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PHYSICAL ACTIVITY, HEALTH AND EXERCISE

Immunomodulatory effects of fucoidan in recreationally active adult males undertaking 3-weeks of intensified training

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ABSTRACT

The aim of the current study was to determine whether daily fucoidan supplementation (*Undaria pinnatifida* extract containing >85% fucoidan, 1 g/day) for three-weeks in a double blind-placebo controlled cross-over trial (ACTRN12621000872831) could modulate alterations in faecal (calprotectin, lysozyme and IgA) and salivary (lactoferrin, lysozyme and IgA) markers of mucosal immune competence typically observed in response to both acute physical activity, and a period of intensified exercise training, in healthy recreationally active men ($n = 12$). Participants responded positively to the intensified training with 16–19% improvement in mean power that was not different between supplement groups. Faecal biomarkers and concentrations of lactoferrin, lysozyme and IgA from resting saliva samples were largely stable over the supplementation period. Concentrations of salivary biomarkers varied significantly over time in response to acute exercise, however differences between supplementation groups were modest. For salivary lysozyme, there was a trend for a lower magnitude of increase post-exercise ($p = 0.08$) and limited return towards pre-exercise in response to fucoidan. For salivary IgA, a greater acute exercise response was noted for IgA in response to fucoidan (~2.7-fold higher; $p = 0.02$). Different dosage and supplementation protocols and inclusion of additional immune markers should be considered in subsequent assessments of any potential benefits of fucoidan supplementation in healthy active adults.

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Mucosa; immune; physical activity; seaweed; fucoidan

Introduction

Seaweeds comprise a diverse range of marine organisms containing biologically active metabolites with purported therapeutic effect(s) on a range of health conditions. Fucoidans are a group of high molecular weight, fucose-based, polysaccharides found in brown macroalgae (Luthuli et al., 2019). The bioactive properties of fucoidan preparations have been assessed in a range of *in vitro* and animal models (Fitton et al., 2019), with demonstrated antimicrobial (Besednova et al., 2015), anti-viral (Ahmadi et al., 2015) and anti-cancer (Kwak, 2014) effects. Evidence from animal models also supports the potential for fucoidans to possess immune-modulating effects (Tomori et al., 2019; Yoo et al., 2019) and more recently, evidence for anti-inflammatory effects in isolated human immune cells has been documented (Ahmad et al., 2021). The ability of fucoidans to modulate mucosal health generally, and mucosal immune function, in humans is of particular interest given the fucose-based structure of fucoidans. Fucose is a terminal sugar found in human mucin glycoproteins (Chow & Lee, 2008), and evidence from *ex vivo* tissue preparations indicates it can regulate gut motility (Bienenstock et al., 2013). Despite this growing base of pre-clinical evidence, there are few human clinical trials examining the impacts of fucoidans on immune markers, and specifically mucosal immune markers.

There is strong interest in the use of naturally occurring supplements to promote immune competence and prevent illness in daily living, as well as in response to periods of increased stress, including intensified physical activity. This interest spans both the general population and higher risk occupation settings including sport, military, agriculture, construction and healthcare workers (Anderson et al., 2023; Derman et al., 2022; Heckenberg et al., 2019). In a pilot study, we observed substantial increases in faecal lysozyme concentrations in professional team sport athletes following 7 days of supplementation with fucoidan (*Undaria pinnatifida* extract, 1 g/day) (Cox et al., 2020). To extend these earlier findings, the aim of the current study was to determine whether daily fucoidan supplementation can positively modulate alterations in markers of mucosal immune competence typically observed in response to both acute physical activity, and a three-week period of intensified exercise training, in healthy recreationally active adults. This type of experimental data is needed to clarify the immunological effects of Fucoidan supplementation, and inform the preparation of clinical guidelines in practical settings.

