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The Effect of Bovine Colostrum Supplementation on Salivary IgA in Distance Runners

Christine V. Crooks, Clare R. Wall, Martin L. Cross, and Kay J. Rutherfurd-Markwick

Secretory IgA in saliva (s-IgA) is a potential mucosal immune correlate of upper respiratory tract infection (URTI) status. Nutritional supplements may improve mucosal immunity, and could be beneficial to athletes who are at increased risk of URTI. In this study, 35 distance runners (15 female, 20 male, age 35 to 58 y) consumed a supplement of either bovine colostrum or placebo for 12 wk. Saliva samples were taken prior to training at baseline, monthly during supplementation, and 2 wk post supplementation. Median levels of s-IgA increased by 79% in the colostrum group after 12 wk intervention, and the time-dependent change from baseline value was significant ($P = 0.0291$). This significance was still apparent after adjusting for training volume and self-reporting of upper respiratory symptoms. This study has demonstrated increased s-IgA levels among a cohort of athletes following colostrum supplementation. While this result is statistically significant, its physiological interpretation must be viewed with caution due to the small numbers in this study and the large variability in s-IgA levels.

Key Words: dietary supplementation, upper respiratory tract infection, upper respiratory symptoms, mucosal immunity.

Exercise when performed at strenuous levels can act as a stressor that may temporarily alter the immune system. The athlete experiences a transient period of depressed immunity that is usually reversed with rest. This stress response is exacerbated if the athlete is under psychological stress, has poor nutrition, is lacking sleep, or is in an unfamiliar environment during competition. Moderate levels of exercise appear to improve the immune response, but when exercise is performed at intensive levels a higher incidence of upper respiratory tract infection (URTI) has been observed (28) which is possibly as a consequence of the transient reduction in immune defense. For the athlete, this is of concern if the illness occurs close to a competition as performance can be affected.

While many immune parameters are altered by strenuous exercise, the association with an increased incidence of URTI has not been conclusive. The exception

The authors are with the Institute of Food Nutrition and Human Health, Massey University, Albany Campus, Auckland, New Zealand.

has been the effect of exercise on mucosal immunity. Some mucosal immune parameters are depressed after exercise, including reduced salivary immunoglobulin A (s-IgA) concentrations and a lower overall secretion rate, and it has been speculated that temporary deficiency of antibodies at the mucosal surfaces could make individuals susceptible to infection from pathogenic viral and bacterial organisms (15-17, 24).

Upper respiratory tract infections in athletes are usually of viral origin and the presentation of symptoms occurs within a few days of a viral infection. The symptoms experienced are a result of disruption to the normal function of infected cells and the body's immune response to rid itself of the infection (17). Upper respiratory symptoms (URS) are typically sore throat, cough, runny nose, fever, and headache (17, 30). In athletes, URS can last from a few days to 1 to 2 wk and many studies of URTI in athletes have generally relied on self recording of URS which have not been verified by a physician. In longitudinal field studies such as this preliminary one it may not always be possible to involve a physician, and so establishing true URS depends on the reliability and accuracy of the records kept by the athlete.

Various nutritional supplements have been studied for their potential to enhance immune function in athletes. Carbohydrate supplementation immediately before, during, and after exercise has shown the most promising results by attenuating the stress response (29). In distance runners, levels of cortisol, interleukin (IL)-6, and IL-1ra were attenuated after 2.5 h running in those runners who were supplemented with a drink containing 6% carbohydrate (27). Modulation of the cortisol response could improve immune function as it is known to have a depressive effect. In addition, adequate dietary carbohydrate intake in the days leading up to a strenuous bout of exercise is also important in attenuating the stress response (1, 3). However, no effect is seen on levels of s-IgA with carbohydrate supplementation in distance runners after completing a marathon (31).

Recently the effect of bovine colostrum supplementation on immune function in athletes has been investigated (5, 25, 26)**.** Anecdotal reports suggest that many athletes take bovine colostrum to help their immune system. Two reports also suggest that bovine colostrum improves the health status in female endurance athletes (25) and in active males (5) although the physiological reason for this was not elucidated. Bovine colostrum has been shown to improve performance in 30 male endurance runners after a second bout of exercise. It was thought that this was due to improved recovery mechanisms (6), which possibly could include improvement of immune function due to less physiological disturbances.

Another study has shown that supplementation with bovine colostrum in a group of athletes for 3 wk resulted in a 33% increase in mean levels of s-IgA (25). However, there were a number of limitations to this study; there was no discussion of how the quality of the saliva was controlled for (e.g., with respect to the effects of dehydration and URS symptoms), the athletes were from a range of sports, and the placebo used was maltodextrin and therefore did not have the same macronutrient profile as the intervention. Nevertheless, as s-IgA plays an important part in mucosal immunity, this is a significant finding and the effect of bovine colostrum on s-IgA levels needs further investigating in specific sports, in a normal training environment, in different age groups, and with various periods of intervention. Because concurrent URS may artificially elevate s-IgA levels and confound the

interpretation of diet-mediated effects, it is also important to establish a methodology to control for this variable.

