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Effect of probiotics and synbiotics consumption on serum concentrations of liver function test enzymes: a systematic review and meta-analysis

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Abstract

Purpose: The gut-liver interaction suggests that modification of gut bacterial flora using probiotics and synbiotics may improve liver function. This systematic review and meta-analysis aimed to clarify the effect of probiotics and synbiotics consumption on the serum concentration of liver function enzymes. **Methods:** PubMed (MEDLINE), Cumulative Index to Nursing and Allied Health Literature, and Cochrane Library (Central) were searched from 1980 to August 2017 for studies where adults consumed probiotics and/or synbiotics in controlled trials and changes in liver function enzymes were examined. **Results:** A total of 17 studies (19 trials) were included in the meta-analysis. Random effects meta-analyses were applied. Probiotics and synbiotics significantly reduced serum alanine aminotransferase (-8.05 IU/L, 95 % confidence interval (CI): -13.07 to -3.04; $p = 0.002$); aspartate aminotransferase (-7.79 IU/L, 95% CI: -13.93 to -1.65; $p = 0.02$) and gamma-glutamyl transpeptidase (-8.40 IU/L, 95% CI: -12.61 to -4.20; $p < 0.001$). Changes in the serum concentration of alkaline phosphatase and albumin did not reach a statistically significant level. Changes to bilirubin levels were in favour of the control group (0.95 μmol/L, 95% CI: 0.48 to 1.42; $p < 0.001$). Subgroup analysis suggested the existence of liver disease at baseline, synbiotics supplementation and duration of supplementation ≥ 8 weeks resulted in more pronounced improvement in liver function enzymes than their counterparts. **Conclusions:** Probiotics and synbiotics may be suggested as supplements to improve serum concentration of liver enzymes, especially when synbiotics administered for a period ≥ 8 weeks and in individuals with liver disease.

Keywords: Liver function; Liver enzyme; Probiotics; Synbiotics; Systematic review

Introduction

The human gastro-intestinal tract is a densely populated ecosystem of microorganisms. A healthy gut is considered to be in symbiosis when the equilibrium of symbionts (i.e. healthy bacteria), commensals (i.e. bacteria with no harm or benefit for the host) and pathobionts (i.e. pathogenic bacteria) exists [1,2]. This symbiosis contributes to the digestion, absorption and synthesis of nutrients, and is the first mechanism of defence against pathogenic bacteria [3,1]. Poor diet (i.e. high saturated fat and low dietary fibre intake, and high alcohol consumption), infections and some chronic conditions (e.g. obesity) may disrupt this equilibrium [4,1], resulting in a disproportionate increase in the number of pathogenic bacteria. While all bacteria can increase the absorption of monosaccharides from the intestine, pathogenic bacteria (mostly gram-negative) can produce and release endotoxins, such as lipopolysaccharide (LPS) and hepatotoxins, which may cause inflammation of the liver [5].

Interactions between the gut and liver are well recognised, owing to the use of the term ‘gut-liver axis’. Liver diseases, such as alcoholic liver disease (ALD) and liver cirrhosis (LC), are associated with changes in gut flora [1,5,6]. However, it is unclear if changes in gut flora are the cause or consequence of liver conditions [7,8]. Nonetheless, health and function of the liver appear to be in a synergistic relationship with gut flora. For example, in individuals suffering from a nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH), a dysbiosis of gut flora towards increased pathogenic Bacteroides and decreased healthy firmicutes is observed [9-11]. Furthermore, endotoxins (e.g. LPS) produced by pathogenic bacteria of the gut can increase cytokine production, leading to inflammation of the liver [12,13]. Conversely, healthy bacteria may assist the removal of cholesterol from bile [14], reduce the production of LPS and hepatotoxins by their competitive nature [15] and reduce intestinal permeability and bacterial translocation to extra-intestinal sites such as the liver [16,17].

This gut-liver interaction has led to the development of interventions aiming to modify the gut bacterial flora, to improve liver function and reduce or reverse the progression of chronic liver diseases [18-20]. Supplementation of probiotics and synbiotics are one of these proposed interventions. Probiotics are defined as live microorganisms that can have health benefits for the host if provided in adequate amounts and duration [21-23]. Synbiotics are defined as dietary supplements with a combination of probiotics and prebiotics (fermentable dietary fibres that stimulate the growth and survival of probiotics) [24]. However, results of studies employing probiotics and synbiotics interventions are inconclusive, with some suggesting significant improvement

82 [25,19,26] and others reporting negligible changes or no effect [27,28] on metabolic factors of liver function.
83 Therefore, this study aimed to clarify the effect of consumption of probiotics or synbiotics on serum
84 concentrations of liver enzymes (namely aspartate aminotransferase [AST], alanine aminotransferase [ALT],
85 alkaline phosphatase [ALP], gamma-glutamyl transpeptidase [GGT], albumin, and bilirubin) in adults
86 participating in randomised controlled trials or quasi-experimental (non-randomised) controlled trials, using a
87 systematic review and meta-analytic procedures. A complete PICOS approach (population, intervention,
88 comparison and outcome) following the 'Preferred Reporting Items for Systematic Reviews and Meta-Analysis'
89 (PRISMA) guidelines [29] is presented in Table 1.

90

91 **Methods**

92 *Literature search*

93 The online databases PubMed (MEDLINE), Cumulative Index to Nursing and Allied Health Literature
94 (CINAHL), and Cochrane Library (Central) were searched for relevant studies. Following the PICOS approach
95 combinations of the following terms (including MeSH terms) were used to search for relevant publications from
96 1980 to August 2017: Probiotics, Prebiotics, Synbiotics, Lactobacillus, Bifidobacterium, Liver, Hepatic,
97 Aspartate aminotransferase (AST); Alanine aminotransferase (ALT); Alkaline phosphatase (ALP); Gamma-
98 glutamyl transpeptidase (GGT); Albumin; and Bilirubin. An example of the search strategy used is presented in
99 Supplemental Material. Reference list of included studies was also checked manually. During the preparation
100 and presentation of this review, the PRISMA guidelines were followed [29]. Methodology for this systematic
101 review was registered with the International Prospective Register for Systematic Reviews (PROSPERO)
102 (registration number: CRD42016051573).

103

104 *Study eligibility*

105 Studies were included if they: (1) were randomised controlled trials or quasi-experimental (non-randomised
106 controlled trials), (2) included adults older than 18 years of age, (3) used live bacteria (probiotics) alone or in
107 combination with prebiotics (synbiotics), and (4) had accessible full-text articles in English. Studies were
108 excluded if probiotics were combined in a mixture with substances other than prebiotics (i.e. if there was no

separate arm to control for the mixed substance); the post-prandial or immediate post-surgery effect of supplementation was studied; or if pregnant women were included as participants. For duplicated publications, the study with complete patient follow-up and outcome measures was included. Publications were discarded if they did not meet the review's initial objective.

The screening process commenced with a review of the title and abstract of searched literature. The next phase involved a review of full texts of all potential records. Two researchers conducted the literature search and screened the literature based on the eligibility criteria independently. The final decision regarding the eligibility of articles was made through an agreement between the two researchers, and any disagreement resolved by involving a third researcher. Figure 1 presents the PRISMA flow diagram of the review summary and procedure.

Data extraction and quality assessment

Methodologic quality of the included studies was examined using both the Rosendal scale [30], and Cochrane risk of bias assessment tools [31]. Studies were not discarded based on their methodology quality rating. However, a sensitivity analysis was performed to check the robustness of the meta-analysis results to the quality of included studies (details are presented in the *Sensitivity and subgroup analysis* section below). Relevant data on the methodology characteristics of included studies and their results were extracted following the *Cochrane Handbook for Systematic Review of Interventions* 'checklist of items to consider in data collection' [32].

Data synthesis and analysis

The effect of probiotics and synbiotics on the markers of liver function was defined as the mean difference of changes observed in the intervention group compared to the control group. The *Cochrane Handbook for Systematic Review of Interventions* [32] was used as the guideline to perform statistical analysis. Three studies reported standard deviation (SD) of change [18,19,33]. The missing SD of change for the remainder of studies were imputed using a correlation coefficient (r) [32]. Only one study [19] provided enough data (Mean and SD of baseline, final and change) to impute the correlation coefficient [32]. The coefficients of 0.75, 0.73 and 0.52 were calculated for ALT, AST and GGT, respectively using the following formula [32]:

$$r = \frac{SD^2_{Baseline} + SD^2_{Final} - SD^2_{Change}}{2 \times SD_{Baseline} \times SD_{Final}}$$

For ALP, bilirubin and albumin a coefficient of 0.6 was assumed (as there was not enough data to calculate the correlation coefficient). The above-mentioned correlation coefficients were used to calculate the missing SD of change using the following formula [32]:

$$SD_{Change} = \sqrt{SD^2_{Baseline} + SD^2_{Final} - (2 \times r \times SD_{Baseline} \times SD_{Final})}$$

RevMan software (Cochrane Review Manager, version 5.2) was used to perform the meta-analysis of data. A DerSimonian and Laird random effect model was used [34]. Heterogeneity was assessed using the I^2 index. The I^2 analysis values <40%, 40-75%, and >75% correspond to low, moderate to substantial, and considerable heterogeneity, respectively [32]. A *p*-value of less than 0.05 was considered a statistically significant effect, differing from zero using a *Z*-test analysis and interpreted as strong evidence of an effect [32].