Materials and methods

Study design and participants

A randomised, double-blind, placebo-controlled, cross-over trial involving two 21-day supplementation phases separated

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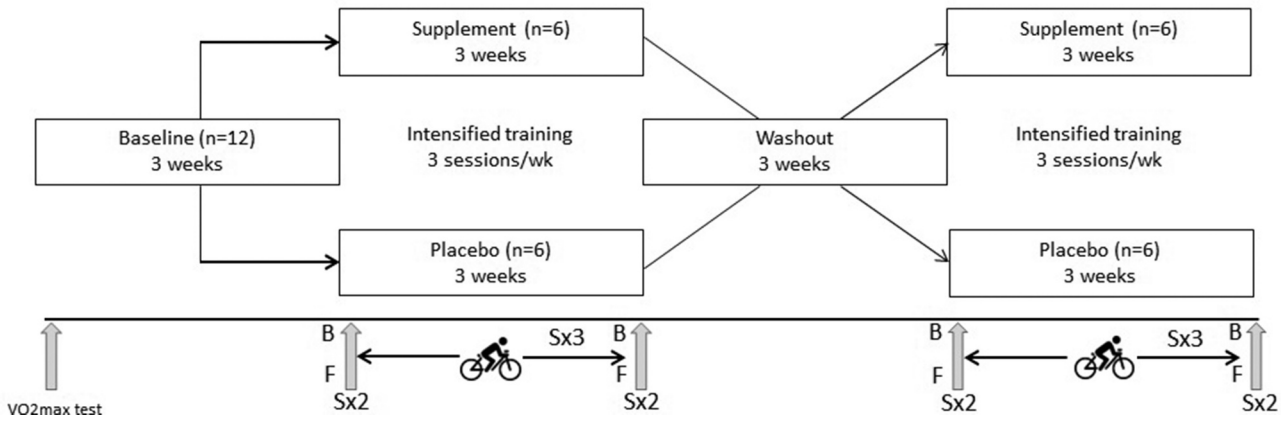

Figure 1. Schematic diagram of the randomised controlled experimental design comparing fucoidan and placebo supplementation. Sample collection points include blood (B) and faecal (F) samples at the beginning and end of each intervention period; pre- and post-exercise saliva samples (Sx2) at the first and last supervised training sessions of each intervention period; and pre-, post- and 1 h post-exercise saliva samples (Sx3) collected during the fifth out of nine scheduled supervised training sessions.

by a minimum 21-day washout was employed (Figure 1). Participants were required to be male, aged 18–45 years, and recreationally active (McKay et al., 2022) with a 12-month history of at least 3.5 h of physical activity per week. Individuals with a history of inflammatory bowel disease, asthma, diabetes, renal disease, cardiovascular disease, arthritis, or use of immune-modulating medications were excluded from participating. Supplementation involved daily ingestion (one capsule, twice daily) of either (i) fucoidan supplement – *Undaria pinnatifida* extract (Marinova Pty Ltd, Tasmania, Australia) containing 87% fucoidan (with 5% alginate, 4% mannitol, ~1% free minerals and ~1% polyphloroglucinol) at a dose of 1 g.day^{-1} or (ii) placebo containing microcrystalline cellulose, and identical in appearance to the fucoidan capsules. Participants ($n = 12$, age: 31.0 ± 9.6 yrs; BMI: 24.8 ± 2.4 kg/m^2) were allocated to supplementation phases in a double-blind, randomised and counterbalanced manner and compliance (%) was assessed via the return of supplements at the conclusion of each training/supplementation phase. Blood and faecal samples were collected at the beginning and end of each supplementation phase for monitoring of a panel of immune biomarkers. A series of saliva samples to assess mucosal immunity were collected in conjunction with scheduled training sessions over the 21 days in each supplementation phase. The study was approved by institutional ethics committees at the University of Canberra (ref #7048) and Griffith University (ref #2021/521) and registered with the Australia and New Zealand Clinical Trials Registry (ACTRN12621000872831). All participants provided written and informed consent prior to participation.

