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Efficacy of Inferrin™ and Lactoferrin on Symptoms of Irritable Bowel Syndrome in Otherwise Healthy Adults: A Randomised, Double-Blind, Placebo-Controlled Study

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Abstract

Background: Irritable bowel syndrome affects one in five Australians, with abdominal pain as one of the main symptoms. Lactoferrin, found in milk, is known for its anti-inflammatory properties, and the development of a novel microencapsulated form, Inferrin™, may be useful in the treatment of irritable bowel syndrome symptoms. The current study aimed to study the effectiveness of Inferrin™ compared to lactoferrin and a placebo on irritable bowel syndrome symptoms. **Methods:** Sixty-eight male and female participants over 18 years of age were recruited to complete 8 weeks of supplementation with either Inferrin™, lactoferrin, or a matched placebo. Outcomes were measured at baseline, week 4, and week 8. **Results:** There was a decrease from baseline across all groups in IBS symptom severity at weeks 4 and 8, as well as improvements in QOL scores. Lactoferrin and Inferrin™ groups had a significant reduction from baseline to week 8 in weekly stool frequency. **Conclusions:** Overall, lactoferrin and Inferrin™ appeared to have an effect in decreasing symptoms of IBS and weekly stool frequency.

Keywords

Inferrin, Lactoferrin, Irritable Bowel Syndrome

1. Introduction

Lactoferrin is a multifunctional protein that occurs in biological secretions, including milk. Lactoferrin has been shown to possess a variety of properties that include iron binding, antibacterial, and anti-inflammatory [1]. Lactoferrin is consumed in foods and complementary medicines for maintaining healthy iron

homeostasis [2] and for the properties listed previously. A novel form of lactoferrin, Inferrin™, has been developed and contains lactoferrin (minimum 45%) microencapsulated in calcium alginate to gastro-protect the lactoferrin and delay release until the small intestine is reached.

Previous research indicates lactoferrin may support digestive health and comfort, including reduction of intestinal polyps, promotion of healthy gut microflora, reducing pathogenic organisms such as *Heliobacter pylori* [3], and supporting intestinal cell health and inflammatory status [2] [4].

Irritable bowel syndrome (IBS) is the most common gastrointestinal disorder, affecting as many as one in five Australians, and is twice as likely in women as men [5]. The main symptoms of IBS include abdominal pain or discomfort that is often relieved by the passing of wind or faeces, stomach bloating, and chronic diarrhoea or constipation (or alternating between the two) [6]. Other symptoms can include tiredness, headache, and nausea. The precise cause of IBS isn't known, but factors that appear to play a role include muscle contractions in the intestine, nervous system, inflammation in the intestines, dietary intake, infections, or changes in the bacteria in the gut (microflora) [7].

The ability of lactoferrin to positively impact inflammation, the gut microflora, and infection in the gut makes it an attractive candidate as a potential treatment for relieving IBS and associated symptoms. Similarly, the ability of Inferrin™ to deliver a higher amount of fully intact lactoferrin to the small intestine, and over a prolonged period, is thought to further improve lactoferrin's ability to combat IBS. The aim of this study was to assess the efficacy and safety of Inferrin™ and lactoferrin in patients with IBS. It was hypothesised that Inferrin™ and lactoferrin would relieve symptoms of IBS to a greater extent compared to placebo.

2. Methods

This study was a randomised, double-blind, placebo-controlled trial with an 8-week intervention period and three groups: one active Inferrin™ group, one active lactoferrin group, and one placebo group. Following preliminary screening via telephone, potentially eligible participants attended the clinic for an information session. Once all criteria were met, eligible participants were required to provide their written informed consent for inclusion in the trial.

