Gastroenterology and Hepatology From Bed to Bench.

SYSTEMATIC REVIEW

The ketogenic diet: its impact on human gut microbiota and potential consequent health outcomes: a systematic literature review

Leslyn Rew, Miranda D Harris, Joanna Goldie

The School of Allied Health and Community, University of Worcester, Henwick Grove, WR2 6AJ, United Kingdom

ABSTRACT

Aim: This systematic review examined the diet's impact on the human gut microbiota to identify potential consequent health outcomes.

Background: The extreme macronutrient profile of the ketogenic diet (KD) instigates compositional shifts in the gut's microbial community.

Methods: In this systematic literature review, an evidence-based and methodical approach was undertaken, which involved systematic searches of the Medical Literature Analysis and Retrieval System Online (MEDLINE), PubMed and Cumulative Index to Nursing and Allied Health Literature (CINAHL) databases, generating a total of 263 relevant research papers. Following the application of inclusion and exclusion criteria, eight papers were deemed suitable for inclusion. These papers were critically appraised using a checklist tool adapted from the National Institute of Care and Excellence (NICE). The findings were analysed using a simplified thematic analysis.

Results: The results provide strong evidence for a persistent reduction in *Bifidobacterium* abundance following KD adherence. A reduced abundance of key *Firmicutes b*utyrate-producing bacteria was found to be a likely impact, although two studies with extended intervention periods indicate this may be time-limited. Studies investigating short-chain fatty acids (SCFA's) indicate KD reduces total faecal SCFA's, acetate, and butyrate.

Conclusion: Changes to microbial communities resulting from KD adherence are potentially detrimental to colonic health. The persistent reduction in *Bifidobacterium* abundance was concerning, with obesity, type-2 diabetes, and depression highlighted as potential consequent risks. For nutrition and healthcare professionals, the findings emphasize the importance of considering KDs microbial effects and resulting health implications at an individual level.

Keywords: Ketogenic diet, Gut microbiota, Human.

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Introduction

The ketogenic diet (KD), characterized by a macronutrient composition of low carbohydrates, high fat, and adequate protein (1), has traditionally been used to treat epilepsy (2), but evidence indicates its therapeutic use in other conditions, including cancer, neurodegenerative diseases (3), obesity, and type-2 diabetes (T2D) (4, 5). Additionally, athletes adopt KD to improve performance and reduce body fat (6).

KD may alter the gut's microbial composition that is fundamental to human health (7). As compositional imbalances are implicated in the development of certain diseases (8), it is important to determine the microbial changes induced by KD in order to understand these implications.

Existing reviews examining KD's impact on microbiota have collated evidence from murine and human studies (7, 9-12). However, differences between murine models and human systems limit their ability to recapitulate human microbial changes resulting from interventions, and caution is recommended in drawing conclusions about humans from murine studies (13). Furthermore, previous reviews have often included

Received: 28 Apr 2022 *Accepted*: 21 Sep 2022
Reprint or Correspondence: **Miranda Harris MSc,** *The School of Allied Health and Community, University of Worcester.*
E-mail: m.harris@worc.ac.uk
ORCID ID: 0000-0003-4293-1543

studies of infants. While research is not conclusive on when a child's microbiome reaches maturation, the consensus is the majority of development takes place before three years (14, 15). The exclusion of children under this age, as in this review, avoids bias as a result of developmental changes.

The current systematic review is novel in that it examines KD's impact on human gut microbiota in isolation, without the bias of potentially non-comparable murine models or human developing microbiomes. In another unique approach, this review then identifies potential consequent health outcomes specifically from these microbial impacts in order to make recommendations for practice and enable nutrition and healthcare professionals to make informed decisions when dealing with KD therapeutically.

The ketogenic diet

KD is defined by its ability to induce a state of "physiological ketosis," with higher-than-normal ketone liver production (16, 17). Under normal dietary conditions, the majority of the body's tissues utilize glucose and fatty acids as energy sources, as both are metabolized to acetyl-coenzyme A, which condenses with oxaloacetate to enter the tricarboxylic acid cycle (18). During a KD, however, diminishing glucose blood levels and reserves instigate two processes to provide glucose and FAs for energy production; gluconeogenesis in the liver and lipolysis of FAs from adipose tissue (19). Consequently, the oxaloacetate supply becomes limited for two reasons. Firstly, glycolysis falls to low levels and oxaloacetate production relies on glycolysis generating its precursor pyruvate. Secondly, oxaloacetate is preferentially used in gluconeogenesis (20). Thus, the oxaloacetate supply becomes insufficient to condense all the acetyl-coenzyme A produced from FAs, and the liver diverts it to produce ketones as an alternative extra-hepatic energy source in the biochemical process "ketogenesis" (19, 20).

A KD may also be defined by the macronutrient ratios consumed. For the purposes of treating epilepsy, five categories have been defined: the classic KD, the modified KD, the medium-chain triglyceride (MCT) KD, the modified Atkins diet, and the low glycemic index treatment (LGIT) (1, 21). The ratio of fat types is specified in the MCT KD, and likewise, the ratios of carbohydrate types is specified in the LGIT. However, there is much more variation in the macronutrient ratios seen in KD research and real life. It is accepted that ketosis results from restricting carbohydrates to under 50 grams (g), or 10% of total energy, while keeping protein adequate at 1.2–1.5 g per kilogram (kg) weight/day and making up the remaining energy intake percentage with fat, normally 60% to 90% (22, 23). Elevated serum levels of the ketone 3-beta-hydroxybutyrate (βOHB) indicate ketosis (20), with βOHB $\geq$0.5 millimole (mmol)/litre (l) accepted as the threshold (24-26), although ketone production is subject to individual variability (27, 28) and influenced by fat and protein type (26, 29).

