Amino Acids (2005) 28: 327–335
DOI 10.1007/s00726-005-0179-8

Amino Acids
Printed in Austria

Protein metabolism and strength performance after bovine colostrum supplementation

A. Mero[1], **T. Nykänen**[1], **O. Keinänen**[2], **J. Knuutinen**[3], **K. Lahti**[3], **M. Alen**[4], **S. Rasi**[5], and **J. Leppäluoto**[5]

[1] Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland
[2] Clinical Physiology, Central Finlands Central Hospital, Jyväskylä, Finland
[3] Department of Chemistry, University of Jyväskylä, Jyväskylä, Finland
[4] Kuopio Research Institute of Exercise Medicine, Kuopio, Finland
[5] Department of Physiology, University of Oulu, Oulu, Finland

Received September 30, 2004
Accepted January 4, 2005
Published online March 25, 2005;

Summary. This study was designed to determine the responses of muscle protein, serum amino acids, and strength performance to bovine colostrum supplementation in physically active men. The rest (R) group ($n = 6$) and the exercise (E) group ($n = 6$) carried out twice a 2-week experiment randomly assigned in a double-blind fashion with either placebo (PLA; consuming daily 20 g maltodextrin) or bovine colostrum (COL; consuming daily 20 g colostrum supplement) treatment with one month between. On the test day after the treatment period the measurements were carried out in fasting conditions and E carried out a strength training session (STS). The methods involved the infusion of *ring*-$^2H_5$-phenylalanine, femoral arterial and venous blood sampling, and biopsies from the vastus lateralis muscle. Serum concentration of essential amino acids during recovery was greater ($p < 0.05$) in the COL groups compared with the PLA groups. Both muscle protein synthesis and breakdown increased ($p < 0.05$) with COL. There were no differences in phenylalanine net balance or strength performance between the PLA and COL groups. It was concluded that a 2-week supplementation with bovine colostrum in physically active men increases serum concentration of essential amino acids but has no effect either on strength performance or protein net balance in fasting conditions during recovery after STS.

Keywords: Amino acids – Protein synthesis – Protein breakdown – Physical training

Introduction

Skeletal muscle growth is dependent on the relationship between the rates of muscle protein synthesis and muscle protein breakdown. When muscle protein synthesis exceeds breakdown it is possible to have muscle hypertrophy. Consequently, muscle metabolism is anabolic. The important factors influencing muscle metabolism are exercise, nutrition, and hormonal status. Nutritional substrates especially daily protein (e.g. beef, fish, poultry, casein, whey, egg and soy) have a strong effect on muscle protein metabolism. Free amino acids are precursors for muscle protein synthesis.

The body of a 70 kg individual contains approximately 12 kg of protein, of which 40–45% is located in skeletal muscle and only 200–220 g are as free amino acids within the plasma and intra- and extracellular spaces called ''free amino acid pool'' (Wagenmakers, 1998). Events such as exercise or a change in dietary status can alter the overall size of the free pool and flux of amino acids into and out of the pool (Gibala, 2001). This magnitude cannot be fully understood by simply measuring changes in amino acid concentrations in blood and in muscle but it is necessary to measure also muscle protein synthesis and breakdown.

It has been shown that bovine colostrum (a high biological protein value) supplementation increases serum insulin like growth factor 1 (IGF-1) concentration in male athletes during a short (8 days) resistance and speed training period with no effect on vertical jump performance (Mero et al., 1997). The increased IGF-1 concentration was also observed in male and female athletes during a 2-wk resistance and endurance training period (Mero et al., 2002). Bovine colostrum supplementation associated with running program during 8 weeks improved recovery in endurance performance in physically active men but there were no effects on plasma IGF-1 concentration (Buckley et al., 2002). The researchers concluded that oral supplementation with intact bovine colostrum improves the ability to

perform a second bout of maximal endurance type exercise following a relatively short period of recovery from a prior bout of maximal exercise. This performance enhancement is supported by the study where an 8-wk bovine colostrum supplementation provided a significant improvement in time trial performance in cyclists after a 2-h ride at 65% VO2 max (Coombes et al., 2002). Bovine colostrum supplementation has been shown to enhance also an ability to repeat sprints in elite field hockey players (Smeets et al., 2000). On the other hand feeding colostrum has recently been shown to increase bone-free lean body mass in healthy trained adults (Antonio et al., 2001) and the synthesis of myofibrillar protein in the skeletal muscle of newborn piglets (Fiorotto et al., 2000). These findings suggest that bovine colostrum supplementation may have positive effects on muscle function and performance capacity of physically active people.

The purpose of the present study was to examine the responses of muscle proteins, serum amino acids, and strength performance to a 2-wk bovine colostrum (Dynamic^{TM} Colostrum) supplementation in physically active men. The product is a colostral whey product that has generally a high biological protein value. We hypothesized that if bovine colostrum supplementation increases amino acid and IGF-1 concentrations and given the anabolic nature of that growth factor (Kraemer, 1988), consequently bovine colostrum enhances protein synthesis and may affect strength performance. If this is the case bovine colostrum products may have positive nutritional applications not only in sport but also in the daily life, extreme conditions (e.g. military) and rehabilitation.

