

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

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26 **Abstract**

27 **Purpose:** The gut-liver interaction suggests that modification of gut bacterial flora using probiotics and
28 synbiotics may improve liver function. This systematic review and meta-analysis aimed to clarify the effect of
29 probiotics and synbiotics consumption on the serum concentration of liver function enzymes. **Methods:**
30 PubMed (MEDLINE), Cumulative Index to Nursing and Allied Health Literature, and Cochrane Library
31 (Central) were searched from 1980 to August 2017 for studies where adults consumed probiotics and/or
32 synbiotics in controlled trials and changes in liver function enzymes were examined. **Results:** A total of 17
33 studies (19 trials) were included in the meta-analysis. Random effects meta-analyses were applied. Probiotics
34 and synbiotics significantly reduced serum alanine aminotransferase (-8.05 IU/L, 95 % confidence interval (CI):
35 -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
36 gamma-glutamyl transpeptidase (-8.40 IU/L, 95% CI: -12.61 to -4.20; $p < 0.001$). Changes in the serum
37 concentration of alkaline phosphatase and albumin did not reach a statistically significant level. Changes to
38 bilirubin levels were in favour of the control group (0.95 $\mu\text{mol/L}$, 95% CI: 0.48 to 1.42; $p < 0.001$). Subgroup
39 analysis suggested the existence of liver disease at baseline, synbiotics supplementation and duration of
40 supplementation ≥ 8 weeks resulted in more pronounced improvement in liver function enzymes than their
41 counterparts. **Conclusions:** Probiotics and synbiotics may be suggested as supplements to improve serum
42 concentration of liver enzymes, especially when synbiotics administered for a period ≥ 8 weeks and in
43 individuals with liver disease.

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

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53 **Introduction**

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

64 Interactions between the gut and liver are well recognised, owing to the use of the term ‘gut-liver axis’. Liver
65 diseases, such as alcoholic liver disease (ALD) and liver cirrhosis (LC), are associated with changes in gut flora
66 [1,5,6]. However, it is unclear if changes in gut flora are the cause or consequence of liver conditions [7,8].
67 Nonetheless, health and function of the liver appear to be in a synergistic relationship with gut flora. For
68 example, in individuals suffering from a nonalcoholic fatty liver disease (NAFLD) and nonalcoholic
69 steatohepatitis (NASH), a dysbiosis of gut flora towards increased pathogenic Bacteroides and decreased
70 healthy firmicutes is observed [9-11]. Furthermore, endotoxins (e.g. LPS) produced by pathogenic bacteria of
71 the gut can increase cytokine production, leading to inflammation of the liver [12,13]. Conversely, healthy
72 bacteria may assist the removal of cholesterol from bile [14], reduce the production of LPS and hepatotoxins by
73 their competitive nature [15] and reduce intestinal permeability and bacterial translocation to extra-intestinal
74 sites such as the liver [16,17].

75 This gut-liver interaction has led to the development of interventions aiming to modify the gut bacterial flora, to
76 improve liver function and reduce or reverse the progression of chronic liver diseases [18-20]. Supplementation
77 of probiotics and synbiotics are one of these proposed interventions. Probiotics are defined as live
78 microorganisms that can have health benefits for the host if provided in adequate amounts and duration [21-23].
79 Synbiotics are defined as dietary supplements with a combination of probiotics and prebiotics (fermentable
80 dietary fibres that stimulate the growth and survival of probiotics) [24]. However, results of studies employing
81 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

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

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

119

120 *Data extraction and quality assessment*

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

127

128 *Data synthesis and analysis*

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

136

$$137 \quad r = \frac{SD_{Baseline}^2 + SD_{Final}^2 - SD_{Change}^2}{2 \times SD_{Baseline} \times SD_{Final}}$$

138

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

142

$$143 \quad SD_{Change} = \sqrt{SD_{Baseline}^2 + SD_{Final}^2 - (2 \times r \times SD_{Baseline} \times SD_{Final})}$$

