Metabolism and Metabolic Disorders and the Microbiome: The Intestinal Microbiota Associated With Obesity, Lipid Metabolism, and Metabolic Health-Pathophysiology and Therapeutic Strategies

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Changes in the Intestinal Microbiota Associated With Obesity, Lipid Metabolism, and Metabolic Health and Therapeutic Strategies

Short title: Intestinal microbiota in metabolic health

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Abbreviations: α-MSH: α-Melanocyte stimulating hormone; AMPK: AMP-activated protein kinase; Ahr: aryl hydrocarbon receptor; ATB: antibiotics; BAT: brown adipose tissue; BMI: body mass index; BCAA: branched chain amino acids; CR: caloric restriction; CD: clostridium difficile; CVD: coronary artery diseases; DGAT2: Diacylglycerol O-Acyltransferase 2; DMB: 3,3 dimethyl-1 butanol; HFD: high-fat diet; FA: fatty acids, FMT: fecal microbiota transfer; FXR: farnesoid-X receptor; GE: ginseng extract; GLP-1: glucagon like peptide 1; GPR-43: G-protein-coupled receptor 43; HDL-C: high-density lipoprotein cholesterol; IDO1: indoleamine 2,3 dioxygenase 1; IM: intestinal microbiome; ImP: imidazole propionate; KO: knock-out; LFD: low-fat diet; LBP: lipopolysaccharide binding protein; LDL: low density lipoprotein; LPS: lipopolysaccharide, LPL: lipoprotein lipase; GF: germ-free; MAMPs: Microbe-Associated Molecular Patterns; miR: microRNAs; MUFA: mono-unsaturated fatty acid; NAFLD: non-alcoholic fatty liver disease; PET-CT: Positron Emission Tomography - Computed Tomography;PGC1α : Peroxisome Proliferator-Activated Receptor-Gamma Coactivator-1α; PPARγ: peroxisome proliferator-activated receptors; PRMD16: PR domain containing 16; PUFA: polyunsaturated fatty acid; RCT: randomized control trial; RT: room temperature; SFA: saturated fatty acids;

SIRT-1: Sirtuin 1; SCFA: short chain fatty acids; SPF: specific pathogen free; T2D: type 2 diabetes; Tg: triglycerides, TGR5: G-protein-coupled bile acid receptor; TLR: toll-like receptors; TMAO: Trimethylamine N-oxide; Trp: tryptophan; TpH1: tryptophan hydroxylase 1; UCP1: uncoupling protein 1 ; VLDL: very low density lipoprotein; WAT: white adipose tissue; WT: wild-type;

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Abstract:

Changes in the intestinal microbiome have been associated with obesity and type 2

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diabetes, in epidemiological studies and studies of the effects of fecal transfer in germ-free mice. We review the mechanisms by which alterations in the intestinal microbiome contribute to development of metabolic diseases, and recent advances, such as the effects of the microbiome on lipid metabolism. Strategies have been developed to modify the intestinal microbiome and reverse metabolic alterations, which might be used as therapies. We discuss approaches that have shown effects in mouse models of obesity and metabolic disorders, and how these might be translated to humans to improve metabolic health.

Key words: intestinal microbiome, insulin resistance, obesity, lipids, microbial-derived metabolites

Introduction

Obesity prevalence reached 40% in the United States in 2016, with major inter-individual socio-economic disparities and is predicted to further increase[1], together with its associated-metabolic comorbidities[2]. To date, while a few anti-obesity medications are available and efficient, on top of lifestyle interventions, to achieve 5% weight-loss, they present several limitations: they are reserved for individuals with already existing overweight/obesity[3], they can induce adverse events leading to treatment discontinuation and finally their cost is significant. Thus, to bend the worldwide obesity epidemic curve and its associated-management costs, safe and inexpensive public heath interventions need to be developed and implemented in adults as was done in children, which led to decreased or plateaued prevalence in individuals below the age of 11[2]. Furthermore, trying to decipher novel patho-physiological mechanisms involved in obesity and related-disease might help develop new preventive or therapeutic strategies in the future.

The intestinal microbiome (IM), is mostly shaped by the environment[4,5], in particular the diet, and varies across ethnicities[6], maybe in links with differences in food cultural habits since genetic does not appear to strongly influence the IM composition in large human studies[4]. The IM is involved in several major physiological functions that maintain metabolic homeostasis. Among others, IM processes and digests nutrients, produces metabolites[7] and shapes the immune system[8]. This field has been revolutionized by high-throughput sequencing techniques such as 16S-sequencing approach, which delivers valuable composition information, and metagenomics which provides additional knowledge on microbial genes and their potential functions[9–11]. Methodological pros and cons of both technics are detailed in[9]. Complementary metabolomics analysis enables to dive

deeper into functionality assessment when combined to metagenomics[11]. These tools led to the discovery of major compositional changes in IM during metabolic disorders (i.e. obesity, insulin-resistance, type-2 diabetes (T2D), dyslipidemia and non-alcoholic fatty liver disease (NAFLD)[9,12–18]), which suggest its involvement in their physiopathology.

Impact on weight and metabolic disorders

In-vivo models, such as co-housing experiments[19] or comparison of conventional mice and germ-free (GF) ones[20], post-weaning pumps[21] and/or antibiotic-treated mice[22], to whom fecal microbiota transplantation (FMT) is performed from mice or humans donors[23] enables to further dig into causality. Translation to humans of results obtained in animals are also possible using the *in-vitro* gut stimulator model[24] and/or intervention trials such as FMT from human to human[25,26], antibiotic-treatment[27] or diet interventions[15]. Although these techniques have their own advantage or drawback to infer causality, they nevertheless enabled to progress in the understanding of IM contribution[28] in metabolic diseases with the discovery of new mechanistic pathways.

Results from GF and FMT on body weight in mice and humans

Firstly, GF mice have lower body weight and white adipose tissue (WAT) than conventional ones[29], on chow or high-fat diet (HFD), despite increased calorie intake[20,30,31]. Their colonization with a normal IM for 14 days enable them to reach similar weight than conventional mice[29]. Noteworthy, while conventionalized mice gain significantly more weight upon HFD than low-fat (LFD), GF remain weight stable whatever their diet, pointing at the IM contribution to handle properly energy storage from food intake[32]. Secondly, FMT from obese conventional mice (either diet-induced

or genetically obese animals (ob/ob)) into GF recipient upon chow-diet, leads to higher weight-gain and WAT depot, than FMT from lean mice[12,20,33,34]. Thirdly, FMT from obese human into GF recipients translates into higher weight gain than FMT from their lean twins[35].

Importantly, differences in food qualitative intake modulate the IM, its implantation after FMT and its capacity to store energy from food leading to differential transferred phenotype[35,36]. While recipient mice submitted to HFD display a microbiome similar to the obese twin, upon LFD, the dominant colonized microbiota resembles the lean twin's[35]. Moreover, upon isocaloric diet containing saturated (lard) or poly-unsaturated fat (fish oil), the lard-fed group develop increased food intake leading to higher weight and adiposity, more inflamed WAT and worse metabolic alteration[36]. Likewise, the two groups display major differences in their IM[36] which is responsible for the clinical phenotype. Indeed, FMT from fish-fed mice into antibiotic-treated recipient on a lard-diet, results in lower weight-gain and WAT inflammation, than FMT from lard-fed animals.

Several human FMT case reports corroborate mouse observations. FMT from a normal weight donor (i.e. BMI=25kg/m²) in an underweight anorexic women enabled a modest weight gain and weight stabilization[37]. Likewise, a women receiving FMT from her overweight daughter, to treat clostridium difficile infection, developed obesity[38]. These observations led an international consensus to propose drastic selection for human donors prior to FMT and exclude those with overweight or obesity[39]. This cautiousness was probably wise since patients receiving FMT for CD infection do not gain more weight than those upon conventional therapy[40] after a mean of 3.8 years of follow-up. Overall, while these FMT experiments using GF mice

or human recipient show that IM can transmit weight gain, even upon chow-diet feeding, human data remain less conclusive to date.

Results from GF and FMT on lipid profile in mice

Compared to conventional mice, GF upon chow-diet[30] display reduced fasting systemic triglyceride[30], total cholesterol[30] and HDL-cholesterol levels[30], reduced portal triglyceride[31], concomitant with increased liver cholesterol and decreased triglyceride content[30]. This phenotype is explained by the enhancement of liver cholesterol synthesis (i.e increased liver gene expression of hydroxyméthylglutaryl-CoA reductase[30,41] and protein level of the nuclear transcription factor Sterol regulatory element-binding proteins (SREBP2)[30], involved in the up-regulation of sterol biosynthesis). Similar to mechanisms involved in weight-storage, the diet and the quality of its lipid content[42], modulates the IM and its associated-lipid phenotype. Indeed, upon HFD, GF mice display increased triglyceride concentration compared to conventional mice as seen with either direct measures[43] or lipidomic analysis[44]. However, the genetic background[45] also influence IM-lipid profile interactions. Indeed, atherosclerotic prone mice (i.e. ApoE-/-) fed a chow-diet and depleted from their IM by broad-spectrum antibiotic display increased levels of cholesterol (specifically in VLDL and LDL particles) compared to conventionally raised ApoE-/- mice[41]. Furthermore, FMT from human with high systemic cholesterol concentration into antibiotic-depleted ApoE-/- mice induced a higher cholesterol concentration and intestinal expression of genes involved in cholesterol absorption in the recipient than similar recipient receiving FMT from human donors with low cholesterol levels[41]. Importantly, the IM composition from donors with high or low cholesterol levels were significantly different[41], suggesting the impact of the modified IM in adapting intestinal cholesterol absorption

Results from human cohorts on the microbiome-lipid profile interaction

Large cohort studies examined the relationships between the variation in IM composition and systemic lipid levels[46,47]. In 800 individuals from the Life-LinesDEEP study, IM explained 6.0% and 4.0% of triglyceride and HDL level variation respectively, while IM had hardly any significant impact on LDL-C levels[46]. In contrast, within the Metacardis study, triglycerides significantly explained 0.39% of IM composition variation, in 764 individuals without any lipid-lowering drugs[48]. Studies examining the effect of statin[48–53] on metabolic health also provides insights into the role of IM in lipid metabolism and regulation (detailed in **Table 1**).

LPS effects on insulin-resistance in mice and humans

Since GF mice display an immature immune system, which plays an important role in metabolic alteration development, the role of the IM in metabolic disease has rather been studied in conventional mice submitted to infusion of bacterial membrane (Lipopolysaccharide (LPS)) and in several genetic models knockout (KO) for its Toll-like receptors (TLRs).