Bovine colostrum is the first milk produced during lactation in cows and contains many bioactive factors and immunoglobulins which may be relevant to mucosal immunity, such as cytokines and transforming growth factor (TGF-β). Further, in vitro studies have shown that the addition of TGF-β as found in bovine colostrum can stimulate human lymphocyte cultures (38). To date the mechanism for how bovine colostrum could affect the immune system in humans has not been elucidated, although in animal model studies dietary bovine whey proteins have been shown to elevate secretory immunoglobulin levels in the gut mucosal environment (22, 37) suggesting the establishment of an enhanced mucosal immune state by bovine milk proteins. Additionally, enhancement of the specific IgA response to a bacterial pathogen was seen in 18 healthy humans (9 females and 9 males age 20 to 50 y) after supplementation with bovine colostrum (19). This response also suggests that there has been an immunomodulatory effect of orally administered bovine colostrum rather than an antimicrobial effect.

In this preliminary study investigating the effects of bovine colostrum on levels of s-IgA in distance runners in their normal training environment, the aim was to establish a valid saliva sampling and test methodology and to control for known variables that can affect levels of s-IgA. As in other studies (30) URS days have only be considered as true URS if two or more consecutive days of URS were reported. Because the immune response can result in local inflammation and systemic consequences (ache, fever, and fatigue) these latter symptoms may also be present days after the initial symptoms disappear (17), therefore it is appropriate to include the reporting of these systemic symptoms in a wellness record. However, if any of these symptoms reoccurred within a week after the end of the initial episode they were considered to be a complication of the first episode and not considered to be a second episode (18).

Methods

Subjects

Thirty-nine runners (22 men and 17 women) between the ages of 35 to 58 y volunteered for the study. These were recreational distance runners who were mostly members of the Auckland YMCA marathon club (New Zealand) or associated with it, working full time and were preparing for a marathon being held in April of 2002 (Rotorua Marathon, New Zealand). Inclusion criteria for the study stipulated that the participants must:

1) Meet weekly either at the marathon clubrooms on Sunday morning or at a specified site for Saturday morning pack runs
2) Be less than 60 y old
3) Be training for a marathon or marathon type events for the last 5 y
4) Not be lactose intolerant or have any other known allergy to cow's milk
5) Not taking whey protein supplements; and
6) Not be receiving treatment for any known medical condition (including asthma).

All subjects were fully informed verbally and in writing about the nature of the study, any known risks, and their right to terminate participation at will before signing a formal consent form. Ethical approval for this study was sought and given by the Auckland Ethics Committee (AKY 2001/305).

Study Design

The study was a randomized double blind, placebo-controlled design and required 14 wk participation (Figure 1). It was attempted to pair match subjects for gender but due to the small numbers it was not possible to assign equal numbers. Each subject was required to consume daily 250 mL of colostrum product or placebo and at four weekly intervals provide a saliva sample for IgA analysis. Training and wellness diaries were completed each day and a 7 d diet record at week 6 and 11.

Intervention

A chocolate drink powder containing colostrum was used as the intervention (Immulac; NZMP Ltd., Auckland, New Zealand). The athletes were given two tubs of chocolate drink powder each (2.2 kg of powder in total). The tubs were identified with a three-digit random number and this number became the identification code for the athlete. Each participant was supplied a scoop and was required to add two level scoops of powder (equivalent to 26 g) to 125 mL of cold water in the 250 mL drink shaker provided. The athletes began taking the supplement a day after the first saliva sample was supplied.

The placebo blend contained skim milk**,** matched to the test intervention for equivalent digestible protein content. Flavors and colors were added to achieve blends that were similar in taste and color. As colostrum powder has a strong distinctive flavor, these blends provided the best combination to minimize the difference in taste and color (Table 1).

Secretory IgA Assessment

Saliva Collection. Saliva samples were drawn, stored, and analyzed using handling procedures that had been suggested by standard diagnostic protocols

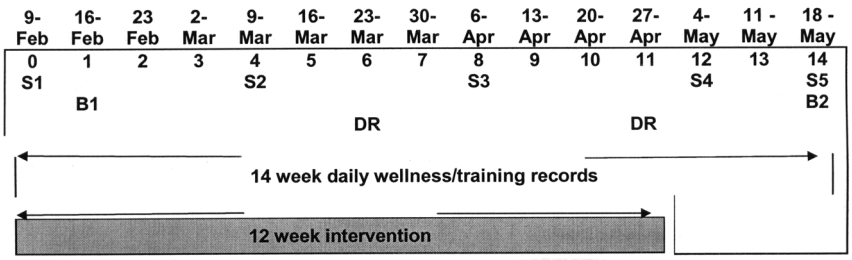

Figure 1 — Schematic summary of the trial design and sampling time points. S, saliva sample taken on the Saturday or Sunday at the beginning of that week; B, blood sampled before breakfast on a day convenient to participant in nominated week; DR, 7-d diet record.

