Effects of bovine colostrum supplementation on immune variables in highly trained cyclists

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COLOSTRUM IS THE FIRST MILK produced by mammals after parturition. In addition to being a source of carbohydrates, protein, fat, minerals, and vitamins, it also contains high concentrations of growth and immune factors that are essential to the physiological maturation and development of the immune system of the newborn. Colostrum contains immunoglobulins, cytokines, lactoferrin, and lactoperoxidase, each of which are active components of the immune system (2, 23, 46).

The importance of human colostrum for the development of the neonate immune system is well recognized (22), and the presence of closely homologous bioactive components in bovine colostrum has led to the use of bovine colostrum to treat and prevent infectious diseases in humans (10, 18). Bovine colostrum contains immune factors that modulate cytokine production in mice (49), enhance bovine leukocyte phagocytosis (42), and modulate the humoral immune response to vaccine administration in healthy humans (20). These findings provide evidence to suggest that bovine colostrum may also strengthen the immune function of athletes involved in high-intensity exercise. High-intensity exercise impairs immune function for up to several hours postexercise (32). Immune alterations that occur after intense exercise include reduced salivary IgA secretion rate (34), depressed natural killer (NK) cell numbers and cytotoxicity (39), decreased cell-mediated immunity, and increased humoral immunity (44). These alterations may contribute to increased susceptibility to illness in well-trained athletes (17, 27, 28, 39).

During periods of normal exercise training in athletes, 12 wk of bovine colostrum supplementation (12.5 g/day) has been associated with an increase in resting salivary IgA levels (9) and a reduced incidence of upper respiratory tract infection (6, 9). To date, in humans, the influence of bovine colostrum on immune variables of athletes is unclear. Whether bovine colostrum supplementation can enhance an athlete’s immune system, particularly during short periods of intense training, is yet to be established.

The aim of the present study was to investigate the influence of bovine colostrum protein concentrate (CPC) on a variety of immune parameters in a group of highly trained endurance cyclists over an 8-wk period. We investigated the effects of colostrum on NK cell cytotoxicity, salivary IgA, and cytokine and immunoglobulin concentrations, as well as expression of neutrophil and lymphocyte surface markers. Components of bovine colostrum have been shown to enhance neutrophil phagocytosis and oxidative burst capacity of bovine polymorphonuclear leukocytes (25, 41). Therefore, we chose to investigate neutrophil surface marker expression of CD35 and CD11b, which facilitate the uptake and phagocytosis of opsonized bacteria, and CD89, which binds IgA complexes to initiate phagocytosis and release of soluble mediators. Changes
in lymphocyte populations were also assessed because colostrum preparations have been shown to upregulate surface expression of CD4 in immunocompromised patients (14). We hypothesized that bovine CPC supplementation would reduce postexercise immune suppression and maintain healthy immune function over an 8-wk training period that included 5 consecutive days of high-intensity training (HIT).

METHODS

Subjects. Twenty-nine highly trained male road cyclists volunteered to participate in the present investigation. The cyclists had been racing competitively for at least two seasons and had maintained consistent training volumes for at least 2 mo before the study. Before acceptance into the study, the cyclists completed a medical history questionnaire and gave their written consent; the experimental protocol was approved by the Medical Research Ethics Committee at The University of Queensland. Mean (±SE) age, weight, height, maximal O2 consumption (V\textsubscript{O2 max}), peak power output, and training volume for the cyclists are shown in Table 1 (there were no significant differences between groups for these variables, P > 0.05).

Experimental overview. All testing was completed in the Exercise Physiology Laboratory at The University of Queensland. The investigation was randomized, double-blind and placebo controlled. Cyclists completed 1 wk of familiarization testing that consisted of a V\textsubscript{O2 max} test (in which their ventilatory threshold was estimated), a time to fatigue test at 110% of ventilatory threshold (TTF), and a 40-km time trial (TT\textsubscript{a0}). Figure 1 provides an overview of the experimental protocol that followed familiarization. The familiarization tests were repeated the following week (in the same order), and the data from this week were used as baseline measures (V\textsubscript{O2 max-1}, TTF1, TT\textsubscript{a0-1}). After 5 wk of supplementation and regular training, cyclists returned to the laboratory to complete another series of performance tests (V\textsubscript{O2 max-2}, TTF2, TT\textsubscript{a0-2}). During the following week, the cyclists completed 5 consecutive days of HIT (which included TT\textsubscript{a0-3}), followed by a further series of performance tests (V\textsubscript{O2 max-3}, TTF3, TT\textsubscript{a0-4}). Blood and saliva were collected before and immediately after each TT\textsubscript{a0} (TT\textsubscript{a0-1}, TT\textsubscript{a0-2}, TT\textsubscript{a0-3}, TT\textsubscript{a0-4}). Cyclists avoided all strenuous physical activity for 24 h before they performed all tests. Changes in exercise performance over the supplementation period are described elsewhere (38).

