

ARGININE AND ORNITHINE SUPPLEMENTATION INCREASES GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR-1 SERUM LEVELS AFTER HEAVY-RESISTANCE EXERCISE IN STRENGTH-TRAINED ATHLETES

ADAM ZAJAC,¹ STANISŁAW POPRZECKI,² ALEKSANDRA ŻEBROWSKA,³ MAŁGORZATA CHALIMONIUK,⁴ AND JOZEF LANGFORT^{3,5}

¹Department of Sports Training, Academy of Physical Education, Katowice, Poland; ²Department of Biochemistry, Academy of Physical Education, Katowice, Poland; ³Department of Physiology, Academy of Physical Education, Katowice, Poland; ⁴Department of Cellular Signaling, Mossakowski Medical Research Center, Polish Academy of Sciences, Warsaw, Poland; and ⁵Department of Experimental Pharmacology, Mossakowski Medical Research Center, Polish Academy of Sciences, Warsaw, Poland

ABSTRACT

Zajac, A, Poprzęcki, S, Żebrowska, A, Chalimoniuk, M, and Langfort, J. Arginine and ornithine supplementation increases GH and IGF-1 serum levels after heavy-resistance exercise in strength-trained athletes. *J Strength Cond Res* 24(4): 1082–1090, 2010—This placebo-controlled double-blind study was designed to investigate the effect of arginine and ornithine (arg and orn) supplementation during 3-week heavy-resistance training on serum growth hormone/insulin-like growth factor-1/insulin-like growth factor-binding protein 3 (GH/IGF-1/IGFBP-3), testosterone, cortisol, and insulin levels in experienced strength-trained athletes. The subjects were randomly divided between a placebo group ($n = 8$) and the L-Arg/L-Orn-supplemented group ($n = 9$), and performed pre and posttraining standard exercise tests with the same absolute load, which consisted of the same exercise schedule as that applied in the training process. Fasting blood samples were obtained at rest, 2 minutes after the cessation of the strength exercise protocol, and after 1 hour of recovery. The resting concentrations of the investigated hormones and IGFBP-3 did not differ significantly between the study groups. In response to exercise test, all the hormones were elevated ($p < 0.05$) at both time points. Significant increases ($p < 0.05$) were observed in both GH and IGF-1 serum levels after arg and orn supplementation at both time points, whereas a significant decrease was seen in IGFBP-3 protein during the recovery

period. Because there was no between-group difference in the remaining hormone levels, it appears that the GH/IGF-1/IGFBP-3 complex may be the major player in muscle tissue response to short-term resistance training after arg and orn supplementation.

KEY WORDS strength training, amino acids, endocrine response

INTRODUCTION

Primarily synthesized through several reactions from aspartate and citrulline in the kidney and liver, L-arginine (Arg) plays an important role in multiple physiological phenomena, including the production of nitric oxide, creatine, ammonia, urea, and protein and growth hormone (GH) release (2,50). In healthy adults with adequate protein intake, endogenous synthesis is sufficient to meet physiological needs (56). However, the metabolic demand for Arg may exceed the body's ability to synthesize Arg under the conditions of intensive exercise or other severe stress; therefore, Arg is considered a semi-essential or conditional amino acid. Insufficient supply of endogenous Arg was documented under some pathological and physiological conditions in humans (3,43,62), and strategies with Arg supplementation have been documented to provide functional benefits in such cases (38,62).

One of the most striking and common effects of exogenous Arg is the stimulation of GH secretion, with the same phenomenon being observed after ornithine (Orn) administration (13,15). The metabolic effects of GH are complex and involve increased lipolysis, hyperinsulinemia, and elevated blood levels of insulin-like growth factor-1 (IGF-1), all of which have protein anabolic effects (4,20,26). The GH-IGF-1

Address correspondence to Dr. Adam Zajac, a.zajac@awf.katowice.pl.
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axis controls the growth of many tissues in most species (37). Insulin-like growth factor-1 has also a number of autocrine and paracrine functions (1). The aforementioned knowledge provides strength athletes with the rationale to believe that Arg possesses ergogenic potential (7). This belief finds additional support in GH having direct anabolic effects (49).

The response of the GH/IGF-1 axis to exercise might depend on the subject's training status (5,32,42). However, other factors, such as training-induced proteolysis of IGFBP-3, inflammation, malnutrition, and overtraining may also be involved (30,44,64).