Sixty-eight male and female participants aged over 18 years old were recruited from databases and public media outlets. Eligible participants were included if they were generally healthy, able to provide informed consent, and had normal dietary habits with no changes in the past month. Participants also had to meet the Rome IV IBS diagnostic criteria, which is defined as recurrent abdominal pain or discomfort at least 1 day per week in the last 3 months (onset at least 6 months ago), associated with two or more of the following criteria: related to defecation, associated with a change in frequency of stool, associated with a change in form (appearance) of stool. Females were also required to be using a

prescribed form of birth control (e.g., oral contraceptive). Exclusion criteria included regular intake of nonsteroidal anti-inflammatory drugs including COX-2-inhibitors (exception: acetylsalicyclic acid for cardiovascular prevention up to 100 mg daily) or medications that could influence immune function, intake of products containing lactoferrin including sports nutrition products within the last 2 months, known hypersensitivity to any component of the trial products, including cow milk allergy, and a history of eating disorders. Other exclusion criteria included participants with a history of diseases with abdominal symptoms that could resemble IBS, presence of any other acute or chronic gastrointestinal disorders, history of abdominal surgery (cholecystectomy and appendectomy were tolerated when performed at least one year previously), unstable or serious illness that included renal, hepatic, gastrointestinal, cardiovascular, endocrine, or diabetes, pregnant or breastfeeding mothers, malignancy or treatment for malignancy within the previous 2 years, receiving or prescribed warfarin, heparin, daltaparin, enoxaparin or other anticoagulation therapy including lose dose aspirin, active smokers, nicotine, alcohol, or drug abuse, chronic past and/or current alcohol use (>14 alcoholic drinks per week), and participants who had participated in any other clinical trial during the past month.

Consenting participants underwent a health assessment that included lifestyle, current medications, weight and height assessment, blood pressure, heart rate, IBS questionnaires, and medical history. A sample of blood was collected for analysis of biochemistry markers. Once all baseline assessments were completed, participants were randomly allocated using randomisation software to one of the three groups. Randomisation was conducted by someone not involved in the study. The initial randomisation code was generated by random allocation software (sealedenvelope.com) and patients were allocated to one of the three groups at time of enrolment. All trial participants, investigators conducting the trial and the biochemist analysing the samples were blinded to who was on what product.

Those in the Inferrin™ group were required to take 120 mg of Inferrin™ (equal to 50 mg of lactoferrin) twice per day for a total of 240 mg (100 mg of lactoferrin). The lactoferrin group was required to take 50 mg lactoferrin with 70 mg micro-crystalline cellulose powder twice per day for a total of 240 mg (100 mg of lactoferrin). The placebo group was required to take 120 mg of micro-crystalline cellulose twice per day for a total of 240 mg (0 mg of lactoferrin). All trial products were encapsulated in identical capsules and contained in trial product containers that appeared identical. Participants were asked to take the allocated product for 56 days and attend the study site at day 28 (4-weeks) for a progress assessment, and day 56 (8-weeks), for a final assessment. At each visit, all baseline tests were re-administered.

The primary outcome measure was the IBS symptom severity scale (range 0 - 40). Secondary measures included score on IBS-QOL scale, stool consistency (IBS-D: decrease in weekly average of >1 in terms of Bristol Stool Scale), stool frequency (IBS-C: increase of 1 or more complete spontaneous bowel move-

ments per week compared with baseline), blood chemistry (fasting glucose, total serum iron, serum ferritin, haemoglobin), inflammation and immunity (IL-6, CRP, IFN-γ, TNF-α, IgA, IgM, IgG), enzyme and liver function test (E/LFT; iron, ALT, AST, ALP, GGT), granulysin, full blood count, intestinal permeability (zonulin), vital signs (blood pressure, heart rate, body weight, BMI), and global assessment of tolerability on a 5-point Likert scale by investigator and participant. Participant diets were monitored throughout the study for any change. If a participant changed their diet from what they typically ate, it was recorded and taken into consideration when analysing the data.