Table 1 Composition of Chocolate Powder Blends

	Placebo (Control)	Colostrum (intervention)
Immulac %w/w	–	40
Skim milk powder %w/w	70	30
Sucrose %w/w	30	30
Protein g/26 g	7.8	8.6
Carbohydrate g/26 g	14.3	13.6

developed at the Hunter Immunology Pathology Service, Newcastle, Australia [now the Hunter New England Pathology Service (13)].

Athletes were instructed to eat and drink at least 30 min before the sample was provided. They were also taught to produce a sample by gently spitting saliva collected underneath the tongue into a collection tube, to provide whole mixed unstimulated saliva. Approximately 1 mL of saliva was collected which took between a minute and 10 min to produce, depending on the individual.

Samples were collected before the start of the longest run of the nominated collection week. Five samples from each athlete were collected on separate occasions over a 14-wk period. The first sample was taken at baseline, the second, third, and fourth samples at monthly intervals (the fourth sample was also taken 1 wk after the New Zealand marathon which 21 athletes competed in). The athletes stopped taking the intervention the day after the fourth saliva sample. The fifth saliva sample was taken 2 wk later. Samples were placed immediately into dry ice until transported to a –70 °C freezer.

S-IgA Analysis. Saliva samples were excluded from analysis if there was insufficient sample and/or if the subject appeared to be dehydrated or fasted at the time of sampling. Dehydration and fasting can elevate levels of s-IgA and this was monitored by measuring salivary albumin and determining osmolality. Elevated levels of salivary albumin can indicate dehydration due to altered saliva flow rates or an increase in tissue permeability. Monitoring levels of salivary albumin is useful to identify differences between groups or time-points (10).

Salivary albumin was measured on the same day on a Roche Cobas Fara II by turbidimetry, using Dako human serum albumin standards (15 to 300 mg/L) and rabbit anti-human serum albumin. High osmolality can indicate dehydration as saliva flow rate is reduced or that there has been recent consumption of a drink high in electrolytes (e.g., a sports drink). Saliva osmolality was measured using a freezing point depression osmometer (Advanced Digimatic Osmometer 3D2, Advanced Instruments, Norwood, MA). The assay coefficients of variation for albumin were < 5% for values >30 mg/L (< 10% for values < 30 mg/L) and for osmolality were between 1.8 to 2.5%.

S-IgA was measured in diluted samples by ELISA, using unconjugated and biotin-conjugated polyclonal goat F(ab')2 antibody fragments, specific for human

IgA (Biosource International, Camarillo, CA). These reagents have no stated cross-reactivity against bovine immunoglobulins, and were further demonstrated in our laboratory to be non-reactive against samples of bovine colostrum containing bovine IgA. To assess levels of human IgA the assay used a two-step "sandwich" enzyme immunoassay, where unconjugated monoclonal antibody was coated onto a 96 well immunoplate (Nunc, Roskilde, Denmark) at a concentration of 0.5 μg/mL overnight. Plates were washed with PBS (pH 7.2, 0.05% Tween 20) and unbound sites were blocked with skim milk powder (5% in PBS). Pre-diluted standards (myeloma-derived human IgA; Dako X0908, Denmark, range 0 to 80 ng/mL) and test samples were added to each well and incubated for 2 h, followed by extensive washing and the addition of biotin-conjugated detection antibody at 1 μg/mL for a further 2 h. Streptavidin-conjugated horseradish peroxidase enzyme (Silenus 988210010 1/2000) was then added for a further 1 hour, before final washing and addition of reactive chromogenic substrate (ABTS - 2-2 Azino-bis-3 ethyl benzthiazoline 6 sulphuric acid). After acidification to stop the reaction the plates were read on a plate reader at 405 nm (CERES 900C Bio Tek microtitre plate reader). The ELISA used in this study to measure s-IgA detects both IgA_1 and IgA_2 subclasses.

Training and Wellness Records

Athletes kept a daily log of the distance run (kilometres) and time run (min). Time spent in other physical activities was also recorded.

Daily records of health problems were kept on the same log. The format for the wellness log was based on a log used for self-recording of health in rowers (30).