Tissue remodeling, because of resistance exercise and its anabolic effect, predominates in the recovery period (34). The complex anabolic effects leading to tissue growth, repair, and remodeling processes are also regulated by changes in testosterone and insulin levels, whereas elevated glucocorticoids and catecholamines levels have direct catabolic effects and induce muscle protein loss (52).

Previous studies demonstrated an increase in plasma resting GH levels in nontrained subjects after oral administration of Arg. A combination of oral Arg and exercise attenuates the GH response, but this increase may be less than that seen with exercise alone (12,29). Still, more controversy surrounds the effect of chronic Arg supplementation during strength training programs on GH response to acute strength exercise. Oral amino acid supplementation over several days did not alter plasma GH levels in highly trained weight lifters (18,19). This phenomenon might be explained, at least partially, by reduced serotonin synthesis, a potential stimulus for GH release, because of elevation in serum branch-chain amino acids (55). Another possibility is that under conditions of intense strength training, the majority of plasma Arg is shifted toward catabolic processes. The subsequent reduction in Arg might be, at least in theory, compensated by Orn administration because Orn can serve as a precursor for Arg synthesis via ornithine α -ketoglutarate formation (35). Additionally, Orn is known to release GH by stimulating the pituitary gland (15).

Growth hormone is a primary hormone that affects adaptation to resistance training. Its response to acute resistance exercise depends on the work-rest interval and the load and frequency of the resistance protocol used. After the increase in GH secretion associated with a bout of exercise, GH released transiently decreases, and as a result, 24-hour integrated GH concentration is not usually elevated by a single bout of exercise (59,60). Some studies employing a varied resistance protocol have reported increases in GH release similar to those observed in aerobic exercise (33). Given these similarities, one may postulate that in experienced strength athletes, GH release is decreased after resistance training of the same absolute workload. This effect has been documented, either after long-term submaximal aerobic exercise (58) or in middle-aged, recently untrained men after long-term resistance training (6). Conceptually, such a scenario may apply to the first

transition phase of a resistance training program in experienced strength athletes, and one may postulate that this effect might be at least partially overcome by arg and orn supplementation.

Thus, the present investigation evaluates the hypothesis that oral arg and orn supplementation in experienced strength-trained athletes stimulates the GH/IGF-1 axis during the first transition phase of an annual training cycle. Keeping in mind that testosterone (T), insulin (I), and cortisol (C) may affect the GH/IGF-1 axis, we have additionally measured plasma concentrations of these hormones.

METHODS

Experimental Approach to the Problem

To address the question of arginine and ornithine intake influence on endocrine responses following resistance exercises a 3 week experiment was conducted with well trained strength athletes. The athletes were randomly divided into a placebo and supplemented group and followed an identical exercise program 3 times per week, which included 5 sets of the back squat with 5 repetitions at 80% of 1RM and 5 min rest periods. The supplemented group received 3000 mg of arginine and 2200 mg of ornithine twice daily for 3 weeks. Body mass and body composition as well as resting and post exercise concentrations of lactate, LDH, GH, IGF-1, IGFBP-3, cortisol, testosterone and insulin were evaluated before and after the 3 weeks of the experiment.

Subjects

Seventeen strength-trained young male athletes (weight lifters and body builders) were randomly assigned to either a placebo group (P, $n = 8$) or the arg and orn-supplemented group (S, $n = 9$) in a double-blind, placebo-controlled study. Participants had the following characteristics: P group, age: 22.8 ± 1.7 years, body height: 174.9 ± 3.8 cm, pretraining body mass: 82.9 ± 3.9 kg, and training experience: 5.6 ± 1.1 years and S group, age, 23.5 ± 2.1 years, body height: 176.3 ± 4.6 cm, pretraining body mass: 85.3 ± 4.3 kg, and training experience: 6.2 ± 0.9 years. There was no significant between-group difference in physical characteristics ($p > 0.05$). There was no change in body mass in either group over the study period (data not shown). The participants volunteered for the study and signed an informed consent after a comprehensive explanation of the whole procedure. The project was approved by the Local Ethics Committee of the Academy of Physical Education in Katowice, Poland and conformed to the standards set by the Declaration of Helsinki (1983).

The tested subjects were asked to stop using all medications and nutritional supplements, 2 weeks before the study. For the entire duration of the experiment, they were placed on a mixed, isocaloric diet that included 60% carbohydrates, 25% protein, and 15% fat and were obligated to register all their meals during this period. The composition of the diet was

calculated with the computer program Dietus BUI InFit 1995 (Warsaw, Poland).

