Bovine Colostrum Supplementation’s Lack of Effect on Immune Variables During Short-Term Intense Exercise in Well-Trained Athletes

Arnoud Carol, Renger F. Witkamp, Harry J. Wichers, and Marco Mensink

The purpose of this study was to investigate the potential of bovine colostrum to attenuate postexercise decline in immune function. The authors evaluated the time course of a number of immune variables after short-term intense exercise in 9 male athletes after 10 d of supplementation with either colostrum or skim-milk powder. To increase the stress on the immune system subjects performed a glycogen-depletion trial the evening before the endurance trial (90 min at 50% \( W_{\text{max}} \)). Blood samples were taken before the glycogen-depletion trial, before and after the endurance trial, and the next morning, ~22 hr after cessation of the exercise. Plasma cortisol levels increased over time, reaching the highest level directly after exercise, and were still elevated ~22 hr after exercise compared with baseline values (\( p < .001 \)). Neutrophil cell count was increased after exercise and dropped below starting values 22 hr after exercise (time effect \( p < .001 \)). Circulating immunoglobulins did not change over time. A significant time effect was seen for interleukin (IL)-6, IL-10, IL-1-receptor agonist, and C-reactive protein, with levels being higher directly after exercise (\( p < .05 \)). Other cytokines (interferon-\( \gamma \), IL-1\( \alpha \), IL-8, tumor necrosis factor-\( \alpha \)) did not show a time effect. No differences were seen between colostrum and skim-milk powder in any of the investigated variables. Our results are consistent with the hypothesis that intense exercise affects several variables of the immune system. Colostrum did not alter any of the postexercise immune variables compared with skim-milk powder, suggesting no role for bovine colostrum supplementation in preventing postexercise immune suppression after short-term intense exercise.

Keywords: immune function, URTI, URTI risk

Strenuous exercise can temporarily impair immune function (Gleeson, 2007; Nieman, 1997a). This effect is most pronounced with prolonged (>1.5 hr) continuous exercise of moderate to high intensity performed in a fasted state (Gleeson, 2007). Typical features of an exercise-induced response of the immune system are an increase in circulating leukocytes, mainly neutrophils, and an increase in plasma concentration of various substances that influence leukocyte function, including inflammatory cytokines. The latter category includes tumor necrosis factor (TNF)-\( \alpha \), macrophage inflammatory protein-1 and interleukin (IL)-1\( \beta \), and anti-inflammatory cytokines such as IL-6, IL-10, and IL-1-receptor antagonist (IL-1ra; Gleeson, 2007). In addition, the number of natural killer cells is reduced after exercise and their cytotoxic activity is depressed (Shephard & Shek, 1999), and salivary immunoglobulin A (s-IgA) secretion rate, a marker of mucosal immunity, is decreased (Bishop & Gleeson, 2009). Taken together, strenuous exercise generally affects compartments of both the innate and the adaptive immune system, creating a so-called open window of ~3–24 hr after exercise during which athletes are vulnerable to infections, especially upper respiratory tract infections (URTIs; Nieman, 2003). Indeed, several reports suggest a higher incidence of infectious episodes during periods of heavy training and 1–2 weeks after strenuous exercise such as a race event (Nieman, 1997b).

Various nutritional strategies and supplements have been investigated for their potential to enhance immune function and decrease the prevalence of URTI among athletes (Akerstrom & Pedersen, 2007; Gleeson, 2006; Nieman, 2008). One of the proposed nutritional supplements is colostrum, the first milk made by mammals after parturition. Bovine colostrum is available as a food supplement and not only is a source of carbohydrates, protein, fat, and minerals but also contains high concentrations of growth and immune factors such as insulin-like growth factors I and II, immunoglobulins, lactoperoxidase, lactoferrin, and several cytokines (Kelly, 2003; Stelwagen, Carpenter, Haigh, Hodgkinson, & Wheeler, 2009). Bovine colostrum preparations are being considered for prevention or treatment support of infections and inflammatory diseases in children and adults. The main focus is on disorders of the gastrointestinal tract (Kelly, 2003; Solomons, 2002), although some studies suggest other health benefits including the enhancement of specific immune responses after vaccination (He, Tuomola, Arvilommi, & Salminen, 2001).

Bovine colostrum is of interest to athletes because it might enhance resistance to the development of URTI.
reported symptoms. In healthy, physically active males, 8 weeks of supplementation resulted in significantly fewer self-reported symptoms of URTI for up to 7 weeks after cessation of the intervention, compared with skim-milk powder (Brinkworth & Buckley, 2003). In addition, several studies reported increased resting s-IgA levels in athletes after colostrum supplementation (Crooks, Wall, Cross, & Rutherford-Markwick, 2006; Mero et al., 2002).

