A kick in the guts

Insulin resistance, gut microbiota and cardiometabolic diseases

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Publication date
2024

Chapter 2

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

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Gastroenterology
January 2021

"Er zijn geen shortcuts voor gezondheid."
Max Nieuwdorp

Abstract

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

Introduction

Obesity prevalence reached 40% in the United States in 2016, with major interindividual socioeconomic disparities, and is predicted to further increase,[1] together with its associated metabolic comorbidities.[2] To date, while a few antiobesity 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] or to genetic forms,[4] they can sometimes induce adverse events leading to treatment discontinuation, and their cost is significant. Thus, to bend the worldwide obesity epidemic curve and its associated management costs, safe and inexpensive public health interventions need to be developed and implemented in adults as was done in children, which led to decreased or plateaued prevalence in individuals aged younger than 11 years.[2] Furthermore, trying to decipher novel pathophysiological mechanisms involved in obesity and related disease might help develop new preventive or therapeutic strategies in the future.

The intestinal microbiome (IM), which is mostly shaped by the environment,[5,6] in particular the diet, and varies across ethnicities,[7] maybe in link with differences in food cultural habits, because large human studies have shown genetics do not appear to strongly influence the IM composition.[5] The IM is involved in several major physiological functions that maintain metabolic homeostasis. Among others, the IM processes and digests nutrients, produces metabolites,8 and shapes the immune system.[9]

This field has been revolutionized by high-throughput sequencing techniques, such as the 16S-sequencing approach, which delivers valuable composition information, and metagenomics, which provides additional knowledge on microbial genes and their potential functions.[10-12] Methodologic pros and cons of both techniques are detailed in a previous study.[10]

Complementary metabolomics analysis enables researchers to dive deeper into functionality assessment when combined with metagenomics.[12] These tools led to the discovery of major compositional changes in the IM during metabolic disorders, such as obesity, insulin-resistance, type 2 diabetes (T2D), dyslipidemia, and nonalcoholic fatty liver disease (NAFLD),[10,11,13-18] which suggest its involvement in their physiopathology.

While most studies have used IM originating from feces in human and animal studies, some have used IM from the cecum or jejunum. Since it is known that the IM composition strongly differs according to the different parts of the digestive tract,[19] as well as its function and most probably its effects on host health, the IM origin when different than fecal will be specified in this review.

Intestinal Microbiome Contributes to Metabolic Disorders

In vivo models, such as cohousing experiments[20] or comparison of conventional and germ-free (GF) mice,[21] postweaning pups,[22] antibiotic-treated mice,[23] or all 3, that undergo fecal microbiota transplantation (FMT) from mice or humans donors,[24] enables investigators to further dig into causality. Translation to humans of results obtained in animals is also possible using the in vitro gut stimulator model,[25] intervention trials, such as FMT from human to human,[26,27] antibiotic treatment,[28] or diet interventions.[15] Although these techniques have their own advantage or drawback to infer causality, they nevertheless advanced progress in the understanding of the IM contribution[29] in metabolic diseases with the discovery of new mechanistic pathways.

Intestinal Microbiome Affects Body Weight

Firstly, GF mice have lower body weight and white adipose tissue (WAT) than conventional mice[30] fed a chow or high-fat diet (HFD), despite increased calorie intake.[21,31,32] Their colonization with a normal IM for 14 days enables them to reach similar weight than conventional mice.[30] Noteworthy, while conventional mice gain significantly more weight on the HFD than the low-fat diet (LFD), the weight of GF mice remains stable, whatever their diet, pointing at the IM contribution to properly handle energy storage from food intake.[33]

Secondly, the increase of body weight in GF mice depends on the source of the FMT. In-

deed, FMT from obese conventional mice (diet-induced or genetically obese animals [ob/ob]) into GF recipients fed a chow diet leads to higher weight gain and WAT depot than FMT from lean mice.[13,21,34,35]

Thirdly, FMT from obese humans into GF recipients translates into higher weight gain than FMT from their lean twins.[36]

Importantly, differences in food qualitative intake modulate the IM, its implantation after FMT, and its capacity to store energy from food, leading to different transferred phenotypes.[36,37] Dietary fat content modulates IM, which affects body weight and inflammation in WAT. While recipient mice fed an HFD display a microbiome similar to the twin with obesity, upon being fed a LFD, the dominant colonized microbiota resembles the lean twin's.[36] Moreover, upon being fed an isocaloric diet containing saturated (lard) or polyunsaturated fat (fish oil), the lard-fed group shows increased food intake, leading to higher weight and adiposity, more inflamed WAT, and worse metabolic alterations.[37] Likewise, the 2 groups display major differences in their IM,[37] which is responsible for the clinical phenotype. Indeed, FMT from fish-fed mice into antibiotic-treated recipients fed 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 (ie, body mass index [BMI] of 25 kg/m2) to underweight anorexic women enabled a modest weight gain and weight stabilization.[38] Likewise, obesity developed in a woman who received FMT from her overweight daughter to treat Clostridium difficile (CD).[39] These observations led an international consensus to propose drastic selection for human donors before FMT and to exclude those with overweight or obesity.[40] This caution was probably wise, because patients receiving FMT for CD infection do not gain more weight than those receiving conventional therapy[41] after a mean of 3.8 years of follow-up. Overall, whereas these FMT experiments using GF mice or human recipients showed that IM can transmit weight gain, even with chow diet feeding, human data remain less conclusive to date.

Intestinal Microbiome and Genetics Affect Lipid Profile in Mice

Compared with conventional mice, GF fed a chow diet[31] display reduced fasting systemic triglyceride,[31], total cholesterol,[31] and high-density lipoprotein-cholesterol (HDL-C) levels,[31] and reduced portal triglycerides,[32] concomitant with increased liver cholesterol and decreased triglyceride content.[31] This phenotype is explained by the enhancement of liver cholesterol synthesis (ie, increased liver gene expression of hydroxymethylglutaryl-coenzyme A [CoA] reductase)[31,42] and protein level of the nuclear transcription factor sterol regulatory element-binding proteins[31] involved in the upregulation of sterol biosynthesis. Similar to mechanisms involved in weight storage, the diet and the quality of its lipid content[43] modulates the IM and its associated lipid phenotype. Upon being fed an HFD, GF mice display increased triglyceride concentration compared with conventional mice as seen with direct measures[44] or lipidomic analysis.[45] However, the genetic background[46] strongly influences IM lipid profile interactions. Indeed, atherosclerotic-prone mice (ie, apolipoprotein E knockout [ApoE−/−] mice) fed a chow diet and with their IM depleted by broad-spectrum antibiotics display increased levels of cholesterol (specifically in very low-density lipoprotein and low-density lipoprotein cholesterol [LDL-C] particles) compared with conventionally raised ApoE−/− mice.[42] Furthermore, FMT from humans with a high systemic cholesterol concentration into antibiotic-treated ApoE−/− mice induced a higher cholesterol concentration and intestinal expression of genes involved in cholesterol absorption in the recipient than in a similar recipient receiving FMT from human donors with low cholesterol levels.[42] Importantly, the IM composition from donors with high or low cholesterol levels was significantly different,[42] suggesting the impact of the modified IM in cholesterol absorption.

