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Fasting intervention and its clinical effects on the human host and microbiome

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Experimental trials in organisms ranging from yeast to humans have shown that various forms of reducing food intake (caloric restriction) appear to increase both overall and healthy lifespan, delaying the onset of disease and slowing the progression of biomarkers of aging. The gut microbiota is considered one of the key environmental factors strongly contributing to the regulation of host health. Perturbations in the composition and activity of the gut microbiome are thought to be involved in the emergence of multiple diseases. Indeed, many studies investigating gut microbiota have been performed and have shown strong associations between specific microorganisms and metabolic diseases including overweight, obesity, and type 2 diabetes mellitus as well as specific gastrointestinal disorders, neurodegenerative diseases, and even cancer. Dietary interventions known to reduce inflammation and improve metabolic health are potentiated by prior fasting. Inversely, birth weight differential host oxidative phosphorylation response to fasting implies epigenetic control of some of its effector pathways. There is substantial evidence for the efficacy of fasting in improving insulin signaling and blood glucose control, and in reducing inflammation, conditions for which, additionally, the gut microbiota has been identified as a site of both risk and protective factors. Accordingly, human gut microbiota, both in symbiont and pathobiont roles, have been proposed to impact and mediate some health benefits of fasting and could potentially affect many of these diseases. While results from small-N studies diverge, fasting consistently enriches widely recognized anti-inflammatory gut commensals such as *Faecalibacterium* and other short-chain fatty acid producers, which likely mediates some of its health effects through immune system and barrier function impact.

Keywords: fasting, gastrointestinal tract, immune system, metabolism, microbiota

Clinical indications for calorie restriction

The relevance of processes potentially impacted through restricted feeding is major, as the examples mentioned in the literature—immune system, metabolism, breakdown and repair, nervous system signaling, proliferation, compensatory processes—together comprise most core actors in maintaining homeostasis, which, when lost, results in broad and diverse (yet substantially comorbid) gradual progression of chronic (with acute escalation following various breakpoints) diseases. This underlies indications for the utility of restricted feeding under diverse indications including hypertension [1–7], dyslipidemia [1, 4–8], glycemic dysregulation and diabetes [1, 9–16], cardiovascular diseases [1, 8, 17–20], liver disease [7, 21–25], as well as autoimmune and inflammatory diseases [11, 26, 27] including rheumatoid arthritis (RA) [28], endocrine disorders [29], even mood and pain conditions [30], neurological diseases [31–33], and possibly cancer [34–36] (Fig. 1). It is fair to say that with a perfectly balanced and individually tailored diet, most human deaths today would take place at a later age than currently, with the impact on a healthy lifespan being larger still. Accordingly, the goal of refining, understanding, and deploying dietary intervention and prevention

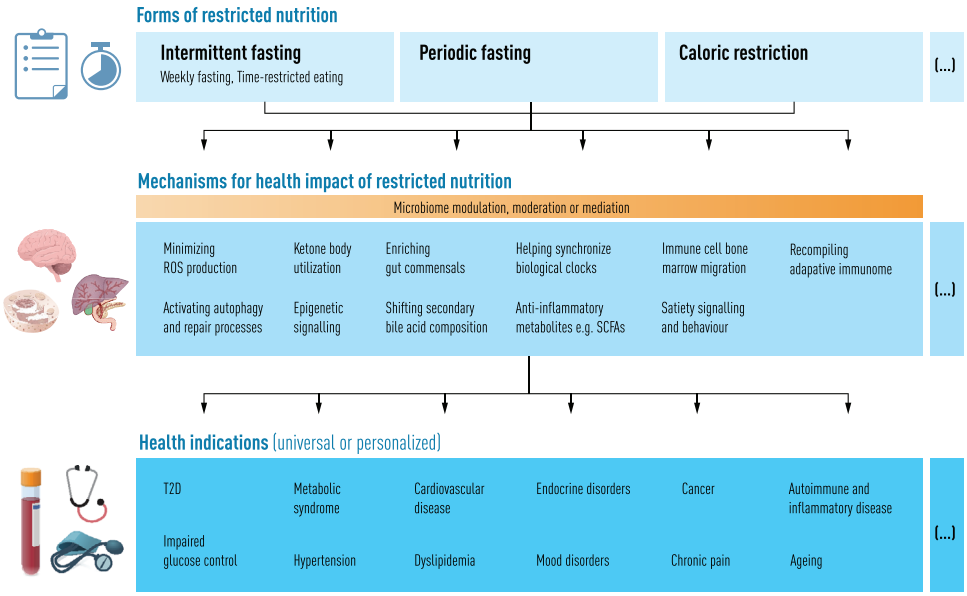

Fig. 1 *Clinical indication for calorie restrictions.*

strategies, while always recognized as relevant, is a key target for translational and personalized omics-informed medicine.

In the wild, animal populations will increase to the level set by limiting factors; frequently, those result in food scarcity within habitats. It can be expected that before various human technologies of civilization, food production, and storage, our evolutionary history will have included recurring periods of food scarcity or even starvation, including during cold or dry seasons, as a given [37]. Accordingly, we should expect animal biology to be profoundly adapted to these pressures [17, 38, 39], and in some form, gene expression programs for adjusting to changes in nutrient availability are ubiquitous already in microbial life. A system under external constraints can rely on those for some of its regulation, meaning there is little fitness gain from evolving or retaining costly internal regulatory mechanisms, though high variability of habitat conditions will again promote some regulatory sophistication. As such, to the extent recurring starvation to a greater or lesser extent has been a fact of a form of life, we should expect systems to cope with it—functions for acquiring, consuming, and storing nutrients in excess to weather deprivation when it comes—to lack internal counterbalances in the organism. An analogy can be made to cancer—freed from those restrictions imposed to impede proliferation in the uninjured adult, uncontrolled growth will result (and accordingly, restricted diets are an active area of research and discussion in cancer treatment [34, 40, 41]).

Calorie restriction as controlled starvation

The discovery nearly a century ago that various animals kept at substantially restricted caloric intake experienced extended lifespans and improved health spurred further inquiry into potential mechanisms [21, 42–46]. Many follow the same principle—the more metabolic turnover, the more molecular and tissue-level wear and tear an organism experiences, including through reactive oxygen species and other potentially toxic side products of its metabolism, driving the accumulation of damage at different scales underlying both disease risk and more general processes of aging, with protective mechanisms in turn activated under functioning homeostasis [1, 38, 47]. Even where damage is repaired, such reparation in turn involves increased metabolic turnover and ultimately contributes to the same process, as do compensatory phenomena where some processes escalate to account for deficits in others. It has been further suggested that calorie restriction (CR) induces a more efficient use of limited resources in homeostasis maintenance through molecular-level adaptations and conservation of stem cell

reserves [48]. While true CR has been difficult to leverage in the same way in the human setting, it has informed dietary advice and offers an arena for understanding downstream mechanistic bases for health consequences of varying metabolic activity directly or indirectly [18, 38]. Some trials have been undertaken on human CR and have revealed improvement in aging-related biomarkers [49], and observed typically long-lived human populations have also been documented as maintaining low-calorie diets [50]. Studies comparing very old and less old adults revealed a complex pattern of gut microbiome differences, where the very old harbor, for example, more *Akkermansia* bacteria, but with inconsistent results for short-chain fatty acid (SCFA) producers [51]. Interpreting these studies faces the complexity that signatures of healthy aging will load onto a survivor bias difficult to disentangle from any microbiome derailment with age, with both factors likely playing parts.

Fasting as a human historical practice

Many human cultures practice some form of a fasting tradition, often serving functions of ritual purification or ordeal, and considered within their system to promote the health of the body and mind [30, 52–54]. Passed down, these practices have inspired complementary or alternative health approaches that have gained more public awareness in modernity, with widespread belief in their utility for a variety of aspects of well-being [9, 52], a progression they also share with practices stemming from ancient food preservation such as fermentation where microbes or microbial metabolic products play a role. An exciting task of modern molecular medicine is to evaluate what effects this may have on the commensal gut microbiome, where so far little research has been done. However, a recent study showed increased gut community diversity and enrichment in taxa, including from the *Roseburia*, *Lachnospira*, *Ruminococcus*, *Streptococcus*, and *Faecalibacterium* genera, under a fermented food intervention [55].

Fasting in context of host and microbiome multicellularity

Reflecting an evolutionary strategy of increasing sophistication, specialization, and regulation, complex multicellular life forms can be thought of as (largely clonal within a body) ecosystems of independent, yet interdependent cells differentiated into tissues and organs. From this perspective of organisms as communities, the integral presence and role of diverse mobile cells in this system are unsurprising. Circulating blood and/or immune cells form distinct and branching subpopulations moving around the human body, especially inhabiting interface surfaces, and frequently perform intracommunity functions such as defense against foreign or rogue cells. To allow selective uptake through such surfaces, mucosal barriers involve a concerted expression of proteins and polysaccharides by adjacent cells forming these tissues and maintaining the integrity of this barrier while still permitting necessary transport, thus requiring careful gene regulation reactive to a variety of signals. Both microbial metabolites and microbial surface components activate a variety of signaling pathways that can either strengthen or weaken this barrier [56] alongside the impact on host cell proliferation [57] and through bacterial degradation of the mucin layer above the epithelium. While the long-standing claim that host-associated bacteria by far outnumber host-derived cells has been updated to be roughly 1:1, that still makes for around 30 trillion bacterial cells in each of us [58]. Far from being sterile, our skin as well as all external and internal mucosal surfaces are home to a diverse community of bacteria and other microbes, acquired and accumulated from birth (and before) and in a constant state of compositional flux reflecting processes of motility, translocation, introduction, competition, extinction, and differential capacity in making use of available nutrients [59, 60]. Work to date on the impact of fasting and fasting-like [61, 62] interventions broadly point to a scenario where nutrient restriction initially drives a shift from adaptive to innate immune reliance [2, 63]. Immune cell populations shrink in the gut and other secondary lymphoid organs but expand, also through migration, in the bone marrow [64–66] while also shifting transcriptional programs. This process has been described as optimization and reorganization for greater sustainability [64] of the adaptive immune repertoire and forms a credible mechanism for observed improvements in autoimmune diseases such as both RA and multiple sclerosis (MS) by CR, ketogenic diet, or intermittent fasting (IF; in MS); by periodic fasting (RA); and by fasting-mimicking diets (RA and MS alike). [62] Refeeding again allows an expansion of the cellular immune arsenal, reverting many of the changes to this repertoire as different subpopulations grow more numerous again [2].

