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Leading Edge

Review

Digesting the complex metabolic effects of diet on the host and microbiome

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SUMMARY

The past 50 years of interdisciplinary research in humans and model organisms has delivered unprecedented insights into the mechanisms through which diet affects energy balance. However, translating these results to prevent and treat obesity and its associated diseases remains challenging. Given the vast scope of this literature, we focus this Review on recent conceptual advances in molecular nutrition targeting the management of energy balance, including emerging dietary and pharmaceutical interventions and their interactions with the human gut microbiome. Notably, multiple current dietary patterns of interest embrace moderate-to-high fat intake or prioritize the timing of eating over macronutrient intake. Furthermore, the rapid expansion of microbiome research findings has complicated multiple longstanding tenets of nutrition while also providing new opportunities for intervention. Continued progress promises more precise and reliable dietary recommendations that leverage our growing knowledge of the microbiome, the changing landscape of clinical interventions, and our molecular understanding of human biology.

INTRODUCTION

Molecular nutrition, which addresses the effects of diet on whole-organism physiology at the molecular level, is a relatively young field, rising to prominence in the early 20th century.[1] By the 1950s, all the essential vitamins and minerals had been discovered and linked to diseases such as scurvy and neural tube defects in infants.[1] However, research into vitamins and other micronutrients declined due to the advent of multivitamin supplements and the micronutrient fortification of staple foods.[1]

Subsequently, nutrition researchers began to turn their attention to macronutrients and chronic disease. For instance, dietary saturated fat has been studied in depth due to its reported correlation with cardiovascular disease (CVD) in epidemiological studies.[2] Yet, randomized controlled trials (RCTs) aimed at limiting saturated fat intake have had more mixed results.[3] Despite some notable early successes,[4,5] a recent 8-year RCT in >48,000 subjects resulted in modest improvements in CVD risk markers but no change in disease endpoints.[6] Similarly, a recent massive epidemiological study failed to replicate the original associations.[7] High-fat diets are also insufficient to elicit CVD in laboratory mice, requiring the use of transgenic animals, surgery, or drugs.[8] Together, these results indicate that dietary fat is just one piece in a more complex puzzle that includes dietary sugar[9] and a broader consideration of the types and sources of macronutrients.

Given the challenges in identifying individual macronutrients that are consistently associated with chronic disease risk across populations, the research community began to move away from reductionist approaches toward higher-level dietary patterns and total caloric intake (Figure 1A).[10,11] The earlier focus on CVD was overshadowed by the dramatic rise in obesity, which is associated with a marked increase in the risk of several debilitating diseases, including but not limited to type 2 diabetes (T2D), cancer, CVD (e.g., stroke), liver disease, and sleep apnea.[12] Since 1970, the rate of obesity in the US has tripled, representing an increase in the average body mass index (BMI) of 16% (from 25.7 to 29.8 kg/m^2).[13] Women, children, and certain racial/ethnic groups have been disproportionately affected.[14] This shift is often attributed to a combination of increased caloric intake (~200 kcal/d)[15] and decreased work-related energy expenditure (~100 kcal/d; Figure 1B)[16]; however, the underlying etiology is likely multifactorial.

Given the major health complications of obesity, there is widespread interest among the public and scientific community in identifying effective dietary interventions to prevent or treat excess adiposity. In this Review, we discuss some of the surprising shifts in our concept of a healthy diet over the past 50 years and how the advent of a new generation of obesity medications is changing the landscape of nutrition research and clinical practice. We highlight the emerging links between dietary interventions and the trillions of microorganisms that inhabit the human gastrointestinal tract (gut microbiota), their aggregate genomes and metabolic products (gut microbiome), and the effects of diet-induced changes in the gut microbiome on human energy metabolism.

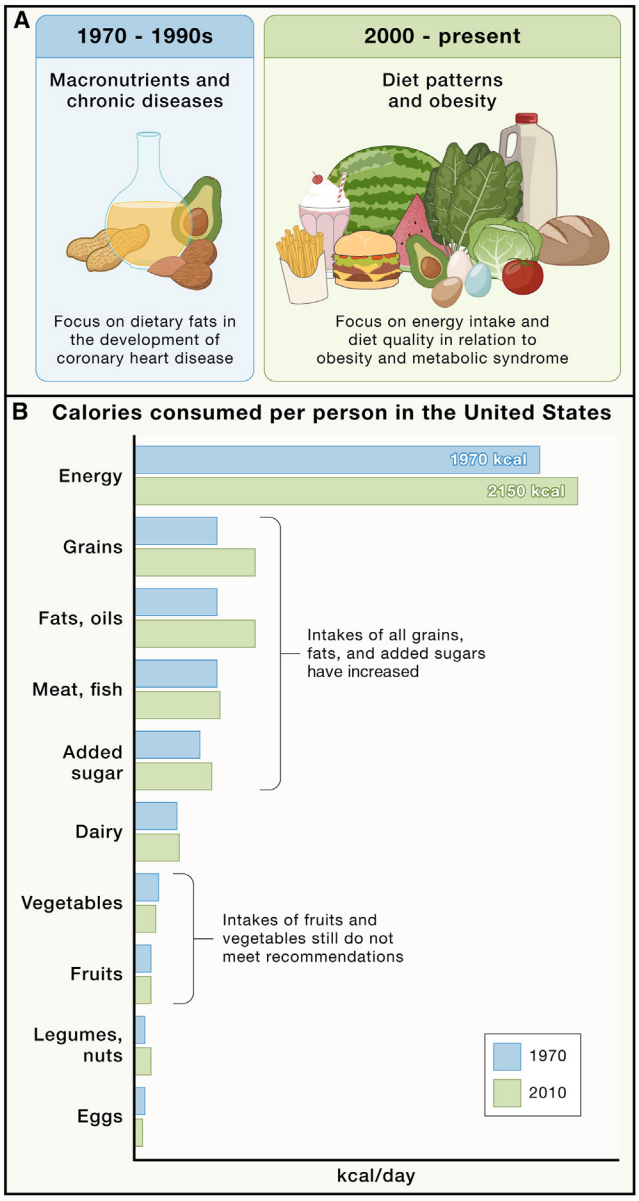

Figure 1. Nutrition research and diet quality changes over the past five decades

(A) The past 50 years of research primarily focused on the role of specific macronutrients (especially dietary fats) in the development of chronic diseases, such as coronary heart disease. By the 2000s, nutrition research shifted toward examining how overall diet quality impacted health metrics with a focus on obesity and metabolic syndrome.

(B) Shifts in the US diet over the past 50 years.[17] Adults consume ~200 more calories per day, consume more refined grains and added sugars, and still do not meet recommended levels of fruit and vegetable intake. Created with BioRender.com.

In just the past two decades, our understanding of human nutrition and energy balance has been revolutionized by rapidly emerging knowledge of the gut microbiome and its intimate, manifold influences on physiology. Work in model organisms has implicated the gut microbiome in the regulation of hunger and satiety, nutrient digestibility, and the allocation of energy across tissues and to alternative fates such as storage versus thermogenesis.[18,19] Human studies have also suggested a role for the gut microbiome in the host response to many popular dietary interventions, including very-low-calorie diets (VLCDs) and more moderate calorie restriction[20,21] as well as Mediterranean,[22] ketogenic,[23] and intermittent fasting[24] dietary patterns. The effects of diet on the gut microbiome and its potential downstream consequences for host energy metabolism have also challenged several of the deepest-rooted ideas in nutrition, including the basic concept of caloric value, the biological importance of assimilated versus unabsorbed dietary components, and the components of diet generally regarded as safe for consumption.

Ultimately, advancing knowledge of molecular nutrition that encapsulates both human and microbial contributions provides new targets for precision nutrition—personalized dietary advice based on an individual's genetic background, microbiome, health status, and lifestyle. Precision nutrition acknowledges that each person's metabolic response to diet will differ, and therefore broad dietary guidelines targeting population-level health will be suboptimal at the individual level. Indeed, large-scale studies such as the Personalized Responses to Dietary Composition Trial (PREDICT) have emphasized that individuals fed identical meals can exhibit substantial differences in postprandial metabolic response, including differences in blood glucose, insulin, and triglycerides.[25]

Over the past 50 years, dramatic improvements in the efficiency and cost of DNA sequencing have made it feasible to develop individual genome-informed dietary recommendations, such as gluten-free diets for individuals carrying human leukocyte antigen (HLA) DQ2 or DQ8 alleles that confer increased risk for celiac disease,[26] phenylalanine-free diets for individuals homozygous for inactivating mutations in the phenylalanine hydroxylase (PAH) gene,[27] and low-lactose diets for individuals lacking one of several genomic mutations enabling the continued expression of lactase into adulthood.[28] Human-genome-informed recommendations are likewise being pursued for T2D, obesity, and hypertension.[29] Additionally, several large-scale studies have begun to incorporate microbiome concepts into precision nutrition. For instance, a study of postprandial blood glucose response found that predictive models incorporating gut microbiome composition substantially outperformed those based on host, dietary, and physical activity factors alone.[30] Microbiome profiling in the PREDICT study also revealed widespread associations between the relative abundance of microbial taxa; the metabolic response to various diets, food groups, food items, and nutrients; and their interaction with biomarkers of metabolic health.[31] The field of precision nutrition awaits additional large-scale trials establishing efficacy, development of diagnostic tools, integration of relevant concepts and methods into medical training, and attention to ethical issues of access and privacy. However, these early demonstrations that the black box surrounding interindividual variation in dietary response can be cracked open, with the information effectively leveraged to improve postprandial health outcomes (e.g., glucose response[30]), suggest enormous potential for nutrition to contribute meaningfully to broader efforts toward personalized medicine.