A total of 90 participants (30 per group) were required based on the power to detect a change of 20% reduction in primary outcome (absolute values). Effect size: 0.7, α probability error: 0.05, power: 0.8. To allow for a 25% drop out rate, the study had a recruitment target of 120 participants (40 per group). Analysis was conducted using either GraphPad Prism 7.0 or SPSS 25. All results were first tested for normality before any other test was conducted. Based on the distribution of the data, the appropriate statistical test (t-test or ANOVA with a post hoc test) was used as required to compare distribution of data and differences between groups and within group variables. Differences between groups were assessed using t-test and covariates were accounted for with an ANCOVA. Some cross-sectional analysis using t-test was also performed on participant data to compare the group dynamics. Results were considered statistically significant if $p < 0.05$. Statistical tests were used to compare differences between groups for changes in: IBS-SSS, score on IBS-QOL, stool consistency & frequency, blood analysis and global assessment of tolerability.

3 Results

Sixty-eight participants were enrolled in the study, and of those 53 completed the entire study (week 8). Fifteen participants withdrew or were lost to follow-up, including 2 that withdrew due to an adverse event. Of the withdrawn participants, 4 completed week 4 collection data and were included in the analysis as intention to treat. Of the 57 participants analysed, 18 participants were in the lactoferrin group, 20 in the Inferrin™ group, and 19 in the placebo group. All groups were well matched at baseline and there were no statistical differences between groups (Table 1).

All groups decreased in IBS symptom severity at week 4 and week 8. There was, however, no statistical difference between treatments at week 4 ($p = 0.934$) or week 8 ($p = 0.350$) (Table 2).

The IBS-QOL results were similar between groups at baseline. All groups improved the QOL score at week 4 and week 8, however no differences were seen between groups at any timepoint. Stool movement frequency analysis was only conducted on participants with a stool movement frequency of greater than 9 per week. The lactoferrin and Inferrin™ groups had a statistically significant reduction from baseline to week 8 in weekly stool frequency of −2.58 and −5.07, respectively (Table 3). There was also a statistically significant difference between

Table 1. Summary of baseline demographics.

	Inferrin™	Lactoferrin	Placebo
Age (years)	48.80 ± 13.98	40.17 ± 15.16	49.47 ± 15.73
Waist circumference (cm)	86.46 ± 11.83	85.76 ± 13.75	83.94 ± 13.89
Hip circumference (cm)	101.78 ± 7.28	105.36 ± 9.02	102.08 ± 11.51
Systolic BP	120.78 ± 13.24	115.88 ± 16.02	116.11 ± 19.35
Diastolic BP	81.78 ± 8.10	76.44 ± 7.44	74.28 ± 8.29
Heart rate	69.39 ± 10.30	68.38 ± 9.42	68.17 ± 9.34
Weight (kg)	73.96 ± 13.01	74.66 ± 15.60	71.20 ± 15.06
Height (cm)	162.89 ± 38.47	160.88 ± 41.09	160.58 ± 39.64
BMI (kg/m^2)	27.47	29.02	27.34

Data shown as mean ± SD.

Table 2. Change from baseline for total IBS-SSS.

	Inferrin™	Lactoferrin	Placebo
Week 4 (day 28)	−5.42 ± 7.81$^{\#}$	−4.78 ± 7.12$^{\#}$	−5.68 ± 7.79$^{\#}$
Week 8 (day 54)	−4.80 ± 9.46$^{\#}$	−4.67 ± 8.50$^{\#}$	−7.37 ± 7.60$^{\#}$

Data shown as mean ± SD. $^{\#}$Significantly different to baseline values.

Table 3. Weekly stool frequency change from baseline.

	Inferrin™ (n = 15)	Lactoferrin (n = 13)	Placebo (n = 13)
Week 4	−3.67 ± 4.98$^{\#}$	−0.33 ± 3.96	−2.17 ± 4.82
Week 8	−5.07 ± 5.54$^{a,\#}$	−2.58 ± 4.36	−0.08 ± 3.32

Data shown as mean ± SD. $^{\#}$Significantly different to baseline values; ^{a} Significantly different to placebo group.

treatments at week 8 [F(2, 38) = 3.942, p = 0.028]. Post hoc tests indicated that the Inferrin™ group was statistically different compared to the placebo group (p = 0.024).

