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

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Mero, Antti, Heidi Miikkulainen, Jarmo 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 125-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; immungolobulin G and immuno-globulin 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 ^{125}I-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
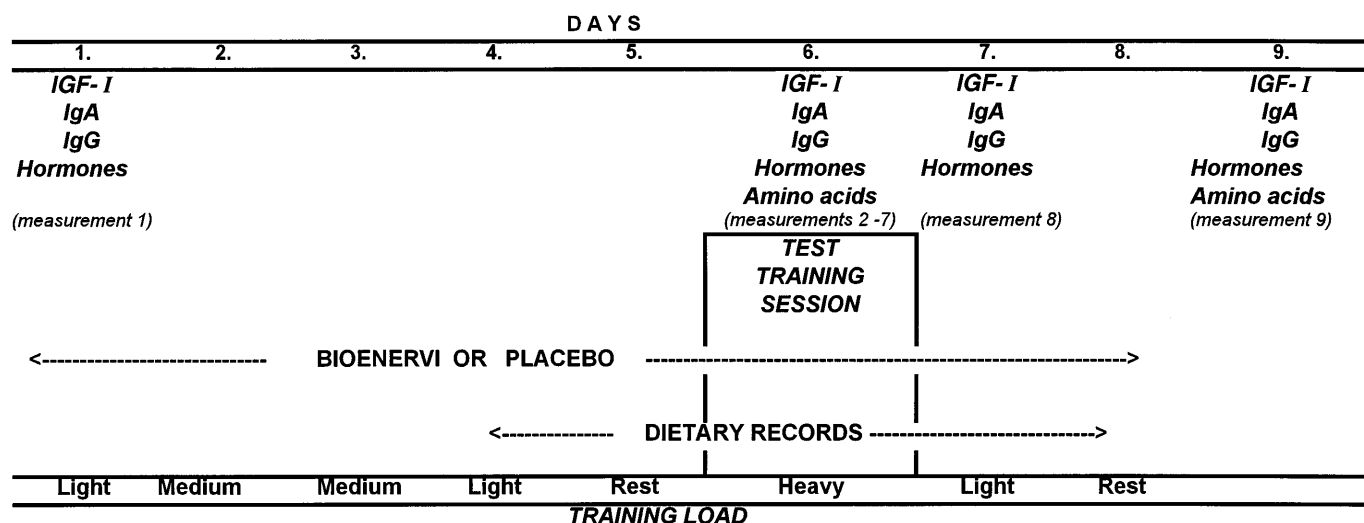
DAYS

| | 1. | 2. | 3. | 4. | 5. | 6. | 7. | 8. | 9. |

IGF- I   IGF- I   IGF- I   IGF- I
IgA   IgA   IgA   IgA
IgG   IgG   IgG   IgG
Hormones   Hormones   Hormones   Hormones
                                          Amino acids   Amino acids
(measurement 1)   (measurements 2 -7)   (measurement 8)   (measurement 9)

TEST
TRAINING
SESSION

<---------------------------- BIOENERVI OR PLACEBO --->

<--------------- DIETARY RECORDS -------------------->

| Light | Medium | Medium | Light | Rest | Heavy | Light | Rest |
TRAINING LOAD

Fig. 1. Experimental study design during an 8-day period. IGF-I, insulin-like growth factor I; IgA, immunoglobulin A; IgG, immunoglobulin G.