Importantly, our decision to focus the scope of this Review on recently proposed diets, energy balance, and the gut microbiome

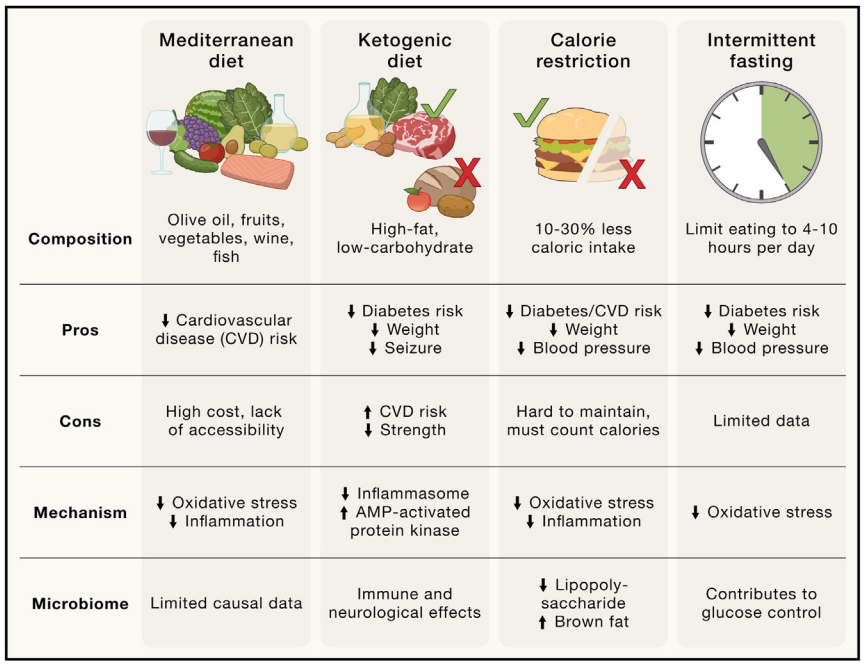

Figure 2. Key aspects of the four eating patterns highlighted in this Review

The eating patterns discussed herein accommodate moderate-to-high fat intake but differ in their guidelines, benefits, risks, mechanisms of action, and reported contributions of the gut microbiome. Created with BioRender.com.

has precluded the ability to more comprehensively describe other major developments since *Cell* was launched. To name a few, these include a detailed discussion of dietary sugars and more complex oligosaccharides and plant polysaccharides; the increased adoption of plant-based diets; the advent of genetically modified crops, livestock, and microorganisms; and ongoing debates over the health relevance of different forms of macronutrients (e.g., between omega-3 and omega-6 polyunsaturated fatty acids[32]). Our Review also does not address critical advances in dietary and microbiome-directed interventions targeting undernutrition, which affects over half a billion people globally and contributes to 45% of all deaths among children under 5 years of age.[33] The field of nutrition is massive and spans multiple scales from chemicals to populations. We hope that the limited areas that we touch on here will provide a view of the rapid pace of progress and more generalizable concepts that could be applied to these other areas of study.

EMERGING DIETARY INTERVENTIONS

One of the most surprising developments in human nutrition, given the long history of guidelines to restrict fat intake, is the recent emergence of multiple dietary interventions that embrace moderate to high levels of dietary fat. In this section, we will discuss four examples of dietary patterns that do not require strict limits on fat intake, their conceptual bases, their potential utility in mitigating the rise in obesity, their proposed mechanisms of action, and their links to the gut microbiome.

The Mediterranean diet (MD)

The Mediterranean eating pattern is inspired by the dietary traditions of Greece and Italy and is rich in minimally processed whole grains, legumes, fruits, vegetables, nuts, seeds, and olive oil (Figure 2). RCTs support a beneficial impact of the MD on multiple markers of disease, including body weight, BMI, blood pressure, LDL-cholesterol, triglycerides, insulin resistance, and the circulating inflammatory markers interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α).[34,35] A prospective study of 25,315 women in the US revealed a 23% reduction in all-cause mortality among those who adhered to a Mediterranean eating pattern over a 25-year follow-up.[36] Data from this study also revealed that higher MD intake was associated with a 30% reduction in future risk of T2D over a 20-year follow-up.[37] The MD eating pattern may also be protective against cancer. Indeed, high adherence to this diet has been shown to be associated with lower cancer mortality in the general population, reduced all-cause mortality among cancer survivors, and lower risk of developing colorectal, head, neck, respiratory, gastric, liver, and bladder cancers.[38] Taken together, these results support the pathophysiological importance of the specific types of foods consumed, as opposed to simply focusing on summary statistics such as caloric intake or macronutrient levels.

Despite these encouraging results in large-scale clinical trials, the mechanisms through which the MD acts to prevent disease remain poorly understood. Many interrelated and overlapping mechanisms have been hypothesized to play a role, including a lipid-lowering effect; protection against oxidative stress, inflammation, and platelet aggregation; modification of hormones and growth factors involved in the pathogenesis of cancer; and the inhibition of nutrient-sensing pathways by specific amino acid restriction.[39]

The role of the gut microbiome in the beneficial effects of the MD also remains poorly understood. Adherence to the MD has been associated with an increased relative abundance of fiber-degrading taxa such as *Faecalibacterium prausnitzii*, *Roseburia*, *Eubacterium eligens*, and *Bacteroides cellulosilyticus*.[40–42] These and other microbiota differences are associated with beneficial metabolic phenotypes such as decreased plasma cholesterol in individuals with excess body weight,[42] improved cognitive function and reduced inflammation and frailty in older adults,[41] and reduced risk of myocardial infarction in individuals simultaneously harboring low levels of *Prevotella copri*.[40] However, consistent associations between adherence to the MD and specific gut bacterial taxonomic abundances have been elusive.[43,44] This work is complicated by the fact that multiple

components of the MD can impact the gut microbiota, including fiber,[45] polyphenols,[46,47] and overall fruit and vegetable intake.[48]

A recent study provided a proof-of-concept for identifying specific components of the MD sufficient to induce the metabolic effects typically attributed to the whole dietary pattern. In humans, weight regain after weight loss was suppressed to a greater extent by autologous fecal microbiota transplantation (aFMT) if microbiota were conditioned on an MD containing green tea and *Wolffia globosa* (duckweed) versus an MD lacking these items.[22] Follow-up mouse experiments confirmed an independent effect of the *Wolffia globosa* Mankai strain; mice treated with Mankai during weight loss and aFMT during subsequent high-fat-diet feeding exhibited lesser weight regain and glucose intolerance than no-Mankai controls, despite both groups losing similar amounts of weight during the weight loss phase.[22] The role of specific dietary components may explain why a similar study in humans that used heterologous FMT (from a lean donor) paired with a generalized MD failed to detect any synergy,[49] although it may also be that exposure to strains adapted to each recipient via aFMT contributes to enhanced effects.

Ketogenic diets (KDs)

Another surprising development over the past 50 years is the broader application of extremely low-carbohydrate, high-fat diets characterized by their ability to induce ketogenesis—the oxidation of lipid stores to produce ketone bodies that support organ function when carbohydrate intake is limited.[50] These ketone bodies (KBs) include β-hydroxybutyrate (βHB), acetoacetate, and acetone and are produced primarily in the liver.[50] In humans, the induction of ketosis requires severe restriction of carbohydrate intake (5%–10% kcal/d), moderate protein intake (30%–35%), and high fat intake (55%–60%).[51]

KDs have been used since 1921 to treat epilepsy but were not widely considered for metabolic disease until the 1970s, based on the hypothesis that lowering carbohydrate intake would improve glycemic control in the management of T2D.[52] Hundreds of clinical trials have tested the effects of KDs; an umbrella review concluded that KDs produce slightly more weight loss (1%–2%) over 6–12 months compared to low-fat diets (Figure 2).[53] Benefits for T2D-related endpoints have been observed,[53] including decreased HbA1c, decreased insulin resistance, and even diabetes remission over 6–12 months compared to low-fat diets.[54]

Yet, KDs have multiple potential downsides, including an elevated risk of CVD, nutrient deficiencies, or other complications of high protein intake. KDs have been reported to alter CVD risk markers, with a marked increase in LDL-cholesterol and a more subtle increase in HDL-cholesterol.[53] Other potential adverse effects of KDs can include inadequate intake of essential vitamins and minerals due to reduced fruit and vegetable intake.[55] Muscle wasting has also been observed, which can negatively impact athletic performance.[56] High levels of methionine and branched-chain amino acid (BCAA) intake could increase risk of metabolic disease due to the activation of the mammalian target of rapamycin (mTOR) pathway.[57] Finally, increased consumption of meats and dairy products could promote the production of potentially damaging gut bacterial metabolites, such as trimethylamine[58] (discussed in detail later in this Review) and hydrogen sulfide.[59]