The Role of the Intestinal Microbiome on the Lipid Profile in Humans

Large cohort studies have examined the bidirectional relationships between the variation in IM composition and that of blood lipid levels[47,48]; that is, how much one explained the variability of the other and vise versa. In 800 individuals from the LifeLines DEEP study, the IM composition explained 6.0% and 4.0% of triglyceride and HDL-C level variation, respectively, whereas IM hardly

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 a-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-a and IL1b 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 b-diversity and a-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 (r > 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 b-diversity - Rosuvastatin restored HFD dysbiosis (ie, in- crease the ratio of Firmicutes/Bacteroidetes), which did not occur upon atorvastatin- Increase in *Bacteroides* and *Butyricimonas* (phylum Bacteroidetes), *Oscillospira* (phylum Firmicutes), and *Mucispirillum* (phylum Deferribacte- res) - Increase solely upon Atorvastatin: Anaero- truncus, Bacteroides, Butyricimonas, Dorea, Mucispirillum and Turicibacter - Increase solely upon Rosuvastatin: B*acteroides*, *Butyricimonas*, *Clostridium*, and *Mucispi- rillum* 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: Anaero- truncus, Bacteroides, Butyricimonas, Dorea, Mucispirillum and Turicibacter - Increase solely upon rosuvastatin: *Bacteroides*, *Butyricimonas*, *Clostridium*, and *Mucispi- rillum* In bold, similar changes upon atorvastatin and rosuvastatin.	- Decrease in total cholesterol, reduc- tion of fasting glycemia, and improve- ment of glucose tolerance - Increased TGF-b1 and decreased IL1b ileum gene expression upon both atorvastatin and rosuvastatin - Positive correlation between TGF-b1 and *Dorea* upon atorvastatin - Negative correlation between IL1b 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-b1 and the decrease in IL1b 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 16Ssequencing.	- Increased a-diversity in the good-responder group - difference in b-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, Firmi- cutes and Fusobacteria negatively correlated with LDL-C, while Cyanobacteria and Lentisphaerae positively correlated with LDL-C

Treatment	Study duration	Groups	Changes	Host changes
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	
Metformin Animal studies Metformin Shin et al,[7] 2014	6 weeks	C57BL/6 mice fed a normal chow or HFD received met-formin 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 16Ssequencing.	- Metformin administration increased relative abundance of *Verrucomicrobiaceae*, *Prevotellaceae*, *Porphyromonadaceae* and *Rikenellaceae* whereas *Lachnospiraceae* and *Rhodobacteraceae* classes were reduced	- Metformin's effect on host biology was not evaluated.

Table 1. Continued

Treatment	Study duration	Groups	Changes	Host changes
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 ¼ 22) vs placebo (n ¼ 18). A subset of the placebo group (n ¼ 13) further started metformin after 6 months and were analyzed 6 months afterward. Assessed by shotgun sequencing þ 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 HbA1c reduced only in the metformin group - A muciniphila increase in metformin group was not correlated to HbA1c - Negative correlation between B adolescentis and Hba1c - Colonization of GF mice with feces of metformintreated 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 HbA1c

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 ¼ 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

HbA1c, 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.

had any significant impact on LDL-C levels.[47] On the other hand, the European Union-supported Metagenomics in Cardiometabolic Diseases (MetaCardis) study evaluated which clinical or biological factors explained IM composition variation and to what degree. Therein, triglycerides concentrations significantly explained 0.39% of IM composition variation in 764 individuals without any lipid-lowering drugs.[49] Studies examining the effect of statins[49-54] on metabolic health also provide insights into the role of the IM in lipid metabolism and regulation (detailed in Table 1).

Effects of Bacterial Components, Lipopolysaccharide, Flagella, and DNA on Metabolic Diseases

Because GF mice display an immature immune system, which plays an important role in metabolic alterations, the role of the IM in metabolic diseases has rather been studied in conventional mice who have received infusion of bacterial membrane molecules (lipopolysaccharide [LPS]) and in several genetic models such as knockout (KO) for LPS Toll-like receptors (TLRs).

Long-term LPS subcutaneous infusion in mice recapitulates the altered phenotype of HFD mice: increfased weight gain, insulin resistance, WAT inflammation, increased systemic LPS,[55] and increased intestinal permeability, thus linking the IM to metabolic health.[56,57] Again, the diet modulates the IM and its associated metabolic health.[58,59] For example, palm oil gavage in mice induces a rapid disruption of the cell-cell junction within the intestine, an increased gut permeability, and inflammation[60] before any significant weight gain. Noteworthy, some microbial-produced metabolites (microbe-associated molecular patterns) 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.[20] LPS binds to TLR4, a pattern recognition receptor, which activates the innate immune system[61] and is highly expressed in the WAT of obese mice, where it induces a proinflammatory response. Compared with WT mice, TLR4-KO mice fed an HFD display lower weight and hepatic steatosis, decreased WAT inflammation, and a switch toward alternative macrophage polarization.[62] Importantly, the specific expression of TLR4 on hematopoietic cells is mandatory to induce WAT inflammation as well as liver and WAT insulin resistance.[63] Several studies further confirmed the protective metabolic effects of TLR4 deficiency,[64-67]as reviewed in Warmbrunn et al.[68] Finally, the relevance of TLR4 was suggested in humans, because TLR4 expression, protein content, and signaling are higher in the muscle tissue of individuals with obesity and T2D than in lean controls.[69]