Box 1 Some forms of fasting and related practices

- Periodic fasting: Food intake is low or zero (water fast) for a period of several consecutive (commonly 5–14) days. The Buchinger protocol and certain forms of religious fasting are examples of this and may be undertaken at an interval from monthly to one or a few times yearly.
- Intermittent fasting: Maintaining an ongoing fasting pattern at all times, including:
  - Weekly fasting (e.g., 5:2 or alternate-day fasting): Periodic fasting for a shorter interval, restricting to at most ~500 calories for 2 days per week with unrestricted eating otherwise (5:2), with similar restrictions on every other day (alternate), and so on.
  - Time-restricted fasting (e.g., 16:8 or Ramadan fasting): Limiting food intake within each day to a shorter interval (e.g., 8 out of 16 hours, or from sundown to sunup).
- Calorie restriction: General term for a nutritionally complete but calorically restricted diet. "Grazing" eating patterns of frequent but small meals would not trigger fasting responses but may still constitute CR.

At least following the introduction of solid food, these microbiomes show stability over time [67], with communities from the same sample donor being more similar over time than those from other donors, in both of which bacterial taxa are represented and in their relative abundances [60]. This stability should be seen in context to substantial variability between individuals [68], with large-scale community structure falling within certain broad patterns each seen in samples from donors from different cultures [69], and also substantial variability within the gut. For the latter, different taxa tend to dominate the lumen of the small intestines (e.g., *Lactobacillaceae* and *Enterobacteriaceae* species) versus the colon (e.g., *Bacteroidaceae* and *Prevotellaceae* species), whereas relatively few species can colonize the mucosa and the epithelial structures [70, 71]. Stool, while often the only accessible sample matrix, thus integrates and flattens this spatial (and temporal, reflecting intestinal transit time) organization of the gut microbiome, providing a simplified and sometimes biased view [72]. The origin of microbiome stability over time is not completely known but will reflect the action of the host by way of individual patterns of immune tolerance versus vigilance. Animals born and raised truly sterile reveal immune abnormalities (partly reverted by subsequent colonization by microbiota) reflecting underdevelopment of gut-associated lymphoid tissue and morphological differences compared to control animals, resulting in impaired antibody production and both innate and adaptive immune response to later bacterial infection being compromised [73–75]. This thus reflects a process of immune training in wildtype development where the presence of bacteria as (controlled or uncontrolled) foreign guests and invaders primes the immune system cells to respond to their recognition by specific increased or decreased inflammation, with particular developmental windows where this learning process most easily occurs. In this manner, host immune and microbial cell populations interact bidirectionally [70, 76–80], with these patterns of favored coexistence versus antagonism also seen within the microbial part of the resulting ecosystem alone. Among interesting patterns seen are recurring anticorrelation between, on one hand, a module of anti-inflammatory commensals like *Faecalibacterium prausnitzii* and *Eubacterium rectale*, and on the other hand inflammation-associated bacteria like *Ruminococcus gnavus*, further supporting the role of microbial immunomodulation in the formation of these networks [68]. Bacteria coordinate, especially when forming biofilms as is the case also in the gut [81], forming trophic webs where metabolic capacities are distributed between cohabiting species to form complete pathways; signal to each other through metabolite secretion; and use specialized peptides and small molecules to fight each other, exerting lethality or imposing growth disadvantage on their rivals while their allies are protected through resistance gene systems [82, 83]. Finally, to a great extent, the microbiome is affected by host-external influences where the nutrients supplied to our internal soil gardens reflect the changing diet of the host [84–86], as well as any medications, whether antibiotic or otherwise [87], that the host is taking.

Microbial metabolites as potential fasting mediators

The totality of host and microbial cells has been termed a holo-organism or holobiont [88–90]. With microbes especially in the intestines performing

many functions of nutrient processing into better host-accessible forms, this symbiosis, which is seen in the internal (micro-)environment, mediates, modulates, and moderates influences of the external (macro-)environment, for example where a dietary factor such as insoluble fiber is differentially produced into SCFAs (primarily acetate, butyrate, and propionate), which in turn has protective effects on the mucosal barrier. The gene functional capacities of the microbiome, reflective of collective gene repertoire and reactivity, vary substantially between individuals, meaning the resulting nutrient availability to the host will differ from person to person under the same diet, leading to individual dietary responses (including, e.g., post-prandial glucose in circulation [91, 92]), suggesting the potential for personalized dietary interventions reflecting gut microbiome composition. Several central products of bacterial metabolism in the gut not only respond to host diet but also to the ability of a particular microbiome to mediate that response and have a complex impact on the host. Particular attention has been paid to SCFAs acting as preferred nutrients for particular host cell types, activating (usually anti-inflammatory) immune gene programs (e.g., in macrophages [93]), triggering a variety of host signaling cascades (several passing through chromatin modification and so acting through epigenetic effects) or affecting the permeability of mucosal membranes to reduce the degree to which bacteria may enter the systemic circulation and end up within remote tissues, where they can cause further (including low-grade and chronic) inflammation, which in turn triggers further responses from the host. One important mechanism possibly enabling further feedback loops is that SCFAs can induce the production of antimicrobial peptides including various C-lectins, defensins, and cathelicidins in cells in the intestinal lining [94].

As noted in the systematic overview of findings below, both fasting and refeeding are linked to the expansion of different species known to produce SCFAs and we have reported the same being seen for propionate on the level of direct readouts of gene functions integrated across species [2]. Another relevant potential mechanism, supported by animal studies [95–97] but as yet relatively unexplored in human studies, is for fasting to impact host health and homeostasis (e.g., with regard to blood pressure [96] or diabetic sequelae [95]) through altering the pool of secondary bile acids produced by the gut microbiota in the course of enterohepatic circulation [98–102]. Increased permeability of the gut will release a heterogeneous class of compounds into circulation that, among other functions, activates an immune response, termed alarmins in this capacity, with some being directly antimicrobial and several useful as gut permeability markers [103]. While such a release may serve to raise vigilance against extraintestinal infection through concordantly translocated bacteria, the resulting state of low-grade inflammation appears to form a contributing risk factor for a diverse set of health conditions [104–108], representing a body in a state of chronically elevated alertness to the perceived foreign threat. A Mediterranean diet (which substantially overlaps with the Dietary Approaches to Stop Hypertension diet) has been linked to reduced inflammation and improved metabolic health compared to a Western diet [109–112], also in our work [2]. Several nutrient categories, including host-indigestible fibers, form good substrates for SCFA production by a capable gut microbiome, and they are accordingly central to prebiotic practices [113], where dietary supplements of particular nutrients aim to increase levels of their bacterial metabolism products. Together with probiotic [114] supplementation of live bacteria and the postbiotic [115] supplementation of products of external microbial fermentation, such biotic functional food approaches (found, as noted above, often as a consequence of premodern food storage techniques) can be contrasted with the antibiotics that revolutionized 20th-century medicine, in that while the latter seem to induce a microbiome pattern also associated with low-grade inflammation [87], the former seem in various regards able to prevent or reverse such changes.

Other fasting targets in the human host

Aside from microbiome-mediated modes of action, forms of fasting have several other possible ways to exert systemic impact alone or in interaction with the former. CR tends to involve a shift towards ketone metabolism [116, 117], which in turn triggers complex changes in gene expression and epigenetic modifications [21, 118]. Several forms of epigenetic modification, including DNA methylation, histone methylation, and histone acetylation, have been demonstrated to follow from forms of CR in human or animal models across a range of tissues, including but not limited to the liver, pancreas, and adipose tissue. Epigenetic markers of metabolic disease within these tissues are

known and linked to genes where expression is normalized upon CR but large-scale data are as yet sparse on how often epigenetic normalization also drives this change, though with substantial indications, for example, for the sirtuin family of histone deacetylases [119, 120] and also in human impacts through DNA methylation on leptin and adiponectin expression [121] and downregulation of genes involved in oxidative phosphorylation [122], though in a manner complexly depending on birth weight still in the adult. Epigenetic signatures of aging have been increasingly identified and refined, and animal studies on CR have shown a slower accumulation of age-related epigenetic markers [123]. With nutrient gathering being perhaps the most central activity required to stay alive, it has been conducive to fitness to build and maintain ways by which hungry animals will seek out food and will be effective in doing so. The intestines as a site of digestion are the site to sense fullness or conclude hunger, and by that complex set of pathways comprising the gut–brain axis [124–126], involving direct nervous connections as well as immune, endocrine, and circulating metabolite signaling, activity elsewhere in the body will shift at all levels. Accordingly, we expect and observe sweeping and wide-ranging effects of reduced nutrition, and as an emergency signal of sorts, for these pathways to have been less optimized for carefully avoiding off-target or secondary effects on the organism. Mood strongly reflects eating and hunger, directly and profoundly involving central nervous system reward mechanisms and triggering instinctive behaviors. These mechanisms in turn can drive a feedback loop over time and dominate habitual behaviors, with these processes likely underpinning some of the anecdotal utility of fasting as a psychological tool. Fasting has been shown to reduce levels of leptin, a satiety signal, beyond its regulation from adiposity itself [127–129], and there are indications that prolonged CR may revert resistance to leptin, which has been linked to overeating [130]. Slightly weaker evidence supports CR increasing adiponectin levels [128]. At least one trial showed increases in hunger suppressing GLP-1 and peptide YY levels following seasonal fasting [131]. Further studies into the impact of different forms of fasting on these signaling systems are needed.