Multiple human studies have suggested that KDs can alter the structure and function of the gut microbiome,[59,60] but these dietary interventions involved substantial concomitant changes in caloric and macronutrient intake that make it hard to disentangle the effects of ketogenesis from those of the underlying dietary change. By contrast, a recent study found that KDs altered the gut microbiome in humans and mice and that these effects were distinct from those observed in non-ketogenic high-fat diets.[23] In an 8-week inpatient study, 17 non-diabetic men with overweight or obesity were fed a 4-week baseline diet followed by a 4-week KD that predictably increased plasma KBs. The KD led to changes in the relative abundance of several bacterial taxa and an altered fecal metabolite profile compared with the baseline diet. Follow-up experiments in mice fed matched semi-purified ketogenic versus high-fat diets or gradient diets with progressively decreasing levels of carbohydrates found that ketogenic and high-fat diets alter *Firmicutes* and *Bacteroidetes* abundances in opposite directions (ketogenic favoring *Bacteroidetes*, high-fat favoring *Firmicutes*), with KDs uniquely reducing bifidobacteria.[23]

KDs also have applications outside of metabolic disease, including epilepsy and autoimmune disease. A KD protected from seizures in conventional but not antibiotic-depleted or germ-free (GF) mice.[61] Transplantation of the KD-associated mouse or human gut microbiota or individual KD-associated members of the *Akkermansia* and *Parabacteroides* genera was sufficient to protect from seizures in mice.[61,62] The KD-altered human gut microbiota also have a diminished ability to induce small intestinal T helper 17 (Th17) cells,[23] which are broadly relevant for multiple immune-related diseases. Work in an experimental autoimmune encephalomyelitis (EAE) mouse model of neuroinflammation revealed that a KD protects against EAE in a microbiota-dependent manner.[63] The effects of a KD on the immune system and gut microbiome have also been studied in human subjects, following 2 weeks of a vegan diet versus a KD.[64] Consistent with prior work,[59] these highly divergent diets led to distinct microbial taxonomic and gene abundance signatures alongside concomitant shifts in immune profiles in whole blood.[64]

There is also increasing interest in studying the capacity of dietary KB supplementation to recapitulate aspects of KDs. In addition to supplying a substrate for cellular metabolism, KBs exert polypharmacological effects on many systems throughout the body, including the immune system[50] and the microbiome.[23,63] Data from humans, rodents, and cell culture support the ability of βHB to inhibit the NLRP3 inflammasome.[65] High levels of KBs can decrease blood pressure and increase vascular function.[66] Augmented levels of circulating KBs can also reduce cardiac inflammation and the probability of heart failure.[66,67] KBs may also improve insulin sensitivity by stimulating the insulin receptor via inducing AMP-activated protein kinase (AMPK) and downregulating mTOR.[68] In addition, high levels of KBs can potentially decrease appetite, thereby promoting weight loss.[68,69]

Calorie restriction (CR)

Another diet therapy that has garnered tremendous attention in the past 50 years is daily CR, defined as a reduction in dietary

intake below energy requirements for weight maintenance while maintaining adequate nutrition. Findings from observational, preclinical, and clinical trials have suggested that CR may increase lifespan by 1–5 years while also improving healthspan and quality of life.[70]

The most rigorous randomized trials of CR come from the National Institute of Aging-sponsored CALERIE (Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy) Consortium.[70,71] The study consists of CALERIE PHASE 1 (three small 6- to 12-month pilot studies of CR) followed by CALERIE PHASE 2 (one large, multi-site, 2-year randomized trial of CR). Each of these studies recruited normal weight adults who were otherwise healthy.[70,71] The degree of CR implemented in each trial varied but generally involved daily decreases in energy intake ranging from 10% to 30% with adequate intake of other key nutrients.[70,71] Findings from the CALERIE studies reveal that both short- and long-term CR can reduce body weight, subcutaneous fat, visceral fat, and intrahepatic lipid content.[70,71] These improvements were associated with several cardiometabolic benefits, including increased insulin sensitivity, enhanced pancreatic β cell function, and reduced fasting insulin.[70,71] Targeted metabolomic studies also showed that CR improved metabolic flexibility by augmenting fatty acid oxidation intermediates from the fasting to postprandial state. Reductions in blood pressure, LDL-cholesterol, and triglyceride levels and increases in HDL-cholesterol levels were also observed after 2 years of CR.[70,71] In addition, 6 months of CR induced a 29% reduction in the 10-year risk for developing CVD.[70,71] This risk reduction was attributed to decreases in oxidative stress and inflammation as well as the preservation of endothelial nitric oxide functions that occur with CR.[70,71]

A study in mice suggested that the gut microbiome is an important mediator of CR-induced metabolic benefits, including weight loss, insulin sensitivity, and adipocyte browning.[72] CR decreased microbial expression of enzymes enabling lipid A biosynthesis, thus limiting lipopolysaccharide (LPS) production and suppressing the LPS-TLR4 pathway in a manner that has been shown pharmacologically to stimulate adipocyte browning and reduce visceral fat. Correspondingly, GF, antibiotic-treated, TLR4$^{-/-}$, and LPS-supplemented mice exhibited resistance to CR-induced metabolic effects. Critically, transplantation of CR-conditioned versus control gut microbiota into untreated GF mice led to lower gains in weight and body fat, higher insulin sensitivity, and increased UCP1^{+} (i.e., brown/thermogenic) adipocytes, demonstrating that CR-induced changes in the gut microbiome contribute causally to these effects.

It remains unclear whether the gut microbiome contributes similarly to CR phenotypes in humans. CR studies in humans have reported various changes to gut microbiome composition and function,[21,73–75] but to our knowledge, none have demonstrated that these changes underpin observed metabolic benefits. A recent RCT comparing the effects of 12 weeks of intermittent versus continuous CR among 147 adults with overweight or obesity found no associations between weight loss and changes in bacterial relative abundance, community α-diversity, or circulating microbial metabolites (e.g., short-chain fatty acids [SCFAs]).[21] Nevertheless, baseline microbiome composition—specifically, the relative abundance of *Dorea*—weakly predicted CR-induced weight loss. Similarly, a prospective study involving 14 weeks of CR among 80 adults with overweight or obesity found that ≥5% weight loss was positively correlated with *Collinsella* and *Christensenellaceae* abundance and negatively correlated with *Escherichia/Shigella*, *Klebsiella*, *Megasphaera*, *Sellimonas*, and *Lactobacillus* abundance.[73] Various associations between microbiome features and specific metabolic health markers have also been reported—for instance, between *Akkermansiaceae* and *Christensenellaceae* and HOMA-IR-based insulin sensitivity.[21] Additional functional studies are required to test whether these links between microbiome profile and metabolic response are causal versus collinear outcomes of other physiological states.

Intermittent fasting

One potential solution for the challenges of initiating and maintaining a major shift in dietary pattern comes from encouraging data that intermittent fasting can lead to significant weight loss.[76,77] The most common form of intermittent fasting is time-restricted eating (TRE), which involves limiting the eating window to 4–10 h and fasting for the remaining hours of the day (14–20 h fast).[78] During the eating window, individuals are not required to count calories or monitor food intake in any way, and this simplicity likely accounts for recent rises in TRE popularity.[76,78] During the fasting window, individuals are encouraged to drink plenty of water and may also consume energy-free beverages such as tea and coffee without additives. When adults with obesity limit their eating window to 4–10 h per day, they typically reduce energy intake by 200–550 kcal/d, a degree of energy restriction on par with that of daily CR.[76]

Data from RCTs suggest that TRE is effective for lowering body weight and improving some markers of cardiovascular health.[76,78] Body weight is typically reduced by 3%–5% after 2–12 months of TRE, with reductions coming mainly from decreases in fat mass and visceral fat mass rather than lean mass.[76,78] However, not all studies of TRE in humans have reported weight loss. Lowe and colleagues[79] demonstrated that 3 months of an 8 h TRE (12–8 p.m. eating window) regimen had no effect on body weight in adults with obesity compared to no-intervention controls. However, this study was conducted in free-living participants who had minimal contact with the research team throughout the trial, highlighting the importance of regular interactions with clinicians to bolster weight loss success.[79,80] Even when weight loss is achieved, not all subjects exhibit metabolic improvements.[81] Blood pressure is typically lowered by 5–10 mmHg after 2–12 months of TRE, but these effects were generally only noted when eating windows were situated earlier in the day (i.e., before 2 p.m.).[76,78] Early eating windows may improve blood pressure by facilitating natriuresis (sodium excretion in the urine via the kidneys) by shifting salt consumption to earlier in the day when sodium excretion is upregulated by the circadian system.[82] TRE does not appear to impact LDL-cholesterol, HDL-cholesterol, or triglyceride levels relative to controls.[76,78] Circulating inflammatory markers, such as c-reactive protein (CRP), IL-6, and TNF-α, are also not impacted by TRE, although data are limited.[76]

However, TRE appears to have important effects on glycemic control.[83] Clinical trial findings demonstrate fairly consistent

TRE-induced improvements in fasting insulin and insulin sensitivity in individuals with prediabetes and obesity.[83,84] TRE also improves glucose tolerance and decreases serum glucose excursions. These improvements are noted more often with early eating windows (i.e., eating all food before 3 p.m.) and with shorter eating windows (4–6 h). In adults with T2D, TRE improves HbA1c levels to the same extent as daily CR and does not increase the risk for hypoglycemia.[85] Interestingly, meal skipping has been associated with all-cause and CVD mortality[86]; however, the relevance of these data to intentional TRE remains unclear.

