IGF-I, IgA, and IgG responses to bovine colostrum supplementation during training

**Mero, Antti, Jonne Kähkönen, Tarja Nykänen, Tapani Parviainen, Ilmari Jokinen, Timo Takala, Tuomo Nikula, Simo Rasi, and Juhani Leppäluoto.** IGF-I, IgA, and IgG responses to bovine colostrum supplementation during training. *J Appl Physiol* 93: 732–739, 2002. First published March 15, 2002; 10.1152/japplphysiol.00002.2002.—This study examined the effect of bovine colostrum (Dynamic colostrum) supplementation on blood and saliva variables (*study 1*) and the absorption of orally administered human recombinant insulin-like growth factor (IGF)-I (rhIGF-I) labeled with $^{123}$I ($^{123}$I-rhIGF-I) (*study 2*). In *study 1*, adult male and female athletes were randomly assigned in a double-blind fashion to either an experimental (Dynamic; $n = 19$) or a control (Placebo; $n = 11$) group. The former consumed daily 20 g of Dynamic supplement, and the latter 20 g of maltodextrin during a 2-wk training period. After bovine colostrum supplementation, significant increases were noticed in serum IGF-I ($P < 0.01$) and saliva IgA ($P < 0.01$) in Dynamic compared with Placebo. In *study 2*, gel electrophoresis was carried out in 12 adult subjects with serum samples taken 60 min after ingestion of $^{123}$I-rhIGF-I and showed peaks at 0.6 and 40–90 kDa, with the former inducing 96% and the latter 4% of the total radioactivity. It was concluded that a long-term supplementation of bovine colostrum (Dynamic) increases serum IGF-I and saliva IgA concentration in athletes during training. Absorption data show that ingested $^{123}$I-rhIGF-I is fragmented in circulation and that no radioactive IGF-I is eluted at the positions of free, or the IGF, binding proteins, giving no support to the absorption of IGF-I from bovine colostrum.

*Physical training; athlete health; human recombinant insulin-like growth factor-I absorption*

**IT HAS BEEN SHOWN THAT BOVINE colostrum supplementation increases serum insulin-like growth factor (IGF)-I concentration in male athletes during a short strength and speed training period, with no effect on vertical jump performance (32). There is also a positive association between bovine colostrum supplementation and health status in female endurance athletes during a winter competition period, although no physiological explanations were found for that (33). Buckley et al. (5) showed that bovine colostrum supplementation associated with a running program during 8 wk improved endurance performance in physically active men, but there were no effects on plasma IGF-I concentrations. The researchers concluded that oral supplementation with intact bovine colostrum improves the ability to perform a second bout of maximal endurance-type exercise after a relatively short period of recovery from a prior bout of maximal exercise. This is partly supported by Smeets et al. (42), who showed that bovine colostrum supplementation increased the ability to repeat sprints in elite field hockey players. Feeding colostrum has recently been shown to increase bone-free lean body mass in healthy trained adults (1) and the synthesis of myofibrillar protein in the skeletal muscle of newborn piglets (15). These findings suggest that bovine colostrum supplementation may have positive effects on muscle function, performance capacity, and health status of physically active people.

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 (10, 37). The most abundant and well-characterized growth factors in bovine colostrum are probably IGF-I and IGF-II (16). They simulate cell growth and are proposed to act both as endocrine hormones via the blood and as paracrine and autocrine growth factors locally (10, 19). IGF-I is a major form in bovine colostrums, and the concentration is 7–67 nmol/l (40), whereas normal milk contains <0.3 nmol/l (7). In normal adult humans, IGF-I occurs at a concentration of ~7 nmol/l in serum (19). IGF-I has a strong anabolic effect on muscle tissue (25, 44), and it is associated with regulatory feedback of growth hor-
monone (25, 38). IGF-I can mimic most, but probably not all, effects of growth hormone (9). The effects of growth hormone on skeletal muscle are thought to be mediated by IGF-I (22).