144

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

150

151 *Sensitivity and subgroup analysis*

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

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

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

166

167 **Results**

168 *Overview of included studies*

169 Twenty-one studies were included in the qualitative synthesis for the effect of probiotics and synbiotics on
170 metabolic factors of liver function (Table 2). Of these, 17 studies (a total of 19 trials: Two studies [39,40] had
171 two arms eligible for the meta-analysis) were eligible for the meta-analysis. Four studies were excluded from the
172 quantitative analysis [41-44]. One study did not report the actual measures for liver enzymes (values were
173 estimated from figures) [41]. In the remainder, values were presented as median (percentile or range) and/or
174 changes were presented as a percentage change [42-44]. Attempts to acquire usable measures were not
175 successful. Of the 19 trials, 16 reported changes in ALT and AST, six reported changes in ALP, eight in GGT,
176 11 in albumin and 13 in bilirubin.

177 All twenty-one studies reported employing a randomised design. All studies, except one (cross-over design) [43]
178 followed a parallel design. Fourteen studies reported using a double-blinded protocol, and one study used a
179 single-blinded study design (Table 2). Three studies followed an open-label protocol [26,33,28] and two did not
180 report blinding [45,46]. Of the 14 double-blinded studies, 11 reported similarities between intervention and
181 placebo supplements but three did not report further information [25,18,47]. The methodologic quality
182 assessment of studies is presented in Supplemental Table 1. The highest Rosendal score of 87% was achieved
183 by four studies [48,42,40,44]. Overall, 16 out of 19 studies had good methodology quality with a Rosendal score
184 $\geq 60\%$ [30]. Similar findings were reported from the Cochrane risk of bias assessment tool (Supplemental Table
185 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].

186

187 *Participants and study protocols*

188 Table 2 presents the characteristics of included studies. Participant's age ranged from 23 – 70 years old. Of the
189 21 studies, five reported using synbiotics [19,41,47,33,39], one had both synbiotics and probiotics arms [39],
190 and the remainder used a probiotic intervention. Four studies used one strain [18,47,27,28], one study had two

191 separate arms with single and multiple strains [40], and the remainder used multiple strains of probiotic bacteria
192 in their supplements. Synbiotic interventions used fructo-oligosaccharides (FOS) [19,47,33], arabino guard [39]
193 or a combination of beta-glucan, inulin, pectin and resistant starch [41]. The duration of supplementation varied
194 from 6 days [26] to 28 weeks [19]. Two studies used yoghurt as the probiotics medium [45,42], and capsules or
195 sachets were used to deliver probiotics or synbiotics in the other studies. Daily probiotics doses varied from 3
196 $\times 10^6$ CFU [28] to 5×10^{10} CFU [18].

197 Participants in the majority of studies had different extents of liver disease, including NAFLD [25,19,42],
198 NASH [47,33], ALD [26,20], HE [18,41,46,28], primary sclerosing cholangitis (PSC) [43], LC [44,50] and
199 chronic liver disease (not further specified) [51,45]. One study included participants with type 2 diabetes
200 mellitus [48], one included patients infected with human immunodeficiency virus (HIV) [27], and three studies
201 included healthy participants [39,49,40]. Only ten studies reported baseline body mass index (BMI) of
202 participants [25,18,19,48,47,42,33,39,49,40], and all except for two study [39,40] reported mean BMI ≥ 25
203 kg/m². Nine studies reported changes in body weight (BW) or BMI [25,18,19,48,47,42,27,33]. Of these, two
204 reported a significant decrease in BW in both intervention and control groups [19,47], one observed a reduction
205 in the intervention group [42] and five reported no changes in BW or BMI after the intervention period
206 [25,18,27,33,39] (Supplemental Table 3).

