Effects of short-term supplementation with bovine lactoferrin and/or immunoglobulins on body mass and metabolic measures: a randomised controlled trial

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ABSTRACT
Given the role of the intestinal microbiota in obesity and related disease, strategies to modulate the composition of the intestinal microbiota may augment traditional weight-management approaches. Here, we examined the safety and tolerability of 28 days of supplementation with bovine whey-derived lactoferrin and immunoglobulin supplements in a cross-sectional cohort of free-living adults. Participants (n = 20 each group) received enteric-coated whey-derived bovine lactoferrin (200 mg), immunoglobulin (200 mg or 800 mg), combination lactoferrin/immunoglobulin supplements (200 mg/200 mg, 200 mg/800 mg) or placebo in a double-blind design. Supplement use was generally well tolerated and routine haematology, and clinical chemistry measures were largely unchanged following supplementation. Measures of body composition remained stable and indices of glycaemic control and blood lipids revealed fluctuations of <5% but were not significantly different between groups. Overall, short-term lactoferrin/immunoglobulin supplementation was well tolerated in this cohort; use of these types of supplements to enhance other weight management strategies should be investigated over extended periods.

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Introduction
The role of the gut and its extensive population of commensal microorganisms in the regulation of metabolic pathways is increasingly recognised. Microbial dysbiosis may increase intestinal permeability and contribute to inflammatory and metabolic aberrations as a result of the translocation of intestinal microbes into the systemic circulation (Cox et al. 2015). The potential for the intestinal microbiota to contribute to energy harvest via fermentation of non-digestible starches may also be relevant in the context of energy balance and excess body mass (Guarner & Malagelada 2003; Tilg et al. 2009). Indeed, data from animal studies support the contribution of the intestinal microbiota to body mass. Lower body mass/body fat has been reported in germ-free mice compared to wild-type counterparts (Backhed et al. 2004), even after exposure to a high-fat and sugar-rich model of a Western style diet (Ding et al. 2010), suggesting that the capability for energy harvest is diminished in the absence of a colonised gastrointestinal tract. Further, transplantation of microbiota from obese mice results in an increase in fat mass in germ-free animals (Turnbaugh et al. 2006) suggesting a unique microbial composition among obese animals that favours accumulation of excess body weight. Differences in the composition of the intestinal microbiota between healthy-weight and overweight cohorts have been reported (Ley et al. 2006; Turnbaugh et al. 2009; Schwieritz et al. 2010), and the potential for modulation of the intestinal microbiota through various nutritional intervention approaches is of interest given the increasing prevalence of obesity and the associated health implications.

Knowledge of immune mechanisms at the mucosal surfaces of the gastrointestinal tract provides insight into strategies that may be effective in modulating the intestinal microbiota and intestinal permeability in obesity and associated disease. Secreted immunoglobulins and antimicrobial peptides, such as lactoferrin, provide local defence mechanisms important in both acute infection and in regulating the commensal microbiota (Ward et al. 2005; Ling et al. 2006). In a murine model of colitis, dietary supplementation with
serum-derived bovine immunoglobulins was associated with reductions in local inflammatory markers and improvements in intestinal barrier function, suggesting a possible role for immunoglobulin supplementation in modulating intestinal permeability (Perez-Bosque et al. 2015). In human clinical studies, an association between lactoferrin consumed in breast milk and the relative abundance of beneficial bifidobacteria and lactobacilli recovered in infant faeces (Mastromarino et al. 2014) provides initial evidence that lactoferrin supplementation may be a strategy to modify the microbiota more broadly. Further, bovine lactoferrin supplementation (200 mg/day for seven days) in eight healthy adult males has been reported to induce increases in peripheral blood T-lymphocyte activation and antioxidant capacity that was interpreted as reflecting the potential for lactoferrin to modulate immune activity (Mulder et al. 2008).

