Randomised clinical trial: *Bifidobacterium lactis* NCC2818 probiotic versus placebo, and impact on gut transit time, symptoms and gut microbiology in chronic constipation"

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Short running title

Effect of a probiotic in constipation

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ABSTRACT (250 words)

Background: Constipation is a prevalent gastrointestinal disorder. Patient dissatisfaction with prescribed medications is common, and there is need for alternative management strategies. Evidence shows that *Bifidobacterium* species may be beneficial in constipation.

Aim: To investigate changes in physiological and clinical measures of gut function in patients with chronic constipation following the consumption of *Bifidobacterium lactis* NCC2818, compared to placebo.

Methods: Participants were randomised to a 4 week supplementation with *B. lactis* NCC2818 or placebo. Gut transit time was measured using the radio-opaque marker technique, while symptoms and quality of life were assessed using validated questionnaires. Gut microbiota composition was assessed using quantitative polymerase chain reaction. Analysis of covariance was used for normally distributed variables, and Mann-Witney test for non-normally distributed variables.

Results: Seventy five participants were randomised. There was no significant difference between the probiotic and placebo groups in gut transit time change from baseline to week 2 (-11.7 h, SD 33.0 h *vs* -12.9 h, SD 33.6 h; p=0.863) or to week 4 (-20.4 h, SD 32.5 h *vs* -8.7 h, SD 33.8 h; p=0.103). There were also no improvements in stool output, symptoms or quality of life. No differences were found in *Bifidobacterium* concentrations between the probiotic and placebo groups at week 4 (9.5 $\log_{10}$/g dry faeces, SD 0.3 *vs* 9.4 $\log_{10}$/g, SD 1.0; p=0.509).

Conclusions: *B. lactis* NCC2818 was not effective in the management of mild chronic constipation. This study highlights the importance of further studies and their publication to better understand the strain-specific effects of probiotics.

INTRODUCTION

Constipation is a common burdensome functional bowel disorder in which symptoms of difficult, infrequent, or incomplete defecation predominate [1]. Management remains challenging, with constipation representing a significant healthcare burden costing up to $7,522 per year per patient [2]. Despite the range of treatments available including laxatives and fiber supplements, approximately half of patients are dissatisfied with current management strategies, with the main complaint relating to limited efficacy [3]. Hence, there is an unmet need for alternative treatments for the management of constipation-related symptoms.

Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit to the host" [4]. In the past decade, research has focused on their effectiveness in chronic constipation, possibly mediated through an effect on regulating gut dysmotility by impacting the gut microbiota, with the subsequent release of metabolites, including short-chain fatty acids (SCFA) during fermentation [5-7], that are known to interact with the enteric nervous system and the immune system [5].

A systematic review and meta-analysis revealed 14 RCTs investigated the effect of probiotics in adults with chronic constipation [8]; this suggested that probiotics may improve whole gut transit time (WGTT), stool frequency and stool consistency, with sub-group analysis based upon species indicating significant effects in favour of *Bifidobacterium lactis* [8]. However, despite favourable results reported, the clinical importance of probiotics in constipation remains uncertain, due to small study sample sizes, high heterogeneity in the study design of the individual studies, and limitations in study methodologies, including the use of non-validated assessment tools. Nevertheless, the promising findings of the meta-analysis, justify further research to identify *B. lactis* strains that may be effective in the management of chronic constipation.

Therefore, the aim of this study was to investigate changes in gut transit time and gastrointestinal symptoms following 4 weeks consumption of a probiotic strain in a randomized, double-blind, placebo-controlled manner, in adults with constipation.

MATERIAL AND METHODS

Protocol

The study was a randomized, parallel-group, double-blind, placebo-controlled, adaptive randomised controlled trial (RCT). The intervention period was four weeks, while the primary outcome (change in WGTT) was assessed at week 2 (i.e. mid-intervention). Participants were recruited from the community and were required to attend five study visits: the screening visit, where informed consent was taken, inclusion/exclusion criteria assessed, and eligible participants enrolled to the study; the baseline visit, where final eligibility tests and randomisation were undertaken, and assessment of clinical, physiological and stool microbiological outcomes were performed; the mid-intervention (week 2) and end of intervention (week 4) visits, where assessment of clinical, physiological and stool microbiological outcomes were also performed; and the follow-up (week 8) visit, where evaluation of clinical outcomes was performed. The trial took place within Queen Mary University of London from January 2014 to October 2015.

Participants

Adults from the general population with mild constipation were enrolled to this study. Inclusion criteria were: 18-65 years old; self-reported stool frequency of 3 or less bowel movements per week; self-reported stool consistency of type 1-4 on the Bristol Stool Form Scale; fulfilment of modified Rome III diagnostic criteria for functional constipation [9]; mild constipation determined using a score of 8-15 on the Cleveland Clinic Constipation Score (CCCS: max score = 30) [10]; body mass index (BMI) of 18.5-29.9 kg/m^2, and willingness to use effective contraception for the duration of the trial (for females only).

Exclusion criteria were: gastrointestinal, neurological, cardiovascular, endocrine, renal or other chronic diseases likely to affect gut motility; prior abdominal surgery; ongoing therapy with medications known to affect gut motility; alarm features and moderate or severe anorectal problems; lactose intolerance, cow's milk allergy and soya allergy; regular consumption of probiotics, prebiotics, fiber supplements and laxatives; use of antibiotic within 4 weeks of study onset; high fibre intake, defined as >18 g/d assessed using the Block Fibre Screener [11]; high anxiety and depression score defined as a score >11 on the Hospital Anxiety and Depression Score (HADS) questionnaire, and ongoing alcohol, drug or medication abuse. At the baseline visit and prior to randomization, participants with short whole gut transit time (WGTT), defined as <24 h measured using radio-opaque markers were also excluded, as were participants reporting being 'markedly better' between screening and baseline (score +3 on the Global Constipation Symptom Score at baseline visit).

Randomization, blinding and study products

After fulfilling eligibility criteria at the baseline visit, participants were randomly allocated to either the probiotic or placebo. Dynamic allocation (minimisation) was chosen, and randomisation was performed through Medidata Balance (www.imedidata.com) by the study team at baseline visit. The latter was set-up independently and was not accessible by the study site staff except when receiving an allocation assignment for a specific patient at the baseline visit, ensuring allocation concealment. Randomisation was stratified by gender and, for females, by menstrual cycle phase at baseline (mid-follicular/mid-luteal/post-menopausal), as both have been shown to influence WGTT [12 13]. Once randomisation was performed online, Medidata Balance would display the product code (PrXXX) the participant had been allocated to. Patients and investigators were blinded to the treatment allocation and blinding was maintained until the end of the data analysis.