Gut microbiota and health

The gut microbiota refers to the bacteria, archaea, and eukaryotes residing in the gastrointestinal tract (30). The majority colonize the colon, with bacterial numbers estimated at 3.8×10^{13}; this review uses the term microbiota to refer specifically to these (31). Microbiota are predominantly from two phyla, *Bacteroidetes* and *Firmicutes*, while *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* are present in smaller proportions (32). The principal genera comprise *Alistipes*, *Bacteroides*, *Faecalibacterium*, *Bifidobacterium*, *Eubacterium*, *Dorea*, and *Ruminococcus* (32). Despite the constancy of these constituents, dramatic differences are present among individuals in terms of relative proportions and species (33). It has been proposed that microbial composition can be categorized into three "enterotypes," namely *Bacteroides*, *Prevotella*, and *Ruminococcus*, distinguishable by the dense population of these genera (34). Microbiota population begins in utero and experiences volatile compositional shifts until around three years of age, when the diversity and composition resemble those of an adult (35, 36). Through adulthood, it is a relatively stable, core community of bacterial strains, with changes normally only in abundances (37, 38). Factors influencing composition include age, sex, geography (35, 39, 40), stress (41), drug use (42, 43), and diet (44, 45).

A symbiotic relationship exists between an individual and their microbiota. Microbiota are able to break down undigested macronutrients, producing an array of metabolites which, in turn, exhibit multifarious

effects on the host (46). Metabolites communicate locally and systemically through metabolic, immune, and neuroendocrine crosstalk with the host (47). Short-chain fatty acids (SCFAs) such as butyrate, propionate, and acetate are predominant metabolites produced mainly through carbohydrate fermentation by specific species (36, 48).

It is well recognized that microbiota are fundamental to human health, with their metabolites playing a key role in modulating disease risk (8, 49). Many metabolites, particularly SCFAs, are beneficial, supporting gastrointestinal integrity and immune system regulation (50, 51). Dysbiosis, where the microbiota's configuration adopts an abnormal state (52), is implicated in the development of cardiovascular disease, metabolic disorders, inflammatory bowel disease (IBD), and some cancers (49, 53).

Impact of KD on gut microbiota

Diet is instrumental in shaping the microbiota's composition and activity (45, 54). Long-term dietary patterns are strongly associated with enterotype (55) and acute dietary interventions, as short as 24 hours, can instigate compositional changes, although it remains unknown what intervention length would translate to durable changes (56, 57). Diet type, for example, plant- versus animal-based, as well as specific dietary components, particularly macronutrients, have profound microbial impacts (8, 49, 56). Given KD's extreme alterations to dietary macronutrient ratios, it follows that this diet may instigate shifts in an individual's microbial composition.

Carbohydrates are commonly classified as either digestible or non-digestible carbohydrates (NDC). Reaching the colon in large quantities for fermentation, NDCs particularly influence microbiota, with variable and complex differences in impact depending on NDC type (8, 49, 58). Unsurprisingly, research thus indicates that a diet low in NDCs, such as KD, reduces bacterial abundances (9, 59).

Dietary fats are mainly absorbed in the small intestine, following emulsification by bile acids (58, 60). Small amounts reach the colon (61), with potentially potent antimicrobial properties (62, 63). Animal studies indicate a high-fat diet has a negative impact on microbiota composition (49, 63). However, a human study that administered a lipase inhibitor to increase fat reaching the colon demonstrated no significant changes to microbial composition (61). Furthermore, the type of fat consumed appears to have differing microbial impacts (49, 63).

Protein is mainly digested and absorbed in the small intestine, yet factors such as source, processing, and macronutrient ratios affect its digestibility and the quantity and type of amino acids reaching the colon for bacterial fermentation, thus resulting in compositional changes (64, 65). Generally, consumption correlates with improved microbial diversity (49).

There are numerous other possible mechanisms by which KD influences metabolic and endocrine functions that may, in turn, impact gut microbiota (12).

Methods

Research strategy

The methods outlined by the Centre for Reviews and Dissemination (CRD) (2009) (66) guidance for undertaking systematic reviews in healthcare were followed in this study, which included quasi-experimental designs and RCTs, due to the limited amount of relevant research.

Data collection and search terms

The Medical Literature Analysis and Retrieval System Online (MEDLINE), PubMed, and the Cumulative Index to Nursing and Allied Health Literature (CINAHL) were searched systematically (66, 67). The Cochrane Library was searched but did not offer any further relevant literature. Backward citation searches were performed on reference lists of selected studies (68). The terms used to locate literature relevant to the research objectives were "Low-carbohydrate" OR "low carbohydrate" OR "low carb" OR "carbohydrate restricted" OR "carbohydrate-restricted" OR ketogenic AND microbiome OR microbiota OR flora OR microbial.

Inclusion and exclusion criteria

Table 1 details the inclusion and exclusion criteria of this review.

Study selection

Figure 1 summarizes the study selection process using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram (69).

Table 1. Inclusion and exclusion criteria.

	Include	Exclude
Evidence type	Peer-reviewed empirical research, published in English	Systematic/literature reviews Meta-analyses Grey literature
Population	Humans, 3 years or older	Children below 3 years Animals or other organisms
Intervention	Ketosis demonstrated by blood βOHB ≥0.5mmol/l and/or KD, with composition fat ≥60%, carbs ≤50g/day or 10% energy and protein making up difference	KD not meeting the inclusion criteria Any other intervention
Comparator	Any non-KD	Diet that meets KD inclusion criteria
Outcomes	Microbiota composition	Microbiota composition unreported
Study design	RCTs Quasi-experimental designs	Non-experimental designs

