A Review of Probiotics Supplementation in Healthy Adults: Helpful or Hype?

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Abstract

Probiotic supplements have a positive impact on several health outcomes. However, the majority of published studies have focused on populations with specific health pathologies. Therefore, this study reviewed the current literature on the health effects of probiotics consumption in 'healthy adults'. The findings from this review may help guide consumers, researchers and manufacturers regarding probiotics supplementation. Relevant literature published between 1990 and August 2017 was reviewed. Studies were included if they were experimental trials, included healthy adults, used live bacteria and had accessible full-text articles published in English. Included studies were classified according to common foci that emerged. Forty-five studies were included in this review. Five foci emerged: gut microbiota changes (*n*=15); immune system response (*n*=16); lipid profile and cardiovascular disease risk (*n*=14); gastrointestinal discomfort (*n*=11); health of female reproductive health (*n*=4). Results suggest that probiotics supplementation in healthy adults can lead to transient improvement in gut microbiota concentration of supplement-specific bacteria. Evidence also supports the role of probiotics in improving immune system responses, stool consistency, bowel movement, and vaginal lactobacilli concentration. There is insufficient evidence to support the role of probiotics to improve blood lipid profile. Probiotics consumption can improve in the immune, gastrointestinal and female reproductive health systems in healthy adults. However, this review failed to support the ability of probiotics to cause persistent changes in gut microbiota, or improve lipid profile in healthy adults. The feasibility of probiotics consumption to provide benefits in healthy adults requires further investigation.

Keywords: probiotics; health; supplement; review; gut microbiota; immune system response; lipid profile; cardiovascular disease; gastrointestinal discomfort; female reproductive health.

Introduction

The use of fermented products can be traced back to ancient Egyptian and Middle-Eastern civilizations, when fermentation was a method of food preservation (1). However, it was not until the early 1900's that associations between human longevity and yoghurt consumption (containing lactobacilli strains of bacteria used for fermentation) were observed (2, 3). It was also around this time when belief originated that fermented products could alter the microflora of the large intestine and reduce toxin production in the intestine (2, 3). In the early 1900s *Bifidobacterium* was first isolated and was hypothesized to have anti-pathogenic effects (1). The next major advancement was in the 1960's, when the term 'probiotic' was first coined for bacterial species deemed to be beneficial to the gastrointestinal (GI) tract. However, it was not until 2001 that the World Health Organization formally defined probiotics as "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host" (4).

The ability of probiotics to impart health benefits has prompted increased scientific interest for several decades. Evidence from animal and human studies has demonstrated potentially favourable benefits of probiotics, including modulating the number and diversity of beneficial gut microbiota (5, 6); reducing symptoms associated with various GI disorders (7, 8); improving blood cholesterol levels and blood lipid profile (9, 10); removal of mycotoxins (11); reducing blood pressure and hypertension (12); improving blood glucose tolerance and diabetes control (13, 14); and enhancing mental state and cognitive function (15).

The translation of these health benefits in the public forum has led to increased demand for probiotic products/supplements over the last decade. This has prompted a rapid increase in the development of new probiotic containing foods and supplements for the consumer market (16). While research has demonstrated positive effects of probiotic consumption on several health outcomes, the majority of the published literature is in populations with underlying pathologies. Evidence supporting the health-promoting effects of probiotics in healthy adults is limited and less consistent (17, 18). Despite this, probiotic manufacturers promote the use of their product to a broader consumer market than those with specific health conditions. Whether probiotics supplementation conveys benefit in healthy individuals is questionable. Therefore, this study aimed to review the literature on the health effects of probiotics consumption in 'healthy adults'. The results of this review may guide the decision-making of consumers, researchers and manufacturers regarding probiotics supplementation.

Methods

Literature search

Due to the number of outcomes included and the wide variety of studies varying in study design, experimental setting and dependent measures (e.g. immune response to different viruses or a different population of older and younger adults), a systematic review was not performed. However, a systematic approach was employed to search and review the relevant literature. The online databases PubMed (MEDLINE), *Cumulative Index to Nursing and Allied Health Literature* (CINAHL), and Cochrane Library (Central) were searched from 1990 through to August 2017. The reference lists of included studies were also manually searched for relevant studies. Following a PICOS (Population, Intervention, Comparison, Outcome, Setting) approach, the online literature search used a combination of the following basic and MeSH (Medical Subject Headings) terms: 'healthy volunteers' or 'healthy adults' as the population, 'probiotics', 'lactobacillus' or 'bifidobacterium' as the intervention, 'placebo' or 'control' as the comparison, 'controlled trials' or 'non-controlled trials' as the study design. During the preparation and presentation of this review, the PRISMA guidelines were followed (19).

Study eligibility and selection

Studies were included if they: (1) were experimental trials, (2) included adults, aged 18 years and older, (3) used live bacteria (probiotics), (4) included healthy adults, and (4) had accessible full-text publications in English. Healthy adults were defined as individuals with no reported status of the chronic or acute diseases, including cardiovascular disease, obesity (Body Mass Index (BMI) $\geq$ 30 kg/m^2), liver disease, diabetes, chronic GI problems, autoimmune disease, cancer, psychological disorder, etc. Adults who reported having symptoms consistent with the common cold, who were overweight (BMI 25 – 29.9 kg/m^2) or smokers, were not excluded. Studies were excluded if probiotics treatment was mixed with other ingredients, or if pregnant women or both healthy and unhealthy adults were included as participants in one group.

The searched literature was initially screened by reviewing titles and abstracts. The full text of all relevant records was then reviewed. Two researchers were involved in the screening process and review of the literature. Eligible articles were only included when agreement was reached between the two researchers. In the case of any disagreement, a third reviewer was involved.