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. Bioenervi and placebo contained undetectable amounts of bovine IgA. 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 ^{125}I 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,

<---------------------- R E C O V E R Y ----------------------->

IGF-I   IGF-I
IgA   IgA
IGF-I   IGF-I   TEST   IgG
IgA   IgG   TRAINING   Hormones   IGF-I   IGF-I   IGF-I
IgG   Hormones   SESSION   Amino acids   IgG   IgG   IgG
Hormones   Amino acids   Hormones   Hormones   Hormones
Amino acids   Lactate   90 min   Lactate
-fasted   standard   -before   -after 5 min   -30 min   -60 min   90 min
           breakfast

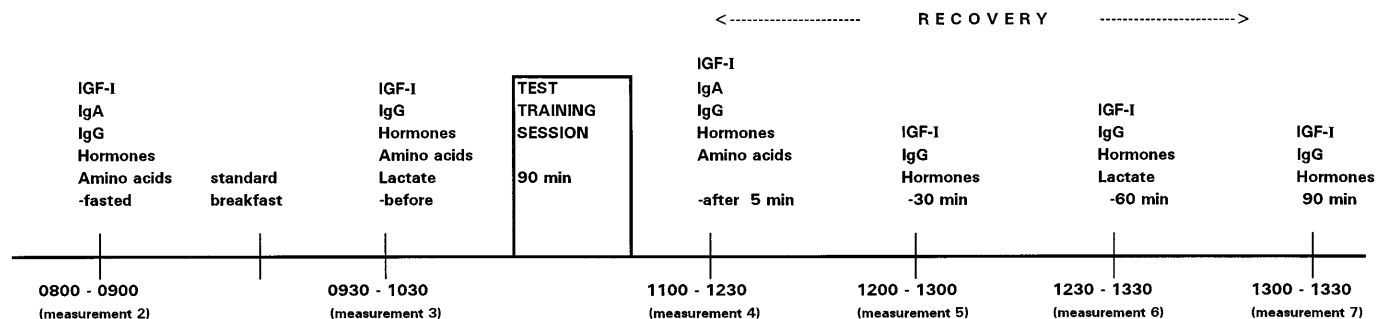| 0800 - 0900 | | 0930 - 1030 | | 1100 - 1230 | 1200 - 1300 | 1230 - 1330 | 1300 - 1330 |
| (measurement 2) | | (measurement 3) | | (measurement 4) | (measurement 5) | (measurement 6) | (measurement 7) |

Fig. 2. Time line for test training session.

Uppsala, Sweden). The assay was sensitive to a detection limit of <2.5 mU/l. Serum samples for testosterone were determined with a solid-phase ^{125}I RIA (Spectria testosterone-coated tube RIA Kit, Orion Diagnostica, Turku, Finland). The assay was sensitive to a detection limit of <0.1 nmol/l. Serum concentrations of cortisol were determined with ^{125}I RIA (Cortisol RIA kit, Orion Diagnostica). The assay was sensitive to a detection limit of 4–7 nmol/l. Serum concentrations of growth hormone were determined with an ^{125}I liquid-phase double-antibody procedure (human growth hormone RIA, Pharmacia Diagnostics, Uppsala, Sweden), and the assay was sensitive to a detection limit of <0.2 μg/l.

IgA was analyzed in duplicate from saliva samples (8 ml) by using IgA-specific enzyme-linked immunosorbent assay (29).

All of the samples from an individual were run in the same assay to avoid any changes in interassay variability. Intra-assay coefficients of variation were 2–5% and interassay coefficients of variation were 4–10% for the blood and saliva variables.

Blood samples (100 μl) were drawn from the fingertip in EDTA-containing tubes to determine lactate concentration (18) before and after the test training session.

Jumping test. Speed strength of the leg extensor muscles was evaluated before and after the test training session by using a countermovement jump (22) on a contact mat (Digi-test, Muurame, Finland) connected by a cable to a digital timer (±0.001 s). The timer was triggered by the feet of the subject at the moment of release from the mat and stopped at the moment of touchdown. Thus the flight time of the subject during the jump was recorded. The height of the rise in center of gravity in a jump was then calculated from the measured flight time. The reproducibility of the jump ($r = 0.99$; coefficient of variation = 3.5 ± 0.4%) has been studied earlier (39).