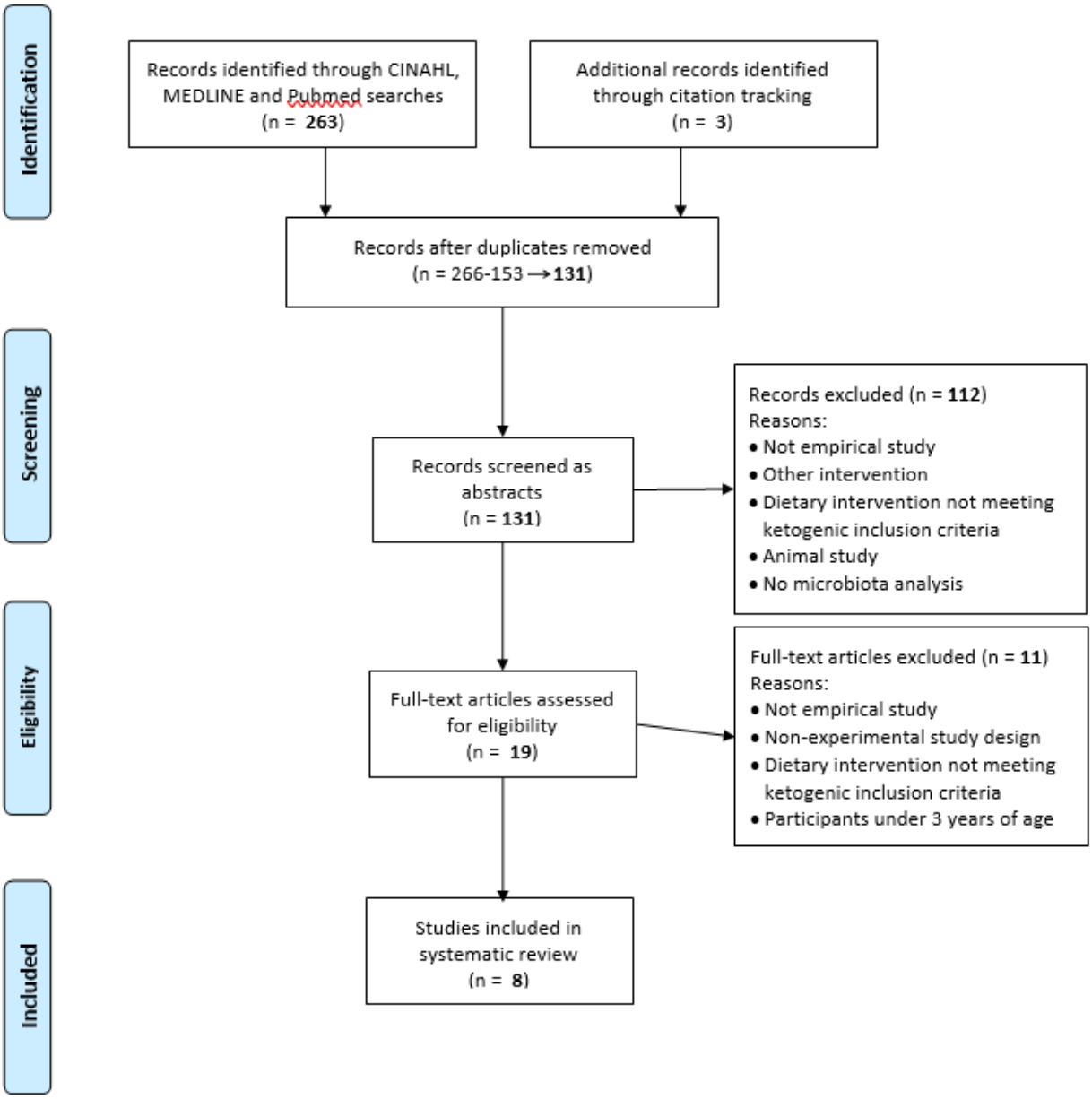


Figure 1. Study selection flow diagram [Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)].

Appraisal and thematic analysis

The quality appraisal checklist developed by the National Institute of Care and Excellence (NICE) (2018) (70) for the development of public health guidance was adapted and utilized. No studies were excluded based on their quality because of the limited number of relevant papers and their possible value for investigating the research objectives (71). An adapted thematic analysis was undertaken to extract and collectively interpret relevant data from the complex information presented across the included studies (71, 72). To minimize errors, guidelines advise multiple researchers carry out the screening, selection, and data extraction (66, 73); this was not permitted for this Master's dissertation, however, the author built in checking processes.

Results

Study Characteristics

A summary of the characteristics of the eight included studies is presented in Table 2.

Thematic analysis

The thematic analysis revealed seven themes, as detailed in Figure 2.

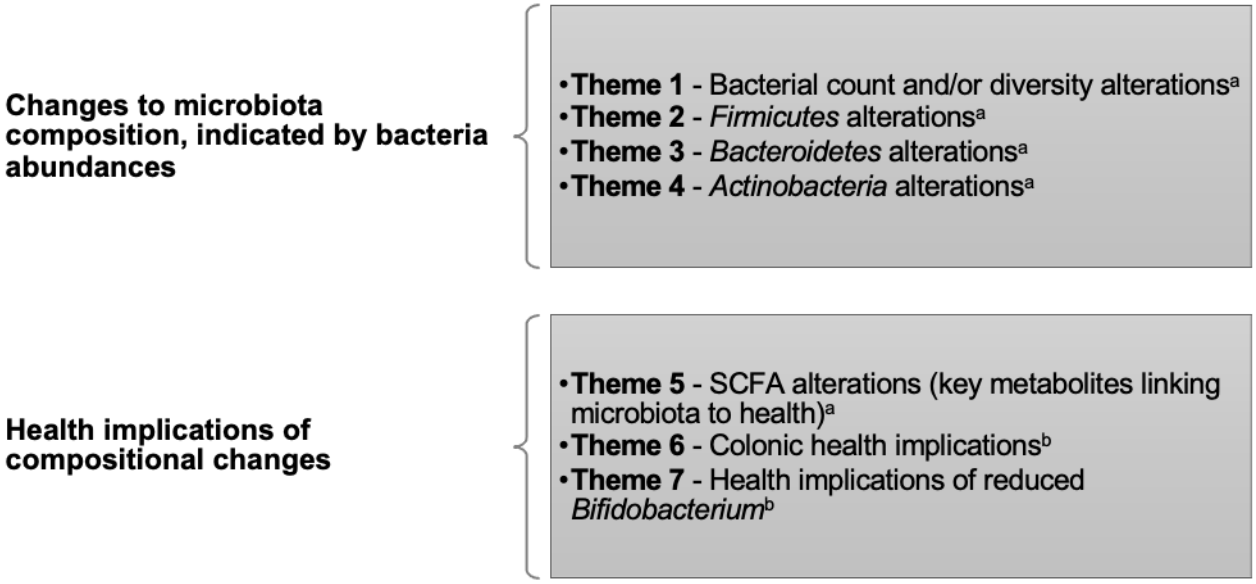
Summary of results

Following their KD intervention, all included studies reported significant alterations to microbiota composition, and four studies, i.e. those investigating SCFAs, found disruptions to faecal SCFAs. This review focused on the phyla, genera, and species reported by multiple papers in order to synthesise and draw conclusions supported by a weight of empirical evidence (81). Table 3 displays a summary of the

Table 2. Summary of human studies investigating KD effects on faecal microbiota.