Another mechanism that should not be underestimated either on shorter or longer time scales is the turnover of tissue-bound energy storage. As circulating blood glucose is essential to maintain at all times for the processes required for even vegetative survival, a calorie deficit must swiftly and decisively be countered by catabolic processes, recycling especially adipose and muscle tissue for energy. If the body cannot eat elsewhere, it must eat itself; analogous to how excess in turn is stored through anabolic processes gradually increasing the mass of those same tissues in different proportions. The autophagic process eventually takes place [38, 53, 132], releasing various compounds into circulation as anatomy begins to reshape. The steps needed for this to occur may serve the purposes of recycling and clearing out damaged cellular components or misfolded proteins [133], and as such, affect a broad range of processes across many tissues and cell types. It is further important to consider the context wherein fasting and catabolism are often followed by refeeding and anabolism, which in turn involve activation of regulatory programs and compensatory processes throughout the organism.

Fasting in the context of modern life

Given the way human bodies reflect adaptations to a (diverse, rather than monolithic) premodern past of recurring food scarcity, the changed conditions beginning with settled agricultural or pastoral lifestyles and proceeding to life in the industrialized modern world, at least in the global north, results in a major homeostatic challenge. For many people alive today, high-fat, high-salt, high-carbohydrate food, if monotonous, is available ad libitum. With bodies evolved to recognize conditions of plenty as an opportunity to build up energy stores for the inevitable winter, dry season, or period of poor fortune, overeating and the resulting weight gain are epidemic and resistant to most interventions [37, 38, 53]. Many modern processed foods are lower in pre- and postbiotic components than traditional fare, hampering the ability of microbiota to exert beneficial aspects, with this state also possibly linked, alongside changes in hygiene and sterility of our habitats, to a loss of co-evolved commensal microbiota, further compounding increased susceptibility to slowly progressing systemic loss of homeostasis and to disease. Comparisons of affluent populations from the global north to some remaining indigenous populations maintaining premodern practices, while heavily confounded by many other factors, indicate an accordingly elevated risk of many diseases in the former. As social and economic transitions take place in the developing world, changes in diet and

lifestyle accompany them. For this process, predictions were made and epidemiologically confirmed of a gradual increase in the same diseases, which can be projected onto a global shift in sources of mortality over the upcoming decades [134, 135]. Yet another intriguing mechanism for the potential impact of restricted feeding (especially IF) on homeostasis occurs through circadian rhythms. Most life is adapted to a day–night cycle and a review of available literature [136, 137] reveals evidence that time-restricted feeding can amplify fluctuations over that cycle in the composition and activity of the gut microbiome, interacting with host intestinal sensors, in turn driving signal cascades. Essentially, adaptations for within-day cyclicity may exist similar to seasonal adaptations, such that maintaining homeostasis is more challenging in its absence, and possible to further through its induction. Disruptions of the circadian rhythms exhibited and also mediated by the microbiota [138] and the immune system [10] are associated with an increased risk of cardiovascular and metabolic illness as well as cancer [138]. Microbial SCFA production plays a role in maintaining this homeostasis, forming another mechanism by which fasting may reduce disease risks. Moreover, especially IF may help strengthen or stabilize impaired cyclicity so that beneficial processes active during the non-feeding phase become more prevalent again [139], also with time-restricted feeding such that endogenous cyclicity is optimally aligned with nutrient availability from food intake [140]. Genes connected with the circadian clock also affect the regulation of production of insulin, thyroid hormones, and glucocorticoid, among others, with varying degrees of support for an impact of restricted feeding (for insulin, summarized below), but with as yet more research needed to establish under which circumstances these effects are beneficial [141]; but this again suggests a mechanism by way of which restricted feeding contributes to increased longevity [140].

Open questions: Locating the site of fasting action

One important largely unresolved question is to what extent the spatial heterogeneity of both host gene expression and microbiome composition/activity along the gastrointestinal tract [70–72] are relevant for mechanisms of fasting. Available restricted diet trial data in humans are largely always based on stool samples representing a rough summary of the gut as a whole, making spatially resolved changes upon dietary intervention thus far largely the domain of a very small number of animal studies, though with the expectation of substantial differences between responses in proximal and distal gut. Fourteen days of CR in a mouse model did show differentiated activation of host genes as well as differential microbial metabolite responses along the gut [142]. Novel sampling approaches for the human setting may here eventually complement what otherwise becomes a reliance on animal models with concomitant limitations.

Fasting and impact on the gut microbiota

With the above context in mind, the present review aims to summarize the main trends of the state of the art of fasting specifically (broadly defined as restricted feeding, see Box 1 for an overview of common forms and Fig. 2 for an overview of fasting mechanisms) as a tool for health improvement, with a particular focus on its interlocking impact on the human gut microbiome and immune cell populations, and with a particular focus on high-throughput ("-omics") studies that can support computational systems analysis. A summary of key findings (aiming primarily to identify recurring patterns as indicators of phenomena robust enough to be reproduced) is given, alongside some discussion of clinical relevance, translational potential, and remaining major knowledge gaps.

We summarize here the state of the art of human microbiome studies on the impact of fasting or CR diets. For a description of inclusion criteria and the comparison approach resulting in the articles shown in Table 1 and the results shown in Table S1 and Fig. 3, please see Supplementary Methods.

Overall, the literature on microbiome changes under any kind of fasting diet skews towards relatively small studies, and while most report significant changes in host health and metabolism, particularly weight loss, most are poorly powered to conclude specific changes robustly and exhaustively in microbiome composition. In those study designs where a fasting period is followed by a maintenance or refeeding period, as a rule, microbiome changes largely revert, suggesting changes are transient. Studies with no significant microbiome impact (Louis et al. [143], Cignarella et al. [144], and Heinsen et al. [145]) under these criteria were omitted from further discussion.

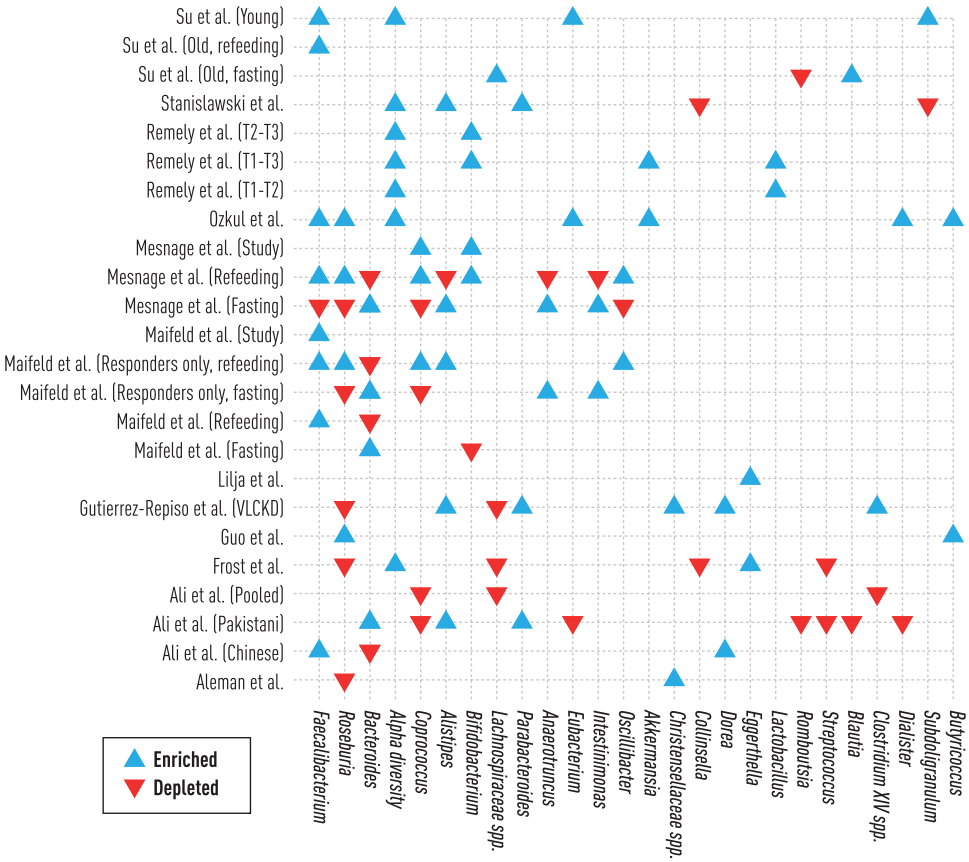

Fig. 2 *Schematic for fasting as an intervention in the context of an overview of fasting mechanisms.*

Effect of periodic fasting

Lilja and coworkers [146] investigated the gut microbiome and host gene expression before and after a Buchinger fast using 16S sequencing, revealing an overall change in microbiome structure yet with little that replicates in other available studies.

Maifeld and coworkers [2] carried out Buchinger fasting in metabolic syndrome patients followed by 3-month refeeding on a Mediterranean diet, assessed through shotgun and 16S sequencing of the gut microbiome, linking findings to changes in immune cell subpopulation proportions while, uniquely, adjusting the analysis for changing medication in many subjects throughout the follow-up period. Individuals in whom blood pressure control either improved or was maintained at a reduced medication dose were considered responders and differed already at baseline from nonresponders in immune profile and some microbiome features, including propionate production capacity. This deficit was normalized during fasting itself, whereas a relative lack of other SCFA-producing commensals, especially butyrate-producing *Faecalibacterium* but also *Coprococcus* and *Roseburia*, normalized during refeeding, particularly in responders. No effects on alpha diversity were seen, and most microbiome alterations had reverted to baseline by 3 months follow-up.