However, while increased levels of LPS are mandatory to induce major WAT macrophage infiltration, altered glucose and insulin tolerance occur after the sole colonization of GF mice by IM, irrespective of the level of microbiota-related LPS production.[30] Going further, the comparison of conventional or GF mice proved that the IM regulates numerous liver gene expressions, in particular those related to LPS transport through Myd88.[70] Furthermore, LPS-binding protein (LBP) impairs insulin signaling in hepatocytes in the presence of low LPS doses in vitro, while by contrast, pharmacologic LBP blocking improves insulin signaling in vitro and glucose homeostasis in vivo.[70] Flagella, another bacterial component, influences metabolic diseases. TLR5-KO mice display hyperphagia[71] and develop low-grade inflammation and metabolic syndrome as well as modification of their IM composition compared with their WT counterparts. FMT from TLR5-KO mice into GF mice replicates the metabolic alterations in the recipients,[71] demonstrating the importance of the IM through flagellin-TLR activation to modulate host metabolism. Importantly, metabolic alterations and modified IM composition originated from TLR5 activation upon intestinal epithelial cells[72] but not dendritic cells. [72]However, TLR5-KO in dendritic cells abrogated the intestinal production of interleukin (IL) 22,[72] a cytokine involved in intestinal health.[73] Yet, a recent study comparing TLR5-KO and WT mice did neither confirm the difference in metabolic alteration upon being fed the chow diet or HFD nor the differences in IM composition,[74] suggesting the need to further investigate the TLR5 pathway. The IM composition in mice from both genotypes in this study was considerably different from the original study, possibly pointing at a major impact of the environment[74] in IM-host phenotype interactions. Noteworthy, a previous study on the impact of IM and TLRs in the context of liver metabolic diseases (ie, NAFLD/nonalcoholic steatohepatitis) also concluded that TLR5 deficiency-related microbiota dysbiosis was not involved in the exacerbation of NAFLD to nonalcoholic steatohepatitis.[20] Nevertheless, dysbiotic microbiota are involved in NAFLD physiopathology through several mechanisms, including LPS and other TLRs activation, as reviewed in detail in a previous study.[10]

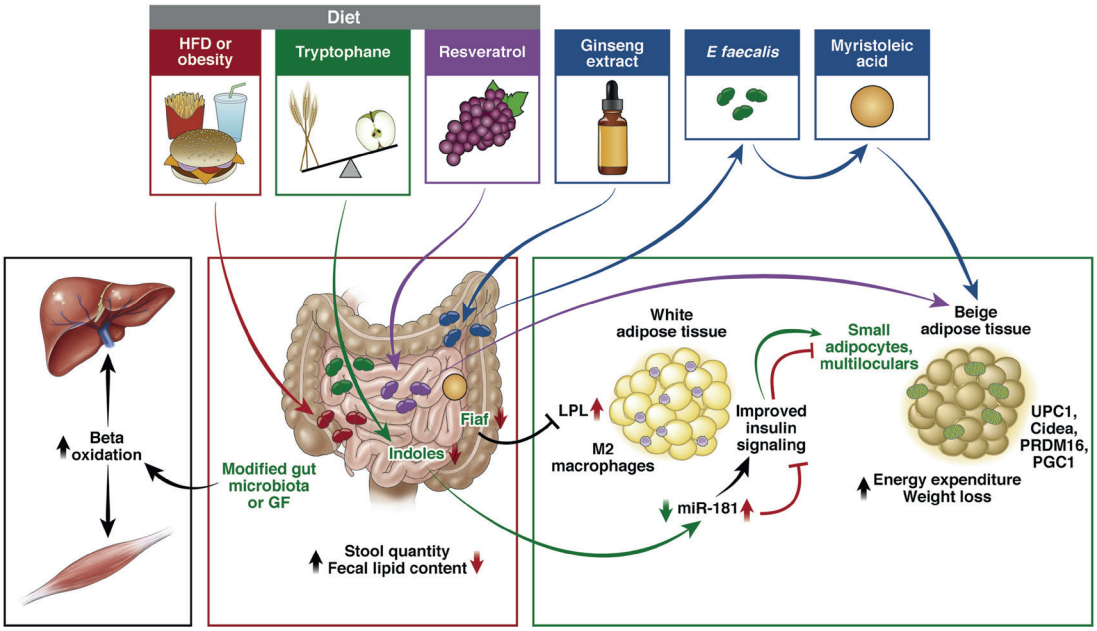

Figure 1. Intestinal microbiota, weight storage, and metabolic health. HFD in conventional mice, depicted in red, induces intestinal microbiome dysbiosis, decreases fecal content, reduces Fiaf, increases LPL activity, decreases indole production, thus upregulating miR-181, and decreases insulin signaling. 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 b-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.

Translating these results in human studies is challenging. Nevertheless, some recent indirect evidence confirms the interaction between the microbiome and metabolic diseases in humans. While the presence of bacteria had previously been suggested in the blood[75] or within metabolic tissues,[76,77] probably due to increased intestinal permeability, these features were recently confirmed by an independent group and showed associations with metabolic alterations.[78] A carefully controlled study confirmed the higher presence of bacterial DNA in the liver and omental WAT of individuals with morbid obesity[78] compared with subcutaneous WAT and mesenteric WAT. Indeed, several types of controls during each analysis step 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 Shannon's index) was significantly lower within the mesenteric WAT of obese individuals with T2D than those without,[78] mirroring the decreased IM bacterial diversity in individuals with obesity and metabolic alteration.[11,15,16] Furthermore, the mesenteric WAT bacterial signature of T2D individuals (ie, increased Enterobacteria[78]) also mirrors that of the IM from patients with insulin resistance.[12] Overall, these studies indicate that the IM, some of its components, or both are modified by the qualitative aspects of the diet and may be involved in weight storage, lipid profile, and insulin resistance.

Mechanisms of Intestinal Microbiome in Weight Regulation

Energy Extraction From Food, Handling, and Storage

IM produces enzymes to break down indigestible carbohydrates by the host. Compared with conventional mice, GF mice fed an HFD or antibiotic-treated mice fed a chow diet[79,80] display de-

creased digestive absorption, as shown by increased 24-hour stool quantity[32] and caloric fecal content.[31,32] Interestingly, decreased digestive absorption is a common mechanism involved in the IM-lipid profile interaction, because GF mice also display a 40% higher lipid (ie, total, cholesterol and triglyceride) fecal content[31,32] (Figure 1). This differential energy extraction from food partly originates from IM functional properties, which may differ according to the donor corpulence.[11,16,81] Compared with lean mice, cecal IM from obese mice are enriched in enzymes breaking down indigestible carbohydrates by the host,[35] leading to increased production of short-chain fatty acids (SCFA), the end-products of the fermentation process[35] involved in energy storage.[82] Nevertheless, conflicting results[11,16,81,83] are found, thus warranting more research in the field.

