohort studies of prebiotic-containing food (whole grains, fruits and vegetables) unequivocally associate higher intake with decreased mortality risk in populations without and with diabetes (61-65). Probiotics are more difficult to assess due to variable fat content in fermented dairy and variable salt and acidity content in fermented vegetables. A few large prospective studies have evaluated relationship between fermented food intake and mortality. In a prospective cohort of 34,409 Dutch men and women followed for ~15 years, higher intake of fermented foods (predominantly dairy)

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was associated with moderately decreased risk of CVD mortality (66). In this study CVD mortality, and particularly stroke mortality was reduced in highest vs. lowest quartile of fermented milk intake with hazard ratio [HR] 0.6, 95% CI 0.38-0.92 (p for trend 0.046) (66). Similarly, meta-analysis of prospective cohort studies (764,635 participants) showed that higher intake of fermented dairy was associated with reduced risk of stroke (relative risk 0.8, 95% CI 0.71-0.89) (67). Fermented dairy intake was inversely associated with all-cause mortality in a prospective cohort of 4,526 participants followed for 10 years from Whitehall London civil servants study (68) but no relationship was observed in Dutch cohort (66). Non-fermented soy was associated with lower risk of gastric and prostate cancer while fermented soy was neutral in a large prospective cohort (30,792 participants followed for 16 years) (69) and in a meta-analysis of epidemiologic studies (70). A relatively large prospective cohort study (3,158 participants followed for 18 years) suggested lower risk of cancer with higher intake of pickled vegetables (71). There were no prospective cohort studies evaluating relationship between fermented soy or pickled vegetables and CVD or all-cause mortality.

RECOMMENDATIONS FOR INTAKE

Fiber is a main source of prebiotics in American diet. Average reported fiber and inulin intakes are 12.5 -18 g/day (72) and 1.3 - 3.5 g/day (73), respectively, both lower than recommended. For fiber the Recommended Adequate Intake (RAI) is 25 - 38 g/day (14 g/1,000 kcal/day) for all adults (74) and 25 - 50 g/day for DM2 (75) corresponding to the AACE-recommended 7 - 10 servings/day of “healthful” carbohydrates (2). In

DOI:10.4158/EP151157.RA

comparison, the prehistoric hunter-foragers have estimated use of inulin-type prebiotic fibers of 135 g/day (76). For vegetables and grains, the main sources of fiber, United States Department of Agriculture (USDA) recommends 2 - 3 cups and 3 - 8 once (1 once is ½ cup of cooked grains) daily, respectively (77). For dairy, the main source of probiotics in the American diet, the USDA recommends 3 cups daily (77). There are no recommendations for adequate intake of probiotics, as evidence of probiotic safety and health benefits requires further proof. Contrary to the officially recommended use, popular use of potential '–biotics' is involved in traditional recipes for preparation and preservation of food. For example, a species of yeast Saccharomyces cerevisiae has been instrumental to winemaking, baking, and brewing since ancient times. Vinegars and wines among many products of fermented grapes and grains have also been used since antiquity as a popular remedy for various disease states including infections and gastrointestinal problems.

Caution should be added on specific recommendations. There is no governmental agency that regulates health supplements in the U. S. These supplements purporting to contain pre- and probiotcs and to provide health benefits are not standardized and not definitively proven to be beneficial. The '-biotic' supplements are popular and widely sold in the stores, pharmacies and internet. According to industry expert, Eric Pierce, director of strategy and insights at New Hope Natural Media, probiotic sales in the U.S. is expected to grow from 1.5 bln (2013) to 2.5 bln in 2018 (http://www.nutraingredients-usa.com/ Markets/ Probiotics). There are multiple organizations that may provide useful information to physicians interested in specific products and/or supplements. Among these organizations are the National Center for

DOI:10.4158/EP151157.RA

Complementary and Integrative Health (NCCIH, https://nccih.nih.gov/ health/probiotics) and the International Scientific Association for Probiotic and Prebiotic (ISAPP, www.isapp.net).

CONCLUSIONS

Available data suggest that dietary pre- and probiotics from natural foods could have, at least to some extent, beneficial effect on gut microbiota and health. At present only dietary fiber together with increased energy expenditure from physical activity offer attainable and cost-effective approach to obesity and diabetes prevention and are included in mainstream recommendations. Further efforts from all strata of society including researchers, regulatory authorities, food industry, health care providers and media are needed to improve integration of these simple measures in daily routine.