Reference	Study Design	Population/Characteristics	N	KD	Intervention Length	Microbiota Analysis Method
74	RCT – Parallel	Overweight/ obese Age: 24–64 Sex: M & Fe	KD: 48 Control group: 43	C 4%, F 61%, P 35% Diet designed for weight loss, ≈ 30% energy restricted.	8-week	Selective culture media
75	RCT - Crossover	Obese Age: 23–57 Sex: M	18	C 4%, F 66%, P 30% Diet designed for weight loss, ad libitum intake.	4-week	FISH
76	RCT - Crossover	Obese Age: unreported Sex: M	15	C 4%, F 66%, P 30% Designed for weight loss, ad libitum intake.	4-week	FISH
77	Experimental ITS	Obese & NAFLD Age: 50-58 Sex: M & Fe	10	C 4%, F 72%, P 24% Diet designed to maintain weight.	2-week	Whole genome sequencing
78	Non-randomised controlled trial	Elite race walkers Age: 20-35 Sex: M	KD: 10 Control groups: 9 and 10	C 5%, F 78%, P 17% Diet designed to maintain weight.	3-week	16S-rRNA gene amplicon sequencing
79	RCT - Crossover	Overweight/ obese Age: 21-74 Sex: M	17	C 5%, F 66%, P 29% Diet designed for weight loss, fixed intake.	4-week	FISH
80	Experimental ITS	Multiple sclerosis Age/Sex: Unreported	10	C <50g, F >160g, P <100g to achieve βOHB ≥500µmol/L (0.5mmol/L)	6-month	FISH
28	Experimental before and after study	GLUT1-DS Age: 8-34 Sex: M & Fe	6	1:1 ratio KD increased to 2:1, 3:1 or 4:1 as required to produce βOHB ≥2.0 mmol/l.	3-month	qPCR analysis

16S-rRNA: 16S ribosomal ribonucleic acids; βOHB: beta-hydroxybutyrate; µmol/L; Micromoles per litre; C: Carbohydrate (as % energy); F: Fat (as % energy); Fe: Female; FISH: Fluorescence in situ hybridization; GLUT1-DS: Glucose transporter 1 deficiency syndrome; ITS: Interrupted time series; KD: Ketogenic diet; M: male; mmol/L: millimoles per litre; N: number of participants; NAFLD: Non-alcoholic fatty liver disease; P: Protein (as % energy); RCT: Randomised control trial; qPCR: real-time quantitative polymerase chain reaction



Notes: a. relevant results identified within included studies. b. relevant discussions/risks identified within included studies.

Figure 2. Themes identified through thematic analysis.

included studies' results. Table 4 displays a summary of these results according to themes 1-5.

Discussion

Mechanisms behind KD's impact on gut microbiota

This review indicates that KD has a general "anti-microbial" effect, reducing bacterial count, although potentially only in the short-term. Given the microbiota's role in the breakdown of NDCs, research has predominantly allocated cause to the reduction in carbohydrate intake (82-84). Despite using inconsistent unitary measures of fibre, four studies (72, 74, 76, 79) examined NDC intake, and all confirmed intake on a KD is significantly reduced. NDC intake directly correlates with the amount of carbohydrate reaching the colon for bacterial fermentation (85).

This review's most striking and conclusive finding relates to KD's persistent negative impact on *Bifidobacterium*, which strictly metabolises carbohydrate substrates as its energy source (46, 86). Their central fermentation pathway, the fructose-6-phosphoketolase or "bifid shunt," provides them with an ecological advantage in the presence of carbohydrates, because it produces more energy compared with the fermentation pathways of other bacterial species (87, 88). These insights may explain its decreased abundance in response to the reduced NDC intake of a KD.

Furthermore, in addition to the liver, intestinal epithelial cells are able to produce ketones (89), and a KD results in increased βOHB within intestinal tissues (90). Recent in vitro and in vivo experiments have demonstrated that βOHB directly inhibits *Bifidobacterium* growth (91).

This review also provides reasonable evidence of a reduction in the butyrate-producing *Firmicutes, Eubacterium rectale, Roseburia,* and *Faecalibacterium prausnitzii,* although again, the results indicate this may not be a long-term concern due to potential microbial adaptation. *Eubacterium rectale, Roseburia,* and *Faecalibacterium prausnitzii* ferment NDCs directly. They also utilize Bifidobacterium's fermentation metabolites in a process called substrate cross-feeding to produce butyrate (86, 92). Thus, a reduction in these bacteria could be explained by the concomitant decrease in both NDC intake and *Bifidobacterium.* Research has demonstrated high dietary fibre intake increases these bacteria (93). As more acidic conditions favor their growth, this is likely due to fermentation producing acidic end-products, such as SCFAs and lactic acid (94, 95). It follows that the fibre-restricting KD may have the opposite effect. Thus, there is a physiological basis for the KD causing a reduction in these bacteria.

Colonic health implications

Colonic health was the main concern raised by the included studies relating to the KD's microbial impacts, primarily ascribed to the negative impact on SCFA production (96). While this review may not provide conclusive evidence for this risk, the findings warrant further consideration.

SCFAs act through complex mechanisms to exert

Table 3. Summary of included study results relating to bacterial abundances and SCFAs.