Data collection and synthesis

Eligible literature was classified based on the most prominent themes that emerged from the literature search. Studies with similar outcomes were grouped if more than three trials were classified in the relevant group [Gut microbiota changes, Immune system response, Lipid profile and cardiovascular disease risk, GI discomfort and Female reproductive health]. In each group, methodology characteristics and outcomes were extracted. A narrative synthesis was used to review the effect of probiotics consumption on outcomes in each classification. The *Cochrane Handbook for Systematic Review of Interventions* (20) was followed as a guideline to review literature.

Results

Forty-five studies were included in the overall review. A summary of the review process and study selection criteria is presented in Figure 1.

Gut microbiota colonization

The proposed health benefits of probiotics are often initially measured at the gut microbiota level (6). Changes in the concentration and composition of intestinal microorganisms would suggest that probiotics are effective, at least in terms of colonization (6). Fifteen studies from the literature search were included in this classification group. Findings from these studies are summarised in Table 1. Of the fifteen studies include, fourteen suggested that probiotics supplementation is likely to increase the fecal count of specific bacterial strains administered in healthy adults. However, changes in the total count, diversity and composition of gut microbiota were only reported in three studies (6, 21, 22). It also appears that changes in the gut microbiota of healthy adults following probiotic supplementation are temporary and return to pre-treatment levels within one to three weeks once supplementation has ceased (23, 24).

No obvious conclusions regarding the effects of dose, duration or strain of probiotics on changes in gut microbiota can be made based on the studies included in this review (Table 1). In order to transition through the GI tract and colonise the gut, probiotics need to be viable (i.e., contain adequate live bacteria) and resistant (i.e., survive) to stomach acidity and bile salts (25, 26). Some probiotic strains of the *Bifidobacterium* and *Lactobacillus* species such as *B. animalis lactic* (27), *L. acidophilus johnsonii* (27, 28) and *L. casei Shirota* (29) are

reported as being resistant to low pH environments, thus appear to have good survival rates. In addition, *L. acidophilus, B. longum* and *B. infantis* have demonstrated good resistance to bile salts (30, 31). Encapsulation and microencapsulation manufacturing techniques have provided more efficient ways of delivering probiotics and preserving their viability (32). Although these methods may improve the survival of bacteria, they do not guarantee colonization of the probiotic bacteria in the intestine. The type of bacteria and the GI environment (33) also influence bacterial colonization. However, colonization may not necessarily be required for changes in the gut microbiota (31). The passage of probiotic bacteria (e.g. bifidobacteria) itself through the gut may be sufficient to reduce colonies of pathogenic bacteria by reducing their adhesion and competitive nature (34).

An ideal environment modulating the colonization of probiotics is one with adequate food to support the growth of healthy bacteria and reduce competition (25). The host's diet is an important determinant of gut microbiota biodiversity. Fermentable carbohydrate supports the growth and colonization of selective bacteria in the gut. The term 'prebiotics' (35) is used in reference to these fermentable fibers that resist gastric acidity and are able to stimulate the growth and activity of beneficial bacteria in the gut (36). Although commercially available (i.e., inulin, fructo-oligosaccharides, galacto-oligosaccharides), these fibers are abundant in natural foods such as fruit and vegetables (36). Evidence suggests that prebiotics consumption can improve the faecal count of beneficial bacteria (especially bifidobacteria) (37, 38) and maintain gut health.

When gut microbiota dysbiosis exists (e.g., diarrhea), prebiotics alone may not be able to return gut microbiota to its equilibrium. Despite skepticism about the influence of probiotics on the gut microbiota of healthy adults, probiotics have proven beneficial effects when dysbiosis exists (7, 39). Aging is also associated with a relative dysbiosis in gut microbiota. Reductions in bifidobacteria count, diversity and an increase in pathogenic bacteria are observed in the elderly (40, 41). Lahti et al. (23) reported that probiotic supplementation in healthy elderly adults could improve this age-related dysbiosis (Table 1). Overall, it seems that probiotic supplementation in healthy adults can lead to an increase in the colonization of specific probiotic strains. However, this increase may be transient and return to baseline after supplementation stops. Further studies need to focus on the sustainability of probiotics colonization in gut microbiota.

Immune system response

Probiotics have been proposed to improve intestinal defense mechanisms against pathogenic microorganisms, enhance the immune system and reduce the likelihood of respiratory infections (42, 43). Sixteen studies from the literature search were included in this classification group, with findings summarized in Table 2. Eight studies reported the effect of probiotic consumption on common respiratory infections such as cold and flu. All of the three studies reporting on the effect on the common cold support a beneficial effect of probiotics supplementation at increasing immunity against the common cold in healthy adults (albeit in a limited number of studies) (Table 2). Reductions in the incidence (44, 45), duration (44, 46) and symptoms (44, 46) associated with common cold are commonly observed in healthy adults when a probiotics intervention is administered.

In contrast, the effect of probiotics supplementation on immune responses against influenza infection in healthy adults is less consistent (43, 47, 48). In vitro exposure of T cells collected from 10 individuals (supplied with probiotics for 30 days) to the influenza A virus showed a significant increase in TNF-α (43). This suggests enhancement of T cell responses to a respiratory tract infection. These findings are supported in a larger study (n = 465 participants supplemented with probiotics for 150 days), which indicated a significantly reduced risk of respiratory illness (49). However, these findings are in contrast with the results of several other studies indicating that probiotic supplementation had no influence on the incidence (47, 48, 50) or severity (47, 48) of the flu.

The effect of probiotics on immune function of healthy adults are summarised in Table 2. Ten studies reported improvement in the immune function by activating T lymphocytes, including cytotoxic plus and T helper cells (CD8+ and CD4+) after probiotics consumption (46, 51, 52), increase natural killer (NK) cell activity (45, 51, 53, 54), reduce the pro-inflammatory cytokines IL-12, IL-6 and IL-4 (51, 53, 55) and increase anti-inflammatory cytokines IL-10 (53, 56). However, there is no consistent and clear relationship between the effects observed and the dose, duration and type of probiotic strains administered. Overall, it appears that probiotic supplementation in healthy adults can improve immune function and the immune response to common cold infections. However, the immune response to influenza infection and the effective duration, dose and type of probiotics supplementation require further investigation.