Antimicrobial factors in bovine colostrum include immunoglobulins, lactoperoxidase, lysozyme, and lactoferrin. Bovine colostrum is an extremely rich source of immunoglobulins. The concentrations of IgG, IgM, and IgA in bovine colostrum are ~100-fold higher than in normal milk (27).

The purpose of the present study was to examine the effect of bovine colostrum supplementation (Dynamic Colostrum, which is a colostrum whey product sold in some European countries, but other colostrum products are approved for sale in the United States; Dynamic Colostrum is not on the banned drug list of the International Olympic Committee) on the concentration of serum IGF-I and serum and salivary immunoglobulins. We further hypothesized that the bovine colostrum supplement enhances serum IGF-I and immunoglobulin concentrations in athletes, both in men and in women, during training. This hypothesis is supported by the IGF-I results in male athletes (32) and in calves (17, 39). In addition, orally administered 125I-labeled IGF-I (125I-IGF-I) has been demonstrated to be transported into circulation in calves (3) and neonatal pigs (47). Because the mechanism of the increased serum IGF-I concentration with the supplementation is not defined, we tried additionally to investigate the absorption of orally administered human recombinant IGF-I (rhIGF-I) (labeled with 125I, 125I-rhIGF-I) in human subjects. The hypothesis of the enhancement of immunoglobulins is partly supported by the notion that transforming growth factor-β1 (TGF-β1) found in bovine colostrum increases mucosal IgA production in vitro (6).

METHODS

Subjects

Thirty active adult athletes [track and field athletes, cross-country skiers, and orienteers; average training history 6.5 ± 1.6 (SE) yr] were recruited to participate in study 1. In the randomized and double-blind experiments, there were 19 athletes in the experimental group (10 men and 9 women) and 11 athletes in the control group (6 men and 5 women) (Table 1). All subjects were drug free, which was tested by the International Olympic Committee) on the concentration of serum IGF-I and serum and salivary immunoglobulins. We further hypothesized that the bovine colostrum supplement enhances serum IGF-I and immunoglobulin concentrations in athletes, both in men and in women, during training. This hypothesis is supported by the IGF-I results in male athletes (32) and in calves (17, 39). In addition, orally administered 125I-labeled IGF-I (125I-IGF-I) has been demonstrated to be transported into circulation in calves (3) and neonatal pigs (47). Because the mechanism of the increased serum IGF-I concentration with the supplementation is not defined, we tried additionally to investigate the absorption of orally administered human recombinant IGF-I (rhIGF-I) (labeled with 125I, 125I-rhIGF-I) in human subjects. The hypothesis of the enhancement of immunoglobulins is partly supported by the notion that transforming growth factor-β1 (TGF-β1) found in bovine colostrum increases mucosal IgA production in vitro (6).

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Experimental Protocol in Study 2

On the day before the measurements, the subjects performed no physical activity. On the measurement day, each subject underwent a $^{125}$I-rhIGF-I treatment (50 MBq) in the morning, several blood samples, and gamma-camera imaging and ate a standard breakfast (2.0 MJ energy, 60% carbohydrate, 25% fat, and 15% protein) and lunch (2.5 MJ energy, 60% carbohydrate, 25% fat, and 15% protein) during 7 h

Blood and Saliva Collection and Analysis in Study 1

Blood samples (5 ml) for IGF-I, IgA, and IgG were drawn from the antecubital vein. The sample in the morning at 0700–0800 was taken after 10 h of fasting. Serum samples were immediately stored in plastic Eppendorf tubes and were stored at $-20\, ^\circ\, C$. The samples that required storage were frozen for no longer than 3 mo and thawed only once for analysis. A saliva sample (8 ml) for IgA was also taken in the morning.