Results of the stool consistency as measured by the Bristol stool scale indicated that the average baseline stool consistency was type 4-5 (normal to lacking fibre). No significant changes were seen from baseline or between groups at week 4 and week 8. During the study, no participant reported any change to their usual diet.

At baseline, the lactoferrin group had a significantly higher plasma ALP concentration compared to the Inferrin™ and placebo groups (Table 4). This effect was maintained throughout the study with the concentration of plasma ALP still significantly higher in the lactoferrin group at week 8 (Table 4). No other differences were seen in either the baseline, week 4 or week 8 pathology data for any groups (Table 5, Table 6). When pathology change from baseline was compared between groups, the only significant difference between groups was plasma iron levels between the lactoferrin and placebo groups (Table 4, Table 5).

Table 4. Pathology measures.

	ALP (U/L)				Ferritin (ng/mL)				GGT (U/L)		
	Inferrin™	Lactoferrin	Placebo		Inferrin™	Lactoferrin	Placebo		Inferrin™	Lactoferrin	Placebo
Baseline	67.7 ± 24.2	89.9 ± 32.6[a,b]	68.5 ± 22.1	Baseline	91.1 ± 75.2	67.2 ± 57.2	71.6 ± 71.6	Baseline	17.9 ± 12.4	13.4 ± 8.4	14.2 ± 7.6
Week 4	73.1 ± 17.0	76.3 ± 21.6	66.0 ± 20.5	Week 4	77.0 ± 82.3	62.1 ± 46.6	76.0 ± 74.1	Week 4	19.5 ± 11.4	13.7 ± 13.3	16.2 ± 8.4
Week 8	71.9 ± 19.4	82.9 ± 25.9[a,b]	59.6 ± 23.1	Week 8	80.8 ± 76.3	49.6 ± 40.0	75.2 ± 85.3	Week 8	17.2 ± 7.7	12.7 ± 8.4	16.4 ± 9.8
Change	4.3 ± 28.1	−7.1 ± 32.5	−8.8 ± 32.7	Change	−10.2 ± 30.1	−17.6 ± 48.1	3.6 ± 25.9	Change	−0.7 ± 8.6	−0.7 ± 5.6	2.2 ± 7.2
	ALT (U/L)				IgA (mg/dL)				Glucose (mmol/L)		
	Inferrin™	Lactoferrin	Placebo		Inferrin™	Lactoferrin	Placebo		Inferrin™	Lactoferrin	Placebo
Baseline	19.7 ± 10.2	17.2 ± 6.3	16.7 ± 6.2	Baseline	277.7 ± 69.3	335.3 ± 76.2	318.6 ± 73.2	Baseline	5.4 ± 0.4	5.3 ± 0.6	5.4 ± 0.5
Week 4	17.0 ± 8.3	18.4 ± 10.4	17.7 ± 6.4	Week 4	274.7 ± 70.9	354.8 ± 42.8	322.3 ± 72.7	Week 4	5.5 ± 0.6	5.4 ± 0.8	5.3 ± 0.7
Week 8	19.3 ± 8.2	15.5 ± 5.7	18.6 ± 11.8	Week 8	280.4 ± 67.6	334.5 ± 76.6	319.7 ± 74.5	Week 8	5.6 ± 0.4	5.3 ± 0.6	5.3 ± 0.5
Change	−0.4 ± 11.5	−1.7 ± 6.3	1.9 ± 11.5	Change	2.7 ± 19.6	−0.9 ± 18.8	1.1 ± 20.7	Change	0.1 ± 0.4	0.0 ± 0.4	−0.1 ± 0.3
	AST (U/L)				IgM (mg/dL)				hsCRP (mg/L)		
	Inferrin™	Lactoferrin	Placebo		Inferrin™	Lactoferrin	Placebo		Inferrin™	Lactoferrin	Placebo
Baseline	23.6 ± 8.4	22.9 ± 5.8	22.6 ± 4.2	Baseline	199.7 ± 48.2	206.7 ± 51.8	199.1 ± 51.2	Baseline	2.2 ± 3.4	3.6 ± 4.6	1.6 ± 3.7
Week 4	22.6 ± 6.5	23.1 ± 4.9	25.4 ± 5.5	Week 4	198.9 ± 46.2	190.7 ± 57.0	194.9 ± 62.5	Week 4	1.6 ± 2.4	2.4 ± 3.4	2.4 ± 4.6
Week 8	33.8 ± 42.5	22.1 ± 5.6	23.8 ± 5.1	Week 8	205.4 ± 43.4	203.8 ± 57.8	196.5 ± 49.2	Week 8	1.6 ± 3.0	2.7 ± 4.2	1.7 ± 3.2
Change	−0.5 ± 6.5	−0.8 ± 3.7	1.3 ± 4.5	Change	5.8 ± 26.9	−2.9 ± 30.2	−2.6 ± 19.5	Change	−0.6 ± 1.5	−0.9 ± 3.0	−0.1 ± 1.2
	Fe (umol/L)				IgG (g/L)						
	Inferrin™	Lactoferrin	Placebo		Inferrin™	Lactoferrin	Placebo				
Baseline	12.2 ± 4.7	12.6 ± 6.9	9.9 ± 4.3	Baseline	16.4 ± 2.1	16.6 ± 2.6	17.3 ± 3.3				
Week 4	10.6 ± 3.4	8.9 ± 5.0	11.3 ± 4.7	Week 4	16.3 ± 2.2	16.4 ± 2.1	16.9 ± 3.1				
Week 8	10.0 ± 5.1	9.1 ± 3.4	10.5 ± 6.3	Week 8	16.1 ± 2.2	16.3 ± 2.3	16.8 ± 3.1				
Change	−2.2 ± 6.9	−3.5 ± 7.1[a]	0.6 ± 3.6	Change	−0.2 ± 0.8	−0.3 ± 0.8	−0.5 ± 0.6				