The athletes were required to record the absence or presence of any of the following symptoms:

Symptom	
No health problem	
"Cold" symptoms (runny and or stuffy nose, sore throat, coughing, sneezing, colored nasal and/or saliva discharge)	
"Flu" symptoms (fever, headache, general aches and pains, fatigue, weakness, chest discomfort, cough)	
Gastrointestinal (nausea, vomiting, diarrhea)	
Physical (muscle, joint or bone problems/injury)	
Other	

The total number of days with upper respiratory symptoms (URS) was calculated for each athlete. Days were only considered as true URS if two or more consecutive days of URS symptoms were reported. In this study no other attempt has been made to verify that a true URTI occurred.

Dietary Assessment

Participants were asked to complete two sets of 7-d food diaries. Instructions on how to complete the diaries were given in writing and verbally by the investigator. The first record was kept 6 wk into the marathon build-up (week 6) to determine reported dietary intake during heavier training weeks and the second was kept in the week prior to the marathon (week 11) to determine the reported dietary intake during the taper period. The food records were analyzed for intake of carbohydrate $g^{-1} \cdot kg^{-1}$ body mass $\cdot d^{-1}$, protein $g^{-1} \cdot kg^{-1}$ body mass $\cdot d^{-1}$, contribution of fat to total energy intake, and total energy intake using Foodworks version 2.10 with the NZ Food Files 2000, and NZ Vitamin and Mineral Supplements 1999 (Crop and Food Research, Palmerston North, New Zealand).

Assessment of Intake of Intervention

The daily consumption of the chocolate drink intervention (either placebo or colostrum powder) was recorded on the food records and included in the dietary analysis for each participant. Measuring the amount of remaining chocolate powder gave an indication of compliance.

Body Measurements

Body mass was recorded twice during the study within a week of the diet diaries being completed, using digital scales (Soehnle, accuracy 0.1 kg) that were checked with a calibrated weight after every third participant was measured. Body mass was measured in the morning and post-prandially before the long run on one of the saliva sampling days. Height was measured once using a portable stadiometer (Massey University, New Zealand).

Statistical Analysis

Two-way repeat measures analysis of variance was used to identify the effects of intervention and time on s-IgA levels; Dunnett's post hoc test was used to further identify specific time-points where any significant within-group time-dependent changes occurred (SAS version 8, SAS Institute, Inc., Cary, NC). Between-group analyses were undertaken using non-parametric testing. Multiple regression analysis was used to assess the effect that the independent variables (weekly mileage, gender, average self-reported URS, running the New Zealand marathon and colostrum) could have on levels of log s-IgA (dependent variable) (Minitab version 14, Mintab Inc., State College, PA). Those independent variables that were significant were investigated further in a general linear model. Any data that were non-normally distributed were log-transformed prior to analysis; where transformation failed to

normalize the distribution, non-parametric testing was applied (SAS). In all cases, the level of significance was set at $P = 0.05$.

Results

Characteristics of the Participants

The placebo and colostrum group had similar characteristics (Table 2). Thirty-nine athletes volunteered for the study, and data from 35 were used for analysis. One male participant withdrew 3 wk into the study because of an unrelated illness. Data from three athletes were excluded: one did not return training and wellness records (placebo group); one was breast-feeding (colostrum group); and one was participating in her first marathon (placebo group). Twenty-five athletes indicated they were training for the New Zealand marathon but due to injury, illness, or personal reasons, four did not compete in the event. While these four runners continued to be in the study, they reduced their weekly mileage. In addition, two athletes competed in marathon events early in April, 3 wk before the end of the study, and their weekly mileage was reduced after competition. There was a large range in average weekly distances run within the groups: placebo, 8 to 105 km/wk; colostrum, 18 to 114 km/wk. Non-parametric testing indicated there were no significant differences between the groups.

There was no significant difference between body mass at week 6 and week 11 for the placebo and colostrum groups.

Differences in s-IgA

Median levels of s-IgA increased in the colostrum group by 79% and in the placebo group by 16% after 12 wk intervention (Table 3). RMANOVA showed a main effect of group ($P = 0.0051$) and time ($P = 0.015$) on levels of s-IgA but no effect of gender ($P = 0.389$) or the interaction of group and time ($P = 0.3570$). Further investigation of the effect of time using post-hoc tests demonstrated that there was a significant increase in levels of s-IgA after 12 wk supplementation compared to baseline levels in the colostrum group ($P = 0.029$) but not the placebo group (Figure 2).

Multiple regression analysis was performed on the independent variables and the *P* values showed that there was no effect of weekly mileage, average URS days, or gender on levels of log of s-IgA (Table 4). After removing these variables from the multiple regression model the effect of being in the colostrum group on levels of log s-IgA was highly significant ($P = 0.004$) even after controlling for running the New Zealand marathon (M), which was just significant ($P = 0.045$). The general linear model showed the F-values (M = 4.37, colostrum = 9.60) and *P* values (M = 0.045, colostrum = 0.004) which were significant, this model accounting for about 29% of the variation in levels of log s-IgA.