Sensitivity and subgroup analysis

The influence of individual studies on the overall meta-analysis results was assessed in a one-out method, where the changes in heterogeneity and summary effect were assessed after excluding individual trials. The robustness of meta-analysis to the imputed SD of change was assessed by calculating SD of change using different correlation coefficients (r = 0.2 and 0.8) and observing their influence on the summary effect and heterogeneity. The sensitivity analysis of the overall meta-analysis result to the methodologic quality of included studies was performed by limiting the analysis to studies with a Rosendal score ≥ 60% and a low Cochrane risk of bias.

Subgroup analysis of interventions with probiotics was compared to those with synbiotics. Because liver enzyme levels change greatly in liver disease, a subgroup analysis was limited to trials that included participants with liver disease (e.g. NAFLD, ALD, LC, hepatic encephalopathy (HE)). Some recent systematic reviews and meta-analyses have suggested that the health benefits of probiotics may increase when supplementation continues for ≥ 8 weeks [35,36,21]. To test this, trials with supplementation duration ≥ 8 weeks were compared with those with <8 weeks. Furthermore, as the literature suggests that probiotics should be consumed in a daily

dosage of 10^9 [37,38] to 10^{11} colony forming units (CFU) in order to be effective [21], trials with daily probiotics $\geq 10^9$ CFU were compared to those using lower dosages.

Results

Overview of included studies

Twenty-one studies were included in the qualitative synthesis for the effect of probiotics and synbiotics on metabolic factors of liver function (Table 2). Of these, 17 studies (a total of 19 trials: Two studies [39,40] had two arms eligible for the meta-analysis) were eligible for the meta-analysis. Four studies were excluded from the quantitative analysis [41-44]. One study did not report the actual measures for liver enzymes (values were estimated from figures) [41]. In the remainder, values were presented as median (percentile or range) and/or changes were presented as a percentage change [42-44]. Attempts to acquire usable measures were not successful. Of the 19 trials, 16 reported changes in ALT and AST, six reported changes in ALP, eight in GGT, 11 in albumin and 13 in bilirubin.

All twenty-one studies reported employing a randomised design. All studies, except one (cross-over design) [43] followed a parallel design. Fourteen studies reported using a double-blinded protocol, and one study used a single-blinded study design (Table 2). Three studies followed an open-label protocol [26,33,28] and two did not report blinding [45,46]. Of the 14 double-blinded studies, 11 reported similarities between intervention and placebo supplements but three did not report further information [25,18,47]. The methodologic quality assessment of studies is presented in Supplemental Table 1. The highest Rosendal score of 87% was achieved by four studies [48,42,40,44]. Overall, 16 out of 19 studies had good methodology quality with a Rosendal score $\geq$ 60% [30]. Similar findings were reported from the Cochrane risk of bias assessment tool (Supplemental Table 2), where four studies obtained a low risk of bias in at least five out of six domains of the tool [48,42,39,49].

Participants and study protocols

Table 2 presents the characteristics of included studies. Participant's age ranged from 23 – 70 years old. Of the 21 studies, five reported using synbiotics [19,41,47,33,39], one had both synbiotics and probiotics arms [39], and the remainder used a probiotic intervention. Four studies used one strain [18,47,27,28], one study had two

separate arms with single and multiple strains [40], and the remainder used multiple strains of probiotic bacteria in their supplements. Synbiotic interventions used fructo-oligosaccharides (FOS) [19,47,33], arabino guard [39] or a combination of beta-glucan, inulin, pectin and resistant starch [41]. The duration of supplementation varied from 6 days [26] to 28 weeks [19]. Two studies used yoghurt as the probiotics medium [45,42], and capsules or sachets were used to deliver probiotics or synbiotics in the other studies. Daily probiotics doses varied from 3 $\times 10^6$ CFU [28] to 5 $\times 10^{10}$ CFU [18].

Participants in the majority of studies had different extents of liver disease, including NAFLD [25,19,42], NASH [47,33], ALD [26,20], HE [18,41,46,28], primary sclerosing cholangitis (PSC) [43], LC [44,50] and chronic liver disease (not further specified) [51,45]. One study included participants with type 2 diabetes mellitus [48], one included patients infected with human immunodeficiency virus (HIV) [27], and three studies included healthy participants [39,49,40]. Only ten studies reported baseline body mass index (BMI) of participants [25,18,19,48,47,42,33,39,49,40], and all except for two studiy [39,40] reported mean BMI $\geq$ 25 kg/m^2. Nine studies reported changes in body weight (BW) or BMI [25,18,19,48,47,42,27,33]. Of these, two reported a significant decrease in BW in both intervention and control groups [19,47], one observed a reduction in the intervention group [42] and five reported no changes in BW or BMI after the intervention period [25,18,27,33,39] (Supplemental Table 3).

Seven studies reported a method to measure dietary intake changes during the intervention (food record or recall) and reported no significant changes [25,18,19,48,47,42,39]. One study used a Likert scale to measure food intake and reported an increase in consumption [45], four reported dietary advice and prescription [26,28,49,40] and the remainder did not report using any method for controlling dietary intake. Compliance to supplementation was reported in thirteen studies [18,26,51,45,41,47,42,33,28,39,49,40,44] using the proportion (%) of participants that completed the study and adhered to the supplementation strategy. The majority of studies reported more than 90% completion rate and supplementation was reported to be well tolerated. However, incidence of diarrhoea was observed in four studies [18,39,49,44] and abdominal discomfort in another five studies [19,39,49,40,44]. One study reported high attrition rate (26%) and adverse effects in the intervention group [48] (Supplemental Table 3).

Meta-analysis results

The meta-analysis of the effect of probiotics and synbiotics consumption on liver function tests are presented in Figures 2 to 7. The meta-analysis for the mean difference in serum ALT concentrations showed an overall

significant reduction of -8.05 IU/L (95 % confidence interval (CI): -13.07 to -3.04; p = 0.002; 16 trials, 990 participants) (Figure 2). The observed reduction was significantly more pronounced in the synbiotics subgroup (-20.13 IU/L, 95% CI: -22.47 to -17.80; p < 0.001; 4 trials, 156 participants) compared to the probiotics subgroup (-4.83 IU/L, 95% CI -9.34 to -0.33; p = 0.04; 12 trials, 834 participants) (test for subgroup difference I^2 = 97.1%; ρ <0.001). The meta-analysis showed an overall considerable heterogeneity (I^2 = 93 %; ρ <0.001). The source of this high heterogeneity appeared to be related to the probiotics subgroup (I^2 = 89 %, p<0.00001) as opposed to the synbiotics subgroup (I^2 = 0%, p=0.73) (Figure 2).

The meta-analysis for the mean difference in serum AST concentrations also showed a significant overall reduction with probiotic or symbiotic interventions (-7.79 IU/L, 95% CI: -13.93 to -1.65; p = 0.01; 16 trials, 990 participants) (Figure 3). The significant reduction was only observed in the synbiotics subgroup (-23.61 IU/L, 95% CI: -26.63 to -20.58; p < 0.001; 4 trials, 156 participants). The reduction in AST observed in the probiotics subgroup was not statistically significant. The overall heterogeneity level observed was considerable (I^2 = 97.7 %; ρ <0.00001) and was primarily observed in the probiotics subgroup (I^2 = 96 %; ρ <0.00001) rather than the synbiotics subgroup (I^2 = 0 %; ρ =0.85) (Figure 3).

Only four studies reported changes in serum ALP (Figure 4). The meta-analysis of the effect did not show strong evidence of an effect (-0.27 IU/L, 95% CI: -4.00 to 3.47; p = 0.89; 6 trails, 518 participants).

Meta-analysis for the mean difference in serum GGT levels indicated a significant reduction of -8.40 IU/L (95% CI: -12.61 to -4.20; p < 0.001; 8 trials, 438 participants) (Figure 5). Both probiotics and synbiotics subgroups resulted in a significant reduction in GGT with no subgroup differences (I^2 = 0 %; ρ =0.78). The heterogeneity observed was low in the synbiotics subgroup (I^2 = 0%, respectively), and was moderate to substantial in the overall results (I^2 = 53%; ρ =0.04) and probiotics subgroup (I^2 = 62%; ρ =0.02) (Figure 5).