In addition to the potential benefits that manipulation of immune defences at the gastrointestinal mucosa may have on energy harvest and body composition, the cross talk between immune and metabolic-signalling pathways (Marette 2002; Bastard et al. 2006) implicates the intestinal microbiota as a potentially modifiable factor through which improvements in indices of cardiometabolic risk may also be achieved. To date, very few studies have examined this potential. One study by Ono et al demonstrated significant reductions in total body mass and visceral fat area in viscerally obese adults in response to lactoferrin supplementation over eight weeks, in the absence of lifestyle change (Ono et al. 2010). Similarly, six weeks of supplementation with isolated bovine immunoglobulins in a cohort of adults with mild untreated hypercholesterolaemia resulted in significant decreases in total cholesterol and low-density lipoprotein (LDL) cholesterol (Earnest et al. 2005). However, for both of these studies, direct assessments of any alterations in the composition of the intestinal microbiota were not performed and so the mechanisms underpinning these favourable effects on body composition and metabolism remain unknown.

Despite ongoing primary and secondary health promotion campaigns, the prevalence of obesity and associated disease continues to increase. Accordingly, novel strategies, such as supplementation with lactoferrin and immunoglobulins, need to be tested for their potential to enhance traditional approaches to weight management and cardiometabolic risk factor reduction (Brimelow et al. 2015). This paper reports on assessment of the safety and tolerability of short term (four week) supplementation with lactoferrin and immunoglobulin supplements in a cross-sectional cohort of free-living adults and examines changes in basic measures of body mass/composition and metabolic parameters.

Materials and methods

Study design

This study was designed as a double-blind placebo-controlled 28-day intervention trial to investigate the safety and tolerability of bovine whey-derived lactoferrin and immunoglobulin supplements and assess changes in basic measures of body mass and metabolic parameters. Participants were recruited from the general population and were required to be aged between 18 and 55 years. Exclusion criteria included BMI greater than 35 kg/m², self-reported lactose intolerance, history of kidney/liver/thyroid disease, current use of meal replacement products or adherence to a weight-reducing diet plan, use of anti-inflammatory or immunomodulating medications and/or supplements (including whey proteins, colostrum, and fish/krill oil), and for women, pregnancy or possible pregnancy.

The institutional Human Research Ethics Committee provided ethical approval for this study (MED/32/13/HREC), all study procedures were carried out in accordance with the Declaration of Helsinki, and all subjects provided written informed consent prior to participation. The trial was registered prior to commencement with the Australian New Zealand Clinical Trials Registry (ACTRN12614000462684).

Upon recruitment, participants were required to complete a demographic and health history questionnaire and the Australia type 2 diabetes risk assessment tool (AUSDRISK). Dietary composition was assessed by the completion of the Dietary Questionnaire for Epidemiological Studies, version 2. Participants were asked to complete the short-form International Physical Activity Questionnaire (IPAQ) on three occasions throughout the supplementation period and average patterns of physical activity were determined. Participants were asked to maintain regular dietary and physical activity patterns over the 28-day supplementation period.

Supplement

Following screening and recruitment, participants were randomly assigned to one of six treatment groups using a blinded block randomisation method stratified by gender. Supplement groups were as follows: (A) placebo; (B) bovine whey-derived lactoferrin (200 mg) and immunoglobulins (800 mg);
(C) bovine whey-derived lactoferrin (200 mg) and immunoglobulins (200 mg); (D) bovine whey-derived immunoglobulins only (800 mg); (E) bovine whey-derived immunoglobulins only (200 mg); and (F) bovine whey-derived lactoferrin only (200 mg).

Supplements were enteric coated and formulated with excipients mannitol, calcium carbonate, calcium phosphate, sodium starch glycolate, croscarmellose sodium, magnesium stearate, povidone and macrogol. Supplements were identical in appearance and blister packed for daily doses. Participants were requested to consume the provided supplement with their evening meal. Compliance was assessed based on self-report of the number of doses missed and by return of empty packaging at the completion of the supplementation period. Participants were asked to self-report any perceived adverse events at day 14 and day 28. Reported adverse events were classified as gastrointestinal in nature (including gas, bloating, nausea and change in bowel habit), sensory in nature (including perceived fatigue, perceived thirst and vivid dreams) or systemic in nature (headaches).

