Research Highlight

Effect of the Gut Microbiota on Obesity and Its Underlying Mechanisms: an Update[*]

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Obesity has become one of the most prevalent health issues of our time. According to a 2012 WHO report, around 3.4 million adults die each year as a result of being overweight or obese[1]. Humans are in fact superorganisms composed of both human and microbial cells with 2 sets of genes, those encoded in our own genome and those encoded in our microbiota. All these cells and genes have the potential to influence our health[2-3].

In adults, the commensal microbial communities are relatively stable, but can undergo dynamic changes because of their interactions with diet, genotype/epigenetic composition, and immuno-metabolic function. Moreover, differences in the composition of the microbiota in the distal gastrointestinal tract appear to distinguish lean versus obese individuals, suggesting that intestinal dysbiosis contributes to the development of obesity and its consequences[4-5]. By focusing on gut microbes involved in the pathogenesis of obesity, this review summarizes recent advances in understanding regarding the underlying mechanisms, host-gut metabolic interactions, and intervention methods targeting the gut microbiota in basic and clinical research.

Gut Microbiota

The distal human intestine can be viewed as an anaerobic bioreactor containing trillions of bacteria and archaea that are programmed to perform metabolic functions that we have not needed to evolve on our own, including harvesting otherwise inaccessible nutrients from our diet[6]. The human gut is thought to hold approximately 10^{14} cells (mostly prokaryotic), a number some 10-fold greater than the number of cells constituting the rest of the human body combined[7]. Bacteria are classified from the phylum to species level and the two most abundant bacterial phyla in humans and mice are the Firmicutes (60%-80% of total bacteria) and the Bacteroidetes (20%-40% of total bacteria)[8-9]. The high diversity of organisms in the gut and the infeasibility of standard culture techniques in identifying those organisms historically have limited their study. Only within the past decade, with the advent of shotgun genomic sequencing and array-based microbial identification, the whole breadth of the organismal diversity in the gut has become apparent.

The Gut Microbiota may Have a Causative Role in the Onset and Progression of Obesity in Humans and Animals   Using volunteers who received their own fecal microbiota as controls, researchers showed that obese volunteers who received microbiota donations from lean donors showed significantly improved insulin sensitivity in the serum (although not in the liver) over a 6-week period. This is the first time that the gut microbiota has been shown to have a causative role in the development of insulin resistance in humans[10].

The modified Koch's postulates could be used to identify the correlation between the gut microbiota and obesity and insulin resistance. Evans[11] proposed a modified version of Koch's postulates as a unified concept for establishing causation of a putative cause in infectious or non-infectious diseases. According to this concept, three kinds of evidence are required to support causation: an

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association between the disease phenotypes and the presence of the cells or genetic material of the putative cause, cross-sectionally and/or longitudinally; the reproduction or reduction of the disease phenotypes by experimental addition or removal of the putative cause in humans or animals; and the occurrence of host molecular responses that mechanistically connect the presence of the putative cause to the occurrence of the disease, for the whole concept to make biological or epidemiological sense[12].

Alterations in the compositional patterns of the gut microbiota have been observed in obesity. Several investigations have found that the gut microbiotas of obese humans and obese (ob/ob) mice had a greater ratio of members of the phylum Firmicutes to members of the phylum Bacteroidetes (the F/B ratio) compared to their lean counterparts[13-16]. When obese people lost weight by consuming either a low-fat or low-carbohydrate, calorie-restricted diet, the F/B ratio decreased in association with the percentage reduction in body weight (not caloric intake)[17]. However, other studies in humans and rodents have reported no difference in the F/B ratio in obese versus lean individuals, no effect of weight loss on the F/B ratio, or even a reversed F/B ratio in obese individuals[18-19]. Studying the intestinal microbial compositions of well-phenotyped human subjects enrolled in relatively large metagenome-wide association studies (MGWAS) in both Chinese and European populations has further increased our understanding of the gut microbiota as a contributor to the development of obesity[20-22]. Recently, a study showed that the gut microbial composition differed between obese and non-obese subjects in Japan, suggesting that it was related to obesity[16].

The important question has now become whether we can identify specific members of the gut microbiota that are more relevant than others are to the causative role of the microbiota in human obesity. If so, these members of the gut microbiota might serve as new targets for the control of obesity[4,23].