Data shown as mean ± SD. [a]significant difference to Placebo group; [b]significant difference to Inferrin™ group.

Table 5. Inflammatory marker measures.

	TGFb (ng/mL)				Granulysin (pg/mL)				IFNg (pg/mL)		
	Inferrin™	Lactoferrin	Placebo		Inferrin™	Lactoferrin	Placebo		Inferrin™	Lactoferrin	Placebo
Baseline	25.0 ± 4.9	26.9 ± 6.3	25.5 ± 6.3	Baseline	626.5 ± 261.3	626.6 ± 386.8	677.5 ± 290.0	Baseline	42.2 ± 22.5	40.6 ± 22.0	43.3 ± 28.6
Week 4	27.0 ± 8.1	28.7 ± 5.1	25.6 ± 8.4	Week 4	592.8 ± 230.3	654.9 ± 413.6	725.5 ± 309.3	Week 4	44.0 ± 26.3	43.2 ± 22.1	38.8 ± 22.0
Week 8	28.9 ± 9.3	28.4 ± 8.5	24.0 ± 6.4	Week 8	613.0 ± 238.7	642.4 ± 337.8	667.2 ± 333.0	Week 8	43.7 ± 24.9	42.6 ± 22.8	38.3 ± 23.6
Change	3.9 ± 10.0	1.5 ± 9.1	−1.4 ± 7.2	Change	−13.4 ± 113.0	15.7 ± 149.2	−15.3 ± 133.0	Change	1.5 ± 7.1	2.0 ± 9.0	−5.0 ± 12.0
	IL6 (pg/mL)				TNFa (pg/mL)				Zonulin (ng/mL)		
	Inferrin™	Lactoferrin	Placebo		Inferrin™	Lactoferrin	Placebo		Inferrin™	Lactoferrin	Placebo
Baseline	24.3 ± 38.4	13.5 ± 39.7	20.4 ± 36.5	Baseline	10.9 ± 3.2	11.4 ± 4.1	9.9 ± 2.4	Baseline	40.7 ± 10.8	39.2 ± 7.5	38.1 ± 6.2
Week 4	25.3 ± 38.3	21.0 ± 46.7	13.7 ± 19.8	Week 4	11.1 ± 3.2	11.0 ± 2.3	8.4 ± 2.5	Week 4	38.3 ± 4.2	38.8 ± 8.4	38.9 ± 5.4
Week 8	27.6 ± 43.9	14.0 ± 38.5	13.3 ± 19.6	Week 8	11.5 ± 3.3	11.7 ± 3.5	9.9 ± 6.5	Week 8	36.9 ± 3.0	33.3 ± 9.9	38.9 ± 9.7
Change	3.3 ± 8.1	0.5 ± 2.0	−7.2 ± 22.6	Change	0.6 ± 3.3	0.4 ± 2.4	0.7 ± 5.6	Change	−3.8 ± 9.2	0.1 ± 2.0	0.8 ± 7.4