**Physical/physiological measures**

At day 0 and day 28 participants attended the research clinic for the assessment of body composition and blood pressure measurement. Height was determined to the nearest half centimetre using a wall-mounted stadiometer (Surgical & Medical Products, NSW, Australia). Body mass was determined using a digital body composition scale (model HBF-202, Omron Australia, Melbourne, Australia) capable of also calculating per cent muscle and per cent fat mass. Waist and hip circumference were assessed in accordance with the World Health Organisation STEPwise approach to surveillance protocols using a graduated anthropometric measuring tape (Seca, Germany). Blood pressure and pulse rate were determined using an automatic blood pressure monitor (model HEM-7121, Omron Australia, Melbourne, Australia) with individuals in a seated position.

**Laboratory measures**

Venous blood samples were collected from participants following an overnight fast at day 0 and day 28. Collected samples were analysed within 12 h for routine haematology which comprised a full blood count, with white cell differential, and glycated haemoglobin (HbA1c). This analysis was outsourced to a local pathology provider (QML Pathology, Murarrie, Queensland, Australia).

For clinical chemistry, serum was separated by centrifugation at 3500 rpm for 10 min and stored frozen at $-80^\circ$C until analysis as described below. Routine chemistry analysis was performed on a COBAS Integra 400 system using commercially available reagents, calibrators and controls (Roche Diagnostics). Analytes tested included those listed below with established intra-assay (based on 115 duplicate pairs) and interassay variability shown (both expressed as coefficient of variation; CV%): cholesterol (<1%; <4%), triglycerides (<1%; <2%), high-density lipoprotein (HDL) cholesterol (<1%; <3%), glucose (<1%; <2%), fructosamine (<1%; <2%), lipase (<1%; <6%), sodium (1%; <3%), potassium (<1%; <3%), chloride (<1%; <3%), alanine transaminase (3.7%; <4%), aspartate aminotransferase (2.1%; <4%), alkaline phosphatase (<1%; <8%), gamma-glutamyl transferase (2.6%; <3%), lactate dehydrogenase (<1%; <6%), total bilirubin (2.8%; <8%), albumin (<1%; <5%), creatinine (1.2%; <3%), total protein (<1%; <3%), urea (1.6%; <3%), uric acid (<1%; <4%), iron (1.2%; <5%), unsaturated iron-binding capacity (UIBC; 1.6%; <8%). LDL cholesterol was calculated using the Friedewald equation (Friedewald et al. 1972). Serum proteins were determined on a COBAS Integra 400 system as listed below with established inter-assay variability shown: C-reactive protein (CRP; <5%), Immunoglobulin A (IgA; <3%), Immunoglobulin G (IgG; 3%), Immunoglobulin M (IgM; <4%).

**Data analysis**

Following baseline assessment, dyslipidaemia was determined based on the criteria used in the latest Australian Health Survey (Australian Bureau of Statistics 2013), and metabolic syndrome was classified according to the National Cholesterol Education Programme Adult Treatment Panel III (ATPIII) criteria (Grundy et al. 2004). Differences in key demographic and physical measures between supplementation groups were assessed at baseline using a one-way analysis of variance (ANOVA) for continuous variables, or a Chi-squared test for categorical variables. Pre- to post-intervention changes in physical and laboratory measures were determined and expressed as a percent change relative to baseline. Change scores were compared between the intervention groups using a one-way ANOVA. As a secondary analysis, groups were collapsed to allow for comparison of pre- to post-intervention change scores between (i) placebo, (ii) immunoglobulin-only supplement groups (groups D, E), and (iii)
### Table 1. Demographic, physical and biochemical measures for participants at baseline for the entire cohort and supplementation groups.