Mesnage and coworkers [147] assessed a Buchinger fast and subsequent 3-month refeeding in healthy volunteers, assessing the gut microbiome with 16S sequencing and additionally investigating serum metabolite levels. In many ways, changes during fasting resembled those seen in responders in the work by Maifeld et al., with initial depletion of SCFA producers (e.g., *Faecalibacterium*, *Coprococcus*, *Roseburia*, as well

Table 1. *Primary literature on human fasting interventions impacting the gut microbiome*

Title	First author	Journal	Year	Type	Number of probands	Duration	Microbiome characterization
Caloric restriction							
Characterization of the gut microbial community of obese patients following a weight-loss intervention using whole metagenome shotgun sequencing							
	Louis	PloS One	2016	VLCD, OPTIFAST, 800 kcal	16 (9F)	3 months, 2-year follow-up	Shotgun
Beneficial effects of a dietary weight loss intervention on human gut microbiome diversity and metabolism are not sustained during weight maintenance							
	Heinsen	Obesity Facts	2017	VLCD, 800 kcal	18 (15F)	3 months, 3-month maintenance	16S
Fecal microbiota and bile acid interactions with systemic and adipose tissue metabolism in diet-induced weight loss of obese postmenopausal women							
	Alemán	Journal of Translational Medicine	2019	VLCD, 800 kcal	10 (10F)	Average 46 days	16S
A structured weight loss program increases gut microbiota phylogenetic diversity and reduces levels of *Collinsella* in obese type 2 diabetics: a pilot study							
	Frost	PloS One	2020	VLCD, 800 kcal	12 (8F)	6 weeks, 15-week follow-up	16S
Different weight loss intervention approaches reveal a lack of a common pattern of gut microbiota changes							
	Gutiérrez-Repiso	Journal of Personalized Medicine	2021	VLCKD and others	VLCKD 18 (10F)	2 months	16S
The gut microbiota during a behavioral weight loss intervention							
	Stanislawski	Nutrients	2021	DCR, IMF	DCR 25, IMF 34	3 months	16S
Intermittent fasting							
Intermittent fasting confers protection in CNS autoimmunity by altering the gut microbiota							
	Cignarella	Cell Metabolism	2019	IMF	8 (5F)	2 weeks	Shotgun, 16S
Intermittent fasting improves cardiometabolic risk factors and alters gut microbiota in metabolic syndrome patients							
	Guo	The Journal of Clinical Endocrinology & Metabolism	2020	IMF	21 (11F)	8 weeks	16S
Structural changes in gut microbiome after Ramadan fasting: a pilot study							
	Ozkul	Beneficial Microbes	2021	Ramadan IMF	9	29 days	16S
Ramadan fasting leads to shifts in human gut microbiota structured by dietary composition							
	Ali	Frontiers in Microbiology	2021	Ramadan IMF	34	29 days	16S

(*Continued*)

Table 1. *(Continued)*

Title	First author	Journal	Year	Type	Number of probands	Duration	Microbiome characterization
Remodeling of the gut microbiome during Ramadan-associated intermittent fasting							
	Su	The American Journal of Clinical Nutrition	2021	Ramadan IMF	57 (17F)	29 days, 2-month follow-up	16S
Periodic fasting							
Increased gut microbiota diversity and abundance of *Faecalibacterium prausnitzii* and *Akkermansia* after fasting: a pilot study							
	Remely	Wiener Klinische Wochenschrift	2016	Buchinger	13	1 week, 6-week follow-up	16S qPCR
Changes in human gut microbiota composition are linked to the energy metabolic switch during 10 d of Buchinger fasting							
	Mesnage	Journal of Nutritional Science	2020	Buchinger	15 (15M)	10 days 3 months	16S
Fasting alters the gut microbiome reducing blood pressure and body weight in metabolic syndrome patients							
	Maifeld	Nature Communication	2021	Buchinger	35 (23F)	1 week, 3-month follow-up	Shotgun, 16S
Five days periodic fasting elevates levels of longevity related *Christensenella* and sirtuin expression in humans							
	Lilja	International Journal of Molecular Sciences	2021	Buchinger	20 (15F)	1 week	16S

Abbreviations: M, male participants; F, female participants; IMF, Intermittent Fasting; DCR, Daily Calorie Restriction; VLCD, Very Low Calorie Diet; VLCKD, Very Low Calorie Ketogenic Diet.

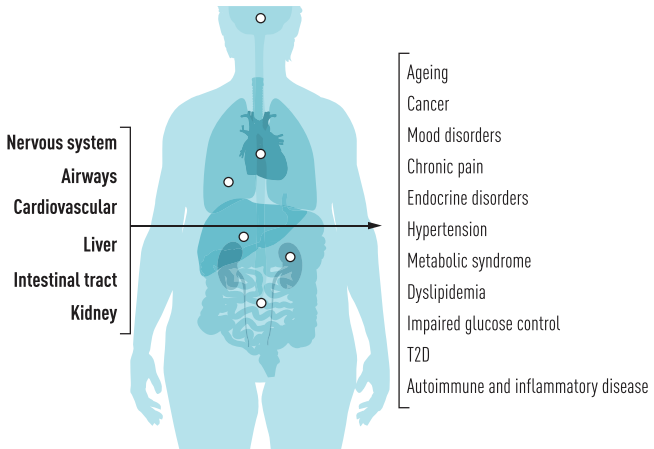

Fig. 3 *Consensus gut microbiome taxonomic composition changes under fasting interventions. The direction of the reported significant change in gut microbiome alpha diversity or genus abundance is shown as marker hue and direction.*

as *Oscillibacter*) then reversed during refeeding; and the opposite pattern was seen for potentially opportunistic taxa (including *Bacteroides*, *Alistipes*, *Intestinimonas*, and *Anaerotruncus*).

Remely and coworkers [148] report on a combined small-sample pilot trial where subjects undergo a Buchinger fast followed by refeeding with additional treatment with probiotics. Increased alpha diversity (reflecting more different taxa present in each ecosystem as opposed to bloom or monoculture) and carriage of the probiotic genera involved are the best-supported results on the microbiome level. This study also reports an increase in SCFA-producing mucus-associated *Akkermansia*, and *Faecalibacterium prausnitzii* at the species but not the genus level.

Effect of IF

Stanislawski et al. [149] report results of the DRIFT2 trial, which is a 12-month weight loss intervention in overweight subjects. Microbiome analysis through 16S sequencing was done for the first 3 months, with subjects randomized to CR or 4:3 IF. Multivariate compositional change of the gut microbiome is reported alongside increased alpha diversity, though no specific microbiome changes replicate in any other considered study.

Su and coworkers [150] followed an old and a young cohort undergoing yearly Ramadan IF, with an additional longer follow-up in the older cohort, analyzing the microbiome through 16S sequencing. Multivariate analysis revealed overall gut remodeling in both groups, with support for increased alpha diversity only in the younger group, and with an increase in *Faecalibacterium* being the main signal shared by both.

Ali and coworkers [151] similarly assessed microbiome changes throughout Ramadan IF in two different cohorts from two different countries using 16S sequencing of stool samples. No effect on alpha diversity was seen, and changes in other regards under the fast differ substantially between the subcohorts, with some signatures resembling that seen in Buchinger fasting, and some resembling refeeding.

Ozkul and coworkers [152] also investigated microbiome alterations in a small sample of volunteers undergoing Ramadan fasting using 16S sequencing. A clear shift towards gut eubiosis as previously described in the literature was seen, revealing elevated alpha diversity as well as elevated levels of SCFA producers including *Faecalibacterium*, *Roseburia*, *Eubacterium*, and *Akkermansia*. The best-known species within the latter, *A. muciniphila*, is a mucin degrader strongly associated with metabolic health in a variety of studies [153].

Guo and coworkers [154] investigated gut microbiome changes after 8 weeks of IF using 16S sequencing. Increases in SCFA producers including *Roseburia* and *Butyricoccus* were seen, alongside microbiome changes towards eubiosis on other taxonomic levels as well. *Butyricoccus* was depleted in inflammatory bowel disease patients and demonstrated protection in a rat colitis model [155], leading to suggestions for its probiotic use.

Effect of CRs

Alemán and coworkers [156] report from one of several overall restricted daily calorie diets, assessing gut microbiome composition through 16S sequencing under a weight loss intervention. The very limited sample number may underlie the largely negative findings in the microbiome space (while the intervention as such was effective).

Frost and coworkers [157] placed a small number of type 2 diabetic obese subjects on a low calorie diet followed by a food reintroduction period, investigating gut microbiome composition using 16S sequencing. From the reported results, alpha diversity, as a marker of eubiosis, increased, along with depletion of some pathobiont taxa. Most microbiome changes had reverted by the time of follow-up though sustained weight loss remained visible.

Gutiérrez-Repiso and coworkers [158] in one study compared bariatric surgery, the Mediterranean diet, and a CR diet to understand the possible scope of CR intervention in humans. While microbiome changes occurred in each study arm, signatures largely did not overlap, indicating that the mode of dietary intervention rather than the weight loss itself is what is most salient in accompanying gut microbiome alterations. The ketogenic CR arm is what most resembles other studies included in this review and has some overlap with taxa seen altered elsewhere (especially *Roseburia*, *Parabacteroides*, and *Alistipes*) in either fasting or refeeding stages, though the resulting heterogeneity suggests specifics of intervention and the

starting point may represent different aspects of an overall more complex process of nutrition-induced microbiome change. All three diets lowered blood sugar, though significance was achieved only in the bariatric surgery arm, in line with improved insulin sensitivity accompanying the microbiome changes.

Consensus findings on fasting impact on the gut microbiome

The most frequently found microbiome impact of fasting interventions, whether periodic or intermittent, is an enrichment of *Faecalibacterium* (resolved further as *F. prausnitzii*), well known to produce anti-inflammatory SCFA from dietary fiber and for being protective against both metabolic and inflammatory disease. Where the study design allows distinguishing of a fasting phase from a refeeding phase, this enrichment takes place during refeeding, sometimes following an initial suppression during fasting itself. *Roseburia*, *Butyricoccus*, and *Coprococcus*, also genera populated by core gut SCFA producers, display similar patterns, with evidence for depletion during fasting and enrichment during refeeding.