Lipid profile and cardiovascular disease risk

Elevated low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG), low levels of high-density lipoprotein cholesterol (HDL-C), increase in body mass index (BMI), blood glucose and pro-inflammatory markers are major risk factors of cardiovascular disease (CVD) (57). The potential of probiotics to support a reduction in inflammation markers in healthy adults has been discussed in the previous section. The effect of probiotics consumption on changes to blood lipid profile has been investigated in several systematic reviews and meta-analyses (9, 58-60). Collectively, findings from these reviews and meta-analyses suggest an improvement in blood lipid profile, especially in total cholesterol (TC) and LDL-C levels. However, these studies included both healthy and unhealthy adults (including conditions such as hypercholesterolemia, CVD, diabetes, obesity). Thus, conclusions regarding the influence of probiotics on the blood lipid profile of healthy adults cannot be reliably determined from this work.

A summary of results from the present review of literature for the effect of probiotics consumption on blood lipid profile of healthy adults is presented in Table 3. Of the fourteen studies (fifteen trials, one study with two separate arms (61)), seven reported no significant changes in the concentration of various blood lipid profile markers following probiotics consumption (18, 55, 61-65). Three studies reported a significant reduction in TC (61, 66-68). Reductions in TG (68-71), LDL-C (61, 66), and an increase in HDL-C (67, 68) were also reported. Furthermore, in one study, six weeks of probiotics treatment in healthy adults who were heavy smokers (55) demonstrated improvements in markers of oxidative stress (F_2-isoprostanes) and the pro-inflammatory marker IL-6, supporting the cardio-protective effect of probiotics. Despite this, to date only a small number of individual studies have demonstrated benefits of probiotics and the collective evidence is not inclusive for the beneficial effect of probiotics consumption on blood lipid profile of healthy adults.

Changes in BMI were reported in six studies (Table 3). Of these four reported no significant changes (18, 55, 67, 71) and one reported a significant increase in BMI after probiotic supplementation (65). One of the studies assessed the BMI increase after four weeks of high-fat diet and reported a lower increase in BMI in the probiotic group compared to placebo (72). Due to a low number of studies included and inconsistent results, a conclusion cannot be reached for the effect of probiotics on BMI of healthy adults. Seven studies also reported changes in fasting blood glucose (FBG) and insulin levels. Of these, four reported no significant changes in FBG or insulin levels (55, 64, 71, 72), two reported reduction in FBG

and insulin level (62, 68) and one reported an increase in FBG after probiotics supplementation in healthy adults (65). Similar to BMI, the evidence is inconclusive for the beneficial effect of probiotics on FBG and insulin level in healthy adults.

Although underlying mechanisms of the effect of probiotics on lipid profile are not completely understood, the cholesterol binding and assimilation ability of probiotics and their ability to deconjugate bile salt (to reduce its solubility and absorbability) are proposed as the potential mechanisms of action (59). Bacteria in the gut also have the ability to produce short-chain fatty acids (SCFA) (such as acetate, butyrate and propionate) through fermentation of dietary fibers (73). SCFA are shown to lower hepatic cholesterol synthesis and regulate cholesterol metabolism (74). Both lactobacilli and bifidobacteria are able to produce SCFA. Recently proposed next generation of probiotics such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* have also shown to have a high SCFA producing ability from dietary fibers (75, 76).

Gastrointestinal discomfort

Although constipation is often considered as a symptom of a health condition, irregularities in bowel movement, evacuation disorder, abdominal discomfort and bloating may occur in response to aging and changes in dietary, lifestyle and psychological factors in otherwise healthy individuals (77, 78). These changes also influence the bacterial flora of the intestine. A diet high in fat, for example, is known to cause dysbiosis in gut microbiota (79). Aging has also been associated with a decrease in the number and diversity of bifidobacteria (80). Benefits of probiotic supplementation in the treatment and management of many types of diarrhea and constipation have been reported in unhealthy populations (7, 8). Table 4 summarizes the evidence of the effect of probiotics consumption on GI health of healthy adults. Of the four studies included (six trials, two studies had two arms (61, 77)), an improvement in the bowel movement, defection frequency and stool consistency was reported in five studies (61, 77, 78). One study did not observe improvements in the colonic transit time or bowel movement (81). However, this study had the shortest duration of supplementation among the identified trials. Overall, it appears that probiotic supplementation may be effective at improving stool consistency, bowel movement and reducing irritation caused by abdominal bloating. The relevant mechanisms of probiotics in this action remain unclear. However, fermentation of non-digestible carbohydrates and production of short-chain fatty acids and carbon dioxide, removal of other intestinal gases

(77) and the anti-inflammatory effect of probiotics have been suggested (8) as potential mechanisms.

Female reproductive system health

From birth until after puberty, lactobacilli are the predominant microorganisms populating the vaginal microbial environment (82). However, after puberty the microbial environment changes due to menstruation, hormonal changes, intercourse, infections and hygiene (83). This often results in a vaginal environment that is not predominant in lactobacilli bacteria for the majority of women (82), increasing susceptibility to urogenital infections such as urinary tract infection and bacterial vaginosis (84). The effect of probiotics supplementation on maintaining female reproductive health is summarised in Table 5. Four studies (five trials, one study had two arms (85)) have examined the effects of oral supplementation or vaginal suppositories with lactobacilli as a means of improving the vaginal environment. Among these four trials have suggested there was a significant increase in the level of vaginal lactobacilli (82, 85-87). Supplementation with *L. acidophilus, L. rhamnosus* or *L. fermentum* increased vaginal lactobacilli levels in healthy women (82, 85-87). The increase in vaginal lactobacilli population seems to prevent and reduce the incidence of vaginal infections in otherwise healthy adult women (83).