However, results on the potential of bovine colostrum in sport nutrition are far from clear. Moreover, there is only limited knowledge on the exercise-induced alterations in immune variables. Several studies evaluated mucosal immunity by assessing s-IgA levels or secretion rates. In contrast to the resting situation, changes in s-IgA concentration (Mero et al., 1997; Shing et al., 2007) and secretion rate (Shing et al., 2007) during exercise were not affected significantly by colostrum. So far, only one study has investigated the effect of colostrum supplementation on immune functions during and after a period of exercise stress in more detail (Shing et al., 2007). Eight weeks of colostrum supplementation prevented the postexercise decrease in IgG2 and cytotoxic/suppressor T-cell count and elevated the concentration of serum-soluble TNF receptor 1 (Shing et al., 2007). It was concluded that these alterations might be responsible for the trend toward a reduction of URTI symptoms in the bovine colostrum group that was noticed during that study (Shing et al., 2007). However, this effect was seen after a period of prolonged stress—5 consecutive days of high-intensity training. It is important to know whether this effect can also be observed after a shorter period of stress because even a single bout of continuous, prolonged exercise of moderate to high intensity evokes a suppression of the immune system (Gleeson, 2007).

Therefore, the aim of the current study was to investigate the effect of bovine colostrum supplementation on several immune variables after short-term intense exercise in well-trained athletes. To increase the stress on the immune system, the exercise was performed in a glycogen-depleted state. Moreover, the period of supplementation was relatively short (10 days), because earlier work suggested that already after such a short time frame colostrum could affect the immune response in healthy volunteers (He et al., 2001) and improve resting immune variables in athletes (Mero et al., 2002). In the current study, immune function was assessed by measuring plasma responses of neutrophils and lymphocytes, serum immunoglobulins, and several cytokines.

**Methods**

**Subjects**

Ten well-trained male athletes volunteered to participate in this study, of whom 9 completed the protocol (see Table 1). To be included athletes had to have at least 2 years of cycling experience and a training frequency of at least three times a week during at least 9 months a year. Reported training frequency ranged from three to seven times per week; training volume ranged from 5 to 12 hr/week. Training consisted of not only cycling but also other—endurance-type—sport activities such as speed skating and running.

Subjects had no known allergy to cow’s milk or a known immune disease, and they had not shown signs of infection during the month preceding the study. They also did not receive treatment for any medical condition, use any drugs, or consume more than two alcoholic beverages per day. The participants had to refrain from using dietary supplements (including colostrum) from 1 month before Day 1 of the first study period until the end of the study—Day 19 of the second period.

Before entering the study all participants completed a medical questionnaire and gave their written, informed consent. The study protocol was approved by the Medical Ethical Review Committee of Wageningen University (METC-WU 08/15; Oct 31, 2008).

**Study Design**

The study had a crossover design and was double-blind and placebo controlled. Total duration of the study was 2 × 19 days, with a washout period of ~2.5 weeks. Half the subjects were randomly assigned to start with colostrum supplementation and the other half with placebo. Figure 1 gives a schematic overview of the study.

During the first 7 days (presupplementation period) subjects kept a log about dietary habits, training performance, and illness symptoms. Logs were kept until the end of the experimental period.

On Day 8 subjects started using the supplement or placebo for 10 days. At the end of this supplementation period the first series of tests was scheduled (Days 17–19). The experimental protocol consisted of two exercise parts—a glycogen-depletion trial (Day 17) and an endurance trial (Day 18), to evoke a substantial amount of stress on the immune system—and a final blood sample on the morning of Day 19 (see Experimental Protocol for more detail).

After this first period a washout of ~2.5 weeks followed, during which the subjects maintained their normal training workload and dietary habits. After the washout period the whole 19-day sequence of presupplementation, supplementation, and experimental period was repeated, but subjects who started with colostrum now used placebo and vice versa. During this second period, subjects

<table>
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<th>Characteristic</th>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Weight (kg)</td>
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<td>Height (cm)</td>
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<td>BMI (kg/m²)</td>
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<tr>
<td>Maximal power output (W)</td>
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</tr>
</tbody>
</table>

**Table 1 Subject Characteristics, M ± SD, N = 9**
used their dietary and training log of the first period as a guideline to keep their intake and training regimen approximately the same.

**Intervention**

Skimmed and freeze-dried 12-hr colostrum from Belgian cows, with a high IgG content (~65 g/L), was used as the intervention (Colostrum Therapie, Arts Food Products, ’s-Hertogenbosch, the Netherlands). As placebo, skim-milk powder was used (Elk, Campina, The Netherlands).