|                      | All          | A                | B                | C                | D                | E                | F                | p Value |
|----------------------|--------------|------------------|------------------|------------------|------------------|------------------|------------------|---------|
| n                    | 115          | 19               | 19               | 20               | 19               | 19               | 19               |         |
| Age (median ± IQR)   | 35.6 ± 12.8  | 36.3 ± 12.9      | 37.1 ± 14.1      | 34.8 ± 13.9      | 35.0 ± 13.6      | 36.0 ± 12.8      | 34.5 ± 11.2      | .99     |
| Gender (M/F)         | 38/77        | 6/13             | 7/12             | 6/14             | 6/13             | 7/12             | 6/13             | .99     |
| Ethnicity (%Caucasian)| 59%          | 55%              | 61%              | 60%              | 100%             | 85%              | 84%              | .50     |
| Smoking (% current)  | 9 (7.8%)     | 2 (10.5%)        | 2 (10.5%)        | 4 (20%)          | 0 (0%)           | 0 (0%)           | 1 (5.3%)         | .16     |
| Physical Measures    |              |                  |                  |                  |                  |                  |                  |         |
| Body mass (kg)       | 76.1 ± 14.7  | 75.9 ± 15.3      | 72.9 ± 12.2      | 76.8 ± 15.7      | 77.1 ± 16.5      | 75.0 ± 14.5      | 78.8 ± 14.6      | .88     |
| BMI (kg/m²)          | 26.3 ± 4.4   | 26.3 ± 4.7       | 26.3 ± 4.1       | 26.2 ± 4.2       | 26.1 ± 4.2       | 26.1 ± 5.1       | 26.7 ± 4.3       | .99     |
| Physical activity    |              |                  |                  |                  |                  |                  |                  |         |
| IPAQ Met min/wk      | 2871 ± 2959  | 2435 ± 3635      | 3404 ± 3908      | 2377 ± 2611      | 3340 ± 2449      | 2825 ± 2348      | 2781 ± 2670      | .81     |
| Energy intake (kJ/day)| 7151 ± 3456 | 7131 ± 2912      | 6514 ± 2257      | 7300 ± 3088      | 6809 ± 3487      | 7834 ± 3160      | 7309 ± 3484      | .87     |
| % energy as carbohydrate | 40.3 ± 5.7 | 41.1 ± 1.7       | 7.2 ± 8.8        | 5.3 ± 4.4        | 7.0 ± 4.5        | 5.5 ± 4.5        | 5.5 ± 5.1        | .65     |
| % energy as fat      | 38.8 ± 4.9   | 38.9 ± 4.5       | 38.9 ± 6.0       | 38.7 ± 5.1       | 38.2 ± 4.4       | 39.4 ± 3.8       | 38.6 ± 5.5       | .99     |
| % energy as fat      | 38.8 ± 4.9   | 38.9 ± 4.5       | 38.9 ± 6.0       | 38.7 ± 5.1       | 38.2 ± 4.4       | 39.4 ± 3.8       | 38.6 ± 5.5       | .99     |
| Biochemical Measures |              |                  |                  |                  |                  |                  |                  |         |
| Glucose (mmol/L)     | 5.3 ± 0.7    | 5.4 ± 0.5        | 5.2 ± 0.7        | 5.3 ± 0.6        | 5.0 ± 0.3        | 5.2 ± 0.5        | 5.5 ± 1.2        | .34     |
| HbA1C (%)            | 5.5 ± 0.4    | 5.4 ± 0.5        | 5.3 ± 0.3        | 5.5 ± 0.4        | 5.3 ± 0.2        | 5.5 ± 0.2        | 5.6 ± 0.8        | .27     |
| Cholesterol (mmol/L) | 5.2 ± 1.1    | 5.2 ± 1.1        | 5.6 ± 1.4        | 5.2 ± 1.3        | 4.9 ± 1.0        | 4.9 ± 0.9        | 5.2 ± 0.9        | .26     |
| Triglycerides (mmol/L)| 1.12 ± 0.67 | 1.31 ± 0.81      | 1.19 ± 0.51      | 1.13 ± 0.70      | 0.89 ± 0.34      | 1.06 ± 0.44      | 1.18 ± 0.99      | .71     |
| HDL (mmol/L)         | 1.49 ± 0.39  | 1.51 ± 0.42      | 1.43 ± 0.31      | 1.40 ± 0.48      | 1.55 ± 0.39      | 1.53 ± 0.43      | 1.48 ± 0.33      | .93     |
| LDL (mmol/L)         | 3.13 ± 0.95  | 3.11 ± 0.98      | 3.65 ± 1.25      | 3.06 ± 0.78      | 2.94 ± 0.77      | 2.83 ± 0.96      | 3.20 ± 0.78      | .13     |

*For those reporting yes to performing regular physical activity.

Data are presented as mean ± SD or % where appropriate.