Data shown as mean ± SD.

Table 6. Full blood count measures.

	WBC			RBC			HGB		
	Inferrin™	Lactoferrin	Placebo	Inferrin™	Lactoferrin	Placebo	Inferrin™	Lactoferrin	Placebo
Baseline	4.6	4.9	4.4	4.3	4.1	3.9	135.6	130.3	131.4
Week 4	4.2	4.9	4.7	4.2	4.1	4.1	132.6	133.6	131.1
Week 8	4.4	4.9	4.6	4.1	4.2	4.1	134.9	132.3	131.4

Data shown as mean.

4. Discussion

The aim of this study was to analyse the effectiveness of lactoferrin and a novel microencapsulated form of lactoferrin, called Inferrin™, in patients with IBS. It was hypothesised that Inferrin™ and lactoferrin would relieve symptoms of IBS to a greater extent compared to a placebo. Results showed that there was a decrease in IBS symptom severity at weeks 4 and 8 across all groups, as well as improvements in QOL scores. There was also a reduction in weekly stool frequency across the lactoferrin and Inferrin™ groups from baseline to week 8, with the Inferrin™ group being statistically different compared to the placebo group.

The lack of difference between groups for the IBS symptom severity appears to be due to a strong training and/or placebo effect. Within the first 4 weeks of the study, all three groups recorded a significant change in IBS symptom severity scores (Table 2). In the second 4 weeks of the study (weeks 4 to 8), there was very little change from week 4 for the IBS symptom severity scores. This suggests either a rapid improvement that was maintained, or a strong placebo and or training effect. We speculate that by being part of a clinical trial, and asking specific questions relating to their symptoms, participants likely put a greater focus on specific aspects of their IBS based on the questions that were being asked. This may have resulted in greater awareness of the symptom and therefore the scores recorded at weeks 4 and 8 may have been considered more than those at baseline. To help avoid this effect, future research would benefit by having a lead in period to help reduce a potential training effect and minimise any placebo effect.

Whilst lactoferrin is produced naturally by the body and involved in many processes, including anti-inflammatory, there is evidence that increases in lactoferrin levels, which can be achieved through supplementation, have an increased effect throughout the body [8]. One of the main symptoms of IBS is abdominal pain, potentially caused by gut inflammation [9], and in the current study, it was shown that treatment with lactoferrin and Inferrin™ decreased IBS symptom severity. The mechanism of action for lactoferrin may involve recruitment of immune cells, including neutrophils, and decreasing levels of inflammation markers such as TNF-α [10] [11]. Studies have found that lactoferrin can maintain the integrity of the epithelial barrier within the gut, as well as shape the microbiota [10]. This evidence could explain the results of the current study, whe-

reby lactoferrin and Inferrin™ were acting in an anti-inflammatory manner to decrease symptom severity.

An animal study conducted in piglets found that dosing with lactoferrin caused decreases in diarrhoea, with no significant changes being seen in haematological parameters [12], and although it was conducted on animals, showed similar results to the current study. Results from a study conducted on a large paediatric population found that supplementation with lactoferrin was able to decrease severity and duration of diarrhoea episodes, as well as prevent loose stool [13]. It was unable to, however, decrease the incidence of diarrhoea episodes. There is limited evidence on the effectiveness of Inferrin™ and whether there are differences in absorption due to its microencapsulated form. A recent trial that focused on inflammation markers and the absorption and efficacy found that Inferrin™ was not degraded in the gut, and downregulated immune system activation, which would decrease inflammation [14]. These results are somewhat in line with those from the current study, where stool frequency and IBS symptom severity were decreased across Inferrin™ and lactoferrin groups.

