Modification of Faecal Microbiota as a Mediator of Effective Weight Loss and Metabolic Benefits Following Bariatric Surgery

Oduro-Donkor, D[1,2]; Turner, MC[3]; Farnaud, S[3]; Renshaw, D[3]; Kyrou, I[1,2,4]; Hanson, P[1,2,5]; Hattersley, J[2,5]; Weickert, MO[1,2,3,5]; Menon, V[1,2]; Randeva, HS[1,2,4,5]; Barber, TM[1,2,5]

1. Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, UK
2. Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism, University Hospitals Coventry and Warwickshire, Clifford Bridge Road, Coventry, CV2 2DX, UK
3. Centre for Sport, Exercise and Life Sciences, Faculty of Health & Life Sciences, Coventry University, Coventry, UK
4. Aston Medical Research Institute, Aston Medical School, Aston University, Birmingham, UK
5. NIHR CRF Human Metabolism Research Unit, University Hospitals Coventry and Warwickshire, Clifford Bridge Road, Coventry, CV2 2DX, UK.

Corresponding author and to whom reprint requests should be addressed:

Dr Thomas M Barber,
Clinical Sciences Research Laboratories,
University Hospitals Coventry and Warwickshire,
Clifford Bridge Road,
Coventry,
CV2 2DX,
United Kingdom.
E-mail: T.Barber@warwick.ac.uk

Written as an invited review article for ***'Expert Reviews in Endocrinology and Metabolism'***

Word count (abstract): 200

Word count (main article): 6,806

Number of figures: 1

Number of tables: 1

None of the authors has any conflicts of interest.

Abstract

Introduction: Bariatric surgery (primarily Laparoscopic Sleeve Gastrectomy [LSG] and Roux-en-Y Gastric Bypass [RYGB]) is an efficacious and durable therapeutic option for weight loss in obesity. The mechanisms that mediate weight loss following bariatric surgery remain incompletely understood.

Areas Covered: Pubmed search of published data on faecal microbiota, metabolic health, LSG and RYGB. The faecal microbiome plays a key role in the establishment and maintenance of metabolic wellbeing, and may also contribute (through faecal dysbiosis) to metabolic dysfunction. LSG and RYGB both result in characteristic, procedure-specific changes to the faecal microbiota that may mediate at least some of the resultant weight-loss and metabolically beneficial effects, when applied to the management of obesity.

Expert Opinion: The human faecal microbiome, containing around 100 trillion microbes, evolved over millions of years and interacts symbiotically with its human host. Rodent-based studies have provided insights into the complexities of the gut-microbiome-brain axis. This includes the important role of the gut microbiome in the mediation of normal immunological development, inflammatory pathways, metabolic functioning, hypothalamic appetite regulation and the absorption of essential nutrients as by-products of bacterial metabolism. Faecal transformation is likely to provide an important therapeutic target for future prevention and management of obesity and metabolic dysfunction.

Keywords: Faecal microbiota; Obesity; Bariatric Surgery; Microbiome; Incretins; Sleeve Gastrectomy; Roux-en-Y Gastric Bypass

Article Highlights

- The human gut microbiome co-evolved with our hominid ancestors over millions of years. Formed of approximately 100 trillion microbes, the gut microbiome vastly outnumbers our own cells. Over recent years, our understanding of the importance of the gut microbiome for health and wellbeing has been transformed.
- Most of the current literature on the effects of gut microbiota (including faecal transplantation) on metabolic health stems from rodent studies. Data from human-based studies on gut microbiota and metabolic health are largely association-based, enabling only tenuous conclusions regarding causality.
- Most of our current data on the gut microbiome relates to individual bacterial species. Certain gut bacterial species such as *Enterococcus*, *Akkermansia muciniphila* and *F. prausnitzii* have been shown to associate with a favourable metabolic and inflammatory profile. Certain bacterial species release useful by-products of metabolism. For example, *Enterococcus*, which through fermentation of dietary fibre, releases short-chain fatty acids (SCFAs) that confer metabolic benefits. (SCFA also contribute to around 10% of energy intake in humans, and therefore may potentially contribute to weight gain. The role of SCFAs in the establishment and maintenance of metabolic homeostasis therefore remains controversial [1, 2]).
- Some gut bacterial species, such as *Bacteroidetes* and *Firmicutes* associate with human obesity.
- Bariatric surgery is our most effective treatment strategy for obesity. The mechanisms of weight loss following bariatric surgery are incompletely understood, although current dogma implicates earlier release of incretins from the distal gut.
- Based on the current literature (primarily studies on RYGB), characteristic modifications to the gut microbiome occur following bariatric surgery, and appear to be procedure-specific and largely metabolically-favourable (based on known associations of specific gut bacterial species).
- Future studies should focus on attempts to replicate the characteristic modifications to the gut microbiome that normally occur following bariatric surgery. Such studies on patients with obesity would require transformation of the gut microbiome through, for example, faecal transplantation. Confirmation of established metabolic and appetitive benefits (including weight loss) from such an intervention, would provide a novel and promising therapeutic strategy, that could be implemented potentially on a global level for the prevention and management of obesity and metabolic dysfunction.

1. Introduction

The incidence of obesity has tripled since 1975, and affects more than 650 million people globally, with 1.9 billion people being overweight [3]. Obesity is associated with several non-communicable chronic diseases. These include metabolic dysfunction (such as Type 2 Diabetes Mellitus [T2D], Hypertension, Dyslipidaemia, non-alcoholic fatty liver disease [NAFLD], and Obstructive Sleep Apnoea), biomechanical disorders (including osteoarthritis), cardiovascular, respiratory and psychiatric conditions, as well as increased risk of certain malignancies [4]. Obesity-related co-morbidities are likely mediated through complex pathways that implicate insulin resistance, hyperinsulinaemia, oxidative stress and inflammatory processes [5]. For example, 'The Million Women Study' showed a direct association of adiposity, as measured by Body Mass Index (BMI), with increased risk of cancer development (including 10 of 17 specific types of cancers) [6]. Furthermore, the Framingham Heart Study revealed obesity and overweight in adulthood to associate with decreased life expectancy and increased risk of early mortality, reduced life expectancy being similar to that of smoking [7]. The current global obesity epidemic, combined with the tsunami of obesity-related medical co-morbidities and chronic diseases, is one of the most important threats to our health in modern times. The global health-economic implications of obesity are substantial, and therefore the development and implementation of effective preventive and management strategies for obesity are a priority. This should incorporate a variety of approaches including multi-disciplinary team-based care, but also strategies that benefit the wider population. Development of novel therapeutic strategies for obesity will require renewed insight into the complex mechanisms that underlie both weight-gain, and effective weight-loss.

Bariatric surgery represents our best current therapeutic option for establishment and maintenance of effective weight loss in obesity. However, despite its clear clinical and metabolic utility, the mechanisms by which bariatric surgery confer weight-loss benefits are incompletely understood. The 'hind-gut hypothesis' states that following gastric bypass procedures, there is earlier and enhanced release of incretin hormones from the distal ileum, stimulated by faster transit of food. This early incretin release suppresses appetite and augments post-prandial pancreatic response [8, 9]. However, this hypothesis remains speculative. It is important to explore alternate explanations for improvements in body weight and metabolic health following bariatric surgery. Renewed mechanistic insights would potentially enable catalysis of novel therapeutic developments, including non-surgical replication of the weight-loss effects of bariatric surgery.