Testing protocol

The experiment was performed in the precompetitive period in experienced strength athletes during a 3-week transition phase of training, which was preceded by 6 weeks of high-volume and 3 weeks of high-intensity work. During the first 2 weeks of high-volume training, the athletes performed individual total-body resistance exercises with high-volume (6–8 sets, 5–8 repetitions) and medium- to high-intensity workouts (60–80% 1 repetition maximum [1RM]). The volume of work was increased by 15% every 2 weeks. During the 3-week phase of high-intensity training, the power lifters performed only the bench press, squat, and dead lift, using low volume (5–8 sets, 1–3 repetitions) and submaximal, maximal, and supramaximal intensity (90–120% 1RM). After completing this program, subjects were required to arrive at the laboratory in the early morning after an overnight fast, on 2 separate occasions. The first testing session occurred before onset of the training program, and the second testing session occurred 48 hours after conclusion of the 3-week training program. After warming-up, which consisted of a set of 8–10 repetitions at 50–60% of perceived maximum, subjects rested for 3–5 minutes and next performed the exercise protocol, which included 5 sets of 5 repetitions of the back squat, with 5 minutes of rest between sets, with similar exercise loads calculated at 80% of perceived 1RM. The average load for the P and S groups was 146.9 ± 8.6 and 152.8 ± 10.2 kg, respectively and did not differ significantly ($p > 0.05$). Upper body resistance exercises were not performed during the experiment to avoid the influence of small muscle exercise on hormonal response. Muscle mass engaged in the squats is known to be sufficient for significant stimulation of anabolic hormones (34).

Supplementation Procedure and Training Protocol

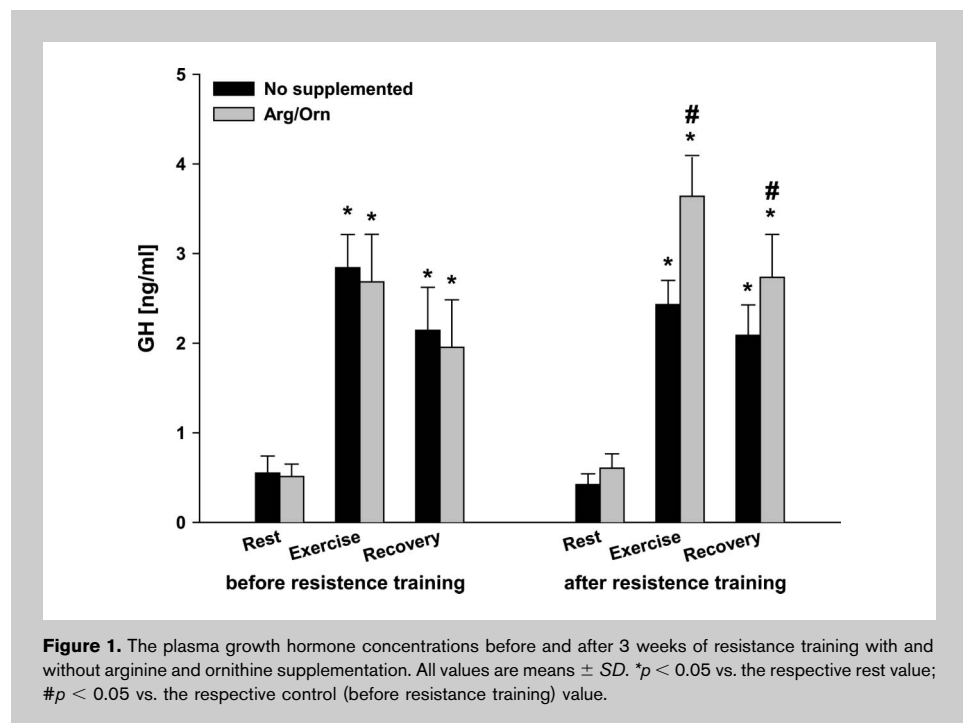
After initial testing, the S group received an oral supplement of 2,200 mg of L-ornithine, 3,000 mg of L-Arg, and 12 mg of vitamin B₁₂ (in gelatin capsules) twice daily for a total of 3 weeks. The dose of Arg was chosen according to the literature data, where a dose range from 5 to 9 g·d⁻¹ was shown to cause a significant GH response with no gastrointestinal distress in young (18–33 years) healthy men (11). The control group