Both colostrum and placebo were supplied in sealed sachets that did not identify the product inside. Subjects and personnel involved in the experiments were not aware of coding of the supplements. The period of supplementation was 10 days, and subjects consumed 12.5 g twice a day mixed in a glass of cold milk or cold buttermilk. They were not allowed to ingest the supplement together with hot food or beverages. The last dose was taken ~2 hr before the start of the exercise tests.

**Pretest**

Before the start of study, subjects performed a graded exercise test to exhaustion to define their maximal aerobic power (\(W_{\text{max}}\)). This maximal-power test was performed on an electronically braked cycle ergometer (Heinz Kettler MX1, Ense-Parsit, Germany) modified with a race saddle and pedals. The positions of the saddle and the handlebars were adjusted to each subject’s own preference. After a short warm-up of 5 min at 100 W the workload was increased every 2 min according to the following protocol: 150–200–225–250–275–300–320–340–360 W and so on until exhaustion. Exhaustion was defined as the point at which subjects were no longer able to keep the pedal frequency above 50 rpm. \(W_{\text{max}}\) was calculated according to the following formula: last completed workload + [(time at final workload/2 min) \times (difference between last completed and final workload)]. \(W_{\text{max}}\) was used to calculate the power output during the exercise parts of the experimental period.

**Experimental Protocol**

The experimental protocol covered 3 days. In the evening on Day 1 a glycogen-depletion test took place, followed by an endurance test the next morning (Day 2). On Day 3 a final blood sample was collected (see Figure 1[b]).

**Glycogen-Depletion Trial.** The glycogen-depletion trial took place in the evening (~7.30 p.m.). Subjects had their last meal of that day at least 2 hr before the start of the test and were asked not to perform any other exercise during that day.

Just before the test, blood was collected (\(T_0\)). After a warm-up of 5 min at 100 W the subjects started with 2-min blocks of exercise alternating between 90% and
50% $W_{\text{max}}$. When the blocks of 90% $W_{\text{max}}$ could no longer be maintained the intensity was reduced to 2-min blocks of 80% $W_{\text{max}}$ alternating with 50%. When 80% $W_{\text{max}}$ could not be maintained, exercise alternating between 70% and 50% $W_{\text{max}}$ was performed until exhaustion, that is, when subjects were unable to continue cycling at 70% $W_{\text{max}}$. This protocol has been shown to substantially reduce muscle glycogen stores (Kuipers, Saris, Brouns, Keizer, & ten Bosch, 1989). After termination, subjects ate a light snack (two crackers with cheese: 881 kJ, 22 g carbohydrates, 9.5 g fat, and 9.4 g protein) before returning home for the night. They were asked to fast until they reported back to the laboratory the next morning. During and after the test they were allowed to drink water ad libitum.

**Endurance Trial.** Subjects reported back to the laboratory the next morning in a fasted state to perform a 1.5-hr cycling test at 50% $W_{\text{max}}$. Although this is a slightly lower intensity than recommended in the literature (Gleeson, 2007), when performed with reduced glycogen stores the exercise was expected to evoke sufficient stress to suppress the immune system. Before the start of the cycling test another blood sample was collected ($T_1$), after which subjects again ate a snack (two crackers with cheese). Water was available ad libitum throughout the test.

On completion of the 1.5 hr of cycling, a third blood sample was collected ($T_2$), and breakfast was provided. Participants were instructed to maintain their normal daily pattern for the rest of that day, without eating extraordinary amounts, and to refrain from exercise. On the morning of Day 3 subjects reported back, after an overnight fast, for a final blood collection ($T_3$).

**Dietary, Training, and Illness Logs**

All logs were kept during both 19-day study periods, during the presupplementation period, supplementation period, and the days of the experimental protocol.

**Dietary Log.** During the first period, subjects had to indicate briefly what they ate and drank during the day. This log was used in the second period as a reference. During both periods subjects had, on 2 separate days, to note down in detail their dietary intake. These data were used to calculate their exact energy and macronutrient intake, using a computer program (Komeet, version 4.0.58, BaS Nutrition Software, Arnhem, The Netherlands).

**Training Log.** For every day, training sessions had to be written down. Subjects were asked to keep the amount and intensity of the training comparable throughout the whole experiment. The log of the last few days before the start of the experimental period was reviewed to see if there was a difference in training intensity. If that was the case we checked whether this had any influence on the number of completed steps of the glycogen-depletion test.