Chronic LPS subcutaneous infusion in mice recapitulates the altered phenotype of HFD mice: increased weight-gain, insulin-resistance, WAT inflammation, increased systemic LPS[54] and increased intestinal permeability, thus linking the IM to metabolic health[55,56]. Again, the diet modulates the IM and its-associated metabolic health[57,58]. For example, palm-oil gavage in mice induces a rapid disruption of cell-cell junction within the intestine, an increased gut permeability and inflammation[59] before any significant weight gain. Noteworthy, some microbial-

produced metabolites (MAMPs) are transferred from the gut into the host and recognized by the innate immune system, mainly through TLRs to activate inflammatory and adverse metabolic outcomes[19]. LPS binds to TLR4, a pattern recognition receptor, which activate the innate immune system[60] and is highly expressed in the WAT of obese mice, where it induces a pro-inflammatory response. Compared to WT mice, TLR4-KO mice upon HFD display lower weight and hepatic steatosis, decreased WAT inflammation and a switch towards alternative macrophage polarization[61]. Importantly, the specific TLR4 on hematopoietic cells is mandatory to induce WAT inflammation as well as liver and WAT insulin-resistance[62]. Several studies further confirmed the protective metabolic effects of TLR4 deficiency[63–66], as reviewed in[67]. Finally, the relevance of TLR4 was suggested in humans, as TLR4 expression, protein content and signaling is higher in muscle tissue of individuals with obesity and T2D than in lean controls[68]. However, while increased level of LPS is mandatory to induce major WAT macrophage infiltration, altered glucose and insulin tolerance occur after the sole colonization of GF, irrespective of the level of microbiota-related LPS production[29]. Going further, the comparison of conventional or GF mice proved that the IM regulates numerous liver gene expression, in particular those related to LPS transport through Myd88[69]. Furthermore, LPS-binding protein (LBP) impairs insulin signaling in hepatocytes in the presence of low-dose of LPS *in-vitro*, while by contrast, pharmacologic LBP blocking improves insulin signaling *in-vitro* and glucose homeostasis *in-vivo*[69].

Other TLRs are also involved since TLR5-KO mice display hyperphagia[70] and develop low-grade inflammation and metabolic syndrome as well as modification of their IM composition compared to their WT counterparts. FMT from TLR5-KO mice into GF replicates the metabolic alterations in the recipients[70] demonstrating the

importance of the IM through flagelin-TLR activation to modulate host metabolism. Importantly, metabolic alterations and modified IM composition originated from TLR5 activation upon intestinal epithelial cells[71] but not dendritic cells[71]. However, TLR5-KO in dendritic cells abrogated the intestinal production of IL-22[71] a cytokine involved in intestinal health[72]. Yet, a recent study comparing TLR5-KO to WT mice did neither confirm the difference in metabolic alteration upon chow-diet or HFD, nor the differences in IM composition[73] suggesting the need to investigate further TLR5 pathway. The IM composition in mice from both genotypes in this study was very different from the original study, possibly pointing at a major impact of the environment[73] in IM-host phenotype interactions. Noteworthy, a previous study on the impact of IM and TLRs in the context of liver metabolic diseases (i.e. NAFLD/NASH) also concluded that TLR5 deficiency-related microbiota dysbiosis was not involved in the exacerbation of NAFLD to NASH[19]. Nevertheless, dysbiotic microbiota is involved in NAFLD physiopathology through several mechanisms including LPS and other TLRs activation as reviewed in detail[74].

Turning to human research, the suspected presence of bacteria in the blood[75] or within metabolic tissues[76,77] (probably due to increased intestinal permeability) has recently been confirmed and further linked to metabolic alterations[78]. The higher presence of bacterial DNA was confirmed in liver and omental WAT of individuals with morbid obesity[78] as compared to SAT and mesenteric WAT (MAT). Importantly, several types of controls during each analysis steps demonstrated that bacterial DNA presence in WAT was not due to environmental contamination, by contrast to plasma. Moreover, microbial species evenness (determined by α-diversity using Shanon index) was significantly lower within the MAT of obese individuals with T2D than those without,[78] mirroring the decreased IM bacterial diversity of individuals with

obesity and metabolic alteration[10,15,16]. Furthermore, the MAT bacterial signature of T2D individuals (i.e. increased Enterobacteria[78]) also mirrors that of IM from patients with insulin-resistance[11].

Overall, these studies indicate that IM and/or some of its components, modified by the qualitative aspects of the diet, are involved in weight-storage, lipid profile and insulin-resistance.

Functional mechanisms involving IM in weight regulation

Energy extraction from food, handling and storage

Compared to conventional mice, GF on HFD or antibiotic-treated mice upon chow-diet[79,80] display decreased digestive absorption, as shown by increased 24h stool quantity[31], caloric fecal content[30,31]. Interestingly, decreased digestive absorption is a common mechanism involved in IM-lipid profile interaction, since GF mice also display a 40%-higher lipid (i.e. total, cholesterol and triglyceride) fecal content[30,31] (**Figure 1**). This differential energy extraction from food partly originates from IM functional properties which may differ according to the donor corpulence[10,16,81]. Compared to lean, caecal IM from obese mice are enriched in enzymes breaking down indigestible carbohydrate by the host[34], leading to increased production of short chain fatty acid (SCFA), the end-products of fermentation process[34] involved in energy storage[82] Nevertheless, conflicting results[10,16,81,83] are found thus warranting more research in the field.

Compared to conventional mice, GF display increased expression of intestinal and WAT *fiaf*, an inhibitor of Lipoprotein lipase (LPL) activity[20]. Upon microbiota conventionalization, *fiaf* decreases thus enhancing LPL activity, which leads to WAT

lipid storage[84]. Moreover, while *fiaf-KO* GF mice for are no longer protected from diet-induced obesity[43], transgenic mice overexpressing *fiaf* display lower adiposity than their wild type (WT) littermate[85]. These results highlight the important dialog between the IM, the intestine and WAT to store energy.

GF mice are also protected from diet-induced obesity through increased muscle and liver β-oxidation[43]. First, GF display increased phosphorylated AMPK[43]; second, *fiaf* increases PGC-1α which regulates positively β-oxidation genes[86]. Likewise, *fiaf*-KO GF mice shows increased genes involved in fat oxidation[43].

<u>Beiging of the white adipose tissue</u>

Recent discovery suggest that IM regulate body weight through its role in WAT beiging and increased energy expenditure[87] (**Figure 1**), a mechanism common to its role on insulin-resistance. Compared to room temperature (RT), cold-exposed mice (i.e. 4°C) modify their IM composition, increase energy expenditure and reduce body weight despite higher caloric intake[79]. FMT from cold-exposed mice into GF recipient on chow-diet, recapitulates the decrease in body weight and fat mass, improved insulin-sensitivity, increased energy expenditure and development of WAT beiging (histological changes and increased UCP1)[79], compared to FMT from RT-exposed mice. These data suggest an interplay between cold modified-IM and WAT beiging, where the role of LPS and LBP axis has been emphasized. Indeed, cold-exposed mice display reduced LBP and increased WAT expression of UCP1[88]. LBP-KO mice have increased WAT beiging and decreased body weight on both chow and HFD as compared to WT mice[88]. Noteworthy, after initial weight-loss, weight from cold-exposed conventionalized mice stabilized in the longer-term, and originated from intestine adaptation[79], namely a major increase of the digestive absorptive surface. This intestinal adaptation was also replicated upon "cold" microbiota transfer to GF

mice, suggesting the importance of the cross-talk between IM and the host to maintain body weight[79].

GF or antibiotic-treated conventional lean or obese (either *ob/ob* or HFD-induced) mice, raised at RT (i.e. 22°C) or thermo-neutrality (i.e. 30°C), also display reduced adiposity and a switch towards decreased number of large adipocytes, increased number of small adipocytes together with functionally active beige adipocytes within WAT[89] with increased thermogenic capacity[89]. By contrast, FMT from conventional mice into GF led to the reverse phenotype.

This induced beiging originates from increased M2-macrophages and their related-cytokine production in WAT (IL4, IL5 and IL13)[89] confirming previous findings[90]. Microbiota-depleted mice KO for type-2 signaling are not able to induce beiging and display adverse metabolic alterations, suggesting that IM is involved in this beiging effect through anti-inflammatory type-2 cytokine production in WAT[89]. Nevertheless, a recent study challenged those results and rather observed that IM depletion negatively regulated WAT beiging, both in antibiotic-treated mice or in GF either at RT or at thermo-neutrality[80]. The high variability in IM composition across different settings, could partly explain these discrepant results. Whether IM plays a role in beiging still warrants more studies.

Indoles, tryptophan-derived microbial metabolites control adiposity via microRNAs in WAT

Some microbial-produced metabolites control the expression of microRNAs in WAT, namely the miR-181 family, which in turn regulates energy expenditure and body weight[91] (**Figure 1**). miR-181 is notably induced in the WAT of diet-induced obese mice and in obese individuals. By contrast, compared to WT mice, miR-181-

KO mice upon HFD are protected from developing obesity. They display reduced WAT, smaller adipocytes and increased energy expenditure[91]. miR-181 controls the expression of genes involved in metabolic fitness[92], adipocyte function and insulin signaling. Furthermore, GF mice have lower miR-181 in their WAT than conventional mice. FMT from conventional to GF mice induces the increase of miR-181 in the WAT of recipients, suggesting a role of IM in these miR regulation. Finally, reduced tryptophan-derived microbial metabolites (i.e. indoles) during obesity as detailed below, leads to increased miR-181 in WAT. By contrast, indole administration decreases miR-181 within the WAT and protects against diet-induced obesity, a phenotype not seen in miR-181-KO mice demonstrating the obligatory role of the cross talk between the IM, its produced-metabolites and miR within the WAT to control weight[91].

Altogether, accumulating evidences suggest a role of the IM in weight storage, with detailed mechanisms studies in animal models, yet warrants their evaluation and confirmation in humans.

Some indirect evidence have tried to address the relevance of mice studies in humans. In a cohort of obese individuals with or without insulin-resistance assessed by the euglycemic-hyperinsulinemic clamp, insulin-sensitivity associated IM composition was correlated with WAT gene expression involved in beiging (UCP-1 and PRDM16). However, this study did not evaluate whether these BAT genes were also correlated with body weight or adiposity[93]. Second, in morbid obesity, LBP gene expression negatively correlates with UCP-1 and PRDM16 within the WAT[88]. Future human research will need to confirm the link between the IM and the presence of WAT beiging markers as well as the BAT activity by positron emission tomography imaging with radiotracers[94,95]. This could be adressed before and after a 7-days ATB

cocktail as previously described in the TMAO story[96,97]. Previous studies however using solely one ATB for 7 days (i.e. vancomycine or amoxiciline) led to only modest changes in WAT[27] (i.e. no change in total body weight, no effect in adipocyte size, yet increased expression of genes involved in increased oxidative metabolism). Markers of beiging were not assessed. Finally, interesting line of future translational research is to explore in humans whether strategies such as polyphenol use modifying IM composition lead to decreased weight through increased beiging.

