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Review

Intermittent fasting modulates human gut microbiota diversity in a phenotype-dependent manner: a systematic review

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Cumulative evidence suggests that intermittent fasting (IF) has beneficial effects on human metabolic health. It has been indicated that its impact on the gut microbiota may mediate these beneficial effects. As a result, we hypothesized that IF may impact the human gut microbiota. A systematic review was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) protocol using the PubMed, Scopus, and CINAHL databases. We registered our systematic review protocol in PROSPERO under registration number CRD42021270050. Human intervention studies published until April 30, 2023, were included. The quality of the included studies was assessed using National Institutes of Health (NIH) quality assessment study tools for intervention studies. The search in the database returned 166 studies, of which 13 matched all criteria for the final qualitative analysis. The body of evidence suggests that IF modulates human gut microbiota alpha and beta diversity in lean (relatively healthy) and relatively healthy overweight/obese individuals but not in individuals with metabolic syndrome. Furthermore, IF also alters human gut microbiota composition in all phenotypes. Of interest, the gut microbiota taxa or microbial metabolites after an IF intervention are associated with metabolic markers. According to this review, IF influences the diversity and taxonomic levels of the human gut microbiota. Individual metabolic phenotypes may alter the effect of IF on the diversity and taxonomic levels of the gut microbiota.

Key words: intermittent fasting, gut microbiota, diversity, human, systematic review

INTRODUCTION

The human body has a distinctive form made up of human cells and microorganisms [1]. It has been shown that a complex ecological community of microbiomes coexists with the human ecosystem [2]. Cumulative evidence suggests that the gut microbiome affects host physiology and metabolism [3]. The gut microbiota is an ecosystem that includes all bacterial species that colonize the gastrointestinal tract permanently, as well as a huge number of additional microorganisms from the environment [4]. Firmicutes (which contains primarily the *Clostridium, Enterococcus, Lactobacillus*, and *Faecalibacterium* genera) and Bacteroidetes (which includes notably the *Bacteroides* and *Prevotella* genera) dominate the gut microbiota of a healthy human adult. Actinobacteria (primarily *Bifidobacterium*), Proteobacteria, Verrucomicrobia, and Euryarchaeota are represented in lower numbers [5].

A review by Lynch and Pedersen described how gut microbiome diversity has been linked to the health and diseases of the host [2]. An *in vivo* animal model and human studies

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support the link between the microbiome and metabolic diseases, such as obesity [6], type 2 diabetes (T2D) [7], insulin resistance [8], and hypertension [9]. The gut microbiota may influence host metabolism through a variety of mechanisms. These mechanisms include the synthesis of microbial metabolites of short-chain fatty acids (SCFAs) [10] and the balance between the gut microbiota and immune system [11]. Other examples include the role of the gut microbiota in the synthesis of micronutrients such as vitamins, which are of great value for both microbial and host metabolisms, and its essential role in the co-metabolism of bile acids with the host [12].

The normal gut microbiota performs particular functions in host nutrition metabolism, xenobiotic and drug metabolism, gut mucosal barrier structural integrity, immunomodulation, and pathogen defense. A number of factors influence the normal gut microbiome. These include (1) mode of delivery (vaginal or caesarean), (2) food throughout infancy (breast milk or formula feeds), and (3) use of antibiotics or antibiotic-like compounds originating from the environment or the gut commensal community [13]. It has been proposed that a greater composition, diversity, and functionality [14] of species associated with the production of SCFAs [10] (such as *Faecalibacterium prausnitzii*, *Bacteroides* spp., *Bifidobacterium* spp.) are perhaps the hallmarks of a microbial community associated with better health outcomes. However, peptide and protein fermentation in the gut (proteolytic fermentation) may produce primarily toxic chemicals, such as ammonia, phenols, and branched-chain fatty acids. These may be harmful to the host's digestive and metabolic health [10].

A comprehensive review by Singh *et al.* indicated the effects of dietary intake/components on the gut microbiome [15]. In addition, there has been growing interest in identifying alternative dietary modifications that involve restricting energy intake to specific periods of the day or prolonging the fasting interval between meals, called intermittent fasting (IF) [16, 17]. Time-restricted feeding (TRF), a type of IF, is the only eating pattern that does not necessitate calorie restriction [17]. Regarding TRF, religious fasting, which can be found in several religions (such as Ramadan fasting in Islam, which has been recognized for its similarities to TRF), could also be considered a form of TRF [18].

IF is one of the diet regimens that may also promote fat mass loss, reduce body weight, and improve metabolic health [19]. Modulation of the gut microbiota may mediate these beneficial effects. There is evidence (primarily from rat models) suggesting that fasting affects the gut microbiome [20–23]. Given the apparent role of IF and the microbiome in human metabolic health, we hypothized that IF influences the diversity and (relative) abundance of distinct bacterial taxa in the human gut microbiota, as well as their functionality, in humans.

MATERIALS AND METHODS

This systematic review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [24] and is registered in the PROSPERO database under registration number CRD42021270050.

Search strategy

Three electronic databases (PubMed, Scopus, and CINAHL) were screened for original articles published up to December 31, 2021 (updated on April 30, 2023), using the following main keywords: "intermittent fasting", "Ramadan fasting", "time-restricted feeding", "time-restricted eating", "time-restricted fasting", "gut microbiome", "gut microbiota", "gut microflora", and "gut bacteria". These keywords were combined using the Boolean operators AND and OR and constructed for each database. The specific combinations of keywords used for the searches in each database are listed in Supplementary Table 1. In addition, relevant articles from previously published reviews are reviewed under the subject categories below. Only articles published in English were eligible. The reference lists of previous reviews and articles were further checked for additional articles.

Study selection, inclusion, and exclusion

The PICOS (Population, Intervention, Comparison/Control, Outcome, Study Design) framework was used to develop inclusion criteria. The population (P) included in this study comprised adults (≥18 years) with no specific criteria for body mass index (BMI; in kg/m^2). The intervention (I) was any type of IF as described by Petterson and Sears [25] (including complete alternate-day fasting, modified fasting regimens, time-restricted feeding, religious fasting, Ramadan fasting, and other religious fasting). No minimum intervention duration criterion was applied. The primary outcome (O) was a measure of the gut microbiome, including (but not limited to) alpha diversity (a measure of variability within a sample), beta diversity (a measure of between-sample variability in microbial composition), species richness, any prevalence or (relative) abundance of bacterial taxa, Firmicutes/Bacteroidetes ratio, and functions of the gut microbiome. The secondary outcome was gut microbiota metabolites.