Diet, training, and illness logs. After the first 2 wk of performance testing, cyclists completed a daily log to obtain information on training, quality of sleep, and daily food intake. Dietary records were analyzed with computer software (Foodworks, Xyris, Brisbane, Australia), and from this analysis daily average macro- and micronutrient intakes were calculated. Cyclists were required to refrain from taking any dietary supplements 1 mo before and for the duration of the study. Training logs provided information that was collated to provide the total number of training kilometers and total training time per week. This was further expressed in five categories of training intensity from each cyclist’s self-selected pace for 5 min, cyclists completed a graded exercise test to exhaustion. An electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) modified with clip-in pedals and low-profile racing handlebars was used for exercise testing; the saddle and handle bar positions of the cycle ergometer were adjusted to resemble each cyclist’s own bike. Each cyclist wore a heart rate monitor (Vantage NV, Polar Instruments) for the duration of the V\textsubscript{O2 max} test. Height (cm) and weight (kg) were measured when the cyclists first arrived at the laboratory. Then, after a warm-up at a selected self-paced speed, cyclists completed a graded exercise test to exhaustion. The ventilatory threshold was estimated by following the first clear breakpoint in the minute ventilation/CO2 production relationship (8).

Table 1. Athlete characteristics

<table>
<thead>
<tr>
<th>Category</th>
<th>Bovine CPC (n = 14)</th>
<th>Placebo (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>29 ± 1</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.1 ± 1.9</td>
<td>77.6 ± 2.2</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.80 ± 1.1</td>
<td>1.79 ± 1.4</td>
</tr>
<tr>
<td>V\textsubscript{O2 max} (mL/kg·min\textsuperscript{-1})</td>
<td>68.8 ± 1.5</td>
<td>69.3 ± 1.3</td>
</tr>
<tr>
<td>Peak power output, W</td>
<td>446 ± 9</td>
<td>461 ± 12</td>
</tr>
<tr>
<td>Training volume, km/wk</td>
<td>463 ± 9</td>
<td>422 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE. V\textsubscript{O2 max}, maximal O2 consumption.

Fig. 1. Experimental overview. The first week of testing involved a maximal O2 consumption (V\textsubscript{O2 max}) test, a 40-km time trial (TT\textsubscript{a0-1}), and a time to fatigue test at 110% of ventilatory threshold (TTF). These tests were repeated in week 2 (TT\textsubscript{a0-2}) and week 9 (TT\textsubscript{a0-9}). After 5 wk of normal training (weeks 2–6) and 1 wk of repeat testing (week 7), cyclists (n = 29) performed 5 consecutive days of high-intensity training (HIT) that included a TT\textsubscript{a0} on the last day (TT\textsubscript{a0-3}).
TTF test. On a separate day (24–72 h after \( \dot{V}O_2 \text{max} \) test), cyclists reported to the laboratory to complete a TTF test. After a self-selected warm-up, cyclists exercised on the cycle ergometer at a constant power output until they were no longer able to maintain pedal revolutions >60 rpm. The power output used for each cyclist’s TTF test corresponded to 110% of the power output at each cyclist’s ventilatory threshold.

40-km time trial. On a third occasion (2–3 days after the previous visit), cyclists completed a laboratory-simulated TT40. Cyclists were instructed to avoid any physical activity for 24 h before each TT40 and to arrive at the laboratory well hydrated and fasted (for a minimum period of 8 h). The cyclists rode their own road bicycle mounted to a stationary windtrainer (CatEye cyclosimulator CS-1000). The rear tire was inflated to 120 pounds/in.\(^2\) and placed against the friction device before being secured; the spring-loaded release brake was removed, placing a wind-regulated friction load against the rear wheel. The cyclists’ same rear wheel was used for each TT40. Performance time was blinded to the cyclist during the TT40 and revealed to them on completion of the ride. Cyclists were allowed to consume water ad libitum during exercise; intake was monitored and recorded to ensure that there were no differences between trials.

Supplements. After the second week of testing, cyclists were randomly assigned to one of two groups using randomly generated number allocation software (GraphPad, San Diego, CA). Cyclists (\( n = 15 \)) in one group consumed 10 g whey protein concentrate (placebo, Alacen 80; Fonterra Co-op Group Limited, Auckland, New Zealand) per day, whereas cyclists (\( n = 14 \)) in the other group consumed 10 g intact bovine CPC (Numirex Research Australia, South Australia, Australia) per day. Intact CPC is a standardized, low-heat, low-fat, low-lactose colostrum powder containing 20% IgG with retained casein and whey proteins. The period of supplementation was 8 wk and 1 day. Both the protein powder and colostrum were supplied to the investigators in sealed sachets that did not identify the contents. Sachets were numbered, and the contents of each were revealed on completion of data analysis. Cyclists took the supplement at the start of each day after it was mixed with 50 ml of water and 100 ml of skim milk.

High-intensity training. After testing in week 8 was completed, cyclists completed 5 consecutive days of HIT. All training sessions involved cycling in the laboratory at or above ventilatory threshold. The first day of HIT involved 20 × 1-min efforts at peak power output (the peak power reached at the end of the \( \dot{V}O_2 \text{max} \) test completed in week 7) with 2 min of recovery at 50 W between each effort. Training on day 2 involved 60 min of cycling at 100% ventilatory threshold (comparable to TT40 intensity). Day 3 involved 12 × 30-s sprints at 175% of peak power output with 4.5 min of cycling at 50 W between sprints. HIT on day 4 involved 30 min of cycling at 80% of the cyclist’s ventilatory threshold following 45 min at 100% of their ventilatory threshold. On the final day of HIT, the cyclists performed a TT40 (TT403).