Functional mechanisms involving IM in lipid metabolism

Clearance and intestinal absorption

The use of a lipid challenge enabled to demonstrate that GF mice upon HFD have increased triglyceride concentrations due to reduced post-prandial triglyceride clearance[43,44]. This originates from LPL inhibition secondary to increased *fiaf* in the absence of the IM[43]. Recent data, using radiolabeled lipid challenge[31,41], have now also demonstrated that the IM is involved in small intestine lipid[31] and cholesterol[41] digestive absorption. Indeed, after LPL inhibitor treatment enabling to solely study the absorption pathway, triglyceride and cholesterol absorption is decreased in GF mice as compared to conventional ones submitted to LFD. Since HFD is not able to restore systemic lipid levels in GF mice upon LFD, this proved the obligatory role of the IM in lipid absorption[31]. Importantly, HFD modifies IM composition within both ileum and jejunum. Subsequently, FMT using jejunum IM from HFD mice, into GF recipient (either on LFD or HFD[31]) restored lipid absorption to the same extend as that seen in conventional mice. This experiment demonstrates the importance of the small IM and the diet (herein HFD, which modulate the IM) in its related-lipid

absorption (**Figure 2**). Furthermore, HFD-induced changes in IM (i.e. for example *L.rhamnosus* and *C.bifermentans*) are involved in microbes-host interaction to increase lipid absorption in the digestive tract[31], through bioactive mediators[98] able to increase the expression of DGAT2, an enzyme involved in triglyceride biosynthesis[99].

Microbial-signals involved in lipid profile

IM-produced metabolites or IM-modulated signals are involved in lipid metabolism (**Figure 2**). Bile acids, which are modulated by the IM, are involved in lipid metabolism through host farnesoid-X-receptor (FXR) and TGR5, which have already been reviewed in detail elsewhere[100].

SCFA, derived from dietary fiber digestion by IM, serve as fuel for host lipid synthesis[101]. A recent study comparing GF and conventionalized mice, using lipidomic, liver gene expression and liver proteome analysis confirmed that pathways involved in lipid metabolism were increased in GF mice[102], which translated into increased circulating levels of saturated (SFA) and polyunsaturated fatty acid (PUFA), while conventional mice had increased levels of mono-unsaturated fatty acids (MUFA), thus improved lipid profile. Indeed, compared to SFA, MUFA decreases postprandial triglycerides and induce a shift from small dense LDL-C particles to lager less atherogenic ones[103], leading to reduced cardiovascular events in human RCT. By contrast, while omega-3 PUFA are beneficial on CVD health and lipid profile, omega-6 PUFA are either associated with no change or to increased LDL-C particle size[103]. Radiolabeled studies confirmed that microbial-derived acetate is involved in increased FA de-novo synthesis. Interestingly, antibiotic-treated mice displayed decreased FA de-novo synthesis[102]. The importance of IM in these mechanisms involving SCFA, was further confirmed when specific pathogen free (SPF) mice were fed either cellulose or fiber, where solely the latter is degraded into

SCFA by the IM. Indeed, upon fiber supplementation and not cellulose, SPF mice displayed increased levels of MUFA and decreased levels of PUFA[102]. Importantly, SCFA play their role through GPR43 activation. Indeed, whereas acetate suppresses insulin-induced glucose and FA uptake in adipocyte of WT mice, this is not the case in GPR43-KO mice[104]. Furthermore, while WT mice have normal WAT LPL activity, it is significantly increased in GPR43-KO mice and by contrast, decreased in mice with GPR43 over-expression. These difference in LPL activity are abolished in WT or GPR43-KO GF mice, confirming that insulin-signaling suppression in the WAT alters lipid metabolism through IM-acetate dependent GPR43 pathway[104]. Finally, a recent human study confirmed that circulating triglyceride levels were negatively correlated to butyryl-CoA-acetate CoA-transferase pathway within the IM, the most common butyrate production pathway in colon bacteria[48], again confirming the link between SCFA, IM and lipid concentrations in humans. Interestingly, intervention studies have shown that oral butyrate supplementation affects plasma lipids and IM differentially in lean vs metabolic syndrome subjects[105].

Functional mechanisms involving microbial-metabolites in insulin-resistance

The development of insulin-resistance is orchestrated by a complex interplay of different metabolites that influence insulin signaling and inflammatory processes. As already described in the lipid section, several IM-derived metabolites (namely amino-acids and their downstream metabolites) influence insulin-resistance[106] (**Figure 3**).

Imidazole propionate

Imidazole propionate (ImP), produced by the IM from the amino-acid histidine degradation, is increased (i) *in-vivo*, in diabetic compared to healthy individuals or

those with glucose intolerance, and (ii) *in-vitro*, in a gut stimulator where feces from diabetic individuals are challenged with histidine compared to feces from non-diabetics. Furthermore, injection of ImP in mice increases fasting and postprandial glucose levels through impaired insulin signaling[24].

Tryptophan-derived metabolites

Tryptophan (Trp) is another important amino-acid that influences host metabolism through its metabolites produced by three major fermentation pathways orchestrated by both the IM and gastrointestinal cells. As reviewed in[107], Trp can (i) be broken down by IM into indoles and its derivative known to be Aryl hydrocarbon receptor (Ahr) ligand; (ii) be metabolized through the kynurenine pathway in immune and epithelial cells through the enzyme indoleamine 2,3-dioxygenase 1 (IDO1), whose activity is modulated by the IM; and (iii) can lead to serotonin production by tryptophan hydroxylase 1 (TpH1) in enterochromaffin cells. These pathways are altered in metabolic diseases.

Indoles

Comparing individuals with obesity and metabolic syndrome to healthy ones[108], indoles are reduced while kynurenine is increased in the feces. In agreement, *in-vitro* studies objectified a decreased AhR feces activity during metabolic diseases. These results were confirmed in HFD and *ob/ob* mice compared to controls along with the observation that IM composition significantly differed between groups. IL22 intestinal expression, the end-product of AhR activity[109] is also decreased in the colon of HFD mice[108]. FMT from HFD mice into GF recipient recapitulated the decreased AhR fecal activity in the recipient when compared to FMT from controls. By contrast, HFD mice treated with AhR agonist or with a bacteria

able to produce high AhR ligand[110], improved their glucose metabolism and rescued IL22 intestinal expression, albeit with no changes in IM composition[108]. These data demonstrate the role of altered IM composition in defective AhR activity during metabolic disorders. Interestingly, during obesity, intestinal inflammation, evaluated by CD3 infiltration within the epithelium is increased[111] and negatively correlates with AhR and IL22 gene expression[112]. Furthermore, while palm-oil feeding disrupted epithelial tight junction and induced epithelial inflammation, treating those mice with an AhR agonist restored tight junctions[112] and improved intestinal inflammation, yet was not sufficient to prevent palm-oil induced increased intestinal permeability.

Progresses in mechanistic understanding have been made. The use of AhR agonists improved HFD-induced intestinal permeability[56,113]. Likewise, while HFD mice displayed reduced GLP-1 production, it increased upon AhR agonist treatment both *in-vivo* and *in-vitro*, and in contrast was completely abolished *in-vitro* upon AhR antagonist treatment[108]. Indole metabolites, derived from the Ahr pathway, stimulate the release of GLP-1 after a short exposure *in-vitro*, yet decrease its production after longer exposure[114]. Overall, this shows how bacterial metabolite can modulate host metabolism, through GLP-1 effects on satiety and insulin release by pancreatic beta cells[115]. A recent study further demonstrated that supplementing HFD mice with a plant-based ArH agonist, improved glucose and insulin tolerance, together with reduced intestinal and WAT inflammation, improved intestinal permeability and increased intestinal IL22 production as compared to control HFD mice[116].

Kynurenine

Trp is also metabolized into kynurenine via the rate-limiting enzyme IDO. Compared to lean individuals, obese patients display reduced circulating levels of Trp[117], increased kynurenine/Trp ratio[117–119] indicating increased IDO activity. The

increased kynureine/Trp ratio is confirmed in overweight/obese individuals with the metabolic syndrome[118]. In obesity, systemic inflammation correlates positively with kynurenine/Trp ratio and negatively with Trp[117] and indoles, suggesting that IDO is induced during inflammation as demonstrated *in-vitro*[120]. Furthermore, IDO1 inhibits the anti-inflammatory cytokine IL-10 in mice, and double deficient IDO1/IL-10 mice develop severe colitis, further linking Trp metabolism to inflammation[121]. Moreover, IDO1 is activated in the WAT of obese individuals[122,123] and HFD mice[124]. By contrast, IDO1 deficient mice upon HFD, are protected against obesity, WAT inflammation, liver steatosis and insulin resistance. Pharmacologic inhibition of IDO1 leads to similar findings. By contrast, antibiotic-treated IDO1-deficient or WT mice upon HFD do not display the previously observed phenotype difference, pointing at the IM contribution in those outcomes[124]. Furthermore, upon cohousing, the dominant phenotype is the protective one displayed by IDO1-deficient mice rather than that of WT upon HFD. IDO1-deficient mice upon HFD also show a profoundly different IM composition which results in differential functionality: HFD mice (with increased IDO1 activity) display increased kinureic acid and less indoles as compared to IDO1-deficient mice[124]. Overall, HFD dysbiosis induce a shift in the Trp degradation process towards increased kynurenine pathway. IDO1-deficient mice maintain intestinal barrier function by IM-dependent IL-22 production, thus linking altered IM composition, metabolites and metabolic health[124].

Interestingly, mice prone to develop atherosclerosis (LDL-R KO) upon HFD display increased kinurenin/Trp ratio, which is suppressed in double KO mice (LDL-R and IDO-KO), thus displaying a link between the altered Trp pathway and cardiovascular complications[121]. In humans, kinureic acid is increased in patients with obesity[122–124], metabolic alterations[122,125] and in patients with coronary artery

diseases[126] and is a good predictor of increased risk of acute angina[125,127]. This could explain recent findings where patients with coronary artery disease display severe IM dysbiosis in terms of composition[128] and function as seen with enhanced tryptophan metabolism in patients with CVD[129].

Serotonin

Finally, during obesity and metabolic diseases, Trp conversion towards serotonin precursor (5-HT) synthesis is decreased, due to Trp activated transformation through the kynurenin pathway[117]. This could be a common mechanisms involving IM to obesity since serotonin and its precursors are involved in satiety in the brain[130]. Serotonin cannot pass the blood brain barrier; therefore, the brain depends on distribution of tryptophan and the intermediate precursor 5-hydroxytryptophan (5-HTP) by blood. In agreement, serotonin levels correlates negatively with BMI in a cohort composed of lean to overweight individuals[131]. Literature remain scarce on the relation between IM, circulating serotonin concentration and weight and metabolism, nevertheless with existing conflicting results[132]. Therefore, that field still warrants more in depth mechanistic studies in mice and their subsequent translation in humans.