The probiotic group received spray-dried *B. lactis* NCC2818 at a dose of 1.5×10^{10} CFU/d, with a minimum end of shelf-life count of 8×10^{9} CFU/d, while the placebo received maltodextrin powder. The study

products were supplied by Nestlé (Switzerland) in a milk powder-based format and packaged in aluminium sachet sticks, with each stick containing 24 g of powder. The base composition of the study products was matched and final *B. lactis* and placebo products were indistinguishable in terms of appearance, colour, texture and flavour. Participants were instructed to consume the contents of one stick mixed with 200 ml of water each day for 4 weeks. All study products were refrigerated (4-8^{o}C) both at the study site and by the participants throughout the intervention period. Viability of the probiotic in sachets was assessed and confirmed during and at the end of the study by Nestlé.

Clinical outcomes

The primary outcome was change in WGTT after 2 weeks (i.e. baseline vs mid-intervention) between probiotic and placebo. The secondary clinical outcomes included stool output, constipation symptoms and severity, and constipation-related quality of life. Palatability and compliance with the study product, as well as dietary intake, physical activity, and anxiety and depression, were also assessed.

Whole gut transit time

Whole and regional gut transit times were assessed using a standard radio-opaque markers (ROM) technique [14]. Participants were provided with a box containing 12 capsules, each containing 6 ring markers (PRODIMED®, Plastimed, France). Participants ingested two capsules per day (total of 12 ROM per day) for six consecutive days prior to the baseline, mid-intervention and end of intervention visit. On the seventh day (study visit day), an abdominal x-ray was performed to determine location of any retained markers from which both regional and WGTT could be calculated.

Stool output, constipation outcomes and gut symptoms

Stool frequency and stool consistency were assessed using a daily stool diary and the Bristol Stool Form Scale [15], respectively, throughout the study. The total number of bowel movements (TBM), the number

of spontaneous bowel movements (SBM), and the number of complete spontaneous bowel movements were calculated based on the averages over the seven days prior to the baseline, mid-intervention and end-of-intervention visits.

Participants were also required to complete the validated CCCS [10], Patient Assessment of Constipation Symptoms (PAC-SYM) [16], and Patient Assessment of Constipation Quality of Life (PAC-QoL) [17] at each study visit. In addition, the Global Constipation Symptom Score (GCSS) was completed at all visits except for the screening visit, to assess current symptom severity compared to those experienced at baseline on a scale from -3 (markedly worse) to +3 (markedly better) [16]. Both PAC-SYM and PAC-QoL were used to define responders from non-responders. A reduction of ≥1 point in PAC-SYM or PAC-QoL score was considered a minimal clinically important difference [17 18].

Bloating was assessed at all visits other than the screening visit using a 100 mm visual analogue scale (VAS) anchored by the word descriptions “not at all” and “all the time” at each end. A number of GI symptoms were also measured in order to assess tolerance of the study product (abdominal discomfort, abdominal bloating/distention, flatulence, nausea) by means of a composite GI tolerance score, 6 days after the start of product consumption, and at the end of the intervention period. Participants were asked to rate the frequency and severity of these symptoms on a 5-point Likert scale ranging from 0 (absent) to 4 (very severe) over the past 24 h, as well as over the past 6 days [19 20]. Responses to the questions were used to calculate a mean GI tolerance score for each visit using the following formula:

$$\text{Mean GI tolerance score} = \frac{\text{Sum of (response to individual question x corresponding frequency)}}{\text{Sum of frequency for all answered questions}}$$

Palatability and compliance

Palatability of the study product was assessed 6 days after baseline and at the end of intervention visit using a VAS anchored by the word descriptors “worst” and “best” at each end. Diaries were completed every day during the 4 week intervention period to report intake, and hence compliance, of the

probiotic/placebo. Non-compliance was defined *a priori* in the following three ways: (a) participants with >2 consecutive days without any probiotic/placebo intake during the first two weeks of the intervention period (i.e. between baseline and mid-intervention visits); or (b) >3 consecutive days without probiotic/placebo intake during the last two weeks of the consumption period (i.e. between mid and end of intervention visits); or (c) no probiotic/placebo consumption on all 3 days prior to mid-intervention visit or end of intervention visit.

Confounding factors

A three day unweighed food and drink diary was completed prior to each study visit to assess dietary intake. Food diaries were analysed for nutritional composition using the Nutritics Professional Nutrition Analysis software (Nutritics v3.74, Ireland). Physical activity was also measured at each study visit using the validated International Physical Activity Questionnaire (IPAQ) [21]. Anxiety and depression were assessed at each visit via the HADS questionnaire which contains two subscales, with seven items for anxiety (HADS-A) and seven for depression (HADS-D) [22].

Microbiological outcomes

One fresh stool sample was collected at baseline, mid-intervention (week 2) and end of the intervention (week 4) from each participant and used for microbiological analysis. Participants were provided with a bespoke stool collection kit and were instructed to provide a fresh stool sample at the next scheduled visit or at a convenient time within 5 days from the scheduled study visit. The stool sample had to be returned to the study site no more than 3 hours following defaecation. Participants were instructed to store the fresh stool sample in a Styrofoam box with frozen gel packs (provided by the researchers) to preserve the sample in a cold temperature and minimise loss of cell viability [23]. Stool sample collection was an optional task for participants in order to reduce the burden of study participation; hence microbiology data were not available for all participants.

Quantitative polymerase chain reaction (qPCR) was used to quantify the gut microbiota in this study from phylum to species level. Fresh stool was diluted 1:3 with sterile phosphate buffered saline/30% glycerol buffer, homogenised and frozen at 80 °C until analysis. DNA extraction was performed using the FastDNA SPIN Kit for Soil (MP Biomedicals Europe, Illkirch, France). The reporter system used was SYBR Green Supermix (Biorad, California, USA), and primers were purchased for the following assays: universal bacteria, *Lachnospiraceae, Roseburia* spp. (includes *Eubacterium rectale*), *Faecalibacterium prausnitzii, Bacteroides* spp., *Prevotella* spp., *Bifidobacterium* spp. and *Archaea* (Sigma-Aldrich Company Ltd, Dorset, UK) (Supplementary Material). These target bacterial groups were selected based on bacterial groups and species that have been previously shown to either be different between healthy and constipated populations or to be affected by the consumption of probiotics [5]. Standards were provided by the Rowett Institute, University of Aberdeen, UK.