Material and methods

Subjects

Twelve healthy, physically trained men, who participated only in recreational noncompetitive athletic activity, volunteered as subjects for the study. Their (mean ± SD) age was 27 ± 5 yr, mean body height was 1.82 ± 0.05 m, and mean body mass was 84.6 ± 7.4 kg. All subjects were drug free, which was evaluated by interviews and questionnaires during medical examination. Furthermore, none of the subjects used supplements of amino acids, vitamins, minerals, creatine or any other supplement during the study phase which was instructed before the study and recorded from the food diaries. After a medical examination, the protocol and the potential benefits and risks were fully explained to each subject before he signed an informed consent document. This study was approved by both the University Ethical Board and the Hospital Ethical Board.

Experimental design

This study was a double-blind, placebo controlled trial in which subjects were divided in two groups (Fig. 1). In the rest (R) group six subjects did twice a 2-wk experimental treatment with either placebo (RPLA) or bovine colostrum (RCOL) with one month between. We chose as long as one month wash-out period to be sure that the muscles were washed (Mero et al., 1997). In the exercise (E) group six subjects did twice a 2-wk experimental treatment with either placebo (EPLA) or bovine colostrum (ECOL) with one month between. In the R group the measurements at the end of the 2-wk period were done in fasting and resting conditions whereas in the E group the measurements were done in fasting conditions but included a strength training session (STS). It was expected that the chronic effects of the supplementation would be clearer in the fasting conditions. Total fasting time was 15 ± 3 hours including overnight fasting time of 10 ± 3 hours and total experiment time of 5 hours. On the test

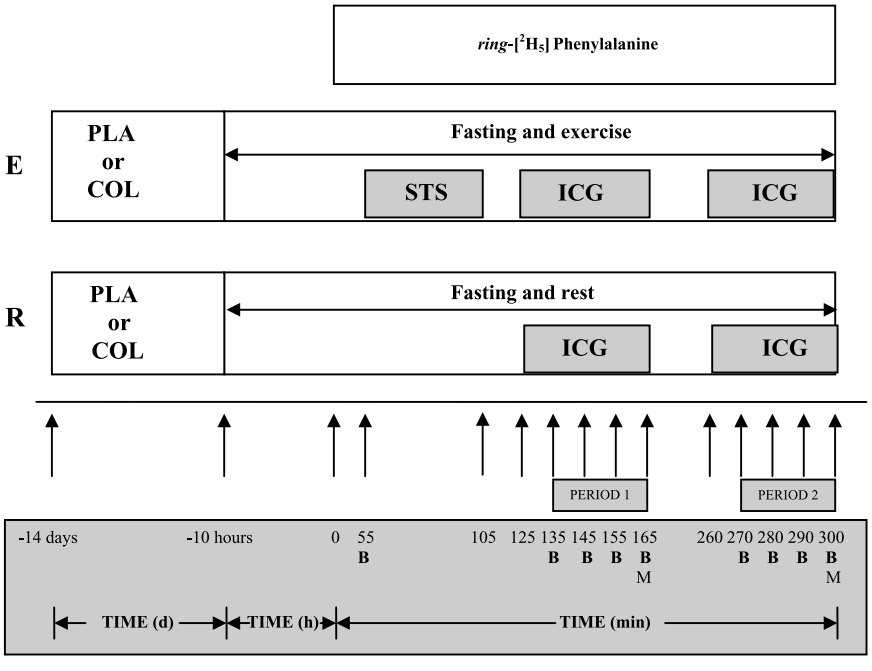

Fig. 1. Experimental design. *E*, exercise; *R*, rest; *PLA*, placebo; *COL*, colostrum; *STS*, strength training session; *ICG*, indocyanine green; *B*, blood sample; *M*, muscle biopsy

day the subjects were allowed to drink water (1500 ml in E and 1000 ml in R; 500 ml more in E because of the evaluated effect of exercise on need of water). All subjects were instructed not to change their dietary habits during 2 weeks before the test day. The subjects in the COL groups consumed 20 g Dynamic^{TM} Colostrum (it is a colostral whey product and is not on the banned substance list of the International Olympic Committee) supplement in 4 parts (the first at 0800 AM and then the next ones at 4 h intervals so that the last one was taken at 0800 PM) during every day of the 2-wk period. The daily amount of 20 g colostral whey powder (total energy 340 kJ a day) consisted of 6 g proteins (mostly immunoglobulins (4.5 g IgG and 0.3 g IgA), lactalbumin, lactoglobulin, lesser amounts growth factors (74 μg IGF-1) and antimicrobial proteins such as lactoferrin), 13 g carbohydrates (10 g maltodextrin and the rest 3 g galactose, glucose and some oligosaccharides), 1 g free amino acids and related compounds (as taurine), no casein or fat and electrolytes (Na, Ca, K, Mg, Mn). The total amount of free amino acids was 62 mg of which 25 mg were essential. In the PLA treatments the subjects consumed 20 g maltodextrin (total energy 340 kJ a day), respectively. The taste and color of the supplements were indistinguishable. All supplements were donated by Hi-Col Ltd (Oulu, Finland).

Training

All subjects were instructed to maintain current levels of exercise training during the first 2-wk period and repeat the training similarly during the second 2-wk period. Training was not allowed on the day before the measurements. The subjects kept training diaries which were included in the analysis of training.