Several possible mechanisms may explain how TRE improves glucoregulation. Data from human trials show that the body undergoes a metabolic switch during TRE. After 12 to 36 h of fasting, the body switches from utilizing glucose (from glycogen stores) to utilizing fatty acids and fatty-acid-derived KBs for energy.[83,87] Adipocytes liberate free fatty acids, which are then transported to the liver and converted into KBs, leading to mild ketonemia and improved insulin sensitivity.[88] Specifically, cells adapt to mild ketonemia by activating signaling pathways that augment antioxidant defenses, leading to a subsequent decrease in oxidative stress, downstream changes in serine/threonine kinase-mediated stress response pathways, and ultimately improvement in both β cell function and insulin signaling.[83,87] For instance, human trials show that TRE decreases circulating levels of 8-isoprostane (a marker of oxidative stress to lipids) and that these reductions are associated with improvements in insulin sensitivity.[89,90] Other studies have shown improvements in insulin sensitivity when administering antioxidants such as vitamin E.[91] Therefore, it is possible that TRE improves insulin sensitivity by decreasing oxidative stress in response to flipping the metabolic switch.

Studies in mice have revealed that time-restricted feeding (TRF) can attenuate the effects of high-fat, high-sugar (HFHS) diets on the gut microbiota.[92] Consumption of HFHS diets disrupts diurnal variation in the relative abundance of gut bacterial taxa.[92,93] A model of TRF in which food was removed during the light phase rescued some of these oscillations and protected against diet-induced obesity.[92] These effects on the gut microbiota were most marked within the distal small intestine (ileum) and corresponded to an increased expression of the proglucagon gene *Gcg* and elevated plasma levels of the hormone glucagon-like peptide-1 (GLP-1).[94] Data from antibiotic-treated and GF mice support a causal role of the gut microbiota in diurnal GLP-1 release[95]; however, the microbial effectors remain unclear. A proof-of-concept comes from studies of the gut commensal *Akkermansia muciniphila,* which secretes an 84 kDa protein (P9) that is sufficient to induce GLP-1 secretion due to interacting with the intercellular adhesion molecule 2 (ICAM-2).[96] More work is needed to understand the full range of microorganisms involved in glucoregulatory and other beneficial effects of TRE, as well as their clinical relevance. Notably, a recent clinical study comparing CR versus an energy-matched TRE plus protein pacing (defined as four evenly spaced meals/day; TRE-P) regimen in adults with overweight or obesity found TRE-P to be associated with more pronounced changes in gut microbiome composition, including enrichments in taxa previously linked to weight loss and protein consumption, such as *Christensenellaceae*.[97,98] Additionally, differences in gut microbiome composition and functional capacity were observed between participants showing high versus low weight loss during the TRE-P intervention, but whether these microbiome changes contribute causally to observed TRE-P-induced improvements in metabolism remains unclear.

A NEW GENERATION OF WEIGHT LOSS MEDICATIONS

Given the many challenges and limitations of dietary interventions for obesity and its associated metabolic diseases, there has long been interest in developing effective pharmaceuticals for weight loss. Multiple prior attempts have been made, including the infamous fenfluramine-phentermine (Fen-Phen) treatment introduced in the 1990s that was later linked to severe heart valve defects.[99] Alternatives include orlistat, phentermine/topiramate, and bupropion/naltrexone; however, their widespread adoption has been limited by numerous common adverse effects, especially related to the gastrointestinal (GI) tract.[100]

More recently, multiple GLP-1 receptor agonists have been introduced that were originally developed to treat T2D but have also shown efficacy in weight management.[100] The primary mechanism driving weight loss appears to be the inhibition of postprandial gastric emptying and concomitant increases in satiety.[101] Data from large-scale RCTs show that GLP-1 agonists produce up to 15% weight loss in individuals with obesity and T2D and that these reductions are sustained for up to 15 months.[102]

Given their recent clinical rollout, the long-term efficacy and side effect profiles of GLP-1 agonists for obesity remain unknown. As expected, drug response has varied tremendously from patient to patient, and the drivers of inter-individual variation in weight loss, glucose control, and weight regain remain to be determined.[103] Based on established links between the gut microbiome and GLP-1 secretion (discussed above and below), it seems reasonable to hypothesize that the microbiome may contribute, at least in part, to these patient outcomes. Data in mice with diet-induced obesity support this hypothesis; the GLP-1 receptor agonist liraglutide led to a significant increase in *A. muciniphila*, potentially leading to a positive feedback loop promoting further host GLP-1 release.[104] On the other hand, a comparable study from an independent group revealed much more modest microbiota shifts in response to liraglutide, potentially due to baseline differences in the gut microbiota.[105]

UPDATING THE ENERGY BALANCE PARADIGM

The microbiome has far broader implications for nutrition than just its role in mediating the effects of dietary and pharmaceutical interventions, including multiple distinct molecular mechanisms that together alter both sides of the energy balance equation: caloric intake and energy expenditure. In this section, we dive deeper into the role of the gut microbiome in energy balance and how it provides a valuable context in which to consider obesity and related diseases.

Digestion as a host-microbiome system

Before the 1970s, it was already clear that the human gut housed a diverse array of microbes with broad enzymatic repertoires that could digest nutrients inaccessible to mammalian enzymes, including many plant polysaccharides.[106,107] Microbial fermentation of indigestible carbohydrates was known to produce SCFAs—including acetate, butyrate, and propionate—which can be assimilated and used as energetic substrates by host tissues.[18,108] Although important for energy salvage, SCFAs were known to deliver fewer calories to the host than the carbohydrate sources from which they are derived, with the remainder of energy fueling microbial functions or lost in heat. For instance, carbohydrates digested by the host in the small intestine deliver ~4 kcal/g, whereas SCFAs produced from microbial fermentation of these carbohydrates typically deliver <2 kcal/g.[109] Nevertheless, SCFAs account for an estimated 5%–10% of daily energy requirements in healthy humans living in industrialized contexts and likely more in patients with malabsorption or populations consuming more dietary fiber.[109] Thus, human adaptations such as pH gradients, sphincters, and antimicrobial peptides were understood as important mechanisms for restricting the bulk of microbes to the colon, ensuring our primary access to nutrients available for small intestinal digestion and thereby maximizing direct energy uptake.[110] It was also appreciated at the time that the gut microbiome could synthesize some essential micronutrients, such as vitamin K and B-group vitamins, including B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), B9 (folate), and B12 (cobalamin).[111]

However, our view of the gut microbiome 50 years ago was limited by a paucity of methods with which to survey microbial diversity beyond the small fraction of taxa capable of growing in culture and with which to dissect the cellular and molecular mechanisms driving microbiota response to diet and their downstream impacts on host health. The advent of efficient high-throughput sequencing of microbial DNA and RNA, coupled with advances in bioinformatics and metabolomics, vastly expanded our insight into the composition, function, and metabolic output of the gut microbiome and its interactions with diet. Moreover, expanded use of gnotobiotics (animal models reared in GF conditions or colonized with defined microbial communities) has allowed us to disentangle correlation from causation and illustrate the direct metabolic contributions of the gut microbiome to host digestion and metabolism. These advances have confirmed ecological predictions that the gut microbiome is highly sensitive to macronutrient composition,[31,59] dietary processing,[112] and diet-derived xenobiotics such as emulsifiers.[113,114] Moreover, these effects are dynamic, with changes in diet triggering concomitant changes in gut microbiome structure and function within 24–48 h of effluent reaching the colon in both humans and mice.[59,115] Moreover, cross-sectional human studies,[116] twin studies,[117] and experimental studies in mice[115] have all found that environmental factors such as diet exert stronger effects on the gut microbiome than host genetics. Thus, diet represents a powerful lever for manipulating the gut microbiome in the service of health, including by perturbing pathways of microbial control over host energy metabolism.

Effects of the gut microbiome on host energy metabolism

The impact of the gut microbiome on host energy status has been recognized to some extent since the late 1940s, when researchers at Lederle Laboratories discovered that antibiotic residues could promote growth, and there was a subsequent rapid rise in global feed conversion efficiency that accrued from supplementing livestock diets with low (subtherapeutic) doses of antibiotics.[118] However, critical gnotobiotic studies conducted in the past two decades have driven home the extent to which host energy balance depends on the presence of the gut microbiome, its compositional profile, and its manifold influences on host energy metabolism.

A seminal study in 2004 demonstrated that colonization of GF mice with gut microbiota harvested from conventional mice led to a 60% increase in body fat over a period of 2 weeks, despite lesser food consumption and increased energy expenditure.[119] Myriad subsequent studies have since replicated the basic finding that harboring a gut microbiota generally has net-positive effects on host energy status.