We only included human studies that were published in an English-language, peer-reviewed journal. Electronic items were permitted ahead of print. Reviews, editorials, letters, and comments were not considered due to the fact that they lacked original data. Conference abstracts and protocols were also omitted, as they had not undergone the same level of peer review as full-text articles. Two authors (AP and MA) separately assessed abstracts and complete texts for eligibility, with any doubts about eligibility discussed among the authors.

Data extraction and synthesis

Two authors (AP and MA) extracted data using standardized forms, including study characteristics, PICOS details, biological specimens and techniques used to assess the microbiome, and all intervention-outcome effect measures. The characteristics of each included article, such as references, study design, ethnicity, and number of participants, were included in the intervention and control groups. Furthermore, the details included patient characteristics (age, BMI, % female, comorbidities), descriptions of interventions (type of IF and duration), comparisons, and settings (laboratory or free living). Other details included outcomes (the primary outcome was gut microbiome diversity and composition; the secondary outcome was gut microbiota-derived metabolites) and durations of follow-up.

Quality assessment

The authors applied a previously reported tool created by the National Heart, Lung, and Blood Institute in the United States to assess a study's quality. This original assessment form was adopted because it has previously been utilized in controlled trials

and single-group intervention studies [26]. Four assessment items represented fatal flaws if the answers for the following criteria were "no/not reported/can't determine": 1) randomization, 2) dropout rate of less than 20%, 3) valid/reliable outcome measures, and 4) intent-to-treat analysis in random/cross-over trials. For single-group interventions, the criteria were 1) eligibility criteria pre-specified, 2) adequate sample size, 3) valid/reliable outcome measures, and 4) dropout rate of less than 20% or intent-to-treat analysis. A global rating was determined based on the number of fatal flaws: good quality (0 fatal flaws), acceptable quality (1 fatal flaw), or poor quality (≥2 fatal flaws). Quality assessment was conducted independently by two reviewers (AP and MA). Any disagreement between the reviewers was resolved through discussion (with the third author, EKSL).

RESULTS

Characteristics of the included studies

Figure 1 shows an article identification, screening, and final selection flow chart. The search identified 166 unique publications. Following the removal of articles with duplicate titles and full-text screening, the authors included 13 studies [27–39] in this systematic review. The characteristics of the included studies are summarized in Table 1.

Table 1 shows the characteristics of the included studies concerning the populations/participants, age, nutritional status (such as BMI), sex, sample size, type of fasting interventions, duration, and methodology used to perform the gut microbiome analysis. Eight studies enrolled healthy participants [27–30, 33, 34, 36, 37], three studies were performed on overweight or obese people [31, 32, 38], and two studies enrolled individuals with metabolic syndrome [35, 39]. Several types of IF were used in the included studies. Four studies [32, 35, 38, 39] used an IF with or without modification, such as pairing it with a Dietary Approaches to Stop Hypertension (DASH) diet. Furthermore, five studies [28, 33, 34, 36, 37] evaluated the effects of Ramadan fasting on the gut microbiota, while one study [25] investigated the effect of Buchinger fasting on the gut microbiota [27]. Other studies reported the effect of TRF only (2 studies) [30, 31]. The durations of the interventions in the included trials ranged from 7 days to one and a half years [27–39]. Microbiome characteristics were reported in all of these studies, including diversity, changes, or both at the genus, phylum, and species levels. A few studies also reported on the impact of fasting on the levels of gut microbiota metabolites such as SCFAs.

Qualitative analysis of the effects of fasting on the gut microbiota

Effect of IF on the gut microbiota's alpha-beta diversity

In general, the majority of the included studies (8 out of 13) found significant differences in microbial diversity between baseline and after their interventions (key findings are shown in

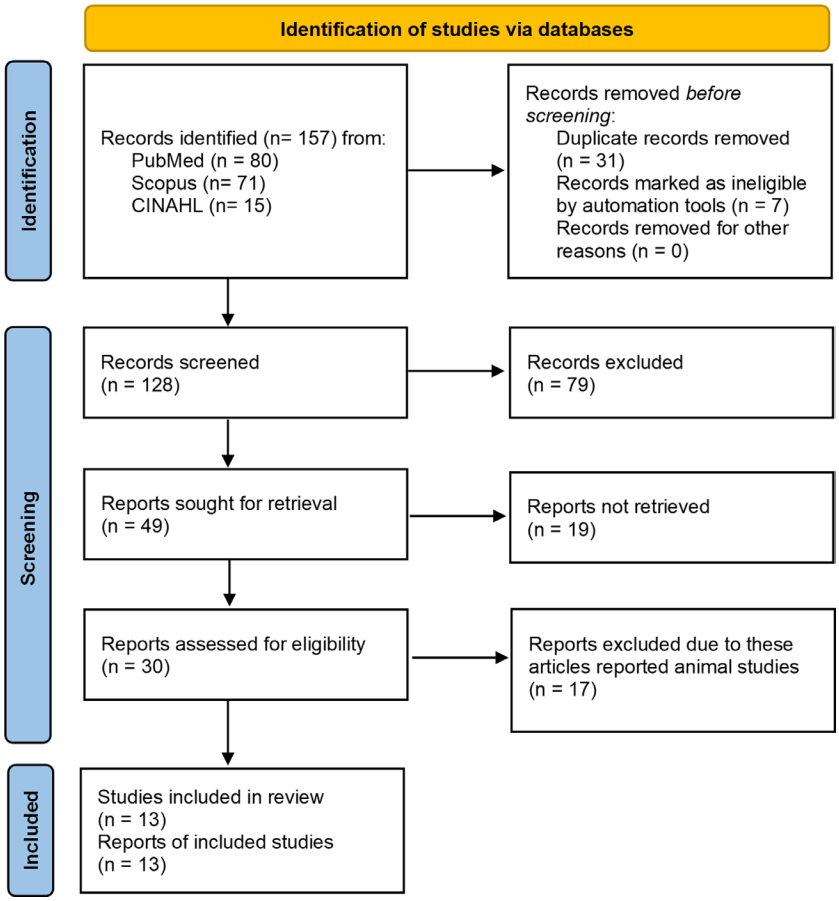

Fig. 1. The PRISMA flow diagram represents the different stages of article selection in this systematic review.