DOI:10.4158/EP151157.RA

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Table 1. Randomized controlled trials of Prebiotics, Probiotics, and Synbiotics for metabolic disease*

Study	Popu-lation. Sample size (n)	Dura-tion, (wks) De-sign	Treatment: daily dose	Control: daily dose	Results and study details Comparisons are between intervention vs. control after intervention ($p < 0.05$) unless specified**
Prebiotics					
Parnell & Reimer, 2009 (19)	OW/OB M/F n = 37	12 DB	OF 21 g n = 20	MD 21 g n = 17	Decreased: BW, FBG, Fasting insulin, Calorie intake, Ghrelin Increased: Protein-YY NS: TC, LDL, HDL, TG, GLP-1
Genta et al, 2009 (20)	OB F n = 35	16 DB	Yacon syrup, containing 0.14 g of FOS/kg (~10 g/70 kg BW) n = 20	Placebo syrup n =15	Decreased: BW, BMI, WaistC, FBG (-11.9 mg/dl), HOMA-IR Decreased: Fasting insulin (-9.9 mU/mL), LDL (-35.2 mg/dL) NS: TC, HDL
Tovar et al, 2012 (21)	OW/OB F n = 59	12 OL	LCDiet + Inulin 10 g n = 30	LCDiet n = 29	NS: BW, WaistC, FBG, TC, LDL, HDL, TG
Dewulf et al, 2013 (22) Salazar et al, 2015 (23)	OB F n = 30	12 DB	Inulin + OF 50/50% mix 16 g n = 15	MD 16 g n = 15	NS: BW, BMI, FBG, fasting insulin, A1c, TC, LDL, HDL, TG, LPS Decreased: Bacteroides, Propionibacterium Increased: *Bifidobacterium*, *Faecalibacterium prausnitzii* Decreased: Fecal: Total SCFA, acetate & propionate Increased: B. longum, B. pseudocatenulatum, B. adolescentis Negatively correlated: B. longum with LPS and endotoxin ($p < 0.01$). Positively correlated: Fecal Total SCFA, acetate & propionate with BMI, fasting insulin and HOMA-IR
Vulevic et al, 2013 (24)	OW/OB & MetS M/F n = 45	12 CO	GOS 5.5 g n = 45	MD 5.5 g n = 45	Decreased: Fasting insulin (-1.7μU/ml), TC (-11.6 mg/dL), TG (-8.9 mg/dL) Decreased: CRP, fecal calprotectin NS: BW, BP, FBG, LDL, HDL
Gargari et al, 2013 (25) Dehghan et al, 2013 (26) Dehghan et.al., 2014 (27)	OB & DM2 F n = 49 OB & DM2 F n = 49 OB & DM2 F	8 DB 8 DB 8 DB	Inulin 10 g n = 24 Inulin 10 g n = 24 OF-enriched Inulin 10 g n = 27	MD 10 g n = 25 MD 10 g n = 25 MD 10 g n = 25	Decreased: FBG (-8.5%), A1c (-10.4%) Decreased: marker of oxidative stress malondialdehyde (-37.2%) Increased: antioxidant defense (total antioxidant capacity 18.8% & SOD 4.36%) NS: Fasting insulin, HOMA-IR, anti-oxidant catalase and GSH Decreased: TC (-12.9%), TG (-23.6%), LDL (-35.3%) Increased: HDL (19.9%) Decreased: hsCRP (-35.6%), TNF-α (-23.1%), and LPS (-27.9%) Decreased: FBG (19.2 mg/dL; 9.50%), A1c (1.0%; 8.40%), IL-6 (1.3 pg/mL; 8.15%), TNF-α (3.0 pg/mL; 19.80%) & LPS (6.0 EU/mL; 21.95%) NS: hsCRP, IFG-γ, IL-10