Nutrition

The subjects were instructed to eat normally during the first 2-wk period. During the second 2-wk period they were advised to repeat the nutrition program they had followed. The subjects kept food diaries during 5 days before the testing day 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).

Strength training session and strength measurement

The subjects in E carried out a heavy hypertrofic strength training session (STS) of 50 minutes for lower extremities. It was started with a 10 min warm up on a bicycle and stretching exercises. Then in order to measure maximal strength and possible change in maximal strength after STS they performed 3 times a bilateral maximal voluntary isometric leg extension (MVC; maximal voluntary contraction) on a leg dynamometer before and after STS. In both cases 3 MVCs were performed allowing a 2 min recovery between trials. This measure of maximal strength was carried out on a sitting position on a chair so that the knee and hip angles were 90 and 110 degrees, respectively. The mean value of 3 trials was used both as a maximal strength measure and to calculate muscle fatigue during STS. In STS the subjects performed normal leg exercises (deep squat, hip extension, leg press) with a 10-repetition maximum (10-RM) load which was tested in the beginning of the 2-wk period. All repetitions were instructed to perform maximally and the amount of sets and repetitions were similar in both treatments.

Data collection protocol

In the beginning of the 2-wk period basal blood samples were taken from a left antecubital vein (AV) in the fasting conditions to determine IGF-1 concentration which was repeated after 2 weeks in the morning of the measurement day. One subject was measured per day using the same study protocol. The subjects fasted overnight and arrived the laboratory in the morning, where their body mass was measured. An 18-gauge polyethylene catheter was inserted into a left AV to take basal blood samples. After taking basal blood samples, a primed, continuous infusion of L-[*ring*-$^{2}H_5$] phenylalanine was started and maintained throughout the measurements. The prime was 2 μmol/kg and the infusion rate was 0.05 μmol/kg/min. At 125 minutes polyethylene catheters (20-gauge) were inserted into the right femoral artery and femoral vein, as well as an 18-gauge catheter was inserted into a right AV for drawing blood samples. The arterial catheter was used for the infusion of indocyanine green (ICG) for measuring blood flow (Fig. 1).

Collection of blood and muscle samples

The ICG infusion (0.5 mg/ml; 60 ml/h) was started 10 min before the first blood sample during recovery period 1 and 2. In period 1, the blood samples for isotopic measurements and blood flow were drawn at 135, 145, 155 and 165 min (period 1; 30, 40, 50, and 60 min following STS) after the initiation of L-[*ring*-$^{2}H_5$] phenylalanine infusion. In period 2, the blood samples for isotopic measurements were taken 270, 280, 290, and 300 min (165, 175, 185, and 195 min following STS) after the initiation of the infusion. Blood samples for free amino acid concentrations were taken at 135, 165, 270, and 300 min after the initiation of infusion. Blood flow samples were simultaneously drawn from a femoral vein (FV) and a right AV. The ICG infusion was briefly halted to allow sampling from the femoral artery (FA) for isotopic measurements. Blood for amino acid concentrations and enrichments was placed into preweighed tubes containing 2 ml sulfosalicylic acid and known amount of internal standard ($^{13}C_6$ phenylalanine; 50 μmol/l). Samples were mixed carefully and stored in ice. Blood flow samples were stored at room temperature until analysis. After the last blood sample in the two periods (165 min and 300 min) a muscle biopsy was taken for isotopic measurements and for analysis of phenylalanine concentration from the vastus lateralis (VL) muscle under local anesthesia. With the use of sterile technique, the skin and subcutaneous tissue were anesthetized and a 6–7 mm incision was made. A 4-mm biopsy needle was advanced 3–5 cm into the muscle with the closed cutting window. The cutting cylinder was opened and closed 2–4 times and a sample of 30–50 mg was obtained. Visible fat and connective tissue were removed and the samples were rinsed with ice-cold saline before storing into tubes in liquid nitrogen.

Analysis of enrichment and concentration of phenylalanine

Enrichment and concentration of phenylalanine in whole blood were measured by gas chromatography-mass spectrometry (GC/MS; Hewlett Packard Agilent 5973N, GC 6890 Plus + , USA). In order to determine the enrichment of infused amino acid in whole blood, the *tertiary*-butyl dimethylsilyl derivative was made. Isotopic enrichments were expressed as a tracer-to-tracee ratio. The concentration of phenylalanine was determined with an internal standard solution as previously (Wolfe, 1992; Biolo et al., 1995) described. Because the tube weight and the amount of blood were known, the blood amino acid concentration was determined from the internal standard enrichment on the basis of the amount of blood and internal standard added. Appropriate corrections were made for any spectra that overlapped, contributing to the tracer-to-tracee ratio (Wolfe, 1992).

Muscle tissue samples were analyzed for phenylalanine enrichment. On thawing, the tissue was weighed and the protein was precipitated with 0.8 ml of 14% percholoroacetic acid. The tissue was then homogenized and centrifuged, and the supernatant was collected. This procedure was repeated one more time and the collected supernatant was processed like blood samples. The concentration of phenylalanine in muscle was then analyzed using HPLC equipped with fluorescent detector similarly to blood samples (see below).