Branched-chain amino acids

Branched chain amino acids (BCAA) (i.e. leucine, isoleucine and valine) are partly produced and metabolized by the IM[133], but their pathophysiological involvement in insulin-resistance are not entirely elucidated[134]. Increased BCAA circulating levels are associated with insulin resistance[134,135]. Mice on a BCAA-restricted diet lose weight and improve their glycemic control[136,137]. Moreover, a recent RCT including T2D individuals submitted to a BCAA-restricted diet who

displayed decreased systemic BCAA levels, improved oral glucose sensitivity index, decreased post-prandial insulin secretion and modified the IM composition[138] compared to individuals on a normal control diet.

Noteworthy, increased circulating BCAA levels could arise from an inability to sufficiently catabolize BCAA[139], as shown in WAT of humans with insulin-resistance [140,141]. Newgard proposed another possible mechanism, where the increased BCAA pool spills over into catabolic pathway within the liver and muscle. Therein, the produced metabolites would reduce the efficiency of FA and glucose oxidation[142]. While this shift between substrate for oxidation is mandatory to maintain healthy metabolic flexibility[143], metabolic inflexibility occurs in obese individuals[144].

Patients with insulin-resistance or obesity display dysbiotic IM with increased capacity of BCAA synthesis, and decreased BCAA catabolism[11,35,145], however, if and how IM can influence circulating BCAA levels still remains unclear. Noteworthy, GF mice receiving FMT from an obese twin, whose gut microbiota is enriched in genes involved in BCAA biosynthesis, display higher BCAA circulating levels, than GF receiving FMT from the lean twin. Likewise, FMT from insulin-resistant individuals into GF mice replicates the insulin-resistance profile with increased circulating BCAA levels[11]. Furthermore, individuals with dysbiotic IM with decreased capacity to catabolize BCAA display higher levels of circulating BCAA[145], suggesting that IM is partly responsible for the circulating levels of BCAA during obesity[35]. Exercise intervention studies, with modulate the IM composition[9] corroborate this. Individuals with pre-diabetes submitted to a 12-weeks intensive exercise training program display heterogeneous responses. Those with improved insulin-sensibility displayed significant IM changes compared to non-responders, namely a decrease in *Prevotella Copri* (involved in BCAA synthesis), an increase in genes involved in BCAA

catabolism, which translated into reduced circulating BCAA levels. Finally, FMT from responders leads to reduced circulating BCAA in GF recipient as compared to FMT from non-responders[146]. Likewise, berberine which has shown its beneficial effects on insulin-resistance and its ability to modify IM[147] was tested in HFD mice. Berberine supplementation leads to reduced weight-gain, improved insulin-sensitivity along with modified IM functions towards reduced BCAA synthesis and increased BCAA catabolism. This translated into reduced circulating BCAA levels[148].

SCFA

By contrast, SCFA are amongst microbiota-derived metabolites with beneficial effects on host metabolism. Microbiome-wide association studies in human confirmed the beneficial effects of SCFA on insulin-sensitivity[149]. Butyrate oral supplementation improves insulin-sensitivity, decreases weight through increased energy expenditure in HFD mice[150]. The effect of SCFA on the improvement of insulin sensitivity has been reviewed in detail elsewhere[151].

Strategies modifying the intestinal microbiome to improve metabolic alterations

A number of strategies aiming at modifying the IM are available to improve metabolic heath as reviewed in details[152–155]. They include probiotics[152], multi-strain probiotic cocktails[156–161], 3rd generation probiotics, prebiotics, symbiotic and nutrients with pre or probiotic activities[153]. Importantly, while their efficacy and mechanism of action have been relatively well proven in animal studies, translation to humans sometimes display controversy or positive yet minor effects. By contrast, other therapeutics such as FMT[154] now represent a novel therapeutic tool in metabolic diseases that has been tested in several human studies, where it objectified its

effects on improved insulin-sensitivity, yet not on weight-loss as detailed in **Table 2**. Bariatric surgery which drastically improve weight and insulin-resistance represent another example where numerous human studies have demonstrated its effect on modifying the IM[162]. Furthermore, some of these microbial changes are associated with weight-loss or improvement insulin-resistance or T2D[163,164]. They are summarized in **Table 2.** Finally, some drugs, such as statin or metformin, also modify the IM, which in part explain their related-clinical improvement (summarized in **Table1**)

Thereafter, we chose to only focus on recent dietary interventions which induce metabolic improvements and changes in IM composition, through the above-described mechanisms.

Caloric restriction

Lean mice submitted to a 40% caloric restriction (CR) lose weight and adiposity while stabilizing their lean-mass. They display a switch towards M2-macrophage polarization within the WAT, improvement in insulin-sensitivity, together with major changes in the IM composition and functionality[165,166] (**Figure 1**). FMT from CR mice into GF recipients replicates beneficial phenotypes compared to FMT from controls, despite no difference in food intake, both at room temperature or thermo-neutrality. Mechanistic studies[166,167] showed that GF mice receiving FMT from CR mice developed WAT beiging phenotype. By contrast, GF or ATB-treated mice submitted to similar CR do not display these improvements, pointing at the importance of the modified IM in these CR-induced beiging effects[166]. This beiging phenotype probably involves decreased LPS biosynthesis, leading to reduced TLR4 activation since mice treated with TLR4 inhibitor or in TLR4-KO mice display the same phenotype observed upon CR. These recent data partly confirm previous

findings, where mice were submitted to a life-long CR inducing improved lifespan, weight and metabolic health, both under LFD and HFD[168]. CR durably modifies IM composition together with reducing circulating LBP[169], yet LPS/TLR4 pathway and WAT beiging were not assessed therein[169]. While a first human trial did not confirm those findings, WAT beiging was solely evaluated upon WAT biopsy performed at room temperature[170], emphasizing on the need for more translational research in this field.

Intermittent fasting

Animal studies support the notion that Intermittent fasting or time-restricted feeding improves metabolic health[171]. Human studies performed in overweight or obese individuals replicate these beneficial effects[172,173]: In a recent RCT, CR and intermittent fasting similarly induced weight-loss and metabolic improvement[174]. Mice studies further substantiated the role of the modified IM in these improvements. Compared to *ad-libitum fed* mice, lean ones submitted to 15-cycle of Intermittent fasting display reduced body weight and adiposity despite similar food intake[175]. This was due to increased energy expenditure through lipid utilization, and WAT beiging (demonstrated by increased multi-locular adipocytes and UCP-1 gene expression), which occurred prior to weight loss. Similar findings were replicated in diet-induced obese mice. The obligatory role of the IM in intermittent fasting-induced weight-loss via WAT beiging[175] was proposed based on several observations. First, intermittent fasting modifies the IM composition. Second, FMT from intermittent fasting mice into microbiota-depleted mice replicates the beneficial phenotype as compared to FMT from *ad-libidum* mice. Third, microbiota-depleted mice submitted to intermittent fasting do not display this beneficial phenotype. Likewise, 28 days intermittent fasting submitted to *db/db* mice induced weight and adiposity reduction and improvement in

insulin-sensitivity despite no change in caloric intake[22]. Circulating LPS decreased and gut permeability improved. Concomitantly, diabetic-induced anxious behavior as well as synapse ultrastructure and insulin brain signaling improved highlighting the importance of a gut-brain axis in these improvements. Antibiotic treatment partly abrogated these intermittent fasting-induced beneficial effects, substantiating the role of IM in these phenomenons[22].

Translational research in human is now warranted to evaluate whether intermittent fasting or time-restriction feeding also modulates human IM explaining the metabolic health improvements. Only one pilot study in humans with obesity submitted to 12 weeks of time restriction feeding observed a significant yet small weight-loss. Nevertheless, using 16S-pyrosequencing, no significant change in IM diversity or composition at the phylum level was observed. Whether some modifications occurs at a lower taxonomic level has not been evaluated[176]. Two RCT are currently registered in Clinical.trials.gov to further substantiate the effect of intermittent fasting or time restriction feeding on metabolic health and the IM (NCT04355910, NCT03608800).

<u>Dietary supplementation recapitulates beiging of adipose tissue in mice</u>

Poplyphenol supplementation also provide evidence of the link between IM and WAT beiging (**Figure 1**). First, compared to controls, resveratrol-supplemented lean mice display increased energy expenditure, BAT gene expression (i.e. UCP-1, cidea[177], PRMD16[177], PGC1α[178]) and decreased WAT depots [179]. Similar results were obtained after a 10-weeks resveratrol supplementation in *db/db* mice along with IM modifications, as well as in a model of diet-induced obesity[180]. By contrast, treating those mice with antibiotics abrogated the increased WAT beiging and BAT activity, confirming the role of the IM in these phenomenon[181]. Finally, FMT from

resveratrol-supplemented mice into recipient mice replicated the increased WAT beiging capacity, whereas no change was observed upon FMT from control mice[181]. Interestingly, resveratrol supplementation also protected mice from major HFD-induced weight gain, despite similar energy intake to control mice on HFD. Similarly, resveratrol supplementation reversed HFD-induced gut microbiota alteration towards a composition similar to chow-fed mice. FMT from resveratrol-supplemented mice (upon HFD or chow-diet), replicated in recipients the decrease in body weight, the reduced adiposity and increased WAT beiging capacities (i.e. increased markers of BAT within the WAT: UCP-1, PGC-1α, PPARγ[182] and SIRT-1[183] gene expression as well as protein content)[180].

Concordant results were replicated in HFD mice supplemented with other polyphenol extracts (i.e. grape fruit from cabernet sauvignon wine)[184]. Compared to controls, polyphenol reduced body weight and WAT depots, increased energy expenditure and restored HFD-induced IM dysbiosis. Polyphenols induced-beiging occurred through modulation of bile acids that up-regulates TGR5 (both at the gene and protein level) in the BAT, together with genes involved in thermogenesis. Finally, upon cold exposure, this polyphenol-induced BAT increase was indeed functional as displayed by increased thermogenesis and PET-CT measured glucose uptake[184].

Ginseng extract (GE), which modulates the IM[185] in rats, also induce WAT beiging, thus limiting weight gain. GE supplementation in db/db mice resulted in decreased weight and adiposity, and increased energy expenditure as compared to control mice despite similar energy intake[186]. These phenotypes were accompanied by increased BAT activity (i.e. increased UCP-1 and OXPHOS staining in BAT and WAT), a phenomenon that was absent at thermo-neutrality. Interestingly, GE supplementation led to increased *E. fecalis* in the feces, which in turn, when

supplemented to HFD-fed mice replicated the beneficial phenotype observed upon GE treatment. This phenotype was not observed at thermo-neutrality, again suggesting the implication of BAT. Finally, GE supplementation also induced a 12-fold increase in systemic Myristoleic acid, a long chain fatty acid that *E. fecalis* is able to produce thanks to its genetic machinery. Myristoleic acid supplementation in db/db mice replicated the beneficial phenotype observed after both GE or *E. fecalis,* notably its role in inducing BAT activity and WAT beiging. Importantly, the beneficial effects observed upon GE, *E. fecalis* or myristoleic acid supplementation were abrogated in a model of mice KO for the enzyme able to synthetize myristoleic acid, thus firmly confirming the role of IM-produced metabolites in reducing weight through increased BAT activity[186].

