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

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ABSTRACT

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 IGF-1 (rhIGF-1) labeled with ^{123} iodine (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 Dynamic supplement and the latter 20 g maltodextrin during a 2 week training period. After bovine colostrum supplementation significant increases were noticed in serum IGF-1 ($p < 0.01$) 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 at 60 min after ingestion of ^{123} I-rhIGF-1 and showed peaks at 0.6-kDa and at 40 - 90-kDa, 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-1 and saliva IgA concentration in athletes during training. Absorption data shows that ingested ^{123}I-rhIGF-1 is fragmented in circulation and no radioactive IGF-1 is eluted at the positions of free or the IGF binding proteins giving no support to the absorption of IGF-1 from bovine colostrum.

Keywords: physical training; health of athletes; rhIGF-1 absorption

INTRODUCTION

It has been shown that bovine colostrum supplementation increases serum IGF-l 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 among bovine colostrum supplementation and health status in female endurance athletes during a winter competition period although any physiological explanations were not found for that (33). Buckley et al. (5) showed that bovine colostrum supplementation associated with running program during eight weeks improved endurance performance in physically active men but there were no effects on plasma IGF-1 concentrations. 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 is partly supported by Smeets et al. (42) who showed that bovine colostrum supplementation increased 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 insulin-like growth factors 1 and 2 (IGF-1 and IGF-2, respectively; 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-l is a major form in bovine colostrum and the concentration is 7-67 nmol/l (40), whereas normal milk contains < 0.3 nmol/l (7). In normal adult humans, IGF-l occurs at a concentration of ~ 7 nmol/l in serum (19). IGF-1 has a strong anabolic effect on muscle tissue (25, 44), and it is associated with regulatory feedback of growth hormone (25, 38). IGF-1 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-1 (22).

Antimicrobial factors in bovine colostrum include immunoglobulins, lactoperoxidase, lysozyme, and lactoferrin. Bovine colostrum is an extremely rich source of immunoglobulins. The concentrations of immunoglobulin G (IgG), M (IgM) and A (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-1 and serum and salivary immunoglobulins. We further hypothesized that the bovine colostrum supplement enhances serum IGF-l and immunoglobulin concentrations in athletes, both in males and in females during training. This hypothesis is supported by the IGF-1 results in male athletes (32) and in calves (17, 39). In addition, orally administered ^{125} iodine-labeled IGF-1 has been demonstrated to be transported into circulation in calves (2) and neonatal pigs (47). Because the mechanism of the increased serum IGF-1 concentration with the supplementation is not defined we additionally tried to investigate the absorption of orally administered rhIGF-1 (labeled with ^{123} iodine) 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) years) 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 using questionnaires. Furthermore, none of the subjects used supplements of amino acids, vitamins, minerals, or creatine monohydrate or any other supplement during the study phase. The athletes were members of the track and field association, ski association and orienteering association and they could have been tested for doping by either national or international testing groups. No doping tests were carried out during the study period. **In study 2** there were six male (age 29.1±2.8(SE) yr; mass 75.8±3.0 kg; height 1.80±0.01 m) and six female (age 23.9±0.6 yr; mass 63.0±4.0 kg; height 1.69±0.03 m) subjects. All 12 subjects participated only in recreational noncompetitive athletic activity. **TABLE 1 HERE**

The protocol and the potential benefits and risks associated with participation were fully explained to each subject before he or she signed an informed consent document. The study was approved by the University Ethical Board.

Experimental protocol in study 1. All subjects were initially familiarized (4 wk) with the following important parts (training and nutrition) of the study. In the

protocol (Figure 1) each subject underwent 2 weeks experimental treatment including 4 measurement day for fasting blood and saliva samples. During the familiarization period and the study phase the subjects were advised to eat according to the principles of the general nutritional recommendations with some extra carbohydrates, sport drinks and water. The subjects were not allowed to consume caffeine, alcohol, or specific nutritional supplements (e.g. creatine, multivitamins, multiminerals). The subjects in the experimental group consumed 20 g Dynamic™ Colostrum supplement in four parts during every day of the 2 week period. The amount of 20 g contained 74 µg IGF-1, 4.5 g IgG and 0.3 g IgA (total energy 340 kJ: 6 g protein, 14g carbohydrates). In the placebo treatment the subjects consumed 20 g maltodextrin (total energy 340 kJ), respectively. The taste and color of the supplements were indistinguishable. All supplements were donated by Hi-Col Ltd (Oulu, Finland).

Training in study 1. All subjects were active athletes and the volume and intensity of their training was planned and supervised by the researchers and coaches in collaboration. The study period took place during a training season. During the 4-wk familiarization period the volume and intensity were similar to that in the study phase. The subjects kept training diaries which were included in the analysis of training.