Effect of probiotics on psychological health

Although focus of this review thus far has been on physiological health outcomes, it is important to acknowledge that probiotics consumption may also influence psychological health – an essential domain of overall well-being. Conceptualization of the brain-gut axis has introduced a link between psychological health and gut microflora (88, 89). Psychological distress can reduce the number and diversity of intestinal microorganisms by changing intestinal transit time, acidity, mucus secretion, stress hormones and immune response (88). Conversely, the gut microbiota can influence a hosts nervous system (gut-brain axis) by producing signalling molecules (e.g. polypeptides), modulating neuronal signalling mechanisms (88). A recent meta-analysis examining the effect of probiotics supplementation on depression score suggests that probiotics supplementation can induce a significant reduction in depression in both healthy adults and those with major depressive symptoms (90). Similarly, a recent systematic review and meta-analysis indicates significant improvement in subclinical symptoms (including reduced stress, depression and anxiety) can be achieved in healthy adults following probiotics supplementation (91). Collectively,

evidence from these studies suggests that probiotics supplementation may improve psychological symptoms in healthy adults.

Discussion

Demand for probiotics food and supplements has increased over the past few decades. Globally, the probiotics market is expected to have up to an 8% compound annual growth rate increase from 2014 to 2020 (92). This prompts continued interest in research regarding the health benefits of probiotics. Although consumption of probiotics has beneficial effects on several health outcomes, the majority of findings are related to populations with specific health conditions or disease. This raises an important question as to whether the health benefits provided by probiotics are limited to individuals with underlying pathologies. Thus, in the current review, we explored the effects of probiotics consumption in otherwise healthy adults. Overall, results from this review suggest that probiotics supplementation in healthy adults generate an improvement in gut microbiota. However, despite gut microbiota changes occurring with probiotics, these changes appear to be limited to a transient increase in the bacterial count of the specific strain administered. This implies that supplementation with probiotics may need to be an ongoing process in order to maintain gut microbiota changes in healthy adults. Gut microbiota is sensitive to multiple factors, such as lifestyle, aging and disease. Even in apparently healthy individuals, changes in diet quality and alcohol intake can significantly affect gut symbiosis. A diet poor in fruit and vegetable intake (as a good source of prebiotics) may not provide the food required for probiotics survival and maintenance. This may explain the constant need for probiotic food and supplements to maintain gut symbiosis and health. Furthermore, in older adults with age-related dysbiosis, probiotic supplementation appears to improve and reduce dysbiosis.

The transient effect of probiotics on gut microbiota may also be explained by the viability of probiotic microorganisms. Evidence suggests that viability of probiotics could differ from the number of viable cells declared on the product label (93). Although drying processes during probiotic production may have a negative impact on viability, different drying methods (air-drying, freeze-drying and spray-drying) have diverse effects on probiotic strains in terms of both viability and functionality (94). Although viability is acknowledged as a prerequisite for the health benefits of probiotics, few interventions have reported the viability of probiotics during the period of supplementation. Evidence suggests that non-viable probiotic strains

may also confer some positive health outcomes (93) however, this requires further investigation.

The current review also suggests that probiotics consumption in healthy adults may improve immune function, particularly in response to common upper respiratory infections; reducing their incidence and/or symptom severity. This is particularly important since improved immune function via probiotics may reduce the antibiotic needs in infections, thus reduce the risk of antibiotic resistance (95) – one of the greatest global threat of present decade (96). Probiotics may enhance the immune response by activating T lymphocyte cells, increasing NK cell activity and anti-inflammatory cytokines (e.g. IL-10) and reducing pro-inflammatory cytokines (e.g. IL-12 and IL14). These findings are in agreement with a recent meta-analysis (including both healthy and unhealthy populations) indicating that probiotics consumption may have a protective effect against the common cold (97). As gut microbiota is typically considered the first line of defense against pathogenic microorganisms in the intestine, maintaining symbiosis through regular consumption of probiotics foods and/or supplements is essential. This is particularly important in older adults, where age-related decreases in immune function (98) may increase vulnerability to a variety of infections.

The present review did not find sufficient evidence to support a blood lipid profile lowering effect of probiotics in healthy adults. These findings are in contrast with several systematic reviews and meta-analyses suggesting probiotics consumption facilitates improvements in blood lipids (9, 58-60). This disagreement is likely due to the inclusion of both healthy and unhealthy (participants with a high baseline level of blood lipids) populations, hence a greater opportunity for improvements in lipid profiles was afforded in these previous reviews. Similarly, BMI, FBG and insulin level of healthy adults were not significantly affected by probiotics consumption in the present review. These findings, however, are in contrast to previous systematic review and meta-analysis of the effect of probiotics on BMI (99) and FBG (13). This can be explained by the inclusion of participants with underlying pathologies (obese or diabetic). For example, Nikbakht, Khalesi (13) included trials with high and normal FBG participants with an overall significant effect of probiotics consumption on FBG reduction. However, their subgroup analysis results showed no significant changes in participants with normal FBG level. This can explain the findings of the current review reporting no significant effect of probiotics on FBG of healthy adults.

The current review also found some evidence for the effect of probiotics as a means of relieving abdominal discomfort and improving bowel movement irregularities in healthy adults. Although probiotics have been reported as beneficial for these conditions (78), definitive conclusions cannot be made and further research is required to clarify these findings in healthy adult populations. Probiotics consumption also appears to offer some benefits in maintaining female reproductive health by improving the lactobacilli count of the vaginal environment to prevent urogenital infections. Again however, the low number of eligible studies included in this review suggest that further investigation is required to support any dietary guidance provided for these conditions.

Although the effect of probiotics supplementation on psychological symptoms of healthy adults was not thoroughly reviewed in this paper, results of recent meta-analyses suggest improvements in depression, stress and anxiety following probiotics supplementation (90, 91). However in most studies included in these meta-analyses, changes in gut flora were not reported. Furthermore, psychological symptoms are typically measured using subjective self-reported scales and questionnaires, with different scales often administered across studies. Thus, it is important to consider these limitations when translating the findings. Nevertheless, probiotics supplementation may confer psychological benefits in healthy individuals and further exploration of these effects is warranted.