207 Seven studies reported a method to measure dietary intake changes during the intervention (food record or
208 recall) and reported no significant changes [25,18,19,48,47,42,39]. One study used a Likert scale to measure
209 food intake and reported an increase in consumption [45], four reported dietary advice and prescription
210 [26,28,49,40] and the remainder did not report using any method for controlling dietary intake. Compliance to
211 supplementation was reported in thirteen studies [18,26,51,45,41,47,42,33,28,39,49,40,44] using the proportion
212 (%) of participants that completed the study and adhered to the supplementation strategy. The majority of
213 studies reported more than 90% completion rate and supplementation was reported to be well tolerated.
214 However, incidence of diarrhoea was observed in four studies [18,39,49,44] and abdominal discomfort in
215 another five studies [19,39,49,40,44]. One study reported high attrition rate (26%) and adverse effects in the
216 intervention group [48] (Supplemental Table 3).

217

218 *Meta-analysis results*

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

221 significant reduction of -8.05 IU/L (95 % confidence interval (CI): -13.07 to -3.04; $p = 0.002$; 16 trials, 990
222 participants) (Figure 2). The observed reduction was significantly more pronounced in the synbiotics subgroup
223 (-20.13 IU/L, 95% CI: -22.47 to -17.80; $p < 0.001$; 4 trials, 156 participants) compared to the probiotics
224 subgroup (-4.83 IU/L, 95% CI -9.34 to -0.33; $p = 0.04$; 12 trials, 834 participants) (test for subgroup difference
225 $I^2 = 97.1\%$; $\rho < 0.001$). The meta-analysis showed an overall considerable heterogeneity ($I^2 = 93\%$; $\rho < 0.001$).
226 The source of this high heterogeneity appeared to be related to the probiotics subgroup ($I^2 = 89\%$, $p < 0.00001$)
227 as opposed to the synbiotics subgroup ($I^2 = 0\%$, $p = 0.73$) (Figure 2).

228 The meta-analysis for the mean difference in serum AST concentrations also showed a significant overall
229 reduction with probiotic or symbiotic interventions (-7.79 IU/L, 95% CI: -13.93 to -1.65; $p = 0.01$; 16 trials, 990
230 participants) (Figure 3). The significant reduction was only observed in the synbiotics subgroup (-23.61 IU/L,
231 95% CI: -26.63 to -20.58; $p < 0.001$; 4 trials, 156 participants). The reduction in AST observed in the probiotics
232 subgroup was not statistically significant. The overall heterogeneity level observed was considerable ($I^2 = 97.7$
233 $\%$; $\rho < 0.00001$) and was primarily observed in the probiotics subgroup ($I^2 = 96\%$; $\rho < 0.00001$) rather than the
234 synbiotics subgroup ($I^2 = 0\%$; $\rho = 0.85$) (Figure 3).

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

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

242 No significant differences were observed between the intervention and control groups for serum levels of
243 albumin (Figure 6). However, the results were in favour of placebo (control) for bilirubin changes (0.95 $\mu\text{mol/L}$,
244 95% CI: 0.48 to 1.42; $p < 0.001$; 13 trials, 806 participants; $I^2 = 4\%$). Although meta-analysis results of the
245 synbiotics subgroup also suggested an increase in bilirubin, the difference did not reach a statistically significant
246 level (Figure 7).

247

248 *Sensitivity and subgroup analysis*

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

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

270 Sensitivity to the methodology quality of included studies was also conducted by excluding studies with <60%
271 Rosendal scores [52,53] or those with a high risk of bias in the Cochrane assessment tool. Excluding two studies
272 [26,46] from ALT and AST, one study [26] from GGT, three studies [45,46,28] from albumin, and two studies
273 [45,46] from bilirubin analyses, did not result in significant changes to the overall meta-analysis results or
274 heterogeneity.

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

277 participants with some degree of liver disease at baseline had a more pronounced improvement in ALT, AST
278 and GGT levels compared to their otherwise healthy (no reported liver disease) counterparts. However, the
279 bilirubin reduction was more favourable in the placebo arm of liver disease subgroup compared to the otherwise
280 healthy subgroup (although the subgroup difference was not significant). On the other hand, the magnitude of
281 albumin reduction was greater in the otherwise healthy subgroup compared to the liver disease subgroup (Table
282 3).