Blood collection. Blood was sampled from a forearm vein by venipuncture before the start of TT40 (after 5 min of rest) and immediately after TT40. Blood (10 ml) was collected and separated into different collection tubes: 3 ml into a tube containing EDTA for cell counts, 5 ml into a heparinized tube for the determination of NK cell cytotoxicity and phenotyping of lymphocytes and neutrophils, and 2 ml into a serum separator tube for determination of cytokines and immunoglobulins.

Hematological profile. Full blood cell counts, hemoglobin, and hematocrit were obtained via an automated cell analyzer (Sysmex SE-9000, TOA Medical Electronics, Kobe, Japan). The concentration of cytokines and immunoglobulins, full blood cell counts, and cell numbers for lymphocyte and neutrophil surface receptors were adjusted to account for changes in plasma volume according to the methods of Dill and Costill (12).

Lymphocyte and neutrophil immunophenotyping assay. Three-color flow cytometry was used to determine lymphocyte and neutrophil subpopulations. Briefly, 50 \( \mu l \) of whole blood was incubated at room temperature for 20 min with 10 \( \mu l \) of antibody cocktail in polypropylene tubes. Each cocktail contained combinations of mouse anti-human monoclonal antibodies for the following receptors: phycoerythrin (PE)-conjugated CD89, PE-conjugated CD16, PE-conjugated CD56, FITC-conjugated CD18, FITC-conjugated CD11b, PE-conjugated CD3, FITC-conjugated CD11c, allophycocyanin-conjugated CD4, PE-conjugated CD8, allophycocyanin-conjugated CD19, peridinin-chlorophyll protein-conjugated CD33, and peridinin-chlorophyll protein-conjugated CD3. FITC-conjugated IgG1 and PE-conjugated IgG2 were used as controls. All antibodies were purchased from Becton-Dickinson (San Jose, CA). Erythrocytes were then lysed with 1 ml of FACSlyse and incubated for a further 9 min. Finally, the tubes were centrifuged at 2,500 rpm for 3 min before the supernatant was removed, and cells were resuspended in 250 \( \mu l \) of 1% formalin until analysis. The samples were analyzed with a FACSScan flow cytometer (Becton-Dickinson) located in a commercial pathology laboratory (Sullivan Nicolaides, Taringa, Queensland, Australia). The flow cytometer was calibrated daily using different microbeads [QC Windows and Quantum 1000 (Flow Cytometry Standards, San Juan, PR) and CaliBRITE (Becton-Dickinson)], as per usual methods for quantifying cellular antigens by flow cytometry. A total of 20,000 events were collected, and the data were recorded as mean fluorescence intensity and percentage of positive cells.

Determination of NK cell cytotoxicity. NK cell cytotoxicity was determined by a modified version of a previously described method (3). Briefly, peripheral blood lymphocytes (effector cells) were separated from whole blood by Ficoll-Hypaque separation. Cells were resuspended in RPMI 1640 at 1 × 10^6/ml, and 5 \( \mu l \) of a fluorescent probe (DiI; Sigma-Aldrich, Castle Hill, NSW, Australia) were added per milliliter of cell suspension. K562 cells (target cells) were suspended in RPMI 1640 at a concentration of 1 × 10^6/ml. Effector cells were added to target cells at a concentration of 50:1. Target cell death and apoptosis were determined on annexin V buffer (Becton-Dickinson) vs. 7-amino actinomycin D quadrants.

Saliva collection. Each cyclist was asked to sit up with his head tilted forward and to expectorate saliva over 5 min into pre-weighted 50-ml tubes. He was asked not to force salivary production and was told he could fill the tubes at intervals of his own choice. Samples were then placed on ice and frozen at −80°C until they were analyzed for IgA and albumin.

Salivary IgA analysis. Saliva samples were analyzed for IgA and albumin with a BN ProSpec nephelometer (Dade Behring, Marburg, Germany). For the measurement of salivary IgA, diluent, reaction buffer, and N latex IgA test kits were all purchased from Dade Behring. These kits are made up of the following items: 1) an IgA reagent, consisting of freeze-dried polystyrene particles coated with rabbit anti-human IgA; 2) an IgA standard (consisting of a mixture of human sera), which is referenced to the International Federation of Clinical Chemists Certified Reference Material-470; 3) an IgA supplementary reagent A, consisting of an aqueous solution of pyrrolidone (maximum 100 g/l) and Tween 20 (maximum 10 g/l); 4) an IgA supplementary reagent B, consisting of animal and human proteins as well as of pyrrolidone (−50 g/l); and 5) an IgA control (human), consisting of a mixture of human serum. Salivary albumin was measured with an antiseraum for human albumin, a protein standard, and various protein controls (Dade Behring). All reagents, controls, and standards were reconstituted according to the manufacturer’s instructions with ultrapure water obtained from a MODULAB analytical-research grade UP/polishing system (Continental Water Systems, San Antonio, TX). Any samples that contained mucus or blood were excluded from analysis.

