Less is more: effectiveness and feasibility of a fasting-mimicking diet programme in persons with type 2 diabetes in primary care

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Chapter 4

Impact of dietary carbohydrate, fat or protein restriction on the human gut microbiome: a systematic review

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Abstract

Restriction of dietary carbohydrates, fat, and/or protein is often used to reduce body weight and/or treat (metabolic) diseases. Since diet is a key modulator of the human gut microbiome, which plays an important role in health and disease, this review aims to provide an overview of current knowledge of the effects of macronutrient-restricted diets on gut microbial composition and metabolites. A structured search strategy was performed in several databases. After screening for in-and exclusion criteria, 36 articles could be included. Data are included in the results only when supported by at least three independent studies to enhance the reliability of our conclusions. Low-carbohydrate (<30 energy%) diets tended to induce a decrease in the relative abundance of several health-promoting bacteria, such as *Bifidobacterium,* as well as a reduction in short-chain fatty acid (SCFA) levels in faeces. In contrast, diets low in fat (<30 energy%) increased alpha diversity, faecal SCFA levels, and abundance of some beneficial bacteria, including *F. prausnitzii.* There was insufficient data to draw conclusions concerning the effects of low-protein (<10 energy%) diets on gut microbiota. Although the data of included studies unveils possible benefits of low-fat and potential drawbacks of low-carbohydrate diets for human gut microbiota, the diversity in study designs made it difficult to draw firm conclusions. Using a more uniform methodology in design, sample processing and sharing raw sequence data could foster our understanding of the effects of macronutrient restriction on gut microbiota composition and metabolic dynamics relevant to health. This systematic review was registered at https://www.crd.york.ac.uk/prospero as CRD42020156929.

Introduction

A wide range of diets has been developed over the last decades to reduce weight and/or to improve health(1-7). Reducing the amount of any of the macronutrients, fat, carbohydrate, or protein, is often used as a dietary strategy(1, 2, 4, 8). Such dietary alterations have been applied for the treatment of several diseases, including type 2 diabetes (T2D)(9-11), chronic kidney disease(12-15), epilepsy(16-18), and inflammatory bowel disease(19-21). It has been suggested that an important effect of diet on health is mediated via the gut microbiome(22), and evidence is emerging that microbial metabolites may affect health by acting as signalling molecules(23). The gut microbiome, also referred to as the forgotten organ(24), is an essential component of the human body. The human digestive tract harbours a diverse community of primarily anaerobic microorganisms. The conditions, as well as the numbers of bacteria differ considerably in the various sections of the gastrointestinal tract, which hosts up to 10^3 colony-forming units (cfu) per millilitre (cfu × mL–1) in the stomach and duodenum, while the numbers increase in jejunum and ileum (10^4–10^8 cfu × mL–1), and rise to even higher levels in the colon (10^9–10^{12} cfu × mL–1)(25). Hundreds of different bacterial species can be present in a single individual, of which particular species are present in most individuals. Approximately 94% of all species in healthy adults belong to the phyla *Bacteroidetes (new nomenclature; Bacteroidota), Firmicutes (Bacillota), Actinobacteria (Actinomycetota),* or *Proteobacteria (Pseudomonadota)*(26, 27).

Faecal samples can be collected after bolus transit through the gastrointestinal tract to characterise the gut microbiome. Interindividual variability and plasticity of the gut microbiota composition make identifying a 'healthy' microbiome profile challenging, which remains a heavily debated topic(28). However, richness and diversity generally provide the gut ecosystem with stability and resilience and are therefore associated with health(29, 30). Richness can be quantified as the total number of bacterial species in a sample; alpha diversity further incorporates relative abundance profiles (microbiota diversity within an individual sample), whilst beta diversity reflects the diversity between samples (inter-variability)(31). Healthy individuals generally have higher richness and diversity than people with metabolic dysfunction or chronic diseases(28). Reduced gut microbiome diversity and richness are associated with a myriad of diseases, including T2D, rheumatoid arthritis, inflammatory bowel disease, and several types of cancer(32).

Not only diversity but also the relative abundance (distribution of individual bacterial taxa within a sample) of individual bacterial taxa in the gut may be associated with health or disease(32). Some bacteria are assumed to be primarily health-promoting, like *Lactobacillus* and *Bifidobacterium*, which are known to produce microbial compounds important for healthy gut function(33). Other bacteria may confer pathogenic effects since their abundance is related to adverse health outcomes(34). Several diseases

are associated with an alteration in the abundance of specific bacteria. For example, people with T2D have lower faecal numbers of at least one of the genera *Bacteroides, Bifidobacterium, Roseburia, Faecalibacterium, and Akkermansia* as compared to healthy controls(35), whereas colorectal cancer has been associated with an increase in the relative abundance of a core set of 29 bacterial species(36).

The complex bacterial ecosystem in the human digestive tract has a myriad of functions, including vitamin synthesis(37), provision of colonisation resistance against incoming pathogens(34), mediation of immune responses, and digestion of macronutrients into metabolites by the production of a great array of enzymes(27). The processing of macronutrients starts in the upper gastrointestinal tract. Carbohydrates are partly digested by salivary amylase, pancreatic enzymes and enzymes on the surface of small intestinal cells and subsequently absorbed by the small intestine wall(38). Some carbohydrates are easily digested in the small intestine, while others are more difficult to digest(38, 39). The non-digestible carbohydrates (NDCs) thus largely pass through the small intestine into the colon, where they are fermented by the intestinal microbiota. Some NDCs are associated with health benefits, such as laxation or lowering of blood cholesterol or glucose levels(39, 40). They are primarily metabolised by the gut microbiome into short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate(26). SCFAs are partly consumed by the colonic mucosa and absorbed by intestinal cells, where they confer local effects. Some of the SCFAs are partly transported through the basolateral membrane towards the bloodstream and can act on receptors at different body sites. The rest of the SCFAs are excreted in the faeces. SCFAs appear to regulate hepatic lipid and glucose homeostasis by decreasing glucose output, lipogenesis, and free fatty acid accumulation. Also, associations with adipocyte lipolysis and adipogenesis have been reported(41, 42). Moreover, they affect appetite regulation by increasing anorexigenic signalling in appetite centres and affect energy homeostasis through several metabolic pathways activated in parallel(42-44).

Fat can be digested and absorbed in the small intestine after it is partially emulsified by bile acids and broken down into smaller fragments by pancreatic and intestinal lipases(38). A small part of ingested fat is not absorbed in the small intestine and can be metabolised by gut microbiota or excreted(26, 45). The gut microbiome can convert bile acids into secondary bile acids, which are suggested to play a role in epithelial cell integrity, host immune response and gut bacterial composition(46). Proteins are broken down by gastric, pancreatic, and intestinal proteases into smaller protein fragments, tripeptides, dipeptides, and individual amino acids, which are partly absorbed by the small intestine(38). In the colon, protein fermentation produces diverse metabolites, including SCFAs, ammonia, tryptophan metabolites, and the branched-chain fatty acids (BCFA) isobutyrate, 2-methylbutyrate, and

isovalerate(26, 47, 48). Tryptophan is a precursor for crucial compounds, including serotonin and kynurenine, which are important for neurobiological functions, gut-brain signalling, gut motility, platelet functions, and immune homeostasis(48). Macronutrient processing thus leads mostly to the absorption of metabolites by the gut, and only a minority of metabolites is excreted in the faeces. These metabolites can be used as an approximate indication for carbohydrate, fat, and protein metabolisation by the microbiota(26, 49).