In recent years, the gut microbiome has assumed major interest within the scientific community. Current biomedical literature provides compelling support for the importance of a healthy colonic microbiome in the establishment and maintenance of health and wellbeing, and on gut microbial dysbiosis as an originator of much ill-health. This includes links to multiple chronic diseases, including T2D, autism, depression, auto-immunity, atopy, food intolerances and obesity. Indeed Hippocrates, the father of modern medicine, suggested that the root of all disease lies within the gut. There are increasing data in the literature, particularly from rodent and other animal models, that associate certain species of gut microbiota with body weight, metabolic profile and modulation of the immune system [10-15]. This raises a possible future role for faecal modulation (through, for example, faecal transplantation), for facilitating weight loss and improved metabolic profile. Currently, implementation of faecal transplants within the NHS is solely reserved for the management of patients suffering from intractable colonic colonisation with *Clostridium difficile* [16]. Future clinical indications for faecal transplantation are likely to diversify, given the emergent data that links the colonic microbiome with a spectrum that encompasses health and wellbeing, and chronic ill-health at opposite ends.

In this concise review, we provide an overview of the gut microbiome and consider its relevance for maintenance of health and wellbeing, and as an originator of chronic ill-health, including obesity. We then consider a possible role for changes in the gut microbiome as a mediator of weight loss, and other metabolically beneficial effects following bariatric surgery. We review critically the available evidence from the literature, using a Pubmed search of published data on faecal microbiota, metabolic health, LSG and RYGB, and provide suggestions for future directions in this important and emerging field.

2. The Gut Microbiome

2.1 The microbiome as we know it: There are approximately 100 trillion microbes within the human gut [17]. This number is well beyond our comprehension, although another perspective is that we are numerically more microbe than human (probably by an order of magnitude), if only taking into account numbers of cells. Whilst this may seem alarming or even disgusting to some, it is worth considering the essential role that the gut microbiome plays in the establishment and maintenance of health and wellbeing. Our gut microbiota co-evolved with us and our ancestors over hundreds of millions of years. Accordingly, our immune system is intricately interlinked with our gut microbiome. The gut is the ultimate 'training ground' for our immune system that empowers our immune defence following its

effective graduation. Without proper training from a rich and diverse gut flora, the immune system remains under-developed. In such a scenario, auto-immunity and atopy can ensue. Our gut microflora should be considered almost like an organ in its own right, with the capacity to make us unwell if not properly nurtured, or conversely to promote health and wellbeing if we nurture it correctly. Only around 1,000 microorganisms have been identified in the human body to date, of which 70% reside in the colon [18]. This probably represents a very small proportion of the total microbes within the gut. 'Microbiome' is an umbrella term that incorporates all non-Eukaryotic cells that associate with the human body, including locations such as skin, respiratory epithelia, genitourinary tract, and of course the entire gastrointestinal tract. However, the vast majority of microbes reside within the colon, which forms the main focus for this review.

It is important that future studies focus on a more in-depth and complete understanding of the gut microbiome, not just through the identification of novel gut microbes, but also key insights into the mechanisms by which these microbes affect us. One of the challenges of this complex field relates to not just how individual microbes interact with their host, but also to how groups of different microbes interact with each other. It is possible, indeed likely that the beneficial or deleterious effects of a particular gut microbe is influenced by its immediate microbial environment.

2.2 We are what our gut microbes eat: Our diet influences our gut microbiome. Typical modern-day western diets that are highly processed, sterile and often fat- and carbohydrate-rich, ultimately impoverish our gut microbiome. Conversely, natural, unprocessed and fibre-rich foods (including leafy green vegetables), encourage, nurture and facilitate development of a healthy and diverse gut microbiome. Although we are perhaps more what our gut microbiota eat than what we ourselves eat, we may never fully understand the mechanisms by which our gut microbiome influences our position on the health-disease spectrum. This field is inherently complex, and our understanding of how the gut microbiome mediates health and chronic disease remains in its infancy.

2.3 The microbiome and macronutrients: An important function of the human gut pertains to nutrient and energy harvesting from macronutrients, including non-digestible macronutrients such as cellulose, through the action of intestinal enzymes from the host. For example, vitamins including biotin, folic acid, and pantothenate are synthesized by several intestinal genera, including *Bacteroides*, *Eubacterium*, *Propionibacterium*, and *Fusobacterium* [19]. Conversely, some gut microbes (eg. *B. thetaiotaomicron*) can actually

compete with the host for essential nutrients, such as B12. Indeed, Wexler and colleagues, using Western blot analysis from custom-made rabbit anti-BtuG2 polyclonal antibody, showed that some gut microbes such as *B. thetaiotaomicron*, actually utilize vitamin B12 on transit through the digestive tract. BtuG (a surface-exposed lipoprotein essential for B12 transport in *B. thetaiotaomicron*), binds with great affinity to B12, and even sequesters B12 from intrinsic factor in humans [20]. Accordingly, BtuG reduces the host's own absorption of B12. This highlights how some gut microbes appear to play a duplicitous role with regards to certain macronutrients, including both provision of essential nutrients such as biotin and folic acid, and competition with the host for other essential nutrients such as B12.

2.4 Identification of the gut microbiome: Accurate analysis of gut microbiota composition is important to enable insights into associations between gut microbiota signatures and disease. There are two main approaches: i) Shotgun next generation sequencing metagenomics analysis of random DNA fragments, and; ii) 16S ribosomal RNA gene (rDNA) amplicon sequencing [21-24]. Laudadio and colleagues reported on one of the few studies to directly compare application of these two approaches on the same faecal samples. It was demonstrated that compared with 16S rDNA amplicon sequencing, the Shotgun metagenomics approach provided a much deeper characterization of the microbiome complexity, with identification of a larger number of species [24]. A further means of assessing the human gut microbiome is through 'quantitative microbiome profiling' that enables measurement of overall absolute microbial abundance, (rather than relative abundance that is simply reported as fractions within each faecal sample) [25]. Data from quantitative microbiome profiling provides insights into how *quantitative* changes in microbial species may influence metabolite concentrations and physiological parameters [25].

Comparison of the microbiome across two or more subjects has proved challenging. Accordingly, the microbial signature is broken down into two main categories: the number of different species in a faecal sample, and the abundance with which each species appears. Within a faecal sample from a single human, microbial signature is referred to as 'α-diversity'. When faecal samples from two different human sources are compared, the difference between the two samples is referred to as 'ß-diversity' [26]. Data from such studies reveal that most human gut microbiota are members of the *Firmicutes* and *Bacteroidetes* phyla [27-30]. Overall, there are six main bacterial clusters that inhabit the gut of healthy individuals. These include *Firmicutes* (gram-positive strains of *Clostridium, Eubacterium, Ruminococcus, Butyrivibrio, Anaerostipes, Roseburia* and *Faecalibacterium*);

Bacteroidetes (gram-negative strains of *Bacteroides, Porphyromonas* and *Prevotella*); *Proteobacteria* (gram-negative strains such as *Enterobacteriaceae*); *Actinobacteria* (gram-positive *Bifidobacterium* genus); *Fusobacteria*, and; *Verrucomicrobia* (*Akkermansia*) [31, 32].

3. Clinical Relevance of the Human Gut Microbiome for Obesity and Metabolic Status

Most of the existing data on the gut microbiome and its association with body weight and metabolic health stems from rodent-based studies. There are relatively few human-based studies reported. Thus, it is important that we conduct future well-designed studies in humans to corroborate the rodent-based data, to develop novel insights into a potential future therapeutic role for gut microbiota as an important mediator of a healthy metabolism and body weight. Most of the human-based literature to date report on association between BMI and groups of bacterial species. We know relatively little about the metabolic effects of, and interactions between individual bacterial species within the human gut.

