Effects of bovine colostrum supplementation on serum IGF-I, IgG, hormone, and saliva IgA during training

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Mero, Antti, Heidi Mikkulainen, J Armo Riski, Raimo Pakkanen, Jouni Aalto, and Timo Takala. Effects of bovine colostrum supplementation on serum IGF-I, IgG, hormone, and saliva IgA during training. J. Appl. Physiol. 83(4): 1144–1151, 1997.—The purpose of this study was to examine the effects of bovine colostrum supplementation (Bioenervi) on serum insulin-like growth factor I (IGF-I), immunoglobulin G, hormone, and amino acid and saliva immunoglobulin A concentrations during a strength and speed training period. Nine male sprinters and jumpers underwent three randomized experimental training treatments of 8 days separated by 13 days. The only difference in the treatments was the drink of 125 ml consumed per day. Posttraining increases were noticed for serum IGF-I in the 25-ml Bioenervi treatment (125 ml contained 25 ml Bioenervi) and especially in the 25-ml Bioenervi treatment (125 ml contained 125 ml Bioenervi) compared with the placebo (normal milk whey) treatment (P < 0.05). The change in IGF-I concentration during the 8-day periods correlated positively with the change in insulin concentration during the same periods with 25-ml Bioenervi treatment (r = 0.68; P = 0.045) and with 125-ml Bioenervi treatment (r = 0.69; P = 0.038). Serum immunoglobulin G, hormone, and amino acid and saliva immunoglobulin A responses were similar during the three treatments. It appears that a bovine colostrum supplement (Bioenervi) may increase serum IGF-I concentration in athletes during strength and speed training. Insulin-like growth factor I, immunoglobulin G, and immunoglobulin A concentrations.

Bovine colostrum is a milk secreted during the first few days after calving, and its importance for the health of calves has been known for a long time (23). Colostrum contains not only nutrients like proteins, carbohydrates, fat, vitamins, and minerals but also bioactive components like growth factors and antimicrobial factors (12, 33). The most abundant and well-characterized growth factors in bovine colostrum are probably insulin-like growth factors I and II (IGF-I and IGF-II, respectively) (13). They stimulate cell growth and are proposed to act both as endocrine hormones via the blood and as paracrine and autocrine growth factors locally (12, 19). IGF-I is a major form in bovine colostrum and is biologically more potent than IGF-II (13). The concentration of IGF-I in bovine colostrum is 200–2,000 µg/l (36), whereas normal milk contains <10 µg/l (9). In normal adult humans, IGF-I occurs at a concentration of ~200 µg/l in serum (19). IGF-I has a strong anabolic effect on muscle tissue (25, 37), and it is associated with regulatory feedback of growth hormone (25, 34). IGF-I can mimic most, but probably not all, effects of growth hormone (10). The effects of growth hormone on skeletal muscle are thought to be mediated by IGF-I (21).

Consequently, this raises a very interesting question as to whether it would be possible to increase IGF-I concentration in human blood and muscle by drinking colostrum or colostrum supplements. If this occurs, it may have positive effects on human tissues, for example, during strenuous training. This hypothesis is supported by the finding that dietary cow colostrum has been shown to increase blood IGF-I concentration in calves (16, 35). In addition, orally administered 125µl-labeled IGF-I has been demonstrated to be transported into circulation in calves (4). Dietary bovine colostrum may also exert local effects in the gut of human subjects, because it has been shown to promote the growth of small intestine of newborn piglets (38). The increased growth and turnover of intestine may increase uptake of dietary components like amino acids.

Another important group of bioactive components in bovine colostrum is composed of antimicrobial factors, including immunoglobulins, lactoperoxidase, lysozyme, and lactoferrin. Bovine colostrum is an extremely rich source of immunoglobulins. The concentration of immunoglobulin G (IgG) 1 (52–87 g/l), IgG2 (1.6–2.1 g/l), immunoglobulin M (3.7–6.1 g/l), and immunoglobulin A (IgA; 3.2–6.2 g/l) in bovine colostrum is ~100-fold higher than in normal milk (30).

The primary purpose of the present study was to examine the effect of bovine colostrum supplementation (Bioenervi, which is a colostrum whey product sold in some European countries but is not approved for sale in the United States. Bioenervi is not on the banned drug list of the International Olympic Committee) on serum concentration of IGF-I in athletes. A further purpose was to investigate whether there are any other changes in physiological responses during a short-term strength and speed training period when a bovine colostrum supplement that contains not only proteins, carbohydrates, vitamins, and minerals but also IGF-I and immunoglobulins is taken.