URS

The mean number of URS days for the colostrum group was 5.3 d and for the placebo group 8 d. Non-parametric testing showed no significant difference between the groups. Mean URS episodes were 0.8 for the colostrum group and 1.1 for the placebo, and no significant difference was found.

Table 2 Descriptive Characteristics for the Subject Group

	Placebo		Colostrum		
	Male	Female	Male	Female	*P**
Number	8	9	12	6	0.25
Age (y)	48 (36-56)	51 (41-58)	46 (35-57)	43 (30-53)	0.26
Height (cm)	180 (170-192)	160 (152-164)	179 (172-184)	168 (160-174)	0.11
Body mass (kg)	75.4 (70.0–83.8)	60.8 (52.4 – 71.2)	78.7 (66.6 – 83.1)	67.5 (47.7 -87.5)	N/A
% Weight change	-0.14 (-2.2 - 2.4)	-0.49 (-1.1 - 1.0)	0.8 ($P = 0.61$) (-3.0 – 4.5)	-0.38 ($P = 0.97$) (-2.7- 2.8)	N/A
Weekly mileage (km)	43 (19-105)	46 (8-57)	50 (18-114)	58 (26-70)	0.13
Total URS days	12 (0-16)	16 (0-25)	7 (0-23)	12 (0-15)	0.55
G drink/d	25 (20-27)	25 (20-28)	25 (16-30)	25 (16-26)	0.62

Note. * Values are medians and ranges. *P* refers to probability value. The *P*-value shows the statistical significance of the difference between the placebo and the colostrum groups.

Table 3 Saliva Analysis for Absolute Concentration of s-IgA mg/L, Osmolality, s-IgA/Osmolality Ratio, and Albumin

	Absolute IgA conc (mg/L)	Osmolality (mOsmol/kg)	IgA to osmolality ratio	Albumin (mg/L)
Base (sample 1)				
PL	50 ± 6	65 ± 5	1.07 ± 0.04	39 ± 5
BC	68 ± 3	72 ± 7	1.01 ± 0.08	60 ± 8
4 wk (sample 2)				
PL	57 ± 5	67 ± 10	0.86 ± 0.09	46 ± 9
BC	77 ± 7	76 ± 12	1.05 ± 0.09	84 ± 20
8 wk (sample 3)				
PL	55 ± 5	72 ± 7	0.92 ± 0.05	51 ± 9
BC	87 ± 14	77 ± 10	1.14 ± 0.10	70 ± 14
12 wk (sample 4)				
PL	65 ± 4	71 ± 11	1.07 ± 0.06	51 ± 8
BC	123 ± 20	81 ± 12	1.47 ± 0.14	89 ± 18
Post (sample 5)				
PL	58 ± 5	65 ± 9	1.02 ± 0.08	52 ± 15
BC	101 ± 12	74 ± 11	1.38 ± 0.04	91 ± 17

Note. Values are means ± standard error of the mean. s-IgA = salivary IgA; PL = placebo; BC = bovine colostrum

Salivary Albumin and Osmolality

RMANOVA of salivary albumin levels showed there was a marginally significant group effect ($P = 0.044$); however, this did not change with time ($P = 0.12$). There was no effect of gender ($P = 0.68$). RMANOVA of saliva osmolality showed there was no main effect of group ($P = 0.24$), gender ($P = 0.51$) or time ($P = 0.22$). The RMANOVA for the s-IgA/osmolality ratio also showed no main effect of group ($P = 0.11$), gender ($P = 0.16$), or time ($P = 0.37$) (Table 3).

Reported Dietary Intake

Analysis of reported dietary intake was carried out using two 7-d food records. The first record was kept 6 wk into the marathon build-up (week 6) to determine reported

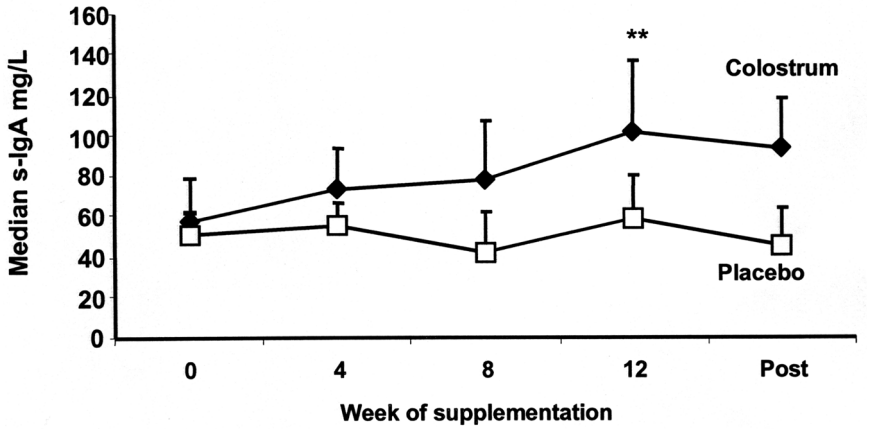

Figure 2—Time-dependent changes in the median absolute concentration of s-IgA and 95% confidence intervals. ** indicates significant increase in levels of s-IgA in colostrum group compared to baseline ($P = 0.0291$).