Serum immunoglobulins. Serum IgG1, IgG2, IgG3, and IgG4 were measured by ELISA (Zymed Laboratories, San Francisco, CA). Serum samples were diluted 1:10,000 for the measurement of IgG1 and 1:3,600 for the measurement of IgG2, IgG3, and IgG4. IgA and IgE were measured in undiluted serum by ELISA (Bethyl Laboratories, Montgomery, TX). All assays were performed according to the
manufacturer's instructions. The serum concentrations of IgG subclasses, IgA and IgE, were calculated by comparison to a standard curve established in the same set of measurements. These measurements were performed with a microplate reader (VERSAMax; Molecular Devices, Sunnyvale, CA).

**Serum cytokines.** Serum IL-10, IL-12p40, IFN-γ, and soluble TNF-α receptor 1 (sTNFR1) were measured by OptEIA kits (Becton-Dickinson). Serum TNF-α and IL-6 were measured by Quantikine high-sensitivity ELISA (R&D Systems, Minneapolis, MN). All assays were performed according to the manufacturers’ instructions. Serum cytokine concentrations were calculated by comparison to a standard curve established in the same set of measurements. These measurements were performed with a microplate reader (VERSAMax; Molecular Devices).

**Statistical analysis.** All statistical analyses were performed with SPSS version 13.0 for Windows (SPSS, Chicago, IL). Data were first tested for normality with the Kolmogorov-Smirnov and Shapiro-Wilk tests. The residuals not normally distributed were serum IL-10 concentration and reported illness incidence. Serum cytokine concentrations were calculated by comparison to a standard curve established in the same set of measurements. These measurements were performed with a microplate reader (VERSAMax; Molecular Devices).

There were no significant differences between the bovine CPC and placebo groups at baseline for total training kilometers, total training time, and training intensity over the experimental period (P > 0.05). As expected, total training time (in minutes) spent in heart rate categories 4 and 5 was significantly greater during the HIT period (week 8), for both the placebo (category 4: 190 ± 14%, P < 0.01; category 5: 75 ± 44%, P < 0.03) and bovine CPC groups (category 4: 160 ± 49%, P < 0.001; category 5: 81 ± 25%, P < 0.001), than during the same 5-day training period for week 7.

**Results**

**Nutrient intake.** Over the experimental period, the average macronutrient intake of cyclists in the placebo group was not significantly different from intake in the bovine CPC group (P > 0.05). For the placebo group, average daily macronutrient intake (as a percentage of total energy intake) was as follows: carbohydrate 56%, protein 20%, and fat 24%, with energy 10,673 kJ. For the bovine CPC group, average daily macronutrient intake was as follows: carbohydrate 55%, protein 23%, and fat 22%, with energy 10,572 kJ. There were no significant differences between groups for micronutrient intake (P > 0.05). Each cyclist’s water intake was similar (±150 ml) during each of their four TT40 across the experimental period (P > 0.05).

**Leukocytes.** There was a significant main effect of time (pre- and postexercise) for white blood cell, neutrophil, lymphocyte, and monocyte counts (P < 0.001) (Table 2); however, there were no significant differences between groups or across the experimental period (P > 0.05). There were no significant main effects for eosinophil count.

**Neutrophil surface markers.** There was a significant main effect of time (pre- and postexercise) for CD89 expression (P < 0.001) (Table 3) and a significant interaction of trial (TT40) × group for CD89 expression over the experimental period (P = 0.027) (Table 3). There was a significant postexercise increase for CD89 expression at TT40 in the placebo group, whereas there was a significant postexercise increase for the bovine CPC group after TT40. There was also a significant main effect of time (pre- and postexercise) for the expression of CD11b (P = 0.014), CD18 (P = 0.017), and CD35 (P = 0.006) (Table 3); however, there was no main effect of trial or group (P > 0.05) for these surface markers.

**Lymphocyte surface markers.** There was a significant main effect of time (pre- and postexercise) for the percentage of CD3+ (T cells), CD3+CD4+ (helper T cells), CD19+ cells (B cells), and CD3−CD16+CD56+ (NK cells) (P < 0.003) (Table 4). However, there were no differences between groups or across trials. There was, nevertheless, a significant interaction