Exercise testing and training intervention

Details on exercise history, including training frequency and intensity, were collected using an Exercise and Sports Science Australia (ESSA) pre-exercise screening questionnaire (https://www.essa.org.au/Public/Public/ABOUT_ESSA/Pre-Exercise_Screening_Systems.aspx). Upon recruitment, all participants completed a cycle-based VO_2 max on an electromagnetically braked cycle ergometer (Velotron, RacerMate zalnc., Seattle, WA, USA) at an initial power output of 100 W with a 25 W.min^{-1} increase until volitional exhaustion. Expired air was analysed by paramagnetic O_2 and infrared CO_2 analysers (ParvoMedics Inc., Salt Lake City, UT, USA). Heart rate was monitored continuously with a Polar transmitter-receiver (*T*-31 Polar Electro, Lake Success, USA) and captured within the Velotron software. Following at least 48 h recovery, participants then completed a repeated-sprint test and familiarisation session on a cycle ergometer (Wattbike Pro, Nottingham, UK) to assess their capacity for high-intensity exercise. During the supplementation phases, each participant completed three sessions of supervised high-intensity repeated-sprint cycle ergometer (Wattbike Pro, Nottingham, UK) training per week in addition to maintaining their regular patterns of physical activity. Supervised training involved a total of 45 min of interval cycling, beginning with a 15-min warm-up at predetermined workloads corresponding to 40% and 50% of the peak power outputs calculated from the VO_{2max} test for the first five, and following 10 min, respectively. At the start of minutes 12, 13 and 14, a brief (~five sec) maximal sprint was performed. Following 3 min of seated rest, the repeated-sprint training consisted of four sets of five × 6 sec sprints on a 30 sec cycle as described previously (Periard et al., 2020). Peak and mean power, heart rate and participant ratings of perceived exertion (RPE; Borg 6–20 scale (Borg, 1982)) were recorded at the end of each sprint set.

Sample collection, handling and analysis

Individuals presented to a local pathology collection centre (Capital Pathology, Canberra, Australia) at the beginning and end of each supplementation phase (a total of four samples) to allow for collection of a venous blood sample (~12 ml) from a forearm vein. Blood samples were used for routine haematology and biochemistry analysis, including full blood count with white cell differential, electrolytes, measures of liver and kidney function, blood lipids and C-reactive protein. These analyses were undertaken by the National Association of Testing

Authorities (NATA) accredited pathology provider (Capital Pathology, Canberra, Australia).

Faecal samples were collected at the beginning and end of each supplementation phase (a total of four samples) using a home-based collection kit (consisting of flushable collection paper and a sample collection cup with a scooped lid). Samples were returned to the laboratory within 24 h of collection and stored frozen (−80°C) until analysis. Faecal samples were used for determination of calprotectin, lysozyme and IgA concentrations using commercially available immunoassay kits. All assay kits were specific for assessment of faecal samples (Immunodiagnostik, Bensheim, Germany) and performed according to the manufacturer's instructions with samples assessed in duplicate. Typical intra-assay variability was 8.8% for calprotectin, 3.0% for IgA and 3.5% for lysozyme. High-range and low-range quality controls were provided for inclusion in each assay; all quality controls were measured within the specified ranges. Typical inter-assay variability was <2% for calprotectin and <7% for both IgA and lysozyme.

Saliva samples were collected at a series of seven time points during each supplementation phase. These time points included at rest (S1) and immediately post-exercise (S2), collected during the first training session of each supplementation phase; at rest (S3), immediately post (S4) and 1 hr post-exercise (S5), collected during the fifth of the nine scheduled training sessions for each supplementation phase; at rest (S6) and immediately post exercise (S7) during the final training session of each supplementation phase. Saliva samples were collected unstimulated using eye-spear absorbent swabs placed in the sublingual region until saturated. Eye-spear swabs were placed in a collection tube and stored frozen (−80°C) until analysis. Saliva samples were used for determination of lactoferrin, lysozyme and IgA concentrations using commercially available immunoassay kits (Abcam, Cambridge, UK). Assays were performed according to the manufacturer's instructions and samples assessed in duplicate. Where possible, samples from a single participant were analysed in a single assay. Typical intra-assay variability was 5.5% for lactoferrin, 4.6% for IgA and 3.9% for lysozyme. Typical inter-assay variability was <10% for lactoferrin, <8% for lysozyme and <14% for IgA.

Statistical analysis

For the exercise data, the mean change score in mean and maximum (peak) cycling power output over the 3-week supplementation period were calculated for each intervention. A Student's-test was used to evaluate the difference in the mean change in power output in a two-sample model assuming equal variance (homoscedastic). Distributions of blood measures and salivary and faecal biomarker data were inspected for normality; data were log-transformed where appropriate to approximate the conditional normality assumption. Blood measures were compared over time using a one-way analysis of variance (ANOVA). The impacts of supplementation on faecal biomarkers were assessed using a two-factor (time × group) ANOVA.