To our knowledge, the present review is first to assess the evidence for a range of health-related outcomes of probiotics consumption in healthy adults. However, the present study does have some limitations. Firstly, it was not possible to employ a complete systematic review approach due to the differences in study design, participant characteristics and dependent measures included in this review. Therefore, it limited the potential of the review to assess the methodological quality and bias risk of included studies. However, of the 45 studies included in this review the majority were randomised (n=38), included blinding (n=32) and had similar control or placebo groups (n=38). Secondly, the low number of studies included in the review of GI health and female reproductive health outcomes limits the conclusions that can be drawn from these research areas. Also, probiotics characteristics are strain-specific and due to variations in the probiotic strains used in the included studies, a conclusion cannot be made on the effective strains.

With ongoing increases in the manufacturing and marketing of probiotics to the general public there is a need for the benefits of probiotics to be better understood. Although this

review suggests that healthy adults (and in particular, older adults) may achieve some health benefits from consistent use of probiotics, probiotics supplementation may have a similar fate to multivitamins. That is, they may be effective in specific cases or conditions. More research on the probiotics use is necessary to develop a stronger body of evidence for their efficacy across both healthy and unhealthy population groups. Additionally, interventions with follow-up periods are required to assess the sustainability of probiotics use and their effect in healthy adults. Until further research is conducted, the benefits and feasibility of probiotics consumption in healthy adults remain uncertain and it would be prudent to advise consumers that supplementation with probiotics may be more effective in specific population groups and those with underlying pathologies.

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Figure Legends:

Figure.1 Flow diagram of the review process for the effect of probiotic supplementation in healthy adults

Table 1. Characteristics of included studies for the effect of probiotics on gut microbiota changes

Study (year)	Design; Location	Intervention/ Control, supplement	Duration; Age; participants (m/f)	Probiotic; Dose (per day), CFU	Outcome effect; reported side-effects
Bjerg, et al. 2015 (69)	RC, DB, P, Denmark	Probiotic capsule/ placebo	4 wk; 20 – 54 y; 64 (32/32)	*L. casei;* 1.0×10^{10}	Significant increase in the specific strain after intervention but no effect on overall composition of gut
Brown, et al. 2005 (100)	CO, RC; USA	Poi probiotics drink (non-dairy based)/ control	14 wk; 18 – 64 y; 18 (8/10)	*L. lactic* + *L. streptococcus;* 1.9 – 3.9×10^{8}	No significant change in the total concentration of gut bacteria; No side-effects
Ferrario, et al. 2014 (6)	CO, RC, DB; Italy	Probiotic supplements/ placebo	4 wk; 23 – 55 y; 34 (19/15)	*L. paracasei DG;* 2.4×10^{10}	Significant increase in healthy bacteria genus, and reduction in bacteria associated with disease (reduction in *Blautia*:*Coprococcus* ratio); No side-effects
Guillemard, et al. 2010 (101)	RC; DB; P; France	Fermented dairy drink/ placebo drink	12 wk; 72 – 80 y; 537 (198/339)	*L. paracasei (L. casei)* + *S. thermophilus* + *L. bulgaricus* 1.0×10^{9}	Significant increase in specific strain during probiotics consumption.
Hanifi, et al. 2015 (102)	RC, DB, P, USA	Probiotic capsule/ placebo	4 wk; 18-50 y; 81 (40/41)	*B. subtilis;* 1.0×10^{10}	Significant increase in the specific strain after intervention; No side-effect
Irwin, et al. 2017 (65)	P, RC, DB; Australia	Probiotic supplements/ placebo	8 wk: 26.2 ± 8.4 y; 19 (10/9)	*L.acidophilus* + *B. lactis;* 2.5×10^{10}	Increase in faecal count of specific strains after intervention. Some GI discomfort
Klein, et al. 2008 (70)	P, RC, CO; Germany	Probiotic yoghurt/ placebo	5 wk; 25 ± 3 y; 26 (13/13)	*L.acidophilus* + *B. lactis;* 9.4×10^{8}	Significant increase in the specific strain after intervention;
Lahti, et al. 2013 (23)	RC, DB, P, Finland	Probiotic drink/ placebo	3 wk; 23 – 44 y; 25 (7/18)	*L. rhamnosus* GG; 1.5×10^{10}	Significant increase in the specific strain after intervention, reduced to pre-treatment 3 weeks follow-up

Mai, et al. 2017 (25)	P, RC, DB, USA	Probiotics capsules in gelatin or Pearls	12 dy; 12	*L. acidophilus* + *B. longum;* 1×10^9	Significant increase in fecal count of specific strains after intervention
Nyangale, et al. 2015 (56)	RC, DB, P; UK	Probiotic capsule	28 dy; 65 – 80 y; 36 917/ 25)	*B. coagulans*; 1.0×10^9	Significant increase in Facalibacterium and Bacillus coagulans;
Plaza-Diaz, et al. 2015 (21)	RC, P, Spain	Probiotic capsule/ placebo	30 dy; 20 – 35 y; 23 (14/9)	*B. breve* +*L. rhamnosus* or *L. paracasei/ B. breve/ L. rhamnosus* separately; 9×10^9	Significant increase in faecal count of specific strains after intervention. *L. rhamnosus* increased all Lactobacillus genus. Most changes remained after 15 days follow-up.
Rampelli, et al. 2013 (22)	RC, DB, P, Italy	Probiotic biscuit/ placebo	30 dy; 71 – 88 y; 32 (13/19)	*B. longum* + *L. helveticus;* 1.0×10^9	Reduced the pathogen bacteria (i.e. *Clostridium* cluster Xi, *C. difficile*, *C. perfringens, E. faecium)* and increase in the probiotics *B. longum* and *Lactobacillaceae.* Improved age-related changes in gut microbiota
Wang, et al. 2015 (29)	SA, China	Probiotic drink	14 dy; 20 – 40 y; 25 (9/16)	*L. casei Shirota;* 1.0×10^{10}	Significant increase in faecal count of specific strain after intervention. The count decreased after follow-up but remained higher than baseline; No side-effects
Wassenaar, et al. 2014 (103)	SA, Germany	Probiotic capsule	Single dose; 5 (3/2)	*E. coli* G1/2, G3/10, G4/9, G6/7, G5 and G8; 2×10^9	High colonization of the specific strains for a period of 10-30 weeks after single dose; Mild GI discomfort
Wind, et al. 2010 (24)	RC, DB, P, The Netherlands	Probiotics sachets/ placebo	3 wk; 42± 16 y; 34 (14/20)	*L. rhamnosus;* 1.0×10^{11}	Significant increase in the specific strain after supplementation, reduced back to pre-intervention within 1 week follow-up; No side-effects