Bacterial Translocation and Inflammation Reveal the Mechanisms and Roles of the Gut Microbiota in Obesity and Insulin Resistance

Increased Gut Permeability, Gastrointestinal Inflammation, and Immune Dysfunction Lead to Bacterial Translocation   The intestinal epithelial cells provide a barrier that prevents the passage of toxic and potential pro-inflammatory molecules into the sub-mucosa and the systemic circulation. The tight junctions between the mucosal epithelial cells constitute the primary physical intestinal barrier towards the lumen, and leakiness can be caused by changes in the distributions of tight junctionproteins due to signaling from inflammatory cytokines[24]. The concept of 'bacterial translocation' is proposed, providing a direct cellular link between the intestinal microbiota and the host, in which intestinal phagocytes such as dendritic cells and macrophages capture bacterial intestinal antigens and transfer them into lysosomes for degradation[25]. Intestinal immune responses, such as the production of IgA antibodies and antimicrobial peptides, influence and are influenced by the gastrointestinal microbiota. Under homeostatic conditions, the intestinal immune system helps to preserve systemic ignorance of intestinal bacteria and regulates its composition over time[26]. Gastrointestinal permeability caused by either intestinal inflammation or intestinal immune dysfunction results in the penetration of bacteria or bacterial products [e.g., lipopolysaccharides (LPS) and DNA] into surrounding tissues and the circulation, which can drive systemic inflammation[26]. The inflammatory process is characterized by an increased production of cytokines and infiltration of macrophages[27]. During obesity, there is a substantial increase in hepatic macrophages[28]. It has also been shown that obesity is associated with hypothalamic inflammation and that the resulting local production of pro-inflammatory cytokines can cause central leptin resistance, a key feature of obesity[29].

The Gut Microbiota Induces Obesity and Insulin Resistance via Toll-like Receptors (TLR) and Nucleotide-binding Oligomerization Domain (NOD) Proteins   A fat-enriched diet induces a low-grade infection that targets mesenteric adipose tissue through bacterial translocation, which is mostly responsible for the low-grade inflammation contributing to obesity[30]. Metabolic endotoxemia is defined as a moderate increase in the circulating concentration of LPS derived from gram-negative bacteria and it develops owing to alterations in the composition of the gut microbiota and an increase in gut permeability[31].

Intraluminal microbial detection requires the recognition of pathogen associated molecular patterns by pattern recognition receptors that are distributed on the cell surface and within the cytosol

of innate immune cells. The TLR and NOD-like receptor families function as extracellular and intracellular pattern recognition receptors, respectively, and trigger innate immune responses[32-33]. In the TLR family, TLR4 is activated by LPS derived from gram-negative bacteria. TLR2, which forms a receptor complex with TLR1 or TLR6, recognizes peptidoglycans and lipoproteins from gram-positive bacteria[34]. Mouse studies have shown that TLR1, TLR5, TLR8, TLR9, and TLR12 are overexpressed in the visceral adipose tissue of diet-induced obese and genetically ob ese ob/ob mice[35].

To date, the precise roles of TLR2 and TLR5 have not been fully elucidated. Animals lacking TLR2 show either higher or lower adiposity/insulin resistance depending on the experimental conditions and their microbiota composition[36-37]. As for TLR5, studies in mice suggest that it is a protective factor. Mice lacking TLR5 (T5KO) exhibit hyperphagia and develop hyperlipidemia, insulin resistance, and increased adiposity[38]. In contrast, in human studies, loss of human TLR5 function protects from weight gain, but analogously to the animal model, the nonsense allele predisposes its carriers to T2DM[39]. A recent human study suggested that bacterial flagellin activated TLR5 inflammatory pathways, decreased insulin signaling, and increased glycerol secretion with an increased abundance of flagellated *Clostridium* cluster XIV bacteria[40]. Further work is needed to clarify how the gut microbiome modulates TLR5 in animals and humans.

NOD1 and NOD2 are intracellular proteins that recognize cell wall peptidoglycan moieties from gram-negative and gram-positive bacteria, respectively[41]. Peptidoglycan-induced activation of NOD1 in adipocytes or hepatocytes[42-43] and NOD2 in muscle cells[44] triggers insulin resistance through the production of inflammatory mediators and the activation of mitogen activated protein kinases signaling, leading to the desensitization of insulin receptor substrate 1 function.