Several interventions that would potentially be capable of altering the gut microbiome composition and/or its products to improve health status include the following: 1) supplements of dietary substrates that are selectively utilised by host microorganisms conferring a health benefit (prebiotics); or 2) live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (probiotics); or 3) a mixture comprising live microorganisms and substrate(s) selectively utilised by host microorganisms (synbiotics); or 4) inanimate microorganisms and/or their components (postbiotics); or 5) faecal microbiota transplantations(33, 50). However, diet is the most natural daily modulator of the gut microbiome and health(51). An elaborate modification of the diet may represent an excellent strategy to alter the microbial community composition and function for improved health. However, little is known about the effects of restriction of macronutrient levels on the gut microbiome. Therefore, this review aims to give an overview of the effects of diets restricted in carbohydrates, fat, or protein on the bacterial composition of the human gut microbiome and on faecal metabolites.

Methods

Eligibility criteria

The study characteristics were defined as human studies with an intervention described as a low-fat diet (LFD), low-carbohydrate diet (LCD), or low-protein diet (LPD) with gut microbiome as an outcome measure. Studies had to be published in English or with an available English translation. Exclusion criteria included animal studies, paediatric studies, studies with no relevant extractable data, or studies with no full text available. The following study designs were included: RCT, non-randomised trials, cohort studies, and observational studies. Reviews and case reports were excluded.

Information sources and search strategy

The search strategy (Supplementary material) was used to search PubMed, Embase, Web of Science, and the Cochrane Library. It was adapted for each dietary intervention (low-carbohydrate, low-fat, and low-protein). Articles were selected for screening on 03-06-2021.

Selection and data collection process

Covidence (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia) was used for the screening process. After removing duplicates, the title and abstract screening and subsequent full-text screening were performed with the pre-defined in- and exclusion criteria by two independent reviewers (MS and NJ or PP). A third review member (HP) was available for discussion in case of inconsistencies. Since there is no worldwide accepted definition of low-fat, low-carbohydrate, and low-fat diets, the following definitions were adopted; low-carb: <30 energy% intake of carbohydrates, low-fat: <30 energy% intake of fat, low-protein: <10 energy% intake of protein(52-54).

Reported data

Outcome domains include changes in alpha diversity, relative bacterial abundance, and/or metabolites between baseline and after intervention. Outcome data are reported as either increased or decreased only when a significant difference from baseline was observed. Data are included in the results section only when reported in at least three independent studies to enhance the reliability of our conclusions. Tables with all outcome data were included in the supplementary file. Furthermore, the macronutrient composition of the dietary intervention, participant characteristics (including age, BMI, gender, and eventual disease), number of participants, intervention time, wash-out period in case of cross-over, and time of analyses (directly after intervention or at a later moment), were extracted and reported in supplementary tables.

Quality assessment

The Cochrane Collaboration risk of bias tool was used to assess the methodological quality of the included studies on outcome level. The ROBINS-I tool(55) was used for non-randomised studies, and the RoB 2.0 tool was used for randomised studies. The risk of bias was independently reviewed by the two reviewers (MP and NJ or PP) and discussed until a consensus was reached. A third reviewer (HP) was available for consultation when consensus was not reached.

Results

Study selection

The literature search resulted in 1178 articles (**Supplementary Figure 1**). After removing 100 duplicates, 1078 articles were screened on title and abstract. 938 articles were deemed irrelevant to the research question and were excluded, for example, due to the inclusion of animals or lack of gut microbiome outcomes. Four reports could

not be retrieved. Full-text screening on eligibility was conducted on the remaining 136 articles, of which 100 were excluded, resulting in the inclusion of 36 articles. Excluded articles often only reported change between intervention groups and did not describe the effect from baseline per individual group. Of the 36 included articles, 19 conducted LCD interventions, 20 conducted LFD interventions, and five conducted LPD interventions. Six studies had LCD as well as LFD intervention groups(56-61), and two had both LCD and LPD intervention groups(17, 62).

Study Characteristics

LCD, LFD, and LPD study features describe the year of execution, design, patient- and intervention characteristics (**Tables 1, 2, and 3**). The LCD studies were published between 2006 and 2021, the LFD studies between 1978 and 2021, and the LPD between 2016 and 2021. Study designs included randomised prospective, randomised cross-over, non-randomised cross-over, and non-randomised trials. Some studies used healthy subjects; however, more often, participants with overweight/obesity or specific diseases were included. In the low-carbohydrate studies, participants were often obese. In the low-fat studies, obese persons and persons with multiple sclerosis (MS) were often included. Low-protein studies often examined persons with chronic kidney disease. Study group size differed from six to 246, with most studies including less than 30 participants. Not all studies reported the number of subjects in the specific diet groups(63-66). Some studies use several study groups, which all undergo either LCD, LFD, or LPD, where interventions differ in the source of the nutrition or additional supplements(26, 59, 67-70). The average age varied between 23.3 and 70.5 years, although the average age was often not reported. Most studies included males and females, while some included only males(59, 63, 71-74). The male/female numbers were not always reported. In papers reporting the average body mass index (BMI), it varied between 21.7 and 35.9 kg/m^2; however, most papers reported an average BMI of >25 kg/m^2 (overweight), and the average BMI was >30 kg/m^2 (obese) in the majority of studies evaluating the effects of LCD. Intervention time varied substantially between studies, with the shortest intervention time of two weeks and the longest of three years, while most studies had an intervention time of fewer than six months. In cross-over studies, wash-out time (if reported) varied from zero days to three months. In the majority of studies, data collected directly after intervention were used for analysis, except in the studies of Pataky, Russell, and Gutierrez-Repiso, where the outcome was measured three weeks(75), five weeks(73), or two months(57) after the end of the intervention.

Author	Year	Set-up	Healthy/ diseased	Number analysed in diet group	Male/ female	Mean age (years)	Mean BMI (kg/cm2)	Intervention time	Wash-out period (crossover)	Timepoint of analysis
Ang (74)	2020	Cohort	Overweight or obese	17	17/0	NI	NI	4 weeks	-	end of intervention
Basciani (67)	2020	RP	Obese	16 (per diet group)	19/29	56.2	35.9	6 1/2 weeks	-	end of intervention
Brinkworth (82)	2009	RP	Obese + >= 1 metabolic risk factor	48	18/30	50.4	33.5	8 weeks	-	end of intervention
Duncan (85)	2007	RC	Obese	20	20/0	36.7	35.4	1 month	3 days	end of intervention
Duncan (72)	2008	NRC	Obese	23	NI	NI	NI	4 weeks	NI	end of intervention
Ferraris (17)	2021	Cohort	DRE	7	NI	NI	NI	1 month	-	end of intervention
Fragiadakis (56)	2020	RP	Healthy	25	5/19	42.6	32.8	3 months	-	end of intervention
Gutierrez-Repiso (57)	2019	RP	Obese	9	4/5	38.2	33.1	2 months	-	2 months after end of intervention
Gutierrez-Repiso (58)	2021	Cohort	Obese	18	8/10	42.6	33.0	2 months	-	end of intervention
Ley (86)	2006	RP	Obese	NI	NI	NI	NI	1 year	-	end of intervention
Lundsgaard (68)	2018	RP	Overweight	9	NI	NI	NI	6 weeks	-	end of intervention
Mardinoglu (83)	2018	Cohort	NASH	10	8/2	54.0	34.1	2 weeks	-	end of intervention
Murtaza (59)	2019	NRC	Healthy	10	NI	NI	NI	3 weeks	-	end of intervention
Nagpal (60)	2019	RC	Healthy or MCI	17	5/12	64.6	NI	6 weeks	6 weeks	end of intervention
O'Keefe (61)	2015	Cohort	Healthy or obese	19	NI	NI	NI	2 weeks	-	end of intervention
Pataky (75)	2016	NI	Overweight or obese + NAFLD	15	11/4	50.0	34.6	3 weeks	-	3 weeks after end of intervention

Author	Year	Set-up	Healthy/ diseased	Number analysed in diet group	Male/ female	Mean age (years)	Mean BMI (kg/cm2)	Intervention time	Wash-out period (crossover)	Timepoint of analysis
Russell (73)	2011	RC	Obese	17	17/0	56.4	35.8	4 weeks	none	5 weeks after end of intervention
Swidsinski (84)	2017	Cohort	MS	NI	NI	NI	NI	6 months	-	end of intervention
Tagliabue (62)	2017	Cohort	GLUT 1DS	6	3/3	NI	NI	3 months	-	end of intervention

Table 1. Study characteristics of included low-carbohydrate intervention studies.