Nutrition in study 1. The subjects kept food diaries during 2-wk study period and 4 days (see Figure 1) were included in the analysis of nutrition. It was performed by using the Micro Nutrica software (version 2.0, Social Insurance Institution, Finland).

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 ^{123}I-rhIGF-1 treatment (50 MBq) in the morning, several blood samples, gamma-camera imaging and ate a standard breakfast (energy 2.0MJ, CHO 60%, fat 25% and protein 15%) and lunch (energy 2.5MJ, CHO 60%. fat 25% and protein 15%) during 7h (Figure 2).

Blood and saliva collection and analysis in study 1. Blood samples (5 ml) for IGF-1, 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 frozen at -20°C. The samples that required storage were stored for no longer than 3 mo and thawed only once for analysis. Saliva sample (8 ml) for IgA was taken also in the morning.

Serum IGF-1 analysis (studies 1 and 2). Serum IGF-1 was analyzed in dublicate with an OCTEIA IGF-1 kit , which is two-site immunoenzymometric assay (IEMA) for the quantitative determination of IGF-1 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 plus 2 standard deviations 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 below 10%, is approximately 1.2 nmol/L.

Analysis of immunoglobulins (study 1). Serum immunoglobulins IgA and IgG and saliva IgA were analyzed with clinical chemistry analyser KONE Specific Supra. The methods are based on measurement of immunoprecipitation enhanced by polyethylene glycol (PEG) at 340 nm. Specific antiserum 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 solution.

Iodination of IGF-1 (study 2). Recombinant human IGF-l (rhIGF-1, R&D System, Minneapolis, USA) was labeled using chloramine-T method with iodine-123 (^{123}I) for oral administration into human subjects. Briefly: 400 MBq of ^{123}I (MAP Medical Technologies Co., Finland) in 0.18 mol/L phosphate buffer pH 7.5 was mixed with 50 µg rhIGF-l and 100 µL freshly prepared chloramine-T solution (1 mg/ mL), and allowed to react and then purified using HPLC with reverse phase chromatography (µ-Bondapak C18 column, 3.9x400 mm, Waters, Milford, USA). 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, Finland) and a UV-detector (Waters model 440, Ma, USA) at 254 nm. The fractions containing radio labeled rhIGF-1 were evaporated under vacuum, dissolved into 2% human serum albumin and filtered (0.22 µm).

Binding of iodinated IGF-1 to receptors (study 2). Biological functionality of the batches of ^{123}I-labeled rhIGF-1 was checked with binding to the membrane receptors for IGF-1 (18, 36). Human placenta, used in 1 hour from delivery, were washed with ice-cold PBS, dissected into 10g samples and stored at -80°C. Placental membranes possessing receptors for IGF-1 were isolated using the method of Pekonen et al. (34). Membranes were stored at -80°C until used for binding studies. Placental membranes (2 mg protein) were incubated over night at +4°C with 10 ng of ^{123}I-labeled rhIGF-1 together with a graded series of unlabeled rhIGF-1 (0.l ng-1000ng). Cold buffer was added and the tubes were centrifuged. After removing the supernatant the radioactivity of the pellets, as well as aliquots of the supernatants, were measured with a gamma counter (RackGamma, LKB Wallac, Sweden). The radioactivity of the supernatant trapped in the pellet was subtracted from radioactivity of each pellet.

Serum radioactivity and TCA-precipitated radioactivity (study 2). The radioactivity of serum samples after intake of ^{123}I-labeled rhIGF-1 were measured. Further, 1 ml of serum was mixed with trichloric acetic acid (TCA, final concentration 10%) and incubated for 60 min at +4°C. Proteins were pelleted with centrifugation 4000 x g for 10 min. The pellets were washed once with 10% TCA, 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-1 were separated using a routine procedure of gel filtration with Sephadex G-100 matrix , pump P1, detector UV-1 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 NaCI and EDTA 1 mg/ml) the column (16x250 mm) was calibrated with marker proteins: aldolase 150-kDa, oval albumin 43.1-kDa, ribonuclease-A 13.7-kDa), aprotinin 6-kDa and tripeptide Pro-Phe-Arg 0.6-kDa. Fractions from eluted serum samples were collected and their radioactivity was measured.

Statistics. Multivariate analysis of variance (MANOVA) 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 using univariate results subsequent to MANOVA, and they provided a measure of significance between pairwise differences. Furthermore, trends over time during the 2-week period were examined separately for each treatment. The level of significance was set at $P<0.05$.