A: placebo; B: lactoferrin and immunoglobulins (800 mg); C: lactoferrin and immunoglobulins (200 mg); D: immunoglobulins only (800 mg); E: immunoglobulins only (200 mg); and F: lactoferrin only.

Results

The recruited cohort was comprised of young to middle-aged adults (mean age: 35.6 ± 12.9 years), was predominantly Caucasian and largely female (67%). On average, participants were considered overweight based on BMI (26.3 ± 4.4 years), despite reporting moderate levels of physical activity (Table 1). Baseline anthropometric measures are also considered for male and female participants separately (Supplementary Appendix A) and further support this tendency towards a predominately overweight cohort. Following baseline assessment, 55% of the cohort had evidence of dyslipidaemia and 12% of the cohort were classified as having metabolic syndrome.

A consort diagram to indicate participant flow and retention is included (Figure 1). Reasons for withdrawal included acute illness/injury (n = 4), inability to swallow tablets (n = 1), unplanned international travel (n = 1) and unexplained loss to follow-up (n = 3). Groups were considered matched at baseline on key demographic, physical and key biochemical measures (Table 1). Supplement use was generally well tolerated and self-reported compliance was assessed as ≥96% of the 28-day dose consumed in all groups (Table 2). All reported adverse events were considered to be mild in nature, having minimal impact on daily activities, were self-limiting and patterns of reporting did not differ between supplementation groups (Table 2).

Activity patterns during the supplementation period were assessed for each individual using the IPAQ Short Form. These data confirmed the self-reported physical activity data collected at baseline; no significant differences in physical activity were noted between groups (Table 1). While we acknowledged the likelihood of energy under-reporting with the Dietary Questionnaire for Epidemiological Studies (Hodge, Patterson, Brown, Ireland & Giles, 2000), neither total energy intake, nor contribution to energy intake by lactoferrin-containing supplement groups (groups B, C, F) and the analysis repeated, again using a one-way ANOVA. All analyses were performed using R version 3.2.0 (R Core Team, 2015). Statistical significance was accepted at p < .05.
Macronutrients was significantly different between groups (Table 1). Measures of body composition were stable across the supplementation period (Table 3). Body mass varied by less than 0.5% and derived fat mass and muscle mass values varied by less than 1.5% for all groups. Similarly, routine haematology and clinical chemistry measures were largely unchanged following supplementation and responses were not significantly different between groups (Supplementary Appendix B). This included both iron and UIBC, which were considered of particular interest given the role of iron in the absorption and utilization of associated macronutrients.

**Figure 1.** Consort diagram to indicate participant flow and retention for 28-day double-blind, placebo-controlled trial of bovine whey-derived lactoferrin and immunoglobulin supplements.

**Table 2.** Self-reported compliance and adverse events over the 28-day supplementation period for the entire cohort and supplementation groups.

| Supplementation Groups | All | A | B | C | D | E | F |
|------------------------|-----|---|---|---|---|---|---|
| n                      | 115 | 19| 19| 20| 19| 19| 19|
| Compliance             | 97% | 97%| 98%| 96%| 97%| 99%| 96%|
| Adverse Events (n, self reported) | | | | | | | |
| Gastro-intestinal      | 27  | 4 | 3 | 6 | 5 | 3 | 6 |
| Sensory                | 7   | 3 | 0 | 2 | 0 | 2 | 0 |
| Systemic               | 2   | 0 | 0 | 1 | 1 | 0 | 0 |

A: placebo; B: lactoferrin and immunoglobulins (800 mg); C: lactoferrin and immunoglobulins (200 mg); D: immunoglobulins only (800 mg); E: immunoglobulins only (200 mg); and F: lactoferrin only.
lactoferrin as an iron-binding protein. Patterns of response were largely similar when groups were collapsed and the immunoglobulin-only supplement groups were compared to lactoferrin-containing supplement groups (Supplementary Appendix C).

Measures of glycaemic control differed by <2.5% across all groups following supplementation, while total cholesterol, HDL and LDL concentrations varied by <5% across all groups (Table 3). Greater fluctuations were noted in triglyceride concentrations with average changes ranging from −3% to 25% for the six supplementation groups (Table 3); here, the variable responses appear to be driven by a small number of individuals showing considerable differences pre- to post-supplementation, although absolute triglyceride concentrations were generally within established reference ranges. Patterns of response in metabolic markers were also largely similar when groups were collapsed and the immunoglobulin-only supplement groups were compared to lactoferrin-containing supplement groups (Supplementary Appendix C). Lastly, IgA, IgG and IgM concentrations were assessed and differed by <3.5% across all groups following supplementation (Table 3).