Moreover, numerous studies have confirmed that gut microbiomes can differ in their contributions to host energy status. Early studies established that gut microbiome profiles associated with obesity could exacerbate host phenotypes: genetically obese (*ob/ob*) mice and their lean littermates were shown to differ in their gut microbiota compositions,[120] with subsequent gnotobiotic transplantation experiments finding that recipients of gut microbiota harvested from *ob/ob* donors gained 74% more body fat over a period of 2 weeks than recipients of a microbiota from genetically lean donors.[121] Additionally, transplantation of murine gut microbiota conditioned on an HFHS diet versus a low-fat, high-plant-polysaccharide diet consistently enhanced fat gain in murine GF recipients fed control chow.[122,123] Jointly, such studies established that obese phenotypes, whether driven by genetics or diet, were transmissible via the gut microbiota. Similar experiments have shown that the gut microbiome potentiates energy gain among human twins who are discordant for obesity[124] and in dynamic states of weight gain, including weight rebound after calorie restriction[125] or weight cycling[126] and among women in late pregnancy.[127]

Conversely, the gut microbiome can exacerbate negative energy balance among hosts who are undernourished. The gut microbiomes of children with kwashiorkor exhibit a stunted developmental profile and contribute causally to undernutrition, as evidenced by impaired nutrient uptake in GF mice following colonization with gut microbiota harvested from children with kwashiorkor versus healthy controls.[128,129] Gut microbiome changes have also been shown to contribute to rapid weight loss following VLCDs[20] and Roux-en-Y gastric bypass surgery.[130,131] For example, administration of an 800 kcal/day VLCD to postmenopausal women with overweight or obesity led to changes in the gut microbiome and improved metabolic phenotypes (e.g., weight loss and decreased adiposity) that could be recapitulated in murine GF recipients of post-diet versus pre-diet gut microbiota.[20]

Changes in the gut microbiome do not always exacerbate host energy imbalance as seen in obesity and undernourishment. For instance, studies in mice suggest that the higher nutrient influx

into the colon associated with a low-digestibility diet can alter the gut microbiome in a manner that potentiates its contributions to host energy status, as evidenced by greater weight gain and adiposity among chow-fed GF recipients of microbiomes conditioned on diets with lower versus higher digestibility.[112] In this example of host-microbiome ecological codependency, low nutrient uptake by the host is partially buffered by nutrient-availability-driven changes in gut-microbiome-derived metabolites and their downstream effects, e.g., increasing host energy intake. Such energy buffering could conceivably benefit host metabolic health under energy-limited conditions but can also be expected to stymie weight management under conditions of energetic excess.

Gut microbiome contributions to host energy balance can be dependent on environmental and dietary context even when host energy balance is not manipulated via diet. For instance, GF murine recipients of gut microbiota from human twins discordant for obesity generally mimicked the metabolic phenotype of their donors, but lean-donor-derived microbes invaded obese-donor-derived microbiomes when differentially colonized recipient animals were cohoused, with the result that both cohoused recipients remained lean.[124] Critically, the transmissibility of the lean-associated microbiota was disrupted when cohoused recipient animals were fed a diet high in fat and low in fruits and vegetables. Such complex interactions emphasize that the effects of diet on host-microbiome metabolic interactions may sometimes be difficult to trace.

Changing perspectives on SCFAs

When *Cell* was launched, our understanding of the pathways of gut microbial influence on human energy metabolism was largely limited to the microbial contribution to dietary energy harvest via fermentation of carbohydrates into SCFAs. SCFAs can be converted to ATP by various host tissues, with butyrate serving as a primary metabolic fuel for the colonic epithelium, propionate used in hepatic gluconeogenesis, and acetate—the most abundant SCFA molecule produced in the colon—supporting the brain, heart, and skeletal muscle.[109]

However, it is now clear that SCFAs have diverse signaling functions affecting both sides of the energy balance equation (Figure 3). For instance, SCFAs can alter energy intake, as evidenced by the exogenous delivery of SCFAs suppressing appetite in humans and mice.[132,133] Anorexigenic effects of SCFAs may arise via several complementary mechanisms. First, experiments combining isotopically labeled acetate and positron emission tomography (PET) show that colonic acetate crosses the blood-brain barrier, inducing satiety by altering the expression of regulatory neuropeptides in the hypothalamus.[134] Second, butyrate and propionate bind to GPR41 and GPR43 receptors on enteroendocrine L cells, triggering the release of GLP-1 and PYY, which promote satiety via endocrine receptors in the hypothalamus and nucleus of the solitary tract.[135,136] Third, SCFAs appear to induce satiety via vagal nerve stimulation, as appetite suppression by SCFAs was attenuated by vagotomy in mice.[133] Nevertheless, the net effects of SCFAs on vagal-nerve-mediated satiety remain unclear, as acetate generated during high-fat diet (HFD) feeding has independently been shown to promote hyperphagia through increases in ghrelin and glucose-sensitive insulin secretion, with these effects abolished in vagotomized rats.[137] These SCFA-mediated mechanisms complement other potential pathways of gut microbiome influence on energy intake, including the microbial synthesis of peptide mimics of hormones regulating hunger and satiety (e.g., insulin and leptin)[138] and the microbial synthesis or modulation of neurotransmitters (e.g., GABA, acetylcholine, dopamine, and serotonin) that can affect satiety and gut motility.[139,140]

SCFAs can also regulate energy expenditure by increasing mitochondrial function and stimulating thermogenesis. For instance, dietary supplementation of butyrate in HFD-fed mice protected against the development of obesity and insulin resistance, a result attributable in part to increased mitochondrial respiration in skeletal muscle and increased proliferator-activated receptor-γ coactivator-1α (*Pgc-1*α) expression in brown adipose tissue (BAT) that in turn upregulated uncoupling protein 1 (*Ucp1*) expression to promote thermogenesis.[141] Similarly, dietary supplementation of acetate, propionate, butyrate, or their combination in HFD-fed mice protected against weight gain in association with white adipose tissue (WAT) beiging,[142] a process involving the growth of *Ucp1*-expressing brown-fat-like adipocytes within WAT that results in increased metabolic rate and thermogenesis.[143] SCFAs may also affect the balance between lipogenesis and lipolysis. For instance, it has been reported that acetate and propionate inhibit lipolysis and promote fat accumulation in WAT,[144,145] while butyrate promotes lipolysis and fatty acid oxidation.[146]

Strikingly, recent evidence in mice suggests that SCFAs may begin to shape host metabolic physiology *in utero* through effects on embryonic tissue development. SCFAs derived from the murine maternal gut microbiome cross the placental barrier, bind to embryonic GPR41 and GRP43 receptors, and thereby trigger changes in gene expression leading to differential development of metabolically expensive neural, pancreatic, and intestinal tissues.[147] These SCFA-mediated effects on tissue development led to persistent consequences for host metabolism, with mice that were exposed to SCFAs *in utero* exhibiting protection from diet-induced obesity as adults.[147] Critically, this protection did not arise due to differential postnatal microbiome exposures, as both groups were surgically delivered and cross-fostered.[147] Therefore, the adult interaction of metabolic phenotype with diet could be ascribed to SCFA-mediated impacts on embryonic tissue development, highlighting that gut-microbiome-mediated metabolic programming may even precede colonization with a gut microbiota.

Other diet-microbiome interactions affecting host metabolic health

The gut microbiome also interacts with a wide array of non-carbohydrate compounds, producing metabolites that, like SCFAs, have important consequences for host metabolism. For example, the gut microbiome plays a critical role in the link between animal-rich diets and both atherosclerosis and thrombosis through its effects on dietary choline (present in red meat, poultry, fish, eggs, and dairy) and carnitine (present mainly in red meat and liver).[58] Members of the gut microbiota (e.g., those possessing trimethylamine [TMA] lyase operons including CutC/D, CntA/B, and YeaW/X) convert dietary choline and