Table 4 Results from Multiple Regression Analysis Investigating the Effects of Independent Variables on Levels of Log S-Iga After 12 Weeks Intervention

Variable	Coefficient (SE coefficient)	*T*-statistic	*P*
Mean km/wk	–0.02 (0.14)	–0.15	0.88
Male	0.24 (0.23)	1.03	0.31
Mean URS	0.21 (0.20)	1.03	0.31
NZ marathon	0.42 (0.23)	1.81	0.08
Colostrum	0.65 (0.24)	2.76	0.01

Note. *Significance level set at $P < 0.05$

dietary intake during heavier training weeks and the second was kept in the week prior to the marathon (week 11) to determine the reported dietary intake during the taper period. Of the 35 participants who met the study criteria, 34 completed two sets of 7-d food records. Repeat measures ANOVA was used to determine differences in the reported dietary intake between the groups. There were no differences between the colostrum and placebo groups for reported daily intake of energy (kJ) ($P = 0.65$) carbohydrate g/kg body mass ($P = 0.27$), protein g/kg body mass ($P = 0.76$), or percent contribution of fat to energy (0.17). Means and standard errors of the mean at week 6 and week 11 are shown in Table 5. The RMANOVA

Table 5 Summary of Mean Daily Intake of Energy, Carbohydrate, Protein, and Fat Contribution to Energy Intake

		Week 6	Week 11
Colostrum	Energy (kJ)	10,058 ± 762	9,517 ± 619
	Carbohydrate (g/kg BM)	4.1 ± 0.33	4.0 ± 0.31
	Protein (g/kg BM)	1.4 ± 0.14	1.3 ± 0.09
	Fat (%)	31 ± 1.8	31 ± 1.3
Placebo	Energy (kJ)	10,162 ± 586	9,522 ± 539
	Carbohydrate (g/kg BM)	4.6 ± 0.24	4.3 ± – 0.27
	Protein (g/kg BM)	1.5 ± 0.07	1.4 ± 0.05
	Fat (%)	29 ± 1	30 ± 0.9

showed a time difference for reported energy intake. At week 11 intake was less than week 6 and this difference was significant for both groups ($P = 0.05$). There was no significant time change for the individual macronutrients.

Compliance and Side Effects

There was no significant difference between the groups in the consumption of drink powder (grams of drink per day). Two athletes started the treatment a week later than the others due to personal reasons.

Of the 17 women who participated in the study, six reported stomach problems after the commencement of the treatment. Of these six, two were on the colostrum treatment. All participants who reported stomach problems found the symptoms disappeared with time except for one of the women on the colostrum treatment. No adverse symptoms were reported by the men.

Discussion

The results in this study have shown that there was an increase of 79% in median s-IgA levels after 12 wk supplementation with bovine colostrum and that this increase was significantly different to the corresponding group's s-IgA levels at baseline. The differences in s-IgA could not be accounted for by other variables under investigation and were still significant after controlling for those athletes who ran the New Zealand marathon. A smaller increase in median s-IgA levels was seen in the placebo group (16%) but the difference after 12 wk supplementation was not significant compared to baseline levels. This study also demonstrated that the sampling and testing methodology established is sufficiently sensitive to detect changes occurring in s-IgA in the field among athletes undertaking marathon-type training.

Levels of s-IgA in athletes participating in other studies using similar laboratory procedures have demonstrated similar findings. These include a study on an elite group of 15 male and 11 female swimmers monitored monthly over a period of 7 months whose pretraining median s-IgA concentrations ranged from 42 to 84 mg/L. Additionally, the pre-training s-IgA levels for males was found to be higher on average than the females (15). In another group of elite swimmers (12 males and 10 females) pre-training s-IgA levels ranged from 41.5 to 71.1 mg/L during a 12 wk training cycle. Results were not separated by gender (14). Salivary IgA levels, from a group of 14 elite male swimmers, were monitored every second to third day over a 30-d period of intensive training. Levels of s-IgA varied significantly in response to training and the appearance of URS with medians ranging from 32.6 to 127.5 mg/L (17). This study also showed the benefit of frequent saliva sampling and verification of URS symptoms to identify the causes of the changes seen in s-IgA levels. Median s-IgA levels in the current study ranged between 41.5 to 54.7 mg/L for the placebo group and 56.8 to 101.5 mg/L for the colostrum group, which compares well with the above studies. This demonstrates that the sampling and testing methodology used were sensitive to changes in s-IgA levels, and commensurate with previous studies regarding exercise and s-IgA.