Serum IGF-I Analysis (Studies 1 and 2)

Serum IGF-I was analyzed in duplicate with an OCTEIA IGF-I kit, which is a two-site immunoenzymometric assay for the quantitative determination of IGF-I in human serum. The method incorporates a sample pretreatment to avoid interference from binding proteins. The absolute sensitivity of the kit, defined as the concentration corresponding to the mean $+2\, \text{SDs}$ of 20 replicates of the zero calibrator, is 0.25 nmol/l. The functional sensitivity, defined as the concentration at which the coefficient of variation falls $<10\%$, is $\sim1.2\, \text{nmol/l}$.

Analysis of Immunoglobulins (Study 1)

Serum IgA and IgG and saliva IgA were analyzed with clinical chemistry analyzer KONE Specific Supra. The methods are based on measurement of immunoprecipitation enhanced by polyethylene glycol at 340 nm. Specific antisera is added in excess to buffered samples. The increase in absorbance caused by immunoprecipitation is recorded when the reaction has reached its end point. The change in absorbance is proportional to the amount of antigen in the solution.

Iodination of IGF-I (Study 2)

rhIGF-I (R&D System, Minneapolis, MN) was labeled by using chloramine-T method with $^{125}$I for oral administration into human subjects. Briefly, 400 MBq of $^{125}$I (MAP Medical Technologies) in 0.18 mol/l phosphate buffer, pH 7.5, were mixed with 50 µg rhIGF-I and 100 µl freshly prepared chloramine-T solution (1 mg/ml), were allowed to react, and were then purified with HPLC with reverse-phase chromatography ($\mu$Bondapak C18 column, 3.9 $\times$ 400 mm, Waters, Milford, MA). The mobile phase was aqua followed with acetonitrile and 0.02% aqueous triethylamine (4:6; pH 7.5). The eluant was monitored with Geiger tubes, which were connected to a radioactivity detector (Wallac Decem Series, Single Channel Analyser AS-11) and an ultraviolet detector (model 440, Waters) at 254 nm. The fractions containing radiolabeled rhIGF-I were evaporated under vacuum, dissolved into 2% human serum albumin, and filtered (0.22 µm).

Binding of Iodinated IGF-I to Receptors (Study 2)

Biological functionality of the batches of $^{125}$I-rhIGF-I was checked with binding to the membrane receptors for IGF-I (18, 36). Human placenta, used within 1 h from delivery, was washed with ice-cold PBS, dissected into 10-g samples, and stored at $-80\, ^\circ\, C$. Placental membranes possessing receptors for IGF-I were isolated by using the method of Pekonen et al. (34). Membranes were stored at $-80\, ^\circ\, C$ until they were used for binding studies. Placental membranes (2 mg protein) were incubated overnight at $4\, ^\circ\, C$ with 10 ng of $^{125}$I-rhIGF-I, together with a graded series of unlabeled rhIGF-I (0.1–1,000 ng). Cold buffer was added, and the tubes were centrifuged. After removal of the supernatant, the radioactivity of the pellets, as well as aliquots of the supernatants, was measured with a gamma counter (RackGamma, LKB Wallac). The radioactivity of the supernatant trapped in the pellet was subtracted from the radioactivity of each pellet.

Serum Radioactivity and TCA-precipitated Radioactivity (Study 2)

The radioactivity of serum samples after intake of $^{125}$I-rhIGF-I was measured. Furthermore, 1 ml of serum was mixed with TCA (final concentration, 10%) and incubated for 60 min at $4\, ^\circ\, C$. Proteins were pelleted with centrifugation at 4,000 $g$ for 10 min. The pellets were washed once with 10% TCA and centrifuged as above, and their radioactivity was counted.

Gel Chromatography of Serum (Study 2)

Proteins of serum sampled 60 min after ingestion of labeled rhIGF-I were separated by using a routine procedure of gel filtration with Sephadex G-100 matrix, pump P1, detector ultraviolet-l, and a fraction collector (materials and instruments from Pharmacia, Uppsala, Sweden). After balancing with buffer (0.1 M Tris-HCl, pH 7.2, 0.2 M NaCl, and 1 mg/ml EDTA), the column ($16 \times 250\, \text{mm}$) was calibrated with marker proteins: 150-kDa aldolase, 43.1-kDa ovalbumin, 13.7-kDa ribonuclease A, 6-kDa aprotinin, and 0.6-kDa tripeptide Pro-Phe-Arg. Fractions from eluted serum samples were collected, and their radioactivity was measured.