Training. All subjects were active athletes, and their training was supervised by the researchers. The study period took place during an indoor training season. All subjects trained according to the same program. The volume and intensity of the program (Table 1) were carefully calculated and planned by researchers and coaches in collaboration. On the sixth day of each period the subjects carried out the test training session (Table 2). It was a heavy session and lasted 90 min. All exercises were performed with maximal speed (maximal effort) controlled by performance time and distance. During the 4-wk familiarization period and during the "washout" periods of 13 days, the volume and intensity were similar to that in the study phase.

Nutrition. During the familiarization period and the study phase the subjects were advised to eat according to the principles of the general nutrition recommendations in athletes. Each subject was also advised to use the same nutrition program during the three 8-day periods. The subjects were

Table 1. *Strength and speed training during each 8-day period*

Variable	Volume	Intensity
Training times	6	
Strength training*		
Leg extensors	6.1 tons	Maximal speed
Leg flexors	2.5 tons	Maximal speed
Other muscles	27.8 tons	Maximal speed
Jumps (takeoffs)	165	Maximal speed
Speed training running (30–60 m†)	1,100 m	90–100%‡
Speed endurance running (100–400 m†)	1,500 m	60–80%‡
Aerobic running (warm-up jogging)	7,000 m	<60%†

*Loads from 30 to 90% of 1 repetition maximum. †Distances used in training. ‡Percentage of 1 repetition maximum.

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

Exercise Order	Sets or Distance	Repetitions	Recovery Between Sets and Exercises, min
Sprint coordination/speed			
Skipping	25 m	4	2
Knee flexion	25 m	4	2
Bounding	25 m	4	2
Acceleration	25 m	4	2
Speed strength			
Hurdle jumps	5	10	3
5 Jumps	6	5	3
Heavy resistance			
Deep squat	4	10 RM	3
Calf raises	4	20 RM	3
Bench press	4	10 RM	3

RM, repetition maximum.

not allowed to consume caffeine, alcohol, or nutritional supplements (e.g., multivitamins, multiminerals). All subjects kept food diaries during 5 days of each period, and they were included in the analysis of nutrition. It was performed by using the Micro Nutrica software (version 2.0, Social Insurance Institution, Finland).

Statistics. Multivariate analysis of variance (MANOVA) to produce the *F*-statistic was used to detect the presence of a significant difference within the three treatments. As post hoc methods, additional examinations were performed by contrast examination using univariate results subsequent to MANOVA, and they provided a measure of significance between 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 breakfast, decreased ($P < 0.001$) 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 between pretraining and posttraining (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
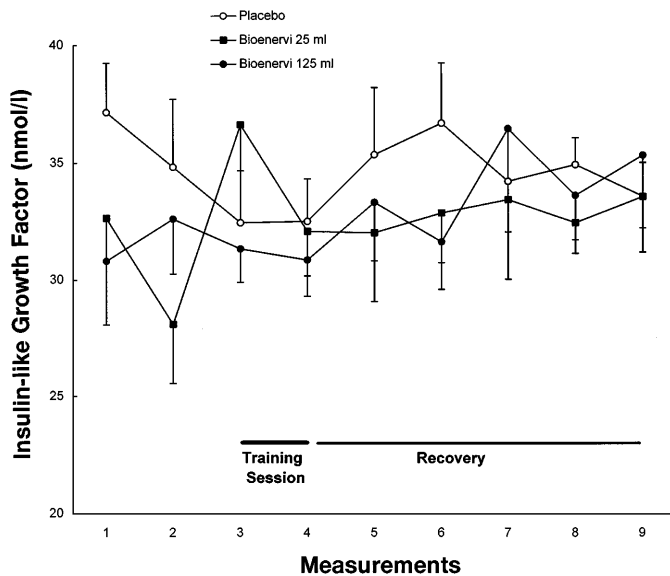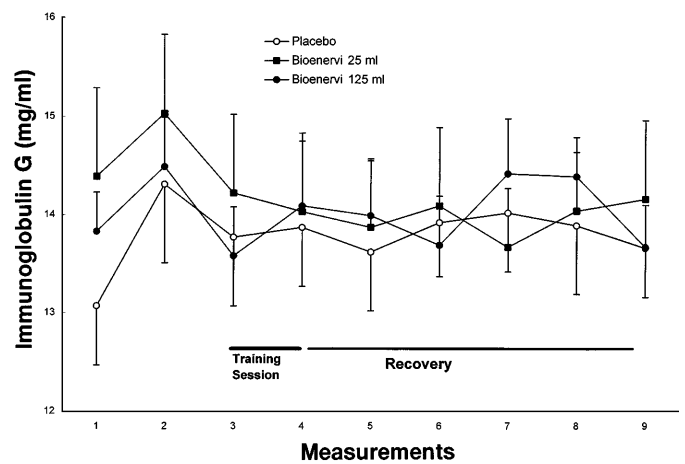
Fig. 3. Responses of serum IGF-I concentrations with various treatments during 8-day periods. Data are means ± SE.