| Table 2. Full blood count pre- and postexercise for each TT40 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | TT401 | TT402 | TT403 | TT404 |
|                 | Pre    | Post   | Pre    | Post   | Pre    | Post   | Pre    | Post   |
| **WBC, 1 × 10^9/liter** |          |        |        |        |        |        |        |        |
| Placebo         | 5.98±0.48 | 6.20±0.37 | 6.22±0.42 | 5.93±0.37 |          |        |        |        |
| Bovine CPC      | 5.97±0.27 | 6.44±0.32 | 6.76±0.82 | 6.07±0.31 |          |        |        |        |
| Neutrophils, 1 × 10^9/liter | 2.72±0.3 | 2.73±0.18 | 2.61±0.23 | 2.5±0.25 |          |        |        |        |
| Placebo         | 2.72±0.3 | 3.13±0.32 | 3.33±0.46 | 2.76±0.17 |          |        |        |        |
| Bovine CPC      | 2.73±0.18 | 3.49±0.24 | 4.1±0.68 | 3.41±0.28 |          |        |        |        |
| Lymphocytes, 1 × 10^9/liter | 2.44±0.18 | 2.52±0.17 | 2.4±0.15 | 2.5±0.21 |          |        |        |        |
| Placebo         | 2.44±0.18 | 2.36±0.14 | 2.31±0.14 | 2.25±0.12 |          |        |        |        |
| Bovine CPC      | 2.35±0.12 | 4.2±0.23 | 4.09±0.27 | 4.13±0.28 |          |        |        |        |
| Monocytes, 1 × 10^9/liter | 0.58±0.04 | 0.6±0.04 | 0.65±0.07 | 0.65±0.06 |          |        |        |        |
| Placebo         | 0.58±0.04 | 0.74±0.05 | 0.75±0.05 | 0.78±0.06 |          |        |        |        |
| Bovine CPC      | 0.58±0.04 | 0.68±0.05 | 0.78±0.07 | 0.79±0.06 |          |        |        |        |
| Eosinophils, 1 × 10^9/liter | 0.24±0.03 | 0.29±0.04 | 0.26±0.04 | 0.27±0.04 |          |        |        |        |
| Placebo         | 0.24±0.03 | 0.26±0.05 | 0.25±0.04 | 0.24±0.03 |          |        |        |        |
| Bovine CPC      | 0.26±0.05 | 0.2±0.03 | 0.29±0.04 | 0.34±0.07 |          |        |        |        |

Values are means ± SE. TT401 was completed at baseline (before the supplementation period), TT402 was performed after 5 wk of supplementation, TT403 was performed on the last of the 5 consecutive days of HIT, and TT404 was performed during the last week of performance testing. WBC, white blood cell; CPC, colostrum protein concentrate; TT40, 40-km time trial; pre and post, preexercise and postexercise, respectively.
Table 3. Neutrophil surface markers

<table>
<thead>
<tr>
<th>Antigen</th>
<th>TTα1</th>
<th>TTα2</th>
<th>TTα3</th>
<th>TTα4</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td>CD89+</td>
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<tr>
<td>Placebo</td>
<td>327±26</td>
<td>354±24*</td>
<td>333±18‡</td>
<td>349±24</td>
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<tr>
<td>Bovine CPC</td>
<td>380±36</td>
<td>407±34</td>
<td>394±22</td>
<td>400±26</td>
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<tr>
<td>CD11b</td>
<td></td>
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<tr>
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<td>111±9</td>
<td>140±7</td>
<td>117±9</td>
<td>156±14</td>
</tr>
<tr>
<td>Bovine CPC</td>
<td>118±24</td>
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<td>118±17</td>
<td>145±17</td>
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<td>CD35+</td>
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<td>Placebo</td>
<td>53±14</td>
<td>60±16</td>
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<td>Bovine CPC</td>
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<td>CD18+</td>
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<td>121±8</td>
<td>135±8</td>
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<td>128±8</td>
</tr>
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</table>

Values are mean ± SE. *Significantly different from preexercise; †significantly different from TTα1; ‡significantly different between groups at specified time point (P < 0.05).

of time × group for the percentage of CD3+CD8+ (cytotoxic/suppressor T cells) (P = 0.048). The exercise-induced change in CD3+CD8+ cells was significantly smaller in the bovine CPC group than in the placebo group at the end of the HIT period (TTα3: bovine CPC = −1.0 ± 2.7%, placebo = −9.2 ± 2.8%; P = 0.017) and the following week (TTα4: bovine CPC = 1.4 ± 2.9%, placebo = −8.2 ± 2.8%; P = 0.004). As a result of changes in the percentage of CD3+CD8+ cells, there was a significant main effect of time (pre- and postexercise) (P < 0.001) and interaction of time × group (P = 0.049) for CD4/CD8. Post hoc analysis revealed a significantly greater postexercise decrease in CD4/CD8 for the bovine CPC group after TTα2 and TTα3 (at the end of the HIT period).

NK cell cytotoxicity. There were no significant main effects (P > 0.05) for NK cell cytotoxicity over the experimental period (data not shown).

Salivary IgA. There was no main effect of time, trial, or group for absolute IgA concentration (P > 0.05) (Fig. 2A); however, there was a significant main effect of time (pre- and postexercise) for IgA-albumin (P = 0.002) (Fig. 2B). There was also a significant main effect of trial and significant interaction of trial × group for saliva flow rate (P = 0.044 and 0.024, respectively). Although there was a greater increase in preexercise saliva flow rate over the experimental period for the bovine CPC group than for the placebo group (bovine CPC group = 173 ± 116%, placebo group = 75 ± 25%), this was not significantly different between groups (Fig. 2C). The interaction of trial (TTα) × time (pre- and postexercise) × group for IgA secretion rate approached significance (P = 0.068) (Fig. 2D). Compared with the placebo group, there was a trend in the bovine CPC group toward a greater increase in preexercise IgA secretion rate from baseline to the end of the supplementation period (bovine CPC = 131 ± 67%, placebo = 74 ± 38%), although these changes were not statistically significant (P > 0.05).