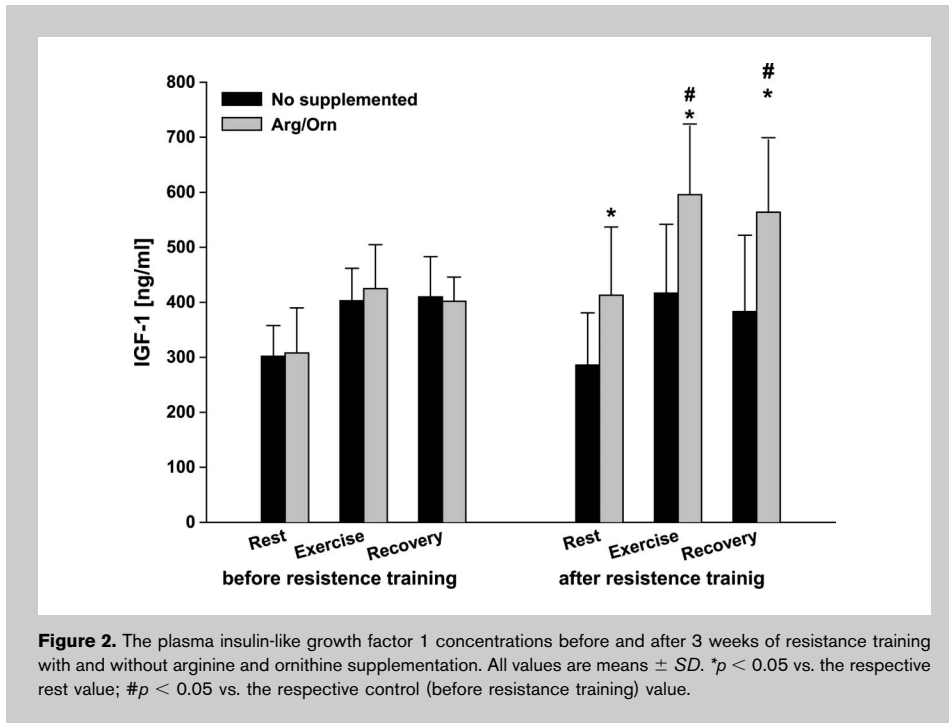
received a placebo in the form of gelatin capsules. The first dose was administered at least 2 hours after breakfast (to avoid competition for absorption with other amino acids) and 60 minutes before the training session. The second dose of the supplement was taken at least 2 hours after the last meal, but 30 minutes before bedtime. Throughout the experiment, the athletes performed a training program that consisted of squats with the same schedule as in the testing trial, performed in 3 training sessions per week (on Monday, Wednesday, and Friday). Proper technique and a complete range of motion were required for each trial during testing and during training sessions.

Measurements and Blood Collection

Body composition was evaluated with the use of electrical impedance (TB-300 Tanita body composition analyzer, Tokyo, Japan).

For hormonal and plasma lactate dehydrogenase (LDH) activity evaluation, antecubital venous blood samples were drawn at rest, 2 minutes after the cessation of the strength-exercise protocol, and after 1 hour of recovery, at the same time of the day for each testing session, with the subject in a seated position. Blood was allowed to clot at room temperature and then centrifuged at 1,500g for 15 minutes. The resulting serum was aliquoted and stored at -80°C for later analyses. Additional blood samples were taken from the fingertip, at rest, 2 minutes after the cessation of the strength exercise protocol, and after 1 hour of recovery for the evaluation of plasma lactate (LA) concentrations.