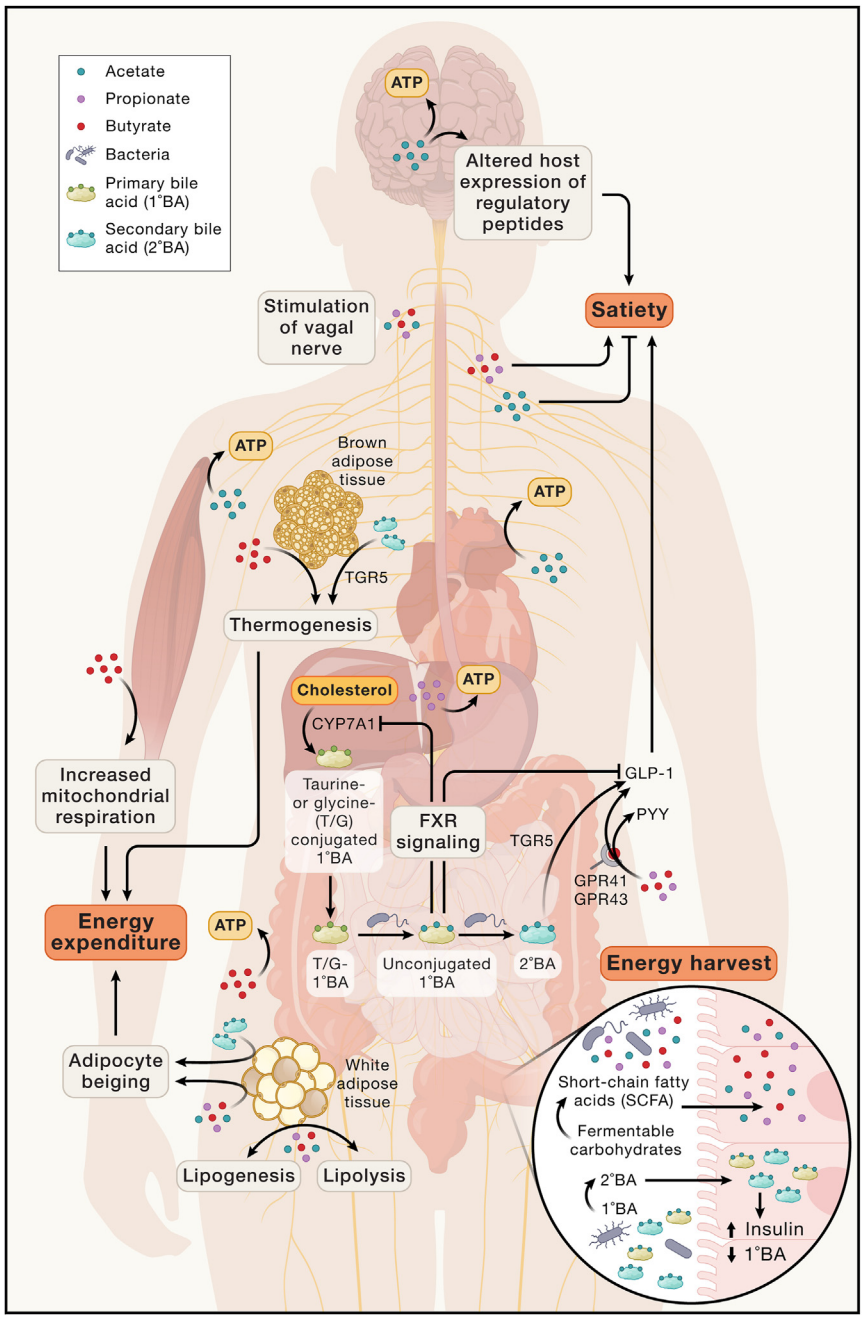

Figure 3. Microbial metabolite-mediated effects on host energy balance

Short-chain fatty acids (SCFAs) generated during the microbial fermentation of dietary carbohydrates reaching the colon can affect both sides of the energy balance equation through effects on energy harvest, energy intake, energy expenditure, and allocation of energy to lipogenesis versus lipolysis. SCFAs can be assimilated and converted to ATP by diverse host tissues, thus enabling the host to salvage energy from nutrients that would otherwise have escaped metabolism. Acetate (blue dots) supplies energy to the brain, heart, and skeletal muscle. Propionate (purple dots) is a substrate for hepatic gluconeogenesis. Butyrate (red dots) is the principal metabolic fuel for the colonic epithelium. SCFAs affect energy intake through various methods, including acetate crossing the blood-brain barrier and mediating the expression of regulatory neuropeptides, propionate and butyrate binding to GPR41 and GPR43 receptors in enteroendocrine L cells and stimulating the release of the anorexigenic hormones GLP-1 and PYY, and gut-brain signaling via the vagus nerve that may be mediated differentially by acetate versus SCFA mixtures. SCFAs affect energy expenditure by promoting thermogenesis in brown adipose tissue, beiging in white adipose tissue, and mitochondrial respiration in skeletal muscle. SCFAs can also affect the dynamics of lipogenesis and lipolysis, with butyrate reported to promote lipolysis while acetate and propionate promote lipogenesis. Additionally, the gut microbiome can deconjugate and dehydroxylate the taurine- or glycine-conjugated primary bile acids (T/G-1°BA) secreted by the host liver, generating unconjugated primary bile acids (1°BA) and secondary bile acids (2°BA) that modulate diverse aspects of host energy metabolism. Unconjugated primary bile acids signal via the farnesoid X receptor (FXR) to inhibit CYP7A1, the rate-limiting step in primary bile acid synthesis, with potential downstream consequences for dietary fat absorption. Secondary bile acids activate TGR5, contributing to thermogenesis in brown adipose tissue, beiging in white adipose tissue, and insulin production in pancreatic β cells. Gut microbial bile acid metabolism may also affect energy intake through contrasting effects on the anorexigenic hormone GLP-1, with 2°BA-activated TGR5 signaling promoting the secretion of GLP-1 by L cells and 1°BA-activated FXR signaling shown in mice to inhibit GLP-1 activity. These pleiotropic effects underscore a changing view of SCFAs and bile acids from vehicles for energy harvest to master metabolic regulators capable of both net-positive and net-negative effects on host energy status. Created with BioRender.com.

carnitine to TMA, a gaseous intermediate that is absorbed into circulation and converted by hepatic flavin monooxygenases (FMOs) to the proatherosclerotic and prothrombotic metabolite trimethylamine *N*-oxide (TMAO).[58] Prospective studies in humans have found plasma TMAO levels to be highly predictive of future major adverse cardiac events,[148] with experimental administration of TMAO promoting CVD phenotypes in mice.[148–151] The identification of the bacterial enzymes responsible for the production of TMA from dietary precursors has provided opportunities for intervention.[152,153] Notably, inhibition of microbial TMA lyases by chemical mimics of choline (e.g., 3,3-dimethyl-1-butanol [DMB]) protected against diet-induced atherosclerosis in mice.[154]

Additionally, the gut microbiome can affect host metabolism via indirect interactions with dietary compounds. For example, synthetic emulsifiers are commonly added to processed foods to increase shelf stability and palatability by inhibiting separation. Emulsifiers have been shown to increase bacterial translocation across epithelia *in vitro*,[155] as well as bacterial encroachment from the lumen toward the epithelia, translocation of

proinflammatory bacterial products (e.g., LPS), and inflammation *in vivo*.[113] For instance, administration of the emulsifier compounds carboxymethylcellulose and polysorbate-80 to mice at diet-relevant doses altered gut microbiota composition and thinned the mucus lining of the proximal colon, promoting bacterial encroachment toward epithelial cells and concomitant low-grade inflammation.[113] Comparisons of conventionalized and GF mice showed that these microbiota-linked effects were necessary and sufficient to promote obesity and insulin resistance in wild-type mice as well as colitis in genetically susceptible (*IL-10*$^{-/-}$) mice.[113]

In addition to direct and indirect interactions with dietary substrates, gut microbes may also modify diet-induced host-derived secretions, producing secondary metabolites that shape host metabolism. For instance, primary bile acids secreted by the host liver have long been understood to function as emulsifiers in dietary fat digestion and as antimicrobial compounds that help minimize microbiota encroachment into the small intestine.[110] However, we now know that certain members of the gut microbiota (e.g., those possessing bile salt hydrolase [BSH] and bile acid inducible [*bai*] genes) can deconjugate and dehydroxylate the taurine- or glycine-conjugated primary bile acids secreted by the host liver, generating unconjugated primary bile acids and secondary bile acids with narrow-spectrum antimicrobial properties and diverse signaling functions.[156] Microbially deconjugated primary bile acids are ligands for the farnesoid X receptor (FXR), a transcription factor known to modulate lipid and glucose homeostasis and inhibit hepatic expression of CYP7A1, the rate-limiting enzyme in primary bile acid synthesis.[156–158] This remarkable example of gut microbial control of host physiology may benefit gut microbes by dampening the antimicrobial effects of primary bile acids and reducing lipid absorption in the small intestine, thus increasing the quantity reaching the colon.[159] Additionally, secondary bile acids are ligands for Takeda G-protein receptor 5 (TGR5),[160,161] which exerts effects on both sides of the energy balance equation. Activation of TGR5 increases energy expenditure by upregulating *Ucp1* expression, leading to WAT beiging and BAT thermogenesis.[162,163] TGR5 activation stimulates insulin secretion by pancreatic β cells and increases insulin sensitivity by enhancing glucose uptake in skeletal muscles.[164,165] TGR5 activation also enhances GLP-1 production by enteroendocrine L cells, which can lower caloric intake by inducing satiety and contribute to glucose homeostasis by upregulating mitochondrial oxidative phosphorylation.[162,163] However, the secondary-bile-acid-mediated effects of TGR5 on GLP-1 may be balanced by FXR-mediated inhibition of GLP-1 induced by microbially deconjugated primary bile acids,[166] illustrating the delicate balance between differential gut microbial metabolism of bile acids and host phenotype.[159]

In some cases, the mechanisms of interaction between diet and gut-microbiome-mediated effects on host metabolism remain unclear, even when such interactions appear highly reproducible. For instance, several independent studies in mice have reported that consumption of dietary polyphenols results in rapid and substantial enrichment of the mucin-degrading bacterium *A. muciniphila*,[46,47] a species repeatedly linked to metabolic health in mice and humans.[130,167–169] Polyphenol-induced blooms of *A. muciniphila* precede anti-inflammatory changes in cytokine expression and have been associated with robust protection from biomarkers of HFD-induced metabolic syndrome.[46,47] Enrichment of *A. muciniphila* has been reported to promote metabolic health in part by enhancing gut barrier integrity, thus reducing systemic inflammation and its metabolic sequelae.[167] GLP-1 release triggered by the *A. muciniphila*-derived P9 protein may also contribute.[96] However, the mechanisms governing the links between polyphenol intake and *A. muciniphila* enrichment, including whether these effects are direct or host-mediated, remain unknown.

Finally, in many cases, substantial and metabolically important interactions between diet and the gut microbiome are predicted yet remain understudied. For instance, protein fermentation has been given an experimental backseat to carbohydrate fermentation, likely due to the comparatively high small intestinal digestibility of protein. Yet, protein uptake in the small intestine may be overestimated. Studies of ileostomy patients and healthy controls eating isotopically labeled eggs found that just 51%–65% of raw egg protein was absorbed in the small intestine, in contrast to its >95% predicted bioavailability.[170,171] Studies in animal models have reported similarly low small intestinal protein uptakes, e.g., 63%–89% in rats.[172] Moreover, the minor fraction of dietary protein reaching the colon is then combined with proteins from endogenous (e.g., secretions and sloughed epithelial cells) and microbial sources. The result is that protein fermentation may be surprisingly substantive: among industrialized populations, an estimated 12 g of protein is fermented in the colon daily compared with 30–40 g of carbohydrates,[109] a proportion expected to rise for people following ketogenic and other high-protein diets.