Serum immunoglobulins. There was a main effect of time (pre- and postexercise) for serum IgE, IgA, and IgG3 concentration (P < 0.02), but there were no significant differences between groups or over the experimental period (Fig. 3, A and B).
There was a significant main effect of trial (TT40) for serum IgG1 \((P = 0.021)\) and serum IgG4 concentration \((P = 0.015)\), but there was no main effect of time or group (Fig. 3, C and F).

There was a significant main effect of trial (TT40) for serum IgG2 \((P < 0.001)\) and a significant interaction of trial \(\times\) group \((P = 0.004)\) (Fig. 3D). Postexercise serum IgG2 was significantly decreased in the placebo group at the end of the HIT period \((P < 0.05)\), whereas there was no significant decrease in the bovine CPC group \((P < 0.05)\).

Serum cytokines. There was a significant main effect of time (pre- and postexercise) for serum IL-6, IL-10, and TNF-\(\alpha\) concentrations \((P < 0.001)\); however, there was no significant main effect of group or trial (TT40) \((P > 0.05)\) (Fig. 4). There were no significant main effects for serum IFN-\(\gamma\) \((P > 0.05)\). There was, however, a significant interaction of trial \(\times\) time \(\times\) group for serum sTNF\(_{\alpha}\) across the experimental period \((P = 0.023)\). Preexercise serum sTNF\(_{\alpha}\) was significantly greater in the bovine CPC group at the end of the HIT period \((P = 0.023)\), whereas preexercise serum sTNF\(_{\alpha}\) was significantly reduced over the experimental period in the placebo group (Fig. 4E). There was no significant influence of bovine CPC on serum IL-12p40 concentrations; however, there was a significant main effect of trial (TT40) \((P = 0.01)\). Serum IL-12p40 was increased postexercise for TT401, TT402, and TT404, whereas there was a decrease in postexercise serum IL-12p40 at the end of the HIT period. The postexercise increase in serum IL-12p40 for TT404 tended to be greater in the bovine CPC group than in the placebo group \((\text{placebo} = 2.5 \pm 6.7\%, \text{bovine CPC} = 29 \pm 20\%);\) however, this was not significant \((P > 0.05)\).

Illness log. Of the cyclists that recorded an illness, the only symptoms reported were those that have previously been associated with upper respiratory illness (4). The difference between groups in self-reported illness approached significance \((8 \text{ placebo vs. 3 bovine CPC incidences}; P = 0.055)\). Duration of each illness was not different between groups (bovine CPC = 6.6 days and placebo = 4.5 days; \(P > 0.05\)). In the placebo group, each illness was reported as starting in week 4 (2 reports), week 7 (1 report), weeks 8 and 9 (2 reports each), and week 10 (1 report). In the bovine CPC group, the three incidences of illness were reported in weeks 6, 7, and 11.
Although there is scant evidence of compounds actually enhancing immune function in healthy populations, nutritional interventions may benefit particular individuals and populations during periods of immune suppression (16). Changes in the immune response to exercise, particularly during periods of heavy training, may better highlight immune disturbances and the influence of particular nutritional intervention strategies to prevent and/or reduce immune suppression (30). Research on the potential for bovine colostrum to enhance the human immune system is limited, and this is the first investigation to examine the influence of bovine CPC supplementation on immune parameters in highly trained cyclists participating in a period of HIT. Low-dose bovine CPC supplementation enhanced serum concentrations of sTNFr1 at the end of the HIT period and prevented the postexercise suppression of cytotoxic/suppressor T cell counts (CD3⁺8⁺) at the end of the HIT period and the week after compared with the placebo group. There was also a significant postexercise decrease in serum IgG2 concentration at the end of the HIT period in the placebo group, which was prevented with bovine CPC supplementation. These alterations in immune markers following bovine CPC supplementation may combine to offer additional protection against upper respiratory illness symptoms.

Serum sTNFr1 concentration was elevated after 8 wk of bovine CPC supplementation; however, there was no conclusive change in serum cytokine concentrations toward either a cell-mediated or humoral cytokine profile. Bovine colostrum administration in mice at a dose of 35 mg/day for 6 mo has been shown to enhance the secretion of cell-mediated cytokines by intestinal epithelial cells (49). Although circulating cytokine concentrations of IFN-γ, TNF-α, IL-6, IL-10, and IL-12p40 were similar between groups in the present study, it is possible that the cytokine profile of intestinal cells was altered; however, only circulating cytokine concentrations were measured in the present investigation.

The activity of TNF-α signaling events is primarily mediated by the cytokine inhibitor sTNFr1, and an increase in serum sTNFr1 generally exceeds or parallels TNF-α production (47). Preexercise sTNFr1 concentrations were elevated in the bovine CPC group from the commencement of supplementation to the end of the HIT period, whereas serum TNF-α concentration remained unchanged in both groups. sTNFr1 may inhibit the increase of TNF-α or prolong its half-life (1). The maintenance of resting serum sTNFr1 in the bovine CPC group may represent an important poststress response to restrict TNF-α-mediated inflammatory responses and apoptosis and may also reflect an increase in receptor shedding induced by TNF-α (45). Human colostrum contains sTNFr1, which has been shown to reduce the activity of TNF-α in vitro (7), suggesting that the increase in sTNFr1 may represent an anti-inflammatory effect.