Analysis of leg blood flow

Leg blood flow was determined from blood samples collected during a continuous infusion of ICG (Jorfelt and Wahren, 1971). The blood flow

was determined by spectrophotometrically measuring the ICG concentration in serum from the femoral vein and the peripheral vein as described previously (Jorfelt and Wahren, 1971; Biolo et al., 1995). Leg plasma flow was then calculated from steady-state values of dye concentration and converted to blood flow with the hematocrit. Serum from the blood samples was analyzed in a spectrophotometer with absorbance set at 805 nm.

Calculations

The three-compartment model of leg muscle amino acid kinetics has been described previously by Biolo et al. (1995). The use of this model allows to determine the rate of utilization of phenylalanine for muscle protein synthesis and appearance from breakdown, because phenylalanine is neither oxidized nor synthesized in muscle. The average values for blood flow, blood and muscle phenylalanine concentrations, and enrichments were calculated from individual samples drawn during two periods (period 1 and period 2). Muscle protein net balance was determined by calculating the difference between muscle protein synthesis and muscle protein breakdown. The detailed model assumption has been described earlier (e.g. Biolo et al., 1995).

Analysis of serum free amino acid concentrations

Concentrations of free amino acids in serum were determined applying the procedure of Pfeifer et al. (1983) by reversed phase high performance liquid chromatography (RPHPLC) (Waters 501 pumps, Waters 717 autosampler and Zorbax C_{18} column). The 18 essential amino acids and two internal standards (β-Abc and Nor-Valine) were detected by Perkin Elmer LS-4 fluorescent detector using wavelengths 338 nm (exitation) and 455 nm (emission). 100 μl of internal standard solution was added to the serum sample (50 μl) and acetonitrile (100 μl) was used to precipitate the proteins. 750 μl of distilled deionized water was added and the resulted sample was vortexed and allowed to stand on ice bath for 1 h. The 200 μl of the sample was transferred to the ultraspin centrifuge filter and centrifuged. The clear mixture was transferred to the HPLC vial, derivatized with OPA derivatizing solution and analyzed by Waters HPLC system using gradient two-buffer elution.

Analysis of blood lactate and serum IGF-1

Blood samples from a fingertip for peak lactate analysis were drawn immediately, 2.5 and 5 minutes after STS. They were analyzed using a Lactate Pro™ Analyzer (KDK Co, Kyoto, Japan).

Serum IGF-1 was analyzed from the blood samples taken in the morning after 10 h of fasting before and after the 2-wk period in duplicate with an OCTEIA IGF-1 kit, which is a two-site immunoenzymometric assay (IEMA) for the quantitative determination of IGF-1 in human serum. The IGF-1 was dissociated from binding proteins by using the releasing agent according to the instructions of the manufacturer of the OCTEIA IFG-1 Kit. The method incorporates a sample pretreatment to avoid interference from binding proteins. According to the manufacturer the sensitivity (a least detectable dose) of the kit is 0.25 nmol/L and the intra-assay coefficient of variation is below 10% corresponding approximately to 1.2 nmol/L.

Statistics

Multivariate ANOVA to produce the F statistics was used to detect the presence of a significance of the main effects of test group (rest/exercise), vessel (artery/vein), treatment (placebo/bovine colostrum) and time and their interactions. In the analyses the test group factor was analyzed as between subject factor and all the other factors as within subject factors. If statistically significant interactions were found some addition examinations were done. 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. In addition, Student's t-test was used in selected pair comparisons. Statistical significance was set at $p < 0.05$. Data was analyzed using the SPSS 10.1. for Windows software package.

Results

Training and nutrition

There were no differences in the training volume during two weeks before the measurement day between the groups. The amounts of training sessions during the period were 8 ± 3 (mean $\pm$ SD) in RPLA, 9 ± 3 in RCOL, 8 ± 3 in EPLA, and 8 ± 3 in ECOL. One training session lasted 1.2 ± 0.2 h. Total training included 70% aerobic work (running, cycling) and 30% strength training in the gym.

The average daily energy intake during the measured period was similar in all groups (Table 1).

Table 1. Calculated daily energy intake and macronutrient composition in the groups. Energy from bovine colostrum or placebo is not included in the values

Variable	RPLA	RCOL	EPLA	ECOL
Energy (kcal/kg BW)	26.5 ± 5.5	27.0 ± 5.7	28.8 ± 5.3	29.0 ± 5.4
Protein				
g/kg BW	1.2 ± 0.4	1.2 ± 0.3	1.4 ± 0.4	1.4 ± 0.3
%	18.3 ± 6.6	18.1 ± 6.6	18.3 ± 3.9	18.4 ± 4.0
Carbohydrate				
g/kg BW	3.5 ± 1.0	3.7 ± 1.1	3.9 ± 1.4	3.8 ± 1.5
%	52.6 ± 5.4	53.0 ± 5.5	53.7 ± 7.2	53.5 ± 6.9
Fat				
g/kg BW	0.9 ± 0.3	0.8 ± 0.3	0.7 ± 0.4	0.7 ± 0.5
%	29.2 ± 6.4	29.0 ± 6.0	28.0 ± 6.6	29.2 ± 7.0
Body mass (kg)	83.6 ± 7.3	83.9 ± 6.8	85.2 ± 8.0	85.6 ± 7.4

Values are mean ± SD

Strength performance

Maximal isometric strength was in the beginning of STS in EPLA 4880 ± 1774 N and at the end of STS 4724 ± 1699. The respective values in ECOL were 4770 ± 1390 N and 4629 ± 1240 N. There were no differences in maximal isometric strength between the groups. The deterioration of the performance during STS was in both cases similar and significant (3%; $p < 0.05$). Peak blood lactate following STS was similar in the groups (12.8 ± 2.3 mmol/l in EPLA and 12.5 ± 2.8 mmol/l in ECOL).