For salivary biomarkers, the analyses included (i) assessment of the effect of supplementation on resting measures (S1, S3, S6) using a two-factor (time [3] × group [2]) ANOVA; (ii) assessment of the effect of supplementation on the response to exercise (pre, post and 1 h post-exercise) using a two-factor (time [3] × group [2]) ANOVA; and (iii) exploration of the combined effect of training and supplementation on the acute exercise responses determined by calculating the per cent change values at the beginning (S1, S2) and end (S6, S7) of each supplementation phase, and comparing these values within and between (at both Week 1 and Week 3) supplementation phases using a paired t-test. Data are presented as mean ± SD unless specified with a statistical significance accepted a $p < 0.05$.

Results

Compliance, safety and tolerability

Compliance in taking the daily capsules (Fucoidan and placebo) was relatively high with 86% of placebo and 77% of fucoidan capsules consumed over the three-week supplementation phases. There were no self-reported issues by the participants on the tolerability of either supplement. However, one participant withdrew from the second supplementation phase of the study (fucoidan supplement for this participant) due to a positive COVID-19 test. Compliance with the prescribed training protocol was also high. Eleven of the 12 participants completed all nine intensive exercise sessions in the first supplementation phase of the study, with one participant completing a total of eight of the exercise sessions. All participants included in the second supplementation phase of the study ($n = 11$) completed all nine intensive exercise sessions.

There were no changes in routine haematology or basic biochemistry measures (including glycaemic control, blood lipids, measures of liver and kidney function, and inflammation), over the course of the study (Supplementary Table S1), supporting safe use of fucoidan supplementation at a dose of 1 $g.day^{-1}$ in otherwise healthy adults over a period of 3 weeks.

Performance outcomes

Participants were considered recreationally active, partaking in regular combinations of moderate-intensity and vigorous-intensity activity with a mean training history of 6 ± 3 h/wk, comprising a mean training frequency of 3.8 ± 1.0 light or moderate sessions/wk and 2.9 ± 2.9 vigorous sessions/wk. At recruitment, assessment of VO_2max results confirmed moderate levels of aerobic fitness (46.6 ± 9.7 $mL.kg{\cdot}min^{-1}$) and peak power (11.4 ± 3.6 $W.kg^{-1}$) among participants. While marked increases in mean and peak power were observed within-subjects over the 3 weeks of exercise training and supplementation, these were not significantly different between treatment groups (Table 1).

Faecal biomarkers

Substantial inter-individual variation in faecal biomarkers was noted. When using the prescribed cut-off threshold of 100 mg. L^{-1} for a positive result in accordance with the specifications of the assay kit used, it was noted that faecal calprotectin concentrations in the cohort were generally considered negative.

Table 1. Physiological and perceptual measures recorded during high-intensity cycling at the first and last supervised training session during each intervention period. Data are presented as mean ± standard deviation for absolute units. W = watts, kg = kilograms, AU = arbitrary units (6–20 scale). Reported p-values are from a Student's t-test comparing the session 1 to session 9 changes between interventions.

	Placebo ($n = 12$)		Fucoidan ($n = 11$)		
	Session 1	Session 9	Session 1	Session 9	p-value
Peak Power (W)	916 ± 230	1048 ± 223	884 ± 245	1038 ± 234	0.33
Peak Power (W.kg^{-1})	11.6 ± 2.6	13.5 ± 2.5	11.4 ± 3.0	13.4 ± 2.9	
Mean Power (W)	759 ± 188	866 ± 183	744 ± 203	862 ± 194	0.42
Mean Power (W.kg^{-1})	9.6 ± 2.2	11.1 ± 2.0	9.6 ± 2.5	11.1 ± 2.2	
Heart Rate (bpm)	160 ± 10	155 ± 10	160 ± 15	162 ± 14	0.65
Rating of Perceived Exertion (AU)	17.6 ± 2.1	17.9 ± 2.1	17.4 ± 1.8	17.3 ± 2.3	0.23

Table 2. Faecal biomarker concentrations in healthy recreationally active adults at the beginning (wk 1) and end (wk 3) of each supplementation phase. Data are presented as mean ± SD. P-values are reported for two-factor (time[2] × group[2]) analysis of variance.