BMI, body mass index. DRE, drug-resistant epilepsy. GLUT 1DS, Glucose transporter 1 deficiency syndrome. MCI, mild cognitive impairment. MS, Multiple Sclerosis. NAFLD, Non-alcoholic fatty liver disease. NASH, non-alcoholic steatohepatitis. NI, not indicated. NRC, non-randomised cross-over. RC, randomised cross-over. RP, randomised parallel.

Author	Year	Set-up	Healthy/ diseased	Number analysed in intervention group	Male/ female	Mean age (years)	Mean BMI (kg/cm2)	Intervention time	Wash-out period (crossover)	Timepoint of analysis
Cuevas-Sierra (78)	2021	RP	Overweight or obese	97	27/70	NI	31.9 (w), 32.1 (m)	4 months	-	end of intervention
Cummings (71)	1978	NRC	Healthy	6	6/0	NI	NI	4 weeks	none	end of intervention
Fava (69)	2013	RP	Healthy	21 (HC/HGI), 17 (HC/LGI)	43/45	56.0	28.8	24 weeks	-	end of intervention
Fragiadakis (56)	2020	RP	Healthy	24	5/19	39.2	33.7	3 months	-	end of intervention
Fritsch (20)	2021	RC	Ulcerative colitis	17	NI	41.7	27.4	4 weeks	2 weeks	end of intervention
Guevara-Cruz (79)	2019	RP	Healthy, obese and/or MS	42	NI	NI	NI	2,5 months	-	end of intervention
Gutierrez-Repiso (57)	2019	RP	Obese	9	4/5	38.2	33.1	2 months	-	2 months after end of intervention
Gutierrez-Repiso (58)	2021	Cohort	Obese	18	8/10	42.6	33.0	2 months	-	end of intervention
Haro (63)	2016	RP	Healthy or MS	NI	198/14	61.2	NI	2 years	-	end of intervention
Haro (65)	2016	RP	Healthy or MS	NI	20/0	61.4	31.6	1 year	-	end of intervention
Haro (64)	2017	RP	Healthy or MS	NI	106/0	61.5	30.5	2 years	-	end of intervention
Kahleova (87)	2020	RP	Overweight	84	15/69	52.9	32.6	16 weeks	-	end of intervention
Liu (136)	2020	Cohort	Healthy or T2D	16	8/8	50.3	26.4	6 months	-	end of intervention
Murtaza (59)	2019	Cohort	Healthy	8 (LFD), 10 (LFD per)	21/0	NI	NI	3 weeks	-	end of intervention
Nagpal (60)	2019	RC	Healthy or MCI	17	5/12	64.6	NI	6 weeks	6 weeks	end of intervention
O'Keefe (61)	2015	Cohort	Healthy or obese	14	NI	NI	NI	2 weeks	-	end of intervention
Ren (80)	2020	RP	T2D	23	11/12	70.5	NI	12 weeks	-	end of intervention
Santos-Marcos (76)	2019	RP	Healthy or MS	246	123/123	61.8	31.2	3 years	-	end of intervention
Sugawara (137)	1992	NRC	Healthy	8	6/2	NI	NI	10 days	30 days	end of intervention
Wan (81)	2019	RP	Healthy	73	35/38	23.3	21.7	6 months	-	end of intervention

Table 2. Study characteristics of included low-fat intervention studies.

BMI, body mass index. DRE, drug-resistant epilepsy. GLUT 1DS, Glucose transporter 1 deficiency syndrome. HC, high carbohydrate. HGI, high glycemic index. MCI, mild cognitive impairment. MS, Multiple Sclerosis. NAFLD, Non-alcoholic fatty liver disease. NASH, non-alcoholic steatohepatitis. NRC, non-randomised cross-over. LFD, low fat diet. LFD per, low fat diet periodized. LGI, low glycemic index. RC, randomised cross-over. RP, randomised parallel. T2D, type 2 diabetes.

Author	Year	Set-up	Healthy/ diseased	Number analysed in diet group	Male/ female	Mean age (years)	Mean BMI (kg/cm2)	Intervention time	Wash-out period in (crossover)	Timepoint of analysis
Di Iorio (77)	2019	RC	CKD	60	46/14	68.4	26.8	6 months	3 months	end of intervention
Ferraris (17)	2021	Cohort	DRE	7	NI	NI	NI	1 month	-	end of intervention
Lai (70)	2019	Cohort	CKD	7 (LPD) 9 (LPD + inulin)	NI	NI	NI	6 months	-	end of intervention
Rocchetti (66)	2021	RC	CKD	NI	NI	NI	NI	6 months	3 months	end of intervention
Tagliabue (62)	2017	Cohort	GLUT1 DS	6	3/3	NI	NI	3 months	-	end of intervention

Table 3. Study characteristics of included low-protein intervention studies.

BMI, body mass index. CKD, Chronic Kidney Disease. DRE, drug-resistant epilepsy. GLUT 1DS, Glucose transporter 1 deficiency syndrome. NI, not indicated. RC, randomised cross-over. RP, randomised parallel .

Macronutrient composition

The macronutrient composition of the diet was very heterogeneous among the included studies (**Tables 4, 5, and 6**). Not all percentages add up to 100%, often without an explanation from the authors. Macronutrient content was sometimes reported in grams, so for this review, the percentage was calculated using the formula “grams*energy per gram*100)/consumed kcal/day”. The energy per gram of carbohydrate and protein is 4 kcal (16.7 kJ) and, per gram of fat, 9 kcal (37.7 kJ). The macronutrient composition of the LCD, LFD, and LPD diets will be described.

In the LCD interventions (**Table 4**), the carbohydrate content varied between 4% and 25% of total calories, fat content between 14% and 87% of total calories, and protein between 9% and 68% of total calories. The calorie content varied between 600 and 2526 kcal/day; however, only nine out of nineteen papers reported calorie content. In the paper by Gutierrez-Repiso(57), the number of grams of carbohydrates, fat, and protein was only reported for the first two months of the intervention, and the number of calories derived from additional vegetables was not reported. The following two months of intervention were not specified, although calorie intake was higher than the first two months (800-1500 kcal/day). The studies of Basciani(67) and Lundsgaard(68) used several study groups; in the trial of Basciani, the protein source differed between groups, and Lundsgaard supplemented either polyunsaturated fatty acids (PUFA) or saturated fatty acids (SFAs). Overall, LCD studies were very diverse in regard to dietary composition.