3.1 Association of the gut microbiome with obesity: Human obesity associates with two main bacterial species predominant within the gut: *Bacteroidetes* and *Firmicutes* [33, 34]. In one study reported by Ley and colleagues, genetically obese mice (ob/ob) were compared with lean (ob/+) and wild-type siblings, that were fed on the same diet and had their gut microbiomes sequenced via 16S rRNA gene sequences. As expected, *Firmicutes* and *Bacteroides* predominated within the gut microbiome of the obese (ob/ob) mice. There was also a 50% reduction of *Bacteroides* and proportional increase of *Firmicutes* in the ob/ob mice compared to the ob/+ mice. [35]. Human-based studies show that obesity associates with other gut microbiota that include the genera *Alistipes*, *Anaerococcus*, *Corpococcus*, *Fusobacterium* and *Parvimonas* [36].

3.2 The gut-microbiome-brain axis: Much of our insights into the complexities of the gut-microbiome-brain axis originate from metabolomics data. Such studies target small molecule metabolites through a variety of mass spectrometry-based techniques, including gas chromatography, liquid chromatography and capillary electrophoresis [37]. Metabolomics data from rodent-based studies underlies much of our current understanding of the gut-microbiome-brain axis, with implication of neuro-immune, neuroendocrine and autonomic (primarily vagal) pathways [38]. The actual mechanisms involved are incompletely understood. It is possible that direct communication between the gut microbiome and brain occur, through the release of chemicals [38, 39]. Direct microbiome-

brain communication may be mediated through release of by-products from gut microbes such as SCFAs, secondary bile acids and tryptophan metabolites [38, 40]. These molecules may initiate signals via enteroendocrine cells, enterochromaffin cells and the mucosal immune system. Some microbial by-products (such as SCFAs) cross the intestinal barrier to enter the systemic circulation, and some may also cross the blood-brain barrier to exert direct effects on hypothalamic regulation of appetite and metabolic processes [41, 42]. Secondary bile acids are products of the gut microbiota following their metabolism of bile acids (synthesized from cholesterol within the liver). These gut microbiota-induced bioconversions are known to modulate the signalling properties of bile acids through the nuclear farnesoid X receptor and the G protein-coupled membrane receptor 5 [43]. These pathways regulate multiple metabolic pathways in the host. Furthermore, the gut microbiota composition can itself be regulated through the action of bile acids (including through modulation of the intestinal innate immune response) [43]. Therefore, secondary bile acids play an important role in the maintenance of a healthy gut microbiota, insulin sensitivity, innate immunity and balanced lipid and carbohydrate metabolism [44, 45]. In addition to metabolic dysfunction, aberrant bioconversion of bile acids into secondary bile acids resulting from faecal dysbiosis, has also been linked mechanistically with gastrointestinal carcinogenesis (including colorectal cancer and hepatocellular carcinoma) [44].

Observations consistent with a role for gut wall ‘leakiness’ in metabolic health include those reported in a human-based study by Chassaing and colleagues [46]. In colonic biopsies, bacterial-epithelial distance (the distance between the gut microbiota and the gut epithelial lining and a marker of colonic mucus production), was shown to inversely correlate with BMI, fasting glucose levels and HbA1C. These data are consistent with a local effect of gut microbiota on colonic mucus production and permeability of the gut wall. Therefore, mucus-mediated gut wall permeability may influence metabolic status through physical protection of the gut epithelial lining from gut microbiota [46]. Gut microbiota are likely to play an important role in the control of secretion and thickness of gut mucus and its subsequent metabolic implications, although this hypothesis requires confirmation.

The realm of interconnections and communications within the gut-microbiome-brain axis should provide a focus for future research, to provide novel insights into the regulation of appetite, metabolism and even emotional regulation and mental health. Evidence from the literature provides useful insight into the potential effects of some species of human gut microbiota on body weight and metabolic status, mediated through the gut-microbiome-

brain axis (outlined in *Table 1*). To further explore the intricacies of the gut-microbiome-brain axis, we outline here some insights into potential roles of individual species of gut microbiota and some of their metabolic by-products.

3.3 Akkermansia muciniphila: This is a mucin-degrading bacterium that may play a role in human gut barrier function, and thereby influence host metabolism through modulation of translocation of microbial molecules across the gut wall [47]. In one study reported by Clarke *et al*. on gut microbiota from professional rugby athletes compared with controls, it was shown that athletes and the low BMI controls had significantly higher proportions of the genus *Akkermansia* than the high BMI controls [48]. Through the reduction of intestinal mucin to propionic and acetic acid, *Akkermansia muciniphila* engages in symbiosis with its host, providing nutrients accessible for other resident gut bacteria [49, 50]. Interestingly, colonic *Akkermansia muciniphila* levels have also been shown to be decreased in adults with obesity and T2D [50].

3.4 Enterococcus: Gut microflora may influence appetite through direct or indirect communication with the hypothalamic appetite centres. One such colonic bacterial species is *Enterococcus*, a gram-positive facultative anaerobe of the genus *lactobacillus*, and phylum *Firmicutes*. *Enterococcus* ferments dietary fibre, producing SCFAs in the process. Levels of colonic *Enterococcus* and its by-product SCFAs directly correlate with appetite inhibition. Future studies should explore further the causal mechanisms (direct and/or indirect) between colonic *Enterococcus* and SCFAs and appetite regulation [23].

3.5 Faecalibacterium prausnitzii: It has been demonstrated that some species of colonic bacteria, including the gram-positive anaerobic *Faecalibacterium prausnitzii* associate with anti-inflammatory effects post-bariatric surgery [51]. Mediation of anti-inflammatory effects of *F. prausnitzii* may occur through blockade of nuclear factor-kB activation, and subsequent inhibition of secretion of pro-inflammatory mediators [51-53]. Other studies have shown possible effects of Gram-positive anaerobic bacteria on the production of butyrate, a SCFA. An increased proportion of butyrate-producing bacteria within the gut microbiome associates with a favourable metabolic profile in humans [54]. The mechanism(s) by which butyrate confers metabolic benefit may include anti-inflammatory effects, and enhancement of intestinal barrier function.

3.6 Short Chain Fatty Acids (SCFAs): The gut microbiome utilizes energy from food products, producing some by-products that are beneficial to the host. SCFAs are one such by-product of microbial respiration. SCFAs are produced by anaerobic microbes within the

caecum during fermentation of dietary fibre (non-digestible carbohydrates). SCFAs appear to provide a source of energy for colonocytes [55]. Furthermore, human-based studies have shown that SCFAs can pass through the colonic epithelium into the bloodstream. Serum SCFAs may then influence lipid, glucose and cholesterol metabolism through effects on G protein-coupled receptors (GPCRs) [55]. GPR41/FFAR3 (free fatty acid receptor 3) and GPR43/FFAR2 are two SCFA-specific GPCRs expressed in the gut entero-endocrine cells, adipocytes, and immune cells [56]. Compared with GPR41 knock-out mice, stimulation of GPR41 on entero-endocrine cells in wild-type mice with SCFAs demonstrated enhanced secretion of peptide YY (PYY), a gut hormone with appetite-suppressant effects. Other effects in the wild-type mice from GPR41 stimulation with SCFAs included increased gut motility, and reduced harvesting of energy via SCFAs from the diet [57]. SCFA-dependent effects of GPR43 signalling in wild-type mice demonstrated enhanced incretin (glucagon-like peptide-1, GLP-1) release, and enhanced insulin sensitivity [58].