No significant differences were observed between the intervention and control groups for serum levels of albumin (Figure 6). However, the results were in favour of placebo (control) for bilirubin changes (0.95 μmol/L, 95% CI: 0.48 to 1.42; p < 0.001; 13 trials, 806 participants; I^2 = 4%). Although meta-analysis results of the synbiotics subgroup also suggested an increase in bilirubin, the difference did not reach a statistically significant level (Figure 7).

Sensitivity and subgroup analysis

The one-out sensitivity analysis for ALT suggested the sensitivity of the probiotics subgroup to the study by Kirpich et al. [26]. Excluding this study reduced the heterogeneity from 89% to 1%, while retaining significant subgroup meta-analysis results. The probiotics subgroup of GGT was also sensitive to the Kirpich et al. [26] study and its exclusion reduced the heterogeneity from 62% to 0% without changing the significance of the meta-analysis results. Albumin results were sensitive to two studies. Exclusion of the study by Bajaj et al. [18] reduced the heterogeneity of the probiotics subgroup (from I^2=52% to 26%) and resulted in a significant reduction of albumin in this subgroup. Excluding the study by Wolf et al. [27] also resulted in a reduction of heterogeneity in the probiotics subgroup (from I^2=52% to 0%), but did not affect the meta-analysis results. Excluding the study by Kirpich et al. [26] resulted in a non-significant increase (p = 0.15) in the meta-analysis of probiotics subgroup for bilirubin. A few differences were observed in the study by Kirpich et al. [26] compared to other studies that may have caused the sensitivity of meta-analysis results. This study recruited alcoholic participants and involved standard treatment (alcohol detoxification therapy) in addition to probiotics or placebo supplementation. The standard treatment itself may affect levels of liver function enzymes. In addition, the short duration of supplementation (5 days) may have influenced the effectiveness of probiotics supplementation and the measurement of liver function enzymes.

Sensitivity analyses of the alternative correlation coefficients (r) are presented in Supplemental Table 4. Overall, the significance and heterogeneity levels of the majority of meta-analysis results were not sensitive to the level of correlation coefficients used. This suggests that the meta-analyses were robust to the imputed SD of change. However, ALP meta-analysis results showed sensitivity to alternative correlation coefficients in the magnitude of the effect and the heterogeneity. This, however, did not change the direction of the effect and may be explained by the low number of studies (n=4) included in the meta-analysis of ALP.

Sensitivity to the methodology quality of included studies was also conducted by excluding studies with <60% Rosendal scores [52,53] or those with a high risk of bias in the Cochrane assessment tool. Excluding two studies [26,46] from ALT and AST, one study [26] from GGT, three studies [45,46,28] from albumin, and two studies [45,46] from bilirubin analyses, did not result in significant changes to the overall meta-analysis results or heterogeneity.

Results of subgroup analyses based on participant liver disease status, intervention duration and the dose and strain of probiotics/synbiotics consumption are shown in Table 3. These results suggest that the subgroup of

participants with some degree of liver disease at baseline had a more pronounced improvement in ALT, AST and GGT levels compared to their otherwise healthy (no reported liver disease) counterparts. However, the bilirubin reduction was more favourable in the placebo arm of liver disease subgroup compared to the otherwise healthy subgroup (although the subgroup difference was not significant). On the other hand, the magnitude of albumin reduction was greater in the otherwise healthy subgroup compared to the liver disease subgroup (Table 3).

Similar results were observed in the intervention duration subgroup. Supplementation with probiotics and synbiotics for ≥ 8 weeks resulted in more pronounced reductions in serum ALT and AST levels. However, a greater magnitude of reduction in serum albumin was observed in the supplementation duration <8 weeks, although the test for the subgroup difference did not result in a statistically significant difference (Table 3). The subgroup analysis of the dose of probiotics did not result in a significant difference between supplementation with dose ≥ 10^9 CFU compared to dose < 10^9 CFU. However, this subgroup difference was significant for ALP, suggesting a difference in the direction of effect (reduction in ALP in dose ≥ 10^9 CFU). The results of subgroup analyses of probiotics strain (single vs multiple) did not show an overall meaningful result, except for Bilirubin level with a higher increase in the concentration of this enzyme in the serum of those consuming probiotics with more than one strain (Table 3).

The sources of high heterogeneity reported for overall results of ALT, AST and ALP were also explored in subgroup analysis results (Table 3). The findings did not suggest any subgroup as a potential source of heterogeneity for ALT. However, AST subgroup analyses suggested lower heterogeneity for the subgroup of studies with supplementation dose of ≥ 10^9 CFU compared to dose < 10^9 CFU. For ALP, the subgroup of participants with liver disease, consuming supplements ≥ 8 weeks, with dose < 10^9 CFU of multiple strains had lower heterogeneity compared to their counterparts. However, the low number of trials in some subgroups limits the interpretation of findings. Similar findings were reported for GGT, except for the subgroup of participants with no reported liver disease, which showed lower heterogeneity compared to their counterparts (Table 3).

Discussion

The results of this systematic review and meta-analysis suggest that probiotics and synbiotics consumption can be beneficial in reducing serum concentrations of liver enzymes, especially ALT, AST and GGT. Reductions were more pronounced when probiotics were consumed concurrently with prebiotics (in the form of synbiotics)

compared to probiotics alone. Since non-digestible but fermentable carbohydrates (such as the prebiotics inulin and oligosaccharides) facilitate the growth and survival of probiotics [54], their synergistic effect may explain the results of subgroup analyses observed in this study.

Although the disruption of gut flora may be both a cause and/or consequence of impaired liver function [7,8], results of this systematic review and meta-analysis confirm that modification of gut flora via probiotics and synbiotics consumption affects liver function. However, the mechanism/s of the effect of gut bacteria on liver function and health are not clear. There are a few pathways suggested for this relationship. Probiotics and synbiotics may enhance the integrity and tightness of the intestinal epithelium [55], thereby modulating chronic damage to these cells (e.g. by ethanol in alcoholic liver disease) and restoring intestinal permeability [56,17]. This may, in turn, reduce bacterial translocation [57] and reduce the production of cytokines, tumour necrosis factor (TNF-α) and hepatotoxins [58,17], which can lead to the inflammation of liver and development of liver disease [19]. Probiotics have also shown potential in the synthesis of vitamins B and K [45,59] and facilitate the breakdown and digestion of polyphenols (e.g. flavanols, flavan-3-ols, tannins, lignans) [59]. These components are effective antioxidants with the potential to moderate the hepatic oxidative stress caused by inflammatory cytokines and hepatotoxins [60]. Furthermore, the gram-negative bacterial overgrowth that exists in more than 50% of cirrhotic patients [56] may increase bacterial translocation and the production of hepatotoxins (LPS and cytokines) [17]. Probiotics and synbiotics may lower gram-negative and pathogenic bacteria through their competitive behaviour [61], and reduce inflammation [62,15]. Based on subgroup analysis results from the present study, reductions in ALT, AST and GGT after probiotics and synbiotics consumption appear to be more pronounced in participants with liver disease compared to their otherwise healthy counterparts.

A controversial finding of the present study was the observation that probiotics and synbiotics consumption increased blood bilirubin levels. However, it is important to note that the meta-analysis results were sensitive to one study [26], such that excluding this study resulted in a non-significant effect of supplementation on bilirubin level. This study was the only trial that investigated the effect of less than one week (5 days) supplementation on liver enzymes. Since the participants had an alcohol-induced liver injury, it is possible that the duration of probiotics consumption was not sufficient to affect bilirubin removal from the body, especially for participants of this study who were heavy alcohol drinkers before the commencement of the trial with their last drink occurring within 48 hours prior to the admission [26]. Chronic alcohol consumption can cause gram-negative bacterial overgrowth and dysbiosis [1], which in turn might potentially affect the ability of the intestinal

335 microflora to reduce and remove bilirubin [65]. This alcohol-induced dysbiosis may take longer than 5 days to
336 manipulate via probiotics and synbiotics consumption. The influence of duration of supplementation was also
337 supported by the subgroup analysis of bilirubin in this systematic review. This was also evident from the greater
338 reductions in serum levels of ALT and AST observed with longer duration of supplementation (≥ 8 weeks) in
339 this study.