In the LFD interventions (**Table 5**), the percentage of fat of total calories varied between 8% and 28%. In two studies, the exact fat percentage was not reported, only that it was below 30%(64, 76). Carbohydrate content varied between 13% and 78%. One study examined two study groups consuming the same energy% of carbohydrates, differing in glycemic index (relative rise in the blood glucose level two hours after consuming that food)(69). Protein content varied between 14% and 68% of total calorie intake in studies where the content was indicated. Eight of 20 papers reported the total calorie intake varying between 600 and 2684 kcal/day. Again, LFD interventions were very heterogeneous in macronutrient composition.

Author	Year	Carbohydrate (%)	Fat (%)	Protein (%)	Kcal/day	kJ/day	Note
Ang (74)	2020	5	80	15	NI	NI	
Basciani (67)	2020	14	40	46	780	3264	3 groups, protein source differed between groups
Brinkworth (82)	2009	4	35	61	NI	NI	
Duncan (85)	2007	4	66	30	NI	NI	
Duncan (72)	2008	4	66	30	NI	NI	
Ferraris (17)	2021	4	87	9	1615 (1200-1675)	6757 (5020-7008)	
Fragiadakis (56)	2020	24	49	26	1485	6213	
Gutierrez-Repiso (57)	2019	13	23	50	600-800	2510-3347	Additional LGI vegetables and supplementation of 250mg DHA in the first 2 months
Gutierrez-Repiso (58)	2021	18	14	68	600-800	2510-3347	
Ley (86)	2006	25	NI	NI	1200-1500 (w); 1500-1800 (m)	5020-6276 (w); 6276-7531 (m)	
Lundsgaard (68)	2018	20	64	16	NI	NI	2 groups, supplementation of either PUFA or SFAs
Mardinoglu (83)	2018	4	72	24	NI	NI	
Murtaza (59)	2019	4	78	17	NI	NI	
Nagpal (60)	2019	10	60	30	NI	NI	
O'Keefe (61)	2015	21	52	27	2526	10569	
Pataky (75)	2016	16	36	47	1059	4430	
Russell (73)	2011	5	66	29	NI	NI	
Swidsinski (84)	2017	11	52	37	NI	NI	
Tagliabue (62)	2017	4	87	9	1892	7916	

Table 4. Macro-nutrient composition of low-carbohydrate diets.

Overview of macro-nutrient composition demonstrating the percentage of carbohydrate, fat, and protein of every intervention. When percentages were lacking, we calculated the percentage from the number of grams per macro-nutrient with the formula “amount of grams*energy per gram*100)/consumed kcal/day”.
DHA, docosahexaenoic acid. LGI, low glycemic index. NI, not indicated. PUFA, polyunsaturated fatty acids. SFA: saturated fatty acids.

Author	Year	Fat (%)	Carbohydrate (%)	Protein (%)	Kcal/day	kJ/day	Note
Cuevas-Sierra (78)	2021	22	60	18	NI	NI	
Cummings (71)	1978	21	68	15	2684	11229	
Fava (69)	2013	28	55	17	NI	NI	2 groups with either high glycemic index or low glycemic index diets
Fragiadakis (56)	2020	23	53	22	1460	6109	
Fritsch (20)	2021	11	64	25	NI	NI	
Guevara-Cruz (79)	2019	25-35	50-60	15	NI	NI	
Gutierrez-Repiso (57)	2021	14	18	68	600-800	2510-3347	
Gutierrez-Repiso (58)	2019	23	13	50	600-800	2510-3347	Additional LGI vegetables and supplementation of 250mg DHA in the first 2 months
Haro (63)	2016	28	NI	NI	NI	NI	
Haro (65)	2016	28	NI	NI	NI	NI	
Haro (64)	2017	<30	55	15	NI	NI	
Kahleova (87)	2020	8	78	14	1294	5414	
Liu (136)	2020	25	50-60	15-20	1800-2200	7531-9205	
Murtaza (59)	2019	20	60	16	NI	NI	2 groups, one with a periodized intervention
Nagpal (60)	2019	15	65	20	NI	NI	
O'Keefe (61)	2015	16	70	14	2206	9230	
Ren (80)	2020	25	59	16	NI	NI	
Santos-Marcos (76)	2019	<30	NI	NI	NI	NI	
Sugawara (137)	1992	14	70	16	1823	7627	
Wan (81)	2019	20	66	14	NI	NI	

Table 5. Macro-nutrient composition of low-fat diets.

Overview of macro-nutrient composition demonstrating the percentage of carbohydrate, fat, and protein of every intervention. When percentages were lacking, we calculated the percentage from the number of grams per macro-nutrient with the formula “amount of grams*energy per gram*100)/consumed kcal/day”.
DHA, docosahexaenoic acid. LGI, low glycemic index. NI, not indicated.

The percentage of protein in LPD interventions varied between 3% and 9% of total calories (**Table 6**). In two out of six papers, the carbohydrate and fat content are not reported(70, 77). The carbohydrate percentage of total calories varied from 4% to 62%, and fat percentages ranged from 32% to 87% of total calories. Furthermore, in two studies, supplementation of keto-analogues was used(66, 77); in another, inulin was supplemented(70).

Author	Year	Protein (%)	Carbohydrate (%)	Fat (%)	Kcal/day	kJ/day	Note
Di Iorio (77)	2019	9	NI	NI	30-35 kg/day	126-146 kg/day	
Di Iorio (77)	2019	3	NI	NI	30-35 kg/day	126-146 kg/day	Supplementation of keto-analogues
Ferraris (17)	2021	9	4	87	1615 (1200-1675)	6757 (5020-7008)	
Lai (70)	2019	7	NI	NI	30-35 kg/day	126-146 kg/day	Supplementation of inulin in one group
Rocchetti (66)	2021	4	62	32	NI	NI	Supplementation of keto-analogues
Tagliabue (62)	2017	9	4	87	1892	7916	

Table 6. Macro-nutrient composition of low-protein diets.

Overview of macro-nutrient composition demonstrating the percentage of carbohydrate, fat, and protein of every intervention. When percentages were lacking, we calculated the percentage from the number of grams per macro-nutrient with the formula "amount of grams*energy per gram*100)/consumed kcal/day".
NI, not indicated.

Risk of bias

The risk of bias was assessed for randomised (**Supplementary Figure 1**) and non-randomised **(Supplementary Figure 2**) studies. Six out of 24 randomised trials were judged to be at high risk, thirteen at moderate risk, and five at low risk of bias. Studies were classified as being at high risk of bias for different reasons, including not reporting potential cross-over effects in a cross-over trial, deviations from the intended intervention, and missing outcome data. Of the twelve non-randomised trials, four were judged as at high risk, four as at moderate risk, and four as at low risk of bias. Most risks of bias were judged as moderate or high due to a lack of reported study procedures by not mentioning any possible confounders or how confounding factors were controlled for. Blinding of dietary interventions is often not feasible, especially when participants must prepare their food. Therefore, the risk of bias arising from the randomisation process was often judged as moderate.

Outcomes

Change in alpha diversity of bacterial gut microbiota

Alpha diversity was reported in seven papers documenting the effects of LCD interventions (**Table 7**). No difference in alpha diversity was found after the intervention compared to baseline in all but one study, which examined just a small group of nine participants(57), where a higher alpha diversity was measured after two months of an LCD. Alpha diversity was documented in eleven LFD intervention groups. Five studies reported increased bacterial diversity(57, 78-81), whereas the other six groups measured no difference in bacterial diversity between baseline and post-intervention. In the study of Cuevas-Sierra(78), only men displayed an increase in diversity in response to LFD,

whereas there was no change in women. Only one paper reported alpha diversity in response to an LPD intervention(66) and found no difference between baseline and post-intervention. Overall, there is not much evidence that LCD or LPD interventions change alpha diversity, while an increased alpha diversity was measured in response to an LFD in several studies.