	n = 52				
Probiotics					
Agerholm-Larsen et al, 2000 (36)	OW/OB M/F n = 70	8 DB	Yoghurt (Y) 450 ml 3 Groups: StLa, StLr, G 10^7-10^{10} CFU	PL 2 groups: PY PP	Comparison G vs. PY, PP: NS: BW, WHR, BP, FatM, TC, HDL, TG Decreased: LDL (-8.4%); Increased: Fibrinogen after adjusting for BW Groups: Gr1 (StLa), n = 16: Y fermented with S. thermophiles (2 stains) + L. acidophilus Gr2 (PY), n = 14: PL y fermented with delta-acid-lactone Gr3 (StLr), n = 14: Yogurt fermented with S. thermophiles (2 stains) + L. rhamnosus Gr4 (G), n = 16: Y fermented with S. thermophiles (2 stains) +Enterococcus faecium Gr5 (PP), n = 10: 2 PL pills daily
Kadooka et al, 2010 (37)	OW/OB M/F n = 87	12 DB	Yogut 200 g with L. gasseri (LG2055) 5 x 10^{10} CFU n = 43	PL Yogurt 200 g (PL) n = 44	Decreased: VisFat -4.6%, SubFat -3.3%, BW -1.4%, BMI -1.5%, WaistC, 1.8%, HipC -1.5%, FatM -0.8 kg Increased : Adiponectin NS: SubFat, Lean mass
Kadooka et al, 2013 (38)	OW/OB M/F n = 210	12 DB	Yogurt 200 g with L. gasseri (LG2055) 2 Groups	PL Yogurt 200 g (PL) n = 70	Gr1 vs. PL Decreased: BMI -1.1%, WaistC -1.4%, HipC -1.5%, VisFat -8.5%, FatM -2.4 kg NS: SubFat, LeanM Gr2 vs. PL Decreased: BMI -1.6%, WaistC -1.2%, VisFat -8.2%, FatM -2.2 kg NS: SubFat, Lean mass Groups: Gr1, n = 69: LG2055 10^7 CFU; Gr2, n = 71: LG2055 10^6 CFU
Zarrati et al, 2013 (39)	OW/OB M/F n = 50	8 DB	LCDiet + L spp-Yogurt with: L. acidophilus La5+ B. Bb12+ L. casei DN001 10^8 CFU n = 25	LCDiet+ Regular yogurt n = 25	NS: BW, BMI, WaistC, HipC, WHR, SBP, DBP, hs-CRP, IL-17, TNF-α
Ivey et al, 2014 (40) 2015 (41)	OW/OB M/F n = 156	6 DB	Yogurt + ProCap Both containing: L. acidophilus La5 + B. animalis subsp. lactis Bb12 3.0 × 10□ CFU 4 Groups	Milk + PL n = 40	Yogurt vs. Milk: increased HOMA-IR (+12.5%) ProCap vs. PL: Increased FBG (+2.8%) NS: A1c, BP, TC, LDL, HDL, TG Groups: Gr1, n = 40: Yogurt + ProCap; Gr 2, n = 37: Yogurt + PL; Gr3, n = 39: Milk + ProCap; Gr4, n = 40: Milk + PL
Chang et al, 2011 (42)	OW/OB & MetS M/F n = 101	8 DB	Functional Yogurt 150 mg BID with S. thermophiles L. acidophilus B. infantis 10^9-10^{10} CFU n = 53	PL- Yogurt 150 mg BID n = 48	Decreased: BW, BMI, LDL NS: WaistC, BP, FBG, A1c, TC, HDL, TG