340 To the best of our knowledge, this is the first study to systematically review the effects of probiotics and
341 synbiotics consumption on serum liver enzyme concentrations by pooling the results of controlled trials.
342 However, the current study does have some limitations that need to be considered when interpreting the overall
343 findings. First, a high degree of heterogeneity was observed in some outcomes. Although the sources of
344 heterogeneity have been explored in this study, the interpretation of findings may be influenced by the level of
345 heterogeneity observed. Second, only a limited number of liver enzymes were selected, based on those
346 commonly used in the diagnosis and reporting of liver function problems. Third, less than half of the included
347 studies reported BW or BMI changes in their intervention. The lack of reporting of changes in BW in the
348 remaining studies may have introduced a bias in interpreting the findings, as the changes in liver enzymes may
349 have been influenced by BW change during the intervention [66,67]. The subgroup analysis of this study also
350 had some limitations. The low number of trials included in some subgroups limited the interpretation of
351 findings. This, was more evident in the subgroup analysis of ALP changes. Also, subgroup analysis based on
352 study design (parallel vs cross-over) was not applicable given that only one study reported employing a cross-
353 over design. Furthermore, the clinical relevance of the reduction observed in the liver enzymes is challenging to
354 be discussed due to the variation in individual's baseline characteristics. However, the high degree of reduction
355 observed in liver enzymes of participants with liver disease (Table 3) can suggest an overall 10 – 30% reduction
356 (depending on baseline values) in liver enzymes after probiotics consumption. Since these reductions observed
357 are generally over a short period of time, they are likely to be clinically relevant.

358 Overall, the results of this systematic review and meta-analysis suggest that probiotics and synbiotics lower
359 serum concentrations of liver enzymes commonly used in clinical practice as biomarkers of liver function. This
360 beneficial effect may be enhanced in individuals with liver disease and when synbiotics are administered for a
361 period ≥ 8 weeks. However, the mechanism of the effect is not clear and requires further investigation. There is
362 also a need for future interventions to examine the effects of different doses and strains of probiotics, prebiotics
363 and synbiotics on liver function test serum biomarkers.

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584 **Figure legends:**

585 **Figure 1.** PRISMA flow diagram for the systematic literature review for the effect of probiotic and synbiotics
586 supplementation on the metabolic factors of liver function.

587 **Figure 2.** The meta-analysis results of the effect of probiotics and synbiotics supplementation on the serum ALT
588 level.

589 **Figure 3.** The meta-analysis results of the effect of probiotics and synbiotics supplementation on the serum
590 AST level.

591 **Figure 4**. The meta-analysis results of the effect of probiotics and synbiotics supplementation on the serum ALP
592 level.

593 **Figure 5**. The meta-analysis results of the effect of probiotics and synbiotics supplementation on the serum
594 GGT level.

595 **Figure 6.** The meta-analysis results of the effect of probiotics and synbiotics supplementation on the serum
596 albumin level.

597 **Figure 7.** The meta-analysis results of the effect of probiotics and synbiotics supplementation on the serum
598 bilirubin level.

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609 **Table 1**. PICOS criteria used to define research question and search literature

Criteria	**Description**
Population	Adults
Intervention	Probiotic; Synbiotic; prebiotic; fermented products; Lactobacillales; Bifidobacterium; Cultured milk products
Comparison	Control group with/without placebo
Outcomes	Liver function test; Aspartate aminotransferase; Alanine Aminotransferase; Alkaline phosphatase; Gamma-glutamyl transpeptidase; Serum Albumin; Bilirubin; Liver failure
Setting	Clinical or non-clinical controlled trials

Table 2. Characteristics of included studies

Study (year)	Design; Location	Intervention/ Control, supplement Duration, wk	Probiotic, prebiotic	Dose (per day)	Participants	Age, y	Intervention n (M/F)	Intervention		Control	
								Baseline[1]	Changes from baseline	Baseline	Changes from baseline
Aller et al. 2011	DB, PC, R, Spain	Probiotic/placebo, Capsule	12	*L. bulgaricus* + *S. thermophilus*	5×10^8	NAFLD	49.4± 10.9	14 (10/4) C:14	ALT: 67.7±25.1 AST: 41.3±15.5 GGT: 118.2±63.1	-7.3±20.24* -5.7±10.63* -10.5±60.73*	ALT: 60.7±32.1 AST: 31.7±13.1 GGT: 82.1±55.1	4.1± 24.11* 4.7± 9.90 1.5± 59.65
Bajaj et al. 2014	DB, PC, R, USA	Probiotic/placebo, Capsule	8	*L. GG*	5×10^{10}	LC, MHE	58.4 ± 3.8	14 (10/4) C:16	BIL: NA ALB: NA	-0.11 ± 0.32 0.01 ± 0.16	BIL: NA ALB: NA	-0.14 ± 0.48 0.04 ± 0.24
Cox et al. 2014 a	DB, R, PC, Australia	Probiotics/ placebo, sachet	~22	*B. animalis lactis*	2×10^9	Healthy	42.2 ± 16.2	39 (23/16) C:45	ALT: 18.7 ±7.4 AST: 21.4 ±5.5 ALP: 61.5 ±14.1 BIL: 9.60 ±3.39	− 0.73±4.86 0.06±4.82 − 5.32 ±9.2* 0.68±3.65	ALT: 25.1 ±16.2 AST: 26.0 ±9.8 ALP: 63.5± 16.9 BIL: 11.9± 9.0	1.72 ±15.23 − 0.81 ±11.34 − 5.10 ±7.72* − 0.31 ±5.47
Cox et al. 2014 b	DB, R, PC, Australia	Probiotics/ placebo, sachet	~22	*L. acidophilus* + *B.animalis*	10^{10}	Healthy	37.3 ± 11.4	45 (23/22) C:45	ALT: 23.0± 11.0 AST: 24.0 ±7.2 ALP: 62.5± 17.0	− 1.74± 7.51 − 6.77± 18.8* − 1.00± 10.3	ALT: 25.1 ±16.2 AST: 26.0 ±9.8 ALP: 63.5± 16.9	1.72 ±15.23 − 0.81 ±11.34 − 5.10 ±7.72*

	ia			*lactis*					BIL: 10.16± 6.14	0.46± 4.26	BIL: 11.9± 9.0	− 0.31 ±5.47
Eslampa	DB,	Synbiotic/pl	28	*L. casei,*	4×10^8	NAFL	32.1 ±	26	ALT: 69.3 ± 2.3	-25.1 ± 2.86*	ALT: 71.5 ± 9.1	-4.7 ± 5.72*
rast et	PC, R,	acebo,		*L.*	Prebioti	D	2.4	(14/12)	AST: 66.4 ± 2.6	-31.3 ± 2.08*	AST: 68.3 ± 9.4	-7.9 ± 8.19*
al. 2014	Iran	Capsule		*rhamnosus,*	c: NR			C:26	ALP: 231.4 ± 10.4	-22.9 ± 9.95	ALP: 229.8 ± 12.1	-22.3 ±13.59
				S.					GGT: 89.5 ± 1.5	-15.08 ± 1.04*	GGT: 89.0 ± 2.5	-5.21 ± 3.51*
				thermophilus					BIL: 16.42 ± 4.45	4.79 ± 5.35	BIL: 16.25 ±3.76	3.25 ± 5.13
				, B. breve, L.								
				acidophilus,								
				B. longum,								
				and L.								
				bulgaricus +								
				FOS								
Firouzi	DB,	Probiotic/pl	12	*L.*	6×10^8	T2DM	52.9 ±	68	ALT: 23.20 ± 9.65	-0.87 ± 6.93	ALT: 32.53 ± 16.10	0.74 ± 11.85
et al.	PC, R,	acebo,		*acidophilus,*			9.2	(37/31)	AST: 20.1 ± 4.7	5.61 ± 4.66	AST: 25.8 ± 7.1	-4.51± 4.97
2015	Malays	Capsule		*L. casei, L.*				C:68	ALP: 68.49 ± 23.19	-1.49 ± 20.14	ALP: 73.42 ± 18.11	-2.13 ± 17.52
	ia			*lactis, B.*					BIL: 9.77 ± 3.50	0.32 ± 3.22	BIL: 10.38 ± 3.92	-0.10 ± 3.24
				bifidum, B.					ALB: 45.64 ± 3.22	-0.16 ± 2.77	ALB: 45.51 ± 2.53	0.43 ± 2.06
				longum and								
				B.								