Author	Year	Alpha diversity change	Method of measuring
Low-carbohydrate intervention			
Fragiadakis (56)	2020	=	Observed number of ASVs in a rarefied sample
Gutierrez-Repiso (57)	2019	↑	Shannon index
Gutierrez-Repiso (58)	2021	=	Shannon index, Faith's PD, observed ASVs and Pielou index
Ley (86)	2006	=	Shannon index
Lundsgaard (68)	2018	=	Shannon index
Murtaza (59)	2019	=	Shannon and Simpson indices
Swidsinski (84)	2017	=	% of substantial bacterial groups positive in each patient
Low-fat intervention			
Cuevas-Sierra (men) (78)	2021	↑	Shannon index
Cuevas-Sierra (women) (78)	2021	=	Shannon index
Fragiadakis (56)	2020	=	Observed number of ASVs in a rarefied sample
Fritsch (20)	2021	=	Faith's phylogenetic diversity
Guevara-Cruz (79)	2019	↑	Shannon index
Gutierrez-Repiso (57)	2019	↑	Shannon index
Gutierrez-Repiso (58)	2021	=	Shannon index
Kahleova (87)	2020	=	Shannon index, Faith's PD, observed ASVs and Pielou index
Murtaza (59)	2019	=	Abundance-weighted PD measure
Ren (80)	2020	↑	Shannon and Simpson indices
Wan (81)	2019	↑	Faith's phylogenetic diversity
Low-protein intervention			
Rochetti (66)	2021	=	Not indicated

Table 7. Alpha diversity change after dietary intervention compared to baseline.

↑ significantly higher diversity post-intervention, = non-significant difference in diversity post-intervention

Change in the relative abundance of gut bacteria

The relative abundance of various bacterial taxonomic groups changed from baseline to post-intervention in response to the various diets (**Supplementary Tables 1, 2, and 3**). However, changes in the abundance of a specific taxonomic group were often reported in just one paper. To provide a more accurate picture of the influence of diet on the relative abundance of bacterial groups as reliably as currently possible, only the taxa that were reported in at least three intervention groups will be discussed.

Eleven bacterial taxa were reported in three or more different LCD intervention groups (**Table 8**). These groups are part of five phyla: Actinobacteria, Bacteriodetes, Firmicutes, and Verrucomicrobia.

Most studies documented a lower relative abundance of the phylum Actinobacteria in response to an LCD. *Bifidobacterium* was reported in nine study groups, of which seven had a relatively lower abundance in response to an LCD(59, 60, 68, 72, 82-84), while it did not significantly change in the other two(62, 85). Bacteria belonging to the phylum Bacteriodetes were often more abundant after an LCD(56, 58, 67, 68, 74, 75, 86). A minority of studies documented a decrease in the relative abundance of the genera *Bacteroides*(73, 75). Bacteria belonging to the Firmicutes phylum were generally reported to decrease after an LCD(58, 59, 67, 72-75, 83, 85, 86). Just the taxonomic sublevels *Lachnospira*(56, 68) and *Streptococcus*(83) were reported to increase in some studies. The phylum Proteobacteria and its taxonomic subgroup *Enterobacteriaceae* were measured in response to LCD in six studies, showing no change in relative abundance except for two studies showing an increase(59, 74). The genus *Akkermansia* from phylum *Verrucomicrobia* was reported in three studies, with one reporting an increase(59) and the others measuring no difference(60, 84) by use of an LCD.

In summary, the currently available evidence suggests that an LCD impacts the relative bacterial abundance in our gut, inducing an overall decrease of Actinobacteria, an increase of Bacteroidetes, and a lower or stable abundance of Firmicutes, while it generally does not appear to affect the relative abundance of Proteobacteria or Verrucomicrobia.

The relative abundance of 23 bacterial taxonomic groups was reported in three or more study groups at baseline and after an LFD (**Table 9**). These 23 groups originate from five phyla: Actinobacteria, Bacteroides, Firmicutes, Proteobacteria, and Verrucomicrobia.

					Author Year
Phylum	**Class**	**Order**	**Family**	**Genus**	**Species**
Actinobacteria (Actinomycetota)	Unspecified				
	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	*Bifidobacterium*	Unspecified
Bacteroidetes (Bacteroidota)	Unspecified				
	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides*	Unspecified
			Tannerellaceae	*Parabacteroides*	Unspecified
Firmicutes (Bacillota)	Unspecified				
	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	Unspecified
			Streptococcus	Unspecified	
	Clostridia	Eubacteriales	Eubacterium + Roseburia	Unspecified	
			Lachnospiraceae	Unspecified	
				Lachnospira	Unspecified
			Oscillospiraceae	*Faecalibacterium*	*F. prausnitzii*
Proteobacteria (Pseudomonadota)	Unspecified				
	Gammaproteo-bacteria	Enterobac-teriales	Enterobacteriaceae	Unspecified	
Verrucomicrobia (Verrucomicrobiota)	Verrucomicrobiae	Verrucomicro-biales	Akkermaniaceae	*Akkermansia*	Unspecified

Table 8. Change in relative abundance of gut bacteria after a low-carbohydrate diet compared to baseline.

Pre-postintervention changes in bacterial taxonomic levels that were reported by three or more studies are included in this table.
↓ significantly lower abundance post-intervention
↑ significantly higher abundance post-intervention
= no significant difference in abundance post-intervention

	2020	2020	2009	2007	2008	2020	2021	2006	2018	2018	2019	2019	2016	2011	2017	2017
	Ang (74)	Basciani (67)	Brinkworth (82)	Duncan (85)	Duncan (72)	Fragiadakis (56)	Gutierrez-Repiso (58)	Ley (86)	Lundsgaard (68)	Mardinoglu (83)	Murtaza (59)	Nagpal (60)	Pataky (75)	Russell (73)	Swidsinski (84)	Tagliabue(62)
	↓	↓										=				
			↓	=	↓				↓	↓	↓	↓			↓	=
	↑	↑				↑		↑				=				=
					=	↑			↑		↑		↓	↓	=	
						↑	↑						↑			
	↓	↓			=			↓								=
			=				↓									=
										↑	↓					=
				↓	↓									↓		
	↓						↓							=		
						↑			↑		↓		↓			
				=						↓				=	=	=
	↑	=										=				
											↑	=			=	=
											↑	=			=	

					Author Year
Phylum	**Class**	**Order**	**Family**	**Genus**	**Species**
Actinobacteria (Actinomycetota)	Unspecified				
	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	*Bifidobacterium*	Unspecified
Bacteroidetes (Bacteroidota)	Unspecified				
	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides*	Unspecified
			Prevotellaceae	*Prevotella*	Unspecified
			Tannerellaceae	*Parabacteroides*	Unspecified
					P. distasonis
Firmicutes (Bacillota)	Unspecified				
	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	Unspecified
			Streptococcaceae	*Streptococcus*	Unspecified
	Clostridia	Eubacteriales	Clostridiaceae	Unspecified	
				Clostridium	Unspecified
			Lachnospiraceae	Unspecified	
				Dorea	Unspecified
				Roseburia	Unspecified
			Oscillospiraceae	Unspecified	
				Faecalibacterium	Unspecified
					F. prausnitzii
				Ruminococcus	Unspecified
Proteobacteria (Pseudomonadota)	Unspecified				
	Betaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unspecified	
Verrucomicrobia (Verrucomicrobiota)	Verrucomicrobiae	Verrucomicrobiales	Akkermansiaceae	*Akkermansia*	Unspecified

Table 9. Change in relative abundance of gut bacteria after a low-fat diet compared to baseline.