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

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

301

302 **Discussion**

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

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

309 Although the disruption of gut flora may be both a cause and/or consequence of impaired liver function [7,8],
310 results of this systematic review and meta-analysis confirm that modification of gut flora via probiotics and
311 synbiotics consumption affects liver function. However, the mechanism/s of the effect of gut bacteria on liver
312 function and health are not clear. There are a few pathways suggested for this relationship. Probiotics and
313 synbiotics may enhance the integrity and tightness of the intestinal epithelium [55], thereby modulating chronic
314 damage to these cells (e.g. by ethanol in alcoholic liver disease) and restoring intestinal permeability [56,17].
315 This may, in turn, reduce bacterial translocation [57] and reduce the production of cytokines, tumour necrosis
316 factor (TNF- α) and hepatotoxins [58,17], which can lead to the inflammation of liver and development of liver
317 disease [19]. Probiotics have also shown potential in the synthesis of vitamins B and K [45,59] and facilitate the
318 breakdown and digestion of polyphenols (e.g. flavanols, flavan-3-ols, tannins, lignans) [59]. These components
319 are effective antioxidants with the potential to moderate the hepatic oxidative stress caused by inflammatory
320 cytokines and hepatotoxins [60]. Furthermore, the gram-negative bacterial overgrowth that exists in more than
321 50% of cirrhotic patients [56] may increase bacterial translocation and the production of hepatotoxins (LPS and
322 cytokines) [17]. Probiotics and synbiotics may lower gram-negative and pathogenic bacteria through their
323 competitive behaviour [61], and reduce inflammation [62,15]. Based on subgroup analysis results from the
324 present study, reductions in ALT, AST and GGT after probiotics and synbiotics consumption appear to be more
325 pronounced in participants with liver disease compared to their otherwise healthy counterparts.

326 A controversial finding of the present study was the observation that probiotics and synbiotics consumption
327 increased blood bilirubin levels. However, it is important to note that the meta-analysis results were sensitive to
328 one study [26], such that excluding this study resulted in a non-significant effect of supplementation on bilirubin
329 level. This study was the only trial that investigated the effect of less than one week (5 days) supplementation on
330 liver enzymes. Since the participants had an alcohol-induced liver injury, it is possible that the duration of
331 probiotics consumption was not sufficient to affect bilirubin removal from the body, especially for participants
332 of this study who were heavy alcohol drinkers before the commencement of the trial with their last drink
333 occurring within 48 hours prior to the admission [26]. Chronic alcohol consumption can cause gram-negative
334 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/pl acebo, Capsule	12	<i>L. bulgaricus</i> + <i>S. thermophilus</i>	5×10^8	NAFL D	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/pl acebo, Capsule	8	<i>L. GG</i>	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, Austral ia	Probiotics/ placebo, sachet	~22	<i>B. animalis</i> <i>lactis</i>	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, Austral	Probiotics/ placebo, sachet	~22	<i>L. acidophilus</i> + <i>B.animalis</i>	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*

<i>infantis</i>														
Horvath et al. 2016 ⁵	DB, PC, R, Austria	Probiotic/placebo, Powder	24	<i>B. bifidum</i> + <i>B. lactis</i> + <i>L. acidophilus</i> + <i>L. brevis</i> + <i>L. Casei</i> + <i>L. salivarius</i> + <i>L. lactis</i> +	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	DB, R, PC, Austral ia	Probiotic/placebo capsule	8	<i>L. acidophilus</i> , <i>B. lactic</i>	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	DB, R, PC, Austral ia	Synbiotic/placebo powder	8	<i>L. acidophilus</i> , <i>B. lactic</i> + Arabino Guard	2.5 × 10 ¹⁰	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	<i>B. bifidum</i>	1 × 10 ⁹	ALD	42.3 ±	32 (32/0)	ALT: 49.84 ± 6.94	-13.15 ± 4.62	ALT: 49.74 ± 7.17