	Placebo ($n = 12$)		Fucoidan ($n = 11$)		
	Wk 1	Wk 3	Wk 1	Wk 3	p-value
Calprotectin (ug/mL)	36.6 ± 33.8	23.8 ± 23.4	22.6 ± 21.0	24.6 ± 25.4	Interaction: F(1, 10) = 1.15; $p = 0.31$ Time: F(1, 10) = 2.43; $p = 0.15$ Group: F(1, 10) = 1.30; $p = 0.28$
IgA (g/L)	1.70 ± 0.97	1.75 ± 0.91	2.17 ± 1.38	1.93 ± 1.13	Interaction: F(1, 10) = 0.12; $p = 0.74$ Time: F(1, 10) = 0.39; $p = 0.55$ Group: F(1, 10) = 0.39; $p = 0.55$
Lysozyme (ng/mL)	565 ± 242	477 ± 263	507 ± 146	472 ± 209	Interaction: F(1,10) = 0.65; $p = 0.44$ Time: F(1, 10) = 1.18; $p = 0.30$ Group: F(1, 10) = 0.05; $p = 0.82$

Absolute calprotectin concentrations were not significantly different in response to fucoidan supplementation (Table 2). Faecal concentrations of IgA and lysozyme were also not significantly different in response to fucoidan supplementation (Table 2).

Salivary biomarkers

Effect of supplementation on resting measures

Salivary lactoferrin and lysozyme concentrations did not vary at rest over the supplementation period nor were there any marked between-group differences (Table 3). For salivary IgA, concentrations were also stable over the supplementation period (Table 3), but tended to be lower (~25–40%) in the fucoidan group both prior to and during the supplementation period.

Effect of supplementation on response to exercise

As was anticipated, concentrations of salivary biomarkers varied substantially over time in response to an exercise training session (IgA, $p = 0.001$; lactoferrin, $p < 0.001$; lysozyme, $p < 0.001$); a marked increase was observed immediately post-exercise with evidence of a return towards pre-exercise concentrations at 1 h post exercise (Figure 2). For both IgA (Interaction: F(2, 8) = 1.23, $p = 0.32$; Group: F(1, 9) = 1.05, $p = 0.33$) and lactoferrin (Interaction: F(2, 8) = 0.82, $p = 0.46$; Group: F(1, 9) = 0.001, $p = 0.98$) the patterns of response were consistent between the supplementation groups. In contrast, for lysozyme there was a trend for a different pattern of response between groups (Interaction: F(2, 8) = 2.98, $p = 0.08$), with a lower magnitude of increase post-exercise and blunted return towards pre-exercise in the fucoidan supplementation group (Figure 2).

Combined effect of training and supplementation on the acute exercise response

When assessing the effect of the training intervention on the salivary biomarker response to acute exercise using within group comparisons, for the fucoidan supplementation, the acute exercise responses for IgA, lactoferrin and lysozyme were not significantly different following the period of intensified training (Figure 3). In the placebo group, there was a trend for an attenuated acute exercise response for IgA after the period of intensified training (~50% lower, $p = 0.06$); this was not evident for lactoferrin

Table 3. Resting salivary biomarker concentrations in healthy recreationally active adults at each week (wk 1, 2, 3) of the two 3-week supplementation phases. Data are presented as mean ± SD. P-values are reported for two-factor (time[3] × group[2]) analysis of variance.

	Placebo ($n = 12$)			Fucoidan ($n = 11$)			
	Wk 1	Wk 2	Wk 3	Wk 1	Wk 2	Wk 3	p-value
IgA (ug/mL)	469 ± 414	434 ± 237	555 ± 762	305 ± 153	327 ± 154	322 ± 236	Interaction: F(2, 9) = 0.13; $p = 0.88$ Time: F(2, 9) = 0.12; $p = 0.88$ Group: F(1, 10) = 7.2; $p = 0.02$
Lactoferrin (ug/mL)	19.9 ± 30.2	7.7 ± 8.1	20.6 ± 31.7	12.4 ± 18.6	8.3 ± 9.0	9.0 ± 7.8	Interaction: F(2, 9) = 0.17; $p = 0.84$ Time: F(2, 9) = 2.28; $p = 0.13$ Group: F(1, 10) = 0.12; $p = 0.73$
Lysozyme (ug/mL)	34.1 ± 33.6	17.5 ± 10.9	49.9 ± 64.5	34.9 ± 44.5	26.4 ± 25.6	32.2 ± 21.2	Interaction: F(2,9) = 0.28; $p = 0.76$ Time: F(2, 9) = 2.36; $p = 0.12$ Group: F(1, 10) = 0.36; $p = 0.56$