Therapeutic innovation

New therapeutic nutritional-derived strategies are also under development to target the IM and its-produced metabolites with subsequent health benefit.

For example, in the atherosclerosis field, whereas TMAO, a microbial metabolite produced from dietary choline or carnitine is involved in atherosclerosis[187], a non-toxic compound found in olive oil or red wine, namely 3,3-dimethyl-1-butanol (DMB), acts as a substrate mimicking choline and functions as a potent TMA lyase inhibitor. DMB prevents TMAO production and leads to reduced atherosclerosis[188,189] in mouse models. Such strategies, targeted at the production of IM-derived indoles, kynurenine, BCAA or ImP, which display adverse metabolic effects, would appear as promising therapeutic perspective to improve metabolic diseases and should be evaluated in mice models before turning to humans.

Another example implies protein produced by bacteria (i.e. CplB) which is an antigen-mimetic of the anorexigenic α-melanocyte stimulating hormone (α-MSH)[190]. Oral gavage with WT *E.coli* (thus producing ClpB) leads to reduced food intake and lower body weight than oral gavage with ClpB-deficient E.coli both in lean[190] and ob/ob mice[191]. *In-vitro* studies display that bacterial-produced ClpB stimulates PYY secretion by intestinal cells[192], suggesting that this anti-obesity effect acts through increased satiety. Interestingly, *Hafnia alvei* HA4597, a bacteria found in raw milk and cheese, produces 10-times more ClpB than *E.coli* and its oral gavage to HFD or ob/ob mice similarly reduces body weight and adiposity as compared to controls[191,193]. In the human MetaHit cohort, BMI correlated negatively with ClpB gene abundance in IM. Therefore, the food-grade status of *Hafnia alvei* HA4597 could lead to its development into 3rd generation probiotic to treat obesity and related-diseases. In this regard, a recent study using different dosages of *Anaerobutyricum Soehngenii* (an anaerobic butyrate producer) improved insulin-sensitivity in humans with metabolic syndrome. Moreover, in this dose finding study viability and growth of this strain in the human intestine could be linked to clinical efficacy[194]. Yet, human RCT is still needed to translate animal beneficial findings, as is currently done for *A.muciniphila*. *A.muciniphila,* associated with improved metabolic phenotype in mice[195] and humans[196]. Its subsequent live or pasteurized use showed minor beneficial outcomes and was safe in humans[197]. Nevertheless, its use as 3rd generation probiotic in overweight/obese metabolic patients still needs deeper investigation[198]. This last study shows how important human RCTs are, to replicate findings demonstrated *in-vivo* or *in-vitro* concerning IM, its-related metabolite and their effects on host health.

Conclusion

High-throughput sequencing coupled with omic analysis, in humans or different models of IM-depleted mice, with or without FMT have shown the important role of IM and its produced-metabolites in maintaining energy homeostasis and metabolic health. Several mechanisms were deciphered highlighting causality aspects. Moreover, examples of therapeutic strategies, targeting directly IM and even its-produced metabolites to improve health outcomes are encouraging in mouse models. Future studies should now focus on translating these discoveries in humans and evaluate their clinical relevance[28,199].

Table 1: Statin and Metformin effects on the intestinal microbiota composition and metabolic health

Table 2: effects of fecal microbiota transfer and bariatric surgery on the intestinal microbiome and metabolic health in human studies

Legend

Figure 1: intestinal microbiota, weight storage and metabolic health

High-fat diet (HFD) in conventional mice, depicted in red, induces gut microbiota dysbiosis, decreases fecal content, reduces Fiaf, increases LPL activity, decreases indole production thus up-regulating miR-181 and decrease insulin signaling. By contrast, in germ-free mice or in conventional mice with beneficially modified IM, weight storage is prevented by (i) the increase in intestinal Fiaf which inhibits LPL in the white adipose tissue (WAT), (ii) the increase in stool quantity and fecal lipid content, (iii) increased beta-oxydation in the liver and muscle, (iv) the increase of tryptophan-derived indoles, which down-regulates miR 181 thus improving insulin signaling and being of WAT, and (iv) WAT undergoes beiging, through M2 signaling, leading to increased energy expenditure. Likewise, resveratrol and ginseng extract are able to modulate beneficially the IM and promote WAT beiging. Ginseng extract increases *E.fecalis* and myristoleic acid, both of which replicate the WAT beiging effects, when they are supplemented to mice.

Figure 2: Role of intestinal microbiota in lipid metabolism.

A. Germ-free mice have reduced lipid and cholesterol absorption and decreased portal triglyceride levels seen together with an increase of HMG-CoA reductase activity and SREBP2 expression. Circulating lipids are decreased but hepatic cholesterol and triglycerides are increased. Germ-free mice also have higher expression of Fiaf in the gut, which inhibits LPL activity, resulting in decreased lipid storage. **B**. Mice colonized with specific bacteria produce SCFA upon fiber supplementation which increases de novo free fatty acid synthesis through GPR43 activation, leading to increased circulating MUFA and decreased PUFA. HMG-CoA reductase: hydroxyméthylglutaryl-CoA reductase, LPL:

lipoprotein lipase, MUFA: mono-unsaturated fatty acids, PUFA: poly-unsaturated fatty acids, SCFA: short-chain fatty acids, SREBP2: sterol regulatory element-binding protein 2

Figure 3:   Summarized effects of intestinal microbiota and microbiota-derived metabolites on metabolic health.

A. High-fat diet results in obesity and altered intestinal microbiome composition termed dysbiosis. It is associated with intestinal inflammation and decreased intestinal tight junctions (i.e. increased intestinal permeability), thus facilitating the translocation of microbiota-derived molecules such as flagellin and LPS into the circulation where LPS is bound to LPS binding protein. LPS activates TLR4 which is associated with liver steatosis and altered insulin signaling. TLR5 activation by flagellin results in hepatic gene expression modulation. LPS activates TLR4 mediated inflammatory response within the white adipose tissue and bacteria traces have been found in subjects with obesity and dysbiosis. **B.** The breakdown of several amino acids is altered in obesity. Histidine is metabolized by the intestinal microbiota into the metabolite ImP which has been shown to result in insulin receptor degradation. Increased levels of circulating BCAA in obesity have been associated with impaired fatty acid and beta-oxidation as well as impaired glucose homeostasis. Tryptophan can be processed by the intestinal microbiota in three different ways. Dysbiosis increases IDO1 activity, leading to increased kynurenine. Dysbiosis during obesity decreases the Ahr pathway, leading to decreased indole production, thus reducing its inhibitory effect on inflammation, and decreased IL-22 levels which facilitates intestinal interstitial inflammation. Dysbiosis also increases the TpH1 pathway resulting in increased serotonin production, which could influence satiety. Ahr: aryl hydrocarbon receptor, BCAA: branched-chain amino acids, HFD: high-fat diet, IDO1: indoleamine dioxygenase 1, ImP: imidazole propionate, LPS: lipopolysaccharides, TLR: Toll-like receptor, TpH1: tryptophan hydroxylase 1, WAT: white adipose tissue

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Table 1. Statin and Metformin Effects on the Intestinal Microbiota Composition and Metabolic Health

Treatment	Study duration	Groups	Changes	Host changes
Statins				
Animal studies				
Control diet + simvastatie vs control diet alone Catry et al,[53] 2014	7 days	Normocholesterolemic male C57Bl6J mice Assessed using DGGE	No change in IM composition	No change in triglyceride or cholesterol level. Increased ileum mRNA expression of HMG-CoA R, LDL receptor and SREBP-2
Rosuvastatin within drinking water vs sterile drinking water Nolan et al,[54] 2017	28 days	Normocholesterolemic Female C57Bl6J mice Assessed with 16S-sequencing	Decreased α diversity Cecum: increased in genera (*Coprococcus*, *Rikenella*, *Lachnospiraceae*), decrease in family (Rf9, Erysipelotrichaceae, and Roseburia). Feces: decrease in phylum (*Proteobacteria*, *Tenericutes*. and *Verrucomicrobia*), family (Desulfovibrionaceae, RF9, Coriobacteriaceae and Akkermansiaeae), and genus (decrease in *Bilophila*, *Erysipelotrichaceae*, *Roseburia*)	Decreased cholesterol level Reduced circulating and plasma TNF-α and IL1β No effect on SCFA in the feces
Atorvastatin At different dosage vs diet alone Kahn et al,[3] 2018	28 days	Rats fed chow diet or HFD Assessed with 16S-sequencing.	Increased β-diversity and α-diversity. Increased phylum (**Proteobacteria**) Increase in families in a dose-dependent manner (Ruminococcaceae, Bacteroidaceae, **Porphyromonadaceae**, **Helicobacteraceae**, Paraprevotellaceae, **Desulfovibrionaceae**, and Alcaligenaceae), a decrease in families (**Clostridiaceae**, **Lachnospiraceae**, Lactobacillaceae, Rikenellaceae, Peptostreptococcaceae, Turicibacteraceae, and Staphylococcacea). An increase in genera (*Bacteroides*, *Oscillospira*, *Paraprevotella*, *Helicobacter*, and *Parabacteroides*) and a decrease in genera (*Turicibacter*, *Clostridium*, *Ruminococcus*, *Coprococcus*, and unclassified SMB53 and YRC22)	Decrease in cholesterol and triglyceride levels. - Negative correlation between LDL-C or triglyceride, or both ($\rho > -0.14$), and *Clostridium*, *Desulfovibrio*, *Roseburia*, *Blautia*, *Helicobacter*, *Ruminococcus*, and *Lactobacillus*, - Positive correlation between LDL-C and *Prevotella*, *Coprococcus*, *Prevotella* [YRC22], *Paraprevotella*, *Clostridia* [SMB53], and *Dorea*