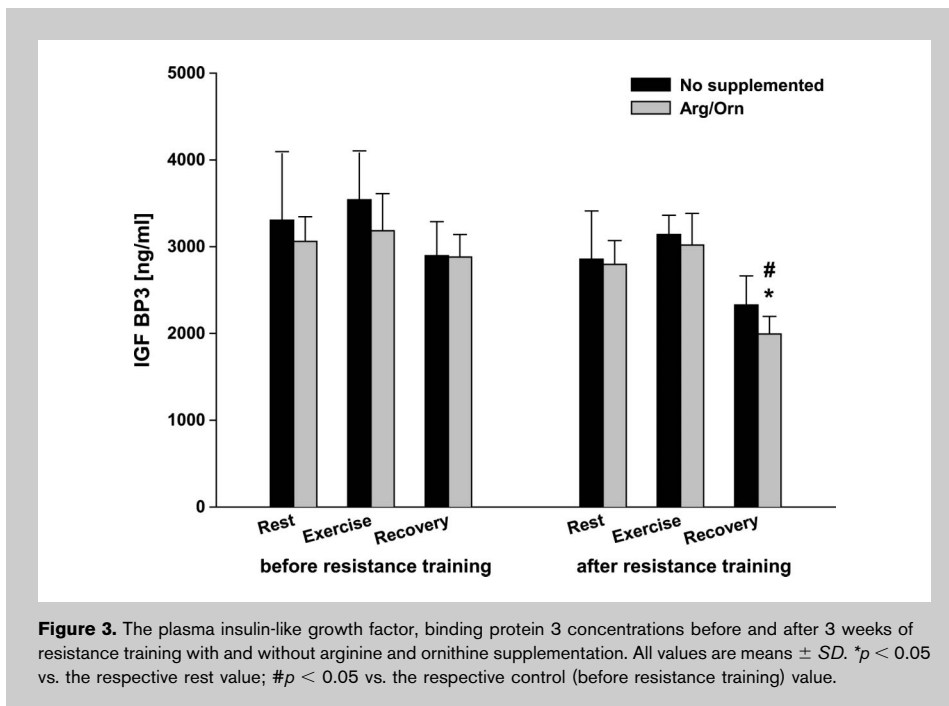
Biochemical and Hormonal Analyses

Serum GH, IGF-1, and IGFBP-3 were assessed using commercial immunoradiometric assay (IRMA, Diagnostic System Laboratories, Webster, TX, USA) kits. Cortisol, testosterone, and insulin were measured with radioimmunoassay kits obtained from Diagnostic System Laboratories (Webster, TX, USA). Blood LA concentration was measured by an enzymatic method using commercial kits (Boehringer,

and bound antigen is achieved using a double antibody system. Intraassay CV for GH ranged from 9.1 to 10.1% on X replicates. Both T and C showed intraassay CV between 5.1 and 10.0%, whereas I ranged from 8.3 to 10.0% on X replicates.

Statistical Analyses

All results are presented as the mean \pm SD. The data were analyzed by 3-way analysis of variance (ANOVA) followed



Manheim, Germany), and LDH activity was measured using an enzymatic method-based kit (Analco, Warsaw, Poland).

The intraassay coefficient of variation (CV) for IGF-I was determined by repetitive measurements of the blood sample and was calculated at 3.9–7.0%. The variance for IGFBP-3 ranged between 5.5 and 6.5%. Serum GH was assayed with the DSL GH IRMA kit, whereas C, T, and I were measured by DSL RIA kits (Diagnostic System Laboratories). The procedure follows the basic principle of radioimmunoassay with competition between a radioactive and a nonradioactive antigen for a fixed number of antibody-binding sites (polyclonal antibodies). The separation of free

and bound antigen is achieved using a double antibody system. Intraassay CV for GH ranged from 9.1 to 10.1% on X replicates. Both T and C showed intraassay CV between 5.1 and 10.0%, whereas I ranged from 8.3 to 10.0% on X replicates.

by the Student-Newman-Keuls test when appropriate. All the analyses were performed using the Statistica v. 7.1 statistical software package (StatSoft, Tulsa, OK, USA). Statistical significance was set at $p < 0.05$. Data estimated in plasma after exercise and recovery were corrected for the after exercise hematocrit change value (Ht, ΔPV) according to the equation: $\Delta PV = (100 - Ht_2)/(100 - Ht_1) \times (P)$, where Ht_1 is the value before exercise, Ht_2 is the value after exercise, and P is a variable.

RESULTS

Average GH level at rest was neither significantly affected by the resistance training nor by the arg and orn supplementation.

TABLE 1. The C, T, and I plasma levels before and after 3 weeks of resistance training with or without Arg/Orn supplementation.*

| Hormone | Supplementation | Before resistance training | | | After resistance training | | |
|----------------------------|-----------------|----------------------------|------------|------------|---------------------------|------------|------------|
| | | Rest | Exercise | Recovery | Rest | Exercise | Recovery |
| C (ng·mL ⁻¹) | None | 388 ± 63 | 453 ± 79 | 466 ± 54 | 372 ± 66 | 491 ± 124 | 446 ± 121 |
| | Arg/Orn | 402 ± 73 | 504 ± 80 | 473 ± 74 | 443 ± 59 | 463 ± 82 | 381 ± 61 |
| T (nmol·mL ⁻¹) | None | 14.7 ± 2.9 | 19.4 ± 3.4 | 16.1 ± 2.9 | 14.1 ± 3.6 | 19.9 ± 4.5 | 14.7 ± 3.6 |
| | Arg/Orn | 15.8 ± 2.8 | 19.1 ± 2.4 | 15.7 ± 2.1 | 16.7 ± 6.1 | 20.6 ± 3.6 | 15.6 ± 6.1 |
| I (mU·mL ⁻¹) | None | 13.2 ± 2.9 | 12.4 ± 3.4 | 11.5 ± 2.9 | 13.4 ± 3.6 | 11.7 ± 4.5 | 10.0 ± 3.6 |
| | Arg/Orn | 13.3 ± 3.9 | 11.2 ± 2.9 | 10.3 ± 2.4 | 13.2 ± 2.8 | 11.1 ± 2.1 | 10.1 ± 1.3 |

*Arg/Orn = arginine and ornithine; C = cortisol; T = testosterone; I = insulin. All values are means ± SD.