Table 1. Characteristics of the fasting studies included in this systematic review on the effect of intermittent fasting on the gut microbiome

First author (year) [References]	Metabolic phenotype of Participants	Age	BMI range/mean BMI (Kg/m^2)	Sex (M/F)	Ethnicity	N (completed)	Intervention (type of intermittent fasting)	Duration (days/ weeks/ months)	Comparator (control/placebo)	Outcome	Study design	Measurement of the gut microbiome
Mesnage *et al.* 2019 [27]	Relatively healthy men	18–70	20–32 (26.5 ± 3.0)	M	Caucasian	15	Natural / a 10-d periodic Buchinger fasting	10 days	No	Gut microbiome analysis	Natural experimental (Buchinger fasting)	16S RNA sequencing regional V3 and V4
Özkul *et al.* 2019 [28]	Healthy	31–56	22.9 ± 1.1	7/2	NA	9	Ramadan fasting	29 days	No	-Gut microbiome analysis -Blood biomarker analysis	Intervention (natural event)	qPCR approach for specific bacteria
He *et al.* 2019 [29]	Apparently healthy individuals	18–40	>18.5/ mean 22.8	6/10	Asian	16	Water-only group (n=6) Juice group (n=10)	7 days	Intermittent fasting (juice)	Gut microbiome analysis	7 days fasting intervention	16S RNA sequencing regional V4[1]
Zeb *et al.* 2020 [30]	Relatively healthy humans	Young aged	TRF group: 24.14 ± 3.5 Non-TRF group: 26.13 ± 5.2	100% men	NA	80 (TRF=56; Non-TRF=24)	TRF: participants were allowed to consume their normal diet with no food restriction for only 8 h per 24 h, that is, from 19.30 to 03.30 hours for 25 d Non-TRF: The non-TRF group continued their regular diet and were not given any specific instructions or time restriction.	25 days	Non-TRF	-Gut microbiome analysis -Blood biomarker analysis -Circadian related genes analysis	A multi-center human intervention study	16S rRNA[2] sequencing regional V1–V3[3]
Gabel *et al.* 2020 [31]	Individuals with obesity	25–56	30–45	NA	NA	14	TRF	12 weeks	No	-Gut microbiome analysis -Blood biomarker analysis	Intervention study	16S rRNA sequencing
Remely *et al.* 2015 [32]	Individuals with overweight	53.33 ± 6.55	28.10 ± 3.50	NA	Caucasian	13	Fasting	7 days	No	-Gut microbiome analysis	Intervention	16S rDNA sequencing[4]
Ozkul *et al.* 2020 [33]	Healthy volunteers	>18 (45.0 ± 9.7)	23.0 ± 1.5	NA	NA	9	Ramadan fasting	29 days	No	-Gut microbiome analysis	Intervention (natural event)	16S rRNA gene sequencing
Su *et al.* 2021 [34]	Healthy	- young = 18.63 ± 1.75 - Adults= Average 40	Normal BMI	NA	100% men Fasting men (70%) Non-fasting men (37%)	67 (young=30; middle-aged=37)	Ramadan fasting	30 days	Young=No control Middle-age= Control no Ramadan fasting	-Gut microbiome analysis	Intervention (natural event)	16S rRNA gene sequencing regional V3–V4[5]
Maifeld *et al.* 2021 [35]	Metabolic syndrome	Fasting +Dash Diet: 58 ± 8 DASH: 62 ± 8	NA	NA	Fasting+Dash Diet: 12/23 Dash Diet: 15/21	71	Fasting+DASH DASH	7 days	Dash Diet	-Gut microbiome -Blood biomarker analysis -Blood pressure	Intervention	16S rRNA gene sequencing regional V4[6]

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Table 1. Continued

First author (year) [References]	Metabolic phenotype of Participants	Age	BMI range/mean BMI (Kg/m^2)	Sex (M/F)	Ethnicity	N (completed)	Intervention (type of intermittent fasting)	Duration (days/ weeks/ months)	Comparator (control/placebo)	Outcome	Study design	Measurement of the gut microbiome
Ali *et al.* 2021 [36]	Healthy	18–40	NA	NA	Multi-ethnic/ Pakistani - Dark Caucasian and Chinese - Asian	34	Ramadan fasting	30 days	No	-Gut microbiome analysis	Intervention (natural)	16S rRNA gene sequencing[7]
Mohammadzadeh *et al.* 2021 [37]	Healthy	25–74	Pre Ramadan: 25.72 ± 0.58 Post Ramadan: 25.25 ± 0.55	NA	24/6 (80%)	30	Ramadan fasting	30 days	No	-Gut microbiome analysis	Intervention (natural)	16S rDNA gene sequencing[8]
Stanislawski *et al.* 2021 [38]	Overweight / Obese	18–55	Overall 33.7 ± 4.4 | DCR (Daily Caloric Restriction) 32.9 ± 4.7 | IMF (Intermittent Fasting) 33.2 ± 4.1	14/45 (31.11%)	White, Black / African American, Asian, Hispanic	59	Comprehensive behavioral wight loss intervention involving an energy restricted diet vs. Intermittent Fasting	12 months with additional 6 months follow up	No	-Gut microbiome analysis	Ancillary to an ongoing randomized lifestyle wight loss trial (DRIFT2)	16S rRNA gene sequencing
Guo *et al.* 2020 (Accepted manuscript) [39]	Metabolic syndrome	30–50	Baseline IF-28 Baseline CD-27.7	IF - 10/11 (47.6%) CD - 11/8 (61.1%)	NA	39 (21 from IF, 18 from CD)	For IF group, 75% energy restriction for 2 nonconsecutive days a week and an ad libitum diet the other five years	8 weeks	No	-Gut microbiome analysis	RCT - Identifier NCT03608800	16S rRNA gene sequencing

[1]by Illumina PE 101 sequencing.
[2]Ribosomal ribonucleic acid.
[3]sequenced at × Illumina Miseq v3 platform (2300 bp paired-end reads) to comprehensively catalogue composition and abundance of the bacteria in the stool samples.
[4]Using TaqMan qPCR and SYBR Green qPCR in a Rotorgene 3000.
[5]Performed by the NovoGene Company.
[6]F515/R806; PCR amplicons were sequenced on MiSeq PE300 platform (Illumina) at Helmholtz Centre for Infection Research, Braunschweig, Germany.
[7]DNA sequencing was conducted by th MiSeq Reagent Kit v3 (Illumina, San Diego, CA, USA).
[8]DNA was extracted using a fecal DNA isolation kit (BioBasic, Canada). QRT-PCR was used to determine the fecal concentration of Bacteroides and Firmicutes. The primers were designed from the variable regions of the 16S rDNA gene sequences from the NCBI GenBank program. PCR amplification and detection were quantified using real-time PCR (Mic -qPCR, Australia).
BMI: body mass index; Dash diet: dietary approaches to stop hypertension diet; DNA: deoxyribonucleic acid; F: female; M: male; NA: not available; RCT: randomized controlled trial; qPCR: quantitative polymerase chain reaction; TRF: time-restricted feeding.