Colonic fermentation of protein generates SCFAs but also branched-chain fatty acids (BCFAs)—mainly isobutyrate from valine, isovalerate from leucine, and 2-methylbutyrate from isoleucine—as well as ammonia, hydrogen sulfide, amines, phenolic, indolic, and *N*-nitroso compounds.[173,174] Physiological consequences of these metabolites are currently not well understood, but abundances have been associated with several metabolic and non-metabolic pathologies, including obesity, T2D, non-alcoholic fatty liver disease, gut permeability, colitis, colorectal cancer, anxiety, and autism.[173] In select cases, experimental evidence supports microbiota-dependent effects. For example, exposure to the phenolic derivative *p*-cresol induced social behavior deficits in mice that were transmissible to GF animals via gut microbiota transplantation.[175] Additionally, GF or antibiotic-treated mice were protected from the increased severity of DSS-induced colitis seen in high-protein-diet-fed conventional animals.[176] Conversely, some metabolites have pleiotropic effects that may be detrimental or beneficial depending on context; for instance, indoles have been shown to promote anxiety-like behavior among gnotobiotic rats colonized with indole-producing *Escherichia coli* versus engineered *tnaA*-null *E. coli* lacking indole-production capacity,[177] but they have also been shown to attenuate EAE through AhR-dependent suppression of Th17 in a mouse model of multiple sclerosis.[178] Products of protein fermentation may either complement or compromise the activity of carbohydrate-derived metabolites. For instance, colonocytes may oxidize BCFAs when butyrate is

Table 1. Three examples of how microbiome research alters our view of nutrition

Area	Current state	Microbiome-aware state	Lesson
Caloric value	Original and modified formulas attribute 0 or 2 kcal/g to resistant starch and fiber, respectively, but these are inconsistently used and oversimplified	Consider the type of resistant starch/fiber, their impacts on energy expenditure, and the metabolic capacity of each individual's gut microbiome	A host calorie is not a host-microbiome calorie
Bioactive nutrients	Unabsorbed dietary components largely treated as waste	Unabsorbed nutrients feed the gut microbiome, which can convert them to bioactive compounds	Unabsorbed nutrients drive host-microbiome interactions in nutrition
Safety	Diet components with low toxicity and/or safe history of use granted "generally regarded as safe" (GRAS) status	Consider the toxicity of microbial metabolites and broader health consequences mediated by the gut microbiome	GRAS is not necessarily safe

unavailable,[174] but research in pigs has also shown that ammonia derived from protein fermentation decreases butyrate uptake by colonocytes and shifts substrate utilization from butyrate oxidation to glycolysis,[179,180] likely promoting gut permeability and inflammation.[173] Work to further elucidate the biological consequences of protein-derived microbial metabolites will contribute fundamentally to our knowledge of human nutrition, physiology, and host-microbial interactions.

CHALLENGES THE MICROBIOME POSES TO THE FIELD OF NUTRITION

The central role of the gut microbiome in human physiology has revolutionized our view of health[181] and is increasingly percolating into nutritional research and recommendations. Currently, there is widespread concordance among global dietary guidelines, but unfortunately, this homogeneity extends to the microbiome, with just a handful (e.g., those in the US and South Africa) explicitly considering diet-microbiome interactions.[182] Recent reviews have addressed how knowledge of the gut microbiome intersects with current nutritional guidelines,[182] opportunities for microbiome-inclusive precision nutrition,[183] and broader considerations for the incorporation of microbiome science into research, education, policy, and communication regarding public health.[184] Virtually all aspects of human nutrition will ultimately need to be reevaluated in light of the direct and indirect consequences of diet-microbiome interactions for human health. As examples, we highlight here how knowledge of the microbiome challenges three tenets of nutritional science: (1) the basic concept of caloric value; (2) the biological importance of assimilated versus unabsorbed dietary components; and (3) the components of diet generally regarded as safe (Table 1).

A host calorie is not a host-microbiome calorie

The metabolizable energy content of the human diet is typically estimated using macronutrient-specific multipliers (e.g., ~4 kcal/g of protein or carbohydrate and ~9 kcal/g of fat). These multipliers, derived from experiments performed in the late 1800s and early 1900s by Wilbur Olin Atwater, reflect the average chemical energy present in a food minus the average fraction excreted in feces, urine, secretions, and gases.[185] The Atwater system provides a simple means of estimating caloric content but is limited in its biological relevance via three key omissions. First, it does not capture the effects of the broader food matrix. For instance, many plant-based macronutrients are contained within cell walls or subcellular structures that remain intact during digestion. Encapsulation of macronutrients explains why effective caloric returns are often markedly lower than those predicted based on Atwater values (e.g., 21% lower in walnuts[186] and 25% lower in almonds[187]) and why returns can be increased by physical disruption of cells (e.g., in peanuts[188]). Additionally, macronutrient digestibility varies based on co-administered substrates. For instance, fecal excretion of fat, nitrogen (an index of protein), and energy all typically rise under conditions of dietary fiber supplementation.[189] Second, Atwater factors do not capture diet-induced thermogenesis, the metabolic cost of digestion, which varies widely based on macronutrient content (~20%–30% of macronutrient caloric value for protein, ~10%–30% for alcohol, ~5%–10% for carbohydrates, and ~0%–3% for fat),[190] perceived meal palatability,[191] or food processing.[192,193] Varying costs across these dimensions reflect the fact that dietary compounds possess diverse physical, chemical, and sensory properties that affect their metabolic fates during ingestion, digestion, absorption, and assimilation. Finally, Atwater factors only minimally distinguish between calories available to humans versus those available to the gut microbiota.

From early experiments on gut microbial fermentation of indigestible carbohydrates[194] through complex cataloging of gut microbiome contributions to human energy balance,[195] progressive research over the past 50 years has underscored an emerging realization that dietary nutrients that are ingested but not excreted do not necessarily benefit humans but may instead be metabolized by the gut microbiome, producing an array of compounds with altered energy availabilities and metabolic consequences for the human host. Despite widespread awareness of this basic principle among scientists and clinicians, conventional tools for measuring dietary value still capture only the most rudimentary host-microbiome dynamics in digestion. Most notably, the original Atwater-based multipliers were modified by Merrill and Watt in 1955 (updated again in 1973) to assign dietary fiber in conventional foods a caloric value of ~2 kcal/g based on its assumed conversion to SCFAs by the gut microbiome.[185] However, food manufacturers may elect to use either the original Atwater or updated Merrill and Watt multipliers, and food labels do not disclose whether original or updated multipliers were used, making it difficult to trace the penetrance of

even this basic nod to host-microbiome interactions. Moreover, we now understand that the metabolic effects of SCFAs are multifaceted and pleiotropic, with the potential for SCFAs to have a net-negative, net-positive, or even net-zero effect on host energy balance depending on the context. For example, exogenous SCFA administration can attenuate rather than potentiate diet-induced obesity.[142]

Additionally, Atwater factors do not account for microbial metabolism of non-carbohydrate dietary compounds or host endogenous secretions, although some of these processes are now known to affect energy balance. For instance, many compounds long assumed to be non-caloric on the basis of their inaccessibility to human digestive enzymes—such as the emulsifier lecithin[113] or the non-caloric artificial sweetener saccharin[196]—have been shown to alter host energy status via microbiome-mediated mechanisms. Similarly, gut microbial transformation of primary bile acids into unconjugated and secondary forms can affect host energy status via pleiotropic effects on energy intake, energy harvest, and energy expenditure (Figure 3).

Part of this lag between discovery and implementation may be attributed to the need for additional replication studies confirming these effects in diverse human populations and to the challenges of grappling with interindividual variation in gut microbiome function. However, similar arguments could be made for the applicability of existing Atwater multipliers to diverse human populations that vary in digestion capacity vis-à-vis genetic differences in factors such as lactase persistence and amylase production.[197,198] Therefore, it may be an opportune time to revisit the way we measure the basic caloric value of the human diet with respect to both host- and microbiome-driven aspects of digestion.

Unabsorbed nutrients drive host-microbiome interactions in nutrition

Quite rationally, the field of nutrition has long focused on the components of diet that are assimilated into human tissues, as these have the potential to shape health directly. However, abundant evidence that the gut microbiome is sensitive to dietary digestibility[112,199] and that diet-induced changes in the gut microbiome can causally shape host health and disease in diverse contexts[18,181] is increasingly highlighting the importance of unabsorbed nutrients. Unlike assimilated nutrients, unabsorbed nutrients reliably reach the densest microbial communities in the colon. Moreover, unabsorbed nutrients are concentrated as effluent progresses down the GI tract by virtue of the disappearance of assimilated nutrients and water. Thus, unabsorbed nutrients might be expected to wield greater power than assimilated nutrients in shaping the gut microbiome and its downstream consequences for health and disease.