Several studies report increase in gut *Alistipes* abundance either in fasting or refeeding state, with a single counterexample of depletion-associated refeeding in a cohort of healthy volunteers. All in all, this suggests a taxon associated with the health improvements of fasting but with more work needed to clarify its specific role. Similar patterns are seen for the *Anaerotruncus* and *Intestinimonas* genera. Enrichment of *Bifidobacterium*, a common probiotic, has also been seen across multiple studies with some ambiguity as to the role of fasting and refeeding stages (and note also its direct use as a probiotic in one of the included studies), and an analogous case holds for *Parabacteroides*. It should be noted that in one of two studies concluding the increase of *Bifidobacterium*, this taxon is also present as a probiotic, so less likely to represent a fasting effect, though its support from another study suggests a protective role.

Inversely, *Bacteroides* is seen enriched during fasting itself in three studies, then depleted during refeeding; its specific impact seems robust nonetheless. As the central Bacteroidetes genus it is core to the Bacteroidetes–Firmicutes phylum ratio often proposed as a biomarker of gut eubiosis and reflected in the high-level summary of gut microbiome composition patterns as enterotypes. Several studies report depletion in the *Lachnospiraceae* taxa. *Eubacterium* is enriched in two but depleted in one intermittent (Ramadan) fasting intervention. Other previously discussed commensal and pathobiont associations reported, such as fasting-associated depletion of *Streptococcus* or *Collinsella* or increase in *Akkermansia*, *Eggerthella*, or *Lactobacillus*, are seen to be replicated but not consistently. Gut alpha diversity, well recognized as a marker of health and homeostasis, increases in several long-term moderate CR and IF scenarios but is generally not seen altered during intensive fasting. In particular, there is no indication of any loss of gut diversity from depriving the gut microbiome of nutrition on a shorter time scale, which might otherwise have been expected.

In summary, there is a good foundation for concluding that a variety of fasting interventions result in the enrichment of various anti-inflammatory core commensals, especially SCFA producers, consistently though with variability, which may reflect a form of fasting, time of follow-up, or state at baseline.

Conclusions

Fasting and refeeding, intermittent and ongoing CR are associated with eventual shifts in the gut ecosystem away from pathobionts and towards major anti-inflammatory commensal taxa. Such shifts seem transient, with the microbiome returning to near baseline within months of cessation of the intervention, but are frequently accompanied by longer-lasting changes to the metabolism and overall health induced when they are visible. There clearly seem to be grounds to adapt fasting-type diets in a variety of health indications, particularly components of the metabolic syndrome and its sequelae, as well as other immune- and inflammation-mediated diseases, especially combined with other modalities such that it may complement and strengthen homeostasis. Interventions showing fasting-induced improvement to a disease entity thus also indicate a potential gut microbiome protective effect or pathological mechanism involvement and can guide the design of further trials to test this. Such fasting-induced microbiome shift typically involves enrichment of bacteria such as *F. prausnitzii* that produce anti-inflammatory SCFA. Indeed, the impact of high fiber on immune homeostasis showed such effects, suggesting possible ways to “prime” an individual

to be more receptive to a wider scope of interventions.

It is also clear that while there exists a broad trend of interventions studied so far—Buchinger fasting, restriction of daily calories to less than 800 (very low calorie diets [159]), and intermittent (including religious) fasting—the specifics of these matter and any comparison of results from studies using different interventions of this kind will diverge reflecting factors of the interventions other than the calories ingested. To what extent do healthy versus metabolically ill individuals respond differently? And to what extent is this—as suggested by our work also in line with other recent studies suggesting individually variable and predictable glycemic responses to different diets—predictable from microbiome baseline, so that it represents a target for personalized nutrition? To address this, it is motivated to run larger-scale harmonized interventions where the individual baseline varies sufficiently within the cohorts.

Going forward, observing emerging standards for systematic data collection [160] and accounting for confounders [161], both in study design and analysis, are likely to further solidify our insights into the potential microbiome-mediated health benefits of fasting. This may guide it to a central role in future interventions, as expected from its anchoring within the scope of our human evolutionary past.

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Conflict of interest

No conflict of interest was declared.

References

1 Dong TA, Sandesara PB, Dhindsa DS, Mehta A, Arneson LC, Dollar AL, et al. Intermittent fasting: a heart healthy dietary pattern. *Am J Med.* 2020;**133**(8):901–7.
2 Maifeld A, Bartolomaeus H, Löber U, Avery EG, Steckhan N, Markó L, et al. Fasting alters the gut microbiome reducing blood pressure and body weight in metabolic syndrome patients. *Nat Commun.* 2021;**12**(1):1970.
3 Li C, Sadraie B, Steckhan N, Kessler CS, Stange R, Jeitler M, et al. Effects of a one-week fasting therapy in patients with type-2 diabetes mellitus and metabolic syndrome—a randomized controlled explorative study. *Exp Clin Endocrinol Diabetes.* 2017;**125**(9):618–24.
4 Kunduraci YE, Ozbek H. Does the energy restriction intermittent fasting diet alleviate metabolic syndrome biomarkers? A randomized controlled trial. *Nutrients.* 2020;**12**(10):E3213.
5 Parvaresh A, Razavi R, Abbasi B, Yaghoobloo K, Hassanzadeh A, Mohammadifard N, et al. Modified alternate-day fasting vs. calorie restriction in the treatment of patients with metabolic syndrome: a randomized clinical trial. *Complement Ther Med.* 2019;**47**:102187.
6 Yang F, Liu C, Liu X, Pan X, Li X, Tian L, et al. Effect of epidemic intermittent fasting on cardiometabolic risk factors: a systematic review and meta-analysis of randomized controlled trials. *Front Nutr.* 2021;**8**:669325.
7 Nicoll R, Henein MY. Caloric restriction and its effect on blood pressure, heart rate variability and arterial stiffness and dilatation: a review of the evidence. *Int J Mol Sci.* 2018;**19**(3):E751.
8 Grundler F, Plonné D, Mesnage R, Müller D, Sirtori CR, Ruscica M, et al. Long-term fasting improves lipoprotein-associated atherogenic risk in humans. *Eur J Nutr.* 2021;**60**(7):4031–44.
9 Skaznik-Wikiel ME, Polotsky AJ. The health pros and cons of continuous versus intermittent calorie restriction: more questions than answers. *Maturitas.* 2014;**79**(3):275–8.
10 Butler TD, Gibbs JE. Circadian host–microbiome interactions in immunity. *Front Immunol.* 2020;**11**:1783.
11 Morales-Suarez-Varela M-A, Collado Sánchez E, Peraita-Costa I, Llopis-Morales A-N, Soriano JM. Intermittent fasting and the possible benefits in obesity, diabetes, and multiple sclerosis: a systematic review of randomized clinical trials. *Nutrients.* 2021;**13**(9):3179.
12 Ganesan K, Habboush Y, Dagogo-Jack S. Calorie restriction and intermittent fasting: impact on glycemic control in people with diabetes. *Diabetes Spectr.* 2020;**33**(2):143–8.
13 Huang YS, Zheng Q, Yang H, Fu X, Zhang X, Xia C, et al. Efficacy of intermittent or continuous very low-energy diets in overweight and obese individuals with type 2 diabetes mellitus: a systematic review and meta-analyses. *J Diabetes Res.* 2020;**2020**:4851671.
14 Steven S, Taylor R. Restoring normoglycaemia by use of a very low calorie diet in long- and short-duration Type 2 diabetes. *Diabet Med.* 2015;**32**(9):1149–55.
15 Sathananthan M, Shah M, Edens KL, Grothe KB, Piccinini F, Farrugia LP, et al. Six and 12 weeks of caloric restriction increases β cell function and lowers fasting and postprandial glucose concentrations in people with type 2 diabetes. *J Nutr.* 2015;**145**(9):2046–51.
16 Saeed M, Ali M, Zehra T, Haider Zaidi SA, Tariq R. Intermittent fasting: a user-friendly method for type 2 diabetes mellitus. *Cureus.* 2021;**13**(11):e19348.
17 Mani K, Javaheri A, Diwan A. Lysosomes mediate benefits of intermittent fasting in cardiometabolic disease: the janitor is the undercover boss. *Compr Physiol.* 2018;**8**(4):1639–67.
18 Crupi AN, Haase J, Brandhorst S, Longo VD. Periodic and intermittent fasting in diabetes and cardiovascular disease. *Curr Diab Rep.* 2020;**20**(12):83.
19 Malinowski B, Zalewska K, Węsierska A, Sokołowska MM, Socha M, Liczner G, et al. Intermittent fasting in cardiovascular disorders—an overview. *Nutrients.* 2019;**11**(3):E673.
20 Maugeri A, Vinciguerra M. The effects of meal timing and frequency, caloric restriction, and fasting on cardiovascular health: an overview. *J Lipid Atheroscler.* 2020;**9**(1):140–52.