Study Reference	Themes 1-4: Changes to bacterial abundances	Theme 5: SCFA alterations (faecal concentrations)
74	↓: *Bifidobacterium* (p<0·001) in KD group but not in control group. No significant change: *Lactobacilli* for either KD or control groups.	↓: Total SCFAs, acetate, butyrate (p≤0.04), in KD group but not for control group.
75	↓: total bacterial count (p<0.001), *Roseburia* and *Eubacterium rectale* (p<0.001), and *Bifidobacterium* (p<0.026) for both medium carbohydrate control and KD groups, with a progressive gradient as dietary carbohydrate reduced. No significant change: *Bacteroides*, *Faecalibacterium prausnitzii*, *Firmicutes*, *Lactobacillus-Enterococcus* group and *Desulfovibrio* genus for either medium carbohydrate control or KD groups.	↓: Total SCFAs, acetate, butyrate, isovalerate, propionate, valerate (p<0.05) for both medium carbohydrate control and KD groups. Butyrate reductions showed significant progressive gradient as dietary carbohydrate reduced.
76	Note: Results combined with 70. Duncan *et al.* (2007). ↓: Total bacterial count (p<0.001), *Roseburia* and *Eubacterium rectale* (p<0.001), and *Bifidobacterium* (p<0.037). No significant change: *Bacteroides*, *Faecalibacterium prausnitzii*, *Firmicutes.*	
77	Note: Significant changes to 94 bacterial strains across 25 genera. 10 most abundant genera reported here. ↓: *Ruminococcus* (p=2.62e-09), *Eubacterium* (p=2.84e-08), *Clostridium* (p=3.73e-12), *Coprococcus* (p=0.0056), *Bifidobacterium* (p=6.77e-14), *Subdoligranulum* (p=0.00039), *Butyrivibrio* (p=1.65e-05). ↑: *Streptococcus* (p=0.0014), *Lactococcus* (p=2.32e-05), *Eggerthella* (p=0.0073).	↓: Total SCFAs (p=0.047).
78	↓: *Faecalibacterium* (p=0.007) *Bifidobacterium*, *Veillonella*, *Streptococcus*, *Succinivibrio*, *Odoribacter* and *Lachnospira* (no p-values provided as identified through LefSe and/or sPLS-DA). ↑: *Dorea* (p=0.007), *Bacteroides* (p=0.002), *Enterobacteriaceae*, *Peptostreptococcaceae*, *Barnesiellaceae* and *Akkermansia* (no p-values provided as identified through LefSe and/or sPLS-DA). No significant change: Alpha-diversity.	
79	↓: Total bacteria count (p=0.013) and *Bacteroides* (P=0.007) for both medium carbohydrate control and KD groups, *Roseburia* and *Eubacterium rectale* (P<0.001) for only KD group. No significant change: *Lachnospiraceae* or *F. prausnitzii.*	↓: Total SCFAs, acetate, butyrate (p<0.001) in KD group only. ↓: Isobutyrate (p=0.002), isovalerate (p<0.001) and propionate (p<0.001) for both medium carbohydrate control and KD groups.
80	At 2 weeks ↓: Diversity (p=0.03-0.05), total bacteria count (p<0.001), *Bacteroides* (p=0.001-0.002)1, *Faecalibacterium prausnitzii* (p=0.001), *Bifidobacterium* (p=0.02-0.03), *Atopobium* cluster (p=0.02), *Ruminococcus albus* (p=0.02) and *Sphaerotilus natans* (p=0.02). At 6 months ↓: *Bifidobacterium* (p<0.001), *Coriobacterium* (p=0.02). ↑: Total bacteria count (p=0.02), *Roseburia* and *Eubacterium rectale* (p=0.03-0.02), *Clostridium viride* (p<0.01), *Eubacterium hallii* (p<0.01), *Ruminococcus productus* (p=0.03). No significant change: Diversity or the other 29 investigated bacterial species.	
28	↑: *Desulfovibrio* (p=0.025). No significant change: *Firmicutes*, *Bacteroidetes*, *Lactobacillus*, *Bifidobacterium*, *Faecalibacterium prausnitzii*, *Clostridium perfringens*, *Enterobacteriaceae and Desulfovibrio*.	

↑: increased abundance; ↓: decreased abundance; LefSe: Linear discriminant analysis effect size; sPLS-DA: Sparse partial least squares discriminant analysis; SCFA: Short chain fatty acid.

beneficial effects in the colon (97). Butyrate, the primary energy source for colonocytes, supports intestinal barrier structure and function and protects from external harm by facilitating epithelial tight-junction assembly, stimulating

Table 4. Summary of results for themes 1-5.