et al.	Russia	standard		<i>and L.</i>		1.1	C:34	AST: 101.06± 4.33	-46.39± 5.42*	AST: 106.80± 12.78	-30.37± 9.72*	
2008		therapy/ standard therapy, capsule		<i>plantarum</i>				GGT: 171.48±26.0	-28.59± 22.81	GGT: 152.51± 20.16	-5.62± 19.03	
								BIL: 20.75± 1.06	-10.24± 0.84	BIL: 24.15± 1.98	-11.67± 1.59*	
Kwak et al. 2014	DB, PC, R, Korea	Probiotic/pl acebo, Capsule	4	<i>B. bifidum,</i> <i>B. lactis,</i> <i>B. longum,</i> <i>L.</i> <i>Acidophilus,</i> <i>L.</i> <i>rhamnosus,</i> <i>and S.</i> <i>thermophilus</i>	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	<i>Bacillus</i> <i>strains</i> <i>(subtilis,</i> <i>coagulans,</i> <i>licheniformis</i>	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

				<i>, cereus,</i>								
				<i>pumilus &</i>								
				<i>clause)</i>								
Liu et al. 2010	C, R, China	Probiotic/control, yogurt	2	<i>L. bulgaricus,</i> <i>L. acidophilus,</i> <i>B. bifidus,</i> <i>and S. thermophilus</i>	3×10^9	CLD	48.62 ± 41 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	$-4.12 \pm 45.72^*$ 0.92 ± 4.18
Liu et al. 2004³	SB, R, PC, China	Synbiotic/Placebo, Capsule	4	<i>Pediococcus pentoseceus,</i> <i>Leuconostoc mesenteroides, L. paracasei</i> <i>and L. plantarum +</i>	4×10^{10}	MHE Prebiotic	55 ± 12	20 (20:0) C: 15	Approx. ³ ALT: 245 ± 25 BIL: 275 ± 25 ALB: 25 ± 5	Approx. ³ $-160 \pm 10^*$ $-140 \pm 10^*$ $5 \pm 5^*$	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								
Malagua rnera et al. 2012	DB, R, PC, Italy	Synbiotic/pl acebo, Capsule	24	<i>B. longum</i> + FOS	5×10^9 Prebioti c: NR	NASH 5.4	$46.9 \pm$ (18/16)	34 C:32	ALT: 101 ± 24.7 AST: 109 ± 23.2 BIL: 10.4 ± 7.9 ALB: 43 ± 8	$-53.9 \pm 16.38^*$ $-69.6 \pm 19.44^*$ $-0.3 \pm 6.71^*$ 1 ± 6.76	ALT: 96.1 ± 24.2 AST: 107.1 ± 21.4 BIL: 10.1 ± 7.6 ALB: 42 ± 7	$-38 \pm 18.38^*$ $-45.9 \pm 17.59^*$ $-0.1 \pm 6.47^*$ 1 ± 6.76
Nabavi et al. 2014⁴	DB, R, PC, Iran	Probiotic/ conventiona l yogurt	8	<i>L.acidophilu</i> s and <i>B. lactis</i>	1.1 $\times 10^7$	NAFL D	$42.75 \pm$ (18/18)	36 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/Pl acebo	7	<i>L. subtilis</i> and <i>S.</i> <i>faecium</i>	6×10^6	AH	$52.7 \pm$ (38/22) ²	60 C:57	ALT: 83 ± 126 AST: 166 ± 213 ALP: 132 ± 54 GGT: 510 ± 629 ALB: 35 ± 7	$-35 \pm 94.95^*$ $-102 \pm 184.58^*$ $-17 \pm 44.73^*$ $-176 \pm 547.03^*$ $2 \pm 5.88^*$	ALT: 93 ± 152 AST: 148 ± 130 ALP: 124 ± 39 GGT: 553 ± 953 ALB: 38 ± 8	$-27 \pm 102.91^*$ $-79 \pm 94.74^*$ $-21 \pm 31.4^*$ $-225 \pm 817.80^*$ 1 ± 6.76
Pereg et all 2011	DB, R, PC,	Probiotic/ placebo,	24	<i>L.</i> <i>Acidophilus</i>	8×10^{10}	LC	$65.9 \pm$ 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		+ <i>L.</i>					BIL: 20.52 ± 8.55	-1.71 ± 7.05	BIL:22.23 ± 10.26	-3.42 ± 9.17