Average GH level was significantly increased in both investigated groups at the end of the last exercise trial and remained significantly above the baseline 1 hour later. The group that received the arg and orn supplementation demonstrated significantly higher GH levels at both time points in comparison with its unsupplemented counterpart. Moreover, the average GH levels in the arg and orn-supplemented group were significantly higher at these time points after resistance training than before the training (Figure 1).

Average IGF-1 level at rest was neither significantly affected by the resistance training nor by the arg and orn supplementation. Exercise trial caused no significant change in average IGF-1 level in either group before the resistance training. There was also no resistance trial-induced change in average IGF-1 levels after resistance training in the unsupplemented group, whereas the arg and orn-supplemented group demonstrated significantly increased IGF-1 levels both at the end of the last resistance trial and 1 hour

later. The latter group also showed significantly higher IGF-1 levels at these time points after resistance training than before the training (Figure 2).

The IGFBP-3 level was not affected significantly by the exercise protocol, nor by the arg and orn supplementation; however, a significant effect of the resistance training program was observed. One hour after the completion of the last exercise trial, the average IGFBP-3 level in the arg and orn-supplemented group was significantly lower than both the respective resting level before the trial and the respective postexercise level before resistance training (Figure 3).

Three-way ANOVA showed no significant effect of resistance training, exercise trial, arg and orn supplementation, or of interactions between these factors on I, T, or C levels (Table 1).

Average LA level at rest was neither significantly affected by the resistance training nor by the arg and orn supplementation. Average LA level was significantly

TABLE 2. The concentration of blood LA and activity of creatine kinase registered before and after 3-week resistance training with or without Arg/Orn supplementation.

| | Supplementation | Before resistance training | | | After resistance training | | |
|-----------------------------|-----------------|----------------------------|---------------|-------------|---------------------------|---------------|-------------|
| | | Rest | Exercise | Recovery | Rest | Exercise | Recovery |
| LDH (ng·mL ⁻¹) | None | 299 ± 23 | 334 ± 16 | 340 ± 22 | 274 ± 35 | 320 ± 56 | 344 ± 60 |
| | Arg/Orn | 296 ± 14 | 315 ± 21 | 322 ± 27 | 303 ± 53 | 341 ± 76 | 342 ± 84 |
| LA (nmol·mL ⁻¹) | None | 1.74 ± 0.35 | 11.12 ± 1.10* | 2.69 ± 0.54 | 1.83 ± 0.12 | 10.63 ± 0.25* | 2.34 ± 1.18 |
| | Arg/Orn | 1.66 ± 0.25 | 10.89 ± 1.29* | 2.55 ± 0.25 | 1.54 ± 0.35 | 8.30 ± 1.25*# | 1.83 ± 0.13 |

LA = lactate; LDH = lactate dehydrogenase; Arg/Orn = arginine and ornithine.

All values are means ± SD.

*p < 0.05 vs. the respective rest value; #p < 0.05 vs. the respective control (before resistance training) value.

increased in both investigated groups at the end of the last exercise trial. The group that received the arg and orn supplementation demonstrated significantly lower LA levels at the end of the last exercise trial in comparison with its unsupplemented counterpart (Table 2).

Three-way ANOVA showed no significant effect of resistance training, exercise trial, arg and orn supplementation, or of interactions between these factors on plasma LDH activities (Table 2).