Stool SCFA were quantified using gas-liquid chromatrography (9890A System, Agilent Technology, California, USA). Stool pH was measured using a pH meter (Seven Compact, Meter Toledo, Leicester, UK) equipped with a glass electrode specifically designed for slurries (pHRESH Electrode, Beckman Coulter, High Wycombe, UK). Water content was determined via lyophilisation of frozen samples (LyolabA; LSL Secfroid SA, Aclens-Lausanne, Switzerland).

Safety outcomes

Adverse events were recorded at each study visit and throughout the study using a pre-specified study document. All adverse events were summarised according to the System Organ Class (SOC) and Preferred Term (PT).

Sample size calculation and interim analysis

The study started with two groups (high dose probiotic group *vs* placebo group), with the plan to perform an interim analysis that would indicate whether a third, low dose probiotic group was to be included.

Hence, the sample size calculation was performed for 3 groups and was based on the primary outcome of change in WGTT at mid-intervention (week 2) in the high dose probiotic group compared to placebo group, using data from a previously published RCT, in which a mean change in WGTT of -28 h (95% CI -38.9 h to -17.3 h) was observed following probiotic supplementation compared to 1 h (95% CI -5.7 to 8.3) following placebo [24]. Assuming a power of 80% and a 2-sided significance level of 5%, the sample size was estimated to be 36 participants per group with a total of 108 for all three groups. The attrition was estimated at 10% leading to an overall sample size of 120 patients across all three groups.

An interim analysis was performed as planned by an independent panel after 39 subjects had completed the study. Based on the observed effect size and the conditional power, the panel decided that the study should continue as a two-arm RCT (high dose probiotic *vs* placebo) with 36 participants per group and a final sample size of 72.

Statistical analysis

Baseline characteristics of study participants were analysed using mean and SD for continuous variables and counts and percentages for categorical variables. Continuous variables were assessed for normality using histograms and Q-Q plots. For normally distributed variables, analysis of covariance (ANCOVA) was used whereas for non-normally distributed variables Mann-Whitney tests were used. Changes in outcomes from baseline were compared between groups using an ANCOVA model, with the baseline measurement as a covariate, and stratification factor (menstrual cycle phase) and study group as fixed effects. Categorical outcomes were analysed using Chi squared test.

The primary outcome was also analysed using ANCOVA, which had baseline WGTT as a covariate, and menstrual cycle phase and study group as fixed effects. The alpha (α) spent at interim analysis was 0.0032. Therefore, to control for multiple comparisons, the α was adjusted for the primary outcome analysis at α=0.0468. Hence, a P value of <0.0468 was considered statistically significant for the primary outcome.

The majority of the statistical analysis was performed using SAS® software (version 9.4, North Carolina, USA) except for the statistical analysis on the absolute values which was performed using IBM SPSS statistics for Windows (version 22, New York, USA).

All analyses were performed in the intention to treat (ITT) population. For the microbiological outcomes, the ITT population included all the participants who provided a stool sample for each analysis and for each study time point. A per-protocol (PP) analysis was performed for the primary outcome analysis only, and consisted of all participants included in the ITT population who adhered to all protocol requirements without any major protocol deviations. Missing data were assumed to be missing at random and no imputation was performed. P values of < 0.05 were considered statistically significant.

Ethical issues

Ethical approval for the study was granted by the Camberwell St Giles NHS Research Ethics Committee (13/LO/0891). The study was registered at ClinicalTrials.gov (registration number NCT01874301).

RESULTS

One hundred and seven participants were eligible following screening and enrolled to the study, of whom 75 were randomised and received the study product (probiotic or placebo) (Figure 1). Demographic and baseline characteristics of study participants included in the ITT population are summarised in Table 1.

Clinical outcomes

Whole gut transit time

In the ITT population, there was no significant difference in the primary outcome of change in WGTT from baseline to mid-intervention between the probiotic (-12 h, SD 33 h) and placebo (-13 h, SD 34 h) (p=0.863).

Similarly, in the PP population, there was no significant difference in the change in WGTT from baseline to mid-intervention between the probiotic (-11 h, SD 32 h) and the placebo (-17 h, SD 33 h) (*p*=0.433).

There was no significant difference in change in WGTT from baseline to end of intervention between the probiotic (-20 h, SD 33 h) and placebo (-9 h, SD 34 h) (p=0.103) (Table 2). There were no significant differences in WGTT at baseline, mid-intervention or end of intervention between groups, although the difference at end of intervention approached significance (p=0.062) (Table 2). Regional gut transit time (left, right and rectosigmoid) were also not significantly different between the groups (Table 2).

No significant difference between the probiotic and placebo groups was shown for the odds of having WGTT <72 h (i.e. upper limit of normal [25]) at mid-intervention (OR 1.8, 95% CI 0.53 -5.0) and end of intervention (OR 0.8, 95% CI 0.2 -2.7).

Stool output, gut symptoms and constipation outcomes (Table 3)

No significant differences were observed between the probiotic and placebo in the change in total bowel movements (TBM) from baseline to mid-intervention (p=0.836) and from baseline to end of intervention (p=0.831). Similarly, no differences were found for spontaneous bowel movements (SBM) and complete spontaneous bowel movements (CSBM). Furthermore, no significant differences were observed between the probiotic and placebo groups in the change in stool consistency from baseline to mid-intervention (p=0.848) and end of intervention (p=0.936). No significant difference was observed for the change from baseline in GI tolerance or bloating between the groups during the intervention period.

There was no significant difference between the probiotic and placebo groups in the change in PAC-SYM global score from baseline to mid-intervention (p=0.474), end of intervention (p=0.780) and follow-up (p=0.907) (Table 4). Similarly, no significant difference was observed between the probiotic and placebo groups in the change in the abdominal, rectal, and stool sub-scores, at any of the study time points (Supplementary Table 1).