Statistics

Multivariate ANOVA to produce the $F$ statistics was used to detect the presence of a significant difference between the treatments. As post hoc methods, additional examinations were performed by contrast examination by using univariate results subsequent to multivariate ANOVA, and they provided a measure of significance between pairwise differences. Furthermore, trends over time during the 2-wk period were
examined separately for each treatment. The level of significance was set at $P < 0.05$.

RESULTS

Study 1

IGF-I, IgA, and IgG. There were no differences in IGF-I, IgA, and IgG between genders, and the results in all subjects are presented in Figs. 3–6. Significant increases were observed in serum IGF-I (17%; $P < 0.01$) and saliva IgA (33%; $P < 0.01$) in the experimental group (Dynamic) but not in the control group (Placebo) during 2 wk, whereas in serum IgA and IgG, there were no differences between the groups.

Nutrition. The average daily energy intake during the measured period was similar in Dynamic (9.57 ± 0.70 MJ) and Placebo (10.05 ± 0.69 MJ), and there were no differences in carbohydrate (58 ± 3% in Dynamic and 56 ± 2% in Placebo), protein (17 ± 1% in Dynamic and 17 ± 2% in Placebo), and fat (28 ± 2% in Dynamic and 30 ± 3% in Placebo) distribution between the groups.

Training. There were 14 ± 2 training sessions in both Dynamic and Placebo during 2 wk. The average duration of one training session was 1.2 ± 0.1 h. Total training included 75% event training and 25% strength training. Event training included 65% aerobic and 35% anaerobic training.

Study 2

IGF-I. There were no differences between genders in serum IGF-I on the test day. During the first 180 min after $^{125}$I-rhIGF-I treatment, there were no changes in the IGF-I concentration, but, after the standard lunch at 420 min, the increase compared with the starting value was significant (17%; $P < 0.001$) in all subjects (Fig. 7).

Binding of $^{125}$I-rhIGF-I to receptors. In each of the batches of $^{125}$I-rhIGF-I, half-maximal inhibition of binding of labeled IGF-I was observed when labeled and unlabeled IGF-I were present at equivalent concentrations (10 ng/ml), indicating that iodination had resulted in labeled rhIGF-I with preserved biological functionality.

Radioactivity of plasma and TCA precipitate radioactivity. Radioactivity after ingestion of $^{125}$I-rhIGF-I appeared soon in plasma, and highest values were
observed at 60-min sampling (Table 2). At any sampling time, only a small amount of serum radioactivity precipitated with TCA, indicating that the main part of radioactivity in serum consisted of low-molecular-mass molecules.

**Gel electrophoresis of plasma proteins.** Electrophoresis was carried out with serum samples taken 60 min after ingestion of $^{123}$I-rhIGF-I. The radioactivity in fractions of eluted samples appeared in two peaks (Fig. 8). The smaller peak of 40- to 90-kDa molecular mass included ~4% of the total radioactivity, and the larger peak of molecular mass <1 kDa contained 96% of radioactivity.

**DISCUSSION**

The results of this study showed that there was a 17% increase in circulating IGF-I and a 33% increase in saliva IgA in the Dynamic treatment during a 2-wk training period. On the other hand, in circulating IgA and IgG, there were no changes. In the other series of the experiments, we showed that ingested $^{123}$I-rhIGF-I was fragmented when in circulation, and only a minor proportion of the broad peak may represent unbound, intact IGF-I.