Although there were no significant differences between groups for serum IL-12p40 concentration, it is interesting to note that this is the first investigation to demonstrate a reduction in serum IL-12p40 with consecutive days of HIT. Serum IL-12p40 concentration was increased postexercise during normal training and during the week following the HIT period. However, after 5 days of HIT, there was a decrease in postexercise serum IL-12p40. It is possible that the extent of the postexercise increase in IL-12p40 may reflect changes in training load and/or fatigue. The postexercise increase in IL-12p40 in the week after the HIT period was moderately greater in the bovine CPC group (29%) than in the placebo group (2.5%) and may represent enhanced recovery from cumulative days of HIT, although changes in IL-12p40 in response to periods of HIT requires further investigation.

At the end of the HIT period and the following week, bovine colostrum supplementation prevented a postexercise decrease in cytotoxic/suppressor T cells, compared with the placebo group. Exercise at an intensity of 70% VO2 max in untrained men resulted in no increase of cytotoxic/suppressor T lymphocytes (24), although Ricken et al. (36) showed an increase in cytotoxic/suppressor cells after 1 h of exercise at 63% VO2 max in active men. Cytotoxic/suppressor T lymphocytes play an important role in cell-mediated immunity against pathogens.
and have been shown to increase at rest 2 wk after an overload period of training (21). Resting cytotoxic/suppressor T lymphocytes remained unchanged in both groups the week follow-
ing the HIT period; however, the greater postexercise increase in the bovine CPC group after exercise may offer enhanced protection from pathogens during the recovery or “open window” period. Cytotoxic/suppressor T cells have been reported to secrete a Th1 cytokine profile (5), which has been associated with the downregulation of the binding site for rhinovirus (37), and may have contributed to the trend for a reduction in reported illness in the bovine CPC group.

Along with cytotoxic/suppressor T lymphocytes, NK cells also play an important role in cell-mediated immunity. Intense exercise has been associated with an increase in NK cell count immediately postexercise, which then decreases to below pre-exercise counts in the subsequent hours (33). Although NK cell counts were significantly elevated immediately postexercise, there were no differences between groups. It is possible that bovine CPC reduced the extent of postexercise depression in NK cell number. However, because we did not investigate the time course of recovery to preexercise NK cell count, this remains speculative. In combination with decreased NK cell counts, periods of intense training are also associated with suppression of NK cell cytotoxicity (43). In the present study, NK cell cytotoxicity was not significantly influenced by bovine CPC supplementation or the HIT period. It is possible that the HIT period was not sufficient to induce a suppression in NK cell cytotoxicity, despite the placebo group showing a decrease in exercise performance at the end of the HIT period and a decrease in ventilatory threshold in the days following, indi-
cating fatigue from HIT (38).

Immunoglobulins play an important role in antigen binding and elimination or inactivation of antigen through interaction with complement or specific receptors (29). Gastrointestinal uptake of Colostral immunoglobulins plays a role in the passive transfer of immunity to newborn calves, whereas immunoglobulins at the mucosal surface are an important first line of defense against invading pathogens (23). Bovine IgG1 comprises 70–75% of Colostral immunoglobulins, followed by IgM, IgA, and IgG2 (23). To date, only one investigation by Mero et al. (31) has measured serum IgA and total IgG over a 2-wk period of Colostrum supplementation, and the authors reported no change in immunoglobulin concentration (31). However, the duration of Colostrum supplementation was 2 wk, compared with 8 wk in the present study (31). In the present study, bovine CPC prevented a postexercise decrease in serum IgG2 concentration during the acute HIT period.

Decreased IgG2 concentration is sometimes associated with an increased risk of bacterial infection (29), and IgG2 concen-
trations are positively correlated with the ability to produce antibodies in response to polysaccharide-coated antigens (40). Although resting (preexercise) IgG2 concentrations were increased from baseline across the experimental period in both groups, postexercise IgG2 was significantly decreased for the placebo group at the end of the HIT period. A study in which cyclists were investigated over a 3-mo training period that involved intensive exercise reported significantly reduced serum IgG2 concentrations at 4 and 12 wk (15). This decrease was prevented in cyclists supplementing with thymomodulin (an immunostimulatory compound) (15). McKune et al. (29) also reported a significant decrease in serum concentration of IgG2 in runners completing 60 min of downhill running, 6 days after completing an earlier bout of downhill running. A decrease in IgG2 concentration may represent a cumulative effect of consecutive days of HIT. It is important to note that, although postexercise concentrations were significantly decreased in the placebo group after the HIT period, postexercise concentrations across the experimental period were similar to baseline postexercise values. The clinical significance of postexercise changes in IgG2 concentrations within normal ranges remains to be determined; however, changes in immuno-
globulin may provide insight into modulation of the humoral immune system (11).