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 ± 1.08 MJ for placebo, 11.55 ± 2.80 MJ for 25-ml Bioenervi, and 11.34 ± 1.54 MJ for 125-ml Bioenervi), and there were

Fig. 5. Responses of serum IgG concentrations with various treatments during 8-day periods. Data are means ± SE.

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 ± 1.0, 3.4 ± 0.6, and 2.8 ± 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 ± 7.9, 54.8 ± 8.0, and 54.6 ± 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 ± 8.2, 51.5 ± 7.4, and 50.4 ± 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.
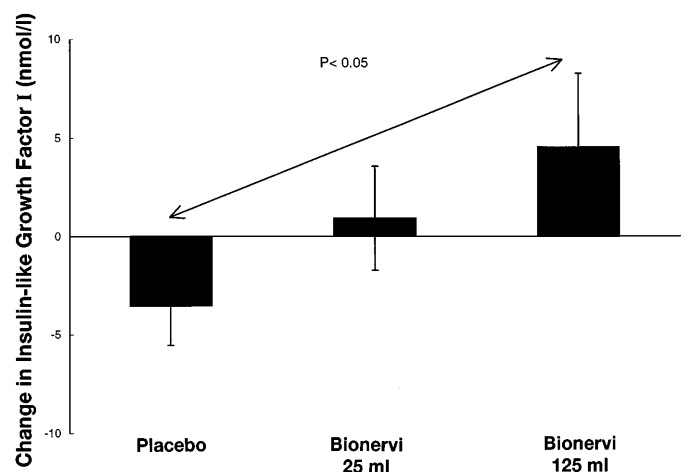

Fig. 4. Responses of saliva IgA concentrations with various treatments during 8-day periods. Data are means ± SE.

Fig. 6. Changes in serum IGF-I concentrations between pretraining and posttraining values with various treatments. Data are means ± SE.
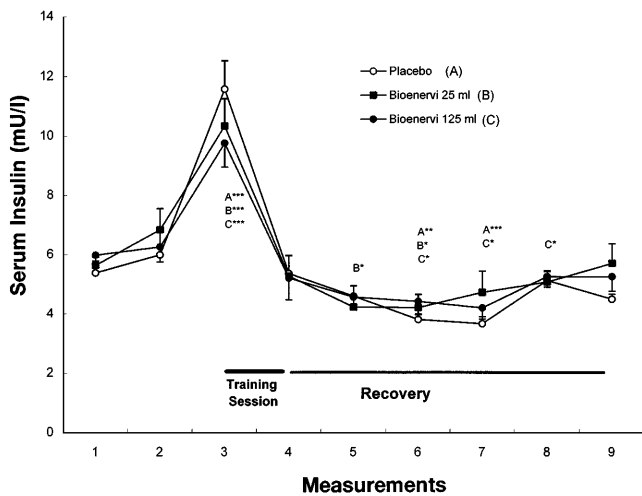
Fig. 7. Responses of serum insulin concentrations with various treatments during 8-day periods. Data are means ± SE. Significantly different compared with pretraining values (*measurement 1*), *P < 0.05; **P < 0.01; ***P < 0.001.