Table 1. Continued

| Treatment	Study duration	Groups	Changes	Host changes
Atorvastatin or rosuvastatin Kim et al,[4] 2019	16 weeks	C57BL/6N mice upon HFD or chow diet Assessed with 16S-sequencing.	- Rosuvastatin increased microbial β-diversity - Rosuvastatin restored HFD dysbiosis (ie, increase the ratio of *Firmicutes/Bacteroidetes*), which did not occur upon atorvastatin- Increase in **Bacteroides** and **Butyricimonas** (phylum *Bacteroidetes*), **Oscillospira** (phylum *Firmicutes*), and **Mucispirillum** (phylum *Deferribacteres*) - Increase solely upon Atorvastatin: Anaerotruncus, **Bacteroides**, **Butyricimonas**, Dorea, **Mucispirillum** and Turicibacter - Increase solely upon Rosuvastatin: **Bacteroides**, **Butyricimonas**, *Clostridium*, and **Mucispirillum** *In bold, similar changes upon atorvastatin and rosuvastatin* - Increase in **Bacteroides** and **Butyricimonas** (phylum *Bacteroidetes*), **Oscillospira** (phylum Firmicutes), and *Mucispirillum* (phylum Deferribacteres) - Increase solely upon atorvastatin: Anaerotruncus, **Bacteroides, Butyricimonas**, Dorea, **Mucispirillu**m and Turicibacter - Increase solely upon rosuvastatin: **Bacteroides**, **Butyricimonas**, *Clostridium*, and **Mucispirillum** In bold, similar changes upon atorvastatin and rosuvastatin.	- Decrease in total cholesterol, reduction of fasting glycemia, and improvement of glucose tolerance - Increased TGF-β1 and decreased IL1β ileum gene expression upon both atorvastatin and rosuvastatin - Positive correlation between TGF-β1 and *Dorea* upon atorvastatin - Negative correlation between IL1β and *Dorea* and *Mucispirillum* upon atorvastatin - FMT from rosuvastatin-treated mice replicated the improvement in glucose level and glucose tolerance and the increase in TGF-β1 and the decrease in IL1β within the ileum
Human studies Rosuvastatin Liu et al,[5] 2018	4-8 weeks	64 patients with hyperlipidemia 2 response groups: 1 achieved LDL target and 1 remained above the target Assessed with 16S-sequencing.	- Increased α-diversity in the good-responder group - difference in β-diversity in the 2 groups - Significant increase in *Firmicutes*, *Verrucomicrobia*, *Tenericutes*, and *Fusobacteria* in the good responder group, while *Bacteroidetes*, *Actinobacteria*, *Cyanobacteria*, and *Lentisphaerae* were increased in the poor responders - 42 taxa were significantly different between the 2 groups	Among the 42 differential taxa, *Firmicutes* and *Fusobacteria* negatively correlated with LDL-C, while *Cyanobacteria* and *Lentisphaerae* positively correlated with LDL-C
Statins in general Vieira-Silva et al,[6] 2020	NA	MetaCardis human transversal study Assessed by shotgun sequencing	- Individuals with obesity and adverse lipid profile displayed decreased diversity and increased prevalence of enterotype B2 - Statin treatment reduced the prevalence of enterotype B2 and improved low-grade inflammation	

Table 1. Continued

| Treatment	Study duration	Groups	Changes	Host changes
Metformin				
Animal studies				
Metformin Shin et al,[7] 2014	6 weeks	C57BL/6 mice fed a normal chow or HFD received metformin treatment Assessed with 16S rRNA gene sequences with 454 pyrosequencing	- Relative abundance of *Akkermansia muciniphila* was increased by the metformin treatment in HFD-fed mice compared with HFD mice on placebo - Oral *A muciniphila* supplementation improved glucose tolerance and reduced WAT inflammation	- Metformin use improved glucose tolerance in HFD mice but did not alter BMI or weight. - Metformin increased the number of intestinal goblet cells upon chow and HFD. - Mice fed a normal chow diet did not show improved glycemic parameters upon metformin treatment
Metformin Ma et al,[8] 2016	30 days	Healthy C57BL/6 mice Assessed with 16S-sequencing.	- Metformin administration increased relative abundance of *Verrucomicrobiaceae, Pre-votellaceae, Porphyromonadaceae* and *Rike-nellaceae* whereas *Lachnospiraceae* and *Rhodobacteraceae* classes were reduced	- Metformin's effect on host biology was not evaluated.
Human studies				
Metformin Wu et al,[9] 2017	4 months	Treatment-naïve T2D participants on a calorie-restricted diet, double-blind randomized trial: metformin (n = 22) vs placebo (n = 18). A subset of the placebo group (n = 13) further started metformin after 6 months and were analyzed 6 months afterward. Assessed by shotgun sequencing + Targeted metabolomics	- 86 bacterial strains relative abundances changed in the metformin group after 4 months such as increased *Escherichia coli*, *Bifidobacterium adolescentis* and *Akkermansia muciniphila* whereas there was a decrease of *B fragilis* and *Intestinibacter,* in contrast, only 1 bacterial strain was changed in the placebo group. - Metformin induced major functional changes in the gut microbiome (KEGG annotation, upon which SCFA metabolism), whereas hardly any changes were seen upon placebo - Fecal propionate and butyrate levels were higher in the metformin group than in the placebo group after the 4-month intervention - Increased concentration of unconjugated bile acids upon metformin treatment - Culture of feces in gut simulator with metformin supplementation induced functional shifts reflected in DNA and RNA changes of several bacterial strains, including up-regulation of *A muciniphila and Lachnospiraceae bacterium.* - Yet, there was a donor specific signature of metformin-induced changes	- BMI decreased in both groups - Fasting glucose and HbA_{1c} reduced only in the metformin group - *A muciniphila* increase in metformin group was not correlated to HbA_{1c} - Negative correlation between *B adolescentis* and Hba_{1c} - Colonization of GF mice with feces of metformin-treated T2D individuals improved glucose tolerance but did not improve body weight, fasting insulin, or body fat composition - Negative correlation between the concentration of unconjugated bile acids and HbA_{1c}

Table 1. Continued

| Treatment	Study duration	Groups	Changes	Host changes
Forslund et al,[10] 2017	Cross-sectional	784 multicountry cohort with T2D individuals either metformin or untreated T2D and non-T2D control individuals. Assessed by shotgun sequencing	- Compared with healthy controls, T2D individuals without metformin use had lower genera of butyrate-producing bacteria such as *Roseburia* spp, *Subdoligranulum* spp, and *Clostridiales* spp. - Comparing T2D with or without metformin treatment, confirmed an increase in *Escherichia coli* and a reduction in *Intestinibacter*	- Effect of metformin on host biology not shown
De la Cuesta-Zuluaga et al,[11] 2017	Cross-sectional	28 T2D (n = 14 on metformin treatment) and 84 nondiabetic individuals	- T2D subjects using metformin had higher *Akkermansia muciniphila* relative abundance than nondiabetic individuals - T2D subjects upon metformin had higher levels of SCFA producing bacteria such as *Bifidobacterium, Butyrivibrio* and *Megasphaera* among others, than nondiabetic individuals. - Compared with TD2 without metformin treatment, T2D with metformin had increased *Prevotella* and *Megaspharea* and decreased *Oscillospira, Barnesiellaceae* and *Clostridiaceae.*	- No effect of metformin on host biology was evaluated