DISCUSSION

In this study, intense resistance training during the first transition phase was combined with oral arg and orn treatment, in a dose known to effectively elevate serum GH (11), to evaluate the response of GH/IGF-1/IGFBP-3 axis to a standard test that consisted of the same exercise schedule as that applied in the training process. This strategy enabled answering the question whether arg and orn supplementation protects experienced strength-trained athletes against the previously documented lowering of posttraining GH concentrations (9). It is well established that intense resistance exercise and arg and orn supplementation both result in substantial increases in plasma GH. In this study, an increase in GH concentration was only found in the arginine and ornithine-supplemented group. No change in GH concentration was observed in the placebo group in response to a standard test, and the lack of differences in the mean concentrations of all measured hormones between the placebo and arg and orn-supplemented athlete groups, allows us to assume that arg and orn supplementation was a sole factor leading to GH elevation. Consequently, IGF-1 levels rose in response to an acute standard resistance exercise test after a short-term resistance training program. In this respect, one should stress the significant decrease in LA levels after arginine and ornithine supplementation, which could also contribute to plasma GH elevation (34). The lowered serum IGFBP-3 level observed in the subjects given arg and orn supplementation during recovery period might suggest increased IGF-1 availability in tissues. Because there was no between-group difference in the levels of other hormones measured in this study, it appears that the GH/IGF-1/IGFBP-3 complex may be an important player in skeletal muscle response to short-term resistance training with arg and orn supplementation.

Changes in total plasma GH concentration after resistance exercise have been shown to be because of increased secretion of various isoforms of the hormone (45). Circulating IGF-1 elevations that have been reported after resistance exercise presumably reflect GH-stimulated hepatic secretion. However, in addition to hepatic IGF-1 production, other tissues, including skeletal muscles, may produce IGF-1 for local autocrine and paracrine actions, and some may contribute to circulating IGF-1 (26,27).

It has been shown that muscle growth depends on the expression of different splice variants, including IGF-1Ea

(which is released from the liver into blood circulation in response to GH), and mechanogrowth factor (MGF) which secretes IGF-1Eb and IGF-1Ec, as well by the physical composition of skeletal muscles (25). Prior studies have also demonstrated that after exercise, the expression of all 3 muscle IGF-1 splice variants are increased; however, the increase is more pronounced for MGF. In the present study, elevated circulating GH concentration in arg and orn-supplemented athletes could lead to further, exercise-independent increases in IGF-1Ea expression within skeletal muscles. This assumption is in agreement with the previously described increase in muscle IGF-1Ea mRNA expressions in older men in response to GH injections (24). However, the polyclonal anti-IGF-1 antibody used for the assessment of IGF-1 in our study does not differentiate between different IGF-1 splice variants. Therefore, the actual source of increased post-exercise IGF-1 remains to be established.

The present study demonstrated that elevated IGF-1 levels accompanied elevated serum GH concentration after oral arg and orn administration. Because GH stimulation is thought to be the primary determinant of circulating IGF-1 levels, our results indicate that exogenous arg and orn stimulates the GH-IGF-1 axis similarly in experienced strength-trained athletes and untrained subjects. As serum IGFBP-3 level decreased in arg and orn-supplemented athletes during the recovery period, our data provided evidence to support regulation of IGF-1 metabolic effect by IGFBP-3 bioavailability. The IGF-1 system is composed of IGF-1 (a 7.6-kDa polypeptide), a family of 6 binding proteins (IGFBPs; i.e., BPs 1–6, ranging in size from 22.8 to 31.4 kDa), and an acid-labile subunit (ALS; 80–86 kDa) (28). Nindl et al. (46) reported that resistance exercise influenced the circulating IGF-1 system, in which IGF-1 is portioned among its family of BPs. Of the 6 IGFBPs, IGFBP-3 has the highest affinity to IGF-1 and is thereby considered an important factor modulating the interaction of IGF-1 with its receptors (17,41). It has been proposed that exercise increases IGFBP-3 proteolysis and lowers ALS concentrations. This proteolysis has been postulated to be a mechanism by which free IGF-1 might be redistributed from the circulation in to tissues (51).

The majority of plasma Arg is derived from protein metabolism and turnover. Studies by Castillo et al. (8) indicated that Arg homeostasis is accomplished by modulation of Arg catabolism rather than of Arg synthesis. Under conditions of intense strength training, a balance between skeletal muscle protein synthesis and breakdown is shifted toward catabolic processes (52). Thus, it is possible that under such conditions, the supply of endogenous Arg from whole-protein turnover, and intestinal and renal de novo synthesis, is insufficient as a consequence of elevated protein demand by muscles. In our study, we administered Arg at a dose known to cause significant GH response (11). Moreover, to maintain an elevated Arg concentration in the extracellular fluid and reduce its conversion to ornithine by urea cycle, we administered Arg in combination with Orn

(48). Ornithine can also serve as a precursor for Arg synthesis via ornithine α -ketoglutarate formation (35).