METHODS

Subjects. Nine male active sprinters and jumpers were recruited to participate in this study. The average age, body mass, body height, and 100-m record time of the subjects were 25.0 ± 2.5 (SD) yr, 76.1 ± 7.9 kg, 1.81 ± 0.09 m, and 10.98 ± 0.39 s, respectively. All subjects were drug free, which was tested by using questionnaires. Furthermore, none of the subjects used supplements of amino acids, vitamins, minerals, or creatine monohydrate or any other sport supplement during the study phase. The athletes were members of the track and field association, and they could have been tested for doping. No doping tests were carried out during the study
period. The protocol and the potential benefits and risks associated with participation were fully explained to each subject before he signed an informed consent document. The study was approved by the University Ethical Board.

Experimental protocol. The study was carried out as a randomized double-blind crossover design. All subjects were initially familiarized (4 wk) with the following important parts of the study: training, nutrition, and a jumping test. In the protocol each subject underwent three randomized experimental treatments (a strength and speed training period of 8 days) separated by 13 days. During each treatment the subjects performed the same strength and speed training program. Each subject was advised to eat according to the same nutrition program during the periods. Before (80 min) the test training session the subjects had a small, standardized breakfast. Figures 1 and 2 show the experimental design. The only difference in the treatments was the drink of 125 ml consumed twice per day (62.5 ml in the morning and 62.5 ml in the evening). The drink was not taken in the morning of the test training session but after the recovery of 90 min after the session. In the treatment with 125-ml Bioenervi the subjects consumed the 125-ml Bioenervi supplement containing 67.6 µg/l IGF-I and 0.390 g/l IgG (total energy 100 kJ). In the treatment with the 25-ml Bioenervi the subjects consumed the drink of 125 ml containing the 25-ml Bioenervi supplement (total energy 20 kJ) mixed with 100-ml placebo including 13.5 µg/l IGF-I and 0.124 g/l IgG. In the placebo treatment the subjects drank 125 ml normal milk whey containing undetectable amounts IGF-I and 0.057 g/l IgG. The taste and color of the three test drinks were indistinguishable. All supplements were donated by Viable Bioproducts (Turku, Finland).

Blood and saliva collection and analysis. Blood samples (5 ml) for IGF-I, IgG, amino acids, and hormones were drawn from the antecubital vein. The sample in the morning at 0800–0900 was taken after 10 h of fasting (for other samples, see Figs. 1 and 2). Serum samples were immediately stored in plastic Eppendorf tubes and frozen at −20°C. The samples that required storage were stored for no longer than 3 mo and thawed only once for analysis. Serum IGF-I was analyzed in duplicate with an 125I liquid-phase double-antibody radioimmunoassay (RIA) with an octadecasilyl-silica preliminary column (acid-methanol) extraction to separate total IGF-I from its binding proteins (Incstar, Stillwater, MN). The assay was sensitive to a detection limit of <2.0 nmol/l. Serum IgG was analyzed in duplicate with ProAna Mabs affinity chromatography system (HyClone Laboratories).

For amino acid analysis, the serum samples were deproteinized with 5% sulfosalicylic acid containing L-2,4-diaminobutyric acid as internal standard, mixed with lithium citrate buffer, and subjected to ion-exchange chromatography by using an automatic Pharmacia LKB Alpha Plus amino acid analyzer (Cambridge Medical Diagnostics) with o-phthalaldehyde derivatization and fluorescence detection.

Insulin, testosterone, cortisol, and growth hormone were determined in duplicate by RIA. Serum concentrations of insulin were determined by using a double-antibody procedure (Phadeseph insulin RIA, Pharmacia Diagnostics, Uppsala, Sweden) with an intraassay coefficient of variation of <8.0%. The assay was sensitive to a detection limit of 0.3 µmol/l. Serum testosterone, cortisol, and growth hormone concentrations were determined by using a radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden) with intraassay coefficients of variation of <10.0% for testosterone, 6.0% for cortisol, and 4.0% for growth hormone. The assay was sensitive to a detection limit of 0.1 µmol/l for testosterone and cortisol and 0.01 µmol/l for growth hormone.