To date, studies of the links between diet and the gut microbiome have largely characterized diet as it appears upon entry into the mouth. However, greater insight into diet-microbiome interactions may come from characterizing diet as the residual fraction of ingested nutrients that resist assimilation by the end (terminal ileum) of the small intestine. While characterizing ileal digestibility has historically depended on *in vitro* models or complicated *in vivo* models, such as cannulated animals, human patients post-ileostomy, invasive naso-ileal or colonic intubation in healthy humans, and detection in plasma of isotopically labeled nutrients,[200–202] new approaches inspired by the microbiome may prove promising. For instance, DNA-based characterization of dietary substrates—a technique known as DNA metabarcoding[203,204]—could potentially be combined with DNA-based microbiome profiling to study direct diet-microbiome interactions in a given sample of effluent. Dual characterization of diet and microbiome signals along the GI tract could be performed either in animal models, whose effluent can be sampled directly, or in humans using new swallowable devices capable of sampling effluent at GI intervals determined by changing pH.[205,206]

GRAS is not necessarily safe

Many dietary substances have been granted "generally regarded as safe" (GRAS) designations by the US Food and Drug Administration (FDA) on the basis of toxicology assays in animals and/or extensive past use producing no known harmful effects in humans.[207] However, GRAS evaluations have not generally considered the impact of these substances on the gut microbiota or the potential for indirect, microbiome-mediated health effects. The dangers of focusing exclusively on host tissues are illustrated by findings that emulsifiers like lecithin and artificial sweeteners like saccharin with GRAS designations may induce obesity and insulin resistance when consumed at diet-relevant levels via their effects on the gut microbiome.[113,196] Similarly, the GRAS compound taurocholic acid and its chemical constituents, the GRAS compounds taurine and cholic acid, may interact with the gut microbiome to promote intestinal pathology.[208,209] Specifically, taurine released in the bacterial deconjugation of taurocholic acid by *Bilophila wadsworthia* generates genotoxic hydrogen sulfide, and the concomitant release of cholic acid serves as a substrate for the microbial production of the proinflammatory secondary bile acid deoxycholic acid. Thus, feeding a taurocholic-acid-supplemented diet led to blooms of *B. wadsworthia* and the development of colitis in genetically susceptible (*IL-10*$^{-/-}$) mice.[208]

Additionally, the gut microbiome may convert dietary compounds or their host metabolites to more detrimental forms using its extensive enzymatic arsenal.[210–212] For instance, bacterial β-glucuronidase enzymes contribute to enterohepatic cycling of carcinogenic heterocyclic amines such as IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline) that are detoxified through hepatic glucuronidation. Upon exposure to IQ, conventional mice showed more DNA adducts and DNA damage than their GF counterparts.[213,214] Rats monocolonized with isogenic *E. coli* carrying functional versus non-functional *uidA*, the gene encoding β-glucuronidase, showed increased colonic genotoxicity coupled to multiple peaks in the excretion of this compound, a profile consistent with enterohepatic circulation.[215]

The gut microbiome has also been linked to renal pathology arising from the dietary contaminant melamine, an additive to plastics used in many food preparation tools. In 2008, melamine contamination of infant formula in China was responsible for ~300,000 cases of kidney stones and several deaths. This devastating outcome was initially puzzling, as melamine displayed very low toxicity in animal studies. However, subsequent *in vitro* and *in vivo* experiments showed that *Klebsiella* bacteria

present in some infant guts can convert melamine to cyanuric acid,[216] a structural analog now known to complex with melamine to form insoluble renal aggregates.[217,218]

On the other hand, biotransformations of unabsorbed dietary compounds by the gut microbiome may contribute to beneficial effects that are missed by focusing only on the direct effects of diet on the host. For instance, gut microbial biotransformations of plant-derived dietary lignans (e.g., those found in whole grains, seeds, legumes, and nuts) are thought to underlie their protective effects against breast cancer. A consortium of gut bacterial taxa (e.g., *Eggerthella lenta*, *Blautia producta*, *Gordonibacter pamelaeae*, and *Lactonifactor longoviformis*) convert the dietary lignan pinoresinol to the cancer-protective estrogen mimics enterodiol and enterolactone.[219] Accordingly, GF rats colonized with a bacterial consortium capable of producing enterodiol and enterolactone from dietary lignan precursors showed lower numbers of tumors and smaller tumor sizes than GF animals upon chemical induction of breast cancer.[220]

Similarly, the gut microbiome may contribute to dietary detoxification to alter disease risk. For example, the gut bacterium *Oxalobacter formigenes* participates in the breakdown of oxalate,[221] a chelating dietary toxin that contributes to kidney stones and renal failure by binding to free metal cations.[222] Lack of *O. formigenes* is associated with increased risk of hyperoxaluria,[223] and its administration in rats reduced diet-induced hyperoxaluria in a dose-dependent manner.[224]

FOOD FOR THOUGHT

In certain ways, conventional nutritional wisdom has not changed much in the past 50 years. Michael Pollan's recent maxim, "eat food, not too much, mostly plants," may be overly simplistic but captures the high-level nutritional messaging over this time period. Responsible levels of caloric intake and physical activity, with increasing emphasis on dietary quality in addition to quantity and composition, remain key components of energy balance and valuable tools for combating disease. Even the microbiome is not entirely new: researchers in the 1970s already appreciated the key role of the microbiome in digestion and its potential to salvage calories from otherwise indigestible components of the diet.

Yet, in many ways, a scientist or member of the lay public when *Cell* was founded might perceive some of today's nutritional concepts as entirely alien. The successful prevention of diseases rooted in micronutrient deficiency coupled with the dramatic rise in obesity has shifted both the research priorities and the healthcare landscape in which nutritional guidelines are implemented. Preclinical and clinical data have bolstered multiple dietary patterns that embrace moderate-to-high fat intake, including MDs, KDs, CR, and TRE. In turn, the rapid expansion of the microbiome field over the past 20 years has broadened our appreciation for the numerous pathways through which the microbiome impacts host metabolic and immunological responses to diet. Microbiome research is forcing us to reconsider the very concept of a calorie; the scope, location, and impact of digestion; and the reliability of GRAS designations.

At the same time, it is important to recognize that diet is just one of numerous lifestyle factors—such as physical activity, environmental exposure, and sleep—that can influence both host energy balance and the gut microbiome. Additionally, the widespread availability of pharmaceuticals has already markedly changed the context in which dietary interventions occur. For example, GLP-1 agonists delay gastric emptying,[100] which has profound implications for digestion, including a change in the substrates available for gut microbial metabolism. While a large body of observational and interventional studies have linked diet to the gut microbiome, the effect size can vary dramatically. A recent meta-analysis found that diet explained <9% of variation in gut microbial community structure, depending on the study design and distance metric analyzed, whereas inter-individual variation typically explained >55%.[18] The sources of these pre-existing variations remain elusive and may be multifactorial; however, pharmaceuticals have emerged as an important contributing factor.[225] This is particularly salient for studies of obesity, where widespread drugs like metformin[226] and statins[227,228] confound attempts to link diet, the gut microbiota, and health outcomes. Moving forward, it will be critical to consider the potential for interactions between diet, other lifestyle factors, and pharmaceutical use to mediate host energy balance via microbiome-dependent and -independent pathways.

Even when considering diet alone, it is now abundantly clear that the diverse pathways of gut microbial influence on host metabolism, coupled with pleiotropic effects of key diet- and microbiome-linked metabolites such as SCFAs, secondary bile acids, and KBs, complicate efforts to predict the metabolic effects of a given diet or microbiota profile. The dream of a microbiome-informed approach to precision nutrition will require experimental studies in humans that measure integrated effects at the whole-organism level[195] and larger-scale cross-sectional studies[31] targeting specific links between dietary components, gut microbiome structure and function, and host health. These data will benefit from the rapid progress in machine learning[30] and artificial intelligence coupled to analogous efforts in the context of implementing precision medicine.

More emphasis should be placed on rigorous RCTs in human subjects paired with tractable model systems that enable rapid reduction of complex systems to their cellular and molecular mechanisms. Only then will we be able to rationally design the next generation of diet-related interventions, hopefully providing an end to the cycle of conflicting nutritional advice from observational human studies. Regardless of the approach, it will be important to interpret results with caution given the potential for the same dietary intervention to show contrasting effects in the short versus long term or in different individuals. A striking example of this dilemma comes from subjects in the "Biggest Loser" competition, who exhibited persistent changes to their resting metabolic rate that likely explained their weight regain over the subsequent 6 years.[229]

Steadfast application of this more mechanistic approach to host-microbiome nutrition research is already bearing fruit. For example, characterization of the enzymes involved in the microbiome-mediated conversion of dietary choline and carnitine to the proatherosclerotic and prothrombotic metabolite TMAO has enabled the development of clinical tests to differentiate patient risk profiles[230] as well as identification of small molecule

inhibitors showing promising protection against the progression of CVD.[154,231] Such data-driven targeting of the microbiome and its interactions with diet will hopefully provide a rich new toolbox for managing human nutrition over the next 50 years.

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AUTHOR CONTRIBUTIONS

Conceptualization, visualization, writing – original draft, and writing – review & editing: R.N.C., K.V., and P.J.T.

DECLARATION OF INTERESTS

P.J.T. is on the Scientific Advisory Boards for Pendulum, Seed, and SNIPRbiome; there is no direct overlap between the current study and these consulting duties.

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