In a study of runners competing in the Los Angeles Marathon it was found that athletes who ran more than 97km/wk in training reported a greater incidence of URTI compared to those running less than 32km/wk (28). In comparison, the mean kilometers run per week by distance runners in this preliminary study was 44.5 km for the placebo group and 54 km for the colostrum group. The difference in training volumes was not significant between the placebo and colostrum groups ($P = 0.19$). Multiple regression showed that the volume of training was not sufficient to have an effect on levels of s-IgA during the study period ($P = 0.88$). The significant increase in levels of s-IgA however was partly due to participating in the New Zealand marathon ($P = 0.045$). It is possible, therefore, that the athletes who ran this marathon experienced moderately elevated levels of s-IgA via a mechanism not linked to dietary intervention. However, even after controlling for the runners (21/35 runners) who participated in the marathon, the increase in levels of log s-IgA was still very significantly associated with being in the colostrum group ($P = 0.004$).

The median incidence of self-reporting of URS over the study period was one episode per person (colostrum group range, 0 to 4; placebo group range, 0 to 3). It is interesting to note that this incidence is similar to that of a group of 26 elite swimmers who were monitored for 7 months (15). The median incidence in the elite swimmers was one episode (range 0 to 5) per male swimmer and three URS episodes (range 0 to 7) per female swimmer (15), the difference in incidence of URS observed between males and females was not significant. The rate of reporting of URS in the distance runners in the current study seems high in relation to the volume of training, as they were not elite athletes (except for two veteran elite males). However, it is possible the participants could have been experiencing combined stress from working full time, family pressures, poor dietary practices (as indicated by reported carbohydrate intake which was lower than that recommended for endurance athletes), and also participating in distance training. It is known that the risk for URTI in athletes is multifactorial in origin (2).

Viral infection is the most common cause of URTI in athletes, but is not the only cause of URS. It is possible that at least some of the symptoms self reported by the runners were due to local or systemic inflammation in response to exercise, rather than infection (33). The cytokine IL-6, for example, is produced during exercise by muscles in response to a lowering of muscle glycogen (34). IL-6 can co-stimulate the production of other anti-inflammatory cytokines (such as IL-1ra and IL-10) and can reduce levels of the pro-inflammatory cytokine TNF-α. Levels of IL-6 also increase as a result of a local response when infection occurs or tissue is damaged, where local inflammation results in a systemic acute-phase response associated with "feelings of sickness" (25), due to the in vivo pyrogenic and febrile nature of the cytokine. Further, high circulating levels of IL-1ra have been reported to be associated with a perception of poor health in 325 volunteers age 18 y and over visiting a primary health care unit (21). In the current study, it was interesting to note that the average total number of URS days reported by the colostrum group was lower than the placebo group; however the difference was not significant. As bovine colostrum contains growth factors that can help with tissue repair it is possible that the recovery process was improved in these runners. This supports the suggestion by Buckley et al. (2002) that colostrum supplementation improved recovery in distance runners as indicated by improved performance after a second bout of exercise (6).

Other methodological constraints that are known to affect levels of s-IgA were controlled for in the present study. This included standardizing the post-prandial sampling time in order to minimize the effects of dehydration. The post-prandial sampling time was set at 30 min to accommodate the athletes; however, several other studies have used 2 h (13, 35), therefore it may not be possible to compare absolute s-IgA concentrations between studies. Controlling for the quality of saliva is also important for cross-study comparisons. Recently the secretion rate of s-IgA and the absolute s-IgA concentration in American footballers have been found as the most useful clinical biomarkers for predicting the risk of contracting URTI (29). It has been suggested that the amount of s-IgA on mucosal surfaces is important in preventing infection (9). In this group of footballers the results for osmolality and s-IgA/osmolality ratio were not associated with increased incidence of URTI. The conclusion was that the absolute s-IgA concentration and s-IgA secretion rate were the most useful predictors when investigating the effects of exercise on URTI incidence in footballers. Therefore the use of osmolality in the preliminary study reported here may not have been the most appropriate method for controlling for saliva quality and may help to explain why no significant changes were seen in the s-IgA/osmolality ratio. However an increase in s-IgA concentration with no change in the s-IgA/osmolality ratio could indicate that at least part of the s-IgA increase was due to the subjects being less hydrated.