The Gut Microbiota Mediate Interactions between the Host Metabolism and the Pathogenesis of Obesity and Insulin Resistance

Short-Chain Fatty Acids (SCFAs) and the Gut Microbiota   Non-digestible carbohydrates including xylans, resistant starch, and inulin are fermented in the colon by microbiota into SCFAs, mainly acetate, propionate, and butyrate, to harvest energy for microbial growth. SCFAs are energy substrates for the colonic epithelium (butyrate) and peripheral tissues (acetate and propionate)[45]. Acetate and propionate are taken up by the liver and used as substrates for lipogenesis and gluconeogenesis. Butyrate has many beneficial effects such as combating inflammation, enhancing epithelial barrier function, and increasing insulin sensitivity[46-48].

The positive effect of butyrate on insulin sensitivity is mediated by stimulating the excretion of gastric inhibitory polypeptide and glucagon-like peptide 1 (GLP-1)[47]. In addition, SCFAs can regulate gene expression by binding to the G-protein-coupled receptors GPR41 (FFAR3) and GPR43 (FFAR2). SCFAs suppress inflammation through GPR43 signaling in immune cells, improve insulin secretion, have anti-diabetic effects, and modulate the secretion of the hormone GLP-1 by stimulating enteroendocrine L-cells. The gut microbiota induces peptide YY expression by L-cells through a GPR41-dependent mechanism[48].

Choline Metabolism and the Gut Microbiota
Alterations in the composition of the gut microbiota that lead to changes in its metabolism of choline have been shown to be associated with obesity, metabolic syndrome, and diseases such as non-alcoholic fatty liver disease and cardiovascular diseases. Choline metabolism by the gut microbiota also plays an important role in the regulation of glucose homeostasis. Choline is an important component of cell membranes that can be obtained from foods such as red meat and eggs and can be synthesized by the host[49]. Microbial and host enzymatic activities interact in choline's transformation into toxic methylamines, which can be further metabolized to trimethylamine-N-oxide in the liver[49]. Plasma levels of trimethylamine-N-oxide and its metabolites are correlated with cardiovascular disease[50].

Bile-Acid Metabolism and the Gut Microbiota
Cholesterol is used in the human liver to synthesize the primary bile acids, cholic acid and chenodeoxycholic acid. Primary bile acids are conjugated to glycine in humans, and are taken up in the distal ileum for transport to the liver. However, these bile acids are deconjugated by bacteria in the ileum and metabolized by the gut microbiota into secondary bile acids. Bile acids also function as signaling molecules and bind to cellular receptors, such as the bile-acid-synthesis controlling nuclear receptor farnesoid X receptor (FXR) and the G-protein-coupled receptor TGR5[48]. FXR is activated

by primary bile acids and impairs glucose metabolism[51], while TGR5 binds secondary bile acids and exerts a beneficial effect by improving liver and pancreatic function and enhancing glucose tolerance through inducing the secretion of GLP-1 by enteroendocrine L-cells[52-53]. Furthermore, TGR5 can increase energy expenditure in brown adipose tissue and can protect against diet-induced obesity[48].

Other Metabolic Processes and the Gut Microbiota
Diet-microbial interactions may affect the metabolome and can affect the insulin sensitivity of the host. For instance, the metabolism of phenolic amino acids to *p*-cresyl sulfates was shown to promote chronic kidney disease-associated insulin resistance[54]. Bacteria belonging to some genera, such as *Bacteroides* spp., *Clostridium* spp., and *Fusobacterium* spp., can promote the biotransformation of phenolic compounds to *p*-cresylsulfate[55-56]. Kuhn et al. found that the gut microbiota metabolized tryptophan to indole-3-propionic acid, which improves insulin resistance[57]. Some vitamins that are essential to the host are derived from metabolites produced by the gut microbiota, such as folic acid and cobalamin. Metabolic syndrome patients treated with these vitamins achieved improvements in their symptoms

of insulin resistance and endothelial dysfunction[58].