Fig. 9. Responses of serum testosterone concentrations with various treatments during 8-day periods. Data are means ± SE. Significantly different compared with pretraining values (*measurement 1*), *P < 0.05; **P < 0.01; ***P < 0.001.

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 ($0.54 \ nmol \cdot l^{-1} \cdot day^{-1}$), 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
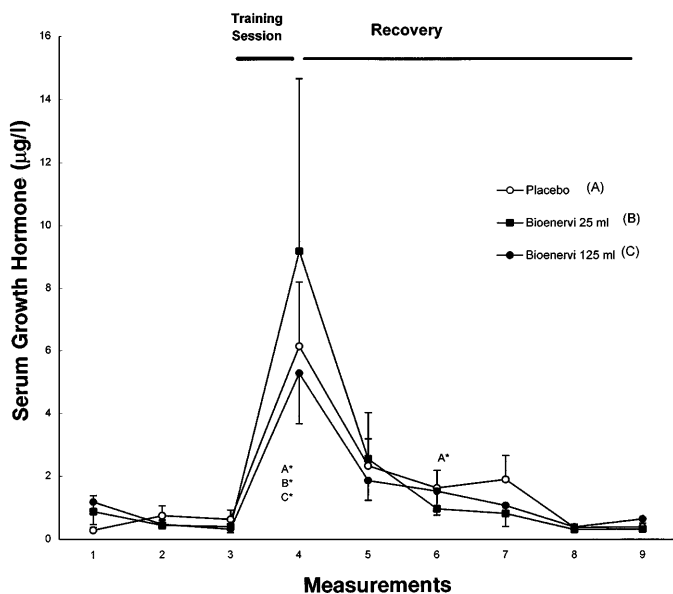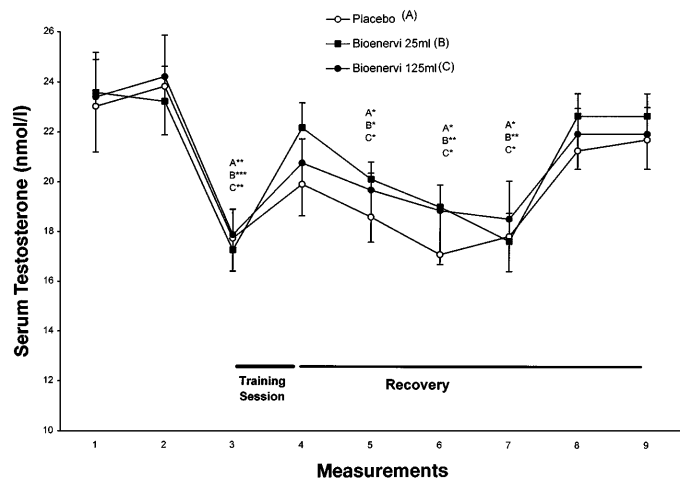
IGF-I (both bovine and human). Thus the possible increase in serum IGF-I can be due to either direct absorption of the growth factor from Bioenervi or enhanced stimulation of human IGF-I synthesis. It should be noted that the initial mean level of IGF-I was somewhat greater in the placebo group. This 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-
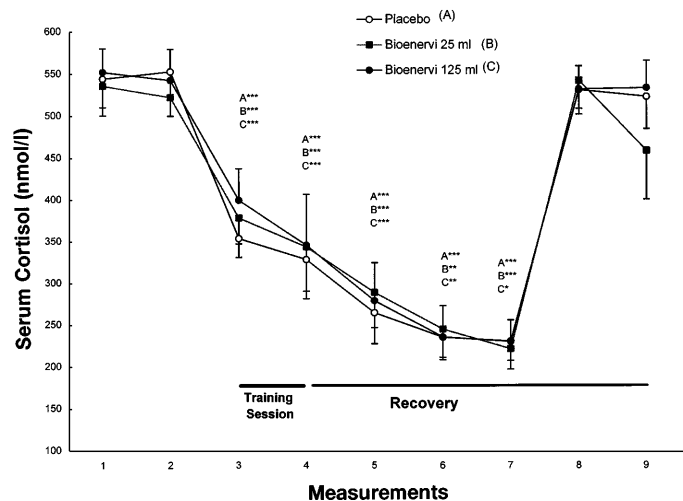