Theme	Summary of results
Theme 1: Bacterial count and/or diversity	Total bacterial count: • Highly significant evidence from four studies of a reduction in total bacterial numbers with KD adherence between 2-12 weeks. [75 (p<0.001), 76 (p=0.013), 79 (p<0.001), 80 (p<0.001)]. • One study indicates longer-term adherence may reverse effect, reporting increased bacterial numbers after 6 months [80 (p=0.02)]. Bacterial diversity: • Inconsistent diversity data from two studies provides insufficient evidence of KD effects [80 reported a reduction in diversity (p=0.03-0.05) at 2-12 weeks but this was reversed to baseline levels by 6 months. 78 reported no change in alpha-diversity, or richness].
Theme 2: *Firmicutes*	*Firmicutes* phylum: • Three studies found no significant changes to *Firmicutes* abundance following a KD. [75;76;28] *Lactobacillus* genus*:* • Four studies found no significant changes to *Lactobacillus* abundances following a KD [75;74;80;28]. *Eubacterium* genus, *Eubacterium rectale* and *Roseburia:* • One study found highly significant reductions of *Eubacterium* genus [77 (p=2.84e-08)] and of *Eubacterium rectale* species [77 (p=4.91e-25)], with KD adherence. • Additional significant evidence from three studies for the reduction of *Eubacterium rectale* and *Roseburia (*collectively) following KD [75;76;79 (p<0.001)] • Conflicting evidence from one study, reporting no change to *Eubacterium rectale* and *Roseburia* abundance at 2 and 12 weeks of KD adherence and a slight increase by 6 months [80 (p=0.03-0.02 compared with baseline, 2 and 12 weeks]. *Faecalibacterium prausnitzii*: • Significant findings for the reduction of *Faecalibacterium prausnitzii* abundance following KD adherence of 2-3 weeks from three studies [77 (p=0.000), 78 (p=0.007), 80 (p=0.001)]. • One study indicates longer-term adherence may reverse effect, reporting reversal to baseline levels after 6 months KD adherence [80]. • Conflicting evidence from three studies reporting no significant change in *Faecalibacterium prausnitzii* abundance following their KD interventions [75;79;28].
Theme 3: *Bacteroidetes*	*Bacteroidetes* phylum: • Only one study reported no significant change to *Bacteroidetes* phylum as a result of their KD intervention [28]. *Bacteroides* genus*:* • One study reported a significant increase in *Bacteroides* [78 (p=0.002)]. • Two studies reported no significant change to *Bacteroides* [75;76]. • Conversely, two studies observed a decrease in *Bacteroides* with KD adherence of between 2-12 weeks [79 (p=0.007), 80 (p=0.002-0.001)] • One study indicates longer-term adherence may reverse any decrease, reporting reversal to baseline levels after 6 months KD adherence [80]
Theme 4: *Actinobacteria*	*Bifidobacterium* genus*:* • Five studies provide highly significant data demonstrating reduced *Bifidobacterium* resulting from short-term and longer-term KD adherence [74 (p<0.001), 75 (p<0.001), 76 p<0.001), 77 (p=6.77e-14), 80 (p=0.02-0.03 at 2 and 12 weeks, p<0.001 at 6 months)]. • One study detected a reduction using Linear discriminant analysis effect size (LefSe) and Sparse partial least squares discriminant analysis (sPLS-DA), but not in their initial multivariate statistical analysis methods, Redundancy Analysis or Anosim [78]. • One study observed no change in *Bifidobacterium* abundance [28].
Theme 5: SCFAs	Total faecal SCFAs: • Supporting evidence from four studies for a reduction in total faecal SCFAs in response to a KD [74 (p≤0.04), 75 (p<0.05), 76 (p=0.047), 77 (p<0.001)]. Acetate and butyrate (faecal): • Three studies demonstrated significant reductions in acetate and butyrate [74 (p≤0.04), 75 (p<0.05) and 79]. Propionate (faecal): • Two studies demonstrated significant reductions in propionate [75 (p<0.05), 79 (p=0.047)]. • One study found no significant reduction to propionate [74].

mucin production, and inhibiting pathogenic bacterial adhesion (99). SCFAs act as the microbiota's link with the host's immune system through several cellular signaling pathways that ultimately modify processes such as gene expression, differentiation, proliferation, and apoptosis (100). They activate G-coupled protein receptors (GPRs), which are expressed in the colonic mucosa and link to downstream signaling pathways involved in gut immune homeostasis (97, 100). They constrain the enzymatic actions of histone deacetylases (HDACs) in colonocytes and mucosal immune cells, which inhibits DNA transcription and the regulation of inflammatory-associated gene expression (97, 100). Through these actions, SCFAs play a preventative role in the development of IBD, Crohn's disease, and ulcerative colitis (97, 101). Reduced faecal butyrate levels have been detected in IBD patients and ulcerative colitis patients in remission compared with controls (102, 103). SCFAs' aforementioned actions protect against colorectal cancer, and they induce apoptosis and inhibit proliferation of colonic cancer cells (97, 101).

Microbiota are considered key in the pathogenesis of IBD and colorectal cancer (104-106) with dysbiotic compositions identified in patients, characterized by reduced butyrate-producing *Firmicutes*, especially *Faecalibacterium prausnitzii* in IBD patients (107) and *Roseburia* in colorectal cancer patients (108).

High *Bifidobacterium* abundance is important for colonic health (109), intestinal barrier function (110), and facilitating healthy microbial composition through cross-feeding metabolites to butyrate-producers and competitive exclusion of pathogenic bacteria (109, 111). Their metabolites have direct benefits, with certain strains prolifically producing conjugated linoleic acid (112), which is anti-inflammatory and inhibits colon cancer cell growth and proliferation (96). Murine models have demonstrated that *Bifidobacterium* provides protection from certain carcinogens (103) and has anti-tumor effects (114). Furthermore, reduced *Bifidobacterium* abundance is found in colorectal cancer patients (115).

Cumulatively, this research suggests that the pattern of microbial alterations following KD adherence, namely decreased *Bifidobacterium* and *Firmicutes* butyrate-producing bacteria, may be detrimental to colonic health and potentially increase the risk of colonic diseases. However, to the best of the authors knowledge, no studies have investigated these risks specifically for KD. There is extensive research into the relationship between diet, microbiota, SCFA alterations, and colonic diseases, with fibre a key focus, given it promotes *Bifidobacterium* and *Firmicutes* abundance as well as SCFA production. However, there remains controversy on its criticality for colonic health; epidemiological studies provide abundant evidence of an inverse association between fibre intake and colorectal cancer risk, yet other cohort studies have found no association (106, 116), and RCTs investigating the effect of supplementation for the prevention of colorectal cancer have yielded inconsistent results (117). A recent study found no association between faecal SCFA concentrations and colonic carcinomas (118). For IBD, the consensus is that fibre reduces the risk of disease development (119), although the beneficial effect of a high-fibre diet or supplementation for IBD patients has not been demonstrated (120). Inconsistency across such research may not be surprising, given fibre is not a homogeneous substance, rather a group of compounds with differing properties and effects on the microbiota and SCFA production, with type, dose, and consumption timing all likely influencing outcomes (120, 121).

For KD, there is a further consideration relating to the fact that ketone, βOHB, has a similar chemical structure to butyrate, as depicted in Figure 3.

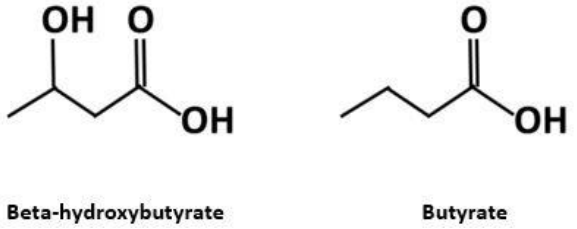


Figure 3. Chemical structures of beta-hydroxybutyrate and butyrate.