Gut Microbes That may Influence Obesity and Insulin Resistance

Summarized from published papers, the phylum Proteobacteria and the species *Faecalibacterium prausnitzii* and *Akkermansiamuciniphila* may be beneficial for weight control and insulin sensitivity. Mice with obesity and insulin resistance induced by a high-fat diet had improved insulin sensitivity concomitant with a significant over representation of the phylum Proteobacteria when given the antibiotics ampicillin, neomycin, and metronidazole by mouth, showing that the abundance of the phylum Proteobacteria and insulin sensitivity may be linked[59]. Rats subjected to Roux-en-Y gastric bypass surgery showed a decreased F/B ratio and a striking 52-fold increase in the abundance of the phylum Proteobacteria[60-61], and Zhang et al. demonstrated similar results[16].

An inverse correlation was found between fecal *F. prausnitzii* and serum concentrations of the inflammatory markers high-sensitivity C-reactive protein, interleukin 6, and orosomucoid[53]. *F. prausnitzii* are thought to exert such anti-inflammatory effects by producing butyrate, and some secreted
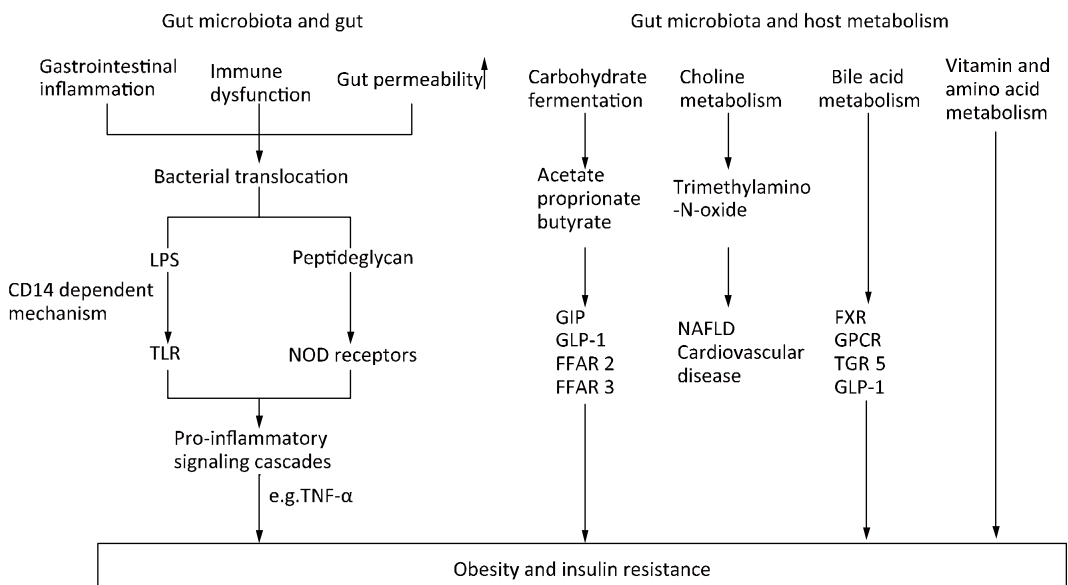

Figure 1. Potential mechanisms linking gut microbiota, host metabolism and obesity and insulin resistance. The disorder of gut microbiota can cause gastrointestinal inflammation, immune dysfunction and an increasing gut permeability, leading to bacterial translocation and the components of bacteria can mediate the TLR and NOD receptors causing systemic inflammation. The gut microbiota can also interact with the host metabolism and influence the pathogenesis of obesity and insulin resistance.

metabolites are able to block nuclear factor κB activation and interleukin 8 production[62].

The abundances of *Clostridium cocleatum* and the mucin-degrading bacterium *A. muciniphila* were demonstrated to increase significantly in mice after metformin treatment[63]. Metformin also significantly improved the glycemic profile of high fat diet fed mice and led toa significant increase in the number of mucin-producing goblet cells in the gut[64].

The beneficial effect of A. *muciniphila* was demonstrated by Everard et al. that probiotic treatment with *A. muciniphila* reversed high-fat diet-induced metabolic disorders, including fat-mass gain, metabolic endotoxemia, adipose tissue inflammation, and insulin resistance[65].

Members of the endotoxin-producing genus *Enterobacter* may causatively contribute to the development of obesity in humans. The strain *E. cloacae* was isolated from a morbidly obese human's gut and transferred to germfree mice, in which it induced obesity and insulin resistance. The relative abundance of *Enterobacter* spp. among the gut microbiota decreased from 35% to an undetectable level after the volunteer lost 51.4 kg of their initial body weight of 174.8 kg. The volunteer also recovered from hyperglycemia and hypertension after 23 weeks of adhering to a dietary intervention[66].