				infantis								
Horvath et al. 2016 [5]	DB, PC, R, Austria	Probiotic/placebo, Powder	24	*B. bifidum* + *B. lactis* + *L. acidophilus* *L. brevis* + *L. Casei* + *L. salivarius* + *L. lactis* +	1.5 ×10^{10}	LC	60 (54; 64)*	44 (32/12) C:36	ALT: 36.5 (27.0; 51.25) AST: 49.0 (37.75; 69.5) ALB:40 (33; 45) BIL: 23.6 (13.3; 41.2)	38.5 (25.8; 52.3) 53.5 (36.8; 70.0)* 40 (34; 45) 22.7 (13.2; 45.9)	ALT: 32.5 (20.75; 46.25) AST: 42.5 (32.5; 56.5) ALB: 43 (41; 47) BIL: 18.9 (10.7; 24.3)	29.5 (22.0; 49.8) 37.5 (30.8; 59.0) 43 (40; 44) 16.2 (11.6; 25.3)
Irwin et al. 2017 a	DB, R, PC, Australia	Probiotic/ placebo capsule	8	L. acidophilus, B. lactic	2.5× 10^{10}	Healthy	27.9±6.5	10 (5/5) C:8	ALT: 18.04±10.61 AST: 23.50±12.26 GGT: 19.32±9.16 ALB: 47.71±1.71 BIL: 10.73±3.81	0.31±7.08 -0.86±8.46 -2.00±9.71 0.30±2.05 0.95±3.73	ALT: 18.13±2.90 AST: 22.09±3.14 GGT: 18.67±6.91 ALB: 46.50±3.18 BIL: 11.09±5.60	10.75±23.07 21.13±48.24 3.11±12.18 0.69±2.72 -1.31±4.48
Irwin et al. 2017 b	DB, R, PC, Australia	Synbiotic/ placebo powder	8	L. acidophilus, B. lactic + Arabino Guard	2.5× 10^{10} Prebiotic: ½ teaspoon	Healthy	26.1±7.7	10 (5/5) C:8	ALT: 23.53±13.37 AST: 33.03±27.10 GGT: 23.51±15.71 ALB: 48.23±1.77 BIL: 8.18±2.55	-3.07±10.15 -5.14±19.95 -0.45±14.40 0.07±1.72 0.97±2.48	ALT: 18.13±2.90 AST: 22.09±3.14 GGT: 18.67±6.91 ALB: 46.50±3.18 BIL: 11.09±5.60	10.75±23.07 21.13±48.24 3.11±12.18 0.69±2.72 -1.31±4.48
Kirpich	C, R,	Probiotic +	<1	*B. bifidum*	1 ×10^{9}	ALD	42.3±	32 (32/0)	ALT: 49.84± 6.94	-13.15± 4.62	ALT: 49.74 ± 7.17	1.52 ± 4.74

et al. 2008	Russia	standard therapy/ standard therapy, capsule		*and L. plantarum*			1.1	C:34	AST: 101.06± 4.33 GGT: 171.48±26.0 BIL: 20.75± 1.06	-46.39± 5.42* -28.59± 22.81 -10.24± 0.84	AST: 106.80± 12.78 GGT: 152.51± 20.16 BIL: 24.15± 1.98	-30.37± 9.72* -5.62± 19.03 -11.67± 1.59*
Kwak et al. 2014	DB, PC, R, Korea	Probiotic/placebo, Capsule	4	*B. bifidum, B. lactis, B. longum, L. Acidophilus, L. rhamnosus, and S. thermophilus*	1×10^{10}	SIBO, CLD	54.4 ± 8.4	25 (18/7) C:25	ALT: 37.4 ± 30.7 AST: 53.6 ± 36.3 BIL: 22.23 ± 20.52	-4.9 ± 22.45 -6.8 ± 25.05 -1.71 ± 16.41	ALT:48.8 ± 47.7 AST: 61.0 ± 34.5 BIL: 20.52 ± 15.39	-9.7 ± 32.63 -13.2 ± 24.02 -6.84 ± 12.43
Lefevre et al. 2017	DB, PC, R, France	Probiotic/ placebo	~ 6	*Bacillus strains (subtilis, coagulans, licheniformis*	2×10^{9}	Health elderly	63.0	50 (10/40) C:50	ALT: 17.65 ± 8.82 AST: 20.59 ± 5.88 GGT: 25.79 ± 18.59	-1.18 ± 5.85 -1.18 ± 4.14 -3.0 ± 16.14	18.82 ± 10.0 20.58 ± 5.29 32.39 ± 24.59	-1.76 ± 6.80 -0.58 ± 3.71 -3.0 ± 22.14

				, cereus, pumilus & clause)								
Liu et al. 2010	C, R, China	Probiotic/control, yogurt	2	*L. bulgaricus, L. acidophilus, B. bifidus, and S. thermophilus*	3×10^9	CLD	48.62 ± 11.11	41 (26/15) [2] C:40	BIL: 59.45 ± 73.63 ALB: 31.91 ± 5.96	-14.2 ± 59.15 0.91 ± 4.81	BIL: 45.25 ± 49.65 ALB: 31.53 ± 4.78	-4.12 ± 45.72* 0.92 ± 4.18
Liu et al. 2004 [3]	SB, R, PC, China	Synbiotic/Placebo, Capsule	4	*Pediacoccus pentoseceus*, *Leuconostoc mesenteroides*, *L. paracasei and L. plantarum* +	4×10^{10} Prebiotic: 10g	MHE	55 ±12	20 (20:0) C: 15	Approx.[3] ALT: 245 ± 25 BIL: 275 ± 25 ALB: 25 ± 5	Approx.[3] -160 ± 10* -140 ± 10* 5 ± 5*	Approx.[3] ALT: 230 ± 25 BIL: 250 ± 25 ALB: 25 ± 5	Approx.[3] -10 ± 10 -25 ± 25 0 ± 5

				beta glucan, inulin, pectin and resistant starch							
Malaguarnera et al. 2012	DB, R, PC, Italy	Synbiotic/placebo, Capsule	24	*B. longum* + FOS	5×10^9 Prebiotic: NR	NASH	46.9 ± 5.4	34 (18/16) C:32	ALT: 101 ± 24.7 AST: 109 ± 23.2 BIL: 10.4 ± 7.9 ALB: 43 ± 8	-53.9 ± 16.38* -69.6 ± 19.44* -0.3 ± 6.71* 1 ± 6.76	ALT: 96.1 ± 24.2 AST: 107.1 ± 21.4 BIL: 10.1 ± 7.6 ALB: 42 ± 7	-38 ± 18.38* -45.9 ± 17.59* -0.1 ± 6.47* 1 ± 6.76
Nabavi et al. 2014 [4]	DB, R, PC, Iran	Probiotic/ conventional yogurt	8	*L.acidophilus* and *B. lactis*	1.1 $\times 10^7$	NAFLD	42.75 ± 8.72	36 (18/18) C:36	ALT: 31.5 (21-49.5)[4] AST: 32.5 (24.2-46.5)[4]	25.5 (20-40.2)*[4] 27.5 (21.2-36.7)*[4]	ALT: 25.5 (20-37)[4] AST: 26 (20.2-36.5)[4]	24.5 (19.2-34.5)[4] 25 (22-35) [4]
Sang Hak et al. 2015	DB, R, PC, Korea	Probiotic/Placebo	7	*L. subtilis* and *S. faecium*	6×10^6	AH	52.7 ± 11.3	60 (38/22) [2] C:57	ALT: 83 ± 126 AST: 166 ± 213 ALP: 132 ± 54 GGT: 510 ± 629 ALB: 35 ± 7	-35 ± 94.95* -102 ± 184.58* -17 ± 44.73* -176 ± 547.03* 2 ± 5.88*	ALT: 93 ± 152 AST: 148 ± 130 ALP: 124 ± 39 GGT: 553 ± 953 ALB: 38 ± 8	-27 ± 102.91* -79 ± 94.74* -21 ± 31.4* -225 ± 817.80* 1 ± 6.76
Pereg et all 2011	DB, R, PC,	Probiotic/ placebo,	24	*L. Acidophilus*	8×10^{10}	LC	65.9 ± 8.4	18 C:18	ALT: 50.2 ± 32.6 AST: 58.4 ± 25.9	-0.6 ± 22.1 -4.0 ± 23.2	ALT: 55 ± 34.5 AST: 62.2 ± 32.2	6.4 ± 23.4 4.2 ± 22.7