IM inhibits fiaf gene expression to increase lipid storage in WAT. Compared with conventional mice, GF mice display increased expression of intestinal and WAT fiaf, an inhibitor of lipoprotein lipase (LPL) activity.[21] Upon microbiota conventionalization, fiaf decreases, thus enhancing LPL activity, which leads to WAT lipid storage.[84] Moreover, while fiaf-KO GF mice are no longer protected from diet-induced obesity,[44] transgenic mice overexpressing fiaf display lower adiposity than their WT littermates.[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.[44] First, GF display increased phosphorylated adenosine 5′ monophosphate-activated protein kinase.[44] Second, fiaf increases peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), which regulates positively β-oxidation genes.[86] Likewise, fiaf-KO GF mice show increased genes involved in fat oxidation.[44]

Beiging of the White Adipose Tissue

A recent discovery suggests that the IM regulates body weight through its role in WAT beiging and increased energy expenditure[87] (Figure 1), a mechanism common to its role in insulin resistance. Compared with room temperature (RT), mice exposed to cold (ie, 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 recipients fed a chow diet recapitulates the decrease in body weight and fat mass, improved insulin sensitivity, increased energy expenditure, and development of WAT beiging (histologic changes and increased uncoupling protein 1 [UCP1]),[79] compared with FMT from RT-exposed mice. These data suggest an interplay between cold-modified IM and WAT beiging, where the role of the 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 compared with WT mice.[88] Noteworthy, after initial weight loss, weight from cold-exposed conventional 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 (ob/ob or HFD-induced) mice raised at RT (ie, 22°C) or thermoneutrality (ie, 30°C) also display reduced adiposity and a switch toward a decreased number of large adipocytes and an 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 the 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 at RT or at thermoneutrality.[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.

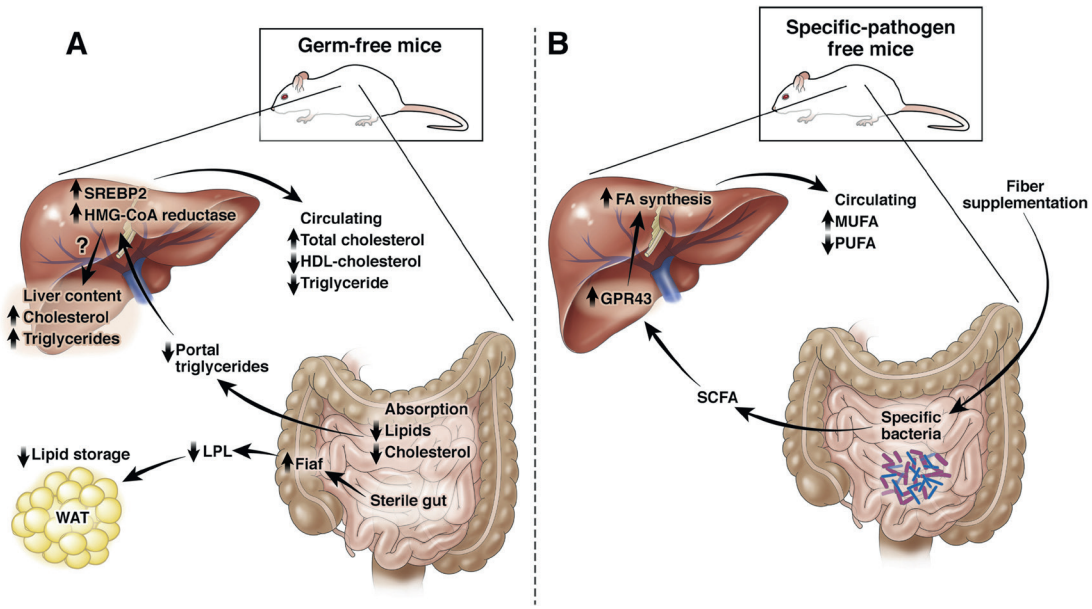

Figure 2. Role of intestinal microbiota in lipid metabolism. (A). GF mice have reduced lipid and cholesterol absorption and decreased portal triglyceride levels seen together with an increase of hydroxymethylglutaryl (HMG)-CoA reductase activity and sterol regulatory element-binding protein 2 (SREBP2) 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.

Indoles, Tryptophan-Derived Microbial Metabolites Control Adiposity via MicroRNAs in White Adipose Tissue

Some microbial-produced metabolites control the expression of microRNAs (miRs) 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 with WT mice, miR-181–KO mice fed an 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 the IM in this miR regulation.

Finally, reduced tryptophan-derived microbial metabolites (ie, 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 evidence suggests 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 has aimed to address the relevance of mice studies in humans. In a cohort of individuals with obesity, 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 PR domain containing 16 [PRDM16]). Howev-

er, this study did not evaluate whether these brown adipose tissue (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 addressed before and after a 7-day antibiotic cocktail, as previously described in trimethylamine N-oxide (TMAO) studies.[96, 97] Previous studies, however, using solely 1 antibiotic for 7 days (ie, vancomycin or amoxicillin) led to only modest changes in WAT[28] and 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, an 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 the Intestinal Microbiome in Lipid Metabolism

Clearance and Intestinal Absorption

The use of a lipid challenge has enabled researchers to demonstrate that GF mice fed an HFD have increased triglyceride concentrations due to reduced postprandial triglyceride clearance.[44, 45] This originates from LPL inhibition secondary to increased fiaf in the absence of the IM.[44] Recent data from a radiolabeled lipid challenge[32,42] have now also demonstrated that the IM is involved in small-intestine lipid[32] and cholesterol[42] digestive absorption. Indeed, after LPL-inhibitor treatment enabling to solely study the absorption pathway, triglyceride and cholesterol absorption was decreased in GF mice compared with conventional mice that received LFD. Because an HFD is not able to restore systemic lipid levels in GF mice after an LFD, this proved the obligatory role of the IM in lipid absorption.