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Table 2). Two studies did not analyze gut microbiota diversity in their research. Meanwhile, three other studies found no changes in microbial diversity following IF regimens. Changes in microbial diversity (alpha and beta diversity) were detected after Ramadan fasting [33, 34, 36] and IF/TRF [27, 29, 30, 32, 38] (Fig. 2).

Effect of IF on the gut microbiota diversity according to metabolic phenotype

The investigations with lean (relatively healthy) and overweight or obese participants demonstrated consistent changes in beta diversity, as described in Table 3. In terms of alpha diversity change, inconsistent results were obtained for both lean (relatively healthy) and overweight or obese people. Two fasting studies (Mesnage *et al.* [27] and Ozkul *et al.* [33]), reported no change in alpha diversity. It could be that these two studies performed their fasting interventions in individuals with characteristics similar to those of the general population with regard to dietary patterns and that they had already gotten used to the types of IF that were used.

Surprisingly, we did not see any alpha or beta diversity changes after IF interventions in adult individuals with metabolic syndrome. These findings may imply that the metabolic phenotype of the individual influences the outcome for gut microbiota diversity after IF.

Table 2. Primary outcomes for gut microbiota diversity following fasting interventions

The first author (year) [References]	Alpha diversity (α-diversity)	Beta diversity (β-diversity)
Mesnage *et al.* 2019 [27]	Not changed (No differences in α-diversity based on Chao1 and Shannon index measurement)	Changed (The comparison of Bray–Curtis distances revealed that the composition of the microbiota changed during the intervention)
Özkul *et al.* 2019 [28]	Not available	Not available
He *et al.* 2019 [29]	Changed (Water-only fasting also affected alpha diversity but heterogeneity between individuals. The Shannon index and PD whole tree increased in one volunteer, while three subjects decreased after fasting)	Changed (Bray Curtis's distance showed that the gut microbiome of different individuals was affected by fasting with different intensities)
Zeb *et al.* 2020 [30]	Changed (Microbial richness was used to determine α-diversity, and increased in fasting group/ significantly different compared to non-fasting)	Changed (α-diversity was also determined based on Principle Component Analysis of OUT richness analysis and was significantly different between fasting vs non-fasting group / no overlapping taxa)
Gabel *et al.* 2020 [31]	Not changed ("Shannon Diversity" was not significantly different between baseline period, week 1, and week 12 of 8-hr TRF (8-hr feeding window/16-hr fasting window)	Not available
Remely *et al.* 2020 [32]	Changed Reported as microbial diversity analyzed by PCR-Denaturing gradient gel electrophoresis (DGGE)/ PCR-DGGE fingerprinting of 16s rDNA	
Ozkul *et al.* 2020 [33]	Not changed (Shannon index was not significantly different)	Changed (Microbial community structure/beta diversity was significantly different between the two time points)
Su *et al.* 2021 [34]	Changed (The α-diversity of gut microbiota by the Shannon-Weaver index was statistically increased following intermittent fasting in the young-aged but not middle-aged, which showed a slight upward trend and was not statistically significant)	Changed (Bray–Curtis Principle Component Analysis (PCA) showed the gut microbiome was significantly remodelled after fasting in both young group and middle-aged group)
Maifeld *et al.* 2021 [35]	Not changed (No significant changes to the microbiome species richness/ α-diversity after either fasting or refeeding)	Not changed (No significant changes of Bray–Curtis index between time points in the intersample gut taxonomic variability/β-diversity)
Ali *et al.* 2021 [36]	Changed (The α-diversity showed significantly different)	Changed (the microbial community composition determined by Bray-Curtis index was significantly different)
Mohammadzadeh *et al.* 2021 [37]	Not available	Not available
Stanislawski *et al.* 2021 [38]	Changed (Shannon index was increased significantly over the first three months)	Changed (Bray–Curtis index suggested a shift from baseline to three months)
Guo *et al.* 2021 [39]	Not changed (Shannon index was not significantly different after fasting)	Not changed (Based on Weighing UniFrac analysis of Bray–Curtis index was significantly different)

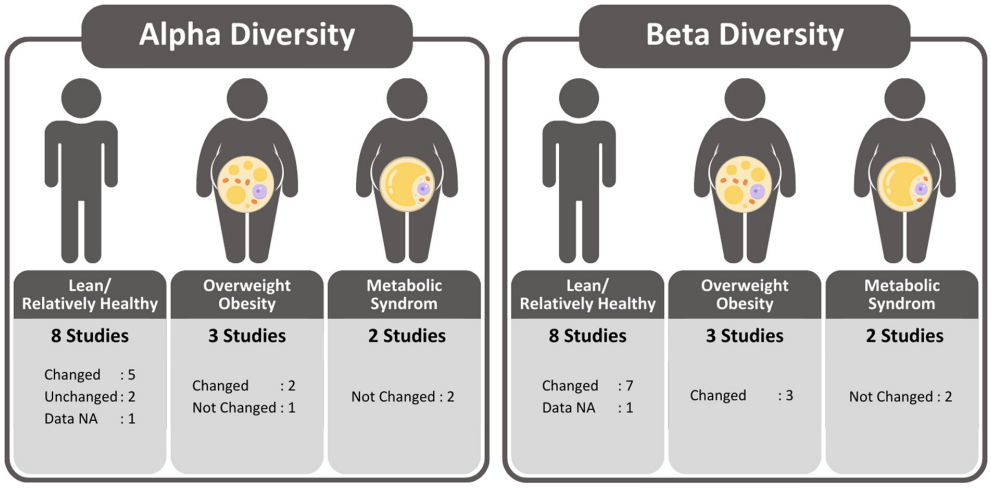

Fig. 2. The gut microbiota diversity as a result of intermittent fasting interventions in humans with different metabolic phenotypes.