Pre-postintervention change in bacterial taxonomic levels that were reported by three or more studies are included in this table.
↓ significantly lower abundance post-intervention
↑ significantly higher abundance post-intervention
= no significant difference in abundance post-intervention
HGI: high glycemic index. LGI: low glycemic index. MetS: metabolic syndrome. OB: obese.

1978 Cummings (71)	2013 Fava (HGI) (69)	2013 Fava (LGI) (69)	2020 Fragiadakis (56)	2021 Fritsch (20)	2019 Gutierrez-Repiso (57)	2021 Gutierrez-Repiso (58)	2016 Haro (65)	2017 Haro (MetS-OB) (64)	2017 Haro (NonMets-OB) (64)	2017 Haro (NonMetS-non OB) (64)	2020 Kahleova (87)	2006 Ley (86)	2020 Liu (134)	2019 Murtaza (non-periodised) (59)	2019 Murtaza (periodised) (59)	2019 Nagpal (60)	2015 O'Keefe (61)	2020 Ren (80)	2019 Santos-Marcos (female) (76)	2019 Santos-Marcos (male) (76)	2019 Wan (81)
			↓	↓							=					=					
	↑	↑	↓								=				=	=					
			↑	↑				↑	=	=	=	↑						=			=
=	↑	=	↑				=	↑	↑	=								=			=
				↑			=	↑	=	=	=					=					
			↑			↑	=														
						↑	=	=	=	=											
			↓		↑						=	↓						↑			=
=						↓												=			
=								↓	=	=					=						
						↑							=	=							
						↑		↓	=	=	=										
			↓			↓					↓			=							=
			↓			↑	=														
						↓	↓	=	=	=	=		=					↓	↓	=	=
					↑						=			=	=						
							=	↑	=	=											↑
	=	↑		↑			↑	=	=	=	↑										
			↓				=	=	=	=								↓			=
					↓						↓					=					
=					↓						=					=					
											=				=	=					

The effect of LFD on Actinobacteria and its subtypes varied, as three papers reported no difference in relative abundance(59, 60, 87), one paper with two study groups reported an increase(69), and two papers a decrease(20, 56). Bacteroidetes, documented at the phylum level, increased in four study groups(20, 56, 64, 86) and were not different in another four groups(64, 80, 87) after the use of an LFD. Fourteen papers reported change within its taxonomic subgroups in response to LFD, of which four (*Bacteroides, Prevotella, Parabacteroides, P. distasonis*) were reported by a minimum of three papers. The majority reported an increase(20, 56, 58, 64, 69) or no change(60, 64, 65, 69, 71, 80, 81, 87) in relative abundance, none reported a decrease. Changes in abundance of the phylum Firmicutes and its taxonomic members in response to LFD differed widely. Members of the family of Oscillospiraceae, *Faecalibacterium,* and *F. prausnitzii*, showed an overall increase(20, 57, 64, 65, 69, 81, 87) or no difference(64, 65, 69), while its member *Ruminococcus* decreased(56, 80) or showed no difference(64, 65). Many genera (*Lactobacillus, Streptococcus*, Lachnospiraceae*, and its taxonomic members Roseburia and Ruminococcus*) decreased or remained unchanged in response to LFD, while an increase, decrease, or no difference in abundance was reported for others, including *Clostridium* and *Dorea* (see **Table 9** for references). Likewise, studies documenting the phylum Proteobacteria and its taxonomic unit Enterobacteriaceae yielded a decrease(57, 87) or no difference in abundance(60, 71, 87) in response to an LFD compared to baseline. The abundance of the phylum Verrucomicrobia after an LFD was reported in three study groups and did not change in any of them(59, 60, 87).

To conclude, current evidence paints a diverse picture of gut bacterial abundance in response to an LFD. Thus, conclusions regarding the impact of an LFD on the gut microbiome are difficult to draw at present, although some trends were observed, including the increase in several Bacteroidetes and its subgroups, a decrease in several Firmicutes subgroups (except for the family Oscillospiraceae and its taxonomic members *Faecalibacterium and F. prausnitzii,* which tended to increase), and a tendency of Proteobacteria and subgroups to decrease.

The change in relative gut bacterial abundance in response to an LPD was measured in only two studies **(Table 10).** One study had two arms using an LPD(70). Its impact on just two bacteria was reported in at least three study groups. Lactobacillaceae from the phylum Firmicutes decreased in response to an LPD in three study groups(70, 77), and Enterobacteriaceae from the phylum Proteobacteria decreased in two out of three study groups(70, 77). Thus, the scarcity of data documenting the gut bacterial response to LPD precludes any conclusion as to the effect of such a diet on the gut microbiome.

						Author	Di Iorio (77)	Lai (no inulin) (70)	Lai (inulin) (70)
						Year	2019	2019	2019
Phylum	**Class**	**Order**	**Family**	**Genus**	**Species**				
Firmicutes (Bacillota)	Bacilli	Lactobacillales	Lactobacillaceae	Unspecified			↓	↓	↓
Proteobacteria (Pseudomonadota)	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unspecified			↓	=	↓

Table 10. Change in relative abundance of gut bacteria after a low-protein diet compared to baseline.

Pre-postintervention change in bacterial taxonomic levels that were reported by three or more studies are included in this table.
↓ significantly lower abundance post-intervention
↑ significantly higher abundance post-intervention
= no significant difference in abundance post-intervention

Change in faecal metabolites

Many metabolites were reported in the included papers **(Supplementary Tables 4, 5, and 6)**. As with the relative abundance of species, we will only report the metabolites that were documented by at least three trials (or trial groups), which specifically concerned SCFAs and lactate. Unfortunately, bile acids and tryptophan/indoles were not reported in three or more papers.

Faecal metabolite concentrations in response to an LCD were documented by seven papers (**Table 11**). The total SCFA concentration was reported in five of them, all showing a decrease after an LCD compared to baseline(17, 73, 82, 83, 85). Six papers reported faecal acetate, propionate, and butyrate concentrations, which consistently decreased in response to an LCD(17, 61, 73, 82, 85). Valerate concentration decreased in two studies(61, 85) while it increased or did not change in one other(73), depending on the measured concentration or proportion of SCFA. Isobutyrate was measured in four studies examining the effects of an LCD. Two studies did not find an effect(74, 85), one study demonstrated an increase in both tested study groups(73), and one showed a decrease in concentration(17) after the intervention. Faecal isovalerate concentration increased in one study in both study groups(73), decreased in one other study(85), and did not change in yet another study(17). Lactate decreased in one trial(61) with no difference in the two other trials(73, 85).

Faecal metabolites were measured in three studies evaluating the effects of an LFD (**Table 12**). Acetate increased after an LFD compared to the baseline in two studies(20, 61) and did not change in one other(69). The quantity of propionate and butyrate increased in one study(61) and did not change compared to the baseline in the two others(20, 69).

Just one study(17) measured metabolites in response to an LPD, so no conclusions can be made concerning the effect of LPD on metabolite concentration.

In concert, the available evidence suggests that faecal SCFA concentrations decline in response to an LCD, while it remains unclear if faecal BCFA concentrations are affected by LCD. An LFD may increase faecal acetate levels. Just one study examined faecal metabolite concentrations in response to LPD, which precludes meaningful conclusions regarding the effects of this dietary intervention.