**Illness Log.** The subjects daily recorded the presence of illness symptoms. The symptoms were ranked as no symptoms today; cold symptoms (runny nose, sore throat, coughing, sneezing, colored discharge); flu symptoms (fever, headache, general aches and pains, fatigue and weakness, chest discomfort, cough); nausea, vomiting, or diarrhea; muscle, joint, or bone problems/injury; allergy (itchy eyes, stuffy nose, clear discharge); or other health problems. With this ranking system an upper respiratory illness (URI) is defined as having occurred when symptoms of flu or a cold were reported for 2 or more consecutive days (Brinkworth & Buckley, 2003). An episode was considered a new URI if it was separated from a previous URI by at least 3 days without any respiratory symptoms.

**Blood Collection and Analysis**

Blood was collected four times during the experimental period: 5 min before the start of the glycogen-depletion trial ($T_0$), before the start of the endurance trial ($T_1$), directly postexercise ($T_2$), and the next morning, ~22 hr after cycling ($T_3$).

Blood was sampled by venipuncture from an antecubital vein and collected in several distinct tubes (Vacutainer, Becton Dickinson, Breda, The Netherlands): EDTA-containing tubes for cell count and cytokines; sodium-fluoride-containing tubes for lactate and glucose; heparinized tubes for IgA, IgG, and IgM; and tubes with a serum separator for cortisol and IgE (Becton Dickinson). Except for cell count, all samples were centrifuged for 10 min at 3,000 rpm (1,020 g) at either 20 °C (immunoglobulins, cortisol) or 4 °C (glucose, lactate, and cytokines). After centrifugation, serum (cortisol and IgE) or plasma (other analyses) was collected and aliquotted into cryo-tubes and stored at −80 °C until further analysis.

Whole-blood cell count of lymphocytes and neutrophils was measured with an automated cell analyzer (Sysmex XE-2100, TOA Medical Electronics, Kobe, Japan). Plasma IgA, IgG, and IgM were measured with an immunoturbidimetric assay on a Cobas c system (IGA-2, IGG-2, and IGM-2 kits, Roche Diagnostics GmbH, Mannheim, Germany). Total IgE was measured with a solid-phase chemiluminescent immunometric assay (Immulite 2000 Total IgE, Siemens, Los Angeles, CA). Serum cortisol level was measured using a solid-phase competitive chemiluminescent enzyme immunoassay (Immulite 2500 Cortisol, Siemens). Plasma glucose and lactate were measured only at $T_1$ and $T_2$, using standard enzymatic techniques on a Cobas c system (GLUC3 glucose HK kit and LACT2 lactate Gen.2 kit, Roche Diagnostics GmbH). All assays were performed according to the manufacturer’s instructions.

A quantitative multiplexed immunoassay panel (humanMAP v 1.6 antigens, Rules Based Medicine, Austin, TX) was used to measure a wide array of parameters at time points $T_0$ and $T_2$. This panel included interferon gamma (IFN-γ), IL-1α, IL-1ra, IL-6, IL-8, IL-10, TNF-α, and C-reactive protein (CRP).

For some of the cytokines several samples could not be quantified because the concentration did not reach the lower detection limit. This was the case for IFN-γ, IL-1α, TNF-α, and IL-6. For IL-1α, 1 subject had values below
the detection limit at all four time points; this subject was not considered in the statistical analysis. For IFN-γ, TNF-α, and IL-6 the lowest measurable concentration was assigned to these missing observations to enable statistical analysis with all 9 subjects.

Statistical Analysis
Statistical analysis was performed with SPSS version 15.0 for Windows (SPSS, Chicago, IL). Data are presented as \( M \pm SD \). Before statistical analysis, variables were tested for normal distribution using a Q–Q plot. All variables appeared to be normally distributed. To determine whether the intakes of energy, carbohydrates, total fat, and total protein were equal in both supplementation periods, paired \( t \) tests were performed. For all other measured parameters, a linear mixed-models procedure was performed to test for time, group (condition), and interaction effects (Time \( \times \) Condition). In case the main model indicated a group (condition) or interaction (Time \( \times \) Condition) effect, paired \( t \) tests were used to compare conditions at each time point. When the main model indicated only a time effect, the linear mixed-model procedure was repeated for each condition separately (colostrum and placebo). Subsequent post hoc comparisons were made by least-significant-difference analysis to locate the time effect. A \( p \) value of <.05 was accepted as statistically significant.

Results

Subjects
One of the subjects became ill just before the second testing period, and we were unable to schedule an alternative testing period. Therefore, the total number of participants for analysis was 9 (see Table 1 for characteristics). Total energy expended and the number of bouts completed at 90% \( W_{\text{max}} \) during the glycogen-depletion trial were not different between conditions, although both tended to be decreased after colostrum supplementation (energy expenditure: 927 ± 153 vs. 1,044 ± 244 kJ, \( p = .09 \); bouts: 11.9 ± 2.5 vs. 13.7 ± 4.2, \( p = .09 \) for colostrum and placebo, respectively).