**Table 2. Radioactivity in plasma and TCA-precipitated plasma proteins after ingestion of $^{123}$I-labeled rhIGF-I in study 2**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Plasma, cpm</th>
<th>TCA Precipitated cpm</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>25,196 ± 3,244</td>
<td>940 ± 131</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>60</td>
<td>26,132 ± 3,225</td>
<td>999 ± 151</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>90</td>
<td>25,014 ± 2,630</td>
<td>809 ± 93</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>120</td>
<td>23,639 ± 2,309</td>
<td>841 ± 104</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>180</td>
<td>21,678 ± 1,999</td>
<td>780 ± 63</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>300</td>
<td>14,452 ± 1,227</td>
<td>629 ± 84</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>420</td>
<td>10,553 ± 896</td>
<td>511 ± 42</td>
<td>4.9 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE ($n = 12$ subjects) of a 1-ml serum sample. cpm, Counts/min. TCA precipitated = cpm in the precipitate × 100% /cpm in plasma.

The concentration of circulating IGF-I increased in Dynamic with increasing usage time (14 days). This result confirms the result with the other bovine colostrum (Bioenervi) supplement (32). The increase per day in the present study was slightly lower (0.38 nmol·l$^{-1}$·day$^{-1}$) than the respective value (0.54 nmol·l$^{-1}$·day$^{-1}$) in the earlier study in which the supplementation period was 8 days. Normally, daily variations of IGF-I are little, or there is none (43). In the earlier study, Bioenervi contained 8.5 μg IGF-I, and the respective value in the present study was greater (74 μg) during a day. Because the amino acid sequences of human and bovine IGF-I are identical (16), the method used in our study measured the total amount of IGF-I (both bovine and human).

The possible increase in serum IGF-I can be due to either direct absorption of the growth factor from Dynamic or enhanced stimulation of human IGF-I synthesis. In animal studies, it has been shown that both dietary colostrum (17, 39) and purified recombinant $^{125}$I-IGF-I (2) increased blood IGF-I concentration in calves. However, in adult rats, it has been observed that most of IGF-I is degraded in the gastrointestinal tract (46). In addition, orally administered $^{125}$I-IGF-I has been demonstrated to be transported into circulation in calves (3). In the present study, after orally administered $^{125}$I-rhIGF-I, radioactivity appeared in circulation very soon, but a major portion (~96%) of the labeled substance was of low molecular mass. The rest was eluted at a broad peak between 40 and 90 kDa. IGF-I is a 7.5-kDa polypeptide, and most of it circulates in a 150-kDa high-affinity complex, which also con-
tains IGF binding protein (IGFBP)-3 and an acid-labile subunit (41). IGF-I also circulates bound to lower molecular mass IGFBPs, including IGFBP-1, a 30-kDa protein, which is produced largely in the liver and is thought to be the major short-term modulator of IGF-I bioavailability (26). Less than 1% of IGF-I is thought to circulate in a free (7.5-kDa) or rapidly dissociable state and is thought to be readily available to mediate the effects on target tissues through an endocrine mechanism, similar to the situation with steroid and thyroid hormones. Therefore, it is not probable that significantly increased amounts of free or bound IGF-I are circulating after the oral intake of the 125I-rhIGF-I compound. We emphasize that we did not find any radioactivity at elution positions of free IGF-I (7.5 kDa).

The hypothesis that fitness training would lead to increases in circulating IGF-I in both humans and animals is not consistently observed (8, 21). Physical activity and maximal O2 consumption have been found to be related positively to serum IGF-I in men (35). The 5 wk of increased physical activity led to drops in circulating IGF-I, despite training-induced increases in muscle volume in adolescent girls (14%; Ref. 12) and in adolescent boys (12%; Ref. 13). In sedentary adult men and women, there has been an increase (20%) in circulating IGF-I after 13-wk resistance training, but no further increases occurred between the 13th and 25th wk, which was the follow-up period (4). In competitive athletes (24), it has been shown that, in the beginning of the training season, IGF-I concentrations are increased and are subsequently maintained at the level. In the present study, the subjects were also competitive athletes and had trained systematically for 6.5 yr. Their training stimulus was not new, the study phase occurred in the middle of their normal training season, and there were no differences in training between Dynamic and Placebo. Thus it seems that a short training period in the present study hardly influenced circulating IGF-I concentration.