[32] Importantly, an HFD modifies IM composition within the ileum and jejunum. Subsequently, FMT using jejunum IM from HFD mice into GF recipient (fed an LFD or HFD[32]) 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 (here the HFD, which modulates the IM) in its related-lipid absorption (Figure 2). Furthermore, HFD-induced changes in the IM (ie, for example Lactobacillus rhamnosus and Clostridium bifermentans) are involved in microbes-host interaction to increase lipid absorption in the digestive tract[32] through bioactive mediators[98.] able to increase the expression of diacylglycerol O-acyltransferase, 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 and G-protein-coupled bile acid receptor (TGR5), which have already been reviewed in detail elsewhere.[100]

SCFAs, derived from dietary fiber digestion by IM, serve as the fuel for host lipid synthesis.[101] A recent study comparing GF and conventional 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), whereas conventional mice had increased levels of monounsaturated fatty acids (MUFA), thus improved lipid profile. Indeed, compared with SFA, MUFA decreases postprandial triglycerides and induces a shift from small dense LDL-C particles to larger less atherogenic ones,[103] leading to reduced cardiovascular events in human randomized controlled trial. By contrast, while ω-3 PUFAs are beneficial on cardiovascular disease health and lipid profile, ω-6 PUFAs are associated with no change or with increased LDL-C particle size.[103]

Radiolabeled studies confirmed that microbial-derived acetate is involved in increased fatty acid de novo synthesis. Interestingly, antibiotic-treated mice displayed decreased fatty acid de novo synthesis.[102] The importance of the IM in these mechanisms involving SCFA, was further

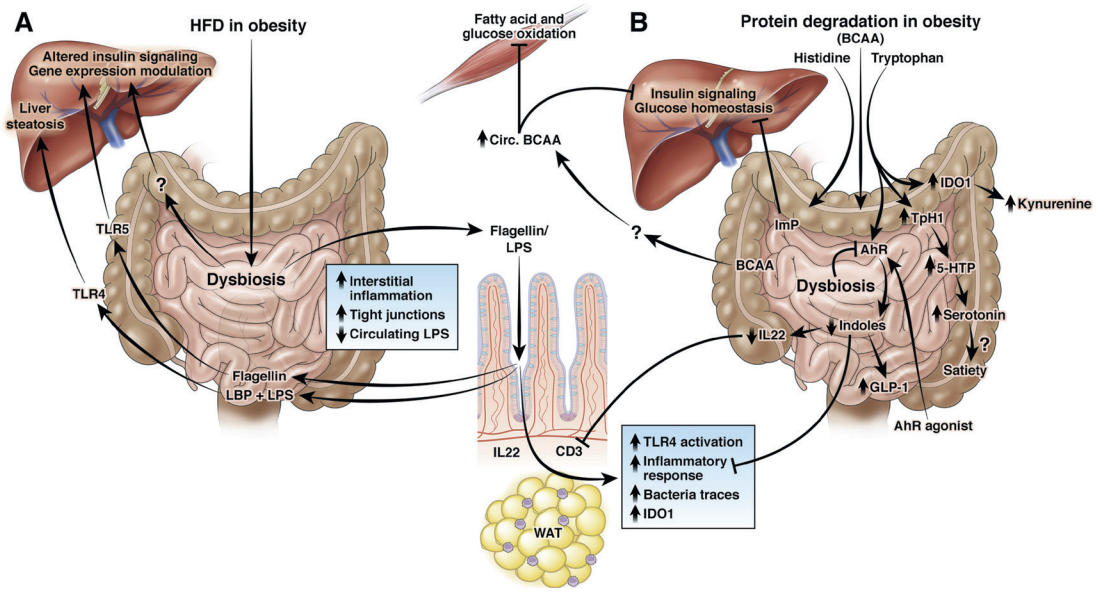

Figure 3. Summarized effects of intestinal microbiota and microbiota-derived metabolites on metabolic health. (A) HFD results in obesity and altered intestinal microbiome composition, termed dysbiosis. It is associated with intestinal inflammation and decreased intestinal tight junctions (ie, increased 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 b- 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.

confirmed when specific pathogen-free mice were fed cellulose or fiber, where solely the latter is degraded into SCFA by the IM. Indeed, upon fiber supplementation and not cellulose, specific pathogen-free mice displayed increased levels of MUFA and decreased levels of PUFA.[102] Importantly, SCFAs play their role through G-protein-coupled receptor 43 (GPR43) activation. Indeed, whereas acetate suppresses insulin-induced glucose and fatty acid uptake in adipocytes of WT mice, this is not the case in GPR43-KO mice.[104] Furthermore, although WT mice have normal WAT LPL activity, it is significantly increased in GPR43-KO mice and, by contrast, decreased in mice with GPR43 overexpression. These differences in LPL activity are abolished in WT or GPR43-KO GF mice, confirming that insulin-signaling suppression in the WAT alters lipid metabolism through the IM-acetate–dependent GPR43 pathway.[104]

Finally, a recent human study confirmed that circulating triglyceride levels were negatively correlated to the butyryl-CoA-acetate CoA-transferase pathway within the IM, the most common butyrate production pathway in colon bacteria,[49] 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 individuals.[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, produced by the IM from degradation of the amino acid histidine, is increased (1) in vivo in diabetic compared with healthy individuals or those with glucose intolerance, and (2) in vitro in a gut stimulator where feces from diabetic individuals are challenged with histidine compared with feces from nondiabetic individuals. Furthermore, injection of imidazole propionate in mice increases fasting and postprandial glucose levels through impaired insulin signaling.[25]

Tryptophan-Derived Metabolites

Tryptophan is another important amino acid that influences host metabolism through its metabolites produced by 3 major fermentation pathways orchestrated by both the IM and gastrointestinal cells. A previous review[107] showed tryptophan (1) can be broken down by the IM into indoles and its derivative known to be aryl hydrocarbon receptor (Ahr) ligand, (2) can 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 (3) can lead to serotonin production by tryptophan hydroxylase 1 in enterochromaffin cells. These pathways are altered in metabolic diseases.

Indoles. A study of individuals with obesity and metabolic syndrome compared with healthy individuals[108] found indoles are reduced, whereas 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 with 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.[109] FMT from HFD mice into GF recipient recapitulated the decreased AhR fecal activity in the recipient compared with 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 the 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.