Fig. 8. Responses of serum growth hormone concentrations with various treatments during 8-day periods. Data are means ± SE. *Significantly different compared with pretraining values (*measurement 1*), P < 0.05.

Fig. 10. Responses of serum cortisol concentrations with various treatments during 8-day periods. Data are means ± SE. Significantly different compared with pretraining values (*measurement 1*), *P < 0.05; **P < 0.01; ***P < 0.001.
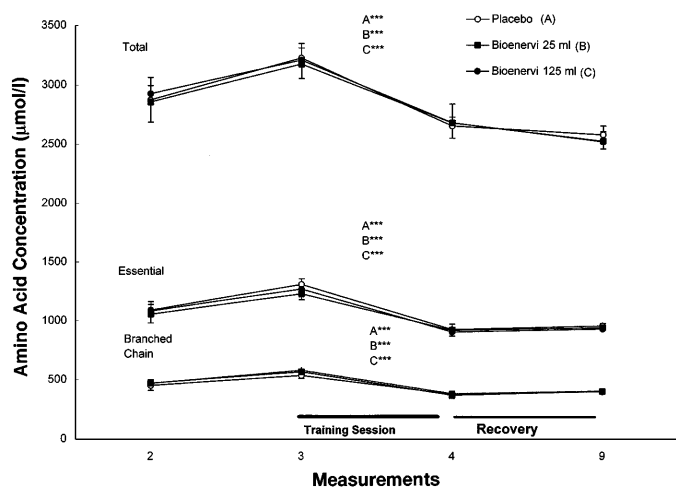
Fig. 11. Responses of serum amino acid concentrations with various treatments during 8-day periods. Data are means ± SE. Significant difference between pre- and posttraining session values, ***$P < 0.001$.

nant natural target of growth factors in colostrum is probably the gastrointestinal tract (38). The increased growth and turnover of intestine can provide for a healthier gut by increasing uptake of dietary components, which may enhance growth generally. Whether the dietary colostrum would have any effects on the gastrointestinal tract in human subject requires further studies.

In our study the training session consisted of not only heavy-resistance exercises but also many speed strength (power) exercises (e.g., jumps). The recoveries were long (from 2 to 3 min), and intensity (effort) was maximal (as fast as possible). No acute increase in serum IGF-I was observed after the training session, which is not consistent with a previous study where an increase in serum IGF-I was detected after a heavy-resistance exercise (27, 28). On the other hand, our protocol resulted in IGF-I and growth hormone responses that were similar to those observed during 23 h of recovery after a moderate-intensity and low-volume heavy-resistance exercise protocol (24). It should also be mentioned that in contrast to our study, the subjects have been nonathletes in previous studies. The negligible change in IGF-I level in our study could be due to the training effect in our subject group. Growth hormone is a primary endocrine stimulus for IGF-I production, and it mediates IGF-I synthesis, synthesis of the components of the 150-kDa complex of IGF-I, and binding protein 3 (20). However, IGF-I concentrations may be independent of growth hormone stimulatory mechanisms after exercise (26). It has been shown earlier (25, 27, 28) that not all heavy-resistance exercise protocols produce the same magnitude of serum growth hormone elevations. It has also been speculated that there may be many unrelated acute and chronic effects of exercise on IGF-I not related to growth hormone production (1, 6).

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. In 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-β (TGF-β) found in bovine colostrum increases both IgG (7) and especially IgA production in vitro (7). In addition, TGF-β 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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