Nutrition is one of the main regulators of circulating IGF-I (43). In humans, serum IGF-I concentrations are markedly lowered by energy and/or protein deprivation (e.g., Ref. 20); therefore, both energy and proteins are critical in the regulation of serum IGF-I concentrations. In the present study, the average daily energy and protein intake were similar in both Dynamic and Placebo groups. The total energy and protein values were in the range of normal active people, and it seems that nutrition without Dynamic supplementation had no influences on circulating IGF-I during the 2-wk training period. In the absorption measurements, there was a strong increase in circulating IGF-I concentration after lunch, which may be due to the effect of insulin or other nutrients, as suggested by Thissen et al. (43).

The observed increase in circulating IGF-I with Dynamic may have influences on muscular function. In the earlier study (32), the strong relationship between IGF-I and insulin (not measured in the present study) was observed with bovine colostrum (Bioenervi) supplementation, which emphasizes the role of IGF-I and insulin in protein anabolism. IGF-I promotes muscle protein synthesis, whereas insulin inhibits proteolysis in human muscle, thereby increasing protein anabolism. Recently, it has been shown that feeding colostrum increases the synthesis of myofibrillar protein in the skeletal muscle of newborn piglets (15) and bone-free lean body mass in healthy trained adults (1). Fiorotto et al. (15) showed that the greater stimulation of muscle protein synthesis in colostrum-fed piglets was restricted entirely to the myofibrillar protein compartment. IGF-I stimulates muscle protein synthesis equally in both the myofibrillar and cytoplasmic compartments (15), and it is, therefore, an open question as to how much the increased (17%) concentration of IGF-I resulting from bovine colostrum supplementation in humans is responsible for any improvements in muscle function.

A novel finding was the 33% increase in salivary IgA concentrations during 2 wk of bovine colostrum supplementation. In the earlier study (32), there was no change in saliva IgA during 8 days. In the present study, the daily amount of IgA was 0.3 g (in 20 g of Dynamic), which is greater than in the earlier study. This may be the main reason for the increase of saliva IgA. The humoral immune response of mucosal surfaces is mediated mainly by antibodies of the IgA class. Secretory IgA has been shown to inhibit attachment and replication of certain viruses and bacteria, thus preventing their entry into the body, to neutralize toxins and some viruses, and to mediate antibody-dependent cytotoxicity, another antiviral defense mechanism (45).

Secretory IgA is important to the host defense against certain viruses that are not carried in the blood, especially those causing upper respiratory tract infections (URTI). The level of secretory IgA contained in mucosal fluids correlates more closely than do serum antibodies with resistance to certain infections caused by viruses, such as URTI (28, 45). Intensive daily training appears to exert a cumulative suppressive effect on saliva IgA levels (29, 30). In a recent study (14), the results indicated that an 8-mo season of college football is associated with a progressive reduction in saliva IgA levels and a subsequent increase in the number of URTI. It should also be noted that TGF-β1 found in Dynamic increases IgA production in vitro (6). In addition, TGF-β1 has been demonstrated to enhance expression of the secretory component in rat epithelial cells, which is responsible for the transport of polymeric IgA into the intestinal lumen (31). In the present study, there were no changes in serum IgA and IgG with bovine colostrum supplementation, which confirms earlier results regarding IgG (32). It is suggested that, because IgA plays a major role in immunological protection of mucous membranes, it could also be possible (at least in theory) that dietary bovine colostrum may activate the immunological defense system against microbes on mucous membranes.

In conclusion, a bovine colostrum supplementation (Dynamic) increases serum IGF-I and saliva IgA concentrations in athletes during training. The increased
concentration of IGF-I may have positive effects on protein synthesis, and the increased saliva concentration of IgA may activate the immunological defense against microbes on mucous membranes.

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