Dietary Interventions, Probiotics, and Fecal Microbiota Transplantation may Treat Obesity and Insulin Resistance by Targeting the Gut Microbiota

Dietary Interventions   Oligofructose intervention was shown to increase the abundance of *A.muciniphila* in obese and type 2 diabetic mice and result in an improved metabolic profile[65]. Oligofructose can also lower plasma LPS and inflammatory cytokine concentrations, and decrease hepatic expression of markers of inflammation and oxidative stress[67]. Feeding a 4-week high-amylose starch diet to male obese Sprague Dawley® rats led to a lower glycemic response and higher insulin sensitivity compared to feeding them with a high-amylopectin starch diet[68]. Dietary supplementation with resistant starch increased the abundance of representatives of the Actinobacteria and Bacteroidetes phyla and decreased the abundance of those of the Firmicutes phylum in humans[69]. A cranberry extract treatment reduced high fat/high sucrose-induced weight gain and visceral obesity, decreased liver weight and triglyceride accumulation, improved insulin

sensitivity, and increased the abundance of the mucin-degrading bacterium *A. muciniphila* in mice[70]. Fei and Zhao used a diet of whole grains, traditional Chinese medicinal foods, and prebiotics to achieve effective weight-loss and benign metabolic results in an obese volunteer[66].

Probiotics   The administration of *Lactobacillus gasseri* was shown to decrease fat mass (visceral and subcutaneous) and body mass index in obese and type 2 diabetic patients[71]. In a double blind randomized study, the administration of *Lactobacillus* spp. was shown to preserve insulin sensitivity as evaluated by euglycemichyperinsulinemic clamp, whereas it decreased in the placebo group[72]. Amar et al. conducted a 1-month probiotic treatment with *Bifidobacterium animalis* (strain B420), which resulted in decreased bacterial translocation and improved insulin sensitivity, suggesting that certain probiotic interventions can reverse the adverse metabolic phenotype induced by a high-fat diet[30].

Fecal Microbiota Transplantation   Fecal microbiota transplantation (FMT) has proven a highly effective and successful treatment for patients with several diseases[73]. In the study conducted by Vrieze et al., male insulin-resistant subjects with metabolic syndrome received solutions of stool samples from lean donors and showed a significant improvement in peripheral insulin resistance and an altered intestinal microbiota composition[74]. In another study, treatment of obese subjects with metabolic syndrome by using vancomycin resulted in impaired peripheral insulin sensitivity and a decreased diversity of the gut microbiota[75].

Human-to-human FMTs have received considerable attention. However, the USA Food and Drug Administration (FDA) recently ruled that FMT has not received approval for any clinical indications at this point[76]. To justify the standard use of FMT, further large controlled studies are needed to demonstrate the efficacy of FMT[77].

Tables 1 and 2 summarize methods that have recently been tested for the treatment of obesity and insulin resistance in animal and human research that have targeted the gut microbiota (Tables 1 and 2).

Conclusion

It can be concluded that the microbial community in the gut is complex and that interplay exists among bacterial translocation, chronic inflammation, the immune system, host material

metabolism, and the composition of the gut microbiota. Additionally, the gut microbiota is involved in the regulation of multiple host metabolic pathways and host-microbiota metabolic and signaling pathways. Modulation of the gut microbiota through dietary interventions, probiotics, and/or fecal microbiota transplantation represents a promising approach for the treatment of obesity and insulin resistance. The naturally existing human gut microbiota may contain beneficial and harmful species, but precisely how the composition of the gut microbiota relates to health has not yet been clearly illuminated.

Considering the close association of the gut microbiota with the pathogenesis of obesity and insulin resistance, its causal role in the development of these conditions needs to be established and clarified. Further well-designed and longitudinal studies focusing on species-level changes in the composition of the gut microbiota are necessary and should examine deeper taxon levels. Such research has the potential to ameliorate the startling contemporary epidemics of obesity and metabolic disease worldwide.