Consequently, they have functional similarities, for example, βOHB activates GPRs, such as GPR109a (122, 123), and inhibits HDACs (124, 125). It has been hypothesized that the increased systemic levels of βOHB induced during KD may lessen the importance of microbial butyrate production (126), and thus, the potential negative impact on colonic health from reduced butyrate may be lessened.

Health implications of reduced *Bifidobacterium*

Bifidobacterium's metabolic activities are considered fundamental to human health (96), and several extraintestinal diseases have been associated with reduced *Bifidobacterium*, namely obesity, T2D (128-131), and depression (132). While currently no research provides definitive evidence of a causal relationship, wider research does provide corroboration and probable physiological mechanisms.

For obesity and T2D, SCFA production and metabolic endotoxemia are two mechanisms postulated to link altered microbiota compositions with obesity and metabolic syndrome (133). *Bifidobacterium* mediates the production of SCFAs, which influence signaling pathways through GPR activation. These actions extend beyond the gut to stimulate production of several appetite regulating hormones, including adipocyte-derived leptin (134). Leptin acts on the hypothalamus to suppress appetite and interacts with insulin signaling (135). SCFAs stimulate two intestinal-derived hormones, glucagon-like peptide and peptide YY (136, 137), which play important roles in glucose homeostasis and promoting satiety (138, 139). Metabolic endotoxemia is defined as an increase in plasma bacterial lipopolysaccharide (LPS) (140). In mice, the link between high-fat diet-induced microbial alterations, including a reduction in *Bifidobacterium*, and a downstream inflammatory response, specifically cytokines linked to insulin resistance, has been demonstrated (140, 141). In humans, high fat consumption has been demonstrated to induce metabolic endotoxemia (142), which in turn induces adipose inflammation and insulin resistance (143) and is associated with increased energy intake (144). LPS plasma levels rise as a result of increased intestinal permeability, allowing the flow of gram-negative bacteria, such as *Bacteroidetes* whose membranes contain LPS, from the intestines into the blood (145, 146). Many factors may contribute to a disrupted intestinal barrier function, but reduced *Bifidobacterium* may be key, given its role in preserving mucosal health. Thus, theoretically, a reduction in *Bifidobacterium* could promote obesity and T2D by instigating hormonal and immune disturbances. This is supported by murine trials, which demonstrate that *Bifidobacterium* probiotics lower plasma LPS (146) and decrease body weight in mice fed a high-fat diet and have a wide range of other anti-obesity effects, including improved glucose homeostasis and decreased serum leptin (147, 148). Recent murine research, however, has highlighted that KD is distinctive in its impact on gut microbiota because of the production of ketone bodies which directly inhibit *Bifidobacterium* growth and, in turn, decreases the pro-inflammatory Th17 cells within the small intestine and possibly also adipose tissues (91). While it is postulated that this may be a possible mechanism in the reduction of body fat associated with KD (77, 91), whether this impact of *Bifidobacterium* on Th17 cells is beneficial or detrimental to gut health and inflammatory-related diseases is dependent on context and requires further investigation (149).

Microbiota are considered key in determining the onset and duration of depression through a complex array of mechanisms (150). Changes in microbiota composition alter the balance of chemicals produced, which in turn impacts the bi-directional communication between endocrine, immune, and central nervous systems, or the "gut-brain-axis" (151). The vagal nerve is the primary communication pathway between the microbiota and the brain, and its afferent receptors are stimulated by SCFAs and LPS (152). As discussed above, these bacterial metabolites are sensitive to *Bifidobacterium* abundance. Depression has been linked to an immune response instigated by increased intestinal permeability and the resulting raised serum LPS (145, 150). Disrupted brain neurotransmitters have been implicated in depression, including both gamma-aminobutyric acid (GABA) and serotonergic systems (151, 153). *Bifidobacterium* is a prolific GABA producer (154). While the ability of such microbiota-produced neurotransmitters to cross the blood-brain barrier is unclear (155), they do influence the central nervous system via the vagal nerve and its afferent receptors (156). Indeed, the administration of *Bifidobacterium longum* in mice displaying anxiety behaviors had anti-anxiolytic effects, which were demonstrated to be dependent on vagal nerve integrity (157). Microbiota modulate the metabolism of serotonin's precursor tryptophan to influence brain levels (150), as demonstrated in a study investigating the anti-depressive effects of *Bifidobacterium infantis* on rats, where administration resulted in reduced

depressive behaviors alongside increased plasma tryptophan (158). Additionally, a human RCT with irritable bowel syndrome patients demonstrated that *Bifidobacterium longum* altered brain activity and reduced depression scores (159). Thus, although the potential preventative effects of a KD on depression via its nutrition and microbiome impacts continue to attract attention (12), it is conceivable that a persistent disruption to *Bifidobacterium* abundance, as a result of longer-term KD adherence, could increase the risk of developing or worsening depression. Summary of findings is in Figure 4.

Research strengths and limitations

The key strengths of the current review are its systematic approach and its exclusive examination of human studies, excluding animal results that may not reflect human microbial responses. The inclusion of differing study designs ensures consideration of all valuable evidence. However, this heterogeneity may hamper comparability and the ability to determine conclusive outcomes, as may the small number of included studies with mostly low participant numbers. The included studies lack consistency across factors that may influence microbiota, such as their KD composition, data collection methods, microbial analysis methods, inclusion and exclusion criteria, along with participant characteristics such as age, health, medication, and baseline diet. Furthermore, no included study demonstrated sufficient power, despite microbiome-specific power analysis approaches being available (160, 161). Microbiota-host interactions are complex and the health effects are not fully understood, limiting the health impact interpretation of microbial changes. KD's effect on mucosal microbiota levels cannot conclusively be determined, as the included studies utilized faecal microbiota as a proxy.