Serum amino acid concentration

There were no differences in amino acid concentrations either between arteries and veins or in resting situation before STS between the groups. The serum concentration (mean value of artery and vein) of essential amino acids (EAAs) (Fig. 2) and branched chain amino acids (BCAAs) (Fig. 3) were greater (treatment effect $p < 0.05$–0.01) in the COL groups compared with the PLA groups. The similar treatment effect was observed in single amino acids leucine (16%; $p < 0.05$), isoleucine (18%; $p < 0.01$), valine (14%; $p < 0.01$) and phenylalanine (19%; $p < 0.05$) concentration which were greater in the COL groups than in the PLA groups. The serum concentration (venous value) of leucine was greater ($p < 0.05$) before STS than during recovery after STS in EPLA but not in ECOL (Fig. 4). The venous concentration of alanine was greater ($p < 0.01$) at 30 min and 60 min of recovery after STS compared with the before value in EPLA (Fig. 5).

Protein synthesis, breakdown and net balance

Protein synthesis and protein breakdown increased ($p < 0.05$) with COL but protein net balance was similar with COL and PLA during 3 h recovery after STS (Fig. 6). Furthermore protein synthesis and protein breakdown were significantly (exercise effect $p < 0.05$) greater (22% and 24%) in EPLA than in RPLA and greater (21% and 25%) in ECOL than in RCOL during period 2.

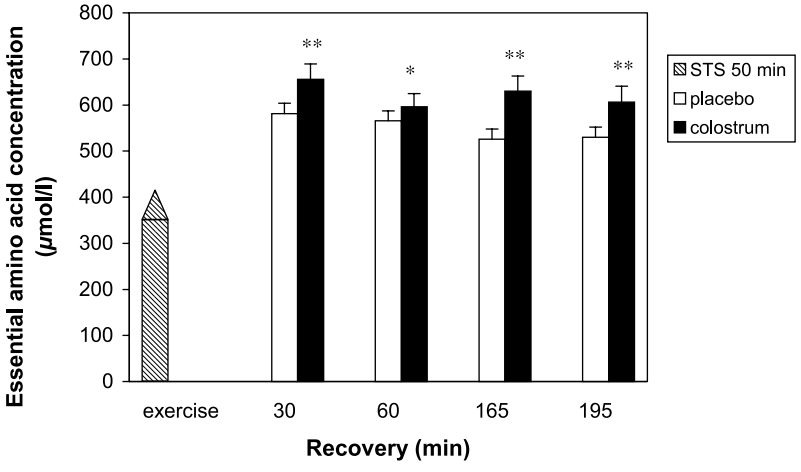

Fig. 2. Responses of serum essential amino acid concentration with placebo and bovine colostrum treatments after 2 weeks in all subjects. Values are mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$. Significantly different from placebo values

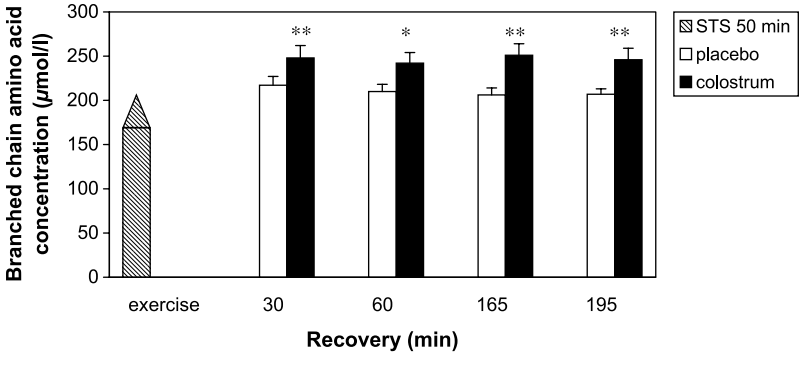

Fig. 3. Responses of serum branched amino acid concentration with placebo and bovine colostrum treatments after 2 weeks in all subjects. Values are mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$. Significantly different from placebo values

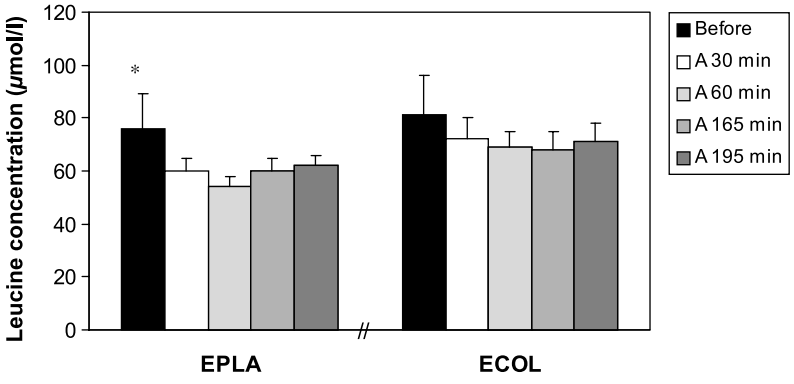

Fig. 4. Responses of serum leucine concentration with placebo and bovine colostrum treatments after 2 weeks before and after STS. Venous values are mean ± SE. A = after, $^{*}p < 0.05$. Before value is significantly different from after values in the exercise placebo group (*EPLA*)