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TABLE 2. Order of exercises performed, distance and number of sets, repetitions, and recoveries during the test strength and speed training session

<table>
<thead>
<tr>
<th>Exercise Order</th>
<th>Sets</th>
<th>Distance</th>
<th>Repetitions</th>
<th>Recovery Between Exercises, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprint coordination/speed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skipping</td>
<td>25 m</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Knee flexion</td>
<td>25 m</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Bounding</td>
<td>25 m</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Acceleration</td>
<td>25 m</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Speed strength</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hurdle jumps</td>
<td>5</td>
<td>10</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>SJ jumps</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Heavy resistance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep squat</td>
<td>4</td>
<td>10 RM</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Calf raises</td>
<td>4</td>
<td>20 RM</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Bench press</td>
<td>4</td>
<td>10 RM</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

RM, repetition maximum.

Trends over time during the three 8-day periods were examined separately for pairwise differences. Furthermore, trends over time during the three 8-day periods were examined separately for each treatment. The level of significance was set at P < 0.05. Spearman correlation coefficients were used to determine whether relationships existed between the variables.

RESULTS

The IGF-I, IgA, and IgG results are presented in Figs. 3–5. The only significant (P < 0.05) difference was noticed in the IGF-I change (from the beginning of the 8-day period to the end of the 8-day period) when the three treatments were compared (Fig. 6). The trend of time with 125-ml Bioenervi showed that the IGF-I values increased in a linear fashion [-0.54 ± 0.26 (SE) nmol/l; P < 0.05].

The serum insulin concentration (Fig. 7) increased (P < 0.001) after the test training session, and was low during the acute recovery in all treatments. At the end of the 8-day period, the insulin concentration was similar to that at the beginning of the period. The change in IGF-I level and IGFBP-1 levels were similar. The change in IGF-I level between pretraining and postraining (change after 8 days) concentrations correlated positively with the change in insulin concentrations between the same time points with placebo (r = 0.60; P = 0.088), 25-ml Bioenervi (r = 0.68; P = 0.045), and 125-ml Bioenervi (r = 0.69; P = 0.038).

The growth hormone concentrations (Fig. 8) increased (P < 0.05) immediately after the test training session and decreased (P < 0.05) thereafter. The testosterone concentrations (Fig. 9) were similar in the
fasting conditions (measurements 1, 2, 8, and 9). Before the test training session the testosterone concentration decreased ($P < 0.01–0.001$), but it increased ($P < 0.01$) after the session. During the acute recovery (90-min) phase the testosterone concentration decreased ($P < 0.05–0.001$). The cortisol concentration (Fig. 10) was similar in the fasting conditions but decreased ($P < 0.001$) strongly from the fasting value to the end of the acute (90-min) recovery during the test training session. There were no significant differences in any hormone concentration between the three treatments.

The average daily energy intakes (5-day periods) were similar during each treatment ($10.50 \pm 1.08 \text{ MJ}$ for placebo, $11.55 \pm 2.80 \text{ MJ}$ for 25-ml Bioenervi, and $11.34 \pm 1.54 \text{ MJ}$ for 125-ml Bioenervi), and there were no differences in carbohydrate, protein, and fat distribution. The serum amino acid concentrations are presented in Fig. 11. There were no differences between the three treatments. The test training session achieved a significant ($P < 0.001$) decrease in the sum of the concentrations of all amino acids, in the essential amino acids, and in the branched-chain amino acids during all treatments.

The test training session slightly increased (not significant) blood lactate concentrations, which were 3.4 $\pm$ 1.0, 3.4 $\pm$ 0.6, and 2.8 $\pm$ 0.6 mmol/l for placebo, 25-ml Bioenervi, and 125-ml Bioenervi, respectively.

The countermovement jump was performed immediately before the test training session (56.3 $\pm$ 7.9, 54.8 $\pm$ 8.0, and 54.6 $\pm$ 7.8 cm for placebo, 25-ml Bioenervi, and 125-ml Bioenervi, respectively), and the values decreased ($P < 0.05$) after the session in all treatments. The values immediately after the session were 51.2 $\pm$ 8.2, 51.5 $\pm$ 7.4, and 50.4 $\pm$ 7.5 cm for placebo, 25-ml Bioenervi, and 125-ml Bioenervi, respectively. There were no measurable training effects in jumping performance during the three treatments.
DISCUSSION