Progress in mechanistic understanding has been made. The use of AhR agonists improved HFD-induced intestinal permeability.[57, 113] Likewise, although HFD mice displayed reduced glucagon-like peptide 1 (GLP-1) production, it increased with AhR agonist treatment both in vivo and in vitro and, in contrast, was completely abolished in vitro with AhR antagonist treatment.[108] Indole metabolites, derived from the Ahr pathway, stimulated the release of GLP-1 after a short exposure in vitro, yet decreased its production after longer exposure.[114] Overall, this shows how bacterial metabolites can modulate host metabolism through GLP-1 effects on satiety and insulin release by pancreatic β-cells.[115] A recent study further demonstrated that supplementing HFD mice with a plant-based AhR agonist improved glucose and insulin tolerance, together with reduced intestinal and WAT inflammation, improved intestinal permeability, and increased intestinal IL22 production compared with control HFD mice.[116]

Kynurenine. Tryptophan is also metabolized into kynurenine via the rate-limiting enzyme IDO. Compared with lean individuals, obese patients display reduced circulating levels of tryptophanTrp[117] and an increased kynurenine/tryptophan ratio[117-119] indicating increased IDO activity. The increased kynurenine/tryptophan ratio is confirmed in overweight/obese individuals with the metabolic syndrome.[118] In obesity, systemic inflammation correlates positively with the ky-

nurenine/tryptophan ratio and negatively with tryptophan
[117]and indoles, suggesting that IDO is induced during inflammation as demonstrated in vitro.[120] Furthermore, IDO1 inhibits the anti-inflammatory cytokine IL10 in mice, and double-deficient IDO1/IL10 mice develop severe colitis, further linking tryptophan 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 fed an 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 fed the HFD do not display the previously observed phenotype difference, pointing at the IM contribution in these outcomes.[124] Furthermore, upon cohousing, the dominant phenotype is the protective one displayed by IDO1-deficient mice rather than that of WT mice fed the HFD. IDO1-deficient mice fed the HFD also show a profoundly different IM composition that results in differential functionality: HFD mice (with increased IDO1 activity) display increased kynurenic acid and fewer indoles compared with IDO1-deficient mice.[124] Overall, HFD dysbiosis induces a shift in the tryptophan degradation process toward an increased kynurenine pathway. IDO1-deficient mice maintain intestinal barrier function by IM-dependent IL22 production, thus linking altered IM composition, metabolites, and metabolic health.[124]

Interestingly, mice prone to develop atherosclerosis (LDL receptor KO) upon being fed the HFD display increased kynurenin/tryptophan ratio, which is suppressed in double KO mice (LDL receptor and IDO-KO), thus displaying a link between the altered tryptophan pathway and cardiovascular complications.[121] In humans, kynurenic 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 an increased risk of acute angina.[125, 127] This could explain recent findings where patients with coronary artery disease display severe IM dysbiosis in composition[128] and function as seen with enhanced tryptophan metabolism in patients with cardiovascular disease.[129]

Serotonin. Finally, during obesity and metabolic diseases, tryptophan conversion toward serotonin precursor 5-hydroxytryptamine synthesis is decreased, due to tryptophan activated transformation through the kynurenin pathway.[117] This could be a common mechanism involving the IM to obesity because 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 by blood. In agreement, serotonin levels correlated negatively with BMI in a cohort composed of lean to overweight individuals.[131] Literature remains scarce on the relation between IM, circulating serotonin concentrations, 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 into humans.

Branched-Chain Amino Acids

Branched-chain amino acids (BCAA) (ie, leucine, isoleucine, and valine) are partly produced and metabolized by the IM,[133] but their pathophysiological involvement in insulin resistance is not entirely elucidated.[134] Increased BCAA circulating levels are associated with insulin resistance.[134,135] Mice fed a BCAA-restricted diet lose weight, and glycemic control is improved.[136,137] Moreover, a recent randomized controlled trial included T2D individuals who consumed a BCAA-restricted diet, which resulted in decreased systemic BCAA levels, improved oral glucose sensitivity index, decreased postprandial insulin secretion, and modified the IM composition[138] compared with individuals who consumed a normal control diet.

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[142] proposed another possible mechanism, where the increased BCAA pool spills over into the catabolic pathway within the liver and muscle. Therein, the produced metabolites would reduce the efficiency of fatty acid and glucose oxidation.[142] While this shift between the 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 a dysbiotic IM with increased capacity of BCAA synthesis and decreased BCAA catabolism[12,36,145]; however, whether and how the IM can

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 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 hyperinsulinemiceuglycemic clamp using [6,6 2H2]-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	- Safety ok - Improvement in insulin sensitivity measured with hyperinsulinemiceuglycemic clamp using [6,6 2H2]-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 HbA1c - Allogenic FMT induced significant increase in Tg post-prandial rise - No effect at week 18 - No effect on weight

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 hyperinsulinemiceuglycemic 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 a-diversity, but an increase in b-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 insulinresistance 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 HbA1c in the FMT group

Table 2. Continued

Treatment	Study duration	Groups	Changes	Host changes
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	Increased *Gammaproteobacteria, Verrucomicrobia, Fusobacteria* Decreased *Clostridia*	
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	Increased *Bacteroides/Prevotella* ratio, *Faecalibacterium prausnitzii, Escherichia* Decreased *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
Patil et al,[8] 2012		5 thin, 5 lean, 5 obese and 5 obese operated-on individuals (3 SG and 2 AGB) Sanger	Decreased *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	Increased *Bacteroides*, *Escherichia*, *Alistipes* Decreased *Lactobacillus, Dorea, Blautia* and *Bifidobacterium* Increased Number of genera and Chao1index	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 ¼ 5 T2D) who underwent RYGB Shotgun metagenomic sequencing	Increased *Proteobacteria, Bacteroidetes/ Firmicutes* ratio, *Verrucomicrobia* Decreased *Firmicutes, Cyanobacteria*	
Ward et al,[11] 2014	Before and 6 months post-BS	8 MO patients who underwent RYGB 16S rRNA(V4) pyrosequencing	Increased *Bacteroidetes, Bacteroidetes/ Firmicutes ratio, Proteobacteria, Verrucomicrobia* Decreased *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)	Increased *Bacteroidetes, Faecalibacterium pausnitzii* Decreased Several *Firmicutes (Eubacterium, Faecalibacterium, Dorea, and Coprococcus), Bacteroides vulgatus, Bacteroidetes/ Firmicutes* ratio	

Treatment	Study duration	Groups	Changes	Host changes
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)	Increased Proteobacteria *(Escherichia, Klebsiella and Pseudomonas)* Decreased *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
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)	Increased Proteobacteria *(including Escherichia coli and Klebsiella pneumoniae), Streptococcus salivarius, Akkermansia muciniphila* Decreased *Faecalibacterium prausnitzii, Anaerotruncus colihominis, Megasphaera micronuciformis* Increased 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)	Increased RYGB: *Firmicutes, Actinobacteria; SG: Bacteroidetes* Decreased 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)	Increased *Bacteroidetes thetaiotaomicron, Akkermansia muciniphila, Clostridiales bacterium* Decreased *Coprococcus comes* and *Dorea longicatena* Increased Gene count, a-diversity	*Bacteroidetes thetaiotaomicron* is associated negatively with BMI and glutamate levels. Glutamate levels are associated with improved hyperglycemia, insulin resistance, and inflammatory mark
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)	Increased GU:99 Roseburia, GU:225 *Butyricimonas virosa*, GU:359 *Butyricimonas* Increased 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)	Increased *Streptococcaceae*, *Enterobacteriaceae* Decreasedd *Bifidobacteriaceae*	