Dietary-Intake and Training Logs
Energy intake, measured at 2 days during the run-in period, was higher in the colostrum condition than with placebo (13.8 ± 2.9 and 11.4 ± 2.9 MJ, respectively, \( p < .01 \)). Contribution of carbohydrates, protein, and fat as percent of energy was comparable during colostrum supplementation and placebo.

Participants wrote down their training activity during both intervention periods. In some cases a difference in training intensity was observed during the last days preceding the experimental period, but this difference had no effect on the number of completed bouts of exercise at 90% \( W_{\text{max}} \) during the glycogen-depletion protocol.

Plasma Glucose, Lactate, and Serum Cortisol
Because of analytical problems a few plasma glucose and lactate levels were missing, resulting in \( N = 7 \) for glucose and \( N = 8 \) for lactate. For both glucose and lactate a time effect was revealed in the main model (\( p = .028 \) and \( p < .001 \) for glucose and lactate, respectively). Plasma lactate levels increased between \( T_0 \) and \( T_2 \) in both the colostrum (0.9 ± 0.5 to 1.9 ± 0.6 mmol/L, \( p = .003 \)) and placebo condition (0.9 ± 0.2 to 1.9 ± 0.6 mmol/L, \( p = .003 \)), whereas plasma glucose levels tended to decrease in both conditions (colostrum 4.7 ± 0.4 to 4.2 ± 0.7 mmol/L, \( p = .123 \); placebo 4.9 ± 0.1 to 4.6 ± 0.4 mmol/L, \( p = .063 \)). Plasma glucose levels were lower after colostrum supplementation, but not significantly (main-model group effect \( p = .088 \)), and lactate levels were comparable.

Changes in serum cortisol levels are depicted in Figure 2. Cortisol increased significantly over time (\( p < .001 \)), with the highest values directly after exercise (\( T_2 \)). Serum cortisol at \( T_3 \), ~22 hr after cessation of the exercise, was still significantly elevated compared with \( T_0 \) (colostrum 550.0 ± 134.3 vs. 206.8 ± 101.4 nmol/L, \( p < .001 \); placebo 465.7 ± 118.8 vs. 200.6 ± 72.1 nmol/L, \( p < .001 \)). No differences between colostrum supplementation and placebo were seen.

Cell Count
A clear time effect was observed for neutrophils (mixed-model \( p < .001 \); Figure 3[a]). Neutrophils increased after 1.5 hr of cycling in the glycogen-depleted state (\( p = .003 \)) and sharply decreased during recovery (\( p < .001 \)). Neutrophil count was significantly lower at \( T_1 \) than at \( T_0 \) in the colostrum condition (3.91 ± 1.41 vs. 2.50 ± 1.03 10⁶/ml for \( T_0 \) and \( T_1 \), respectively, \( p = .029 \)) and tended to be lower in the placebo condition (3.74 ± 1.32 vs. 2.50 ± 1.31 10⁶/ml, \( p = .064 \)). No differences between colostrum supplementation and placebo were observed. Lymphocyte counts showed changes comparable to those seen for the neutrophils, but effects were less pronounced, not reaching statistical significance (Figure 3[b]).

Immunoglobulins
One of the participants had extremely high values for IgE (an average of 6,056 kU/L), most likely indicating some allergic reaction. On his medical questionnaire he wrote that he had experienced milk allergy in 1993, but he recovered from it and never had any problems with milk products since. These high IgE values, however, could indicate that he still has some sort of reaction to milk products, so we decided to omit this subject’s IgE values from the analysis.

No significant time effect or difference between conditions was found for plasma immunoglobulin levels (Table 2).

CRP and Cytokines
CRP and cytokines were only measured twice during the experimental period: at \( T_0 \) and \( T_2 \) (Table 3). At \( T_2 \),
CRP levels were significantly higher than at T₀ (p = .001; Figure 4[a]) for both the colostrum condition (1.01 ± 0.91 and 1.94 ± 0.91 mg/L at T₀ and T₂, respectively, p = .044) and placebo (0.58 ± 0.23 and 1.43 ± 0.65 mg/L, p = .004). There tended to be a difference between conditions (mixed-model group effect p = .07), with CRP levels being higher after colostrum supplementation on both occasions (Figure 4[a]).