In accordance with the lack of difference found for WGTT, stool output and symptom scores, no significant difference was found between the probiotic and placebo in the change with respect to PAC-QoL global score from baseline to mid-intervention (p=0.836), end of intervention (p=0.315) and follow-up (p=0.977) (Table 4). Similarly, no change was observed in the PAC-QoL sub-scores between the two groups at any of the study time points (Supplementary Table 2). Furthermore, there was no significant difference in the change from baseline in CCCS and GCSS scores between the probiotic and placebo group at any study time points (Table 4).

There was no significant difference in the number of responders (defined as a reduction of ≥1 point in PAC-SYM) between the probiotic and placebo at mid-intervention (n=0 in both groups; p=1.000), end of intervention (n=2, 5% vs n=2, 5%; p=1.000) and follow-up (n=4, 11% vs n=1, 3%; p=0.356). Similarly, there was no significant difference in the number of responders (defined as a reduction of ≥1 point in PAC-QoL) between the probiotic and placebo at mid-intervention (n=1, 3% vs n=0, 0%; p=1.000), end of intervention (n=1, 3% vs n=0, 0%; p=1.000) and follow-up (n=1, 3% vs n=0, 0%; p=1.000).

Palatability and compliance

At mid-intervention, no significant difference was observed in palatability between the probiotic and placebo in the ITT population (6.5, SD 4.3 *vs* 6.3, SD 5.5; p=0.861), nor were there differences at the end of intervention (6.3, SD 4.3 *vs* 4.3, SD 6.6) (p=0.107). Thirty-three (89%) participants in the probiotic group and 35 (92%) in the placebo group complied with the study product intake.

Confounding factors

There was no significant difference in energy, protein, carbohydrate, fibre, and non-starch polysaccharide intakes between the probiotic and placebo groups at any of the study time points (Supplementary Table 3). There was a significantly higher fat intake at baseline and the end of the intervention (week 4) in the placebo group compared to the probiotic group (p=0.001 and p=0.036, respectively), although the mean

difference between the two groups was lower compared to baseline and mid-intervention. However, when the percentage of energy from fat was calculated, there was no difference between the probiotic (34%, SD 6%) and placebo (36%, SD 6%) groups at the end of the intervention (p=0.388).

There were no significant differences in physical activity METs between the probiotic and placebo groups at any of the study time points (Supplementary Table 4). There was also no significant difference in the change from baseline for the HADS total score, as well as for the anxiety and depression subscale scores, between the study groups at any of the study time points (Supplementary Material Table 5).

Microbiological outcomes

Fifty-two participants provided stool samples at baseline, 50 at mid-intervention (week 2), and 47 at the end of intervention (week 4). Forty participants provided a stool sample at each time point, while 61 participant provided stool samples at least at one time point.

There were no significant differences in any of the microbiota measured between the probiotic and placebo group at baseline, mid- or end of the intervention (Table 5). There were also no significant differences in any of the SCFA between the probiotic and placebo groups at any of the study time points (Table 5). Similarly, there were no significant differences in stool pH or water between the probiotic and placebo groups at any of the study time points (Table 5).

Adverse events

A summary of all the AEs during the study are presented in Supporting Material Table 6. There was no AE that resulted in interruption or dose reduction of the study product. One participant in the probiotic group and one in the placebo group experienced serious adverse events that were unrelated to the study product (trauma caused by car accident and arm fracture).

DISCUSSION

The aim of this trial was to investigate changes in gut transit time and gastrointestinal symptoms following 4 weeks consumption of a probiotic strain in a randomized, double-blind, placebo-controlled manner, in adults with constipation.

There was no significant difference between the probiotic and placebo in changes in WGTT from baseline to mid-intervention and end of intervention. These results are unexpected, as a previous study has shown a significant decrease in WGTT following the consumption of another *B. lactis* strain, HN019 [24]. Similarly, two systematic reviews and meta-analyses - that investigated the effect of various probiotics on WGTT in healthy and constipated individuals - showed that probiotic consumption significantly decreased WGTT, with *B. lactis* strains HN019 and DN-173010 conferring the largest treatment effects, particularly in constipated individuals compared to healthy people [8 26].

However, it is well known that the effects of probiotics are strain-specific [4]. Some actions of probiotics are common to a range of strains, such as metabolite production [4], whereas others appear to be strain-specific, such as neurological effects [27]. The conflicting results between the lack of effect of the *B. lactis* NCC2818 strain tested in this study and the beneficial effects shown by other *B. lactis* strains [28] could be explained by inter-strain genomic and functional differences, such as immunogenic properties [29 30]. As the immune and nervous systems are key regulators of gut motility, essential to normal gut homeostasis, different strains may have a variable impact on symptoms of constipation. It is therefore possible that, although the probiotic strain used in this study has been shown to be effective in another condition like seasonal allergic rhinitis, via its effect on immunological parameters [31], it has limited impact on gut motility and hence on constipation.

Interestingly, the effect size of the probiotic on WGTT observed in this study at mid-intervention (i.e. -12 h) is similar to the effect size demonstrated by a meta-analysis of RCTs of probiotics in constipation. Probiotics were found to significantly reduce WGTT by -12.4 h, with *B. lactis* HN019 decreasing WGTT by

-13.5 h but without reaching significance [8]. The sample size calculation for the current trial was based upon detecting a more relevant 20h- difference effect between the probiotic and placebo (adapted from Waller *et al*. [24]). In addition, the placebo used in this study also resulted in a similar effect size to the placebo effect observed in many other studies assessing the effect of probiotics in the management of lower GI symptoms [32].

Other factors that could have potentially contributed to changes seen in the placebo group were considered. Firstly, the placebo powder contained maltodextrin, which is a sugar absorbed in the small intestine and, in theory, in high doses it may be less efficiently absorbed resulting in its colonic fermentation; however, this has not been previously proven, and maltodextrin has been successfully used as a placebo in numerous trials in constipation, with a meta-analysis showing no change on WGTT[8]. Secondly, physical activity has been associated with changes in gut motility[33 34]; however, this study showed no differences in physical activity between groups. Thirdly, there was a significantly higher fat intake in the placebo group, compared to the probiotic group. Although an association between fat intake and constipation has been demonstrated[35], no studies have conclusively shown a causative effect of fat in constipation.