CO: cross-over; DB: double blind; P: parallel; RC: randomized controlled trial; SA: single arm

Table 2. Characteristics of included studies for the effect of probiotics on immune system response

Study (year)	Design; Location	Intervention/ Control	Duration; Age; participants (m/f)	Probiotic; Dose (per day), CFU	Outcome effect; reported side-effects
Baron 2009 (43)	SA, USA	Probiotic capsule	30 dy; 33 – 63 y; 10 (5/5)	*B. coagulans;* 2.0×10^{9}	Significant increase in TNF-α after in vitro exposure of T cells to flu A; No side-effects
Berggren, et al. 2011 (44)	RC, DB, P; Sweden	Probiotics capsule/ Placebo	12 wk; 18 – 65 y; 318 (180/92)	*L. plantarum* + *L. paracasei;* 1.0×10^{9}	Significant reduction in the incidence of common cold, the duration and the symptoms of common cold. Reduction in inflammatory B lymphocytes
de Vrese, et al. 2005 (46)	RC, DB, P; Germany	Probiotics with multivitamin/ multivitamin	12 wk; 36 ± 13 y; 242 (94/148)	*L. Gasseri* + *B. longum* + *B. bifidum*; 5.0×10^{7}	Significant reduction in the duration and symptoms of common cold, and days with fever. Increase in CD8+ and CD4+ immune cells
Dong, et al. 2013 (53)	RC, SB, CO; UK	Probiotic drink/ placebo drink	4 wk; 55 – 74 y; 30 (12/18)	*L. casei* Shirota; 1.3×10^{10}	Significant increase in NK cell activity and CD25 in T cells. A trend toward increase in anti-inflammatory cytokine IL-10 to pro-inflammatory IL-12
Gill, et al. 2001 (52)	SA; New Zealand	Probiotics milk	3 wk; 63 – 84 y; 30 (12/18)	*B. lactis;* 5.0×10^{10}	Increase in CD4+; CD25+ and NK cells. Increase in the in vitro phagocytose and tumoricidal activity of natural killer cells
Guillemard, et al. 2010 (101)	RC; DB; P; France	Fermented dairy drink/ placebo drink	12 wk; 72 – 80 y; 537 (198/339)	*L. paracasei (L. casei)* + *S. thermophilus* + *L. bulgaricus* 1.0×10^{9}	Decreased duration of upper respiratory tract infection. No differences in the severity of symptoms.
Harbige, et al. 2016 (51)	SA; UK	Probiotic drink	8 wk; 18 – 49 y; 14 (6/8)	*L. casei* Shirota; 6.5×10^{9}	Significant increase in T cell activation markers and NK cell markers. Significant reduction in inflammatory makers IL-12 and IL-4 and pro-inflammatory cytokines
Jespersen, et al. 2015 (47)	RC, DB, P; Denmark	Probiotic drink/ placebo drink	6 wk; 18 – 60 y; 1104 (453/ 651)	*L. paracasei (L. casei);* 1.0×10^{9}	No effect of probiotics on immune response to flu vaccine. No effect on severity or incidence of flu symptoms. A shorter duration of symptoms
Kekkonen, et al. 2007 (50)	RC, DB, P; Finland	Probiotic drink/ placebo	12 wk; 22 – 69 y; 141 (125/16)	*L. rhamnosus* GG; 4.0×10^{10}	No significant effect on incidence of respiratory infection or GI-symptoms, but shortened the duration of symptoms in healthy marathon runners

Klein, et al. 2008 (70)	P, RC, CO; Germany	Probiotic yoghurt/ placebo	5 wk; 25 ± 3 y; 26 (13/13)	*L.acidophilus* + *B. lactis;* 9.4×10^8	Significant increase in the phagocytic activity of monocytes and granulocytes, but no change in the level of inflammatory markers
Makino, et al. 2010 (45)a	P; R; Japan	Probiotic yoghurt/ placebo	8 – 12 wk; 59 – 84 y; 92	*L. bulgaricus* + *S. Thermophiles;* 2.0 – 8.8×10^8	Significant reduction in the risk of catching cold, and increase in NK cells activity
Naruszewic, et al. 2002 (55)	RC, DB, P; Sweden	Probiotic drink/ placebo	6 wk; 35 – 45 y; 36 (18/18)	*L. plantarum;* 2.0×10^{10}	Significant reduction in markers of oxidative stress (F_2-isoprostanes) and pro-inflammatory marker IL-6 in heavy smokers
Nyangale, et al. 2015 (56)	RC, DB, P; UK	Probiotic capsule/ placebo	28 dy; 65 – 80 y; 36 917/ 25)	*B. coagulans*; 1.0×10^9	Significant increase in anti-inflammatory cytokine IL-10. No effect on NK or TNF-α
Parra, et al. 2004 (54)	RC, DB, P;	Probiotic yoghurt/ placebo	8 w; 51 – 58 y; 23	*L. casei;* 10^8– 10^{10}	Increased in NK cells activity and oxidative burst capacity of monocytes. No change in other immune factors
Van Puyenbroeck, et al. 2012 (48)	RC, DB, P; Belgium	Probiotic drink/ placebo drink	176 dy; 55 – 101 y; 737 (184/553)	*L. casei* Shirota; 1.3×10^{10}	No significant effect on the incidence or symptoms of flu
West, et al. 2014 (49)	RC, DB, P; Australia	Probiotic sachet/ placebo	150 dy; 18 – 60 y; 465 (241/ 224)	*B. animalis* subsp. *Lactis*; 2.0×10^9 or *L. acidophilus* and *B. animalis Lactis*; 5.0×10^9	Significant reduction in the risk of respiratory illness