				<i>Bulgaricus</i> +					ALB: 36 ± 5	1 ± 5	ALB: 37 ± 6	-1 ± 5
				<i>B. lactis</i> + <i>S.</i>								
				<i>thermophiles</i>								
Sharma et al. 2008	R, C, India	Probiotic + Lactulose/ Lactoluse, Capsule	4	<i>S. faecalis,</i>	5 × 10 ⁸	MHE	43.7 ± 10.0	35 (26/9) ²	ALT: 55.0 ± 32.1	-15.3 ± 22.82*	ALT: 42.9 ± 20.9	-8.6 ± 15.1*
				<i>C. butyricum,</i>				C: 35	AST: 51.5 ± 32.8	-14 ± 23.49*	AST: 57.3 ± 23.4	-20.5 ± 16.23*
				<i>Bacillus mesentricus,</i>					BIL: 37.62 ± 20.52	-5.13 ± 17.1*	BIL: 34.2 ± 20.52	-11.97 ± 16.54*
				<i>lactic acid bacillus</i>					ALB: 31 ± 6	1 ± 5*	ALB: 31 ± 5	2 ± 4.47*
Vleggaar et al. 2008⁵	DB, R, PC, CO, The Netherlands	Probiotic/pl acebo, capsule	12	<i>L. acidophilus,</i>	10 ¹⁰	PSC	45 (28-70) ⁶	14 (13/1)	ALT: 119 (35-580) ⁵	-27% (-151, 223) ⁵	ALT: 119 (35-580) ⁵	-26% (-254, 59) ⁵
				<i>L. casei,</i>					AST: 101 (33-423) ⁵	-16% (-207, 57) ⁵	AST: 101 (33-423) ⁵	-15% (-143, 70) ⁵
				<i>L. salivarius,</i>					GGT: 260 (45-581) ⁵	-11% (-52, 26) ⁵	GGT: 260 (45-581) ⁵	-5% (-62, 31) ⁵
				<i>L. lactis,</i>					BIL: 17 (7-58) ⁵	-13% (-57, 42) ⁵	BIL: 17 (7-58) ⁵	-15% (-106, 45) ⁵
				<i>B. bifidum and B. lactis</i>					ALB: 40 (31.7-45) ⁵	0% (-9, 11)	ALB: 40 (31.7-45) ⁵	-1% (-8, 11) ⁵
Wolf et	DB, R,	Probiotic/pl	3	<i>L. reuteri</i>	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,	acebo,					(20/1) ²	AST: 26.35± 3.59	1.79± 3.27	AST: 28.14± 2.40	5.99± 3.45	
	USA	packets					C: 18	ALP: 83.83± 5.99	0 ± 5.35	ALP: 83.83± 5.99	5.99± 5.35	
								GGT: 50.90± 12.57	-8.39± 11.01	GGT: 33.53± 4.79	0.0± 5.01	
								ALB: 46 ± 1	0 ± 0.89	ALB: 44 ± 1	1 ± 0.89	
								BIL: 11 ± 1	1 ± 1.61	BIL: 77 ± 1	1 ± 1.61	
Wong et al. 2013	R, C,	Synbiotic +	24	<i>L. plantarum,</i>	4 × 10 ⁸	NASH	42 ± 0	10 (8/2)	ALT: 96 ± 75	-26 ± 91	ALT: 72 ± 30	2 ± 41
	Hong Kong	lifestyle/ lifestyle, sachet		<i>L. bulgaricus,</i> <i>L. acidophilus,</i> <i>L. rhamnosus,</i> <i>B. bifidum</i> + FOS	Prebiotic c: 3g			C:10	AST: 50 ± 25	-13 ± 31	AST: 38 ± 15	23 ± 32
Ziada et al. 2013	R, C,	Probiotic/ Control, Capsule	4	<i>L. acidophilus</i>	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 $\mu\text{mol/l}$, 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.

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

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

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

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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(((((("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, <i>n</i> =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	I²	
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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