RESULTS

Study 1

IGF-1, IgA and IgG. There were no differences in IGF-1, IgA and IgG between genders and the results in all subjects are presented in Figs. 3-6. Significant increases were observed in serum IGF-1 (17%; $p < 0.01$) and saliva IgA (33%; $p < 0.01$) in the experimental group (Dynamic) but not in the control group (Placebo) during two weeks. 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 in 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 two weeks. The average duration of one training session was 1.2±0.1 hours. Total training included 75 % event training and 25% strength training. Event training included 65% aerobic and 35% anaerobic training.

IGF-1. There were no differences between genders in serum IGF-1 on the test day. During the first 180 minutes following ^{123}I-rhIGF-1 treatment there were no changes in the IGF-1 concentration but after the standard lunch at 420 minutes the increase compared with the starting value was significant (17%; $p < 0.001$) in all subjects (Fig. 7).

Binding of ^{123}I-rhIGF-1 to receptors. In each of the batches of ^{123}I-rhIGF-1 half-maximal inhibition of binding of labeled-IGF-1 was observed when labeled and unlabeled IGF-1 were present at equivalent concentrations (10 ng/ml) indicating that iodination had resulted in labeled rhIGF-1 with preserved biological functionality.

Radioactivity of plasma and TCA precipitate radioactivity. Radioactivity after ingestion of ^{123}I- rhIGF-1 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 weight molecules.

TABLE 2 HERE

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

DISCUSSION

The results of this study showed that there was a 17% increase in circulating IGF-1 and a 33% increase in saliva IgA in the Dynamic treatment during a 2-week 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-1 was fragmented when in circulation and only a minor proportion of the broad peak may represent unbound intact IGF-1.

The concentration of circulating IGF-1 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 $\cdot l^{-1} \cdot$ day $^{-1}$) than the respective value (0.54 nmol$\cdot l^{-1} \cdot day^{-1}$) in the

earlier study in which the supplementation period was 8 days. Normally daily variations of IGF-1 are little or there is none (43). In the earlier study Bioenervi contained 67.6 $\mu g \cdot l^{-1}$ IGF-1 and the respective value in the present study was almost the same (74 $\mu g \cdot l^{-1}$) during a day. Because the amino acid sequences of human and bovine IGF-1 are identical (16), the method used in our study measured the total amount of IGF-1 (both bovine and human).

The possible increase in serum IGF-1 can be due to either direct absorption of the growth factor from Dynamic or enhanced stimulation of human IGF-1 synthesis. In animal studies it has been shown that both dietary colostrum (39, 17) and purified recombinant ^{125}I-IGF-1 (3) increased blood IGF-1 concentration in calves. However, in adult rats it has been observed that most of IGF-1 is degraded in gastrointestinal tract (46). In addition, orally administered ^{125}I-IGF-1 has been demonstrated to be transported into circulation in calves (2). In the present study following orally administered ^{123}I-rhIGF-1 radioactivity appeared in circulation very soon but major portion (about 96%) of the labeled substance was of low molecular weight. The rest was eluted at broad peak between 40 and 90-kDa. IGF-1 is a 7.5-kDa polypeptide and most of it circulates in a 150-kDa, high-affinity complex, which also contains IGF binding protein 3 and an acid-labile subunit (41). IGF-1 also circulates bound to lower molecular weight 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-1 bioavailability (26). Less than 1% of IGF-1 is thought to circulate in a free (7.5-kDa) or rapidly dissociable state and is thought to be readily available to mediate 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-1 are circulating after the oral intake of the ^{123}I-rhIGF-1 compound. We emphasize that we did not find any radioactivity at elution positions of free IGF-1 (7.5-kDa).

In order to test the hypothesis if fitness training would lead to increases in circulating IGF-1 in both humans and animals is not consistently observed (8, 21). Physical activity and VO_2 max have found to be related positively to serum IGF-1 in men (35). The 5 week of increased physical activity led to drops in circulating IGF-1 despite training-induced increases in muscle volume in adolescent females (14%; 12) and in adolescent males (12%; 13). In sedentary adult men and women there has been an increase (20%) in circulating IGF-1 following 13 weeks resistance training but no further increases occurred between 13th and 25th week which was the follow-up period (4). In competitive athletes (24) it has been shown that in the beginning of the training season IGF-1 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 years. Their training stimulus was not new and the study phase occurred in the middle of their normal training season and there were no differences in training between Dynamic and Placebo. So it seems that a short training period in the present study hardly has influenced circulating IGF-1 concentration.

