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Revisión

The effect of probiotic fermented milk that includes *Bifidobacterium lactis* CNCM I-2494 on the reduction of gastrointestinal discomfort and symptoms in adults: a narrative review

Dan L. Waitzberg[1], Flávio A. Quilici[2], Sender Michzputen[3] and Maria do Carmo Friche Passos[4]

[1]University of Sao Paulo, School of Medicine, Gastroenterology Department, LIM 35, Sao Paulo. [2]University Catholic of Campinas, School of Medicine, Digestive Surgery Department. Campinas, SP. [3]Federal University of Sao Paulo, Paulista School of Medicine, Section of Gastroenterology, SP. [4]Federal University of Minas Gerais, School of Medicine, Internal Medicine Department, Belo Horizonte - MG (Brazil).

Abstract

Aim: **determine the effectiveness of fermented milk that included *Bifidobacterium lactis* CNCM I-2429 for reducing gastrointestinal (GI) discomfort in healthy adults.**

Methods: **we conducted a systematic literature search to identify studies reporting the use of *B. animalis spp. lactis* for GI discomfort/comfort in healthy adults. A total of 5329 records were identified, of these 99 full-text articles were assessed. Searches for additional trials were conducted using the names of authors of each identified study and several relevant databases. The study selection was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Studies were included if they were randomized controlled trials; the included subjects were healthy adults; and the intervention group received *B. lactis* CNCM I-2494. Studies were excluded if they were non-randomized trials, if they included adults who were not healthy, if they included the use of any other intervention, or if they compared different products without a placebo group. The methodological quality of the studies was evaluated using the Oxford Quality Scale and the Cochrane Concealment Assessment. A meta-analysis was not possible.**

Results: **the search strategy identified two studies that included a total of 538 healthy women, aged 18–60 years, normal weight or overweight (BMI 18–30 kg/m^2). GI well-being was significantly improved in the Probiotic group vs. the Control group in one study, with no differences in the other. The percentage of responders for GI well-being was higher in the Probiotic group vs. the Control group in the first study but not in the second.**

Correspondence: Dan L Waitzberg.
Rua Maestro Cardim 1175.
01323-001, Sao Paulo, SP (Brazil).
E-mail: dan@ganep.com.br

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EL EFECTO DE LA LECHE FERMENTADA PROBIÓTICA CON *BIFIDOBACTERIUM LACTIS* QUE INCLUYE CNCM I-2494 EN LA REDUCCIÓN DE MOLESTIAS Y SÍNTOMAS GASTROINTESTINALES EN LOS ADULTOS: UNA REVISIÓN NARRATIVA

Resumen

Objetivo: **determinar la eficacia de la leche fermentada con *Bifidobacterium lactis* CNCM I-2429 en la reducción de el malestar gastrointestinal (GI) en adultos sanos.**

Métodos: **se realizó una búsqueda sistemática en la literatura para identificar estudios que informaron del uso de *B. animalis spp. lactis* para molestias/ confort GI en adultos sanos. Se identificaron un total de 5.329 registros, de estos se evaluaron 99 artículos de texto completo. Las búsquedas de ensayos adicionales se realizaron utilizando los nombres de los autores de cada estudio identificado y varias bases de datos relevantes. La selección de los estudios se llevó a cabo de acuerdo con las guías de Artículos de Información Preferidos para Revisiones Sistemáticas y Meta-Análisis (PRISMA). Los estudios eran incluidos si eran ensayos randomizados controlados, si los sujetos de estudio eran adultos sanos y si el grupo de intervención recibió *B. lactis* CNCM I-2494. Se excluyeron los estudios que no eran randomizados, que incluían adultos que no estaban sanos, que incluían el uso de cualquier otra intervención o si comparaban diferentes productos sin un grupo placebo. La calidad metodológica de los estudios se evaluó utilizando la Escala de Calidad de Oxford y la Evaluación Cochrane de ocultamiento. No fue posible un metaanálisis.**

Resultados: **la estrategia de búsqueda identificó dos estudios que incluyeron un total de 538 mujeres sanas, con edades entre 18 a 60 años, de peso normal o sobrepeso (IMC de 18-30 kg/m^2). En uno de los estudios las molestias GI disminuyeron significativamente en el grupo de probióticos frente al grupo control, sin diferencias en el otro. El porcentaje de respondedores para el bienestar GI fue mayor en el grupo de probióticos frente al grupo control en el primer estudio, pero no en el segundo. Los síntomas GI se redujeron significativamente en el grupo probiótico frente al grupo control en ambos estudios.**

GI symptoms were significantly decreased in the Probiotic group vs. the Control group in both studies. Bowel function was assessed by one study; the stool frequency did not differ between the groups, but a decrease in stool consistency was observed in the Probiotic group but not in the Control group. Possible mechanisms of action (gut motility, hypersensitivity, gut permeability, and gut microbiota) were also described.