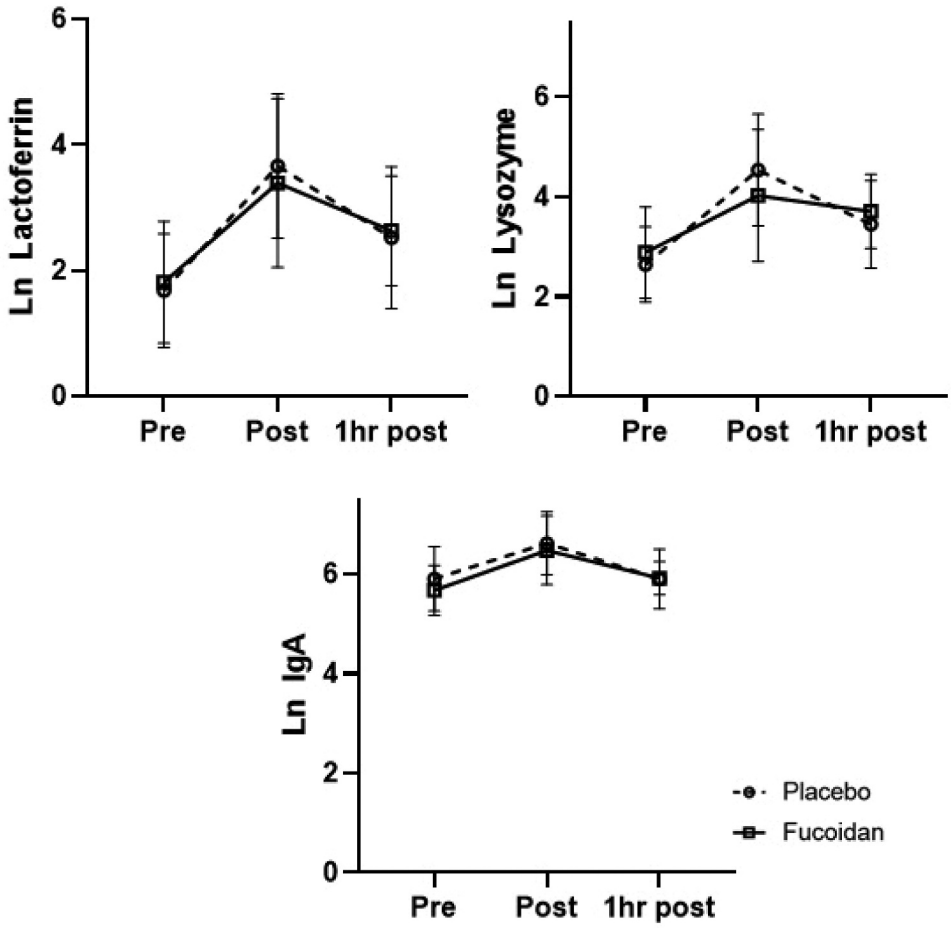

Figure 2. Changes in salivary lactoferrin, lysozyme and IgA concentrations pre, post and 1 h post a supervised exercise training session completed during wk 2 of each 3-week supplementation phase. Data are presented as mean and SD of log transformed (ln) values.