Table 3. Effect of fasting on gut microbiota composition in humans

The first author (year) [References]	Taxa changes following fasting
Mesnage *et al.* 2019 [27]	• A decrease in the abundance of bacteria *Lachnospiraceae* and *Ruminococcaceae* (known to degrade dietary polysaccharides) • There was an inversion of the *Firmicutes to Bacteroidetes* ratio, whereas *Bacteroidetes* (40.7%) became the dominant taxa after the fasting period due to a significant decrease in the relative abundance of *Firmicutes* (39.9%). • There was a concomitant increase in *Bacteroides* abundance (*Bacteroides nordii, Bacteroides fragilis*) and Proteobacteria abundances (*E. coli, Bilophila wadsworthia*).
Özkul *et al.* 2019 [28]	• *A. muciniphila* and *B. fragilis,* were significantly increased after Ramadan fasting. • *Lactobacillus* spp. also increased but not significant after Ramadan fasting. • *F. prausnitzii, Bifidobacterium* spp., and *Enterobacteriaceae* levels decreased but not significant as compared to before Ramadan.
He *et al.* 2019 [29]	• *Phascolarctobacterium, Megamonas, Lachnospira, Veillonella* and *Haemophilus* were observed to be increased by fasting in more than three volunteers. • *Dialister, Fusobacterium, Ruminococcus, Succinivibrio, Oscillospira* and *Faecalibacterium* were found to be depleted through fasting in more than three volunteers.
Zeb *et al.* 2020 [30]	• 34 and 18 bacteria were enriched in the TRF and non-TRF groups at the genus level. • TRF group: *Prevotellaceae (Prevotella_9* and *Prevotella_2)* and *Bacterodetes* were the most abundant. • Non-TRF group: *Escherichia, Shigella* and *Peptosterptococcus* were abundant)
Gabel *et al.* 2020 [31]	• *Firmicutes* and *Bacteroidetes* were the two most common phyla of the total abundance, 61.2% and 26.9%, respectively at baseline. • There were no significant alterations in the abundance of *Firmicutes* and *Bacteroidetes* during the trial. • There were no significant differences in bacterial community composition between baseline, week 1, and week 12.
Remely *et al.* 2015 [32]	• *Faecalibacterium prausnitzii* (the dominant butryrate producer of *Clostridium* cluster IV) was significantly increased between the second (T2: during fasting) and the third time point (T3: after 6 weeks of probiotic intervention). • *Lactobacilli* increased from baseline to T2, and also from baseline to T3. • *Clostridium* cluster IV, *Clostridium* cluster XIVa, *Bacteroidetes, Prevotella abundance* were not significantly altered between the three-time points. • *Bifidobacteria* and *Akkermansia* increased significantly between baseline and T3.
Ozkul *et al.* 2020 [33]	• According to taxa abundance, the most represented phyla were *Firmicutes, Bacteroidetes, Actinobacteria, Verucomicrobia, Proteobacteria,* and *Tenericutes.* • *Butyricicoccus pullicaecorum, Faecalibacterium prausnitzii*, *Roseburia* were the primary species that were significantly increased within *Firmicutes* phylum after Ramadan fasting. • *Akkermansia muciniohila (Verrucomicrobia phylum)* and *Bacteroides* spp. (*Bacteroidetes phylum*) were also significantly more abundant when compared to baseline levels.
Su *et al.* 2021 [34]	Young group: multiple taxonomic differences were found when intermittent fasting in the young adult cohort was contrasted to results from the pre fasting microbiome operational taxonomic units (OTUs) belonging to the phylum *Firmicutes* was upregulated by an increase in OTUs mapping to the order *Clostridiales*. • Family *Lachnospiraceae* did not reach the significance threshold. • OTUs belonging to the phylum *Bacteroides*, especially the family *Prevotellaceae,* were reduced following Ramadan fasting. Middle-aged group: These middle-aged participants were largely similar to those observed in the younger. • The abundance of the order *Clostridiales* was significantly increased after fasting, with this effect being dependent on an increased abundance of the *Lachnospiraceae* and *Ruminococcaceae* families.

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Table 3. Continued

The first author (year) [References]	Taxa changes following fasting
Maifeld *et al.* 2021 [35]	• There were significant differences in microbial composition within individuals during fasting. • *Clostridial Firmicutes* shifted significantly in abundance, with an initial decrease in butyrate producers such as *Faecalibacterium prausnitzii, Eubacterium rectale,* and *Coprococcus comes*, which had also reverted after three months.
Ali *et al.* 2021 [36]	• In the Chinese cohort, *Bacteroidetes* decreased, and *Proteobacteria* increased after fasting. At the genus level, *Dorea, Klebsiella,* and *Faecalibacterium* were all significantly enriched after fasting. • In the Pakistani cohort, *Bacteroidetes* increased, and *Firmicutes* decreased after fasting. *Sutterella,Parabacteroides,* and *Alistipes* were significantly more abundant after fasting, while *Coprococcus, Blautia, Eubacterium, Streptococcus, Romboutsia,* and *Dialister* were more abundant before fasting. • In the total group Cohort, *Proteobacteria* was significant increased after fasting. *Clostridium _XIVa* and *Lachnospiracea incertae sedis* were all significantly more abundant prior to fasting in comparisons between fasting groups. • Before fasting, *Firmicutes* and *Actinobacteria* were significantly higher in the Pakistani group than the Chinese group, while Chinese had higher levels of *Bacteroidetes*. • After fasting, *Lentishpaerae* and *Tenericutes* were significantly more abundant in the Pakistani.
Mohammadzadeh *et al.* 2021 [37]	*Bacteroides* and *Firmicutes* were significantly increased during Ramadan fasting.
Stanislawski *et al.* 2021 [38]	• Baseline and three months were dominated by phyla *Firmicutes* and *Bacteroidetes*, the most common were *Faecalibacterium, Subdoligranulum, Blauita,* and *Bacteroides.* • Five bacterial genera changed in relative abundance from baseline to three months (*Subdoligranulum, Collinsella, Parabacteroides, Alistipes,* and *Bacteroides*)
Guo *et al.* 2021 [39]	• The relative abundances of *Ruminococcus gnavus, Chitinophagaceae bacterium, Roseburia faecis, Paraburkholderia caribensis, Verrucomicrobiae bacterium Ellin516, Neisseria dentiae,* and *Streptococcus ferus* were increased after the intervention. • *Firmicutes* and *Bacteroidetes* are the majority of phyla in the gut microbiota. • Compared to baseline, the relative abundance of *Spirochaetes* was significantly increased after the intervention in the fasting group (FDR=0.026). • *Ruminococcaceae* at the family level and *Roseburia* at the genus level, which both belong to *Firmicutes,* were the most significant increases in abundance after fasting.

Effect of IF on gut microbiota composition

The 13 included human intervention studies showed that fasting altered the gut microbiota at the phylum, genus, or species level. Details of the outcomes of interest are reported in Table 3. There is clear evidence showing that any fasting intervention can modulate the bacterial community composition. The majority of the studies reported that *Firmicutes* is upregulated following a fasting intervention. Mesnage *et al.* showed that ten days of Buchinger fasting resulted in a decreased abundance of *Lachnospiraceae* and *Ruminococcaceae* [27]. However, an increase in Bacteroidetes and Proteobacteria was observed in that study [27]. Özkul *et al.* reported that *Firmicutes*, *Akkermansia muciniphila*, and *Bacteroides fragilis* were significantly increased after Ramadan fasting [28] in Caucasians. In another study, Özkul *et al.* reported similar findings compared with their previous findings [33]. Furthermore, they showed that *Firmicutes* had a relatively higher abundance than *Bacteroidetes* after Ramadan fasting. In addition, they found that *Butyricicoccus pullicaecorum*, *F. prausnitzii*, and *Roseburia* species were the primary species that were significantly increased within the *Firmicutes* phylum after Ramadan fasting in Caucasian volunteers [33].