Conclusion: **probiotic fermented milk containing *B. lactis* CNCM I-2494 by healthy women may improve GI well-being and decrease the frequency of GI symptoms.**

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Key words: *Probiotic. Gastrointestinal discomfort. Healthy subjects. Women.*

La función intestinal solo se evaluó en un estudio; la frecuencia de las deposiciones no difirió entre los grupos, pero se observó una disminución de la consistencia de las heces en el grupo probiótico, pero no en el grupo control. También se describen diferentes mecanismos de acción posibles (la motilidad intestinal, la hipersensibilidad, la permeabilidad del intestino y la microbiota intestinal).

Conclusión: **la leche fermentada con *B. lactis* CNCM I-2494 en mujeres sanas puede mejorar el bienestar GI y disminuir la frecuencia de los síntomas gastrointestinales.**

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Palabras clave: *Probiótico. Malestar gastrointestinal. Sujetos sanos. Mujeres.*

Introduction

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits to the host[1,2]. We are gaining an increased understanding of the importance of having an adequate gut microbiome for optimal health and especially for a healthy gut. The intestinal microbiota comprise a complex and dynamic bacterial community that plays an important role in human health[3]. Changes in microbiota composition (dysbiosis) or function, or altered microbiota/host interactions, directly correlate with several diseases[4], and, conversely, the beneficial effects of specific probiotic strains may be associated with specific health claims[5]. There are often alterations in intestinal *Bifidobacteria* levels, or in the species composition, in patients with intestinal microbiota dysbiosis. Accordingly, the rationale for using microorganisms of the genus *Bifidobacterium* as probiotics is to modulate the intestinal *Bifidobacteria* population to elicit specific responses[3]. For example, *B. longum* strains exhibit differential immunomodulatory properties[6].

There is growing consumer interest in the potential therapeutic and preventive health benefits of probiotics[2], and there is a steady stream of commercially available products that contain probiotics. It is worth noting that beneficial microorganisms have long been a part of the human diet, and Elie Metchnikoff who was awarded a Nobel Prize in 1907 for his studies of phagocytosis and the prolongation of life, was an early pioneer of the empirically beneficial effects of fermented milk (yogurt) in enhancing human health and longevity [7]. For a microorganism be considered a probiotic, it must be genetically defined; have defined phenotypic, morphologic, and biochemical characteristics; be deposited in an internationally recognized culture collection; be non-pathogenic; grow and adhere in the bowel mucosa; compete and prevail over enteropathogenic microorganisms; produce beneficial metabolites for the host (like vitamins and short chain fatty acids); and remain alive through the manufacturing and storage processes[8]. Probiotics may be delivered in different forms, with fermented milk being one such form.

Many disorders affect the digestive tract. Colonic transit disturbances (such as constipation and irritable bowel syndrome, IBS) are common[9] and represent an important target for the probiotic industry[1,10,11,12]. While constipation is a very common disorder in all age groups, its prevalence increases significantly with age[9]. Some healthy subjects presented with important gut symptoms, such as GI discomfort, even after previous diagnostic criteria seemed to rule out a structural disorder. These gut-associated symptoms are very common, they are considered to be part of the normal physiological digestive process[13]. Even in these non-disease subjects having minor digestive symptoms and/or mild form of GI discomfort, quality of life is impaired when compared to true healthy subjects but in a lower extent than in IBS patients [14,15] . This supports the interest in developing appropriate non-drug strategy to relieve these non-disease subjects from their GI symptoms. [14].

Digestive functions play key roles in maintaining and improving health status, so in a general population that has less frequent and less severe symptoms, the target of a probiotic food intervention is to improve the overall sensation of GI comfort or well-being[15]. There is an absence of validated biomarkers for evaluating changes in digestive comfort, so patient-reported outcomes rather than objective measures are used. Data from a study using a validated instrument (a questionnaire about four digestive symptoms: abdominal pain/discomfort; bloating; borborygmi/rumbling stomach; and flatulence) in a non-IBS population found that changes in GI well-being are primarily related to changes in digestive symptoms[15]. The composite score for digestive symptoms showed higher correlation with improvements in GI well-being, while worsening of GI well-being was associated with an increase in digestive symptoms. However, this was not driven by a specific symptom, confirming the multidimensional nature of this concept[15]. This characteristic could allow differentiating this non-disease population from IBS patients where abdominal pain is the predominant symptom.