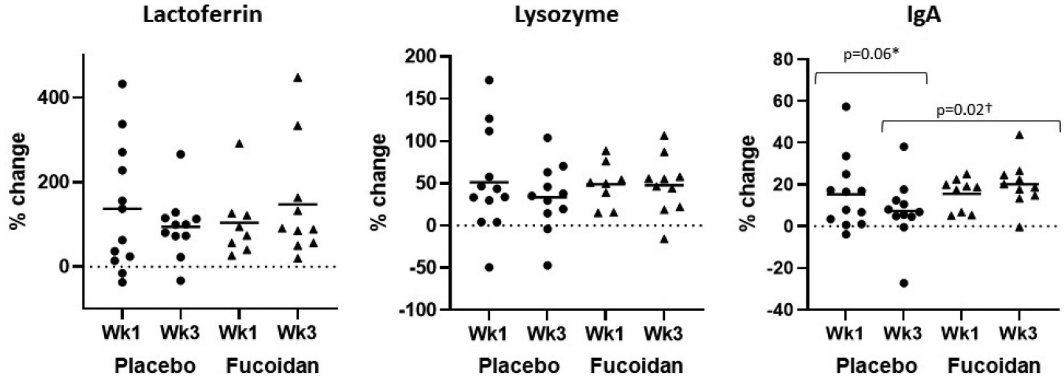

Figure 3. Immediate post-exercise change (expressed as a per cent change pre- to immediately post-exercise) in salivary lactoferrin, lysozyme and IgA concentrations in response to the first (wk 1) and last (wk 3) supervised exercise sessions of the placebo and fucoidan supplementation phases. Data are presented as individual percentage change values with group mean represented by the horizontal bar. *within group comparison and †between group comparison.

and lysozyme (Figure 3). When comparing the acute exercise responses between supplementation groups, no significant differences were observed prior to the period of intensified training (Figure 3). Following the period of intensified training, a greater (~2.7-fold higher; $p = 0.02$) acute exercise response was observed for IgA in response to fucoidan supplementation; this was not evident for lactoferrin and lysozyme (Figure 3).

Discussion

The potential for daily fucoidan supplementation to modulate alterations in markers of mucosal immune competence was explored in a model of 3-weeks of intensified exercise training in healthy recreationally active men. There were no substantial differences in routine haematology or basic biochemistry measures over the course of the study, supporting safe use of

fucoidan supplementation in otherwise healthy adults at a dose of 1 g.day^{-1} over a period of 3 weeks. The participants showed a substantial 16–19% improvement in the ability to generate power on the cycling ergometer over the intervention period, with little apparent (direct) effect of fucoidan supplementation on exercise performance. Similarly, resting faecal and salivary immune biomarkers were largely unchanged in response to supplementation. There were some modest differences in salivary lysozyme response to acute exercise and salivary IgA response following the intensified training between the supplementation groups that require further consideration.

Despite the growing base of pre-clinical evidence supporting potential immune modulating effects of fucoidan, human clinical trials examining the impacts of fucoidans on immune markers and mucosal immune markers specifically remain scarce. An experimental design involving assessment of immune markers in response to both acute exercise, and in response to a period of intensified training, was chosen given the large number of studies documenting the effects of intense physical activity on measures of immunity (Padilha et al., 2022). Given the mechanisms via which fucoidan may influence measures of mucosal immunity are unclear, a range of faecal and salivary markers were selected for assessment in the current study. Biomarkers were selected based on prior reports including lower salivary lactoferrin concentration in a group of rowing athletes compared to controls (West et al., 2010); alterations in salivary lactoferrin concentration in response to an acute bout of exercise (Gillum et al., 2015); decreasing salivary lysozyme in rugby players across a sporting season (Cunniffe et al., 2011); association between lower mucosal (tear) lysozyme concentrations and mucosal infection (upper respiratory tract infection) (Hanstock et al., 2019); and multiple reports that a decrease in salivary IgA can precede the onset of upper respiratory symptoms (Neville et al., 2008; Tiernan et al., 2019).

In this cohort of healthy recreationally active men, resting concentrations of salivary biomarkers appeared largely stable across the study; however, large inter-individual variations in both resting salivary biomarkers and responses to acute exercise were evident. A modest difference in resting salivary IgA concentrations was observed between groups prior to supplementation, and therefore we are unable to attribute the observed effects directly to fucoidan use. A trend for a more modest perturbation in salivary lysozyme concentration in response to acute exercise was recorded during fucoidan supplementation compared to placebo, with a tendency for a lower (~30%) post-exercise increase. Similarly, at the 1 h post-exercise timepoint, the return towards pre-exercise concentrations tended to be reduced for fucoidan. Given the recognised role of lysozyme as an antimicrobial peptide (Antoni et al., 2013) a slower return to pre-exercise concentrations in the post-exercise period may support innate defences at the mucosal surfaces during this time. However, we acknowledge that the absolute concentrations were similar between groups at 1 h post-exercise. Furthermore, lysozyme concentrations were broadly similar to ranges reported by other investigators in otherwise healthy adults (Kmiliauskis et al., 2005; Yang et al., 2002), and higher than the ranges reported in studies examining salivary immune markers in high-performance athletes (Cunniffe et al., 2011; West et al., 2010). Given this variable pattern of results, the clinical relevance of this trend for a more modest post-exercise perturbation is unclear.