Changes in dietary patterns during fasting have been suggested to shape the gut microbiota diversity and abundance. However, some, but not all, types of fasting do not rule out the possibility that dietary patterns may not be changed. For example, Ramadan fasting is a type of IF that requires refraining from food and drink from dawn to sunset. Another method of IF is to limit mealtimes to 8 hr followed by 16 hr of fasting [30]. The beta-diversity of the gut microbiota in the present review study showed consistent changes between these two types of IF, primarily in the *Firmicutes* phylum, which showed significant increases in Ramadan fasting interventions [28, 33, 34, 36, 37] but also in *Lachnospiraceae, Ruminococcaceae, B. pullicaecorum, F. prausnitzii, Roseburia*, and *A. muciniphila*, all of which also showed increases, [5, 28, 33].

Su *et al.* reported similar findings, with the phylum Firmicutes upregulated and the phylum Bacteroides, especially the family *Prevotellaceae*, reduced following Ramadan fasting to a similar extent between young and middle-aged volunteers [34]. According to Ali *et al.*, the abundance of Bacteroidetes decreased after fasting during Ramadan in Chinese people, while the abundance of Proteobacteria increased [36]. In contrast, in Pakistani individuals, the abundance of Bacteroidetes increased after Ramadan fasting, whereas that of Firmicutes decreased [36]. Similar results have been shown for a Buchinger's fasting intervention [27]. In another Ramadhan fasting study, Mohammadzadeh *et al.* showed that *Bacteroides* and Firmicutes were significantly increased during Ramadan fasting [37]. Overall, in this systematic review, we found consistent results about the effects of Ramadan fasting on gut microbiota composition. Ramadan fasting influences the gut microbiota composition in lean (relatively healthy) or overweight/obese individuals. However, the change in gut microbiota composition after Ramadan may also be influenced by the distinct dietary patterns of each ethnicity, at least as indicated by Ali *et al.* [36].

A modified IF (water-only fasting vs. juice-only fasting) suggested that water-only fasting dramatically changed the bacterial community. Individually, the relative abundance of *Fusobacterium* was reduced in four participants who harbored

higher *Fusobacterium* prior to fasting compared with the other two participants. Post-IF, *Fusobacterium* remained consistently low across all six individuals [29]. A pilot study of IF combined with laxative treatment for four weeks and probiotic treatment using capsules containing *Lactiplantibacillus plantarum*, *Streptococcus thermophiles*, *Lactobacillus acidophilus*, *Lacticaseibacillus rhamnosus*, *Bifidobacterium lactis*, *Bifidobacterium longum*, and *Bifidobacterium breve* for six weeks found that the abundances of *Bifidobacterium* and *Akkermansia* were significantly increased but that the abundances of *Clostridium* cluster IV, *Clostridium* cluster XIVa, Bacteroidetes, and *Prevotella* were unchanged [32]. These findings suggest that both medications (a laxative and a specific food type, i.e., a probiotic) may have distinct effects on the IF-related gut microbiota composition.

There were also substantial differences in the microbial composition within individuals during IF combined with a type of diet (the DASH diet), reflecting a characteristic of intervention-induced shift, with partial reversion following a 3-month refeeding period on a DASH diet. There was a significant shift in Firmicutes abundance, with an initial decrease in *F. prausnitzii*, *Eubacterium rectale,* and *Coprococcus* that subsequently reverted after three months [35]. In a study that combined IF and lifestyle changes for three months, there were fluctuations in five bacterial genera (*Subdoligranulum, Collinsella, Parabacteroides, Alistipes,* and *Bacteroides*). *Subdoligranulum* and *Collinsella* decreased in relative abundance, while the other three taxa increased. In that study, the baseline and three-month abundances were dominated by the phyla Firmicutes and Bacteroidetes [38]. Another IF intervention [39] in different ethnic groups was shown to increase the relative abundances of *Ruminococcus gnavus*, *Chitinophagaceae bacterium*, *Roseburia faecis*, *Paraburkholderia caribensis*, *Verrucomicrobiae* bacterium Ellin516, *Neisseria dentiae*, and *Streptococcus ferus* [39].

Zeb *et al.* found that 34 bacteria were enriched at the genus level following a TRF intervention (8 hr per day for 25 days) [30]. *Prevotellaceae* (*Prevotella_9* and *Prevotella_2*) and Bacteroidetes dominated the TRF group, while *Escherichia, Shigella,* and *Peptostreptococcus* were abundant at the genus level in the non-TRF group [30]. In contrast, there was no significant alteration in the abundances of Firmicutes and Bacteroidetes after 12 weeks of TRF, despite Firmicutes and Bacteroidetes being the two most common phyla based on total abundance at baseline, with abundance ratios of 61.2% and 26.9%, respectively [31].

Qualitative analysis of the relationship between the gut microbiota or its metabolites and metabolic health in humans

This review also identified how the gut microbiota and its metabolites may be associated with metabolic health based on the included studies. A Buchinger fasting study [27] showed that SCFA levels were unchanged during fasting. However, the levels of serum brain-chain amino acids (BCAAs) were significantly increased during fasting and significantly decreased after fasting. Interestingly, the abundance of *Lachnospiraceae (Coprococcus_2 eutactus, Fusicatenibacter saccharivorans,* and *Lachnospira pectinoschiza)* was positively associated with plasma glucose levels and negatively associated with BCAA levels. In contrast, *Bacteroidetes (Bacteroides dorei/fragilis* and *Bacteroides thetaiotaomicron)* and a *Proteobacteria (Bilophila wadsworthia)* presented the opposite trend and were negatively and positively associated with plasma glucose levels and BCAA levels, respectively [27]. Özkul *et al.* reported that they did not find any association between bacterial composition and fasting glucose except for a negative correlation between *A. muciniphila* counts and fasting glucose after Ramadan fasting [28]. Ramadan fasting has been shown to upregulate bacterial butyrate producers [34, 36, 37]. More interestingly, at functional levels, Su *et al.* demonstrated that 29 pathways were present in young individuals at the end of Ramadan fasting when contrasted with the pathways present at the start of fasting, whilst 14 pathways were present in their middle-aged cohort [34]. The majority of the pathways presented were associated with host metabolism, genomics, and molecular signaling [34]. These findings indicate that any type of IF (i.e., Buchinger or Ramadan) modulates gut microbiota-derived metabolites, either SCFAs or BCAAs. The duration of IF might differentially affect how gut microbiota-derived metabolites are regulated, especially in those individuals who are overweight or obese.