Independent studies have confirmed that the connection between emotional and physical health involves multiple bidirectional neurocrine and endocrine signaling mechanisms[16,17]. In particular, anxiety can influence gut microbiota[16,17]. Recent advances have provided more detailed insights into the important role of microbiota in human health and disease and in particular into the interactions of gut microbiota and emotional stress and diet. A review of pre-clinical studies note that the disruptive impact of emotional stress on the gut can be prevented, and even partially reversed, by probiotic administration[18]. The authors suggest that pre- and probiotic formulations and fermented food can be used to influence mental health and to control GI symptoms[18]. GI pain can be a good way to characterize dysregulation of the brain-gut axis and, in IBS, the use of a probiotic fermented product may mitigate suffering[16].

Several systematic reviews have looked at the potential beneficial effects of probiotics for IBS, colon transit time, or constipation due to high interest in the use of products that may manipulate gut microbiota that affect these GI conditions[11,19,20]. Pooling data from several studies (eg more than 20) but with different strains and in different patients groups (eg IBS-C, IBS-D, IBS-M) does not allow researchers to determine the efficacy of a single strain or of a specific combination of strains, which is the main limitation of such a global approach. Indeed, no systematic review has been performed to date in non-disease patients despite the interest of using functional probiotic foods in this population.

Here we performed a systematic review of a clearly defined probiotic food that is commercially available (Activia®: Danone), a fermented milk product enriched with *B. lactis* CNCM I-2494 (called *B. animalis* or *lactis* DN-173 010 in some studies), which is associated with two classical yogurt starters, *S. thermophiles* (CNCM strain number I-1630) and *L. bulgaricus* (CNCM strains number I-1632 and I-1519), and with *Lactococcus lactis* ssp. *lactis* (CNCM strain number I-1631)[21]. *B. lactis* DN-173 010 has a high survival capacity in the human GI tract[22] and exhibits probiotic properties in the colon[1]. The effect of this specific probiotic fermented milk product on bowel function and on IBS has been described in the literature. The objective of this review was to narrow the research question and to determine the effectiveness of this commercially-available probiotic food for a specific GI condition, GI discomfort, in a well-defined target population of healthy adults.

Methods

Literature search

York Health Economic Consortium conducted a systematic literature search to identify studies reporting the use of *B. animals spp. lactis* for GI discomfort/comfort that are indexed in the Ovid MEDLINE database. The following search terms were used: "bifidobacterium", "lactis or animalis", "bifidus", "digestivum or regularis or actiregularis or essensis or danregularis", "yogurt or yoghurt or yoghourt or milk or probiotic", "activia or activiaTM or activiaR", "dn173010 or dn-173010", "CNCMI2494 or CNCM I-2494", "colon or colons or colonic", "intestine or intestinal or gastrointestinal", "bowel or gut" or digestive or digestion", "bloat or distend or distension or fullness or satiety or wellbeing", "gas or gaseous or flatulence or flatulent or flatus or belch or burp or eructate", "borborygmi or rumble or rumbling or gurgle or gurgling", "pain or painful or discomfort or uncomfort or comfort or cramp", "stool or defecate or fecal or faeces or constipation or constipated or hypomotility or diarrhea or incontinent", "gastrointestinal tract or intestine or lower gastrointestinal tract", "gastrointestinal diseases or gastrointestinal motility or colonic disease or functional/or irritable bowel syndrome".

Searches for additional trials were conducted using the names of authors of each identified study and several relevant databases: MEDLINE In-process, EMBASE, Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, Database of Abstracts of Reviews of Effects, Health Technology Assessment Database, Science Citation Index, Conference Proceeding Citation Index – Science, OAISTES, OpenGrey, National Technical Information Service, BIOSIS Citation Index, CAB Abstracts, Food Science and Technology Abstracts, Clinical Trials.gov, International Clinical Trials Registry Portal. In addition to searches of bibliographic databases, we searched selected major conference proceedings from the last three years (2012–2014). There was no language restriction for the searches that we performed.