CO: cross-over; DB: double blind; P: parallel; RC: randomized controlled trial; SA: single arm

Table 3. Characteristics of included studies for the effect of probiotics on lipid profile and cardiovascular disease risk

Study (year)	Design; Location	Intervention/ Control	Duration; Age; participants (m/f)	Probiotic; Dose (per day), CFU	Outcome effect; reported side-effects
Agerbaek, Gerdes and Richelsen, 1995 (66)	RC, DB, P; Denmark	Probiotic drink/ placebo	6 wk; 44 y; 58 (28/0)	*E. faecium* + *S. termophilus*	Significant reduction in TC and LDL-C
Bjerg, et al. 2015 (69)	RC, DB, P; Denmark	Probiotic capsule/ placebo	4 wk; 20 – 54 y; 64 (32/32)	*L. casei;* 1.0×10^{10}	Significant reduction in TG
Cox, et al. 2014 (62)	RC, DB, P; Australia	Probiotic sachet/ placebo	150 dy; 18 – 60 y; 465 (241/ 224)	*B. animalis* subsp. *Lactis*; 2.0×10^{9} or *L. acidophilus* and *B. animalis Lactis*; 5.0×10^{9}	No significant change. Significant reduction in insulin after double-strain supplementation
de Roos, et al. 1999 (18)	RC, DB, P; Netherlands	Probiotic yoghurt/ control yoghurt	6 wk; 39.9 ± 8.7 y; 78 (22/56)	*L. acidophilus*; 4.8×10^{9} – 2.7×10^{10}	No significant changes in lipid profile and BMI
Greany, et al. 2008 (63)	RC, SB, P; USA	Probiotic capsule/ placebo	8 wk; 18 – 36 y; 55 (22/33)	*L. acidophilus* and *B. longum*; 1.0×10^{9}	No significant change
Higashikawa, et al. 2010 (61)a	RC, DB, P: Japan	Probiotic yogurt/ placebo yoghurt	6 wk; 37.3 ± 12.2 y; 24 (6/18)	*L. plantarum SN35N;* 2×10^{10}	No significant Change
Higashikawa, et al. 2010 (61)b	RC, DB, P: Japan	Probiotic yogurt/ placebo yoghurt	6 wk; 35.1 ± 11.6 y; 22 (7/15)	*L. plantarum SN13T;* 2×10^{10}	Significant reduction in TC and LDL
Hulston, et al. 2015 (71)	RC, P, England	Probiotic drink/ placebo, + over eating	4 wk; 24 ± 2 y; 8 (7/1)	*L.s casei* Shirota; (CFU not reported)	Significant reduction in TG, No significant changes in BMI, FBG or insulin level
Irwin et al. 2017 (65)	DB, R, P, Australia	Probiotic/ placebo capsule	8 wk 27.9 ± 6.5; 10 (5/5)	*L. acidophilus*, *B. lactic*; 2.5×10^{10}	No significant change. Significant increase in BMI and FBG
Klein, et al. 2008 (70)	P, RC, CO; Germany	Probiotic yoghurt/ placebo	5 wk; 25 ± 3 y; 26 (13/13)	*L.acidophilus* + *B. lactis;* 9.4×10^{8}	Significant reduction in TG
Naruszewicz, et al. 2002 (55)	RC, DB, P; Sweden	Probiotic drink/ placebo	6 wk; 35 – 45 y; 36 (18/18)	*L. plantarum;* 2.0×10^{10}	No significant change in lipid profile, BMI, FBG or insulin level

Osterberg, et al. 2015 (72)	RC, DB, P; USA	Probiotic capsule/ placebo + high fat diet	4 wk; 22.4 ± 1.4 y; 9 (9/0)	*B. longum* + *B.infantis* + *B.breve* + *L. Acidophilus* + *L. paracasei* + L. *Bulgaricus* + *L. plantarum* + *S. thermophiles; 9.0* $\times 10^{11}$	Lower increase in BMI after high fat diet, no differences in FBG
Rajkumar, et al. 2014 (68)	RC, SB, P, India	Probiotic capsule/ placebo	6 wk; 40-60 y; 15	*B. longum* + *B.infantis* + *B.breve* + *L. Acidophilus* + *L. paracasei* + L. *Bulgaricus* + *L. plantarum* + *S. thermophilus*	Significant reduction in TC and TG, increase in HDL-C, and reduction in FBG and insulin sensitivity
Rizkalla, et al. 2000 (64)	RC, CO; France	Yoghurt with live culture/ heated yoghurt	15 dy; 20 – 60 y; 12 (12/0)	*L. bulgaricus* + *S. thermophiles;* ≥ 1.0×10^{7}	No significant changes in lipid profile, FBG or insulin levels
Sadrzadeh-Yeganeh, et al. 2010 (67)	RC, DB, P; Iran	Probiotic yoghurt/ Control	6 wk; 19 – 49 y; 90 (0/90)	*B. lactis* + *L. acidophilus*; 3.9×10^{7}	Significant reduction in TC and increase in HDL-C, No significant change in BMI