**Discussion**

Given the growing prevalence of obesity and associated disease, novel strategies that may augment other weight management approaches are worth further consideration. In this context, the relationships between inflammation, the gut microbiota and excess body mass are also of particular interest (Cox et al. 2010); however, this finding has not yet been replicated. The current study was designed to explore the safety and tolerability of short term (four week) supplementation with bovine whey-derived lactoferrin and immunoglobulin supplements in a cross-sectional cohort of free-living adults and to examine changes in basic measures of body mass/composition and metabolic parameters. A four week supplementation period was selected based on evidence from Mulder et al. bovine lactoferrin supplementation for as little as seven days was able to elicit positive changes in immune cell activation (Mulder et al. 2008).

Short-term supplementation with bovine whey-derived lactoferrin and immunoglobulin supplements was well tolerated in this cohort. The stability of routine haematology and clinical chemistry measures suggests the risk profile is low for short- to medium-term use of lactoferrin and immunoglobulin supplements. In addition, reported adverse events were mild and self-limiting, further supporting the safe use of lactoferrin and immunoglobulin supplements. The reporting of gastrointestinal-type symptoms in the days following commencement of supplementation is of interest and suggests potential effects of the supplements on modulation of the composition of the gut microbiota and/or fermentation of non-digestible starches. The potential for lactoferrin and immunoglobulin supplements to modify the composition of the gut microbiota may be relevant when considering the interplay between the gut microbiota and energy harvest in weight management. Indeed, rapid alteration (within 24 h) in the composition of the intestinal microbiota has been reported previously in response to changes in dietary composition (Wu et al. 2011); however changes in metabolic markers are unlikely to

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**Table 3. Changes in physical and metabolic measures in response to 28-day supplementation.**

| Supplementation groups | A | B | C | D | E | F | p Value |
|------------------------|---|---|---|---|---|---|---------|
| **Physical measures**   |   |   |   |   |   |   |         |
| BMI                    | −0.17 ± 1.38 | −0.12 ± 1.79 | 0.28 ± 1.35 | −0.06 ± 1.70 | 0.03 ± 1.09 | 0.29 ± 1.53 | .86      |
| Muscle mass            | 0.01 ± 1.49  | 0.38 ± 2.23  | −0.46 ± 2.18 | −0.66 ± 3.93 | 1.48 ± 3.17  | 1.06 ± 1.46 | .39      |
| Fat mass               | −0.22 ± 2.12 | 0.56 ± 3.17  | −0.25 ± 4.22 | 0.17 ± 2.90  | −1.45 ± 2.65 | 0.12 ± 2.02 | .40      |
| **Biochemical measures**|   |   |   |   |   |   |         |
| Glucose                | −1.59 ± 5.59 | 1.81 ± 11.06 | 2.17 ± 7.4   | 1.37 ± 4.85  | 1.87 ± 4.09  | −0.52 ± 6.46 | .46      |
| HbA1C                  | 0.88 ± 2.71  | 0.87 ± 2.75  | −1.15 ± 3.3  | −1.16 ± 2.65 | −0.93 ± 3.38 | −0.24 ± 3.33 | .25      |
| Fructosamine           | −1.21 ± 4.43 | −1.62 ± 3.21 | −0.87 ± 4.60 | −0.91 ± 4.40 | −0.52 ± 2.40 | −1.25 ± 3.89 | .98      |
| Cholesterol            | 1.17 ± 9.5   | 3.92 ± 10.5  | −0.61 ± 12.7 | −1.09 ± 9.6  | −2.67 ± 12.1 | −2.02 ± 5.8  | .22      |
| Triglycerides          | 15.0 ± 33.0  | 8.14 ± 12.7  | 4.9 ± 29.8   | 25.6 ± 85.6  | −2.81 ± 30.6 | 15.9 ± 42.0  | .64      |
| HDL                    | −4.11 ± 14.9 | −4.72 ± 12.5 | 2.88 ± 14.5  | −2.74 ± 11.8 | −2.61 ± 11.0 | −0.44 ± 9.0  | .53      |
| LDL                    | −0.48 ± 15.5 | −3.90 ± 16.2 | −2.27 ± 16.2 | −2.17 ± 14.9 | −0.84 ± 19.6 | −4.73 ± 7.2  | .59      |
| **Immunology**         |   |   |   |   |   |   |         |
| IgA                    | −0.36 ± 3.64 | −0.46 ± 3.62 | −0.64 ± 4.88 | −0.05 ± 5.26 | −1.03 ± 4.97 | −3.08 ± 6.91 | .34      |
| IgG                    | −0.31 ± 7.06 | −1.47 ± 6.29 | −0.32 ± 6.12 | −1.44 ± 5.91 | −0.35 ± 5.56 | −2.54 ± 6.10 | .37      |
| IgM                    | 0.80 ± 8.09  | −2.77 ± 6.00 | 1.94 ± 12.12 | −0.28 ± 6.24 | −2.58 ± 5.85 | −3.10 ± 7.11 | .44      |