	Author	Ang (74)	Brinkworth (82)		Duncan (85)	Ferraris (17)	Mardinoglu (83)	O'Keefe (61)	Russell (73)	
	Year	2020	2009		2007	2021	2018	2015	2011	
	Unit of measurement	mg/g	mmol/l	mmol/d	mmol/l	proportion of total SCFA	mg/g	mmol/l	mmol/d	mmol/l	proportion of total SCFA
SCFAs	Total SCFA		↓	↓	↓		↓	↓		↓	
	Acetate	=	↓	↓	↓	↑	↓		↓	↓	=
	Butyrate	=	↓	↓	=	↓	↓		↓	↓	↓
	Propionate	=	=	↓	↓	=	↓		↓	↓	=
	Valerate				↓				↓	=	↑
BCFAs	Isobutyrate	=			=		↓			↑	↑
	Isovalerate				↓		=			↑	↑
	Lactate				=				↓	=	=

Table 11. Change in faecal metabolites after a low-carbohydrate diet compared to baseline.

↓ significantly lower post-intervention, ↑ significantly higher post-intervention, = no significant difference post-intervention
SCFA: short-chain fatty acids, BCFA: branched-chain fatty acids

	Author	Fava (HGI) (69)	Fava (LGI) (69)	Fritsch (20)	O'Keefe (61)
	Year	2013	2013	2021	2015
	Unit of measurement	mmol/l	mmol/l	relative abundance	mmol/d
SCFAs	Acetate	=	=	↑	↑
	Butyrate	=	=	=	↑
	Propionate	=	=	=	↑

Table 12. Change in faecal metabolites after a low-fat diet compared to baseline.

↓ significantly lower post-intervention, ↑ significantly higher post-intervention, = no significant difference post-intervention, SCFA: short-chain fatty acids

Discussion

This systematic review summarises current data documenting the impact of dietary macronutrient composition on human gut microbiota. Gut bacteria play a pivotal role in host health through the biosynthesis of vital nutrients such as vitamins, essential amino acids, and short-chain fatty acids(88). Dietary intake can reproducibly change the human gut microbiome(89), and knowledge of the impact of dietary interventions on gut microbiota composition and metabolic activity is important for understanding their health effects and safety. We summarise available data on the effects of carbohydrate, fat, or protein restriction on alpha diversity, the relative abundance of taxonomic units of the major phyla, and faecal metabolites.

Alpha diversity

There is inconclusive evidence to support the notion that the alpha diversity of human gut microbiota is significantly altered by LCD or LPD. In contrast, diets low in fat increased alpha diversity in five out of twelve study groups, while there was no change in response to LFD in the other seven. Low-fat diets are necessarily (relatively) high in carbohydrate and/or protein content, and indigestible carbohydrates (fibres), in particular, are well known to impact gut microbiota(90). However, the low-fat diets in the studies demonstrating a higher alpha diversity varied widely in macronutrient content, comprising both high or low carbohydrate or protein energy percentage. Therefore, the effect of LFD on alpha diversity cannot (exclusively) be explained by high contents of either (indigestible) carbohydrates or protein, which is in line with a previous review documenting the effects of dietary fibre on the abundance of Bifidobacterium and Lactobacillus spp. without significant impact on alpha diversity(91). Relatively low microbial alpha diversities have been linked to several acute and chronic disorders(28, 29, 92). Thus, the increase in alpha diversity that is generally observed in response to LFD interventions, particularly in people with metabolic disease, may confer health benefits. Notably, four out of five studies demonstrating an increase of alpha diversity in response to an LFD examined overweight or obese participants with or without type 2 diabetes, while only three out of seven showing no effect studied overweight or obese people. Obesity and metabolic disease are well known to be associated with low alpha diversity of the gut microbiome, and low baseline values provide more room for improvement. Thus, the currently available data on the impact of LFD on alpha diversity may well have been confounded by sampling bias.

Relative abundance of taxonomic units

The relative abundance of specific taxonomic units of gut bacteria varies widely between individuals, primarily driven by multiple environmental and lifestyle conditions, and alteration of relative abundance is not necessarily related to health outcomes(93). However, the relative abundance (or absence) of specific bacterial taxonomic units has

been observed to relate to human health. Here, we will discuss our findings concerning the potentially relevant changes in relative microbial abundance in response to dietary intervention for taxonomic units per phylum.

Actinobacteria (Actinomycetota)

Actinobacteria are one of the four major phyla of the gut microbiota and, even though they represent only a small percentage, are pivotal in maintaining gut homeostasis(94). *Bifidobacterium* is a genus that, in healthy breastfed infants, dominates the intestine and has much lower but relatively stable levels in adulthood. The different species of *Bifidobacteria* that are present change with age, from childhood to old age(95). *Bifidobacterium* fulfils important functions in the human gut. Bifidobacterial genera are involved in the protection of the gut mucosal barrier, in the bioavailability of B vitamins, antioxidants, polyphenols, and conjugated linoleic acids, and in the production of several SCFAs(96). Decreased numbers of *Bifidobacterium* have been associated with a variety of disorders(96, 97), although one study also found high numbers of *Bifidobacteria* in an elderly nursing home population(98). In seven out of nine included studies examining *Bifidobacterium* abundance, it declined in response to an LCD, which possibly could have unfavourable effects that could counteract the health benefits of carbohydrate restriction. The studies examining the impact of LFD on *Bifidobacterium* abundance produced highly variable results, while there is a lack of data on the effects of LPD on *Bifidobacterium,* precluding any conclusion as to the effects of either LFD or LPD in this context.

Bacteroidetes (Bacteroidota)

Bacteroides spp., which form ~30% of human gastrointestinal microbiota(93), are acknowledged to play a critical role in gut bacterial colonisation and (host) health through their capabilities to metabolise (host) glycans, their role in protein metabolism, deconjugation of bile acids, modulation of immune responsiveness to infections and protection against various auto-immune disorders(99-105). Because of their broad metabolic potential, the role of the Bacteroidetes in the gastrointestinal microbiota is complex. Reduced abundance of Bacteroidetes and its taxonomic subunit *Bacteroides* have been associated with obesity(86), IBD(106, 107), and asthma(108), while increased abundance is associated with type 1 and 2 diabetes(109). The phylum Bacteroidetes and its taxonomic members were typically reported to increase in response to both LCD and LFD interventions included in this review. It has been speculated that the loss of body weight, which usually accompanies both carbohydrate and fat-restricted dietary interventions, could be responsible for the increase of *Bacteroides* spp. abundance in response to both LCD and LFD(56), but several studies contradict this argument(58, 78). Recent genomic and proteomic advances have greatly facilitated

our understanding of the uniquely adaptive nature of *Bacteroides* species(110, 111). Nevertheless, given the previously mentioned diverse biological features of this phylum, conclusions on health effects from the intervention studies presented here are hampered due to a lack of information.

Firmicutes (Bacillota)

A substantial part (~40%) of the human gut microbiome comprises Firmicutes spp(93). Members of this phylum generally contribute to host health by being involved in gut permeability, inflammation, glucose metabolism, fatty acid oxidation, synthesis, and energy expenditure, partly through the production of butyrate and anti-inflammatory metabolites(112). Indeed, the relative abundance of Firmicutes taxonomic units is decreased in people with several diseases. *Faecalibacterium* was, for example, decreased in non-alcoholic fatty liver disease, hypertension, and gestational diabetes mellitus, and *F. prausnitzii* was decreased in type 2 diabetes, colorectal cancer, coeliac disease, inflammatory bowel disease, and several other auto-immune disorders, and compared to healthy controls(112, 113). The relative abundance of most taxonomic members of the Firmicutes phylum seems to decrease in response to an LCD. The effects of LFD on the relative abundance of Firmicutes vary among taxonomic units of this phylum, with, for example, a decline of *Roseburia* and an increase of *Faecalibacterium* and its species *F. prausnitzii.* As LFD interventions appear to exert mixed effects on the abundance of distinct Firmicutes taxonomic units, their potential impact on (gut) health remains unclear.