For IFN-γ, TNF-α, and IL-6 some samples did not reach the lower detection limit. For both IFN-γ and TNF-α, a single observation at T₀ was below the detection limit, and for IL-6, there were 10 samples at T₀ below the detection limit of this analysis (five for both colostrum and placebo). After exercise (T₂) all samples were in the measurable range.

For IFN-γ, IL-1α, IL-8, and TNF-α, no effects were found for either time or condition or their interaction, although IL-8 and TNF-α tended to increase over time (p = .070 and p = .055, respectively; Table 3). IL-1ra showed an increase over time (p = .035; Table 3), which was significant in the placebo condition (p = .013) but not after colostrum supplementation (p = .17). No differences between conditions were observed.

IL-6 and IL-10 increased more than twofold from T₀ to T₂ directly after exercise (main-model p < .001; Figure 4), with a comparable magnitude in both conditions (IL-6 placebo 0.75 ± 0.16 to 2.95 ± 1.11 pg/ml, p < .01; colostrum 0.72 ± 0.11 to 2.39 ± 1.00 pg/ml, p < .001; IL-10 placebo 17.9 ± 3.8 to 39.6 ± 11.7 pg/ml, p < .001; colostrum 16.9 ± 2.6–43.5 ± 23.8 pg/ml, p = .010). Although the values of IL-6 at T₀ are affected by our substitution, the fact that after exercise all values were in the measurable range, and the observation that all subjects with complete data showed an increase on exercise, indicated to us that IL-6 increased on exercise. For both IL-6 and IL-10, again no effect of colostrum supplementation compared with placebo was seen: Postexercise (T₂) IL-6 and IL-10 levels were not different between conditions (p = .32 and p = .25, paired t test; Figure 4).

**Discussion**

Strenuous exercise can temporarily impair immune function, putting athletes at increased risk of developing an infection during periods of heavy training or in the period after a race event. To investigate the potential of bovine colostrum to attenuate this decline in immune function we evaluated the time course of a number of immune variables after short-term intense exercise. Our results are consistent with the hypothesis that intense exercise affects several markers of the immune system. Bovine colostrum supplementation, however, did not have any effect on the immune variables investigated.

**Exercise-Induced Immune Suppression**

Prolonged strenuous exercise affects features of both the innate and the adaptive immune response, finally resulting in a transient suppression of the immune system, making athletes vulnerable especially for viral URIs (Gleeson, 2007; Nieman, 1997a). Typically, neutrophil count increases during intense exercise, while their activity is generally suppressed (Gleeson, 2007). Indeed, neutrophil count significantly increased after exercise and dropped to values below those at the start of the experimental period at T₃. Neutrophils have several roles in the nonspecific immune response and eliminate microbes by phagocytosis and intracellular killing. A reduced number of neutrophils can easily contribute to the so-called open-window period postexercise.
Figure 3 — Plasma (a) neutrophil cell count and (b) lymphocyte cell count at different time points during the experimental period for placebo (open circles, gray line) and colostrum conditions (closed squares, black line). Data are $M \pm SD$. A main time effect was revealed for neutrophil cell count ($p < .001$) but not for lymphocyte cell count ($p = .17$). Post hoc analysis: *# for colostrum condition only, for placebo $p = .064$. No group effect was seen.

Lymphocyte counts showed the same changes over time as neutrophils, with changes being less profound. It is known that neutrophil and especially lymphocyte activity are important, considering immune function and the open-window theory, not just cell count. These aspects, however, were not assessed in our study.

The exercise protocol also caused the expected increase in the circulating cytokines IL-1ra, IL-6, and IL-10, together with an increase in CRP. TNF-α and IL-8 increased over time, but this was not statistically significant, and other cytokines (IL-1a, IL-8, and IFN-γ) were not affected. As indicated, a substantial number of the preexercise samples of IL-6 were below the detection limit of the analysis. Replacing missing samples with the lowest measured value seems arbitrary, but the fact that after exercise all values were in the measurable range and the observation that all subjects with complete data showed a substantial increase on exercise (~threefold) clearly supports the conclusion that exercise increased IL-6. Moreover, because of the replacement, an underestimation of the real effect most likely occurred, which further strengthens our conclusion. Considering the effect of colostrum, no difference was seen in the postexercise IL-6 level compared with placebo.

It is obvious that changes in cytokine levels only cannot be easily interpreted in terms of susceptibility
were increased directly after exercise. Serum cortisol levels were still increased at T3, ~22 hr after cessation of exercise. Cytokine levels were not measured at that time point.