Tripolt et al, 2014 (43)	OW/OB & MetS M/F n = 28	12 OL	Yakult 195 ml: L. casei Shirota 3 × 6.5 × 10□ CFU n = 13	None n = 15	NS: BW, BMI, FBG, AUC-Glucose, Fasting insulin, HOMA-IR, HOMA-b, ISI NS: LDL, TNF, hsCRP, IL-6 Decreased: sVCAM
Barreto et al, 2014 (44)	OW/OB & MetS F n = 24	12 DB	Yogurt 80 ml with L. plantarum 1.25 x 10^7 CFU n = 12	Milk, 80ml n = 12	Decreased: FBG & homocysteine NS: BW, BMI, WaistC, SBP, DBP, Fasting insulin, HOMA-IR, TC, LDL, HDL, TG NS: CRP, IL-6, TNF-α
Jung et al, 2013 (45)	OW/OB & PreDM M/F n = 48	12 DB	Pill: L. gasseri BNR17 10^{10} CFU n = 22	PL n = 26	NS: BW, BMI, BP, Body Fat (5), WaistC, HipC, VisFat, SubFat, NS: FBG, fasting insulin, A1c, TC, LDL, HDL, TG, BMR, O2 consumption
Hariri et al, 2015 (46)	DM2 M/F n = 40	8 DB	Probiotic soy milk 200 ml with L. plantarum A7 2 x 10^7 n = 20	Soy milk 200 ml n = 20	Decreased: SBP, DBP NS: BW, BMI, WHR
Woodard et al, 2009 (47)	OB & RYGB M/F N = 44	24 DB	ProCap: L. Species 2.4 x 10^9 CFU N =17 at 12 wks N = 15 at 24 wks	PL N = 22 at 12 wks N = 20 at 24 wks	Decreased: BW at 12 wks: ProCap -48% vs PL -39% Increased: B12 at 12 wks & 24 wks NS: BW at 24 wks: ProCap -67% vs PL -60%
Synbiotics					
Lee SJ et al, 2014 (48)	OW/OB M/F n = 50	8 DB	BTS + DUOLAC7: 5x10^9 CFU n = 17	BTS + PL n = 19	NS: BW, BMI, WaistC, FatM (by BIA), LeanM, FBG, LPS, TC, LDL, TG, NS: Gut permeability Increased: HDL Correlations of BW with L. plantarum (r = 0.425), & LPS with B. breve (r = -0.350). GMB change: within DUOLAC7 group: Increased: B. breve, B.Lactis, B. rhamnosus, B. Plantarum, DUOLAC7: L. acidophilus, L. plantarum, L. rhamnosus, B. lactis, B. longum, B. breve, S. thermophiles
Sanchez et al, 2014 (49)	OB M/F n = 93	24 DB	OF 200mg + Inulin 100 mg + L. rhamnosus (LPR) 1.6 x 10^8 CFU 2 pills per day n = 45 (F = 26)	MD 250 mg + PL n = 48 (F = 28)	Comparison Between groups: NS for all markers Comparison of Females: at week 24 Decreased: BW (-5.2kg), FatM (-4.8kg), SBP -1.5 mmHg, Leptin (-11.3 ng/ml) NS: mean daily energy, BMR, RQ respiratory quotient, FBG, Fasting insulin NS: TC, LDL, HDL, TG, Adiponectin, NEFA, Hydroxybutyrate, LBP, CRP Increased abundance of Lachnospiraceae family Phase 1: 12 wks of weight loss (500 kcal energy restriction) Phase 2: 12 wks of weight maintenance
Eslamparast et al,	OB & MetS	28 DB	FOS 250 mg + 7 strains	MD n = 19	Decreased: FBG, TC, TG Increased: HDL

2014 (50)	M/F n = 38		2 x 10^8-10^{10} CFU n = 19		NS: BW, BMI, WaistC, Fasting insulin , HOMA-IR, LDL, MET 7 strains: L. acidophilus, L. casei, L. rhamnosus, L. bulgaricus, B. longum, B. breve, S. thermophilus
Asemi et al, 2013 (51)	OW/OB & DM2 M/F n = 54	8 DB	FOS 100 mg + 7 strains 2 x 10^8-10^{10} CFU	PL	Smaller increase in: FBG, HOMA-IR, hs-CRP, GSH NS: BW, BMI, A1c, Fasting insulin, TC, LDL, HDL, TG, Uric acid 7 strains: L. acidophilus, L. casei, L. rhamnosus, L. bulgaricus, B. longum, B. breve, S. thermophilus
Asemi et al, 2014 (52)	OB & DM2 M/F n = 62	6 DB CO	Inulin 1.08 g + L. sporogenes 2.7 x 10^8 CFU n = 62	PL n = 62	Decreased: hsCRP (-51%) Increased: GSH (46%), Uric acid (12%) NS: FBG, Fasting insulin, TC, LDL, HDL, TG
Malaguarnera et al., 2012 (53)	OW/OB & NASH M/F n= 66	24 DB	FOS 2.5 g + B. longum W11 5 x 10^9 CFU n = 34	FOS 2.5 g + PL n = 32	Decreased: LDL, CRP, TNF-α, LPS NS: BMI, FBG, Fasting insulin, C-peptide, HOMA-IR, TC, HDL, TG Decreased: Steatosis (by liver biopsy)
Mixed trials					
Rajkumar et al, 2014 (54)	OW M/F n = 60	6 OL	Gr1: VSL#3 10^{11} CFU n = 15 Gr2: Omega-3 EPA 180 mg + DHA 120 mg n = 15 Gr3: VSL#3 + Omega-3 n = 15	PL n = 15	Comparison VLS#3 vs. PL: Decreased: FBG, LDL, Fasting insulin, HOMA-IR, hsCRP Change in GMB composition Comparison Omega-3 vs. PL: Decreased: FBG, LDL, HOMA-IR Increased: HDL Comparison VLS#3 + Omega-3 vs. PL: Decreased: FBG, TG, Fasting insulin, HOMA-IR, hsCRP Increased: HDL Change in GMB composition NS (for all comparisons): BW, BMI FBG, Fasting insulin, IL-1β, IL-6, TNF-α VSL#3: L. acidophilus, L. paracasei, L. plantarum, L. bulgaricus, B. longum, B. breve, B. infantis, S. termophilus
Brahe et al, 2015 (55)	OB F n = 53	6 SB	Gr1: Flaxseed mucilage 10 g n = 19 Gr2: L. paracasei F19 9.4 × 10^{10} CFU n = 18	PL n = 16	Comparison each group vs PL: NS: Glycemic (AUC-Glucose, AUC-Insulin, AUC-C-peptide, HOMA-IR, Matsuda index of insulin sensitivity), Lipid (TC, LDL, HDL, TG), inflammatory (hs-CRP, IL-6, TNF-α, LBP, Fecal Total SCFA, Fecal Butyric acid Results for within L. paracasei F19 group (Final vs. Baseline): NS: for all measured biomarkers GMB composition & relative abundance (change as n-fold): Changes in 2 MGS (2,493 bacterial genes)