NOTE: Bold indicates similar changes upon being fed the HFD or chow diet.
HbA$_{1c}$, glycated hemoglobin; HMG, hydroxymethylglutaryl; KEGG, Kyoto Encyclopedia of Genes and Genomes; MetaCardis Metagenomics in Cardiometabolic Diseases; NA, not applicable; T2D, type 2 diabetes; TGF, transforming growth factor; TNF, tumor necrosis factor.
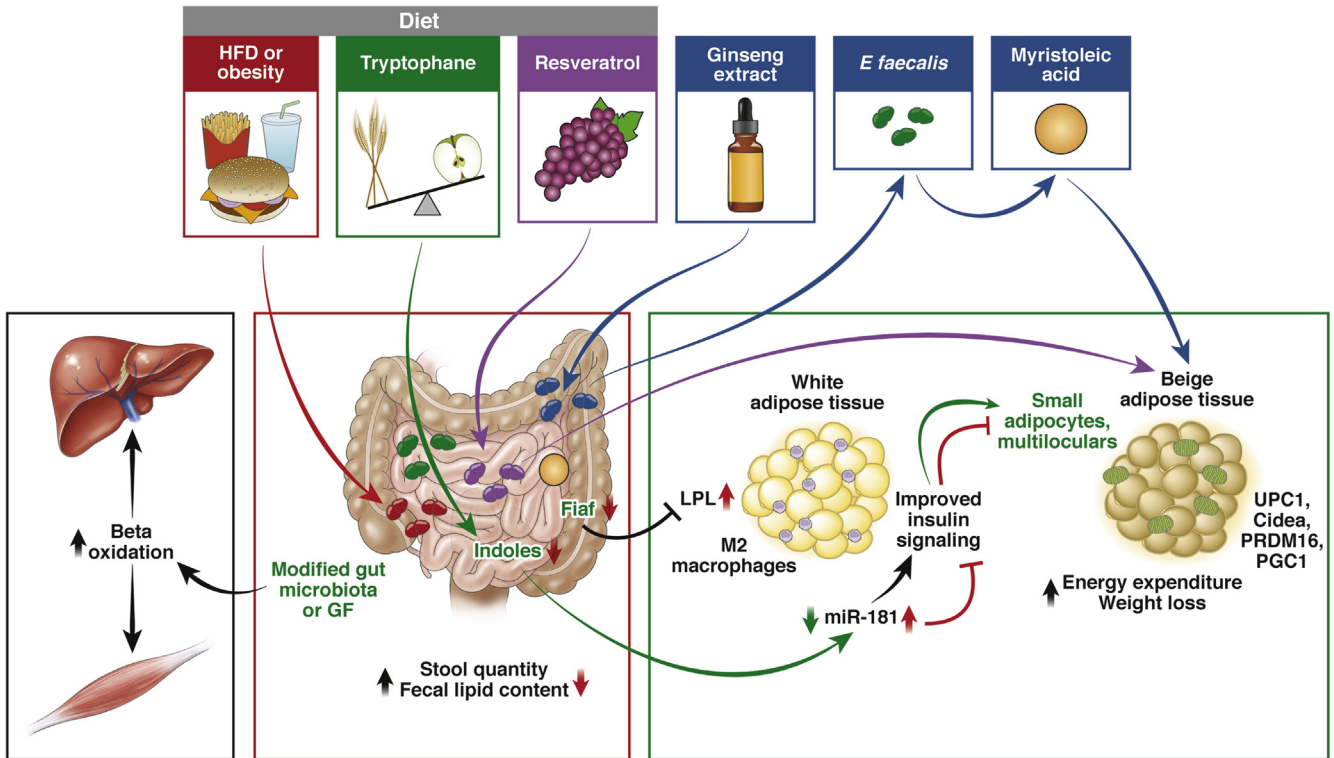
Figure 1. Intestinal microbiota, weight storage, and metabolic health. HFD in conventional mice, depicted in *red*, induces intestinal microbiome dysbiosis, decreases fecal content, reduces Fiaf, increases LPL activity, decreases indole production, thus upregulating miR-181, and decreases insulin signaling. By contrast, in GF mice or in conventional mice with beneficially modified intestinal microbiota, weight storage is prevented by (1) the increase in intestinal Fiaf, which inhibits LPL in the WAT, (2) the increase in stool quantity and fecal lipid content, (3) increased β-oxidation in the liver and muscle, (4) the increase of tryptophan-derived indoles, which downregulates miR-181, thus improving insulin signaling and beiging of WAT, and (4) WAT undergoes beiging through M2 signaling, leading to increased energy expenditure. Likewise, resveratrol and GE are able to modulate the intestinal microbiome beneficially and promote WAT beiging. GE increases *E faecalis* and myristoleic acid, both of which replicate the WAT beiging effects, when they are supplemented to mice.
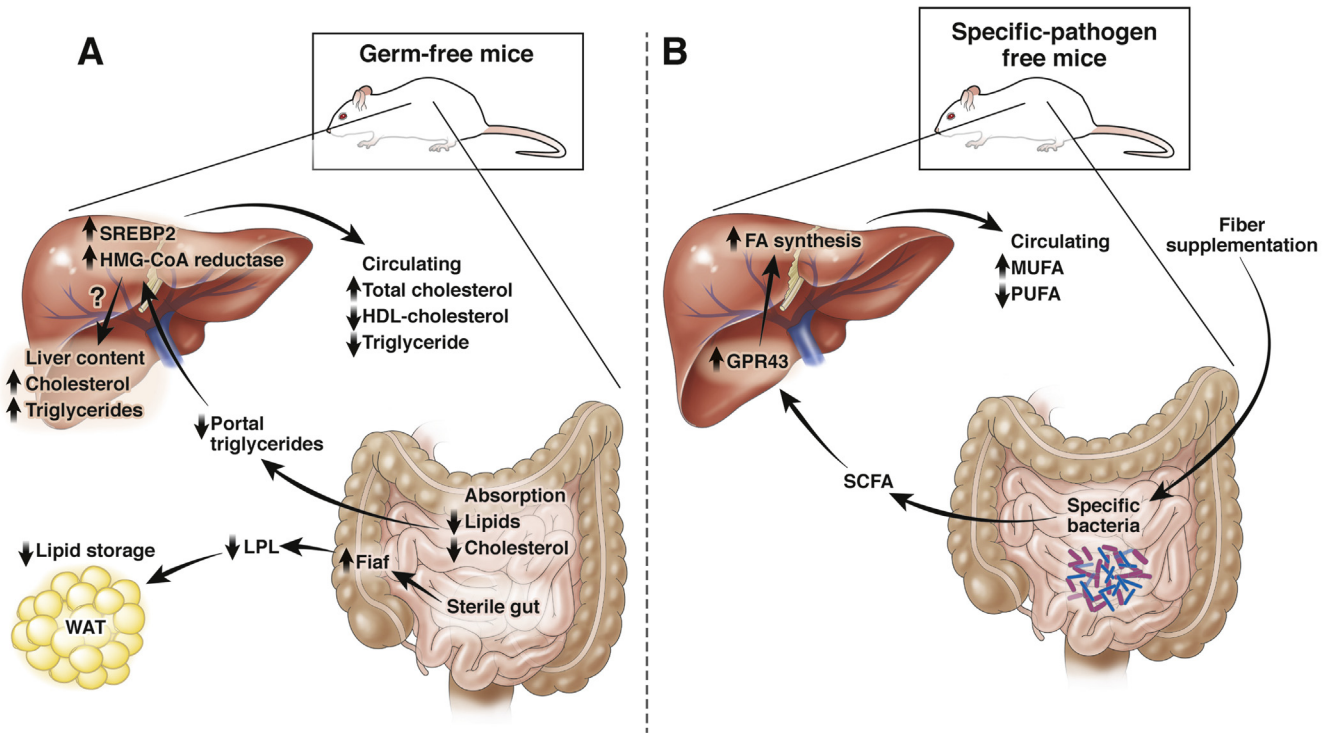
Figure 2. Role of intestinal microbiota in lipid metabolism. (*A*). GF mice have reduced lipid and cholesterol absorption and decreased portal triglyceride levels seen together with an increase of hydroxymethylglutaryl (HMG)-CoA reductase activity and sterol regulatory element-binding protein 2 (SREBP2) expression. Circulating lipids are decreased, but hepatic cholesterol and triglycerides are increased. GF mice also have higher expression of Fiaf in the gut, which inhibits LPL activity, resulting in decreased lipid storage. (*B*) Mice colonized with specific bacteria produce SCFA upon fiber supplementation, which increases de novo free fatty acid (FA) synthesis through GPR43 activation, leading to increased circulating MUFA and decreased PUFA.
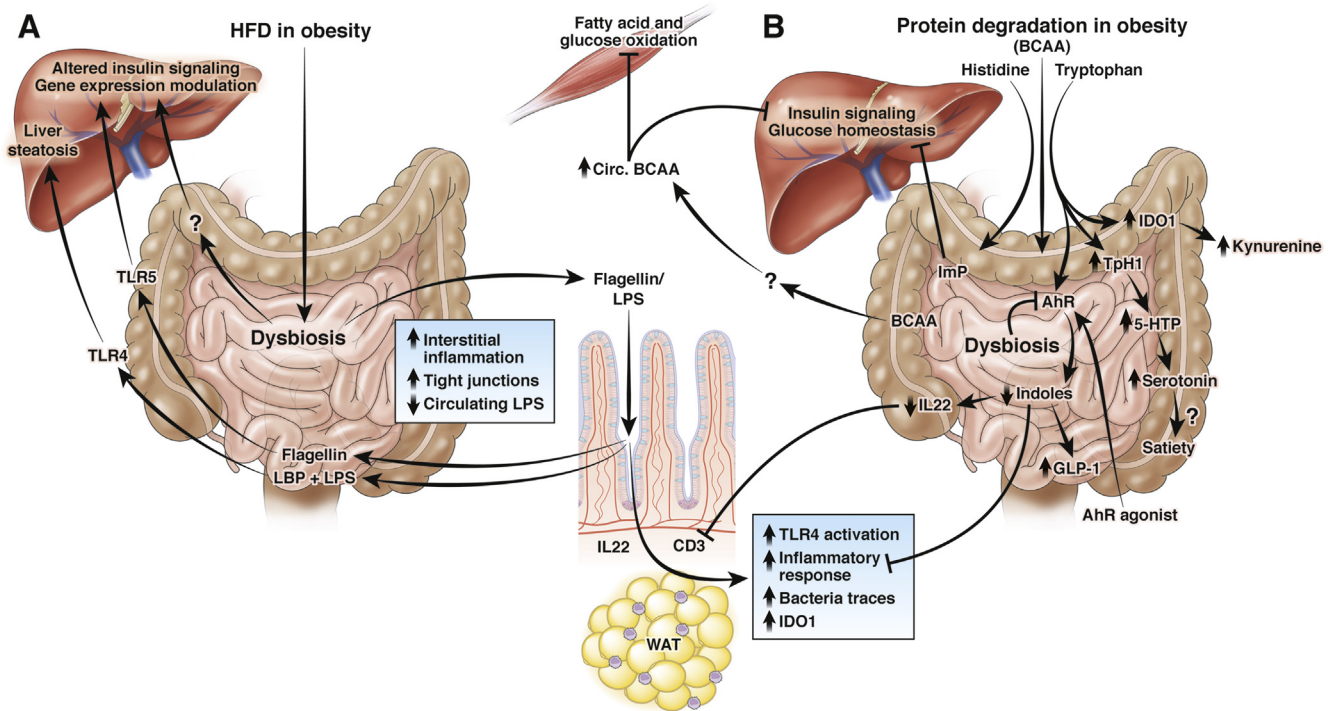
Figure 3. Summarized effects of intestinal microbiota and microbiota-derived metabolites on metabolic health. (*A*) HFD results in obesity and altered intestinal microbiome composition, termed dysbiosis. It is associated with intestinal inflammation and decreased intestinal tight junctions (ie, increased intestinal permeability), thus facilitating the translocation of microbiota-derived molecules such as flagellin and LPS into the circulation, where LPS is bound to LPS-binding protein. LPS activates TLR4, which is associated with liver steatosis and altered insulin signaling. TLR5 activation by flagellin results in hepatic gene expression modulation. LPS activates TLR4-mediated inflammatory response within the WAT, and bacteria traces have been found in individuals with obesity and dysbiosis. (*B*) The breakdown of several amino acids is altered in obesity. Histidine is metabolized by the intestinal microbiota into the metabolite imidazole propionate, which has been shown to result in insulin receptor degradation. Increased levels of circulating BCAA in obesity have been associated with impaired fatty acid and β-oxidation as well as impaired glucose homeostasis. Tryptophan can be processed by the intestinal microbiota in 3 different ways. Dysbiosis increases IDO1 activity, leading to increased kynurenine. Dysbiosis during obesity decreases the AhR pathway, leading to decreased indole production, thus reducing its inhibitory effect on inflammation, and decreased IL22 levels, which facilitates intestinal interstitial inflammation. Dysbiosis also increases the tryptophan hydroxylase 1 (TpH1) pathway, resulting in increased serotonin production, which could influence satiety.

Table 2. Effects of Fecal Microbiota Transfer and Bariatric Surgery on the Intestinal Microbiome and Metabolic Health in Human Studies

Treatment	Study duration	Groups	Changes	Host changes
Human fecal microbiota transfer studies				
Human studies				
Allogenic FMT from healthy donors vs autogenic FMT for metabolic syndrome patients Vrieze et al[1]	6 weeks	9 overweight/obese individuals with metabolic syndrome	- Low microbial diversity in metabolic syndrome patients - Increased microbial diversity after allogenic FMT - Allogenic FMT increased 16 bacterial groups (including butyrate producers: *Roseburia intestinalis*) - Fecal SCFA butyrate and propionate decreased after allogenic FMT	- Safety ok - Improvement in insulin sensitivity measured with hyperinsulinemic-euglycemic clamp using [6,6 ^{2}H2]-glucose, in the allograft group - No effect on weight
Allograft FMT from healthy donors vs autograft for metabolic syndrome patients Kootte et al,[2] 2017	6 weeks and 18 weeks	38 overweight/obese individuals with metabolic syndrome. 26 received allogenic FMT from healthy donors, the rest received autologous FMT	- Allogenic FMT was associated with changes in amino-acid concentrations (measured by metabolomics) - Good responders displayed increased *Akkermansia muciniphila* - Good responders had initial higher abundance of *Subdoligranulum variabile* and *Dorea longicatena*, whereas they had decreased abundance of *Eubacterium ventriosum* and *Ruminococcus torques* compared with poor responders- No change in microbial diversity upon allogenic FMT - No change in plasma SCFA, increased fecal acetate levels after allogenic FMT	- Safety ok - Improvement in insulin sensitivity measured with hyperinsulinemic-euglycemic clamp using [6,6 ^{2}H2]-glucose, in the allogenic group at week 6 yet with major interindividual variability. - Good responders were those with baseline lower microbial diversity - Allogenic FMT induced significant decrease in HbA$_{1c}$ - Allogenic FMT induced significant increase in Tg post-prandial rise - No effect at week 18 - No effect on weight