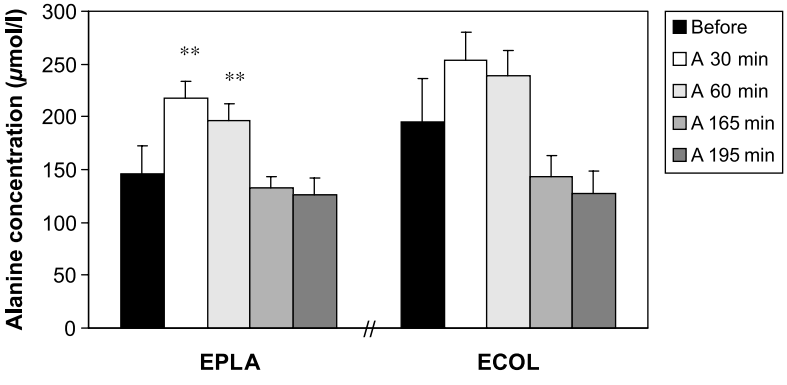

Fig. 5. Responses of serum alanine concentration with placebo and bovine colostrum treatments after 2 weeks before and after STS. Venous values are mean ± SE. A = after, $^{**}p < 0.01$. After 30 min and after 60 min values are significantly different from before value in the exercise placebo group (*EPLA*)

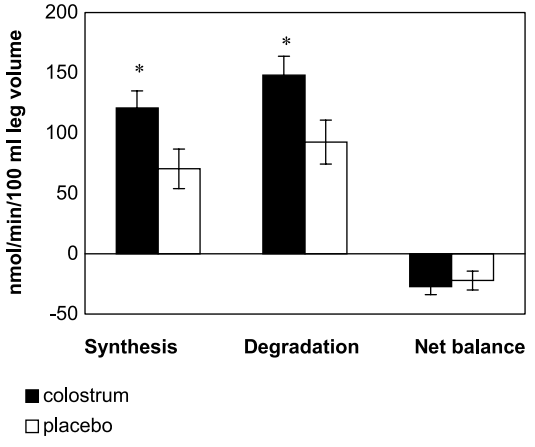

Fig. 6. Protein synthesis, protein degradation and protein net balance with placebo and bovine colostrum treatments after 2 weeks during 3 h recovery (both period 1 and 2) after STS. Values are mean ± SE. $^{*}p < 0.05$

Serum IGF-1

There were no differences in serum IGF-1 concentration between RPLA, RCOL, EPLA, and ECOL when comparing the fasting values before and after the 2-week period. In all placebo subjects (n = 12) IGF-1 concentration was 20.0 ± 7.6 nmol/l in the beginning of the 2-week period and 20.1 ± 7.7 nmol/l at the end of the period. The respective values in all colostrum subjects (n = 12) were 18.7 ± 7.4 nmol/l and 18.8 ± 6.4 nmol/l.

Discussion

The main results of the present study showed that bovine colostrum supplementation in physically active men during 2 weeks increased both serum essential and branched chain amino acid concentration. Furthermore, there was in fasting conditions a subsequent increase in muscle protein synthesis and breakdown but not in protein net balance during 3 h recovery following STS. Bovine colostrum supplementation did not change either strength performance or serum IGF-1 concentration.

Serum amino acid concentration

The concentration of EAAs increased with bovine colostrum supplementation by 13% and the respective increase in BCAA concentration was 15% compared with placebo. These treatment findings are different from the

earlier observation (Mero et al., 1997) which did not show any changes in amino acid concentrations. The daily amount of supplemented bovine colostrum protein (6 g) was over two times greater in the present study compared with the earlier study and the supplementation period was also now longer (14 days vs. 8 days). STS achieved a strong decrease in leucine concentration in EPLA which confirms the earlier studies (Pitkänen et al., 2002). The supplementation period changed the concentration of leucine during recovery and the decrease was not any more significant. Leucine amounts to about 4.6% of all amino acids (Takala et al., 1980) and it has many important roles in the body during intensive exercise (e.g. Mero, 1999). It seems that bovine colostrum supplementation enhances leucine concentration in serum and this may stimulate protein synthesis and leucine oxidation. The concentration of alanine increased strongly immediately after STS in EPLA supporting the earlier finding (Pitkänen et al., 2002) but when supplemented with bovine colostrum the increase was not significant. Alanine is released from the muscles in the greatest amounts during exercise (Felig, 1973). Also the oxidation of leucine in muscle increases the output of alanine which is further used for gluconeogenesis in glucose-alanine cycle (Babij et al., 1983). With bovine colostrum supplementation the increased leucine concentration may have slightly enhanced alanine formation before STS and then the concentration did not change so much during recovery. These results indicate that bovine colostrum supplementation including 6 g protein daily during two weeks increases strongly basic amino acid concentration of EAAs and the changes in the concentration of leucine and alanine during recovery after STS are smaller compared with PLA.