					Increased: *Eubacterium rectale* 3.3-fold & *Ruminococcus torques* 4.5-fold Results for within Flaxseed mucilage group (Final vs. Baseline): Decreased: AUC-Insulin (-12%), AUC-C-peptide (-13%) Increased: Matsuda index of insulin sensitivity (+11%) NS: for all other measured biomarkers GMB composition & relative abundance (change as n-fold): Changes in 33 MGS (41,090 bacterial genes) Increased (9 MGS): Clostridium genus (3 identified species were: *Bilophila wadsworthia* 2.6-fold, *Parabacteroides merdae* 3.6-fold & *Parabacteroides johnsonii* 4.7-fold) Decreased (24 MGS): 8 MGS belonging to Faecalibacterium genus Results for Placebo group (Final vs Baseline): NS: for all measured biomarkers GMB composition & relative abundance (change as n-fold): Changes in 6 MGS (7,436 bacterial genes) Increased: *Eubacterium ventriosum* and one unknown Decreased: *Roseburia hominis*, two Clostridiales and one unknown

*All trials are Randomized Placebo-controlled parallel groups unless specified, only RTC with duration 6 weeks or longer are included. **Biomarkers (lipid profile, hormones, cytokines) are measured in blood unless specified, Bacterial species are measured in fecal samples.
Abbreviations: A1c=glycohemoglobin A1c, AUC=Area under the curve during OGTT, B= Bifidobacterium, BIA=Bioelectrical Impedance Analysis, BP=Blood pressure, BMI=body mass index, BMR=Basal Metabolic Rate, BTS=Bofutsushosan herb, BW=body weight, CFU=Colony Forming Units, CO=cross-over design, DB=double-blind design, DBP=diastolic BP, DHA= docosahexaenoic acid, EPA= eicosapentaenoic acid, F=female, FatM=Fat mass, FOS=Fructo-oligosaccharide, GMB-Gut microbiota, GSH= total glutathione, GOS=galacto-oligo-saccharide, HipC=Hip circumference, HOMA-IR= homeostasis model assessment of insulin resistance, hs-CRP=high sensitivity C-reactive protein, Inulin-TF=Inulin-type fructans, IFG-γ=Interferon-gamma, ISI=Insulin Sensitivity Index, L=Lactobacillus, LCDiet=low calorie diet, LBP= lipopolysaccharide-binding protein, LeanM=Lean mass, LPS= lipopolysaccharide endotoxin, M=male, MD=Maltodextrin, MET=Metabolic Equivalent Task, MGS=metagenomic species, MetS=Metabolic syndrome, NASH=Non-alcoholic steato-hepatitis, NS=no significant difference, OB=Obese, OGTT=Oral glucose tolerance test, OF=oligofructose, OL=open-label design, OW=Overweight, PL=Placebo, ProCap=Probiotic capsule, RQ=respiratory quotient, RYGB=Roux-en-Y gastric bypass, sCD14= (sCD14), SBP=systolic blood pressure, SB=single-blind, SCFA=Short chain fatty acids, SOD= superoxide dismutase activity, SubFat=Subcutaneous Fat, sVCAM-1=Soluble vascular cell adhesion molecule-1,TLR=toll-like receptor expression by flow cytometry, VisFat=Visceral Fat, WaistC=Waist circumference, WHR=waist to hip ratio.