The beneficial effects of SCFAs in rodent-based studies have been corroborated by observations from human-based studies. In one study by Chambers *et al.*, the effects of propionate (one of the commonest SCFAs produced by human gut microbiota) on incretin response (including plasma PYY and GLP-1 excursions), body weight and energy intake were explored. A novel inulin-propionate ester was utilized in a randomised, controlled cross-over design in overweight adults (n=60). Ingestion of propionate resulted in early postprandial release of PYY and GLP-1 from human colonic cells, with associated reduction of subsequent energy intake. Over 24 weeks of regular propionate ingestion (compared with an inulin control group), there was significant weight loss, reduction in intra-abdominal adipose tissue volume and intra-hepatocellular lipid content and preservation of insulin sensitivity [59]. These data provide compelling insight into possible mechanisms by which gut microbiota mediate metabolic health through the effects of SCFAs on incretin release, adipocyte and immune cell functioning. Dietary fibre, as a key source of colonic SCFAs, remains an important component of a healthy diet.

3.7 Succinate: In addition to the association of colonic bacterial species and their by-products (such as SCFAs) on favourable metabolic status, other bacterial species and by-products associate with unfavourable metabolic status. Succinate is a metabolic intermediate by-product produced by some bacterial species. Serum succinate levels in humans, including a predominance of succinate-producing colonic bacterial species such as *Prevotellaceae* and *Veillonellaceae*, associate with obesity and metabolic syndrome. Furthermore, weight-loss has been observed to accompany a reduction in levels of serum

succinate, and increased proportion of succinate-*consuming* colonic bacteria that include *Odoribacteraceae* and *Clostridiaceae* [60]. It is not clear whether succinate plays a causal role in determining body weight and metabolic status, or the nature of such mechanisms.

To summarize this section (outlined in *figure 1*), certain colonic bacterial species (including *Akkermansia muciniphila*, *Enterococcus* and *F prausnitzii*), and bacterial by-products such as SCFAs, appear to associate with a more favourable metabolic status, including lower appetite, lower body weight and reduced overall inflammatory status. Conversely, other bacterial species that include *Prevotellaceae* and by-products such as succinate, associate with an unfavourable metabolic status. It is important to re-emphasize that the studies outlined here on bacterial proportions are mainly based on association data. Causal effects of colonic microbiota and their by-products on regulation of appetite, metabolic and inflammatory milieu, and the mechanisms implicated (including 'gut-microbiome-brain' interlinked communications) are largely speculative. It remains possible that association of colonic microbiota species with favourable body weight and metabolic status, are merely epiphenomena that play no causal role in the establishment of metabolic and inflammatory status. However, evidence exists both from rodent- and human-based studies, to implicate colonic bacterial by-products such as SCFAs, in the mediation of at least some of the metabolic effects of the gut microbiome on human metabolic status.

There is much interest in the interactions between the host mitochondria and microbiota. Complex bi-directional interlinks exist between these two entities, with the gut microbiota implicated in the regulation of key transcriptional co-activators, enzymes and transcription factors involved in mitochondrial biogenesis. SCFAs and secondary bile acids derived from the gut microbiota also influence host energy production and inflammation, with implications for athletic performance [61]. Furthermore, mitochondrial function (including mitochondrial production of reactive oxygen species) play an important role in the regulation of the gut microbiota, including the intestinal barrier function and mucosal immune responses. Such processes may be influenced through genetic variants within the mitochondrial genome [61]. A link between colonic butyrate has been shown with autism spectrum disorder, with mitochondrial dysfunction as a possible mediator [62].

3.8 Metabolic Effects of Faecal Transplantation: To move beyond mere association and address further the important question of causality will require novel investigation. This will include modification of the human gut microbiome through means such as faecal transplantation, and to explore the related metabolic and inflammatory effects. This will

be an important step towards a future that places gut microbiota at centre stage, and one that seeks to improve health and wellbeing of the populace through re-establishment of a healthy and well-nurtured gut microbiome. Important insights into possible metabolic effects of faecal transplantation stem from rodent-based studies. Most reported data to link gut microbiota with obesity stem from murine models, with demonstration of changes in both metabolic status and body weight following faecal transplantation [63]. One important caveat when comparing faecal microbiota between humans and rodents, is that rodents recover some energy requirements from coprophagia that is likely to influence their colonic microbiota in ways that do not apply to humans [64, 65]. Caution should therefore be exercised when extrapolating rodent-based data on faecal transplantation to humans. Although two human studies on faecal transplantation have shown improvements in glucose tolerance in metabolic syndrome, to date there are no current human-based data that demonstrate effectiveness of faecal transplantation in treating obesity [63, 66].

Studies using faecal transplantation in germ-free (gnotobiotic) mice have identified *Prevotella copri* as an important contributor to branched-chain amino acids and insulin resistance. However, in other faecal transplantation studies, *P. copri* was demonstrated to be required for improved insulin sensitivity [1, 67]. In a further study on gnotobiotic mice, colonization with a prominent saccharolytic member of the normal human gut microbiota resulted in a marked improvement in the efficacy of colonic fermentation, increased *de novo* lipogenesis and obesity [64, 68]. Rodent-based studies have also provided insights into interplay between the gut microbiota and the reproduction axis in a rat model of Polycystic Ovary Syndrome (PCOS). Guo and colleagues randomly assigned rats into control and PCOS groups (induced through letrozole treatment, with elevated levels of testosterone and suppressed oestradiol) [69]. One PCOS rat group received colonic *Lactobacillus* that associated with increased levels of oestradiol. A separate PCOS rat group received faecal microbiota transplantation that associated with a reduction in faecal *Prevotella*, and reduced levels of serum testosterone and androstenedione [69]. Interestingly, there were also effects of letrozole treatment (and inducement of PCOS) on faecal microbiota, with lower levels of *Lactobacillus*, *Ruminococcus* and *Clostridium* and higher levels of *Prevotella* (compared with the control group) [69].

Having explored associations of the human gut microbiome with metabolic health and body weight, and the symbiotic relationship of colonic microbiota with the host, we now consider evidence to support modification of the gut microbiome as a mediator of weight loss and metabolic improvement following bariatric surgery.

4. Bariatric Surgery and the Gut Microbiome

4.1 Bariatric Surgical Procedures: Currently, there are three main types of bariatric surgery: Laparoscopic Gastric Band (LGB), Roux-en-Y Gastric Bypass (RYGB) and Laparoscopic Sleeve Gastrectomy (LSG). There is incomplete understanding of the mechanisms of weight-loss and metabolic improvement following each of these procedures, including the degree of mechanistic overlap between them. In recent years, a trend has emerged for LSG, although RYGB also remains a popular choice [70] and both RYGB and LSG have been shown to confer beneficial effects on weight loss and resolution of T2D [71]. For the purposes of this review, we will not consider procedures such as gastric balloon insertion, and only focus on the two most frequently-performed bariatric surgical procedures, RYGB and LSG.

The magnitude of weight loss following bariatric surgery varies according to the type of procedure. Typically, greatest weight loss occurs following RYGB. In one study, percentage of excess weight loss (%EWL) following RYGB was 62.58% at ≥5 years and 63.52% at ≥10 years. Comparative values following LGB were 47.94% and 47.43% respectively. Following LSG, there was 53.25 %EWL at ≥5 years [72]. However, more recent comparisons between weight-loss effects of RYGB and LSG at 5-years post-procedure have shown equivalent effects. Data from the Swiss Multicenter Bypass or Sleeve Study (SM-BOSS) that reported on 217 morbidly obese patients randomly assigned to either RYGB or LSG, showed statistically equivalent weight-loss at 5-years following the procedure (excess BMI loss of 68.3% and 61.1% respectively) [73]. A further independent 'sleeve vs bypass' (SLEEVEPASS) study on 240 morbidly obese patients randomly assigned to RYGB or LSG, also showed statistically equivalent weight-loss (based on pre-specified equivalence margins) at 5-years post-procedure (estimated mean %EWL of 57% and 49% respectively) [74].