IGF-1 concentration

The concentration of circulating IGF-1 did not change with bovine colostrum supplementation during two weeks opposite to what we found in the earlier study (Mero et al., 2002) where there was a 17% increase in IGF-1. In both studies similar amount of supplements were used during two weeks. Nutrition is one of the main regulators of circulating IGF-1. In humans, serum IGF-1 concentration is markedly lowered by energy and/or protein deprivation (e.g. Isley et al., 1983) so both energy and proteins are critical in the regulation of serum IGF-1 concentration. However, in the present study the average daily energy and protein intake values were in the range of normal active people and similar in the PLA and COL groups. So it seems that nutrition affected in the similar way in the groups. Resistance training with low loads and many repetitions (fitness training) can also affect circulating IGF-1 in both humans and animals (Jahreis et al., 1991; Cooper et al., 1994). In the present study physical activity was mainly aerobic and similar (from 8 to 9 times training during 14 days) in both groups. The only difference in the studies (Mero et al., 1997; Mero et al., 2002), where the concentration of circulating IGF-1 has increased with supplementation, is that the subjects have been mainly competitive power-type athletes whose training included more strength and anaerobic exercises. In this respect there were also in the other studies (Buckley et al., 2002; Coombes et al., 2002) a large amount of aerobic type endurance training and there were no changes in circulating IGF-1 after oral bovine colostrum supplementation. Consequently, we can only speculate that if intensive endurance training leads to decreased IGF-1 concentrations in competitive athletes so bovine colostrum supplementation may stimulate circulating IGF-1 concentrations to rise to the normal level.

Protein balance

With the increased concentration of EAAs with bovine colostrum supplementation there was an increase in protein synthesis and in protein breakdown but not in protein net balance between the PLA and COL treatments. This is in part supported by the earlier study (Fiorotto et al., 2000) where colostrum feeding in the newborn pigs resulted in a 2.5- to 3.0-fold increase in total skeletal muscle protein synthesis compared with water feeding. The increase was also 28% greater than in the mature sow's milk fed piglets. The authors concluded that feeding stimulated muscle ribosome and total polyadenylated RNA accretion and ribosomal translational efficiency was similar across all fed groups. The greater stimulation of protein synthesis in colostrum fed pigs was restricted entirely to the myofibrillar protein compartment and was associated with higher ribosome and myosin heavy chain mRNA abundance. Also in active men and women the supplementation with bovine colostrum (20 g/day) in combination with exercise training for 8 weeks increased bone-free lean body mass (Antonio et al., 2001). In our study there were no changes in body mass which partly supports our protein balance result which was negative. But it must be noticed that the supplementation period in the present study was only 2

weeks compared with 8 weeks in the study by Antonio et al. (2001) and the measurements were done in fasting conditions. By flooding with EAAs has been shown to stimulate muscle protein synthesis (Smith et al., 1998) and ingestion of 40 g of amino acids in small increments over 3 h after resistance exercise increased net muscle protein balance, with EAAS providing the same response as mixed amino acids (Tipton et al., 1999). Also EAAs with carbohydrates (6 g EAAs and 35 g sucrose) stimulated muscle protein anabolism by increasing muscle protein synthesis when ingested 1 or 3 h after resistance exercise (Rasmussen et al., 2000). According to the earlier results it seems that EAAs are the primary stimulators of muscle protein synthesis and the effectiveness of the supplement may be increased using carbohydrates. In the absence of an increase in amino acid concentration, an increase in insulin has only a modest effect on muscle protein synthesis (see Tipton and Wolfe, 2001). However, in the present study the supplementation with protein and carbohydrate did not affect protein net balance. One explanation probably is that the measurements were done in fasting conditions as the method has been suggested to carry out earlier (Biolo et al., 1992; Biolo et al., 1995). This changes the metabolic situation and protein net balance is negative as we found. Secondly, there were no changes in circulating IGF-1 concentration with bovine colostrum which consequently did not give any extra increase in stimulation of protein synthesis. In the present study following bovine colostrum supplementation STS increased both protein synthesis and protein breakdown but protein net balance was similar and negative during 3 h of recovery which in part confirms earlier studies in fasting conditions (see Tipton and Wolfe, 2001).

Conclusion

A 2-wk supplementation with bovine colostrum in physically active men results in a statistically significant increase of serum essential amino acid concentration but has no effects on protein net balance or strength performance in fasting conditions during recovery after a strength training session.

Acknowledgements

The authors wish to thank Robert R. Wolfe, Ph.D., for his assistance and guidance to learn a three-compartment model of leg muscle amino acid kinetics in Medical Branch and Shriners Burns Institute, Calveston, Texas 77550. The authors thank also Niina Vehviläinen for help in data collection, Risto Puurtinen for help in blood collection, and Hannu Tuuri for valuable technical assistance in statistical analysis. This study was supported by TEKES grant 40721/00 and a grant from Peurunka Medical Rehabilitation Center, Laukaa, Finland.