21 Ng GY, Kang SW, Kim J, Alli-Shaik A, Baik S-H, Jo D-G, et al. Genome-wide transcriptome analysis reveals intermittent fasting-induced metabolic rewiring in the liver. *Dose Response.* 2019;**17**(3):1559325819876780.
22 Drinda S, Grundler F, Neumann T, Lehmann T, Steckhan N, Michalsen A, et al. Effects of periodic fasting on fatty liver index—a prospective observational study. *Nutrients.* 2019;**11**(11):E2601.
23 Ma J, Cheng Y, Su Q, Ai W, Gong L, Wang Y, et al. Effects of intermittent fasting on liver physiology and metabolism in mice. *Exp Ther Med.* 2021;**22**(3):950.
24 Rohner M, Heiz R, Feldhaus S, Bornstein SR. Hepatic-metabolite-based intermittent fasting enables a sustained reduction in insulin resistance in type 2 diabetes and metabolic syndrome. *Horm Metab Res.* 2021;**53**(8):529–40.
25 Yin C, Li Z, Xiang Y, Peng H, Yang P, Yuan S, et al. Effect of intermittent fasting on non-alcoholic fatty liver disease: systematic review and meta-analysis. *Front Nutr.* 2021;**8**:709683.
26 Kjeldsen-Kragh J, Borchgrevink CF, Laerum E, Haugen M, Eek M, Førre O, et al. Controlled trial of fasting and one-year vegetarian diet in rheumatoid arthritis. *Lancet.* 1991;**338**(8772):899–902.
27 Roman SN, Fitzgerald KC, Beier M, Mowry EM. Safety and feasibility of various fasting-mimicking diets among people with multiple sclerosis. *Mult Scler Relat Disord.* 2020;**42**:102149.
28 Müller H, de Toledo FW, Resch KL. Fasting followed by vegetarian diet in patients with rheumatoid arthritis: a systematic review. *Scand J Rheumatol.* 2001;**30**(1):1–10.
29 Li C, Xing C, Zhang J, Zhao H, Shi W, He B. Eight-hour time-restricted feeding improves endocrine and metabolic profiles in women with anovulatory polycystic ovary syndrome. *J Transl Med.* 2021;**19**(1):148.
30 Michalsen A. Prolonged fasting as a method of mood enhancement in chronic pain syndromes: a review of clinical evidence and mechanisms. *Curr Pain Headache Rep.* 2010;**14**(2):80–7.
31 Phillips MCL. Fasting as a therapy in neurological disease. *Nutrients.* 2019;**11**(10):E2501.
32 Li W, Wu M, Zhang Y, Wei X, Zang J, Liu Y, et al. Intermittent fasting promotes adult hippocampal neuronal differentiation by activating GSK-3β in 3xTg-AD mice. *J Neurochem.* 2020;**155**(6):697–713.
33 Halagappa VKM, Guo Z, Pearson M, Matsuoka Y, Cutler RG, Laferla FM, et al. Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease. *Neurobiol Dis.* 2007;**26**(1):212–20.
34 Sadeghian M, Rahmani S, Khalesi S, Hejazi E. A review of fasting effects on the response of cancer to chemotherapy. *Clin Nutr.* 2021;**40**(4):1669–81.
35 Gabel K, Cares K, Varady K, Gadi V, Tussing-Humphreys L. Current evidence and directions for intermittent fasting during cancer chemotherapy. *Adv Nutr.* 2022;**13**:667–80. https://doi.org/10.1093/advances/nmab132.
36 O'Flanagan CH, Smith LA, Mcdonell SB, Hursting SD. When less may be more: calorie restriction and response to cancer therapy. *BMC Med.* 2017;**15**(1):106.
37 Hammer SS, Vieira CP, Mcfarland D, Sandler M, Levitsky Y, Dorweiler TF, et al. Fasting and fasting-mimicking treatment activate SIRT1/LXRα and alleviate diabetes-induced systemic and microvascular dysfunction. *Diabetologia.* 2021;**64**(7):1674–89.
38 Joaquim L, Faria A, Loureiro H, Matafome P. Benefits, mechanisms, and risks of intermittent fasting in metabolic syndrome and type 2 diabetes. *J Physiol Biochem.* 2022;**78**(2):295–305.
39 Hoddy KK, Marlatt KL, Çetinkaya H, Ravussin E. Intermittent fasting and metabolic health: from religious fast to time-restricted feeding. *Obesity (Silver Spring).* 2020;**28**(Suppl 1):S29–37.
40 Clifton KK, Ma CX, Fontana L, Peterson LL. Intermittent fasting in the prevention and treatment of cancer. *CA Cancer J Clin.* 2021;**71**(6):527–46.
41 Nencioni A, Caffa I, Cortellino S, Longo VD. Fasting and cancer: molecular mechanisms and clinical application. *Nat Rev Cancer.* 2018;**18**(11):707–19.
42 Mendelsohn AR, Larrick JW. Dietary restriction: critical cofactors to separate health span from life span benefits. *Rejuvenation Res.* 2012;**15**(5):523–9.
43 Di Francesco A, Di Germanio C, Bernier M, De Cabo R. A time to fast. *Science.* 2018;**362**(6416):770–5.
44 Hwangbo D-S, Lee H-Y, Abozaid LS, Min K-J. Mechanisms of lifespan regulation by calorie restriction and intermittent fasting in model organisms. *Nutrients.* 2020;**12**(4):E1194.
45 Heilbronn LK, Ravussin E. Calorie restriction and aging: review of the literature and implications for studies in humans. *Am J Clin Nutr.* 2003;**78**(3):361–9.
46 Austad SN, Hoffman JM. Beyond calorie restriction: aging as a biological target for nutrient therapies. *Curr Opin Biotechnol.* 2021;**70**:56–60.
47 Mehdi MM, Solanki P, Singh P. Oxidative stress, antioxidants, hormesis and calorie restriction: the current perspective in the biology of aging. *Arch Gerontol Geriatr.* 2021;**95**:104413.
48 Erbaba B, Arslan-Ergul A, Adams MM. Effects of caloric restriction on the antagonistic and integrative hallmarks of aging. *Ageing Res Rev.* 2021;**66**:101228.
49 Dorling JL, Van Vliet S, Huffman KM, Kraus WE, Bhapkar M, Pieper CF, et al. Effects of caloric restriction on human physiological, psychological, and behavioral outcomes: highlights from CALERIE phase 2. *Nutr Rev.* 2021;**79**(1):98–113.
50 Kebbe M, Sparks JR, Flanagan EW, Redman LM. Beyond weight loss: current perspectives on the impact of calorie restriction on healthspan and lifespan. *Expert Rev Endocrinol Metab.* 2021;**16**(3):95–108.
51 Badal VD, Vaccariello ED, Murray ER, Yu KE, Knight R, Jeste DV, et al. The gut microbiome, aging, and longevity: a systematic review. *Nutrients.* 2020;**12**(12):E3759.
52 Seimon RV, Roekenes JA, Zibellini J, Zhu B, Gibson AA, Hills AP, et al. Do intermittent diets provide physiological benefits over continuous diets for weight loss? A systematic review of clinical trials. *Mol Cell Endocrinol.* 2015;**418**(Pt 2):153–72.
53 Golbidi S, Daiber A, Korac B, Li H, Essop MF, Laher I. Health benefits of fasting and caloric restriction. *Curr Diab Rep.* 2017;**17**(12):123.
54 Longo VD, Mattson MP. Fasting: molecular mechanisms and clinical applications. *Cell Metab.* 2014;**19**(2):181–92.
55 Wastyk HC, Fragiadakis GK, Perelman D, Dahan D, Merrill BD, Yu FB, et al. Gut-microbiota-targeted diets modulate human immune status. *Cell.* 2021;**184**(16):4137–53.e14.