Study selection (Figure 1)

The study selection and the construction of the flow diagram was carried out using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[23]. We selected trials that reported the use of *B. lactis* CNCM I-2494 for GI discomfort/comfort. A total of 5329 records were identified through database searching, and 15 records were identified through other sources; 2219 of these were duplicates. After removing duplicates, 3125 records were screened and assessed for relevance based on their titles and abstracts. Of these, 99 full-text articles were assessed to see if they met the eligibility criteria. Studies were included if they met these criteria: they were randomized controlled trials; the included subjects were healthy adults; and the intervention group received *B. lactis* CNCM I-2494. Studies were excluded if they were non-randomized trials, if they included adults who were not healthy, if they included the use of any other intervention, or if they compared different products without a placebo group. At the end of this process, just two human trials met the inclusion criteria for this systematic review. We

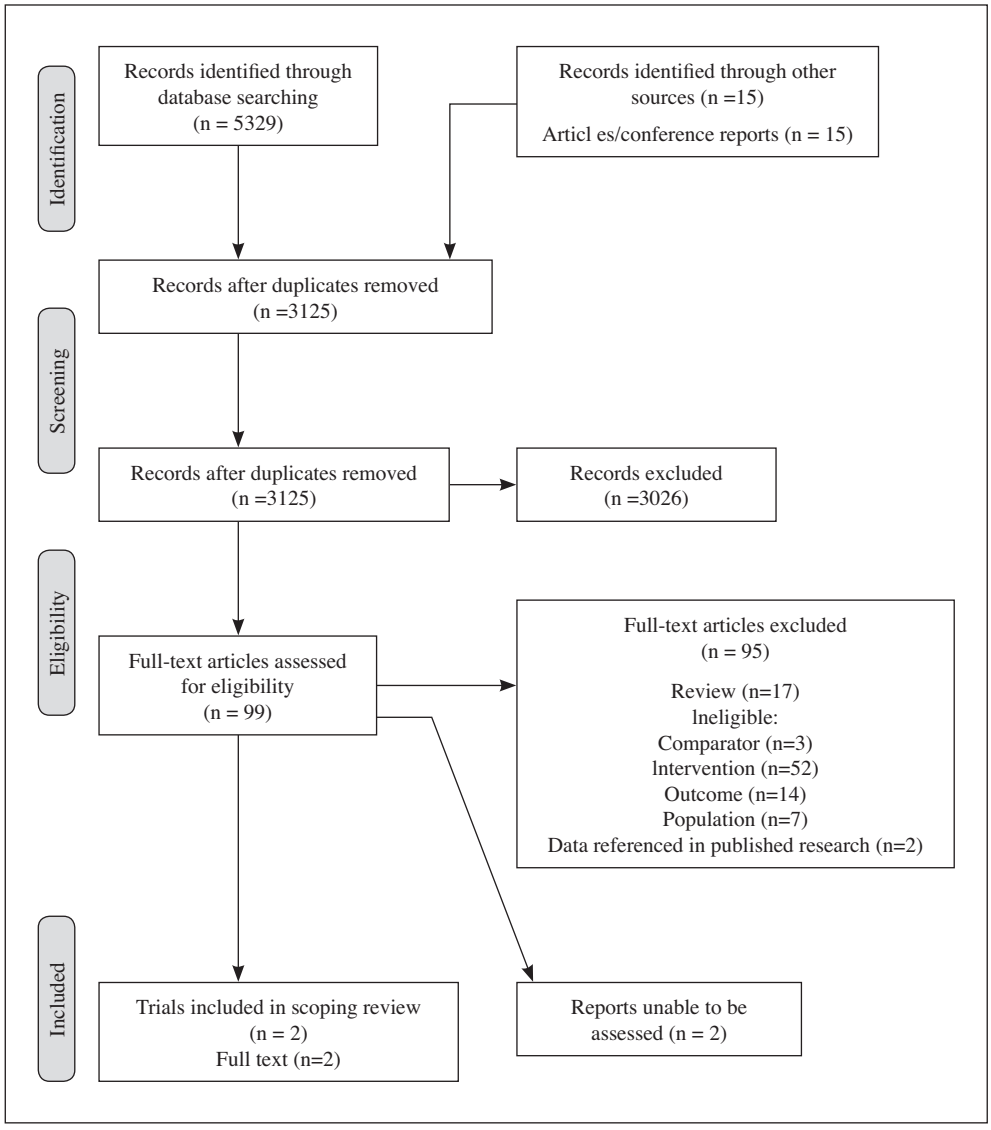

Fig. 1.—Flow diagram of included an excluded studies.

also looked at preclinical studies in animal models and at other human studies to gain insights into the possible mechanisms of action of this probiotic dairy food.