Table 2. Continued

Treatment	Study duration	Groups	Changes	Host changes
Dao et al,[202] 2020	Before, 3 months and 6 months post-BS	65 MO undergoing BS and follow-up n= 10 AGB and 11 RYGB Shotgun metagenomic sequencing (SOLiD) and 16S rRNA qPCR	Increased *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
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	Increased *Verrucomicrobiaceae* and *Streptococcaceae* Deccreased *Bacteroidaceae*	In 10 subjects, increased *Akkermansia muciniphila* was associated with diabetes remission
Farin et al,[204] 2020	Before and 6 months after BS	89 SG and 108 RYGB Shotgun metagenomics sequencing(SOLiD)	Inccreased Shannon's diversity after both surgery and increasedgene richness RYGB : increased *Escherichia coli* andbuccal bacteria (*Streptococus* and *Veillonella*) SG increased *Clostridium* Increased *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)	Increased Shannon's index in whole cohort, Increased 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 correlatedwith BMI, negative correlation between increased *Aeromonas, Akkermansia, Anaeroglobus, Lachnospiraceae_UCG-001* and *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	Increased *Veillonella, Streptococcus, Gemella, Oribacterium, Atopobium*, one unclassified *Lactobacillales* genus, *Leptotrichia, Neisseria,* and one unclassified *Pasteurellaceae* genus and decreased in *Faecalibacterium* at 3 months Increased *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

Table 2. abbreviations: AGB, adjustable gastric band; BS, bariatric surgery; HbA1c, 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.

influence circulating BCAA levels still remains unclear. 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 mice receiving FMT from a lean twin. Likewise, FMT from insulin-resistant individuals into GF mice replicates the insulin-resistance profile with increased circulating BCAA levels.

[12] Furthermore, individuals with a dysbiotic IM with decreased capacity to catabolize BCAA display higher levels of circulating BCAA,

[145] suggesting that the IM is partly responsible for the circulating levels of BCAA during obesity.[36]

Exercise intervention studies that modulate the IM composition[10] corroborate this. Individuals with prediabetes who participated in a 12-week intensive exercise training program displayed heterogeneous responses. Those with improved insulin sensitivity displayed significant IM changes compared with nonresponders, namely, a decrease in Prevotella copri(involved in BCAA synthesis) and 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 recipients compared with FMT from nonresponders.[146]

Likewise, berberine, which has shown its beneficial effects on insulin-resistance and its ability to modify the IM,[147] was tested in HFD mice. Berberine supplementation leads to reduced weight gain and improved insulin sensitivity along with modified IM functions toward reduced BCAA synthesis and increased BCAA catabolism. This translated into reduced circulating BCAA levels.[148]

Short-Chain Fatty Acids

By contrast, SCFAs are among the 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 and 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 health, as previously reviewed in detail.[26,152-154] They include probiotics,[152] multistrain probiotic cocktails,[155-160] third-generation probiotics, prebiotics, symbiotics, and nutrients with prebiotic or probiotic activities.[153] Importantly, although their efficacy and mechanism of action has been relatively well proven in animal studies, translation to humans sometimes results in controversy or positive yet minor effects. By contrast, other therapeutics, such as FMT,[26] 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 can dramatically improve weight and insulin resistance, represents another example where numerous human studies have demonstrated its effect on modifying the IM.

[19] Furthermore, some of these microbial changes are associated with weight loss or improvement in insulin resistance or T2D.[161,162] They are summarized in Table 2.

Finally, some drugs, such as statins or metformin, also modify the IM, which partly explain their related clinical improvement (summarized in Table 1).

We chose to only focus on recent dietary interventions that induce metabolic improvements and changes in IM composition through the above-described mechanisms.

Caloric Restriction

Lean mice fed a diet with a 40% caloric restriction (CR) lose weight and adiposity while stabilizing their lean mass. They display a switch toward M2-macrophage polarization within the WAT, improvement in insulin sensitivity, together with major changes in the IM composition and functionality[163,164] (Figure 1). FMT from CR mice into GF recipients replicates beneficial phenotypes compared with FMT from controls, despite no difference in food intake, both at room temperature or thermoneutrality. Mechanistic studies[164,165] showed that GF mice receiving FMT from CR mice developed WAT beiging phenotype. By contrast, GF or antibiotic-treated mice under similar CR do not display these improvements, pointing at the importance of the modified IM in these CR-induced beiging effects.[164]

This beiging phenotype probably involves decreased LPS biosynthesis, leading to reduced TLR4 activation because mice treated with TLR4 inhibitors or in TLR4-KO mice display the same phenotype observed upon CR. These recent data partly confirm previous findings, where mice maintained on life-long CR showed improved lifespan, weight, and metabolic health, both under LFD and HFD.[166] CR durably modifies the IM composition together with reducing circulating LBP,[167] yet the LPS/TLR4 pathway and WAT beiging were not assessed.[167] Although a first human trial did not confirm those findings, WAT beiging was solely evaluated in specimens of WAT biopsy performed at room temperature,[168] emphasizing 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.[169] Human studies performed in overweight or obese individuals replicate these beneficial effects.[170,171] A recent randomized controlled trial found that CR and intermittent fasting similarly induced weight loss and metabolic improvement.[172] Mice studies further substantiated the role of the modified IM in these improvements. Compared with mice fed ad libitum, lean mice who underwent 15 cycles of intermittent fasting displayed reduced body weight and adiposity despite similar food intake.[173] This was due to increased energy expenditure through lipid utilization and WAT beiging (demonstrated by increased multilocular adipocytes and UCP-1 gene expression), which occurred before 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[173] 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 compared with FMT from ad libitum mice. Third, microbiota-depleted mice submitted to intermittent fasting do not display this beneficial phenotype. Likewise, 28 days' intermittent fasting in db/db mice induced weight and adiposity reduction and improvement in insulin sensitivity despite no change in caloric intake.[23] 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 the IM in these phenomenons.[23]

Translational research in human is now warranted to evaluate whether intermittent fasting or time-restriction feeding also modulates the human IM, explaining metabolic health improvements. Only 1 pilot study in humans with obesity who underwent 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.[174] Two randomized controlled trials are currently registered at ClinicalTrials.gov (NCT04355910 and NCT03608800) to further substantiate the effect of intermittent fasting or time restriction feeding on metabolic health and the IM.