Effects of bariatric surgical procedures on both GI anatomy and physiology influence the speed of food transit from the stomach into the ileum. Both RYGB and LSG diminish the effects of gastric acid on ingested food within the alimentary tract. Furthermore, bariatric surgery often results in changes in dietary habits and eating behaviours. Following bariatric surgery, changes in gastro-intestinal (GI) anatomy and physiology that influence the speed of food transit through the upper alimentary tract and exposure of salivary microbiota to gastric acid, and changes in diet and eating behaviour may all have down-stream effects on the gut microbiome.

4.2 The effects of bariatric surgery on the gut microbiome: The type of bariatric procedure appears to influence the form of modification to the colonic microbiome. Compared with RYGB, LSG does not change the anatomical arrangement of the gut, and therefore results in less dramatic changes in the rapidity and diversity of the adapting gut microbiome. In support of this hypothesis, Sanmiguel *et al*. reported on a study of obese women (n=8) who had undergone LSG. At one month post-LSG, there were no substantive changes in gut microbial diversity compared with pre-LSG [23]. However, LSG does appear to result in colonic microbial changes in some studies, including one in young (age 18-30 years) Chinese obese subjects (n=23), in which the relative abundance of colonic *Bacteroides thetaiotaomicron* increased with weight-loss post-LSG, and continued to increase with further weight-loss [75].

In RYGB, a small gastric pouch is formed with bypassing of the distal stomach and proximal small intestine, through the attachment of the distal end of the jejunum to the proximal gastric pouch. The 'bile and pancreatic limb' is attached along the Roux limb. RYGB results in reduced acidity along the length of the gut, and downstream delivery of bile acids. Changes in gut pH and bile acid exposure following RYGB both likely influence the composition of the gut microbiome [23, 51, 76]. Lee and colleagues performed 16S rRNA amplicon sequencing in women with Diabetes Mellitus (mean age 51 years and 75% black race), to identify gut microbial composition at baseline, and following 10% weight loss (or at 9-months post-bariatric surgery if 10% weight loss was not achieved). Women were randomised to medical weight loss (MWL), LGB or RYGB in a ratio of 1:1:1, each group containing the same number (n=4) of participants. The MWL group received individualized counselling, combined with meal replacements and frequent self-monitoring. Daily caloric consumption was 1200–1500 Kcal for adults weighing <220 lb, and 1500–1800 Kcal for adults weighing >220 lb. Mean weight loss across each of the 3 groups, MWL, LGB and RYGB was 6.3%, 9.9% and 10% respectively. Although relatively little gut microbial diversity was observed at baseline amongst this small sample size, both RYGB and LGB resulted in an increased proportion of *Proteobacteria* within the gut microflora [22], and RYGB also resulted in an increased proportion of *Actinobacteria* [22]. The MWL group showed minimal change in the relative abundance and types of gut microbiota following weight loss, but showed an increase in the relative abundance of *Roseburia* genus compared to RYGB and LGB. There were no changes in the proportion of *Bacteroides* species within the gut microbiome amongst those who had undergone bariatric surgery regardless of type, but an increase in the relative abundance of *Faecalibacterium* in those who had RYGB compared

to LGB and MWL. Also, there was a relative increase in the abundance of *Akkermansia* genus in all three MWL, LGB and RYGB groups [22].

Sanchez-Alcoholado and colleagues reported on the effects of bariatric surgery (RYGB [n=14] and LSG [n=14]) on the colonic microbiome, through sequencing of amplicons from the 16S rDNA gene by next-generation sequencing. Spanish subjects with morbid obesity (n=28) were recruited, with assessments at baseline and at 3-months post-bariatric surgery. Following LSG, there was an increase in the relative abundance of *Verrucomicrobiaceae* species. Conversely following RYGB, there was an increase in the relative abundance of *Enterobacteriaceae, Proteobacteria* and *Fusobacteria* [34]. There was also an inverse correlation between *Enterobacteriaceae* and *Veillonella* and cholesterol levels, and a positive correlation between *Verrucomicrobia* members and high-density lipoprotein cholesterol both pre-and post-bariatric surgery [34]. Interestingly, those subjects who underwent RYGB also had an increase in the relative abundance of facultative anaerobes (that typically originate from the oral tract) in their gut microbiome. These included *Fusobacteria, Veillonella,* and *Granucatiella* [34].

One explanation for the increase in the relative abundance of colonic facultative anaerobes following RYGB includes faster transit of swallowed saliva through the stomach remnant into the ileum, and perhaps reduced exposure of salivary microbes to gastric acid following RYGB. Through this mechanism, it is conceivable that more oral anaerobes within saliva reach the ileum following RYGB and contribute towards modification of the gut microbiome. However, this hypothesis is entirely speculative, and should form a focus for future research. It is also possible that differences in gastric pH following LSG and RYGB (including increased gastric pH following RYGB compromising the gastric pH barrier [77], and resulting in a higher intestinal pH [78]), may influence directly the colonic microbiome, and may explain some of the differences in colonic microbial modification following each of these procedures. It remains possible that changes in salivary transit speed following RYGB may affect the microbiota throughout the entire GI tract, and not just the colon. These potential changes in GI microbiota following RYGB should form a focus for future research.

Changes in the colonic microbiome following bariatric surgery also associate with changes in appetite and measures of food addiction. Using the Yale Food Addiction scale, Sanmiguel and colleagues showed increased levels of certain species of colonic bacteria that associate with a reduction in food addiction following LSG. These species included *Butyricimonas*, *Odoribacter* and *Enterococcoccus*. Conversely, there were reduced levels of other species of

colonic bacteria, such as *Catenibacterium* and *Anaerostipes* [23]. It is possible that causal mechanisms implicating effects of these bacterial species on appetite and food addiction pertain, and should be a focus for future research. In a further study on the effects of LSG in women with obesity (n=8), levels of colonic *Enterococcus* associated inversely with perceptions of hunger post-LSG [23].

To summarize this section, it appears that both RYBG and LSG result in modifications to the colonic microbiome. The nature, diversity and rapidity of such modifications appear to be procedure-specific. Although this review has focused primarily on the colon, it is worth noting that the gut microbiota extends throughout the entire gastro-intestinal tract, from mouth to anus. Most studies to date have focused on the colonic microbiota, partly due to its accessibility through faecal assessment. It remains possible, however, that important changes in the gut microbiota more proximally (such as within the small intestine) occur following bariatric surgery, and play an important role in the mediation of metabolic benefits. This is particularly relevant for RYGB, and the potential impact on the ileal microbiota of the anatomical location of the alimentary limb anastomosis (which in turn influences the degree of food mixing with biliary and pancreatic juices). This should form a focus for future research.

Based on current evidence, generally the changes in the colonic microbiome following RYGB and LSG appear favourable. However, it is not clear whether such changes in the microbiome post-bariatric surgery simply reflect epiphenomena, or are implicated causally with hypothalamic control of appetite and metabolic processes. Further studies on the effects of bariatric surgery on the colonic microbiome in larger sample sizes are required, to validate the findings from the studies outlined here. To prove causality will require interventional studies (such as faecal transplantation) that manipulate the colonic microbiome in some way, with close observation of subsequent effects including appetite, metabolic processes and inflammatory and immune status. Insights gained from such interventional studies will act as a forerunner for future development of novel therapeutic approaches that manipulate the colonic microbiome, to replicate the favourable modifications that occur following bariatric surgery.