CO: cross-over; DB: double blind; FBG: fasting blood glucose; HDL-C: high-density lipoprotein cholesterol P: parallel; RC: randomized controlled trial; TC: total cholesterol; TG: triglyceride;

Table 4. Characteristics of included studies for the effect of probiotics on gastrointestinal discomfort

Study (year)	Design; Location	Intervention/ Control	Duration; Age; participants (m/f)	Probiotic; Dose (per day), CFU	Outcome effect; reported side-effects
Del Piano, et al. 2010 (77)a	RC, DB, P; Italy	Probiotic blend/ placebo	30 dy; 24-71 y; 110 (50/60)	*L. plantarum* + *B. breve;* 5×10^9	Significant improvement in the number of weekly bowel movement and reduction in abdominal irritation
Del Piano, et al. 2010 (77)b	RC, DB, P; Italy	Probiotic blend/ placebo	30 dy; 24-71 y; 110 (50/60)	*B. animalis* subspecies *lactis;* 5×10^9	Significant improvement in the number of weekly bowel movement and reduction in abdominal irritation
Higashikawa, et al. 2010 (61)a	RC, DB, P: Japan	Probiotic yogurt/ placebo yoghurt	6 wk; 35.1 ± 11.6 y; 22 (7/15)	*L. plantarum SN35N;* 2×10^{10}	Significant improvement in the defection frequency
Higashikawa, et al. 2010 (61)b	RC, DB, P: Japan	Probiotic yogurt/ placebo yoghurt	6 wk; 35.1 ± 11.6 y; 22 (7/15)	*L. plantarum SN13T;* 2×10^{10}	Significant improvement in the defection frequency
Merenstein, et al. 2014 (81)	RC, TB, CO, USA	Probiotic yoghurt/ placebo	2 wk; 28.7 ± 10.6 y; 68 (0/68)	*B. animalis* ssp. *lactis* Bf-6; 2×10^{10}	No effect on the colonic transit time, bowel movement or frequency of constipation
Sakai, et al. 2011 (78)	RC, P, Belgium	Fermented milk/ placebo	3 w; 35.4 ± 14.2 y; 19 (11/8)	*L. casei* Shirota; 6.5×10^9	Significant reduction in the incident of hard or lumpy stool

CO: cross-over; DB: double blind; P: parallel; RC: randomized controlled trial; TB: triple blind

Table 5. Characteristics of included studies for the effect of probiotics on female reproductive health

Study (year)	Design; Location	Intervention/ Control	Duration; Age; participants (m/f)	Probiotic; Dose (per day), CFU	Outcome effect; reported side-effects
De Alberti, et al. 2015 (86)	RC, DB, P; Italy	Probiotic capsule/ placebo	14 dy; 33.2 ± 9.7 y; 20	*L. acidophilus* + *L. rhamnosus* 1×10^{10}	Significant increase in vaginal concentration of specific bacteria after intervention and a week follow-up
Reid, et al. 2003 (87)	RC, P; London	Probiotic capsule/ placebo	60 dy; 19 – 46 y; 64	*L. rhamnosus* + *L. fermentum;* 10^9	Significant increase in vaginal lactobacilli and reduce in yeast
Reid, et al. 2001 (85)a	RC, DB, Canada	Probiotic capsule	28 dy; 31 ± 8 ;33	*L. rhamnosus* + *L. fermentum*; 10^8 - 10^9	Significant improvement in the vaginal flora and health
Reid, et al. 2001 (85)b	RC, DB, Canada	Probiotic capsule	28 dy; 31 ± 8 ;33	*L. rhamnosus* GG	No Significant change in vaginal flora
Verdenelli, et al. 2016 (82)	SA, Italy	Probiotic suppositories	7 dy; 29.8 ± 7.1 y; 35	*L. rhamnosus* + *L. paracasei;* 10^9	Significant increase in vaginal lactobacilli

CO: cross-over; DB: double blind; P: parallel; RC: randomized controlled trial; SA: Single arm; TB: triple blind

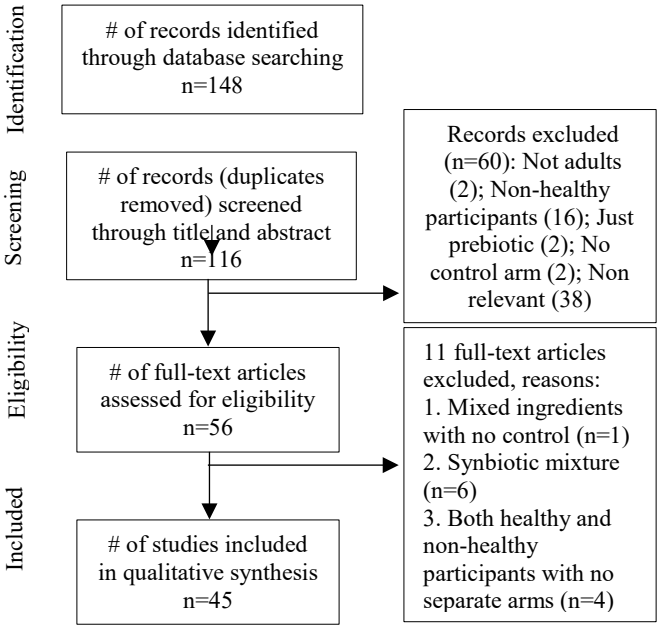
Identification
# of records identified through database searching n=148
Screening
# of records (duplicates removed) screened through titleland abstract n=116
Records excluded (n=60): Not adults (2); Non-healthy participants (16); Just prebiotic (2); No control arm (2); Non relevant (38)
Eligibility
# of full-text articles assessed for eligibility n=56
11 full-text articles excluded, reasons:
1. Mixed ingredients with no control (n=1)
2. Synbiotic mixture (n=6)
3. Both healthy and non-healthy participants with no separate arms (n=4)
Included
# of studies included in qualitative synthesis n=45

Figure.1