Data are percent change relative to baseline and expressed as mean ± SD. A: placebo; B: lactoferrin and immunoglobulins (800 mg); C: lactoferrin and immunoglobulins (200 mg); D: immunoglobulins only (800 mg); E: immunoglobulins only (200 mg); F: lactoferrin only.
be as immediate, and in the current study, metabolic markers were unchanged over the 28-days.

It is also worth noting that the lactoferrin dose used in the current study (200 mg/day) was consistent with the earlier report by Mulder et al. in which indices of T-lymphocyte function were increased (Mulder et al. 2008); however, differed from the previous trial reporting reductions in visceral fat (300 mg/day) (Ono et al. 2010). Further, a clear dose effect of immunoglobulin supplementation has not been established and so both low and high doses were selected for investigation in the current study, with no significant differences noted in measured parameters between the low and high doses. Differences in characteristics of the cohort between the current study and that by Ono et al., (Ono et al. 2010) are also acknowledged. The current cohort was cross-sectional in nature and included individuals with healthy weight, as well as those who were overweight or obese based on BMI, to represent the general population more broadly, whereas the earlier study recruited participants with visceral adiposity specifically (Ono et al. 2010). Given the recognised pro-inflammatory profile of visceral fat (Fontana et al. 2007) and the cross-talk between inflammatory and metabolic-signalling pathways (Marette 2002; Bastard et al. 2006), the underlying inflammatory status may have been markedly different in the current cohort, who were on average younger and with a lower BMI, and these differences may account for the variable findings.

While significant differences in body mass and metabolic measures were not observed overall following the 28-days of supplementation, the potential for individual positive responses cannot be discounted. In this context, reports of associations between genetic polymorphisms in the lactoferrin gene, lactoferrin concentrations and dyslipidaemia (Moreno-Navarrete et al. 2008) are of interest and suggest an increased risk of metabolic disturbance in individuals with an inherited propensity for lower circulating lactoferrin concentrations. It is among these individuals where lactoferrin supplementation may be particularly beneficial. Lactoferrin genotypes were not assessed in this cohort and although group sizes were slightly larger than the earlier study by Ono et al (Ono et al. 2010), they were still considered insufficient to characterise in further detail responders and non-responders.

This study demonstrated that bovine whey-derived lactoferrin and immunoglobulin supplement use was well tolerated in this cross-sectional cohort. The mucosal immune activities of lactoferrin and immunoglobulins, including modulation of the gut microbiota (Ward et al. 2005), lipopolysaccharide scavenging and downregulation of inflammatory-signalling pathways (Latorre et al. 2010), while not assessed here, are plausible mechanisms via which these types of supplements may exert positive health effects in metabolic disease. Measurement of circulating lipopolysaccharide and cytokine concentrations should be considered in future studies, if appropriate, to further resolve these mechanisms. Use of these types of supplements as a way to enhance other weight management strategies should also be further investigated in at-risk populations specifically and over extended time periods.

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**Disclosure statement**

The authors alone are responsible for the content and writing of this article.

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