Metabolites

SCFAs produced by gut bacteria play a pivotal role in the gut- as well as systemic health(114, 115). Distinct SCFA can be fuel for intestinal epithelial cells, strengthen the gut barrier function, have immunomodulatory functions, improve glucose homeostasis, and may play protective roles against cancer and colitis(96, 116). SCFAs are primarily produced by colonic bacteria through anaerobic fermentation of complex carbohydrates that escape digestion and absorption in the small intestine(117). Most of the studies reported a reduction of acetate, propionate, and butyrate concentrations in faeces in response to an LCD, which is in concordance with literature describing an increase in SCFAs by high-carbohydrate interventions(82, 118). However, it should be noted that only SCFAs not absorbed by the (healthy) host can be measured in faeces(49), and these results do not represent all SCFA produced *in vivo*.

LFDs are often (relatively) carbohydrate-rich and, therefore, often (but not always) provide plenty of substrates for SCFA production. SCFA levels were indeed increased or stable in the majority of the included studies documenting the impact of LFD on

faecal metabolite content. This is in accordance with the decrease in SCFAs in high-fat interventions(82, 118). Thus, the fact that SCFAs tend to decline in response to LCD calls for careful consideration of the potential dangers of long-term LCD intervention. In particular, it seems prudent to make sure that the diet provides sufficient fibre (i.e. 25-30 g per day according to the Dietary Guidelines for Americans (https://www.dietaryguidelines.gov/) and many other international guidelines) if carbohydrates are restricted for longer periods to sustain adequate SCFA production.

Limitations

A major difficulty in interpreting the results of studies evaluating the effects of an isolated class of macronutrients in our diet concerns the fact that such a component is never consumed alone. Moreover, the considerable variability of compounds within the macronutrient categories can lead to variable effects, even when macronutrient levels are similar. Within the carbohydrate category, literature has demonstrated differential effects on the microbiome when comparing simple and complex carbohydrates(91, 119, 120). There is increasing but still limited knowledge of the relationship between the physiochemical structure characteristics and functional properties of non-digestible carbohydrates in the gut microbiome(39). Both increases and decreases in fibre content seem to alter the gut microbiota(91, 120, 121), and various types of dietary fibres have exhibited functional distinctions in their impact on the composition of human faecal microbiota(70, 119). In the protein category, the source of protein, whether animal or plant-based, has also been shown to exert varying effects on the gut microbiome(26). Additionally, distinctions emerge when considering the fat content, in which unsaturated versus saturated fats demonstrate differential effects on the gut microbiome(106). Moreover, specific types of polyunsaturated fatty acid or saturated fatty acid(57, 68, 122) can have divergent impacts. Furthermore, dietary availability or supplementation of specific compounds in the diet, such as polyphenols(123, 124) and keto-analogues(66, 77), can affect the composition and function of the gut microbiome. Polyphenols are thought to influence carbohydrate metabolism at many levels, including inhibition of carbohydrate digestion(125), influence fat metabolism via the interaction with bile acids(126) and affect protein metabolism through the phenolic compounds binding influence to protease activity and protein substrate accessibility(127). Caloric content varied across studies, influencing the quantity of consumed macronutrients, and very low caloric content could affect the gut microbiome independent of macronutrients(128). Thus, the type and amount of (other) nutrients and availability of other compounds in each of the specific dietary interventions that were examined in the studies included in this review may have influenced the results. Moreover, the included studies turned out to be very heterogeneous in terms of participant features (healthy or sick, normal weight or obese), age, duration of the

interventions and outcome data (highly variable taxa). In this context, it is also pivotal to acknowledge the gut microbial community the macronutrients are introduced into and the microbiota's metabolic potential to utilise such substrates, as the maturity and metabolic potential of the gut microbiome varies throughout life and with health status(129, 130). There is also accumulating evidence that gut transit time is a key factor in shaping gut microbiota composition and activity, which are linked to human health(131). These factors may have affected the included outcomes and, therefore, complicate drawing uniform conclusions. This review did not differentiate between the methodologies used in relation to collection, fixation, storage, shipping, extraction, library preparation, sequencing, and bioinformatic processing. As none of these steps are standardised, the variability created among different studies for each of these steps may cause bias(132, 133), which made risk of bias assessment of sample collection and processing of samples challenging. Since there is a lack of access to samples from different sites of the intestine and only faecal samples are available, it is not possible to fully unravel the influence of an intervention on the complete gut microbiome(38). Finally, papers often reported only taxonomic units that changed in response to a particular intervention, excluding critical evaluations of unaffected species at the endpoint. Thus, although our review appears to unveil the effects of the restriction of distinct dietary macronutrients despite all these caveats, its results need to be judged in the context of these (partly unavoidable) limitations.

Recommendations for future research

To create a complete overview of the effects of dietary restriction of specific macronutrients on the gut microbiome and its metabolites, it is important to provide a comprehensive and integrated analysis of the microbiome and metabolite changes induced by dietary interventions, where not only taxa and metabolites exhibiting significant change are reported. It is also important to provide detailed information on the diet, including caloric content, the quantity of all macronutrients and the availability of specific compounds like polyphenols. To enhance the adequacy of the interpretation of data from studies examining the effects of macronutrient restriction, it is important to recognise the potential influence of fibre and caloric content on the gut microbiome. Therefore, researchers could strive to maintain fibre and calorie intake close to what is consumed at baseline, thereby minimising the risk of bias by these dietary characteristics. Participant features should also be described in detail, including health status, and preferably, more extended information should be shared, like individual transit time. To reduce bias created by the variability in the methodology of sample processing, it could be interesting to obtain raw sequence data for all the studies and then uniformly process them bioinformatically so that at least variation in that step would be removed. Furthermore, 16S rRNA gene amplicon

sequencing, which was the most often used technique for microbiota profiling in nutritional studies, is somewhat limited, and the implementation of metagenomics for gut microbial community analysis will allow the generation of in-depth knowledge on the microbial community dynamics as well as the metabolic potential of specific microbial communities(134, 135). Thus, future studies should use integrated advanced metagenomics and metabolomics analyses to foster our understanding of the impact of manipulating dietary macronutrients on gut microbiota and its metabolites.

Conclusions

We have reviewed available studies evaluating the impact of the restriction of distinct dietary macronutrient components on gut microbiota composition. The results, which must be assessed in light of certain limitations, suggest that carbohydrate restriction reduces the abundance of several health-promoting bacterial species as well as the faecal concentration of SCFAs. In contrast, low-fat diets appear to have opposite effects on SCFA production and relative abundance of health-promoting bacteria, which is in line with current knowledge on the effect of the fibre content of the diet on the gut microbiome. As to the impact of protein restriction on gut microbiome composition and metabolite production, there is not enough data to draw any conclusions to date.

Declarations

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Conflict of interest

None.

Data sharing

Data described in the manuscript will be made available upon request pending. All proposals requesting data access will need to specify how the data will be used, and all proposals will need the approval of the trial co-investigator team before the data will be released.

Authorship

The authors' responsibilities were as follows – MS, PP, HP, and MN developed the overall research plan; MS devised and executed the literature search; MS and NJ or PP performed the title/abstract as well as the full-text screening; MS and NJ or PP performed the risk of bias; MS was responsible for the construction of data tables and summary and reporting of results; PP, EB, HP, MN, QD and MW reviewed and edited the manuscript; MS had primary responsibility for final content; all authors read and approved the final manuscript.

QR-code to article and supplementary information

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