	Israel	Capsule		+ *L. Bulgaricus*+ *B. lactis* + *S. thermophiles*					BIL: 20.52 ± 8.55 ALB: 36 ± 5	-1.71 ± 7.05 1 ± 5	BIL:22.23 ± 10.26 ALB: 37 ± 6	-3.42 ± 9.17 -1 ± 5
Sharma et al. 2008	R, C, India	Probiotic + Lactulose/ Lactoluse, Capsule	4	*S. faecalis, C. butyricum, Bacillus mesentricus, lactic acid bacillus*	5×10^8	MHE	43.7 ±10.0	35 (26/9)[2] C: 35	ALT: 55.0 ± 32.1 AST: 51.5 ± 32.8 BIL: 37.62 ± 20.52 ALB: 31± 6	-15.3± 22.82* -14± 23.49* -5.13± 17.1* 1± 5*	ALT: 42.9 ±20.9 AST: 57.3 ±23.4 BIL: 34.2±20.52 ALB: 31 ±5	-8.6 ± 15.1* -20.5 ± 16.23* -11.97± 16.54* 2 ± 4.47*
Vleggaar et al. 2008 [5]	DB, R, PC, CO, The Netherlands	Probiotic/placebo, capsule	12	*L. acidophilus, L. casei, L. salivarius, L. lactis, B. bifidum and B. lactis*	10^{10}	PSC	45 (28-70) [6]	14 (13/1)	ALT: 119 (35-580)[5] AST:101 (33-423)[5] GGT: 260 (45-581)[5] BIL: 17 (7-58)[5] ALB: 40 (31.7-45)[5]	-27% (-151, 223)[5] -16% (-207, 57)[5] -11% (-52, 26)[5] -13% (-57, 42)[5] 0% (-9, 11)	ALT: 119 (35-580)[5] AST:101 (33-423)[5] GGT: 260 (45-581)[5] BIL: 17 (7-58)[5] ALB: 40 (31.7-45)[5]	-26% (-254, 59)[5] -15% (-143, 70)[5] -5% (-62, 31)[5] -15% (-106, 45)[5] -1% (-8, 11)[5]
Wolf et	DB, R,	Probiotic/pl	3	*L. reuteri*	10^{10}	HIV	23-50	21	ALT: 31.74 ± 5.55	2.99± 8.38	ALT: 28.74 ± 3.59	6.59± 4.04

al. 1998	PC, USA	acebo, packets						(20/1)[2] C: 18	AST: 26.35± 3.59 ALP: 83.83± 5.99 GGT: 50.90± 12.57 ALB: 46 ± 1 BIL: 11 ± 1	1.79± 3.27 0 ± 5.35 -8.39± 11.01 0 ± 0.89 1 ± 1.61	AST: 28.14± 2.40 ALP: 83.83± 5.99 GGT: 33.53± 4.79 ALB: 44 ± 1 BIL: 77 ± 1	5.99± 3.45 5.99± 5.35 0.0± 5.01 1 ± 0.89 1 ± 1.61
Wong et al. 2013	R, C, Hong Kong	Synbiotic + lifestyle/ lifestyle, sachet	24	*L. plantarum, L. bulgaricus, L. acidophilus, L. rhamnosus, B. bifidum* + FOS	4×10^8 Prebiotic: 3g	NASH	42 ± 0	10 (8/2) C:10	ALT: 96 ± 75 AST: 50 ± 25	-26 ± 91 -13 ± 31	ALT: 72 ± 30 AST: 38 ± 15	2 ± 41 23 ± 32
Ziada et al. 2013	R, C, Egypt	Probiotic/ Control, Capsule	4	L. acidophilus	3×10^6	MHE	50.3 ± 7.8	26 (19/7) C:25	ALB: 26.4 ± 0.39	0.5 ± 3.78	ALB: 26.3 ± 0.27	-0.4 ± 2.73

* Significant change from baseline.

[1] ALT, ALP, AST, GGT in IU/L, BIL in µmol/l, ALB in g/L.

[2] Number of males and females is estimated based on overall percentage of male participants.

[3] Values for liver enzymes are estimated from figures presented in article. Not included in meta-analysis.

[4] Baseline values are presented as Median (percentile) and changes are presented as mean (SD) percentage change. Not included in meta-analysis.

[5] Values are presented as Median (range). Not included in meta-analysis.

[6] Age presented as Median (range).

Abbreviations: AH: alcoholic hepatitis; ALD: alcoholic liver disease; CLD: chronic liver disease; CO: crossover; FOS: fructooligosaccharide; HIV: human Immunodeficiency Virus; LC: liver cirrhosis; M / F: males / females; MHE: minimal hepatic encephalopathy; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; NR: not reported; PSC: primary sclerosing cholangitis; SIBO: small intestinal bacterial overgrowth.

Table 3. Results of subgroup analysis of included randomised controlled trials in the meta-analysis of probiotics and synbiotics and metabolic factors of liver function.

	Subgroups	**Trials (Participant *n*)**	**Mean difference (95% CI, p value)**	**Test for subgroup difference**
ALT	Participants with reported liver disease	*9 (505)*	-13.19 (-17.77, -8.60; ρ< 0.001, I^2=69%)	I^2=92%, ρ<0.001
	Participants with no reported liver disease	*7 (423)*	-2.78 (-5.69, 0.13; ρ=0.06, I^2=57%)	
	Intervention duration ≥ 8 weeks	*10 (548)*	-10.37 (-17.76, -2.99; ρ<0.01, I^2=92%)	I^2=4%, ρ=0.31
	Intervention duration < 8 weeks	*6 (442)*	-4.87 (-12.48, 2.73; ρ=0.21, I^2=94%)	
	Dose of probiotics/synbiotics supplementation ≥ 10^9 CFU	*10 (567)*	-6.91 (-12.24, -1.57; ρ=0.01, I^2=91%)	I^2=0%, ρ=0.59
	Dose of probiotics/synbiotics supplementation < 10^9 CFU	*6 (423)*	-10.44 (-22.22, 1.33; ρ=0.08, I^2=94%)	
	Single strain of probiotic/synbiotics	*3 (189)*	-6.07 (-11.85, -0.29; ρ= 0.04, I^2=75%)	I^2=0%, ρ=0.58
	More than one strain of probiotics/synbiotics	*13 (801)*	-8.48 (-14.67, -2.29; ρ< 0.01, I^2=96%)	
AST	Participants with reported liver disease	*9 (505)*	-12.46 (-19.90, -5.02; ρ<0.001, I^2=86%)	I^2=82%, ρ=0.02
	Participants with no reported liver disease	*7 (485)*	-1.03 (-7.11, 5.04; ρ=0.74, I^2=96%)	
	Intervention duration ≥ 8 weeks	*10 (532)*	-7.70 (-15.35, -0.06; ρ=0.05, I^2=87%)	I^2=0%, ρ=0.51
	Intervention duration < 8 weeks	*6 (442)*	-4.30 (-10.86, 2.26; ρ=0.20, I^2=84%)	
	Dose of probiotics/synbiotics supplementation ≥ 10^9 CFU	*9 (501)*	-3.62 (-7.17, -0.08; ρ=0.05, I^2=57%)	I^2=0%, ρ=0.38
	Dose of probiotics/synbiotics supplementation < 10^9 CFU	*7 (489)*	-10.99 (-27.07, 5.10; ρ=0.18, I^2=98%)	
	Single strain of probiotic/synbiotics	*3 (189)*	-5.05 (-12.22, 2.12; ρ= 0.19, I^2=83%)	I^2=0%, ρ=0.55
	More than one strain of probiotics/synbiotics	*13 (801)*	-8.63 (-17.77, 0.51; ρ= 0.09, I^2=95%)	

ALP	Participants with reported liver disease	*3 (305)*	0.41 (-3.90, 4.72; ρ=0.85, I^2=0%)	I^2=0%, ρ=0.75
	Participants with no reported liver disease	*3 (213)*	-0.75 (-6.56, 5.06; ρ=0.80, I^2=87%)	
	Intervention duration ≥ 8 weeks	*4 (362)*	1.40 (-0.94, 3.75; ρ=0.24, I^2=5%)	I^2=10%, ρ=0.29
	Intervention duration < 8 weeks	*2 (156)*	-3.38 (-11.98, 5.22; ρ=0.44, I^2=46%)	
	Dose of probiotics/synbiotics supplementation ≥ 10^9 CFU	*3 (213)*	-0.75 (-6.56, 5.06; ρ=0.80, I^2=87%)	I^2=75%, ρ=0.02
	Dose of probiotics/synbiotics supplementation < 10^9 CFU	*3 (305)*	0.41 (-3.90, 4.72; ρ=0.85, I^2=0%)	
	Single strain of probiotic/synbiotics	*2 (123)*	-3.15 (-8.81, 2.50; ρ=0.27, I^2=81%)	I^2=67%, ρ=0.08
	More than one strain of probiotics/synbiotics	*4 (395)*	2.51 (-0.33, 5.34; ρ= 0.08, I^2=0%)	
GGT	Participants with reported liver disease	*4 (263)*	-14.71 (-24.82, -4.60; ρ<0.01, I^2=54%)	I^2=65%, ρ=0.09
	Participants with no reported liver disease	*4 (175)*	-5.23 (-9.30, -1.16; ρ=0.01, I^2=9%)	
	Intervention duration ≥ 8 weeks	*4 (116)*	-9.71 (-11.09, -8.32; ρ<0.001, I^2=0%)	I^2=0%, ρ=0.99
	Intervention duration < 8 weeks	*4 (322)*	-9.77 (-20.24, 0.70; ρ=0.07, I^2=77%)	
	Dose of probiotics/synbiotics supplementation ≥ 10^9 CFU	*5 (241)*	-7.86 (-14.92, -0.81; ρ=0.03, I^2=70%)	I^2=0%, ρ=0.58
	Dose of probiotics/synbiotics supplementation < 10^9 CFU	*3 (197)*	-9.87 (-11.28, -8.46; ρ<0.001, I^2=0%)	
	Single strain of probiotic/synbiotics	*1 (39)*	-8.39 (-13.64, -3.14; ρ<0.01)	I^2=0%, ρ=0.99
	More than one strain of probiotics/synbiotics	*7 (399)*	-8.35 (-14.21, -2.49; ρ<0.01, I^2=59%)	
Albumin	Participants with reported liver disease	*7 (451)*	-0.02 (-0.16, 0.12; ρ=0.74, I^2=0%)	I^2=91%, ρ<0.001
	Participants with no reported liver disease	*4 (211)*	-0.84 (-1.28, -0.40; ρ<0.001, I^2=0%)	
	Intervention duration ≥ 8 weeks	*6 (304)*	-0.05 (-0.19, 0.09; ρ=0.52, I^2=0%)	I^2=0%, ρ=0.63