Study quality

The data from the two studies were extracted, and the design quality was evaluated individually by one researcher and confirmed by a second researcher. Discrepancies were resolved through discussion or by consulting a third researcher.

The risk of bias was appraised using criteria for assessing the risk of bias specifically for randomized trials[24]. The methodological quality of the studies was evaluated using the Oxford Quality Scale, with a score of "1" indicating low quality and a score of "5" indicating high quality. The Cochrane Concealment Assessment was also applied: (A=adequate concealment, B=uncertain, C=clearly inadequate)[25]. This includes a criteria evaluation of the randomization procedure, blinding, and individual attrition. A meta-analysis was not possible; pooled analysis of the two selected trials was conducted previously[26].

Results

Figure 1 summarizes the two well-conducted studies that we identified in our literature search[21,26]. These studies had common outcomes of interest, met the selection criteria, and provided data for 538 healthy subjects. These studies were conducted by the same research group, had the same experimental design, and overall showed low risk of bias[24] and adequate concealment (4A)[25].

Each study was a single-center (Munich, Germany[21] and Caen, France[26]), randomized, double-blind, and controlled study that aimed to assess the effect of probiotic

fermented milk containing *B. lactis* CNCM I-2494[26]/*B. lactis* DN-173 010[21] (Probiotic group) compared to a non-fermented dairy product (Control group). All subjects were women aged 18–60 years who were normal weight or overweight (BMI 18–30 kg/m^2) without a diagnosis of any digestive disease and with bowel movement frequency within the normal range (3–21 per week) that complained of digestive symptoms. For inclusion, subjects had to experience a minimal level of digestive symptoms (discomfort or abdominal pain, bloating, flatulence/passage of gas, borborygmi/rumbling stomach) in the past month. The frequency of digestive symptoms and bowel function (movement and stool consistency) were obtained weekly for a 2-week period after inclusion (before intervention). The subjects were randomized to consume two units of product per day for 4 weeks and then underwent a 4-week wash-out period in which they did not consume a specific product[21].

Outcome of two randomized controlled trials (Table 1)
GI well-being

The main outcome was GI well-being in both studies, which was assessed using a 3-point Likert scale (improved, no change, worsened) on a weekly basis. Each subject was classified as a responder or as a non-responder to assess the magnitude of the effect[21,27].

In the Guyonnet et al. (2009) study, the percentage of women who reported an improvement in GI well-being was higher ($P=0.006$; OR = 1.69; 95% CI = 1.17, 2.45) for the Probiotic group vs. the Control group[21], while in Marteau et al. 2013 there was no difference between these groups ($P \geq 0.05$; OR = 1.38; 95% CI = 0.89, 2.14)[26]. The pooled data analyses, conducted for Marteau et al. (2013), showed that the Probiotic group had a significantly greater improvement in GI well-being (OR=1.36; 95%CI= 1.07, 1.73)[26]. Similarly, the percentage of responders for GI well-being was higher in the Probiotic group (52%) vs. the Control group (36.1%) ($P=0.025$; OR = 1.92; 95%CI = 1.09, 3.40) in the first paper[21], but did not differ in the second[26]. A positive effect was observed in the pooled analysis ($P=0.015$; OR = 1.53; 95% CI = 1.09, 2.16), with a difference in the responder rate of 10.6% (Probiotic group, 53.2% vs. Control group, 42.6%) and a Number Needed to Treat (NNT) of 9.5[26].

Frequency of digestive symptoms

Individual digestive symptoms, including abdominal pain/discomfort, bloating, flatulence/passing of gas, and borborygmi/rumbling stomach was evaluated weekly using a five-point Likert scale that ranged from 0 (never) to 4 (every day of the week)[26,21]. The composite score for these 4 symptoms ranged from 0 (none of the symptoms) to 16 (all symptoms, every day).