The current study also revealed no differences in stool frequency, consistency, GI tolerance, QoL and constipation-related symptoms (e.g. CCCS, PAC-SYM). These results are in line with the lack of impact on WGTT, while differing with the findings of the aforementioned meta-analysis, which showed that other *B. lactis* strains significantly increased stool frequency by an average of 1.5 bowel movements per week, and improved stool consistency and other constipation-related symptoms [8]. However, methodological differences between the current and previous studies may account for these findings. For example, Waller *et al.,* measured weekly stool frequency using a VAS score, instead of completing daily bowel diaries [24]. Furthermore, whereas the current study used the internationally accepted Rome III criteria to diagnose

chronic constipation, others have used a variation of less stringent definitions for diagnosing constipation, e.g. based on stool frequency or WGTT alone [24 36 37].

There are also other factors that could justify the lack of effect seen in the current study. Firstly, this study was not powered for any of the clinical endpoints and, hence, the sample size may have been too small to detect a significant difference in the symptoms and quality of life. Secondly, the mean stool frequency at baseline in both groups (assessed from daily bowel diaries) was more than 3 bowel movements per week, which is within the range of defaecation frequency for non-constipated people [38]. This is probably due to participants underestimating their stool frequency at screening. Thirdly, only people with mild constipation, assessed using the CCCS questionnaire (score 8-15), were included in this study. As previous systematic-reviews and meta-analyses have shown a significantly greater effect of probiotics in constipated individuals compared to healthy volunteers, the lack of effect of this probiotic could be potentially due to the presence of only mild constipation symptoms [39]. Moreover, a study in IBS has shown that patients with severe symptoms had the greatest reductions in symptom severity and the largest improvements in QoL following standard medical treatment, when compared to those with mild or moderate symptoms [40]. Therefore, it is likely that the inclusion of participants with mild constipation could have limited the potential of *B. lactis* NCC2818 to reduce constipation-related symptoms, and could perhaps account for the differences in efficacy observed with others using *B. lactis* strains.

Previous studies in chronic constipation have shown that not all treatments work for all pathophysiologies of constipation, likely due to the different underlying mechanisms involved [41]. The absence of severe proctological and evacuatory disorders in the present study was determined based on the participants' reported medical history, rather than on diagnostic investigation, such as proctography. Therefore, the potential heterogeneity in the pathophysiology of the study population may have contributed to the lack of effect.

In line with the findings on the clinical outcomes, there were no significant differences or changes in the stool microbiota following the consumption of the probiotic. Of note, no differences were observed in *Bifidobacterium* concentrations; this is a surprising finding as the probiotic consumed belonged to the *Bifidobacterium* genus and, therefore, an increase in *Bifidobacterium* in the probiotic group was expected. The lack of an increase in bifidobacteria could be explained in several ways. Firstly, it is possible that, although there might have been an increase in the *B. lactis* strain concentration in the gut, this could have been accompanied by a simultaneous decrease in endogenous *Bifidobacterium* species, possibly due to out-competition. However, the current literature does not support this theory; previous studies that have investigated the effect of probiotics on the gut microbiota have shown increases in the species to which the probiotic belonged [6 7]. Secondly, the fact that there was no increase in bifidobacteria in the probiotic group may suggest that this *B. lactis* specific probiotic strain did not survive through the GI tract, although strain survival in the faeces was not measured *per se* due to lack of a validated analytical method for the specific strain. Previous human studies that have administered other *B. lactis* strains have successfully confirmed the strain survival by measuring its stool concentration using culture methods and qPCR [6 42]. Survival of the strain is considered a key requirement of probiotics and can be affected by several host and product-specific factors, such as age, diet and baseline microbiota composition. Probiotic survival can also be influenced by exposure to low pH conditions of the stomach and bile acids, which can be tested *in vitro* [43]. In fact, the survival of the *B. lactis* strain used in this study has been tested and confirmed *in vitro* following simulated gastric and duodenal conditions. Baseline microbiota composition is also a crucial factor for the survival of probiotics due to competition for substrates and binding sites [44]; it is, thus, possible that the probiotic provided in this study was out-competed by the gut microbiota and, hence, was not viable in the colon. However, the lack of effect of the probiotic on the stool microbiota is in agreement with the findings of a systematic review of seven RCTs, which investigated the effect of probiotic supplementation on the stool microbiota and revealed that probiotics had no effects on the stool microbiota composition in terms of α-diversity, richness, or evenness when compared to placebo [45].

Other limitations involve the formula of the probiotic, which has not been previously studied in chronic bowel disorders. A meta-analysis of *Helicobacter pylori* eradication by probiotics delivered in fermented milk products showed that efficacy was better than that of capsule/sachet-based bacteria-only preparations [46]. This is also supported by animal studies that suggest that fermented milk might augment probiotic functionality when compared to water or saline carriers [47 48]. Therefore, the fact that the *B. lactis* was delivered in the form of a powder in the current study could have limited the activity or survival of the probiotic and hence its efficacy. Additionally, the results of the microbiological analysis might not be representative of all stools passed by the participants throughout the study since participants provided only one stool sample per time point.

There were no differences in SCFA concentrations between the probiotic and the placebo groups. Short-chain fatty acids are produced via fermentation by colonic bacteria. This study showed that the microbiota remained stable throughout the intervention period and, therefore, the lack of effect on end-products of bacterial fermentation, including SCFA, is unsurprising. Moreover, determination of SCFA production is challenging because the majority are produced in the proximal colon and more than 95% are rapidly absorbed in that region (i.e. <5% of SCFA are present in faeces); therefore, SCFA concentrations are considerably higher in the proximal colon compared to the distal colon, and the majority are unlikely to be detected in faeces [49].

Notably, the vast majority (92%) of the study population consisted of females, highlighting the fact that the findings of this study cannot be extrapolated to male members of the public. Further, the study population was recruited from the community and, therefore, the findings cannot be generalised to patients in primary, secondary or tertiary care. However, despite the limitations, there are considerable strengths which include the randomised, double-blind design, large sample size, and use of the established formal Rome III diagnostic criteria (modified) and validated outcome assessment tools. Furthermore, an objective measure, WGTT, was used to investigate the primary outcome.

Conclusion

This study has demonstrated that a 4 week intervention of *B. lactis* NCC2818 did not result in greater reduction in WGTT, nor impact other constipation-related outcomes, such as stool frequency and stool consistency, in a strictly-defined population with chronic mild constipation. This is in contrast to previous studies that have demonstrated a beneficial effect of other *B. lactis* strains in chronic constipation. Furthermore, this strain did not result in differences or changes in stool microbiota, including Bifidobacteria concentrations, stool SCFA, stool pH and stool water content. Further studies are needed in order to establish which probiotic strains, if any, are the most efficacious for the management of chronic constipation.