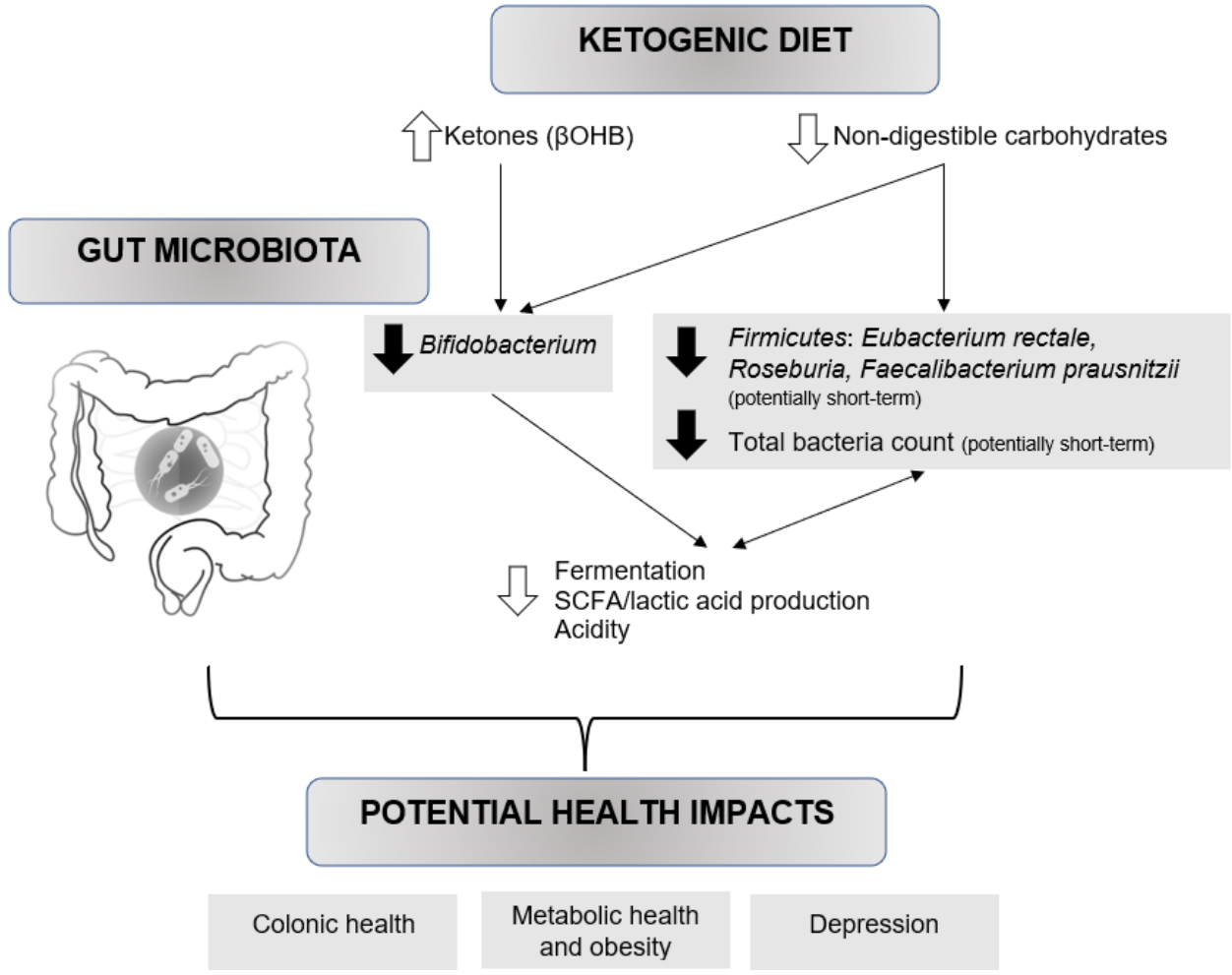


Figure 4. Summary of findings

The ketogenic diet in practice: Key messages for nutrition and healthcare professionals

- Consider faecal testing to assess microbiota composition and ascertain an individual's risks and priorities for microbial support during and/or post-KD adherence. An understanding of the commercial tests available, their interpretation and limitations is essential (163).
- Consider preserving or enhancing microbial communities, for example by:
  - Promoting NDC-containing foods (within constraints of KD, as required) or by supplementation (164).
  - Promoting dietary or lifestyle factors to support a healthy microbiota, such as exercise (165)
  - Discouraging dietary or lifestyle factors with potential negative microbial impacts, such as low-calorie sweeteners (166).
  - Probiotic supplementation may beneficially impact the microbiota's composition and metabolism, albeit transitorily (167;168).
- Tailor approach to individual health, disease status and purpose for KD adherence:
  - For individuals undertaking KD for weight loss, to manage T2D or with a history of depression, *Bifidobacterium* preservation should be a key priority.

Conclusion

This review reveals that in humans, certain bacterial abundances and their metabolites are disrupted by KD adherence, most conclusively a decrease in *Bifidobacterium* and faecal SCFAs as well as short-term reductions in *Firmicutes* butyrate-producing bacteria and that these effects are possibly detrimental to colonic health. The persistent reduction in *Bifidobacterium* abundance may have additional detrimental impacts, with obesity, T2D, and depression as key risks.

Recommendations for future research

Microbiome research is an evolving field, and development is required in key areas, such as cross-study comparability issues, particularly technical variation in sampling and microbial analysis of the observed microbial communities, their structure, and the biological conclusions drawn (162), as well as heterogeneity between participant baseline microbiota compositions (58).

To facilitate cross-study comparison and enable cause-effect conclusions in research into KD's impact, larger-scale, longer-term RCTs in healthy and specific population groups are required, with standardization of KD's composition and controlling of multifarious confounders.

Further research is required to confirm the complex interactions and causal relationships between microbes, their metabolites, and humans. For KD, research is warranted into the biological effects of βOHB and whether these might negate health impacts relating to reduced microbial butyrate production.

Conflict of interests

The authors declare that there is no conflict of interests with respect to the research, authorship or publication of this article.

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