The scores from the 4-week intervention period showed an overall significant decrease in individual digestive symptoms in the Probiotic group vs. the Control group in both studies ($P=0.044$[21] and $P=0.33$[26]), and

Table I
Outcomes of selected studies

Endpoint	*Studies outcomes*	
	(Guyonnet, Schlumberger, Mhamdi, Jakob, & Chassany, 2009) digestive symptoms and health-related quality of life (HRQoL	*(Marteau, Guyonnet, Lafaye de Micheaux, & Gelu, 2013)*
Gastrointestinal Well-being	Percentage of women reporting an improvement was higher (P =0.006) in Probiotic group vs. Control group. Percentage of responders was higher (P =0.025) in Probiotic group vs. Control group.	Percentage of women reporting an improvement was not different between groups. Percentage of responders not different between groups.
Frequency of digestive symptoms	Observe more pronounced decrease (P =0.044) in overall score of 4-week period in Probiotic group vs. Control group. Borborygm frequency showed higher decrease (P = 0.016) in Probiotic group vs. Control group. Flatulence frequency showed higher decrease in Probiotic group vs. Control group in week 1 (P = 0.041), 2 (P = 0.028), 4(P = 0,008). No difference in bloating score, abdominal pain or discomfort score.	Observe more pronounced decrease (P=0.033) in overall score of 4-week period in Probiotic group vs. Control group.
Bowel function	Stool frequency did not differ between groups. Stool consistency decrease (P = 0.02) in Probiotic group vs. Control group.	Results was not measured
Health-related quality of life	Digestive comfort dimension of Food and Benefits Assessment questionnaire increase (P = 0.027) in Probiotic group vs. Control group. Results of Psychological General Well-Being Index Questionnaire not differ between groups.	Results was not measured

this was also found in the pooled analysis ($P=0.003$). Guyonnet et al. (2009) reported all results for changes in each individual symptom over the 4-week period. Borborygmi frequency decreased more in the Probiotic group vs. the Control group over the 4-week period ($P=0.016$); the decrease in flatulence frequency was higher in the Probiotic group than in the Control group in the first ($P=0.041$), second ($P=0.028$), and fourth weeks ($P=0.008$). No significant differences were observed in the bloating score or in the abdominal pain or discomfort score[21].

Bowel function

Subjects reported daily bowel movements according to the Bristol stool scale[21]. Only one study presented results for this secondary endpoint. Stool frequency did not differ between the Probiotic group and the Control group, but a decrease in stool consistency was observed in the Probiotic group vs. the Control group ($P=0.02$)[21].

Health-related quality of life

The Health-related quality of life (HRQoL) was assessed by self-administration of two questionnaires: the Food and Benefits Assessment (FBA) and the Psychological General Well-Being Index (PGWBI) at three time points: baseline, after 4 weeks, and after 8 weeks[21]. Guyonnet et al. (2009) observed an increase ($P=0.027$) in digestive comfort as measured by the FBA questionnaire in the Probiotic group vs. the Control group after 4 weeks; however, the results of the PGWBI questionnaire were no different in the Probiotic vs. the Control groups[21].

Discussion

This review examined whether the consumption of probiotic fermented milk containing *B. lactis* CNCM I-2494/*B. lactis* DN-173 010, together with yogurt symbiosis strains and *L. lactis*, improved minor digestive symptoms in healthy women.

We only included randomized controlled studies that showed high quality methodology and a low risk of bias. These two studies showed that the inclusion of this specific probiotic in the everyday diet of healthy women could improve digestive comfort. Moreover, none of the studies we looked at reported a worse outcome for the interventional group, in agreement with findings of a recent meta-analysis[20]. Ford et al. (2014) examined the efficacy and safety of this probiotic in idiopathic constipation and reported no adverse events in three identified RCTs[20]. Our narrow and specific research question (the combination of 5 bacterial strains in fermented milk; a specific GI condition; and a specific target population) explains the limited number of studies (n=2) included in this review compared to other systematic reviews of probiotics and IBS.

GI well-being was the main outcome. Healthy women in the RTC conducted by Guyonnet et al. (2009) reported improvement in GI discomfort after just one week of probiotic ingestion[21]. Guyonnet et al. (2009)[27]conducted another open-label study that assessed the effect of the same probiotic fermented milk in its commercially available form in real-life conditions i.e. in 371 men and women who consumed one or two portions of probiotics/day for 14 consecutive days[27]. The results were obtained using a self-reported digestive comfort questionnaire. The percentage who reported improvement in digestive comfort was higher in volunteers who consumed this probiotic in low and higher doses (82.5% and 84.3% in the 1-portion and 2-portion groups, respectively) compared with the control group (2.9%)[27]. These results indicate an overall positive effect for probiotics on the promotion of GI well-being.