The rise in IL-6, IL-10, and IL-1ra, just as the increase of cortisol, can contribute to a suppression of Th1 cells in the circulation after exercise, affecting the Th1–Th2 cell balance (Gleeson, 2007; Lancaster et al., 2004; Lancaster et al., 2005). Th1 cells typically activate cytotoxic T cells that drive the immune system toward cell-mediated immune responses, which provides protection against intracellular pathogens such as viruses. Suppression of Th1 cell production by intense exercise may therefore also play a role in the higher susceptibility to infections seen in athletes.

After exercise the secretion of immunoglobulins can be affected. In contrast to regular exercise, strenuous prolonged exercise is reported to reduce circulating immunoglobulins (Pacque, Booth, Ball, & Dwyer, 2007). With our exercise protocol, we did not observe any changes in serum immunoglobulins (IgA, IgE, IgG, and IgM). In line with our data, earlier studies also did not see changes in serum immunoglobulins with a single bout of exercise (Mero et al., 1997; Shing et al., 2007), except for IgG2 after a week of intense training (Shing et al., 2007). The duration of the exercise could be important for immunoglobulins, because changes in immunoglobulins are reported after ultraendurance races (Pacque et al., 2007) and during periods of intense training (Shing et al., 2007). So, for a single bout of exercise, the duration of the exercise could be too short to detect or allow changes in circulating immunoglobulins, notwithstanding the profound changes in other immune variables. Most research has focused on s-IgA. This is the predominant immunoglobulin in mucosal secretion and is hence important in the first defense against pathogens, and it has been identified as a risk factor for infections in athletes.

### Table 2 Serum Immunoglobulin Levels, M ± SD

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<th>IgG (g/L)</th>
<th>IgA (g/L)</th>
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<td>0.75 ± 0.27</td>
<td>0.74 ± 0.27</td>
<td>0.71 ± 0.22</td>
</tr>
<tr>
<td>placebo</td>
<td>198.7 ± 219.0</td>
<td>187.8 ± 196.8</td>
<td>191.1 ± 204.2</td>
<td>196.0 ± 216.8</td>
</tr>
<tr>
<td>colostrum</td>
<td>200.0 ± 219.4</td>
<td>207.6 ± 241.2</td>
<td>202.5 ± 228.0</td>
<td>188.1 ± 199.1</td>
</tr>
</tbody>
</table>

*Note. N = 9 subjects, except where otherwise indicated.*

### Table 3 Plasma Cytokine Levels, M ± SD

<table>
<thead>
<tr>
<th></th>
<th>IFN-γ (pg/ml)</th>
<th>IL-1α (ng/ml; n = 8)</th>
<th>IL-1ra (pg/ml)</th>
<th>IL-8 (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T2</td>
<td>T0</td>
<td>T2</td>
<td>T0</td>
</tr>
<tr>
<td>placebo</td>
<td>5.26 ± 6.40</td>
<td>4.91 ± 5.55</td>
<td>0.014 ± 0.005</td>
<td>0.13 ± 0.004</td>
<td>15.07 ± 5.89</td>
</tr>
<tr>
<td>colostrum</td>
<td>4.35 ± 5.38</td>
<td>5.19 ± 7.11</td>
<td>0.013 ± 0.004</td>
<td>0.014 ± 0.004</td>
<td>14.41 ± 4.88</td>
</tr>
<tr>
<td>placebo</td>
<td>64.5 ± 19.0</td>
<td>110.6 ± 42.5*</td>
<td>70.9 ± 12.9</td>
<td>135.7 ± 128.9</td>
<td>3.23 ± 2.37</td>
</tr>
<tr>
<td>colostrum</td>
<td>70.9 ± 12.9</td>
<td>135.7 ± 128.9</td>
<td>14.41 ± 4.88</td>
<td>17.81 ± 4.73</td>
<td>3.86 ± 2.52</td>
</tr>
</tbody>
</table>

*Note. N = 9 subjects, except where otherwise indicated.*

*p < .05 compared with T0.*
Our exercise protocol consisted of two parts: a glycogen-depletion trial followed by 1.5 hr of cycling at 50% \( W_{\text{max}} \) the next morning. Although 50% \( W_{\text{max}} \) can be considered a moderate intensity, when it was performed with reduced glycogen stores our results show that it evoked sufficient stress to affect the immune system. Cortisol levels were already slightly increased before the 1.5 hr of cycling, reflecting the stress of the glycogen-depletion trial. Neutrophil count was not affected at that time (T1), but after 1.5 hr of cycling at 50% \( W_{\text{max}} \) under conditions of low glycogen availability, a significant increase in neutrophil count was obtained. This and the other changes seen were highly consistent, indicating that our exercise protocol was strenuous enough to induce an activation of the immune system.