Table 1. Summary of Treatment Methods of Obesity and Insulin Resistance in Animals by Targeting Microbiota

Treatment Methods	Ref.	Study Subjects	Results
Diet intervention (*a prebiotic diet*)	[67]	ob/ob mice	prebiotic-treated mice: plasma LPS↓, cytokines↓, hepatic expression of inflammatory and oxidative stressmarkers↓
Diet intervention (*prebiotic treatment*)	[65]	C57BL/6J mice	prebiotic treatment increased Reg3g expression and improved intestinal homeostasis; Gut microbiome at different taxonomic levels were affected
Diet intervention (*a cranberry extract treatment*)	[70]	C57Bl/6J male mice	weight gain and visceral obesity↓, insulin sensitivity↑, intestinal triglyceride content, inflammation and oxidative stress↓, Akkermansia↑
Diet intervention (*1% Concord grape polyphenols*)	[78]	C57BL/6J mice	weight gain, adiposity, TNF-α, IL-6, LPS, glucose intolerance↓, intestinal gene expression of TNFα, IL-6 Glut2↓, barrier function gene(occludin) ↑, Akkermansiamuciniphila↑, Firmicutes/Bacteroidetes↑
Diet intervention (*fermented green tea extract*)	[79]	mice	body weight gain, fat mass ↓, mRNA expression levels of lipogenic and inflammatory genes↓, glucose intolerance and fatty liver symptoms↓, restored the changes in gut microbiota composition (e.g., the Firmicutes/Bacteroidetes and Bacteroides/Prevotella ratios
Probiotics (*ifidobacteriumanimalis subsp. Lactis420 treatment*)	[30]	mice	bacterial translocation↓, insulin sensitivity↑
Probiotics (*Bifidobacteriumpseudocatenulatum CECT 7765*)	[80]	male wild-type C57BL-6 mice	Firmicutes↓, LPS-producing Proteobacteria↓, TNF-α↓, IL-17A↓, pro-inflammatory macrophages↓, body weight gain↓, serum cholesterol, triglyceride, glucose and insulin levels↓, oral glucose tolerance and insulin sensitivity↑
Antibiotics (*vancomycin and bacitracin*)	[81]	diet-induced obesity (DIO) C57BL/6J mice	systemic glucose intolerance, hyperinsulinemia, and insulin resistance in DIO were ameliorated, metabolically beneficial metabolites derived from the gut↑, Firmicutes and Bacteroidetes depleted
Prebiotics (*water extract of Ganodermalucidum*)	[82]	high-fat diet mice	Firmicutes-to-Bacteroidetes ratios↓, endotoxin-bearing Proteobacteria levels↓, metabolic endotoxemia↓, maintains intestinal barrier integrity

Table 2. Summary of Treatment Methods of Obesity and Insulin Resistance in
Humanby Targeting Microbiota

Treatment Methods	Ref.	Study Subjects	Results
Diet intervention (*a WTP diet*)	[66]	Human (*n*=1)	amelioration of hyperinsulinemia, hyperglycemia and hypertension, Enterobacter reduced from 35% to 1.8%
Diet intervention (*flaxseed mucilage*)	[83]	obese postmenopausal women (*n*=58)	C-peptide, insulin release↓, insulin sensitivity↑, relative abundance of 8 Faecalibacterium species↓, alterations in abundance of 33 metagenomic species
Diet intervention (*weight stabilisation diet*)	[84]	overweight and obese adults (*n*=49, including 41 women).	Individuals with higher baseline A. muciniphila displayed greater improvement in insulin sensitivity markers and other clinical parameters after diet intervention. These participants also experienced a reduction in A. muciniphila abundance, but it remained significantly higher than in individuals with lower baseline abundance.
Diet intervention (*L-glutamine*)	[85]	overweight and obese adults (*n*=33)	Firmicutes/Bacteroidetes decreased from 0.85 to 0.57, genus Dialister, Dorea, Pseudobutyrivibrio and Veillonella(belonging to the Firmicutes phylum)↓
Prebiotics (*Rehmannia glutinosa Libosch, ShuDihuang*)	[86]	female middle-aged subjects (*n*=12)	waist↓, phylum Actinobacteria and genus Bifidobacterium↑, phylum Firmicutes and genus Blautia↓ in response to the herbal treatment. B
Probiotics (*Lactobacillus gasseri SBT2055*)	[71]	human (*n*=87)	abdominal visceral and subcutaneous fat areas↓, body weight, BMI, waist, hip↓
Fecal microbiota transplantation	[74]	human (*n*=18)	peripheral and hepatic insulin sensitivity↑, gut microbiota diversity↑, 16 bacterial groups including Roseburiaintestinalis↑

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