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AUTHORSHIP STATEMENT

Guarantor of the article

S. Mark Scott

Specific author contributions

ED, SC, KW, SMS, RC, GB and PM designed the study. ED, AZ and ST organised and performed the study visits and undertook data collection. SC contributed to study organization and data collection. KW and SMS supervised the research. The assessment of gut transit times was performed by ED and SMS. ED performed the microbiological analysis and its interpretation was performed by ED, KW and PL. AD performed statistical design and analysis. ED, KW, SMS, PD, GB and RC interpreted the results. ED drafted the manuscript. All authors approved the final manuscript.

STATEMENT OF INTERESTS

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This study was funded in full by Nestec SA. The funder were involved in the planning of the study, as well as the statistical analysis and interpretation of the data.

Potential competing interests:

NE, RC, AD, PM, PD and GB were or are employees of Nestec SA.

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TABLES

Table 1: Baseline characteristics of participants in the intention to treat population, presented by group.

Characteristics	Probiotic (N=37)	Placebo (N=38)
Age, years	35 (12)	31 (10)
Females, n (%)	34 (92)	35 (92)
Height, cm	165 (6)	166 (7)
Weight, kg	63.9 (9.2)	62.7 (9.1)
BMI, kg/m^2	23.6 (3.1)	22.8 (2.6)
Menstrual cycle, n (%)		
Mid-follicular	18 (49)	20 (53)
Mid-luteal	9 (24)	9 (24)
Post-menopausal	7 (19)	6 (16)
Cleveland Clinic Constipation Score (CCCS) †	12.8 (3.4)	11.3 (3.0)
SBMs†	3.5 (1.4)	3.7 (2.1)
Stool consistency (Bristol Stool Form Scale)‡	2.8 (0.9)	2.9 (1.0)
Hospital Anxiety and Depression Score (HADS) †	7.6 (6.8)	7.8 (6.0)
Fibre intake, g/d	13.2 (2.6)	14.2 (2.3)

Data are mean (SD), unless stated.

† Numbers in each group: n=37 probiotic; n=37 placebo

‡ Numbers in each group: n=36 probiotic; n=38 placebo

Table 2: Whole gut transit time (WGTT) and regionals gut transit time (in hours) in the intention to treat population.

Outcome in hours	Probiotic (N=37) n	Mean (SD)	Placebo (N=38) n	Mean (SD)	P value
WGTT					
Baseline	37	81 (38)	38	72 (30)	0.242
Mid-intervention (week 2)	36	69 (38)	36	61 (34)	0.930
End of intervention (week 4)	35	58 (41)	34	63 (35)	0.062
Δ in WGTT from baseline					
To mid- intervention (week 2)	36	-12 (33)	36	-13 (34)	*0.863*
To end of intervention (week 4)	35	-20 (33)	34	-9 (34)	*0.103*
Left colonic transit time					
Baseline	37	30 (26)	38	22 (17)	*0.116*
Mid-intervention (week 2)	36	24 (21)	36	20 (13)	*0.741*
End of intervention (week 4)	35	21 (22)	34	21 (20)	*0.146*
Δ in left colonic transit time from baseline					
To mid- intervention (week 2)	36	-4 (14)	36	-4 (14)	*0.839*
To end of intervention (week 4)	35	-5 (20)	34	-1 (21)	*0.437*
Right colonic transit time					
Baseline	37	20 (15)	38	22 (10)	*0.552*
Mid-intervention (week 2)	36	22 (19)	36	17 (13)	*0.577*
End of intervention (week 4)	35	20 (17)	34	20 (11)	*0.456*
Δ in right colonic transit time from baseline					
To mid- intervention (week 2)	36	1 (18)	36	-4 (18)	*0.189*
To end of intervention (week 4)	35	-3 (14)	34	-3 (15)	*0.954*
Rectosigmoid transit time					
Baseline	37	31 (20)	38	28 (18)	*0.528*
Mid-intervention (week 2)	36	23 (18)	36	24 (18)	*0.525*
End of intervention (week 4)	35	18 (16)	34	22 (19)	*0.245*
Δ in rectosigmoid transit time from baseline					
To mid- intervention (week 2)	36	-7 (19)	36	-6 (19)	*0.686*
To end of intervention (week 4)	35	-11 (17)	34	-6 (17)	*0.134*

N: Number of participants in groups, n: Number of participants with available data. All p values are obtained from ANCOVA test(s) adjusted for covariates (baseline values, stage of menstrual cycle), except for the p value for baseline data, which is obtained from independent t-test(s). For the primary outcome (Δ in WGTT from baseline at mid-intervention), a P value of <0.0468 was considered statistically significant.

Table 3: Stool frequency, stool consistency, and gut symptoms in the intention to treat population.