Dietary Supplementation Recapitulates Beiging of Adipose Tissue in Mice

Polyphenol supplementation also provides evidence of the link between the IM and WAT beiging (Figure 1). First, compared with controls, resveratrol-supplemented lean mice display increased

energy expenditure, BAT gene expression (ie, UCP-1, cidea,[175] PRMD16,[175] and PGC1α[176]) and decreased WAT depots.[177] Similar results were obtained after 10 weeks of resveratrol supplementation in db/db mice along with IM modifications as well as in a model of diet-induced obesity.[178] By contrast, treating those mice with antibiotics abrogated the increased WAT beiging and BAT activity, confirming the role of the IM in these phenomena.[179] Finally, FMT from resveratrol-supplemented mice into recipient mice replicated the increased WAT beiging capacity, whereas no change was observed with FMT from control mice.[179] Interestingly, resveratrol supplementation also protected mice from major HFD-induced weight gain, despite similar energy intake to control mice on HFD. Resveratrol supplementation similarly reversed HFD-induced gut microbiota alteration toward a composition similar to chow-fed mice. FMT from resveratrol-supplemented mice (fed an HFD or chow diet) replicated in recipients the decrease in body weight, the reduced adiposity, and increased WAT beiging capacities (ie, increased markers of BAT within the WAT: UCP-1, PGC-1α, PPARγ,[180] and SIRT-1[181] gene expression as well as protein content.[178]

Concordant results were replicated in HFD mice supplemented with other polyphenol extracts (ie, grape extract from cabernet sauvignon wine).[182] Compared with controls, polyphenol reduced body weight and WAT depots, increased energy expenditure, and restored HFD-induced IM dysbiosis. Polyphenol-induced beiging occurred through modulation of bile acids that upregulate G-protein-coupled bile acid receptor (TGR5), 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 glucose uptake measured by positron emission tomography-computed tomography.[182]

Ginseng extract (GE), which modulates the IM in rats,[183] also induces WAT beiging, thus limiting weight gain. GE supplementation in db/db mice resulted in decreased weight and adiposity and in increased energy expenditure compared with control mice despite similar energy intake.[184] These phenotypes were accompanied by increased BAT activity (ie, increased UCP-1 and oxidative phosphorylation staining in BAT and WAT), a phenomenon that was absent at thermoneutrality. Interestingly, GE supplementation led to increased Enterococcus faecalis 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 thermoneutrality, 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 faecalis 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 faecalis, notably its role in inducing BAT activity and WAT beiging. Importantly, the beneficial effects observed upon GE, E faecalis or myristoleic acid supplementation were abrogated in a model of mice KO for the enzyme able to synthesize myristoleic acid, thus firmly confirming the role of microbial-produced metabolites in reducing weight through increased BAT activity.[184]

Therapeutic Innovation

New therapeutic nutritionally derived strategies are also under development to target the IM and the metabolites it produces with subsequent health benefit. For example, in the atherosclerosis field, TMAO, a microbial metabolite produced from dietary choline or carnitine, is involved in atherosclerosis,[185] whereas 3,3-dimethyl-1-butanol (DMB), a nontoxic compound found in olive oil or red wine, acts as a substrate mimicking choline and functions as a potent TMA lyase inhibitor. DMB prevents TMAO production and leads to reduced atherosclerosis[186,187] in mouse models. Such strategies, targeted at the production of microbial-derived indoles, kynurenine, BCAA, or imidazole propionate, 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 the caseinolytic protease B (ClpB) protein produced by bacteria, which is an antigen-mimetic of the anorexigenic α-melanocyte stimulating hormone.[188] Oral gavage with WT Escherichia coli (thus producing ClpB) leads to reduced food intake and lower body weight than oral gavage with ClpB-deficient E coli both in lean[188] and ob/ob mice.[189] In vitro studies show that bacterially produced ClpB stimulates peptide YY secretion by intestinal cells,[190] suggest-

ing that this antiobesity effect acts through increased satiety. Interestingly, Hafnia alve 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 compared with controls.[189,191] In the human Metagenomics of the Human Intestinal Tract (MetaHIT) cohort, BMI correlated negatively with ClpB gene abundance in the IM. Therefore, the food-grade status of Hafnia alvei HA4597 could lead to its development into third-generation probiotics 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.[192] Yet, human randomized controlled trials are still needed to translate the beneficial findings of animal studies, as is currently done for Akkermansia muciniphila. This bacterium is associated with improved metabolic phenotypes in mice[193] and humans.[194] Its subsequent live or pasteurized use showed minor beneficial outcomes and was safe in humans.[195] Nevertheless, its use as a third-generation probiotic in overweight/obese metabolic patients still needs deeper investigation.[196] This last study shows how important human randomized control trials are to replicate findings demonstrated in vivo or in vitro concerning the IM, its related metabolites, and their effects on host health.

Conclusion

High-throughput sequencing coupled with omic analysis in humans or in different models of IM-depleted mice, with or without FMT, have shown the important role of the 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 the IM directly and even the metabolites it produces 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.[29,197]

Author Contribution

Moritz V. Warmbrunn, MD, did the literature search and wrote the manuscript. Judith Aron-Wisnewsky, MD, PhD, did the literature search and wrote and edited the manuscript. Max Nieuwdorp, MD, PhD, and Karine Clément, MD, PhD, edited the manuscript.

Conflicts of interest

M.N. is in the Scientific Advisory Board of Caelus Pharmaceuticals, the Netherlands; and of Kaleido USA. M.W. is owner of Nature Plus. K.C. is a consultant for Danone Research and Confo-Therapeutics and on the scientific advisory board of LNC-therapeutics. No personal funding has been received for these activities that would alter the content of this present review. The other authors disclose no conflicts.

Funding

Supported by EU LITMUS (Liver Investigation: Testing Marker Utility in Steatohepatitis) grant to M.N. and K.C. The work of M.N. and K.C. is supported by a grant from the Leducq Foundation, JPI-HDL (Joint Programming Initiative–A Healthy Diet for a Healthy Life) MICRODIET consortium. J.A.W. received a grant from Bettencourt Shueller Foundation. M.N. is supported by a personal ZonMw-Vidi grant 2013 (016.146.327), M.W. is supported by a CVON INCONTROL grant 2018.27 (CVON 2018.27). K.C. and J.A.W. are members

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