In addition, Maifeld *et al.* showed that IF upregulates butyrate and propionate bacterial producers [35]. Moreover, they showed that in subjects with metabolic syndrome, modulation of the gut microbiota composition, such as modulation of *E. rectale*, *Dorea longicatena,* and *Hungatella hathewayi* (acetate producers), was negatively correlated with IL-2-producing CD4+ T cells and the absolute number of IFNgamma+ and TNFalpha-producing mucosal-associated invariant T (MAIT) cells, respectively [35]. Similarly, Guo *et al.* showed that total plasma SCFAs in individuals with metabolic syndrome are significantly increased after IF [39]. This elevation remains significant after being adjusted for baseline SCFA levels [39]. More interestingly, based on the KEGG pathways, the altered gut microbiota was involved in "genetic information processing", "environmental information processing", and "metabolism" after eight weeks of IF. They also found a relationship between the abundances of twenty-three significantly altered gut microbial species and glucose metabolism, lipid profiles, and inflammatory cytokines, independent of weight loss. For instance, *Acidobacteria bacterium* and *Mitsuokella jalaludinii* showed the strongest positive association with homeostatic model assessment for insulin resistance (HOMA-IR), whereas *N. dentiae* was negatively related to serum glucose.

Quality of the included studies

Of the 13 included studies identified as relevant for this review, the methodological quality of one was rated as good; ten were classified as fair, and two were placed as poor. Regarding the prominent flaws, ten studies did not use randomized controlled designs, two studies presented dropout rates above 20%, and 12 studies did not perform an intent-to-treat analysis (Supplementary Table 2).

DISCUSSION

Our systematic review found consistent effects of IF on the human gut microbiota, both on alpha and beta diversity. More interestingly, the alpha or beta diversity changes were different based on human metabolic phenotypes. There was a constant shift in the alpha and beta diversity of the gut microbiome in lean participants (relatively healthy individuals) but not in adult overweight/obese participants with metabolic syndrome. Despite

our findings indicating that IF influences gut microbiota diversity, we were unable to draw conclusions about the effect of IF on specific taxa (gut microbiota composition).

The variability in the studied populations (such as relatively healthy, overweight or obese, and metabolic syndrome populations), the durations of the interventions, the types of IF, the study designs, the methods used to assess the composition of the gut microbiome, and the data analyses all played roles in the synthesis of the results. Furthermore, differences in the sequencing platforms and analyses of hypervariable regions might have contributed to the variances in the abundances of taxa. Two studies [29, 38] did not provide alpha and beta diversity measurements as primary results; however, the primary species that were significantly elevated (*B. pullicaecorum*, *F. prausnitzii*, *Roseburia* sp.) were from the *Firmicutes* phylum following Ramadan fasting. According to both reports, *Bacteroides* spp. (Bacteroidetes phylum) were substantially more prevalent as compared with the baseline levels.

Some of the included studies also analyzed the association between changes in specific taxa after fasting and/or their metabolites with metabolic health parameters related to glucose metabolism, lipid metabolism, insulin sensitivity, and inflammation. Despite heterogeneity in the individual taxa studied, there is a link between the gut microbiota after fasting and metabolic health indices throughout Ramadan fasting. Adjustments for confounding, including baseline values, were made in all trials, with only a few studies adjusting for a limited number of confounders. Although the differences in taxonomic composition and functional potential varied across the studies, *Firmicutes* and *Bacteroidetes* were amongst the most consistently reported and were either upregulated or downregulated after fasting. In adults, it has been shown that *Firmicutes* largely dominate the gut microbiota, followed by *Bacteroidetes* [36]. Some studies in humans have shown that the gut microbiota of obese individuals exhibits a higher *Firmicutes*/*Bacteroidetes* ratio than that of normal-weight individuals and proposed this ratio as an eventual biomarker. As a result, the *Firmicutes*/*Bacteroidetes* ratio is frequently cited in the scientific literature as a marker of obesity [40], although the underlying mechanisms remain unclear.

The changes in microbiota diversity in healthy subjects with lean (relatively healthy) bodies were consistently higher than those in overweight or obese subjects with metabolic syndrome conditions. In their systematic review, Crovesy, Masterson, and Rosado found low abundances of *Lactobacillus* and *Bifidobacterium* in obese individuals at baseline [41], Duan *et al.* further found low abundances of *F. prausnitzii* and *A. muciniphila* and an increased abundance of *Prevotella* in obese individuals at baseline [42]. This may partly be explained by a high fat-carbohydrate diet and low fiber intake in individuals with obesity [43]. Lozupone *et al.* showed that consuming carbohydrate- or fat-restricted low-calorie diets for 1 year or high-fat/low-fiber or low-fat/high-fiber diets for 10 days induces statistically significant changes in the gut microbiota [44]. These baseline gut microbiota characteristics may explain the different responses in individuals with metabolic syndrome [35, 39] than in overweight or obese individuals [31, 32, 38] and healthy individuals after intermittent fasting [27–30, 33, 34, 36, 37].

Study of the gut microbiota and its function in obese and non-obese people has led to the idea of a high gene count (HGC) and low gene count (LGC), both of which have physiological and pathological implications. The defining characteristics of an HGC microbiome in favor of digestive health include an increased number of butyrate-producing species, an increased proclivity for hydrogen production, the establishment of a methanogenic/acetogenic ecosystem, and a decrease in hydrogen sulfide production. Individuals with an HGC have a more functionally robust gut microbiome as well as a decreased prevalence of metabolic diseases and obesity. LGC individuals, on the other hand, have a larger number of pro-inflammatory bacteria, such as *Bacteroides* and *R. gnavus*. Furthermore, a few of the main bacterial metabolites in LGC individuals include modules for glucuronide degradation, aromatic amino acid degradation, and dissimilatory nitrite reduction, all of which are known to be hazardous [13, 45].

Several mechanisms may explain how IF influences the gut microbiota composition and metabolic health. During fasting, there is an increase in intestinal pH, there are changes in mucus production, and there is a decrease in intestinal capacity [46], which could affect the microbial ecosystem. Furthermore, fasting also impacts changes in circadian rhythms via interactions between changes in the gut microbiota composition and gut-derived metabolites, which act as signaling molecules for the peripheral and central clocks of the host [47]. Of interest is the interaction between circadian rhythms and gut microbes, which are intertwined via metabolic regulation, but the mechanisms that underlie their interactions are still not fully understood [48]. The dispositions and functions of microbes can fluctuate within a few hours depending on the timing of a meal, which links the circadian rhythm of the host's behavior with diurnal fluctuations in the composition and function of the microbiota [49].