Outcome	Probiotic (N=37) n	Probiotic (N=37) Mean (SD)	Placebo (N=38) n	Placebo (N=38) Mean (SD)	*P value*
Stool frequency and consistency					
Total bowel movements (TBM)/week					
Baseline	36	3.6 (1.3)	38	4.1 (2.2)	*0.273*
Mid-intervention (week 2)	35	4.2 (1.9)	37	4.7 (3.2)	*0.810*
End of intervention (week 4)	36	4.5 (2.0)	37	4.8(2.8)	*0.879*
Δ in TBM/week from baseline					
To mid- intervention (week 2)	34	0.4 (2.3)	37	0.5 (2.4)	*0.836*
To end of intervention (week 4)	35	0.7 (2.4)	37	0.6 (2.4)	*0.831*
Spontaneous bowel movements (SBM)/week					
Baseline	36	3.5 (1.4)	38	3.7 (2.1)	*0.710*
Mid-intervention (week 2)	34	3.9 (1.9)	37	4.7 (4.0)	*0.853*
End of intervention (week 4)	35	4.5 (1.7)	37	4.8 (3.4)	*0.857*
Δ in SBM/week from baseline					
To mid- intervention (week 2)	33	0.3 (2.3)	37	0.5 (2.4)	*0.712*
To end of intervention (week 4)	35	0.6 (2.4)	37	0.8 (2.4)	*0.638*
Complete spontaneous bowel movements (CSBM)/week					
Baseline	32	2.2 (1.1)	32	2.7 (1.3)	*0.105*
Mid-intervention (week 2)	25	3.1 (1.4)	29	2.8 (1.5)	*0.635*
End of intervention (week 4)	27	3.3 (1.6)	31	3.5 (1.4)	*0.685*
Δ in CSBM/week from baseline					
To mid- intervention (week 2)	23	0.4 (1.4)	28	0.0 (1.6)	*0.351*
To end of intervention (week 4)	27	0.9 (0.4)	28	1.0 (1.6)	*0.868*
Stool consistency					
Baseline	36	2.8 (0.9)	38	2.9 (1.0)	*0.695*
Mid-intervention (week 2)	35	2.8 (1.1)	37	2.8 (1.1)	*0.808*
End of intervention (week 4)	36	2.9 (1.0)	37	2.9 (1.1)	*1.174*
Δ in stool consistency from baseline					
To mid- intervention (week 2)	34	-0.1 (1.2)	37	-0.1 (1.2)	*0.8476*
To end of intervention (week 4)	35	0.1 (1.2)	37	0.0 (1.2)	*0.9364*
Gut symptoms					
GI tolerance					
Baseline	37	1.7 (0.7)	32	1.8 (0.7)	*0.555*
Mid-intervention (week 2)	34	1.6 (0.7)	33	1.5 (0.5)	*0.548*
End of intervention (week 4)	32	1.5 (0.7)	33	1.4 (0.5)	*0.863*
Δ in GI tolerance from baseline					
To mid- intervention (week 2)	34	-0.2 (0.6)	29	0.4 (0.5)	*0.136*
To end of intervention (week 4)	32	-0.4 (0.6)	27	-0.6 (0.5)	*0.151*
Bloating					
Baseline	37	5.8 (2.5)	37	5.0 (2.6)	*0.983*
Mid-intervention (week 2)	36	4.1 (2.5)	36	3.9 (2.7)	*0.146*
End of intervention (week 4)	36	4.1 (2.5)	36	3.8 (2.8)	*0.782*
Δ in bloating from baseline					
To mid- intervention (week 2)	36	-1.3 (2.4)	35	-0.9 (2.4)	*0.466*
To end of intervention (week 4)	36	-1.7 (2.4)	35	-1.5 (2.4)	*0.704*

N: Number of participants in groups, n: Number of participants with available data. All p values are obtained from ANCOVA test(s) adjusted for covariates (baseline values, stage of menstrual cycle), except for the p value for baseline data, which is obtained from independent t-test(s).

Table 4: Constipation-related outcomes, including the Patient Assessment of Constipation Symptoms (PAC-SYM), Patient Assessment of Constipation Quality of Life (PAC-QoL), Cleveland Clinic Constipation Score (CCCS), and Global Constipation Symptom Score (GCSS) in the intention to treat population.

	Probiotic (N=37)		Placebo (N=38)		
Outcome	n	Mean (SD)	n	Mean (SD)	***P value***
PAC-SYM global score					
Baseline	37	1.4 (0.6)	38	1.3 (0.7)	*0.522*
Mid-intervention (week 2)	36	1.0 (0.6)	36	1.0 (0.6)	*0.831*
End of intervention (week 4)	36	0.9 (0.6)	36	0.9 (0.6)	*0.812*
Follow-up (week 8)	35	1.1 (0.7)	34	1.0 (0.7)	*0.372*
Δ in PAC-SYM global score from baseline					
To mid- intervention (week 2)	36	-0.1 (0.6)	36	-0.0 (0.6)	*0.474*
To end of intervention (week 4)	36	-0.3 (0.6)	36	-0.2 (0.6)	*0.780*
To follow-up (week 8)	35	-0.1 (0.6)	34	-0.1 (0.6)	*0.907*
PAC-QoL global score					
Baseline	37	1.1 (0.5)	38	1.0 (0.4)	*0.178*
Mid-intervention (week 2)	36	1.0 (0.4)	36	0.8 (0.3)	*0.606*
End of intervention (week 4)	36	0.9 (0.3)	36	0.9 (0.4)	*0.449*
Follow-up (week 8)	35	0.9 (0.5)	33	0.8 (0.3)	*0.685*
Δ in PAC-QoL Global from baseline					
To mid- intervention (week 2)	36	-0.1 (0.0)	36	-0.1 (0.0)	*0.836*
To end of intervention (week 4)	36	-0.2 (0.6)	36	-0.1 (0.6)	*0.315*
To follow-up (week 8)	35	-0.2 (0.6)	33	-0.2 (0.6)	*0.977*
Cleveland Clinic Constipation Score (CCCS)					
Baseline	37	12.8 (3.4)	37	11.3 (3.0)	*0.999*
Mid-intervention (week 2)	36	10.2 (4.0)	36	10.1 (3.2)	*0.588*
End of intervention (week 4)	36	10.2 (3.5)	36	9.7 (4.0)	*0.861*
Follow-up (week 8)	35	10.3 (3.7)	33	10.1 (3.3)	*0.670*
Δ in CCCS from baseline from baseline					
To mid- intervention (week 2)	36	-2.4 (3.0)	35	-1.2 (3.0)	*0.091*
To end of intervention (week 4)	36	-2.3 (3.6)	35	-1.7 (4.1)	*0.494*
To follow-up (week 8)	35	-2.2 (3.5)	32	-1.3 (3.4)	*0.205*
Global Constipation Symptom Score					
Baseline	37	0.1 (0.5)	37	-0.1 (0.4)	*0.898*
To mid- intervention (week 2)	36	0.7 (0.9)	36	0.7 (0.8)	*0.500*
To end of intervention (week 4)	36	0.9 (1.2)	36	0.8 (1.1)	*0.653*
To follow-up (week 8)	35	0.8 (1.0)	33	0.6 (0.7)	*0.813*
Δ in GCSS from baseline					
To mid-intervention (week 2)	36	0.8 (1.0)	36	0.7 (1.0)	*0.768*
To end of intervention (week 4)	36	0.9 (1.3)	36	0.9 (1.3)	*0.952*
To follow-up (week 8)	35	0.8 (1.0)	33	0.6 (1.0)	*0.497*

N: Number of participants in groups, n: Number of participants with available data. All p values are obtained from ANCOVA test(s) adjusted for covariates (baseline values, stage of menstrual cycle), except for the p value for baseline data, which is obtained from independent t-test(s).
PAC-SYM and PAC-QoL subscores can be found in Supporting Material Tables 1 and 2, respectively.