While overall there were no significant differences in any meaningful pathology data (only iron) for either within or between groups, there were several trends ($p < 0.1$) that may indicate a possible mechanism for the effectiveness of Inferrin™. Serum TFG-b, IFN-g and IL-6 all increased from baseline in the lactoferrin and Inferrin™ groups, while it decreased in the placebo group. Serum zonulin decreased in the Inferrin™ group while remaining relatively stable in the lactoferrin and placebo groups. We theorise that this may indicate a possible change to the inflammatory/immunomodulating response in the lactoferrin and Inferrin™ groups. However, additional data is required to support this theory.

The increase in IL-6 may indicate the start of a change in the immune activity, and when combined with the increase in TGF-b and IFN-g may indicate an initiated change in the immune activity. TGF-b is known as an immunosuppressive cytokine with both pro-and anti-inflammatory activity, depending on circumstances [15]. TGF-b can enhance STAT3 activation, leading to activation of immunosuppressive cells, such as Treg, Th17 and myeloid derived suppressor cells [16] [17]. TGF-b has previously been shown to be dysregulated in people with IBD [18], and this may be due to the sporadic inflammatory nature of IBS. Similarly, IFN-g has long been considered a pro-inflammatory cytokine. And while IFN-g has been shown to be elevated in the gut of patients with IBS [19] [20], it is also now becoming known to promote immune system activation [21] [22] [23]. IFN-g is increasingly becoming thought of more as a regulator of immune response and inflammation [24]. Together, it may be that IL-6, TGF-b and IFN-g are starting to re-regulate the inflammatory effect of IBS. However, additional data is required before this can be proven.

Another possible effect of the increase in IL-6 and TGF-b may be tissue repair. IL-6 and TGF-b have fibrotic properties having been shown to be associated with pathologies associated with fibrosis [25]. This is supported by the decrease

in serum zonulin. Zonulin is a tight junction protein that is responsible for maintaining the junction between epithelial cells and not allowing pathogens to cross into the blood stream. When the junction is compromised, such as in the case of inflammation, the tight junction protein bonds are broken and the proteins themselves enter the blood stream [26] [27] [28] [29]. Therefore, a decrease in serum zonulin may indicate that the epithelial junction integrity is being better maintained as less zonulin is showing in the serum. However, again, further data is required to support this theory. This may mean, that in the case of IBS as presented here, the increase in serum IL-6 and TGF-b and decrease in serum zonulin may indicate a possible tissue repair of the gastrointestinal tract due to a change in inflammatory conditions.

The greatest limitation of this study was participant recruitment. Due to the specific requirements of inclusion into the study, it was very difficult to find eligible participants willing to participate. The main limitation to subject recruitment was that potential participants were already taking a supplement or medication and were unwilling to stop these to be a part of the study. This resulted in the trial having to conclude before the sample size number was reached. Despite the reduced sample size, we were still able to see an effect from the Inferrin™. The full extent of effect Inferrin™ and lactoferrin may have, is unknown due to the reduced sample size. If more people were able to be recruited, a stronger product effect may have been able to have been achieved. Future studies might benefit by being able to recruit matched pairs such as not requiring medications or supplements to be stopped, and therefore make recruitment easier.

Another limitation of this study was that participant diet was not closely monitored in the form of diet recall and/or diet diaries. Participants self-reported any change to their diet from what they typically ate at the start of the study. However, as no participant reported any change to their diet, and people with IBS typically follow relatively consistent diets, this is unlikely to have had any effect on the outcomes of this study.

Based on the evidence and results, supplementation with either lactoferrin or Inferrin™ appeared to have an effect on decreasing IBS symptoms, specifically weekly stool frequency with Inferrin™ superior to lactoferrin.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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