56 Ghosh S, Whitley CS, Haribabu B, Jala VR. Regulation of intestinal barrier function by microbial metabolites. *Cell Mol Gastroenterol Hepatol.* 2021;**11**(5):1463–82.
57 Parada Venegas D, De la Fuente MK, Landskron G, Gonzalez MJ, Quera R, Dijkstra G, et al. Short chain fatty acids (SCFAs)–mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front Immunol.* 2019;**10**:277.
58 Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol.* 2016;**14**(8):e1002533.
59 Tlaskalová-Hogenová H, Stepánková R, Hudcovic T, Tucková L, Cukrowska B, Lodinová-Zádníková R, et al. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. *Immunol Lett.* 2004;**93**(2–3):97–108.
60 Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current understanding of the human microbiome. *Nat Med.* 2018;**24**(4):392–400.
61 Okawa T, Nagai M, Hase K. Dietary intervention impacts immune cell functions and dynamics by inducing metabolic rewiring. *Front Immunol.* 2020;**11**:623989.
62 Choi IY, Lee C, Longo VD. Nutrition and fasting mimicking diets in the prevention and treatment of autoimmune diseases and immunosenescence. *Mol Cell Endocrinol.* 2017;**455**:4–12.
63 Qian J, Fang Y, Yuan N, Gao X, Lv Y, Zhao C, et al. Innate immune remodeling by short-term intensive fasting. *Aging Cell.* 2021;**20**(11):e13507.
64 Collins N, Han S-J, Enamorado M, Link VM, Huang B, Moseman EA, et al. The bone marrow protects and optimizes immunological memory during dietary restriction. *Cell.* 2019;**178**(5):1088–101.e15.
65 Nagai M, Noguchi R, Takahashi D, Morikawa T, Koshida K, Komiyama S, et al. Fasting-refeeding impacts immune cell dynamics and mucosal immune responses. *Cell.* 2019;**178**(5):1072–87.e14.
66 Jordan S, Tung N, Casanova-Acebes M, Chang C, Cantoni C, Zhang D, et al. Dietary intake regulates the circulating inflammatory monocyte pool. *Cell.* 2019;**178**(5):1102–14.e17.
67 Yassour M, Vatanen T, Siljander H, Hämäläinen A-M, Härkönen T, Ryhänen SJ, et al. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Sci Transl Med.* 2016;**8**(343):343ra81.
68 Zhang Z, Geng J, Tang X, Fan H, Xu J, Wen X, et al. Spatial heterogeneity and co-occurrence patterns of human mucosal-associated intestinal microbiota. *ISME J.* 2014;**8**(4):881–93.
69 Costea PI, Hildebrand F, Arumugam M, Bäckhed F, Blaser MJ, Bushman FD, et al. Enterotypes in the landscape of gut microbial community composition. *Nat Microbiol.* 2018;**3**(1):8–16.
70 Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol.* 2016;**14**(1):20–32.
71 Tropini C, Earle KA, Huang KC, Sonnenburg JL. The gut microbiome: connecting spatial organization to function. *Cell Host Microbe.* 2017;**21**(4):433–42.
72 Tang Q, Jin G, Wang G, Liu T, Liu X, Wang B, et al. Current sampling methods for gut microbiota: a call for more precise devices. *Front Cell Infect Microbiol.* 2020;**10**:151.
73 Macpherson AJ, Uhr T. Compartmentalization of the mucosal immune responses to commensal intestinal bacteria. *Ann N Y Acad Sci.* 2004;**1029**:36–43.
74 Fiebiger U, Bereswill S, Heimesaat MM. Dissecting the interplay between intestinal microbiota and host immunity in health and disease: lessons learned from germfree and gnotobiotic animal models. *Eur J Microbiol Immunol (Bp).* 2016;**6**(4):253–71.
75 Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol.* 2009;**9**(5):313–23.
76 Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Res.* 2020;**30**(6):492–506.
77 McDermott AJ, Huffnagle GB. The microbiome and regulation of mucosal immunity. *Immunology.* 2014;**142**(1):24–31.
78 Wiertsema SP, Van Bergenhenegouwen J, Garssen J, Knippels LMJ. The interplay between the gut microbiome and the immune system in the context of infectious diseases throughout life and the role of nutrition in optimizing treatment strategies. *Nutrients.* 2021;**13**(3):886.
79 Shi N, Li N, Duan X, Niu H. Interaction between the gut microbiome and mucosal immune system. *Mil Med Res.* 2017;**4**:14.
80 Bain CC, Cerovic V. Interactions of the microbiota with the mucosal immune system. *Immunology.* 2020;**159**(1):1–3.
81 Motta J-P, Wallace JL, Buret AG, Deraison C, Vergnolle N. Gastrointestinal biofilms in health and disease. *Nat Rev Gastroenterol Hepatol.* 2021;**18**(5):314–34.
82 Hibbing ME, Fuqua C, Parsek MR, Peterson SB. Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev Microbiol.* 2010;**8**(1):15–25.
83 Nadell CD, Drescher K, Foster KR. Spatial structure, cooperation and competition in biofilms. *Nat Rev Microbiol.* 2016;**14**(9):589–600.
84 Wilson AS, Koller KR, Ramaboli MC, Nesengani LT, Ocvirk S, Chen C, et al. Diet and the human gut microbiome: an international review. *Dig Dis Sci.* 2020;**65**(3):723–40.
85 Kolodziejczyk AA, Zheng D, Elinav E. Diet–microbiota interactions and personalized nutrition. *Nat Rev Microbiol.* 2019;**17**(12):742–53.
86 Wolter M, Grant ET, Boudaud M, Steimle A, Pereira GV, Martens EC, et al. Leveraging diet to engineer the gut microbiome. *Nat Rev Gastroenterol Hepatol.* 2021;**18**(12):885–902.
87 Forslund SK, Chakaroun R, Zimmermann-Kogadeeva M, Markó L, Aron-Wisnewsky J, Nielsen T, et al. Combinatorial, additive and dose-dependent drug-microbiome associations. *Nature.* 2021;**600**(7889):500–5.
88 Salvucci E. Microbiome, holobiont and the net of life. *Crit Rev Microbiol.* 2016;**42**(3):485–94.
89 Simon J-C, Marchesi JR, Mougel C, Selosse M-A. Host–microbiota interactions: from holobiont theory to analysis. *Microbiome.* 2019;**7**(1):5.
90 Baedke J, Fábregas-Tejeda A, Nieves Delgado A, Margulis L, Meyer-Abich A. The holobiont concept before Margulis. *J Exp Zool B Mol Dev Evol.* 2020;**334**(3):149–55.

91 Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, et al. Personalized nutrition by prediction of glycemic responses. *Cell.* 2015;**163**(5):1079–94.
92 Rein M, Ben-Yacov O, Godneva A, Shilo S, Zmora N, Kolobkov D, et al. Effects of personalized diets by prediction of glycemic responses on glycemic control and metabolic health in newly diagnosed T2DM: a randomized dietary intervention pilot trial. *BMC Med.* 2022;**20**(1):56.
93 Schulthess J, Pandey S, Capitani M, Rue-Albrecht KC, Arnold I, Franchini F, et al. The short chain fatty acid butyrate imprints an antimicrobial program in macrophages. *Immunity.* 2019;**50**(2):432–45.e7.
94 Zhao Y, Chen F, Wu W, Sun M, Bilotta AJ, Yao S, et al. GPR43 mediates microbiota metabolite SCFA regulation of antimicrobial peptide expression in intestinal epithelial cells via activation of mTOR and STAT3. *Mucosal Immunol.* 2018;**11**(3):752–62.
95 Beli E, Yan Y, Moldovan L, Gao R, Duan Y, Prasad R, et al. Restructuring of the gut microbiome by intermittent fasting prevents retinopathy and prolongs survival in db/db mice. *Diabetes.* 2018;**67**(9):1867–79.
96 Shi H, Zhang B, Abo-Hamzy T, Nelson JW, Ambati CSR, Petrosino JF, et al. Restructuring the gut microbiota by intermittent fasting lowers blood pressure. *Circ Res.* 2021;**128**(9):1240–54.
97 Li M, Wang S, Li Y, Zhao M, Kuang J, Liang D, et al. Gut microbiota-bile acid crosstalk contributes to the rebound weight gain after calorie restriction in mice. *Nat Commun.* 2022;**13**(1):2060.
98 Natarajan N, Hori D, Flavahan S, Steppan J, Flavahan NA, Berkowitz DE, et al. Microbial short chain fatty acid metabolites lower blood pressure via endothelial G protein-coupled receptor 41. *Physiol Genomics.* 2016;**48**(11):826–34.
99 Gill PA, van Zelm MC, Muir JG, Gibson PR. Review article: short chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders. *Aliment Pharmacol Ther.* 2018;**48**(1):15–34.
100 Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. *Adv Immunol.* 2014;**121**:91–119.
101 Blaak EE, Canfora EE, Theis S, Frost G, Groen AK, Mithieux G, et al. Short chain fatty acids in human gut and metabolic health. *Benef Microbes.* 2020;**11**(5):411–55.
102 Chambers ES, Preston T, Frost G, Morrison DJ. Role of gut microbiota-generated short-chain fatty acids in metabolic and cardiovascular health. *Curr Nutr Rep.* 2018;**7**(4):198–206.
103 Yang D, Han Z, Oppenheim JJ. Alarmins and immunity. *Immunol Rev.* 2017;**280**(1):41–56. https://doi.org/10.1111/imr.12577.
104 Minihane AM, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM, et al. Low-grade inflammation, diet composition and health: current research evidence and its translation. *Br J Nutr.* 2015;**114**(7):999–1012.
105 Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. *Br J Nutr.* 2011;**106**(Suppl 3):S5–78.
106 Custodero C, Mankowski RT, Lee SA, Chen Z, Wu S, Manini TM, et al. Evidence-based nutritional and pharmacological interventions targeting chronic low-grade inflammation in middle-age and older adults: a systematic review and meta-analysis. *Ageing Res Rev.* 2018;**46**:42–59.
107 Calder PC, Bosco N, Bourdet-Sicard R, Capuron L, Delzenne N, Doré J, et al. Health relevance of the modification of low grade inflammation in ageing (inflammageing) and the role of nutrition. *Ageing Res Rev.* 2017;**40**:95–119.
108 Bonaccio M, Di Castelnuovo A, Pounis G, De Curtis A, Costanzo S, Persichillo M, et al. A score of low-grade inflammation and risk of mortality: prospective findings from the Moli-sani study. *Haematologica.* 2016;**101**(11):1434–41.
109 Schwingshackl L, Hoffmann G. Mediterranean dietary pattern, inflammation and endothelial function: a systematic review and meta-analysis of intervention trials. *Nutr Metab Cardiovasc Dis.* 2014;**24**(9):929–39.
110 Bonaccio M, Pounis G, Cerletti C, Donati MB, Iacoviello L, De Gaetano G. Mediterranean diet, dietary polyphenols and low grade inflammation: results from the MOLI-SANI study. *Br J Clin Pharmacol.* 2017;**83**(1):107–13.
111 Tsigalou C, Konstantinidis T, Paraschaki A, Stavropoulou E, Voidarou C, Bezirtzoglou E. Mediterranean diet as a tool to combat inflammation and chronic diseases. An overview. *Biomedicines.* 2020;**8**(7):E201.
112 Bailey MA, Holscher HD. Microbiome-mediated effects of the Mediterranean diet on inflammation. *Adv Nutr.* 2018;**9**(3):193–206.
113 Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi S, et al. Prebiotics: definition, types, sources, mechanisms, and clinical applications. *Foods.* 2019;**8**(3):E92.
114 Reid G, Gadir AA, Dhir R. Probiotics: reiterating what they are and what they are not. *Front Microbiol.* 2019;**10**:424.
115 Collado MC, Vinderola G, Salminen S. Postbiotics: facts and open questions. A position paper on the need for a consensus definition. *Benef Microbes.* 2019;**10**(7):711–9.
116 Bland JS. Fasting physiology and therapeutic diets: a look back to the future. *Integr Med (Encinitas).* 2019;**18**(1):16–21.
117 Wilhelmi De Toledo F, Grundler F, Bergouignan A, Drinda S, Michalsen A. Safety, health improvement and well-being during a 4 to 21-day fasting period in an observational study including 1422 subjects. *PLoS One.* 2019;**14**(1):e0209353.
118 Hernández-Saavedra D, Moody L, Xu GB, Chen H, Pan Y-X. Epigenetic regulation of metabolism and inflammation by calorie restriction. *Adv Nutr.* 2019;**10**(3):520–36.
119 Asif S, Morrow NM, Mulvihill EE, Kim K-H. Understanding dietary intervention-mediated epigenetic modifications in metabolic diseases. *Front Genet.* 2020;**11**:590369.
120 Yong-Quan NG, Fann DY, Jo DG, Sobey CG, Arumugam TV. Epigenetic regulation by dietary restriction: part II. *Cond Med.* 2019;**2**(6):300–10.
121 Hjort L, Jørgensen SW, Gillberg L, Hall E, Brøns C, Frystyk J, et al. 36 h fasting of young men influences adipose tissue DNA methylation of LEP and ADIPOQ in a birth weight-dependent manner. *Clin Epigenetics.* 2017;**9**:40.
122 Gillberg L, Rönn T, Jørgensen SW, Perfilyev A, Hjort L, Nilsson E, et al. Fasting unmasks differential fat and muscle transcriptional regulation of metabolic gene sets in low versus normal birth weight men. *EBioMedicine.* 2019;**47**:341–51.
123 Gensous N, Franceschi C, Santoro A, Milazzo M, Garagnani P, Bacalini MG. The impact of caloric restriction on the