Interpreting the magnitude of the effect (responders' rate difference=10.6%; NNT=9.5) is important for assessing the effect of this probiotic food on GI discomfort. There is a lack of guidelines for assessing the effects of functional foods on GI discomfort in the general population, and most of the thresholds/differences considered to be clinically relevant are defined for assessing drugs in diseased populations (e.g. a 10% difference in IBS for the rate of responders). Generally the magnitude of the anticipated effect for food is smaller than for drugs[28]. A recent extensive systematic review of the effects of probiotics on IBS and chronic constipation[20] showed an NNT for the analysis of persistence of symptoms of 7–8, which differs slightly from the rate of responders observed for studies with the probiotic food containing *B. lactis* CNCM I-2494/*B. lactis* DN-173 010 in healthy subjects (NNT=9.5) · Another recent meta-analysis assessed the effect of fiber supplementation on IBS[29], with fiber supplementation showing a significant benefit on a dichotomous outcome for IBS symptoms with an NNT of 10. The evidence for using rifaximin to treat IBS was reviewed recently[30] and showed a therapeutic gain of 9.8% for the responder's rate, corresponding to an NNT of 10.2. The observed improvement in GI well-being with this probiotic food is in the lower spectrum of what is considered clinically relevant for a more severely affected population (i.e. patients with IBS) and therefore could be considered relevant.

Digestive symptom frequency was evaluated by both of the two included trials, and interventional probiotic study groups report decreased symptom severity[21,26]. Although the relationship between the assessed digestive symptoms seems intuitively clear, in the Probiotic group there was a decrease in borborygmi and flatulence but no significant differences in bloating score or in the abdominal pain or discomfort score[21].

Bowel function was an important secondary outcome that was assessed based on decreases in stool consistency[21]. Other studies have assessed effects on bowel function using alterations in colonic transit time to show the mechanism of action. In a double-blind cross-over study of healthy women who consumed three 125-g cups per day of fermented milk containing *Bifidobacteria animalis* DN-173 010 (Probiotic group) or fermented milk without bifidobacteria (Control group), x-ray analysis of the segmental colonic transit time showed that in the Probiotic group, the sigmoid transit time was shorter than for any other colon segment[31]. Bouvier et al. (2001) conducted a double-blind study of healthy men and women that demonstrated that fermented milk containing *Bifidobacteria animalis* DN-173 010 improved colonic transit time and observed that the effect was more pronounced in women than in men[32]. Miller et al. (2013) conducted a meta-analysis to study the short-term (10–28 days) effects of probiotic supplementation on intestinal transit time. They included 13 RCTs involving different probiotic strains and clinical conditions. The authors' general conclusion was that probiotic supplementation was associated with reduced intestinal transit time, with consistent treatment effects in older adults[1]. When a subgroup analysis of the Miller et al. meta-analysis was conducted, only two probiotic strains were associated with treatment effects: *B. lactis* HN019 and DN-173 010[1]. Accordingly, it may be of interest to conduct an RCT with fermented milk containing *B. animalis* DN-173 010 in healthy subjects to assess colonic transit time as measured by radio-opaque markers.

In our investigation, only one of the two included studies assessed the HRQoL to ascertain the relevance of the observed improvement in symptoms and in the comfort dimension of GI well-being. Guyonnet et al. (2009) used two questionnaires, with one, considered by the authors to be the primary endpoint, showing an increased HRQoL score for women that ingested fermented milk containing *B. animalis* DN-173 010[21]. The instrument was developed and validated for patient-reported outcomes to assess specifically the benefits of food or diet on HRQoL[21]. Based on this study, we can conclude that results about probiotics leading to an improvement in digestive comfort dimension seem promising.

Product effects are dose-specific, and a recommendation about an adequate daily amount of probiotic intake should be based in its efficacy in clinical trials[2,8,11]. The two included RCTs[21,26] used two units of fermented milk containing *B. animalis* DN-173 010/daily, so the observed (related) effects are attributed to consuming this amount. Notably, the tested fermented milk product contained the same quantity of the different strains of bacteria. This reinforces the conclusion about the efficacy of this probiotic food, as the same product (matrix and type and quantity of bacterial strains) and same daily dosage (two 125-g servings per day) were tested in both independent RCTs. However, a previous study conducted by Guyonnet et al. (2009) compared groups consuming 1 or 2 portions of fermented milk produt containing *B. animalis* DN-173 010 per day and did not find any significant difference between the low and high intake groups in terms of digestive comfort or digestive symptoms[27].

It is important to keep in mind that this review analyzed a specific probiotic that is included in a commercial fermented milk product in healthy volunteers. Thus, our results could help clinicians make evidence-based decisions about whether this probiotic should be recommended.