	Intervention duration < 8 weeks	*5 (508)*	-14.73 (-27.99, -1.47; ρ=0.03, I^2=41%)	
	Dose of probiotics/synbiotics supplementation ≥ 10^9 CFU	*7 (288)*	-0.33 (-0.94, 0.28; ρ=0.29, I^2=53%)	I^2=0%, ρ=0.76
	Dose of probiotics/synbiotics supplementation < 10^9 CFU	*4 (374)*	-0.16 (-1.03, 0.71; ρ=0.72, I^2=21%)	
	Single strain of probiotic/synbiotics	*6 (440)*	-0.43 (-1.06, 0.19; ρ=0.18, I^2=0%)	I^2=0%, ρ=0.58
	More than one strain of probiotics/synbiotics	*5 (222)*	-0.15 (-0.92, 0.62; ρ=0.70, I^2=70%)	
Bilirubin	Participants with reported liver disease	*7 (421)*	1.42 (0.85, 2.00; ρ<0.001, I^2=0%)	I^2=80%, ρ=0.03
	Participants with no reported liver disease	*6 (385)*	0.45 (-0.18, 1.09; ρ=0.16, I^2=0%)	
	Intervention duration ≥ 8 weeks	*8 (500)*	0.77 (0.02, 1.52; ρ=0.05, I^2=0%)	I^2=0%, ρ=0.50
	Intervention duration < 8 weeks	*5 (306)*	1.09 (0.57, 1.61; ρ<0.001, I^2=58%)	
	Dose of probiotics/synbiotics supplementation ≥ 10^9 CFU	*10 (548)*	1.04 (0.55, 1.53; ρ=<0.001, I^2=1%)	I^2=0%, ρ=0.99
	Dose of probiotics/synbiotics supplementation < 10^9 CFU	*3 (258)*	1.06 (-0.77, 2.88; ρ=0.26, I^2=31%)	
	Single strain of probiotic/synbiotics	*3 (617)*	0.16 (-0.70, 1.03; ρ=0.70, I^2=0%)	I^2=78%, ρ=0.03
	More than one strain of probiotics/synbiotics	*10 (189)*	1.25 (0.76, 1.74; ρ<0.001, I^2=0%)	

Changes in liver enzymes are presented as mean difference and 95% confidence interval. Heterogeneity (I^2) is presented by %. A p-value <0.05 is considered significant

Supplemental Material

Effect of probiotics and synbiotics consumption on serum concentrations of liver function test enzymes: a systematic review and meta-analysis

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An example of search strategy used in PubMed:

((((((("probiotics"[MeSH Terms] OR "probiotics"[All Fields] OR "probiotic"[All Fields]) OR "Fermented"[All Fields]) OR ("lactobacillales"[MeSH Terms] OR "lactobacillales"[All Fields])) OR ("bifidobacterium"[MeSH Terms] OR "bifidobacterium"[All Fields])) OR ("cultured milk products"[MeSH Terms] OR ("cultured"[All Fields] AND "milk"[All Fields] AND "products"[All Fields]) OR "cultured milk products"[All Fields])) OR (("synbiotics"[MeSH Terms] OR "synbiotics"[All Fields] OR "synbiotic"[All Fields]) OR ("prebiotics"[MeSH Terms] OR "prebiotics"[All Fields] OR "prebiotic"[All Fields]))) AND (((((("Liver function"[All Fields] OR ("liver function tests"[MeSH Terms] OR ("liver"[All Fields] AND "function"[All Fields] AND "tests"[All Fields]) OR "liver function tests"[All Fields])) OR ("aspartate aminotransferases"[MeSH Terms] OR ("aspartate"[All Fields] AND "aminotransferases"[All Fields]) OR "aspartate aminotransferases"[All Fields] OR ("aspartate"[All Fields] AND "aminotransferase"[All Fields]) OR "aspartate aminotransferase"[All Fields])) OR ("alanine transaminase"[MeSH Terms] OR ("alanine"[All Fields] AND "transaminase"[All Fields]) OR "alanine transaminase"[All Fields] OR ("alanine"[All Fields] AND "aminotransferase"[All Fields]) OR "alanine aminotransferase"[All Fields])) OR ("alkaline phosphatase"[MeSH Terms] OR ("alkaline"[All Fields] AND "phosphatase"[All Fields]) OR "alkaline phosphatase"[All Fields])) OR ("gamma-glutamyltransferase"[MeSH Terms] OR "gamma-glutamyltransferase"[All Fields] OR ("gamma"[All Fields] AND "glutamyl"[All Fields] AND "transpeptidase"[All Fields]) OR "gamma glutamyl transpeptidase"[All Fields])) OR ("serum albumin"[MeSH Terms] OR ("serum"[All Fields] AND "albumin"[All Fields]) OR "serum albumin"[All Fields])) OR ("bilirubin"[MeSH Terms] OR "bilirubin"[All Fields]))

Supplemental Table 1. Methodology quality assessment summary based on Rosendal scale

Study	Eligibility	Randomisation	Method for Randomisation	Sample Size Calculated	Pre-trial Conditions	Baseline Measures	Blinding of Subjects	Blinding of Investigators	Blinding Method and Evaluation blinding	Non-Completers Described	Stats Described	Measures and Variability Described	Between Group Stats Comparisons	Adverse Effects Described	Reproducibility Reported	Familiarisation Performance Test	% Score
Aller et al. 2011	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**1**	**0**	**0**	**NA**	**67**
Bajaj et al. 2014	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**1**	**1**	**0**	**NA**	**80**
Cox et al. 2014 [a]	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**0**	**NA**	**87**
Eslamparast et al. 2014	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**0**	**NA**	**80**
Firouzi et al. 2015	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**NA**	**87**
Horvath et al 2016	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**NA**	**87**
Irwin et al. 2017	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**NA**	**80**

Kirpich et al. 2008	**1**	**1**	**1**	**0**	**1**	**0**	**0**	**1**	**0**	**NA**	**1**	**0**	**1**	**0**	**0**	**NA**	**47**
Kwak et al. 2014	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**1**	**1**	**0**	**NA**	**73**
Lefevre et al. 2017 [a]	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**NA**	**1**	**0**	**1**	**1**	**0**	**NA**	**73**
Liu et al. 2010	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**NA**	**1**	**1**	**0**	**1**	**0**	**0**	**NA**	**43**
Liu et al. 2004	**1**	**1**	**1**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**1**	**0**	**1**	**1**	**0**	**NA**	**53**
Malaguarnera et al. 2012	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**1**	**1**	**0**	**NA**	**73**
Nabavi et al. 2014	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**NA**	**1**	**1**	**1**	**1**	**0**	**NA**	**87**
Pereg et al. 2011	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**1**	**0**	**0**	**NA**	**67**
Sang Hak et al. 2015	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**1**	**1**	**0**	**1**	**0**	**0**	**NA**	**67**
Sharma et al. 2008	**1**	**1**	**1**	**0**	**1**	**0**	**0**	**0**	**NA**	**1**	**1**	**0**	**1**	**1**	**0**	**NA**	**57**
Vleggaar et al. 2008	**1**	**1**	**0**	**1**	**0**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**0**	**NA**	**73**
Wolf et al. 1998	**1**	**1**	**0**	**1**	**0**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**1**	**1**	**0**	**NA**	**67**

Wong et al. 2013	**1**	**1**	**1**	**1**	**0**	**1**	**0**	**1**	**0**	**1**	**1**	**0**	**1**	**1**	**0**	**NA**	**67**
Ziada et al. 2013	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**NA**	**1**	**1**	**0**	**1**	**1**	**0**	**NA**	**40**