epigenetic signatures of aging. *Int J Mol Sci.* 2019;**20**(8): E2022.
124 Cryan JF, O'riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, et al. The microbiota–gut–brain axis. *Physiol Rev.* 2019;**99**(4):1877–2013.
125 Carabotti M, Scirocco A, Maselli MA, Severi C. The gut–brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol.* 2015;**28**(2):203–9.
126 Osadchiy V, Martin CR, Mayer EA. The gut–brain axis and the microbiome: mechanisms and clinical implications. *Clin Gastroenterol Hepatol.* 2019;**17**(2):322–32.
127 Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. *J Clin Endocrinol Metab.* 1997;**82**(2):561–5.
128 Varkaneh Kord H, Tinsley GM, Santos HO, Zand H, Nazary A, Fatahi S, et al. The influence of fasting and energy-restricted diets on leptin and adiponectin levels in humans: a systematic review and meta-analysis. *Clin Nutr.* 2021;**40**(4):1811–21.
129 Gaeini Z, Mirmiran P, Bahadoran Z. Effects of Ramadan intermittent fasting on leptin and adiponectin: a systematic review and meta-analysis. *Hormones (Athens).* 2021;**20**(2):237–46.
130 Mendoza-Herrera K, Florio AA, Moore M, Marrero A, Tamez M, Bhupathiraju SN, et al. The leptin system and diet: a mini review of the current evidence. *Front Endocrinol (Lausanne).* 2021;**12**:749050.
131 Zouhal H, Bagheri R, Triki R, Saeidi A, Wong A, Hackney AC, et al. Effects of Ramadan intermittent fasting on gut hormones and body composition in males with obesity. *Int J Environ Res Public Health.* 2020;**17**(15):E5600.
132 Rickenbacher A, Jang JH, Limani P, Ungethüm U, Lehmann K, Oberkofler CE, et al. Fasting protects liver from ischemic injury through Sirt1-mediated downregulation of circulating HMGB1 in mice. *J Hepatol.* 2014;**61**(2):301–8.
133 Bagherniya M, Butler AE, Barreto GE, Sahebkar A. The effect of fasting or calorie restriction on autophagy induction: a review of the literature. *Ageing Res Rev.* 2018;**47**:183–97.
134 Misra A, Khurana L. Obesity and the metabolic syndrome in developing countries. *J Clin Endocrinol Metab.* 2008;**93**(11 Suppl 1):S9–30.
135 Saklayen MG. The global epidemic of the metabolic syndrome. *Curr Hypertens Rep.* 2018;**20**(2):12.
136 Daas MC, De Roos NM. Intermittent fasting contributes to aligned circadian rhythms through interactions with the gut microbiome. *Benef Microbes.* 2021;**12**(2):147–61.
137 Patterson RE, Sears DD. Metabolic effects of intermittent fasting. *Annu Rev Nutr.* 2017;**37**:371–93.
138 Bishehsari F, Voigt RM, Keshavarzian A. Circadian rhythms and the gut microbiota: from the metabolic syndrome to cancer. *Nat Rev Endocrinol.* 2020;**16**(12):731–9.
139 Longo VD, Panda S, Circadian rhythms, and time-restricted feeding in healthy lifespan. *Cell Metab.* 2016;**23**(6):1048–59.
140 Acosta-Rodríguez VA, Rijo-Ferreira F, Green CB, Takahashi JS. Importance of circadian timing for aging and longevity. *Nat Commun.* 2021;**12**(1):2862.
141 Kim BH, Joo Y, Kim M-S, Choe HK, Tong Q, Kwon O. Effects of intermittent fasting on the circulating levels and circadian rhythms of hormones. *Endocrinol Metab (Seoul).* 2021;**36**(4):745–56.
142 Duszka K, Ellero-Simatos S, Ow GS, Defernez M, Paramalingam E, Tett A, et al. Complementary intestinal mucosa and microbiota responses to caloric restriction. *Sci Rep.* 2018;**8**(1):11338.
143 Louis S, Tappu R-M, Damms-Machado A, Huson DH, Bischoff SC. Characterization of the gut microbial community of obese patients following a weight-loss intervention using whole metagenome shotgun sequencing. *PLoS One.* 2016;**11**(2):e0149564.
144 Cignarella F, Cantoni C, Ghezzi L, Salter A, Dorsett Y, Chen L, et al. Intermittent fasting confers protection in CNS autoimmunity by altering the gut microbiota. *Cell Metab.* 2018;**27**(6):1222–35.e6.
145 Heinsen F-A, Fangmann D, Müller N, Schulte DM, Rühlemann MC, Türk K, et al. Beneficial effects of a dietary weight loss intervention on human gut microbiome diversity and metabolism are not sustained during weight maintenance. *Obes Facts.* 2016;**9**(6):379–91.
146 Lilja S, Stoll C, Krammer U, Hippe B, Duszka K, Debebe T, et al. Five days periodic fasting elevates levels of longevity related *Christensenella* and sirtuin expression in humans. *Int J Mol Sci.* 2021;**22**(5):2331.
147 Mesnage R, Grundler F, Schwiertz A, Le Maho Y, Wilhelmi De Toledo F, Wilhelmi de Toledo F. Changes in human gut microbiota composition are linked to the energy metabolic switch during 10 d of Buchinger fasting. *J Nutr Sci.* 2019;**8**:e36.
148 Remely M, Hippe B, Geretschlaeger I, Stegmayer S, Hoefinger I, Haslberger A. Increased gut microbiota diversity and abundance of *Faecalibacterium prausnitzii* and *Akkermansia* after fasting: a pilot study. *Wien Klin Wochenschr.* 2015;**127**(9–10):394–8.
149 Stanislawski MA, Frank DN, Borengasser SJ, Ostendorf DM, Ir D, Jambal P, et al. The gut microbiota during a behavioral weight loss intervention. *Nutrients.* 2021;**13**(9): 3248.
150 Su J, Wang Y, Zhang X, Ma M, Xie Z, Pan Q, et al. Remodeling of the gut microbiome during Ramadan-associated intermittent fasting. *Am J Clin Nutr.* 2021;**113**(5):1332–42.
151 Ali I, Liu K, Long D, Faisal S, Hilal MG, Ali I, et al. Ramadan fasting leads to shifts in human gut microbiota structured by dietary composition. *Front Microbiol.* 2021;**12**:642999.
152 Ozkul C, Yalinay M, Karakan T. Structural changes in gut microbiome after Ramadan fasting: a pilot study. *Benef Microbes.* 2020;**11**(3):227–33.
153 Xu Y, Wang N, Tan H-Y, Li S, Zhang C, Feng Y. Function of *Akkermansia muciniphila* in obesity: interactions with lipid metabolism, immune response and gut systems. *Front Microbiol.* 2020;**11**:219.
154 Guo Y, Luo S, Ye Y, Yin S, Fan J, Xia M. Intermittent fasting improves cardiometabolic risk factors and alters gut microbiota in metabolic syndrome patients. *J Clin Endocrinol Metab.* 2021;**106**(1):64–79.
155 Eeckhaut V, Machiels K, Perrier C, Romero C, Maes S, Flahou B, et al. *Butyricicoccus pullicaecorum* in inflammatory bowel disease. *Gut.* 2013;**62**(12):1745–52.
156 Alemán J, Bokulich NA, Swann JR, Walker JM, De Rosa JC, Battaglia T, et al. Fecal microbiota and bile acid interactions with systemic and adipose tissue metabolism in

diet-induced weight loss of obese postmenopausal women. *J Transl Med.* 2018;**16**(1):244.
157 Frost F, Storck LJ, Kacprowski T, GÃ¤Rtner S, RÃ¼Hlemann M, Bang C, et al. A structured weight loss program increases gut microbiota phylogenetic diversity and reduces levels of *Collinsella* in obese type 2 diabetics: a pilot study. *PLoS One.* 2019;**14**(7):e0219489.
158 Gutiérrez-Repiso C, Molina-Vega M-A, Bernal-López MR, Garrido-Sánchez L, Garcã-A-Almeida JM, Sajoux I, et al. Different weight loss intervention approaches reveal a lack of a common pattern of gut microbiota changes. *J Pers Med.* 2021;**11**(2):109.
159 Zubrzycki A, Cierpka-Kmiec K, Kmiec Z, Wronska A. The role of low-calorie diets and intermittent fasting in the treatment of obesity and type-2 diabetes. *J Physiol Pharmacol.* 2018;**69**(5):663–83.
160 Mirzayi C, Renson A, Furlanello C, Sansone S-A, Zohra F, Elsafoury S, et al. Reporting guidelines for human microbiome research: the STORMS checklist. *Nat Med.* 2021;**27**(11):1885–92.
161 Bartolomaeus TUP, Birkner T, Bartolomaeus H, Löber U, Avery EG, Mähler A, et al. Quantifying technical confounders in microbiome studies. *Cardiovasc Res.* 2021;**117**(3):863–75.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supplementary Methods: A systematic review of the fasting literature.

Table S1: Comparison approach resulting in the articles. ■