Table 5: Stool microbiota concentrations, short-chain fatty acids concentrations, pH and water content at baseline, mid- and end of intervention under the intention to treat analysis.

		Probiotic		Placebo		P
	Time point	n	Descriptive	n	Descriptive	**value***
Microbiota concentrations (log10/g dry faeces), median (IQR)						
All bacteria	Baseline	27	11.3 (0.5)	25	11.1 (1.0)	*0.848*
	Mid-intervention (week 2)	23	11.2 (0.5)	25	11.3 (1.1)	*0.828*
	End of intervention (week 4)	22	11.1 (0.9)	24	11.2 (0.7)	*0.860*
Bifidobacterium spp.	Baseline	26	9.4 (0.7)	23	9.5 (0.3)	*0.548*
	Mid-intervention (week 2)	23	9.6 (0.4)	25	9.4 (1.8)	*0.942*
	End of intervention (week 4)	21	9.5 (0.3)	24	9.4 (1.0)	*0.509*
Bacteroides ssp.	Baseline	27	10.1 (0.6)	25	10.1 (0.6)	*0.805*
	Mid-intervention (week 2)	23	10.1 (0.8)	25	10.3 (1.4)	*0.893*
	End of intervention (week 4)	22	10.1 (0.9)	24	10.1 (0.8)	*0.895*
Prevotella ssp.	Baseline	24	7.8 (2.0)	23	7.9 (2.1)	*0.983*
	Mid-intervention (week 2)	20	8.6 (1.9)	24	8.1 (1.3)	*0.077*
	End of intervention (week 4)	19	8.5 (2.5)	22	8.2 (0.9)	*0.433*
Lachnospiraceae	Baseline	27	10.0 (0.7)	24	9.8 (1.0)	*0.850*
	Mid-intervention (week 2)	23	10.0 (0.7)	25	9.7 (1.6)	*0.926*
	End of intervention (week 4)	22	10.0 (1.1)	24	10.0 (0.8)	*0.582*
Roseburia ssp.	Baseline	27	9.7 (1.8)	25	9.0 (1.3)	*0.819*
	Mid-intervention (week 2)	23	9.1 (1.2)	25	9.6 (1.7)	*0.380*
	End of intervention (week 4)	22	9.2 (1.3)	24	9.4 (0.9)	*0.391*
F. prausnitzii	Baseline	27	9.9 (0.7)	25	9.6 (0.8)	*0.680*
	Mid-intervention (week 2)	23	10.1 (0.7)	25	9.5 (1.2)	*0.415*
	End of intervention (week 4)	22	9.9 (0.7)	23	9.8 (0.7)	*0.427*
Archaea	Baseline	20	8.6 (2.0)	13	9.2 (1.0)	*0.181*
	Mid-intervention (week 2)	11	8.5 (1.8)	18	8.6 (0.7)	*0.611*
	End of intervention (week 4)	12	8.4 (1.6)	14	9.0 (0.7)	*0.820*
Short-chain fatty acids (μmol/g dry faeces), mean (SD)						**P value†**
Total SCFA	Baseline	25	374.2 (288.7)	24	294.8 (149.1)	*0.238*
	Mid-intervention (week 2)	18	357.1 (273.1)	20	316.7 (189.6)	*0.853*
	End of intervention (week 4)	16	367.0 (210.8)	18	407.7 (330.3)	*0.501*
Acetate	Baseline	25	206.7 (165.9)	24	157.7 (75.0)	*0.195*
	Mid-intervention (week 2)	18	197.8 (150.7)	20	176.2 (103.9)	*0.913*
	End of intervention (week 4)	16	209.7 (124.9)	18	228.3 (188.2)	*0.625*
Propionate	Baseline	25	65.6 (56.0)	24	52.6 (31.1)	*0.328*
	Mid-intervention (week 2)	18	68.5 (67.4)	20	56.0 (37.2)	*0.915*
	End of intervention (week 4)	16	67.1 (48.2)	18	72.3 (62.0)	*0.474*
Butyrate	Baseline	25	67.1 (61.2)	24	50.1 (32.8)	*0.259*
	Mid-intervention (week 2)	18	56.6 (53.4)	20	51.4 (40.5)	*0.618*
	End of intervention (week 4)	16	61.0 (41.2)	18	76.0 (83.4)	*0.308*
Isobutyrate	Baseline	25	10.6 (6.6)	24	10.1 (5.3)	*0.774*
	Mid-intervention (week 2)	18	10.6 (5.0)	20	9.7 (4.0)	*0.866*
	End of intervention (week 4)	16	8.9 (2.9)	18	9.2 (4.4)	*0.590*
Valerate	Baseline	25	9.0 (5.6)	24	10.1 (7.3)	*0.589*
	Mid-intervention (week 2)	18	8.6 (4.2)	20	9.8 (8.2)	*0.171*

	Time point	Probiotic		Placebo		P value*
		n	Descriptive	n	Descriptive	
Isovalerate	End of intervention (week 4)	16	8.2 (3.3)	18	8.9 (5.9)	*0.409*
	Baseline	25	15.0 (8.6)	24	14.5 (7.9)	*0.835*
	Mid-intervention (week 2)	18	15.0 (6.8)	20	13.5 (4.9)	*0.780*
	End of intervention (week 4)	16	12.1 (3.5)	18	13.1 (6.6)	*0.433*
Stool pH, mean (SD)	Baseline	19	6.9 (0.4)	19	6.9 (0.2)	*0.860*
	Mid-intervention (week 2)	12	7.2 (0.6)	12	7.0 (0.5)	*0.678*
	End of intervention (week 4)	10	7.2 (0.4)	9	6.7 (0.7)	*0.180*
Water content (%), mean (SD)	Baseline	22	68.7 (8.2)	23	64.7 (5.4)	*0.062*
	Mid-intervention (week 2)	17	67.7 (6.3)	17	64.8 (4.2)	*0.339*
	End of intervention (week 4)	4	70.8 (8.0)	14	67.9 (7.1)	*0.332*

*Mann-Whitney p value, †ANCOVA p value using baseline values as covariate, except for the p value for baseline data, which is obtained from independent t-test(s).

FIGURE LEGEND

Figure 1: The CONSORT diagram