The most important finding of this study was that increases occurred in the serum IGF-I concentration during the Bioenervi supplementation. The IGF-I values increased in a linear fashion, which implies that the IGF-I level increased with increasing usage time with 125-ml Bioenervi. However, the sharpness of the increase was quite low (0.54 nmol·l$^{-1}$·day$^{-1}$), especially compared with normally day-to-day variability. On the other hand, during 20 days the IGF-I concentration would increase, for example, to 10.8 nmol/l with 125-ml Bioenervi. Because the amino acid sequences of human and bovine IGF-I are identical (13), the RIA method used in our study measured the total amount of IGF-I (both bovine and human). Thus the possible increase in serum IGF-I can be due to either direct absorption of the growth factor from Bioenervi or enhanced stimulation of human IGF-I synthesis. It should be noted that the initial mean level of IGF-I was somewhat greater in the placebo group. This means the possibility that reasons other than Bioenervi supplementation may have contributed to the difference in the IGF-I concentrations between the groups.

Similar results have been obtained in animal studies. It has been shown that both dietary colostrum (16, 35) and purified recombinant IGF-I (3) increased blood IGF-I concentration in calves. In addition, orally administered $^{125}$I-IGF-I has been demonstrated to be transported into circulation (4). Dietary IGF-I has been shown to suppress erratic insulin secretion and stimulate prolactin secretion in calves (2, 3), indicating that even systemic effects may occur. However, the predomi-
The serum testosterone concentrations were similar in all three treatments, and there were increases in response to the training session as documented earlier (28). The concentrations were recovered at the end of the 8-day period, and there were no significant relationships between the changes in IGF-I and in testosterone during the 8-day period. The serum cortisol concentrations decreased strongly after the morning measurement and were very low during the test day. This could be explained mainly by the daily periodic changes (17), which show that the high concentrations occur in the morning hours and that they decline during daytime.

The serum insulin concentration was high after the standard breakfast, as expected. The test training session decreased the concentration that was recovered at the end of the 8-day period. This insulin curve is typical of physical exercise (15). The strong relationship between IGF-I and insulin observed with Bioenervi supplementation confirms the role of IGF-I and insulin in protein anabolism (14). IGF-I promotes muscle protein anabolism principally by stimulating protein synthesis, whereas insulin inhibits proteolysis in human muscle thereby, increasing protein anabolism. Thus it is possible that Bioenervi supplementation may strengthen the effects of IGF-I and insulin on protein anabolism in athletes.

The serum amino acid concentration decreased strongly during the test sessions. Earlier it has been shown that the essential amino acids show acute decreases during the short intensive anaerobic running exercises but that the concentration of the total amino acids did not change significantly (32). The duration of the training session in the present study (90 min) was longer than those of the running exercises (=30 min). The blood amino acids are transported into muscles where they are mainly needed for the synthesis of tissue proteins, hormones, enzymes, and neurotransmitters. They are also involved in energy metabolism via gluconeogenesis and in the regulation of numerous metabolic pathways. During the training session a small part of the amino acids is used for energy requirements (11), but the main need for amino acids is during the postexercise recovery when the rate of protein synthesis increases. In the study by Chesley et al. (8), it was shown that protein synthesis was significantly elevated 4 h after exercise. The increased protein synthesis rate persisted at least for 24 h. The results were obtained after a resistance session of 4 sets of 6–12 repetitions of various biceps-curl exercises with a resistance equal to 80% of one repetition maximum. During the present study the rate of protein synthesis might have been similar, lasting many hours. This is partly supported by the finding that the amino acid concentrations were slightly lowered at the end of the period. However, there were no significant differences in the amino acid concentrations between placebo and the Bioenervi-supplemented groups, which means that the increased IGF-I did not change the serum amino acid concentrations.
The treatments did not have any significant effects on the saliva IgA and serum IgG concentrations. It should be noted that transforming growth factor-\( \beta \) (TGF-\( \beta \)) found in bovine colostrum increases both IgG (7) and especially IgA production in vitro (7). In addition, TGF-\( \beta \) has been demonstrated to enhance expression of secretory component in rat epithelial cells, which is responsible for the transport of polymeric IgA into intestinal lumen (31). Because it is well known that IgA plays a major role in immunological protection of mucous membranes (5), it could also be possible (at least in theory) that dietary bovine colostrum may activate immunological defense system against microbes on mucous membranes.

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