5. Expert Opinion

The human faecal microbiome, which contains approximately 100 trillion microbes, has co-evolved with our hominid ancestors over millions of years and forms a hugely complex biological system. The colon plays an essential role in the establishment and maintenance

of health and wellbeing, coordinated by its resident myriad microbiota. This includes the mediation of normal immunological development, inflammatory pathways, metabolic functioning, links with hypothalamic appetite regulation and the absorption of essential nutrients as by-products of bacterial metabolism. Such reliance on our prokaryotic cousins is not without precedent. During early eukaryotic cell development, it is thought that mitochondria originated from cellular phagocytosis of a separate prokaryotic cell. Our mitochondria probably originated from a bacterium, and form an essential component of our eukaryotic cells. Our gut microbiome is not part of us in the sense of being within our own cells, but nonetheless plays an essential role in our normal physiological functioning.

Although our understanding of the important symbiotic roles played by the gut microbiome (including the mediation of normal physiological functioning) has been transformed over recent years, it remains in its infancy. The majority of our current understanding stems from rodent-based studies, including demonstration of metabolic changes following faecal transplantation. Data from human-based studies are mainly observational in nature. Such studies usually provide association data, but proof of causality often remains elusive. However, it is clear from human-based studies that certain types of microbiota associate with a healthy body weight and metabolic profile, whilst others associate with obesity and metabolic dysfunction. Furthermore, as outlined in this review, human faecal microbiota are modifiable. Following bariatric surgery (including LSG and RYGB), there are changes in microbiota signature that appear procedure-specific. There is compelling evidence for beneficial effects of certain microbiota species, such as those that produce SCFAs. However, for the majority of identified species of microbiota, it is unclear whether their association with metabolic homeostasis or dysfunction is causal or incidental, the latter possibly as a by-product of other causal mechanism(s).

Rodent-based data provide evidence regarding possible causal roles of the gut microbiome on metabolic health, particularly studies that explore faecal transplantation between rodents, and resultant changes in body weight and metabolic processes. Bariatric surgery remains our most effective treatment strategy for facilitation of weight loss in patients with obesity. However, bariatric surgery cannot be implemented on a population-based level. Furthermore, despite being an established therapeutic option implemented globally for decades, we do not fully understand the precise mechanisms that mediate the weight loss and metabolic benefits of bariatric surgery. The current hind-gut hypothesis, based on enhanced release of incretin hormones from the distal ileum following a meal, seems incomplete and is probably an over-simplification. Data outlined in this review provide

evidence for characteristic modifications to the gut microbiome following bariatric surgery that appear to be procedure-specific. Furthermore, changes in the gut microbiome post-bariatric surgery appear mostly favourable, based on known associations of specific gut bacterial species with metabolic status. It is possible that changes in gastric pH following RYGB and LSG, and the faster transit of salivary bacteria into the ileum, may explain at least partly, the presence of facultative anaerobes in the gut microbiome following these procedures shown in some studies. It is also possible that changes in macronutrient ingestion, and eating-related behaviour (such as slower eating) following bariatric surgery may also contribute towards some of the changes in the gut microbiome.

Ultimately, weight loss and metabolic benefits of bariatric surgery are likely to result from complex mechanisms involving numerous systems. It would perhaps be an over-simplification to consider that such benefits ensue from just one overriding mechanism such as early incretin release, or even modification of the gut microbiome. However, in the context of bariatric surgery, alteration, adaption and transformation of the gut microbiome has received less attention so far. Studying the gut microbial signature prior to and following bariatric surgery may better inform clinical practice, since the gut microbiome appears to play a central role in wellbeing, and manifests intriguing connections with the brain, including hypothalamic regulation of appetite and metabolic processes. We therefore need to further explore the notion that modification(s) of the gut microbiome following bariatric surgery may mediate important metabolic benefits. An important future step will be to replicate such changes in the gut microbiome that occur following bariatric surgery, through faecal transplantation or other means in patients with obesity, and provide evidence to confirm or refute this novel and intriguing hypothesis. If confirmed, modulation and transformation of the gut microbiome would offer a novel and promising therapeutic strategy potentially on a global scale, in the effective prevention and management of obesity and metabolic dysfunction.

It is our view that the human faecal microbiome deserves the focus of future research, since almost the entire spectrum of modern-day chronic cardio-metabolic, and non-communicable ill-health is likely linked to faecal dysbiosis. Our modern, highly-processed, sterilized and fibre-impoverished diets are probably largely to blame. Such diets are the antithesis to what our hominid ancestors would have eaten, and what our species evolved to eat. Perhaps in our drive to avoid gastroenteritis and satisfy our pleasure centres that respond to the hedonic effects of food, particularly the sugar-fat combination frequently contained in our modern diet, we have deviated far from what constitutes a normal/healthy

diet [79]. A healthy faecal microbiome thrives on plant-based fibre, (which most of us simply don't eat enough of), and requires careful nurture. A useful analogy here is providing fertilizer for effective growth of plants in a garden. Fertilizer for our gut microbiome is non-digestible fibre from natural plant foods, but also non-sterilized foods (kefir and sauerkraut being examples). We should view our gut microbiome as a guardian of our health and wellbeing. A guardian that requires constant nurture. Without requisite nurture, our gut microbiome can become an adversary to our health, and instead promote chronic ill health. As we have no option to walk away from our gut microbiome, our health and wellbeing remains utterly dependent upon it. We need to nurture our gut microbiome throughout life, to maintain our friendship with it, so that it can protect our health and wellbeing as we age.

To move beyond data that merely confirm association, there is a strong imperative for the generation of a firm evidence-base from focused research in this field. This should include active transformation of faecal microbiota from dietary change or faecal transplantation, or some other novel future means of modifying the faecal microbiota (such as, for example, a faecal capsule). It remains unclear whether the weight-loss and metabolic benefits of bariatric surgery are conferred through associated changes in the faecal microbiota. To address this question directly will require replication of the characteristic changes in the faecal microbiome that occur following bariatric surgery, followed by careful observation of subsequent changes in body weight and metabolic profile. In this way, it may be possible to re-create some of the beneficial metabolic effects of bariatric surgery, without the need for surgery itself. Bariatric surgery remains our most efficacious and durable therapeutic option for effective obesity management. However, bariatric surgery is a limited resource that cannot be scalable as a viable therapeutic option on a population level. Through bypassing the need for surgery, faecal transformation, if shown to confer beneficial weight loss and metabolic effects, could potentially be implemented as an effective weight management strategy, scalable to the population level.

Funding

This paper was not funded.