Mechanisms of action

The effects of probiotic fermented milk product containing *B. lactis* CNCM I-2494 could be mediated by several mechanisms of action that are involved in the control of GI functions and in interactions with gut microbiota[33].

Effect on colonic transit time

Marteau et al. (2002) studied healthy women who ingested three 125-g cups per day of a fermented milk product containing *B. animalis* DN-173 010 (Activia®) vs. placebo could not attribute the probiotic effect in shorten colonic transit time to fecal pH, fecal weight, bacterial mass and fecal bile acids[31]. However, Agrawal et al (2008) observed different results in a well-controlled double-blind study with female subjects who fulfilled the Rome III criteria for IBS-C. The women were randomized to consume two 125-g cups per day of fermented milk product containing *B. animalis* DN-173 010 vs. placebo. The protocol analysis was conducted with radio-opaque markers (three different types on three consecutive days, and a simple abdominal x-ray was taken on the fourth day)[10]. Colonic transit time was reduced by the probiotic test product (-12.2 h; $P=0.026$)[10].

Effects on hypersensitivity and intestinal permeability

In everyday life, there are many stressful events that can exacerbate digestive symptoms, particularly in IBS patients, and there is evidence of an association between visceral hypersensitivity and some IBS symptoms[10,12]. Moreover, there is frequently an increase in intestinal permeability in stressed patients, and this is associated with digestive disorders like bloating[34]. Experimental results in an animal model of acute stress-induced visceral hyperalgesia and increased intestinal permeability show that fermented milk containing *B. lactis* CNCM I-2494 is beneficial for symptom control. Administration of a fermented milk probiotic at a concentration corresponding to 3.3×10^7

CFU-mL^{-1} clearly inhibited the visceral hypersensitivity response and prevented the increase in intestinal permeability induced by acute stress by normalizing the intestinal epithelial barrier[33].

Gut microbiota and probiotics could influence brain activity via signaling mechanisms and thus modulate behaviour[12]. One interesting study measured the brain's response to an 'emotional faces attention task' vs. the resting brain activity of women that consumed 2 units per day of fermented milk containing *B. lactis* CNCM I-2494 for 4 weeks and compared the data to that from controls and from a non-intervention group[17]. The authors used functional magnetic resonance imaging before and after the intervention and found that probiotic ingestion modulated the responsiveness of an extensive brain network in healthy women, especially changes in midbrain connectivity[17].

Effects on gut microbiota

Gut microbiota includes both resident commensal bacteria and transient microbes introduced by the diet. Little is known about the role of diet on gut microbiota homeostasis. Veiga et al. (2014) used quantitative metagenomics, *in silico* genome reconstruction, and metabolic modeling to examine changes in the gut microbiome induced by a fermented milk product[35]. In a subject with IBS, fermented milk containing *B. animalis* potentiated short chain fatty acid production, especially production of butyrate, and decreased the levels of the pathobiont *Bilophila wadsworthia* compared to a milk product[35]. Another study reported that probiotic-containing food (*B. lactis, L. lactis, L. bulgaricus, and S. thermophiles*) reduces intestinal inflammation in a murine model of colitis[36]. This was associated with an increase in lactate-consuming and butyrate-producing bacteria, a decrease in cecal pH, and an increase in select cecal short chain fatty acids[36].

An interesting study characterized the fecal human microbiomes of twin pairs after they consumed commercially fermented milk containing 5 bacterial fermented milk products and also studied the metatranscriptomes of gnotobiotic mice[22]. The results show that consumption of fermented milk products was not associated with statistically significant changes in the resident community members within and between individuals. They also found that *B. animalis subsp. Lactis* CNCM I-2494 was the most prominent member in the microbiota during the 7-week period of fermented milk product consumption[22]. More research is needed to understand how much influence such factors have on the well-being and digestive comfort (minor GI disturbance) of healthy subjects.

Conclusion

This review found evidence that probiotic fermented milk containing *B. lactis* CNCM I-2494, when consumed by healthy women, can improve GI well-being and decrease the frequency of GI symptoms.

Author contributions

Dan L. Waitzberg, Flávio A. Quilici, Sender Michzputen and Maria do Carmo Friche Passos contributed equally to this work; Dan L.Waitzberg and Maria do Carmo Friche Passos designed the research; Dan L. Waitzberg, Flávio A. Quilici, and Sender Michzputen performed the research; Dan L. Waitzberg and Maria do Carmo Friche Passos and Flávio A. Quilici analysed the data; and Dan L. Waitzberg and Sender Michzputen wrote the paper.

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