References

Antonio J, Sanders MS, Van Gammeren D (2001) The effects of bovine colostrum supplementation on body composition and exercise performance in active men and women. Nutrition 17: 243–247

Babij P, Matthews SM, Rennie MJ (1983) Changes in blood ammonia, lactate and amino acids in relation to workload during bicycle ergometer exercise in man. Eur J Appl Physiol 50: 405–411

Biolo G, Chinkes D, Zheng XJ, Wolfe RR (1992) A new model to determine *in vivo* the relationship between amino acid transmembrane transport and protein kinetics in muscle. J Parenter Enteral Nutr 16: 305–315

Biolo G, Declan Fleming RY, Maggi SP, Wolfe RR (1995) Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. Am J Physiol 268: E75–E84

Buckley JD, Abbott MJ, Brinkworth GD, Whyte PBD (2002) Bovine colostrum supplementation during endurance running training improves recovery but not performance. J Sci Med Sport 5: 65–79

Coombes JS, Conacher M, Austen SK, Marshall PA (2002) Dose effects of oral bovine colostrum on physical work capacity in cyclists. Med Sci Sports Exerc 34: 1184–1188

Cooper DM, Moromisato DY, Zanconato S, Moromisato M, Jensen S, Brasel JA (1994) Effect of growth hormone suppression on exercise training and growth responses in young rats. Pediatr Res 35: 223–227

Felig P (1973) The glucose-alanine cycle. Metabolism 22: 179–207

Fiorotto ML, Davis TA, Reeds PJ, Burrin DG (2000) Nonnutritive factors in colostrum enchance myofibrillar protein synthesis in the newborn pig. Pediatr Res 48: 511–517

Gibala MJ (2001) Regulation of skeletal muscle amino acid metabolism during exercise. Int J Sport Nutr Exerc Metab 11: 87–108

Isley WL, Underwood LE, Clemmons DR (1983) Dietary components that regulate serum somatomedin-C concentrations in humans. J Clin Invest 71: 175–182

Jahreis G, Kauf E, Frohner G, Schmidt HE (1991) Influence of intensive exercise on insulin-like growth factor 1, thyroid and steroid hormones in female gymnasts. Growth Regul 1: 95–99

Jorfelt L, Wahren J (1971) Leg blood flow during exercise in man. Clin Sci 41: 459–473

Kraemer WJ (1988) Endocrine responses to resistance exercise. Med Sci Sports Exerc 20 [Suppl]: 152–157

Mero A (1999) Leucine supplementation and intensive training. Sports Med 27: 347–358

Mero A, Miikkulainen H, Riski J, Pakkanen R, Aalto J, Takala T (1997) Effects of bovine colostrum on serum IGF-1, IgG, hormone, and saliva IgA during training. J Appl Physiol 83: 1144–1151

Mero A, Kähkönen J, Nykänen T, Parviainen T, Jokinen I, Takala T, Nikula T, Rasi S, Leppäluoto J (2002) IGF-1, IgA and IgG responses to bovine colostrum supplementation during training. J Appl Physiol 93: 732–739

Pfeifer R, Karol R, Korpi J, Burgoyne R, McCourt D (1983) Practical application of HPLC to amino acid analysis. Am Lab 15: 78–87

Pitkänen H, Mero A, Oja SS, Komi PV, Pöntinen PJ, Saransaari P, Takala T (2002) Serum amino acid responses to three different exercise sessions in male power athletes. J Sports Med Phys Fitness 42: 472–480

Rasmussen BB, Tipton KD, Miller SL, Wolf SE, Wolfe RR (2000) An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. J Appl Physiol 88: 386–392

Smeets R, Hofman Z, Verlaan G, Lugt R, Verstappen P (2000) Oral supplementation with bovine colostrum (Intact^{TM}) improves sprint

performance in elite hockey players (abstract). J Strength Con Res 14: 370

Smith K, Reynolds N, Downie S, Patel A, Rennie MJ (1998) Effects of flooding amino acids on incorporation of labelled amino acids in the human muscle protein. Am J Physiol Endocrinol Metab 275: E73–E78

Takala T, Hiltunen K, Hassinen E (1980) The mechanism of ammonia production and the effect of mechanical work load on proteolysis and amino acid catabolism in isolated perfused rat heart. Biochem J 192: 285–295

Tipton KD, Ferrando AA, Phillips SM, Doyle D, Wolfe RR (1999) Postexercise net protein synthesis in human muscle from orally administered amino acid. Am J Physiol Endocrinol Metab 276: E628–E634

Tipton KD, Wolfe RR (2001) Exercise, protein metabolism, and muscle growth. Int J Sport Nutr Exerc Metab 11: 109–132

Wagenmakers AJ (1998) Muscle amino acid metabolism at rest and during exercise: role in human physiology and metabolism. Exerc Sports Sci Rev 26: 287–314

Wolfe RR (1992) Radioactive and stable isotope tracers in biomedicine. Principles and practice of kinetic analysis. Wiley-Liss, New York, pp 49–86

Authors' address: Antti Mero, Department of Biology of Physical Activity, University of Jyväskylä, P.O. Box 35, 40014 University of Jyväskylä, Finland,

Fax: 358-14-2602071, E-mail: antti.mero@sport.jyu.fi