Increased neutrophil phagocytosis and oxidative burst ca-
pacity of bovine polymorphonuclear leukocytes may also con-
tribute to enhanced immunity. Furthermore, these immune functions may represent two ways in which Colostrum can enhance immunity (25, 41). In the present study, there was no influence of bovine CPC supplementation on neutrophil surface markers CD11b, CD35, and CD18. Preexercise CD89 was significantly different between groups after 5 wk of bovine CPC supplementation; however, there were no significant differences at the end of the HIT period or the following week. The surface receptor CD89 on neutrophils binds the Fc portion of IgA to induce phagocytosis, superoxide generation, and cytokine release (19), and elevations in CD89 may represent enhanced microbicidal activity of neutrophils. Although CD89 surface marker expression was significantly increased postex-
ercise at baseline in the placebo group, there were no signific-
antly postexercise increases across the supplementation period. Furthermore, postexercise CD89 expression remained unchanged at baseline in the bovine CPC group; however, there was a significant postexercise increase in CD89 at the end of the supplementation period. Although significantly different across the experimental period, changes in CD89 surface marker expression did not follow a specific pattern of change; therefore, the effects of bovine CPC on CD89 remain unclear.

In the present study, the incidence of Upper respiratory illness in the bovine CPC group tended to be lower than that in the placebo group (21% in the Colostrum group vs. 47% in the placebo group), although the duration of illness was similar in both groups. The trend (P = 0.055) toward a decreased incidence of reported upper respiratory illness in the present study is consistent with the work of Brinkworth and Buckley (6) who recently investigated the relationship between bovine Colostrum supplementation and the incidence of upper respira-
atory tract infection. Retrospective self-reported data from daily illness log books were collected from several studies involving resistance training or endurance training interven-
tions in which subjects consumed 60 g/day of bovine Colostrum (n = 93) or a placebo (n = 81) over an 8-wk period. Although data from individual studies were not presented, the combined results suggest that the percentage of subjects who experienced an upper respiratory tract infection was greater in the placebo group than in the bovine Colostrum-supplemented group (32% and 48%, respectively; P = 0.03). For those individuals who experienced an upper respiratory tract infection, the duration was not significantly different between groups. Although it was not measured, the authors speculated that an increase in sali-
vary IgA may have been the primary mechanism responsible for the decreased incidence of upper respiratory tract infection associated with bovine Colostrum supplementation (6). This
would be consistent with the findings of Crooks et al. (9) who reported an increase in IgA concentration in athletes who used 60 g/day bovine colostrum supplementation over a 12-wk period.

In contrast to previous research (9, 31), there was no increase in resting IgA concentration with bovine CPC supplementation in the present study. Although an increase in salivary IgA may be responsible for the reduced upper respiratory tract infection symptoms reported in previous investigations, additional alterations in immune function similar to those changes observed in the present study may also have been responsible. The smaller dose of bovine CPC in the present study (10 g/day) may not have been sufficient to increase salivary IgA concentration. It is possible that other combined mechanisms may provide protection from upper respiratory tract infection, such as increases in cytotoxic/suppressor T cells and maintenance of serum IgG2 concentrations.

Saliva flow rate was significantly different across the experimental period between groups, while there was a trend for changes in IgA secretion rate, with mean saliva flow rate and IgA secretion rate greater in the bovine CPC group at the end of the experimental period. A study by Fahman and Engels (13) established a significant relationship between IgA secretion rate and the incidence of upper respiratory tract infection in American football players over a 12-mo period. Previous studies investigating a relationship between salivary IgA and upper respiratory tract infection have primarily expressed IgA either as an absolute concentration or relative to the protein content of saliva (26). IgA secretion rate may have a closer relationship with the risk of upper respiratory tract infection, as it represents the amount of IgA available at the mucosal surface and, in turn, may be a better predictor of illness during periods of heavy training (13). Bovine colostrum supplementation did not alter IgA concentration in saliva; however, there was a trend for an increase in IgA secretion rate that may be related to the reduced upper respiratory illness symptom reported in the bovine CPC group.

The specific mechanisms responsible for the observed changes in immune parameters remain at present, largely speculative; however, the present investigation shows that bovine CPC supplementation does influence immune parameters of highly trained cyclists, in particular during a HIT period. It is more than likely that colostrum works via a combination of mechanisms as do many components of colostrum such as α1-acid glycoprotein, which both modulates inflammation and binds plasma proteins (48). Bovine colostrum influences the cytokine profile of intestinal epithelial cells in mice (49) and reduces gastrointestinal damage induced by nonsteroidal anti-inflammatory drugs (35). To determine the extent of the influence of bovine CPC on immune function, it will be necessary to investigate compartments other than peripheral circulation, and our group is currently investigating this.

In summary, this is the first investigation to show that low-dose bovine CPC supplementation significantly modulates serum sTNFRI concentrations at the end of a HIT period. The postexercise increase in cytotoxic/suppressor T cell counts following bovine CPC supplementation may offer additional protection against pathogens via an increase in cell-mediated immunity. In combination with the prevention of a postexercise decrease serum IgG2 concentration, the increase in cytotoxic/suppressor T cell counts may be responsible for the trend toward reduced upper respiratory symptoms in the bovine CPC group.

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