Nutrition is one of the main regulators of circulating IGF-1 (43). In humans, serum IGF-1 concentrations are markedly lowered by energy and /or protein deprivation (e.g. 20) so both energy and proteins are critical in the regulation of serum IGF-1 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-1 during the 2-week training period. In the absorption measurements there was a strong increase in circulating IGF-1 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-1 with Dynamic may have influences on muscular function. In the earlier study (32) the strong relationship between IGF-1 and insulin (not measured in the present study) was observed with bovine colostrum (Bioenervi) supplementation which emphasizes the role of IGF-1 and insulin in protein anabolism. IGF-1 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-1 stimulates muscle protein synthesis equally both in the myofibrillar and cytoplasmic compartments (15) and it is therefore an open question how much the increased (17%) concentration of IGF-1 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 two weeks 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 20g 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 anti-viral defence mechanism(45).

Secretory IgA is important to host defence against certain viruses which 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 (45, 30). Intensive daily training appears to exert a cumulative suppressive effect on saliva IgA levels (28, 29). In a recent study (14) the results indicated that that an eight month 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 transforming growth factor-β1 (TGF-β1) found in Dynamic increases IgA production in vitro (6). In addition, TGF-β1 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). 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 immunological defense system against microbes on mucous membranes.

In conclusion, a bovine colostrum supplementation (Dynamic) increases serum IGF-1 and saliva IgA concentrations in athletes during training. The increased concentration of IGF-1 may have positive effects on protein synthesis and the increased saliva concentration of IgA may activate immunological defense against microbes on mucous membranes.

FOOTNOTES

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FIGURE LEGENDS

Fig. 1. Experimental design of study 1. DR, dietary records; BSC, blood and saliva collection.

Fig. 2. Timetable in study 2. BC, blood collection; rhIGF-1, recombinant human IGF-1; γ-C, gamma-camera image.

Fig. 3. Responses of serum IGF-1 concentrations with Dynamic and Placebo treatments during 14 days. Values are mean ± SE. ** Significantly different from Placebo and 0-values, $P < 0.01$.

Fig. 4. Responses of saliva IgA concentrations with Dynamic and Placebo treatments during 14 days. Values are mean ± SE. ** Significantly different from Placebo and 0-values, $P < 0.01$.

Fig. 5. Responses of serum IgA concentrations with Dynamic and Placebo treatments during 14 days. Values are mean ± SE.

Fig. 6. Responses of serum IgG concentrations with Dynamic and Placebo treatments during 14 days. Values are mean ± SE.

Fig. 7. Responses of serum IGF-1 concentrations with rhIGF-1 treatment during the the test day in all subjects (n=12). Values are mean ± SE. *** Significantly different from a pretreatment value (-10 min), $P < 0.001$.

Fig. 8. Elution of plasma (200µl) on a Sephadex G-100 column 60 minutes after the ingestion of ^{123}I labelled rhIGF-1 in study 2; a) radioactivity profile, b) protein concentration monitored as absorption at 280 nm. Molecular weight markers: A, aldolase 150 kD; B, oval albumin 43 kD; C, ribonuclease 14 kD; D, aprotinin 6 kD; E, tripeptide Pro-Phe-Arg 0.6 kD.

Table 1. Subject characteristics in study 1

Variable	Men Dynamic (n=10)	Men Placebo (n=6)	Women Dynamic (n=9)	Women Placebo (n=5)
Age (years)	21.5±0.7	21.7±1.9	22.6±1.6	22.9±2.6
Height (m)	1.83±0.02	1.83±0.04	1.72±0.04	1.71±0.08
Mass (kg)[b]	74.7±2.5	73.6±6.1	62.6±3.7	62.0±5.3
Fat % [a, b]	8.9±0.4	9.0±1.0	19.0±1.1	18.8±2.2

a Durnin and Rahaman (11)
b significant ($P < 0.001$) difference between men and women
Values are mean±SE

Table 2. Radioactivity in plasma and TCA-precipitated plasma proteins after ingestion of ^{123}I -labeled rhIGF-1 in study 2

Time min	Plasma cpm	TCA-precipitated cpm	%*
30	25196 ± 3244	940±131	3.9 ± 0.5
60	26132 ± 3225	999 ± 151	3.8 ± 0.3
90	25014 ± 2630	809 ± 93	3.2 ± 0.2
120	23639 ± 2309	841 ± 104	3.5 ± 0.3
180	21678 ± 1999	780 ± 63	3.7 ± 0.2
300	14452 ± 1227	629 ± 84	4.3 ± 0.4
420	10553 ± 896	511 ± 42	4.9 ± 0.4

Results are expressed as mean ± SE (n=12) of a 1 ml serum sample
* cpm in the precipitate x 100% /cpm in plasma