Declaration of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

***Table 1**: Summary of interactions between the human colonic microbiome and human physiology*

Colonic Bacterial Species	***Association with human physiology (nutrients, metabolism, inflammation)***
Akkermansia muciniphila (*Verrucomicrobia* phylum)	• Modulates translocation of microbial molecules across gut wall [49] • Engages in symbiosis with host • Provision of nutrients accessible for other gut bacteria [51, 53] • Levels decreased in adults with obesity and Type 2 Diabetes [53]
Bacteroidetes and *Firmicutes*	• Levels increased in adults with obesity [35, 36]
Alistipes, Anaerococcus, Corpococcus, Fusobacterium Parvimonas	• Levels increased in adults with obesity [39]
Enterococcus	• Ferments dietary fibre, producing SCFAs in the process [52]
F. prausnitzii	• Associated with anti-inflammatory effects post-bariatric surgery [54] • Blockade of nuclear factor-kB activation • Inhibition of secretion of pro-inflammatory mediators [54-56]
Odoribacteraceae and *Clostridiaceae*	• Succinate-consuming colonic bacteria • Levels associated with weight loss [75]

References

1. Weickert, M.O. and A.F. Pfeiffer, *Impact of dietary fiber consumption on insulin resistance and the prevention of type 2 diabetes*. The Journal of nutrition, 2018. **148**(1): p. 7-12.
2. Weickert, M.O., et al., *Changes in dominant groups of the gut microbiota do not explain cereal-fiber induced improvement of whole-body insulin sensitivity*. Nutrition & metabolism, 2011. **8**(1): p. 90.
3. *Obesity and Overweight*. World Health Organization Factsheet, 2020. *(https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight)*.
4. Pi-Sunyer, X., *The medical risks of obesity*. Postgraduate medicine, 2009. **121**(6): p. 21-33.
5. Fruh, S.M., *Obesity: Risk factors, complications, and strategies for sustainable long-term weight management*. Journal of the American Association of Nurse Practitioners, 2017. **29**(S1): p. S3-S14.
6. Reeves, G.K., et al., *Cancer incidence and mortality in relation to body mass index in the Million Women Study: cohort study*. Bmj, 2007. **335**(7630): p. 1134.
7. Peeters, A., et al., *Obesity in adulthood and its consequences for life expectancy: a life-table analysis*. Annals of internal medicine, 2003. **138**(1): p. 24-32.
8. Mingrone, G. and L. Castagneto-Gissey, *Mechanisms of early improvement/resolution of type 2 diabetes after bariatric surgery*. Diabetes & metabolism, 2009. **35**(6): p. 518-523.
9. Thomas, S. and P. Schauer, *Bariatric surgery and the gut hormone response*. Nutrition in clinical practice, 2010. **25**(2): p. 175-182.
10. Xiang, Q., et al., *Early-Life Intervention Using Fecal Microbiota Combined with Probiotics Promotes Gut Microbiota Maturation, Regulates Immune System Development, and Alleviates Weaning Stress in Piglets*. International Journal of Molecular Sciences, 2020. **21**(2): p. 503.
11. Kim, T.T., et al., *Fecal transplant from resveratrol-fed donors improves glycaemia and cardiovascular features of the metabolic syndrome in mice*. American Journal of Physiology-Endocrinology and Metabolism, 2018. **315**(4): p. E511-E519.
12. Gérard, P., *Gut microbiome and obesity. How to prove causality?* Annals of the American Thoracic Society, 2017. **14**(Supplement 5): p. S354-S356.
13. Zhang, L., et al., *Environmental spread of microbes impacts the development of metabolic phenotypes in mice transplanted with microbial communities from humans*. The ISME journal, 2017. **11**(3): p. 676-690.
14. Clavel, T., et al., *Intestinal microbiota in metabolic diseases: from bacterial community structure and functions to species of pathophysiological relevance*. Gut microbes, 2014. **5**(4): p. 544-551.
15. Guirro, M., et al., *Effects from diet-induced gut microbiota dysbiosis and obesity can be ameliorated by fecal microbiota transplantation: A multiomics approach*. PloS one, 2019. **14**(9): e0218143.
16. Kim, K.O. and M. Gluck, *Fecal Microbiota Transplantation: An Update on Clinical Practice*. Clinical endoscopy, 2019. **52**(2): p. 137-143.
17. Qin, J., et al., *A human gut microbial gene catalogue established by metagenomic sequencing*. Nature, 2010. **464**(7285): p. 59-65.

18. Mueller, N.T., et al., *The infant microbiome development: mom matters*. Trends in molecular medicine, 2015. **21**(2): p. 109-117.
19. Hill, M., *Intestinal flora and endogenous vitamin synthesis*. European journal of cancer prevention: the official journal of the European Cancer Prevention Organisation (ECP), 1997. **6**: p. S43-5.
20. Wexler, A.G., et al., *Human gut Bacteroides capture vitamin B12 via cell surface-exposed lipoproteins*. Elife, 2018. **7**: p. e37138.
21. Shen, N., et al., *Longitudinal changes of microbiome composition and microbial metabolomics after surgical weight loss in individuals with obesity*. Surgery for Obesity and Related Diseases, 2019. **15**(8): p. 1367-1373.
22. Lee, C.J., et al., *Changes in Gut Microbiome after Bariatric Surgery Versus Medical Weight Loss in a Pilot Randomized Trial*. Obesity surgery, 2019. **29**(10): p. 3239-3245.
23. Sanmiguel, C.P., et al., *Surgically induced changes in gut microbiome and hedonic eating as related to weight loss: preliminary findings in obese women undergoing bariatric surgery*. Psychosomatic medicine, 2017. **79**(8): p. 880-887.
24. Laudadio, I., et al., *Quantitative Assessment of Shotgun Metagenomics and 16S rDNA Amplicon Sequencing in the Study of Human Gut Microbiome*. OMICS, 2018. **22**(4): p. 248-254.
25. Vandeputte, D., et al., *Quantitative microbiome profiling links gut community variation to microbial load*. Nature, 2017. **551**(7681): p. 507-511.
26. Kristensen, N.B., et al., *Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials*. Genome medicine, 2016. **8**(1): p. 52.
27. Eckburg, P.B., et al., *Diversity of the human intestinal microbial flora*. science, 2005. **308**(5728): p. 1635-1638.
28. Hold, G.L., et al., *Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis*. FEMS Microbiol Ecol, 2002. **39**(1): p. 33-9.
29. Hayashi, H., M. Sakamoto, and Y. Benno, *Phylogenetic analysis of the human gut microbiota using 16S rDNA clone libraries and strictly anaerobic culture-based methods*. Microbiol Immunol, 2002. **46**(8): p. 535-48.
30. Ottman, N., et al., *The function of our microbiota: who is out there and what do they do?* Frontiers in cellular and infection microbiology, 2012. **2**: p. 104.
31. Lozupone, C.A., et al., *Diversity, stability and resilience of the human gut microbiota*. Nature, 2012. **489**(7415): p. 220-230.
32. Patel, R.M. and P.W. Denning, *Intestinal microbiota and its relationship with necrotizing enterocolitis*. Pediatric research, 2015. **78**(3): p. 232-238.
33. Ley, R.E., et al., *Human gut microbes associated with obesity*. nature, 2006. **444**(7122): p. 1022-1023.
34. Sánchez-Alcoholado, L., et al., *Gut microbiota adaptation after weight loss by Roux-en-Y gastric bypass or sleeve gastrectomy bariatric surgeries*. Surgery for Obesity and Related Diseases, 2019. **15**(11): p. 1888-1895.
35. Ley, R.E., et al., *Obesity alters gut microbial ecology*. Proceedings of the National Academy of Sciences, 2005. **102**(31): p. 11070-11075.
36. Andoh, A., et al., *Comparison of the gut microbial community between obese and lean peoples using 16S gene sequencing in a Japanese population*. Journal of clinical biochemistry and nutrition, 2016. **59**(1): p. 65-70.