Likewise, modest differences in salivary IgA responses should also be considered with caution. A greater post-exercise increase in IgA at the end of an intensified training period with fucoidan compared with placebo ($p = 0.02$; Figure 3) may have been driven, in part, by an attenuated post-exercise change in the placebo group following the intervention when compared to the start of supplementation. Low salivary IgA concentrations have been associated with an increased risk for upper respiratory illness in high performance/elite athletes (Gleeson & Pyne, 2016). However, it is unclear whether the observed greater post-exercise increase following fucoidan supplementation contributed to maintenance of salivary IgA concentrations in the period beyond immediately post-exercise. Again, it is prudent to note that despite this pattern, absolute concentrations were not significantly different between groups, and observed concentrations were not dissimilar to prior studies examining IgA in exercise settings (Nieman et al., 2006; Tiernan et al., 2019).

There were no clear effects of fucoidan supplementation on faecal biomarkers. In a pilot study involving professional team sport athletes, we previously reported both lower faecal lysozyme concentrations in professional athletes compared to healthy adults and substantial increases in faecal lysozyme concentrations following 7 days of supplementation with fucoidan (*Undaria pinnatifida* extract, 1 g/day) (Cox et al., 2020). In the current trial, faecal lysozyme concentrations were more similar to the healthy adults assessed previously (than the professional team-sport athletes). While there was a modest decline over the 3 weeks of intensified training (~15%) in the placebo group, this was not significantly different from the trend in the fucoidan group (~7% reduction). Moreover, calprotectin concentrations (~20–35 ug/mL) were lower than had been observed in the earlier pilot study (mean baseline ~50 ug/mL) (Cox et al., 2020), and generally deemed negative relative to the clinical threshold, indicating that local inflammation at the gut mucosa was unlikely. In this scenario, the potential for supplementation to modulate immune biomarkers at the gastrointestinal mucosa within homoeostatic norms may be low, and any small absolute changes may be difficult to discern in this cohort where large inter-individual variation was evident.

The study was not without its limitations, and it should be recognised that participants in this study were community dwelling and not under dietary control for the course of the study. While compliance with supervised training was excellent, the compliance with fucoidan supplementation was moderately lower (~9%) compared to placebo. We also acknowledge that the panel of immune biomarkers selected for analysis here only provided a limited assessment of innate and adaptive immune system activity. Considering the wide-ranging purported mechanisms of action of fucoidan, this selection of biomarkers may not have captured all immunological effects of supplementation. That said, outcomes from the current study should also be considered in the context of another recent trial exploring the effects of fucoidan supplementation (1 g.day^{-1} for 2 weeks) on acute changes (up to 60

min post exercise) in inflammatory and immune markers following high-intensity interval cycling in a group of 16 recreationally healthy young adults (McFadden et al., 2023). This study also reported no improvements in exercise performance or differences in the acute exercise effects on immune cell counts, including CD4+ and CD8+ T cell subsets, between supplement groups. Small differences (based on reported effect sizes) in the circulating concentrations of two cytokines (IL-6 and IL-10) were observed between fucoidan and placebo groups at 30 min post-exercise, but these differences were ameliorated at 60 min post exercise.

Conclusion

In conclusion, a double-blind randomised placebo-controlled trial investigating the effects of 3 weeks of fucoidan supplementation (*Undaria pinnatifida* extract containing >85% fucoidan at a dose of 1 g/day) had little effect on a suite of serum biomarkers, and faecal and salivary immune markers. There were no reported side-effects, and tolerability was deemed high. Assessment of different dosage and supplementation protocols and consideration of other immune markers should be explored in future studies assessing the potential benefits of fucoidan supplementation in relation to mucosal immune markers in healthy recreationally active adults.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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