[a] Some information obtained from previous publications (1, 2)

1- A clear description of the inclusion and exclusion criteria was provided
2- The trials were randomized
3- The method used to generate the random allocation sequence, including details of any restrictions (e.g. blocking, stratification) was described
4- Sample size was justified (e.g. by power calculation)
5- Attempts were made to control and/or monitor pre-trial condition (e.g. diet, exercise)
6- Design incorporated measures of important baseline variables
7- There was blinding of all subjects
8- There was blinding of all investigators involved in the trials
9- Both the method of blinding and the evaluation of the successfulness of blinding were described
10- Details were provided regarding the inability of subjects to complete study requirements
11- Statistical methods used to compare groups for primary outcome measure, and methods for additional analyses, such as subgroup analyses and adjusted analyses, were described
12- Both point measures and measures of variability for the primary outcome measure were provided
13- The results of between-group statistical comparisons were reported for the primary outcome measure (e.g. an estimated effect size), and its precision (e.g. 95% CI)
14- The method used to assess adverse effects was reported
15- Reproducibility of the primary outcome measures was reported
16- If a performance test was used, a familiarization trial was conducted

Scoring: $\%score = 100 \times \frac{\text{Number of '1'}}{\text{Number of '0'}}$. Number of 'NA' does not count.

Supplemental Table 2. Cochrane risk of bias assessment

Study	Sequence generation	Allocation concealment	Blinding of participants, personnel	Blinding of outcome assessors	Incomplete outcome data	Selective outcome reporting
Aller et al. 2011	LR	LR	UR	UR	LR	LR
Bajaj et al. 2014	LR	LR	UR	UR	LR	UR
Cox et al. 2014 [a]	LR	LR	LR	LR	LR	LR
Eslamparast et al. 2014	LR	LR	UR	UR	LR	LR
Firouzi et al. 2015	LR	LR	LR	LR	LR	UR
Horvath et al 2016	LR	LR	LR	LR	LR	LR
Irwin et al. 2017	LR	LR	LR	LR	LR	LR
Kirpich et al. 2008	LR	LR	HR	UR	LR	UR
Kwak et al. 2014	LR	LR	UR	UR	LR	UR
Lefevre et al. 2017 [a]	LR	LR	LR	LR	LR	LR
Liu et al. 2010	LR	UR	HR	HR	LR	UR
Liu et al. 2004	LR	UR	UR	UR	LR	UR
Malaguarnera et al. 2012	LR	LR	UR	UR	LR	UR
Nabavi et al. 2014	LR	LR	LR	LR	UR	LR

Pereg et al. 2011	LR	LR	LR	LR	LR	LR
Sang Hak et al. 2015	LR	LR	UR	UR	LR	UR
Sharma et al. 2008	LR	UR	UR	HR	LR	LR
Vleggaar et al. 2008	LR	UR	UR	LR	LR	LR
Wolf et al. 1998	LR	LR	UR	UR	LR	UR
Wong et al. 2013	LR	LR	UR	UR	LR	UR
Ziada et al. 2013	LR	UR	HR	HR	LR	UR

[a] Some information obtained from previous publications (1, 2)

Supplemental Table 3. Complementary information on the characteristics of included studies

Study	BMI, change	Intervention/Placebo differentiable	Dietary control, Sig change	Compliance, side-effect
Aller et al. 2011	30.2 ± 4.5, no change	DB, no further information	3-day Food record, no change	Controlled, not mentioned
Bajaj et al. 2014	Baseline BMI not mentioned, no change	DB, no further information	Food recall, no change	95%, higher diarrhea incident in intervention group
Cox et al. 2014	24.6 ± 3.2, 24.4 ± 3.8 & 24.1 ± 3.1, changes NR	DB, identical	Supplements and foods containing prebiotics and probiotics were prohibited, no change [a]	95% compliance, *n*=3 participants on active treatment withdrew due to onset of headaches or uncomfortable GI symptoms
Eslamparast et al. 2014	32.1 ± 2.4, significant decrease in both groups	DB, identical	Food record + Advised to follow diet, no change	Assessed but not reported, abdominal pain in one subject resolved
Firouzi et al. 2015	29.2 ± 5.6, changes NR	DB, identical	3-day Food record, no change	26% attrition rate. Higher incidence of adverse effects with probiotics
Horvath et al 2016	NR	DB, identical	NR, dietary habit did not change	Excellent (more than 90% adherence). Abdominal discomfort and diarrhoea in some patients

Irwin et al. 2016	23.0 ± 3.3 & 24.6 ± 2.7, significant increase in placebo group	DB, identical	24 hour food record and FFQ, no changes	90%, at least 78% of supplements consumed. No serious adverse events, cases of bloating, diarrhoea, gas, stomach cramp reported
Kirpich et al. 2008	NR	Open-label	Prescribed diet, no further assessment	All completed, measurement of compliance or side-effect not mentioned
Kwak et al. 2014	NR	DB, identical	NR	90% compliance, digestive symptoms improved
Lefevre et al. 2017	25.5 ± 22.5 [a], changes NR	DB, identical	Supplements and foods containing probiotics were prohibited. No further assessment	Compliance >99%, well tolerated, mild and moderate cases of abdominal discomfort and diarrhea observed
Liu et al. 2010	NR	NR	Food intake increased (Likert scale), measurement not described	Compliance not reported, digestive symptoms improved
Liu et al. 2004	NR	SB, patients blinded	NR	Well tolerated and complied with no symptoms
Malaguarnera et al. 2012	27.3 ± 1.36, significant reduction in both	DB, no further information	Patients were given similar diet and exercise, food dairy every 2 days	No withdrawal, 100% tolerated
Nabavi et al. 2014	30.1 ± 3.61, significant reduction after intervention	DB, identical	Told not to alter their usual diet or consume any yogurt, 3d diet recall, no change	Good compliance, no adverse effects

Pereg et al. 2011	NR	DB, identical	NR	Two participants in probiotics group lacked compliance. No side effects reported
Sang Hak et al. 2015	NR	DB, identical	Regular diet was given in hospital, no further assessment	NR
Sharma et al. 2008	NR	NR	Some dietary restriction, no further assessment	NR, no side-effects
Vleggaar et al. 2008	NR	DB, identical	NR	Two drop out, no adverse effects
Wolf et al. 1998	NR, BW no change	DB, similar manufacturing information	NR	90%, mild nausea in treatment
Wong et al. 2013	30.2 ± 5.0, no change	Open-label	Diet and lifestyle instructions, no further assessment	80%, Minor dyspepsia in treatment groups
Ziada et al. 2013	NR	Open-label	NR	One patient in probiotics group lacked compliance. No side effects

[a] Information obtained from previous publications (1, 2)

Abbreviation: BMI: body mass index; BW: body weight; DB: double blind; NR: not reported

Supplemental Table 4. Sensitivity analyses of alternative levels of correlation coefficient (r) and their influence on overall meta-analysis results

Sensitivity analysis	**correlation coefficient (r)**		**Mean difference (95% CI), mm Hg**	**p value**	I^2
ALT	Alternative	0.2	-8.18 (-13.77, -2.59)	0.004	89%
		0.8	-8.09 (-12.86, -3.32)	0.001	94%
	Main	0.66	-8.05 (-13.07, -3.04)	0.002	93%
AST	Alternative	0.2	-8.05 (-14.99, -1.12)	0.02	95%
		0.8	-8.85 (-15.88, -1.83)	0.01	98%
	Main	0.69	-7.70 (-13.65, -1.76)	0.01	97%
ALP	Alternative	0.2	-3.53 (-7.26, 0.21)	0.06	0%
		0.8	-1.52 (-5.96, 2.91)	0.50	72%
	Main	0.6	-0.27 (-4.00, 3.47)	0.89	70%
GGT	Alternative	0.2	-8.74 (-12.12, -5.36)	<0.001	26%
		0.8	-9.09 (-17.79, -0.39)	<0.001	78%
	Main	0.81	-8.40 (-12.61, -4.20)	<0.001	53%

Albumin	Alternative	0.2	-0.31 (-0.75, 0.13)	0.17	37%
		0.8	-0.29 (-0.73, 0.15)	0.20	59%
	Main	0.6	-0.29 (-0.74, 0.16)	0.21	40%
Bilirubin	Alternative	0.2	1.00 (0.57, 1.42)	<0.001	0%
		0.8	1.01 (0.28, 1.74)	<0.01	51%
	Main	0.6	0.95 (0.48, 1.42)	<0.01	4%

1- Changes in metabolic factors of liver disease are presented as mean difference and 95% CI. Heterogeneity (I^2) is presented by %. A p-value <0.05 was considered significant.

References

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