37. Chen, M.X., et al., *Metabolome analysis for investigating host-gut microbiota interactions.* J Formos Med Assoc, 2019. **118 Suppl 1**: p. S10-S22.
38. Bravo, J.A., et al., *Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve.* Proceedings of the National Academy of Sciences, 2011. **108**(38): p. 16050-16055.
39. Goehler, L.E., et al., *Activation in vagal afferents and central autonomic pathways: early responses to intestinal infection with Campylobacter jejuni.* Brain, behavior, and immunity, 2005. **19**(4): p. 334-344.
40. Wikoff, W.R., et al., *Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites.* Proceedings of the national academy of sciences, 2009. **106**(10): p. 3698-3703.
41. Yano, J.M., et al., *Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis.* Cell, 2015. **161**(2): p. 264-276.
42. Haghikia, A., et al., *Dietary fatty acids directly impact central nervous system autoimmunity via the small intestine.* Immunity, 2015. **43**(4): p. 817-829.
43. Ramirez-Perez, O., et al., *The Role of the Gut Microbiota in Bile Acid Metabolism.* Ann Hepatol, 2017. **16 Suppl 1**: p. S21-S26.
44. Jia, W., G. Xie, and W. Jia, *Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis.* Nat Rev Gastroenterol Hepatol, 2018. **15**(2): p. 111-128.
45. Weickert, M.O., et al., *Effects of supplemented isoenergetic diets varying in cereal fiber and protein content on the bile acid metabolic signature and relation to insulin resistance.* Nutr Diabetes, 2018. **8**(1): p. 11.
46. Chassaing, B., et al., *Colonic microbiota encroachment correlates with dysglycemia in humans.* Cellular and molecular gastroenterology and hepatology, 2017. **4**(2): p. 205-221.
47. Derrien, M., et al., *Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium.* International journal of systematic and evolutionary microbiology, 2004. **54**(5): p. 1469-1476.
48. Clarke, S.F., et al., *Exercise and associated dietary extremes impact on gut microbial diversity.* Gut, 2014. **63**(12): p. 1913-1920.
49. Hoskins, L.C. and E.T. Boulding, *Mucin degradation in human colon ecosystems: evidence for the existence and role of bacterial subpopulations producing glycosidases as extracellular enzymes.* The Journal of clinical investigation, 1981. **67**(1): p. 163-172.
50. de Vos, W.M., *Microbe Profile: Akkermansia muciniphila: a conserved intestinal symbiont that acts as the gatekeeper of our mucosa.* Microbiology, 2017. **163**(5): p. 646-648.
51. Furet, J.-P., et al., *Differential adaptation of human gut microbiota to bariatric surgery–induced weight loss: links with metabolic and low-grade inflammation markers.* Diabetes, 2010. **59**(12): p. 3049-3057.
52. Remely, M., et al., *Gut microbiota of obese, type 2 diabetic individuals is enriched in Faecalibacterium prausnitzii, Akkermansia muciniphila and Peptostreptococcus anaerobius after weight loss.* Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders), 2016. **16**(2): p. 99-106.

53. Graessler, J., et al., *Metagenomic sequencing of the human gut microbiome before and after bariatric surgery in obese patients with type 2 diabetes: correlation with inflammatory and metabolic parameters.* The pharmacogenomics journal, 2013. **13**(6): p. 514-522.
54. Brahe, L.K., A. Astrup, and L.H. Larsen, *Is butyrate the link between diet, intestinal microbiota and obesity-related metabolic diseases?* Obesity reviews, 2013. **14**(12): p. 950-959.
55. den Besten, G., et al., *The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism.* Journal of lipid research, 2013. **54**(9): p. 2325-2340.
56. Li, X., Y. Shimizu, and I. Kimura, *Gut microbial metabolite short-chain fatty acids and obesity.* Bioscience of microbiota, food and health, 2017. **36**(4): p. 135-140.
57. Samuel, B.S., et al., *Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41.* Proceedings of the National Academy of Sciences, 2008. **105**(43): p. 16767-16772.
58. Tolhurst, G., et al., *Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2.* Diabetes, 2012. **61**(2): p. 364-71.
59. Chambers, E.S., et al., *Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults.* Gut, 2015. **64**(11): p. 1744-1754.
60. Serena, C., et al., *Elevated circulating levels of succinate in human obesity are linked to specific gut microbiota.* The ISME journal, 2018. **12**(7): p. 1642-1657.
61. Clark, A. and N. Mach, *The Crosstalk between the Gut Microbiota and Mitochondria during Exercise.* Front Physiol, 2017. **8**: p. 319.
62. Rose, S., et al., *Butyrate enhances mitochondrial function during oxidative stress in cell lines from boys with autism.* Transl Psychiatry, 2018. **8**(1): p. 42.
63. Lee, P., B.R. Yacyshyn, and M.B. Yacyshyn, *Gut microbiota and obesity: An opportunity to alter obesity through faecal microbiota transplant (FMT).* Diabetes Obes Metab, 2019. **21**(3): p. 479-490.
64. Weickert, M.O. and A.F. Pfeiffer, *Metabolic effects of dietary fiber consumption and prevention of diabetes.* J Nutr, 2008. **138**(3): p. 439-42.
65. Topping, D.L. and P.M. Clifton, *Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides.* Physiol Rev, 2001. **81**(3): p. 1031-64.
66. Kootte, R.S., et al., *Improvement of Insulin Sensitivity after Lean Donor Feces in Metabolic Syndrome Is Driven by Baseline Intestinal Microbiota Composition.* Cell Metab, 2017. **26**(4): p. 611-619 e6.
67. Kovatcheva-Datchary, P., et al., *Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of Prevotella.* Cell Metab, 2015. **22**(6): p. 971-82.
68. Backhed, F., et al., *Mechanisms underlying the resistance to diet-induced obesity in germ-free mice.* Proc Natl Acad Sci U S A, 2007. **104**(3): p. 979-84.
69. Guo, Y., et al., *Association between Polycystic Ovary Syndrome and Gut Microbiota.* PLoS One, 2016. **11**(4): p. e0153196.

70. Alley, J.B., *Comment on: Trends in utilization of bariatric surgery, 2010-2014: Sleeve gastrectomy dominates.* Surgery for Obesity and Related Diseases, 2017. **13**(5): p. 778-779.
71. Han, Y., et al., *Comparative analysis of weight loss and resolution of comorbidities between laparoscopic sleeve gastrectomy and Roux-en-Y gastric bypass: A systematic review and meta-analysis based on 18 studies.* International Journal of Surgery, 2020. **76**: p. 101-110.
72. Golzarand, M., K. Toolabi, and R. Farid, *The bariatric surgery and weight losing: a meta-analysis in the long- and very long-term effects of laparoscopic adjustable gastric banding, laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy on weight loss in adults.* Surg Endosc, 2017. **31**(11): p. 4331-4345.
73. Peterli, R., et al., *Effect of Laparoscopic Sleeve Gastrectomy vs Laparoscopic Roux-en-Y Gastric Bypass on Weight Loss in Patients With Morbid Obesity: The SM-BOSS Randomized Clinical Trial.* JAMA, 2018. **319**(3): p. 255-265.
74. Salminen, P., et al., *Effect of Laparoscopic Sleeve Gastrectomy vs Laparoscopic Roux-en-Y Gastric Bypass on Weight Loss at 5 Years Among Patients With Morbid Obesity: The SLEEVEPASS Randomized Clinical Trial.* JAMA, 2018. **319**(3): p. 241-254.
75. Liu, R., et al., *Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention.* Nature medicine, 2017. **23**(7): p. 859.
76. Damms-Machado, A., et al., *Effects of surgical and dietary weight loss therapy for obesity on gut microbiota composition and nutrient absorption.* BioMed research international, 2015. Article ID: 806248 (12 pages)
77. Zhang, H., et al., *Human gut microbiota in obesity and after gastric bypass.* Proceedings of the National Academy of Sciences, 2009. **106**(7): p. 2365-2370.
78. Stemmer, K., et al., *Roux-en-Y gastric bypass surgery but not vertical sleeve gastrectomy decreases bone mass in male rats.* Endocrinology, 2013. **154**(6): p. 2015-2024.
79. Sonnenburg, E.D., et al., *Diet-induced extinctions in the gut microbiota compound over generations.* Nature, 2016. **529**(7585): p. 212-5.