Prolonged intensive exercise may reduce resting salivary s-IgA levels and salivary s-IgA levels below 40 mg/L at the beginning of a training season have been associated with a higher risk for contracting URTI (15). While only one athlete showed a downward trend in s-IgA levels in the present study, 11 athletes (28.9% of all participants) had baseline concentrations below 40 mg/L. Of the 11 athletes who had s-IgA levels below 40 mg/L at baseline, seven (64%) reported URS later in the study. This observation is consistent with a previous study of the

incidence of URS in elite swimmers compared to preseason s-IgA levels (15) and adds support for the proposal that s-IgA levels below 40 mg/L at the beginning of a training season can place the athlete more at risk of contracting URTI. In this case, colostrum supplementation may be of particular use in increasing s-IgA responses among those individuals who ordinarily express the lowest levels of s-IgA (most likely as a result of exercise-induced transient immune depression). Use of dietary supplements to boost immune parameters among those at-risk individuals who ordinarily express the lowest level of immunity has been reported in other groups, e.g., the elderly (11).

Bovine milk proteins (especially whey) have been reported to demonstrate immunomodulatory effects (12, 23, 32). Therefore the skim milk powder in the colostrum and placebo blends may have had a masking effect on the response of s-IgA levels to colostrum, in that it is possible that inherent immunomodulatory proteins in milk (particularly whey proteins) may have boosted background immune reactivity to a level where the incremental effect of colostrum was no longer detectable. One of the proposed mechanisms for the stimulation of production of s-IgA levels is thought to be through the activity of TGF-β1, which can promote IgA biosynthesis and secretion, at least in vitro (7). Levels of TGF- β2 in the blends were analyzed as they are the larger component of the total TGF-β in colostrum (8). Levels of TGF-β2 in the colostrum blend (8486 ng/26 g) were found to be 61% higher than in the placebo (5241 ng/26 g), the latter due to high levels of TGF- β2 inherent in skim milk. It seems likely, therefore, that any effect from the colostrum blend would not be solely due to increased levels of TGF-β2.

Since bovine colostrum contains secretory IgA of bovine origin, one particular concern in this study was to ensure that measurement of salivary IgA in participants was specific for the human molecule. The immunoglobulin A measured in the saliva samples in this study was undertaken using human-specific antisera, which were shown to exhibit no cross-reactivity against bovine proteins. This suggests that the increases in s-IgA concentration noted in the colostrum group was due to stimulation by some unknown mechanism, and levels have not been increased through absorption of bovine IgA from the colostrum supplement. That bovine colostrum supplementation can act as a stimulant is further supported by a study of 30 athletes who consumed 20 g of bovine colostrum containing labeled insulin-like growth factor 1 (IGF-1) for 2 wk (25). Serum IGF-1 levels increased significantly but radioactive (bovine) IGF-1 was not detected in the serum, further demonstrating that post-prandial uptake of intact bovine growth factors from dietary colostrum is unlikely to occur, as would be expected for polypeptide structures.

As the mucosal immune system is a common network and the B cells responsible for local production of s-IgA come from the mucosa-associated lymphoid tissue (MALT) which includes the tonsils and Peyer's patches in the distal small intestine (4), it seems possible that stimulation in the small intestine could promote increased IgA production in the saliva (36). Peyer's patches make up most of the gut-associated lymphoid tissue (GALT) and from here primed B cells migrate via peripheral blood to mucosa throughout the body including the salivary glands. Although the primary function of GALT is to protect the gut it also acts as the main effector site for mucosal protection for other parts of the body (4, 20). As prolonged periods of intensive exercise have been associated with reduced s-IgA

production, disturbances to the integrated mucosal network (such as alterations in T-cell cytokine control mechanisms) could lead to mucosal suppression. A number of cytokines are involved in the production of salivary s-IgA. Antigen-activated CD4+ T cells can stimulate production of IL-5, IL-10, and IL-6 which may all be involved in terminal differentiation of B cells into IgA secreting plasma cells (4). These potential markers of mucosal immunity were not measured in the present study. Other immune parameters that could help to identify the response of the mucosal immune system include full blood count, lymphocyte differentiation and phenotyping, and serum levels of IgA, IgM, and IgG. Further trials which aim specifically to measure those parameters linked to mucosal immunity following colostrum supplementation are currently being undertaken by our group.

In summary, this study has shown that the sampling and testing methodology established is sufficiently sensitive to detect changes occurring in salivary IgA (in the field) among athletes undertaking marathon-type training. It has also been shown that a change in salivary IgA levels occurred with athletes taking bovine colostrum compared to those taking a placebo. If this effect is real then colostrum could have potential benefits in terms of enhancing mucosal immunity and reducing recurrent infection thus allowing for a reduction in interrupted training schedules. However, due to the small sample size and the heterogenous mix of the participants, further research is needed to determine whether bovine colostrum truly has a physiologically relevant effect. Validating the results from this study with a more homogenous group of endurance athletes in terms of gender, fitness level and type of training program is necessary.

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