Various nutritional strategies and supplements have been investigated for their potential to enhance immune function, including bovine colostrum (Akerstrom & Pedersen, 2007; Gleeson, 2006; Nieman, 2008). Bovine colostrum contains high concentrations of growth and immune factors such as insulin-like growth factor, immunoglobulins, and several cytokines, and there is some evidence that it enhances specific immune responses after vaccination in healthy volunteers (He et al., 2001). In athletes, it is reported to enhance resistance to the development of symptoms of URI (Brinkworth & Buckley, 2003) and increase resting s-IgA levels (Crooks et al., 2006; Mero et al., 2002).

The results of this study indicate that 10 days of bovine colostrum supplementation had no effect on...
exercise-induced changes in the immune variables investigated compared with placebo, which was skim-milk powder. A small, nonsignificant, increase in CRP level was seen after colostrum supplementation, with the exercise-induced increase in CRP not differing between conditions. Earlier studies considering colostrum supplementation in athletes did not report CRP levels. Although we do not have a clear explanation for this difference, we cannot exclude the possibility that a single subject was largely responsible for the increased CRP level, because he had high levels after colostrum supplementation (~3 mg/L) apparently without a clear explanation (e.g., symptoms of an illness).

Only two earlier studies considered the effect of colostrum in athletes during acute exercise. Mero et al. (1997) showed that colostrum increased circulating insulin-like growth factor. However, similar to our findings they did not observe an effect on serum IgG and hormone levels (including cortisol). Shing et al. (2007) investigated in more detail the effect of colostrum supplementation on immune function. Eight weeks of colostrum supplementation prevented the postexercise decrease in IgG2 and cytotoxic/suppressor T-cell count and elevated the concentration of serum-soluble TNF receptor 1 (Shing et al., 2007). However, these effects were seen during acute exercise (40-km time trial) after a period of prolonged stress—5 consecutive days of high-intensity training. In line with our observations, Shing et al. did not observe any effect of colostrum supplementation compared with placebo after acute exercise during periods of normal training. Thus, no alterations were seen in the exercise response of, for example, IL-1ra, IL-6, IL-10, TNF-α, neutrophil, and lymphocyte count (Shing et al., 2007). From these observations, and from the results of the current study, it can be concluded that supplementation with bovine colostrum does not seem to offer clear benefits compared with placebo in preventing postexercise changes in immune variables after short-term intense exercise during periods of normal physical activity. However, our study does not exclude the possibility that colostrum could have an effect during intense exercise during periods of increased physical stress (e.g., training camp). More research is necessary to confirm this. In addition, of primary interest is not whether changes in immune function can be prevented but whether the risk of picking up an infection can be reduced. Earlier studies did report a reduction in URI prevalence (Brinkworth & Buckley, 2003; Shing et al., 2007). A daily dose of ~10–15 g/day is generally used in clinical conditions (Kelly, 2003), whereas in exercise research 20–60 g is used (Brinkworth & Buckley, 2003; Crooks et al., 2006; Kelly, 2003; Mero et al., 2002; Mero et al., 1997; Shing et al., 2007). The composition of the colostrum can also differ considerably depending on the time of collection and treatment (Kelly, 2003). We supplemented athletes for 10 days. In earlier studies with athletes, this period ranged from 8 days to 12 weeks (Brinkworth & Buckley, 2003; Crooks et al., 2006; Mero et al., 2002; Mero et al., 1997; Shing et al., 2007). Changes in s-IgA, the most consistent finding, were already observed after 12 days (Mero et al., 2002). On the other hand after 8 weeks still no effect was seen on many cytokines, including IL-1ra, IL-6, IL-10, CRP, and cell count (Shing et al., 2007). In addition, the potential of bovine colostrum to enhance the immune response to a bacterial pathogen was observed after only 7 days of supplementation (He et al., 2001). Therefore, although the supplementation protocol could have played a role, the lack of effect on any of the investigated parameters does not support the use of bovine colostrum to positively affect immune function during short-term intense exercise.

In conclusion, the current study confirms the impact of strenuous exercise on the investigated immune variables. The exercise protocol used in the current study is a practical and reproducible procedure to induce immune stress. Even a single bout of moderate-intensity exercise, combined with a glycogen-depletion trial, induces significant changes in several immune variables including IL-1ra, IL-6, IL-10, CRP, and neutrophil cell count. After an initial increase, neutrophils were still reduced ~22 hr after exercise. Ten days of colostrum supplementation did not alter any of the investigated postexercise immune variables compared with skim-milk powder, suggesting no role for bovine colostrum supplementation in preventing postexercise immune suppression after short-term intense exercise.

Acknowledgments

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References

Bovine Colostrum and Postexercise Immune Response