Table 2. Continued

Treatment	Study duration	Groups	Changes	Host changes
Allogenic FMT De Groot et al,[3] 2019	2 weeks	22 metabolic syndrome patients received allogenic FMT from patients who had undergone RYGB, or allogenic FMT from other metabolic syndrome patients	- No effect on microbial diversity in any group - Recipients from RYGB FMT displayed increased *Bacteroidetes, Bacteroidales, Haemophililus*, whereas recipients from metabolic syndrome FMT displayed increased *Bacteroides stercoris and Clostridiales*	- Safety ok - Baseline insulin sensitivity is significantly higher in RYGB than metabolic syndrome donors - No improvement in insulin sensitivity measured with hyperinsulinemic-euglycemic clamp using [6,6 ^{2}H2]-glucose, in any group - Significant decrease in insulin sensitivity in patients receiving allogenic FMT from metabolic syndrome patients - No weight effect
RCT comparing capsule FMT from 1 healthy donor to placebo Allegretti et al,[4] 2019	26 weeks	22 metabolically healthy obese individuals 30 capsules at baseline, and maintenance dose of 12 capsules at week 4 and 8	- Change in patient's microbiome towards that of the healthy donor (after capsule FMT) - 200 OTUs engrafted from donor to recipient (many of which were enriched in healthy control and depleted in obese individuals) - No significant change in α-diversity, but an increase in β-diversity (after capsule FMT)	- Safety ok - No significant change in BMI - No significant change in any biomarker (GLP-1, leptin)
RCT comparing oral capsule FMT from healthy donors to placebo Yu et al.[5] 2019	12 weeks	24 obese individuals with insulin-resistance Patients received 30 capsules at baseline followed by 15 weekly FMT capsules until 6 weeks	- Significant change in microbiome composition after capsule FMT as compared to baseline or to the placebo group - Changes toward the composition of the healthy donors, suggesting correct engraftment for the 12- week study period although FMT was performed until 6 weeks	- Safety ok - No significant difference in change of HOMA-IR between groups - No change in fat mass, lipid profile, or body weight - Significant but minor reduction in HbA$_{1c}$ in the FMT group
Human bariatric surgery studies **Human studies** Zhang et al[6], 2009	8 to 15 months post-BS	3 MO individuals, 3 RYGB patients and 3 lean individuals Sanger & 16S rRNA pyrosequencing	↑ *Gammaproteobacteria, Verrucomicrobia, Fusobacteria* ↓ *Clostridia*	

Table 2. Continued

Treatment	Study duration	Groups	Changes	Host changes
Furet et al,[7] 2010	Before, 3 months and 6 months post-BS	30 MO (7 with T2D) patients who underwent RYGB and 13 lean individuals 16S rRNA qPCR	↑ *Bacteroides/Prevotella* ratio, *Faecalibacterium prausnitzii*, *Escherichia* ↓ *Bifidobacterium*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*	Changes in *Faecalibacterium prausnitzii*, *Escherichia coli*, and the *Bacteroides/Prevotella* ratio are associated with improvement in inflammatory parameters, and with changes in weight, BMI, fat mass, leptin concentrations
Patil et al,[8] 2012		5 thin, 5 lean, 5 obese and 5 obese operated-on individuals (3 SG and 2 AGB) Sanger	↓ *Bacteroides* and *Archaea* No change in bacterial diversity	
Kong et al,[9] 2013	Before, 3 months and 6 months post-BS	30 MO patients who underwent RYGB 16S rRNA (V3-V4) pyrosequencing	↑ *Bacteroides*, *Escherichia*, *Alistipes* ↓ *Lactobacillus*, *Dorea*, *Blautia* and *Bifidobacterium* ↑ Number of genera and Chao1 index	Changes in the 14 dominant bacteria are correlated with improvement in HOMA-IR and fat mass
Graessler et al,[198] 2013	Before and 3 months post-BS	6 MO patients (n = 5 T2D) who underwent RYGB Shotgun metagenomic sequencing	↑ *Proteobacteria*, *Bacteroidetes/Firmicutes* ratio, *Verrucomicrobia* ↓ *Firmicutes*, *Cyanobacteria*	
Ward et al,[11] 2014	Before and 6 months post-BS	8 MO patients who underwent RYGB 16S rRNA(V4) pyrosequencing	↑ *Bacteroidetes*, *Bacteroidetes/Firmicutes* ratio, *Proteobacteria*, *Verrucomicrobia* ↓ *Firmicutes*, *Proteobacteria*	
Damms-Machado et al,[12] 2015	Before, 3 months and 6 months post-BS	6 MO patients 3 of which underwent SG and 3 a VLCD Shotgun metagenomic sequencing (SOLiD)	↑ *Bacteroidetes*, *Faecalibacterium pausnitzii* ↓ Several *Firmicutes* (*Eubacterium*, *Faecalibacterium*, *Dorea*, and *Coprococcus*), *Bacteroides vulgatus*, *Bacteroidetes/Firmicutes* ratio	
Tremaroli et al,[13] 2015	Approx 10-year follow-up	7 RYGB vs 7 VBG vs 7 MO patients Shotgun metagenomic sequencing (Illumina, San Diego, CA)	↑ *Proteobacteria* (*Escherichia*, *Klebsiella* and *Pseudomonas*) ↓ *Firmicutes*, *Eubacterium rectale* (VBG), *Roseburia intestinalis* (VBG)	FMT from feces of RYGB into GF mice led to less weight gain than FMT from obese individuals

Table 2. Continued

Treatment	Study duration	Groups	Changes	Host changes
Palleja et al,[199] 2016	Before, 3 months and 1 year post-BS	13 MO patients (n = 7 T2D and n = 1 IGT) who all underwent RYGB Shotgun metagenomic sequencing (Illumina)	↑ *Proteobacteria* (including *Escherichia coli* and *Klebsiella pneumoniae*), *Streptococcus salivarius*, *Akkermansia muciniphila* ↓ *Faecalibacterium prausnitzii*, *Anaerotruncus colihominis*, *Megasphaera micronuciformis* ↑ Gene richness and Shannon's diversity index during the first 3 months and stable afterwards	
Murphy et al,[200] 2017	Before and 1-year post-BS	14 MO patients of which RYGB (n = 7) & SG (n = 7) Shotgun metagenomic sequencing (Illumina)	↑ RYGB: *Firmicutes*, *Actinobacteria*; SG: *Bacteroidetes* ↓ RYGB: *Bacteroidetes*	*Roseburia intestinalis* was increased only in patient undergoing T2D remission
Liu et al,[145] 2017	Before, 1 month and 3 months post-BS	23 MO patients who underwent SG Shotgun metagenomic sequencing (Illumina)	↑ *Bacteroidetes thetaiotaomicron*, *Akkermansia muciniphila*, *Clostridiales bacterium* ↓ *Coprococcus comes* and *Dorea longicatena* ↑ Gene count, α-diversity	*Bacteroidetes thetaiotaomicron* is associated negatively with BMI and glutamate levels. Glutamate levels are associated with improved hyperglycemia, insulin resistance, and inflammatory markers
Aron-Wisnewsky et al,[17] 2018	1, 3, 12 months and up to 5 years post-BS	34 MO patients including 24 RYGB and 10 AGB Shotgun metagenomic sequencing (SOLiD)	↑ *GU:99 Roseburia, GU:225 Butyricimonas virosa, GU:359 Butyricimonas* ↑ Gene richness 3 months after BS, although this increase was similar after both surgery, RYGB started and finished lower than AGB patients. The increase was further stable until 5 years	
Paganelli et al,[201] 2018	Before, 3 months and 6 months post-BS	45 MO patients of which 23 RYGB and 22 VSG 16S rRNA(V3-V4) shotgun sequencing (Illumina)	↑ *Streptococcaceae*, *Enterobacteriaceae* ↓ *Bifidobacteriaceae*	
Dao et al,[202] 2020	Before, 1, 3 and 12 months post-BS	65 MO undergoing BS and follow-up n= 10 AGB and 11 RYGB Shotgun metagenomic sequencing (SOLiD) and 16S rRNA qPCR	↑ *Akkermansia muciniphila* (200-fold) in RYGB No significant change after AGB Correlation between baseline *Akkermansia muciniphila* and bacterial gene richness	No correlation between increase in *Akkermansia muciniphila* and improved glucose homeostasis

Table 2. Continued

Treatment	Study duration	Groups	Changes	Host changes
Mabbey et al,[203] 2020	Up to 13 years post-BS	16 MO individuals underwent BS were compared to 19 MO without surgery 16S rRNA (V4) sequencing	↑ *Verrucomicrobiaceae* and *Streptococcaceae* ↓ *Bacteroidaceae*	In 10 subjects, increased *Akkermansia muciniphila* was associated with diabetes remission
Farin et al, 2020[204]	Before and 6 months after BS	89 SG and 108 RYGB Shotgun metagenomics sequencing (SOLiD)	↑ Shannon's diversity after both surgery and ↑ gene richness RYGB : ↑ *Escherichia coli* and buccal bacteria (*Streptococus and Veillonella*) SG ↑ *Clostridium* ↑ *Akkermansia muciniphila* after both surgery	
Chen et al,[205] 2020	Before and after 9 months follow-up	87 MO undergoing BS (54 SG, 33 RYGB) 16S rDNA (V3 + V4 regions) sequencing (Illumina)	↑ Shannon's index in whole cohort, ↑ Shannon's index after SG (n=33) but not significant after RYGB (n=20) Changes in 33 genera after SG and 19 after RYGB with 11 in common	SG: Changes in 19 genera were correlated with BMI, positive correlation between decrease in BMI and decreased Allisonella and Sutterella RYGB: changes in 5 genera correlated with BMI, negative correlation between increased *Aeromonas*, *Akkermansia*, *Anaeroglobus*, *Lachnospiraceae*_UCG-001and *Veillonella* and decreased BMI
Al Assal et al,[206] 2020	Before and 3 and 12 months after RYGB	25 MO individuals undergoing RYGB, (n = 20 at 3 months and 14 at 12 months) MiSeq Illumina-based V4 bacterial 16S rRNA gene profiling	↑ *Veillonella, Streptococcus, Gemella, Oribacterium, Atopobium*, one unclassified *Lactobacillales* genus, *Leptotrichia, Neisseria*, and one unclassified *Pasteurellaceae* genus and ↓ in *Faecalibacterium* at 3 months ↑ *Veillonella* and *Streptococcus*, and a decrease in *Flavonifractor, Blautia*, and *Butyricicoccus*	At baseline, patient who underwent diabetes remission at 12 months, had significantly lower levels of *Asaccharobacter* and *Atopobium* and higher levels of *Gemella*, *Coprococcus*, and *Desulfovibrio* compared with the baseline signature of patients without remission

AGB, adjustable gastric band; BS, bariatric surgery; HbA$_{1c}$, glycated hemoglobin; HOMA-IR homeostasis model assessment-insulin resistance; IGT, impaired glucose tolerance; MO, morbidly obese; qPCR, quantitative polymerase chain reaction; RCT, randomized controlled trial; RYGB Roux-en-Y gastric bypass; SG, sleeve gastrectomy; VBG, vertical banded gastroplasty; VLCD, very-low-calorie diet; VSG, vertical sleeve gastrectomy.