Furthermore, the beneficial effects of fasting may also be partly explained by an increase in fat oxidation during fasting. It is well established that the rate of carbohydrate utilization is decreased in the fasted state and that the rate of fat oxidation increases to meet energy demands [36]. On the other hand, it is also possible that the SCFAs produced by the gut microbiota are used as a substrate (especially acetate in the overweight/obese phenotype) for host energy metabolism [50]. In addition to this, intermittent fasting may improve insulin regulation, resulting in the maintenance of glucose metabolism, especially in overweight or obese individuals [51]. More interestingly, intermittent fasting may also activate the central metabolic regulation of sirtuins, particularly SIRT1 and SIRT3. The activation of sirtuins by fasting allows them to exhibit effects on the insulin response, antioxidant defense, and glycolysis [52]. A recent review suggests that the effects of fasting on metabolism can be closely associated with alterations in the gut microbiota composition [53].

In their recent meta-analysis, Ejtahed *et al.* identified taxa associated with multiple diseases, including obesity [54]. In this review, the dominance of Firmicutes enrichment as an outcome after IF may be partly associated with an increase in endogenous substrates over a long fasting period [33]. In fact, fasting has been shown to increase endogenous SCFA production as an energy substrate for host metabolism [55], which is supported by the abundances of the butyrate-producing bacterial species *B. pullicaecorum* and *F. prausnitzii* [32, 33]. Furthermore, the abundances of the *Akkermansia* group and Verrucomicrobia phylum also consistently rise after fasting. The ability to dissolve mucin and the inherent features of the mucus layer might cause *A. muciniphila* to be resistant to environmental changes during

fasting, therefore enriching the species group that includes *A. muciniphila*. This species is essential for the proper functioning of intestinal barriers, and its presence defines a healthy gut profile [56, 57].

The metabolic changes during fasting include the dominant use of fatty acids as fuel for synthesizing adenosine triphosphate (ATP), reducing the fat mass, increasing functional capacity, and altering glucose homeostasis [58]. The negative correlation of *A. muciniphila* with fasting glucose levels reinforces the positive effects on glucose regulation after Ramadan fasting [28]. Ramadan fasting also significantly increases butyrate, which can reduce the adverse effects of lipopolysaccharides and simultaneously increase the regulation of intestinal barrier function by stimulating mucin production [59]. In addition, butyrate also plays a role in the activation of G-protein receptors (GPR41 and GPR43) in the colon, stimulating the production of the hormone peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), which further affects glucose homeostasis [60].

In several studies, Bacteroidetes were significantly increased after fasting. A possible mechanism for Bacteroidetes elevation after fasting can be partly explained by the consumption of vegetables and fruits in the diet [61], as shown in the Buchinger and Ramadan fasting studies [27, 28, 37]. Furthermore, the rapid tolerance/adaptation to the host environment during fasting might also explain the higher abundance of Bacteroidetes following fasting. Another possible explanation is its special ability to shift to a transcription profile with glycan derivatives when polysaccharide and glycoprotein supplies are depleted due to extended fasting [62]. Interestingly, both Buchinger and Ramadan fasting interventions gave consistent results in terms of the elevation of *F. prausnitzii* (the dominant acetate producer of *Clostridium* cluster IV). Despite the fact that the composition of the gut microbiota varied across all investigations, the alterations in the microbiota composition could reduce plasma lipopolysaccharide-binding protein and increase butyrate-producing bacteria.

For several types of fasting, including IF (Ramadan, time-restricted fasting), a calorie-restriction fasting regimen provides an overview of the abundance and diversity shift of the gut microbiota taxonomy. However, there is a wide range of outcomes among them. In a TRF intervention, a different type of IF regimen was used in which participants were provided food *ad libitum* from 10:00 to 18:00 and fasted from 18:00 to 10:00 daily. There were no restrictions on the types or quantities of meals ingested throughout the eating window (8 hr), and participants were not required to track their calorie consumption. The results showed no significant association between the diversity or abundance of the gut microbiota and weight loss after TRF treatment for 12 weeks [31]. Those results differed from IF interventions (modified fasting with calorie restriction) in obese-metabolic syndrome individuals, in which BMI and waist circumference were significantly reduced [39]. However, the latter also demonstrated that the link between gut microbiota composition and metabolic improvement depended on body weight change [39]. Several of the included studies [27, 34, 35, 39] indicate an independent association between the gut microbiota and many metabolism pathways, at least in relatively healthy individuals. On the other hand, changes in mealtime during Ramadan fasting could affect the human natural circadian rhythm, which might also have a detrimental effect on health, and this needs further investigation to clarify.

This systematic review has both strengths and limitations. The strengths of our systematic reviews are 1) that we included human intervention studies that addressed the most frequent modalities of intermittent fasting accessible in the field and 2) that we reported the change in diversity and composition of the gut microbiota in great detail. However, there are numerous limitations of this review, including 1) the small number of included studies from which we were able to extract data and 2) the broadness of our population eligibility criteria (healthy, overweight/obese, and metabolic syndrome). Therefore, the results could not be generalized. Another systematic study focusing on specific types of fasting and strict eligibility criteria (i.e., specific demographics) is warranted.

A meta-analysis would be ideal for this systematic review, but the variety of microbiota measures, particularly regional differences, could lead to difficulty. With this consideration, presumably, studies examining the effect of fasting on the gut microbiome could adhere to microbiome analysis best practices [63] and routinely report associations using standard alpha and beta diversity measurements. Taxa investigations and reports should also be consistent.

CONCLUSIONS

In conclusion, this systematic review suggests that IF modulates human gut microbiota diversity (both alpha and beta diversity). Human metabolic phenotype differences may alter alpha/beta diversity after a fasting intervention. Despite their variability, IF affects the gut microbiota at taxonomic levels in all metabolic phenotypes. Nevertheless, given the emerging recognition of the importance of intermittent fasting and the microbiome in physiological and pathological conditions, further investigations are warranted, ideally with adequately powered, long-term, placebo-controlled trials.

AUTHOR CONTRIBUTIONS

A.P., M.A., E.K.S.L., E.R.N., and E.S.L. conceptualized the review. A.P. and M.A. wrote the protocol and performed the search and data extraction. A.P., M.A., and E.K.S.L. performed the assessment of study quality. A.P. wrote the manuscript. M.A., E.K.S.L., E.R.N., E.S.L., and F.M.S. reviewed and revised the manuscript. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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