In general, elevation of GH level can be achieved either by removing the inhibitory effect of somatostatin, or by enhancing the secretion of GH-releasing hormone (14). In our experimental paradigm, increased serum GH level after testing exercise trials was likely because of exercise-induced inhibition of somatostatin secretion (2,40,60). The further increase in GH serum levels evoked by Arg supplementation implies that the inhibitory effect of somatostatin on GH secretion was not saturated under the conditions used in our study. This concept lends much credibility because serum GH levels in our subjects were lower than those documented to maximally stimulate the GH release in the incremental paradigm of the cycle ergometer (40,64). Lack of a plateau in GH release may be also attributed to relatively long, between set rest periods (5 minutes) in our study. As shown by Kraemer et al. (33), high-intensity work interval, with a short rest period (<1.5 minutes), is needed to most effectively stimulate the GH response in heavy-resistance exercise protocols.

Evidence from studies using various physiological models demonstrates that testosterone stimulates GH release in men (21,39,47). Of note, there is evidence for a direct (i.e., GH-independent) upregulation of IGF-1 levels by testosterone in men (22). The sameness of serum testosterone levels before and after Arg supplementation allows the assumption that the mechanism underlying the IGF-1 elevation does not involve direct IGF-1 stimulation by testosterone. Although there is no doubt that IGF-1 plays a role in the hypertrophic response of skeletal muscle, which is synergic with resistance exercise (36), the effect of this peptide is more likely autocrine or paracrine (1,36), and the changes in circulating IGF-1 may not accurately reflect its local effects. However, it is tempting to speculate that elevated IGF-1 level in arg and orn-supplemented athletes might contribute to the stimulation of muscle protein metabolism by increasing IGF-1 bioavailability in the recovery period. Such an effect was documented in the present study by diminished levels of IGFBP-3 after arg and orn supplementation. Proteolysis and/or binding of IGFBP-3 to an unidentified protein in the extracellular matrix reduce its affinity to IGF-1, which increases free IGF-1 and, thereby, stimulates IGF-1 receptors (57).

Enhanced IGFBP-3 proteolysis might also be the result of changes seen in serum C in our subjects, as C level was significantly higher in the recovery period after arg and orn supplementation. Cortisol is known to stimulate protein catabolism (23,34,61). Among the factors that can regulate circulating levels of IGFBP-3, diet (53), type of exercise, and the degree of training were documented (16,31,51). Similarly to our results, enhanced IGF-1 action was achieved by 20% reduction in IGFBP-3 after high-volume (1 vs. 3 sets) resistance training (5). Considering our findings in the light of those reports, it is tempting to postulate that the causative

factor for decreasing IGFBP-3 after high-intensity resistance exercise is training volume. It may be also argued that the use of nonfailure training that was indicated by lack of changes in LDH activity—a marker of muscle damage, might have interfered with arg and orn-induced changes in GH/IGF-1/IGFBP-3 response.

In summary, this study demonstrated that arg and orn supplementation enhanced the elevations in GH and IGF-1 levels that occurred in response to an acute standard resistance exercise test after a short-term resistance training program. The lowered serum IGFBP-3 level observed in the subjects given arg and orn supplementation during the recovery period might suggest increased IGF-1 availability in tissues. The pattern of observed changes in GH/IGF-1/IGFBP-3 axis, which favors an anabolic state, suggests that arg and orn supplementation may provide benefits during strength training.

PRACTICAL APPLICATIONS

It has been postulated that dietary manipulation combined with periodization of a strength training program may optimize the training process and enhance strength development. In general, this strategy is presumed to increase protein availability to enhance muscle protein synthesis. Our results indicate that short-term arg and orn supplementation may be a useful tool for stimulating GH/IGF-1 axis in experienced strength-trained athletes, which is known to promote an anabolic state, and thereby, muscle mass growth. To avoid negative feedback-mediated reduction in Arg transport system capacity in skeletal muscles (10), the supplementation should be recommended for a rather short period of time. Because skeletal muscle injuries are common serious side effects of strength training, Orn (also Arg-derived) can serve as a precursor of proline and thus support formation of proline-rich proteins, such as collagen. Hence, arg and orn supplementation may play a role in tissue remodeling and wound healing (63). Another potential benefit from arg and orn supplementation, which warrants its own study, are alterations in skeletal muscle metabolism brought about by increases in nitric oxide. Of note, dietary Arg supplementation is safe and well tolerated (54).

REFERENCES

1. Adams, GR. Invited review: autocrine/paracrine IGF-1 and skeletal muscle adaptation. *J Appl Physiol* 93: 1159–1167, 2002.
2. Alba-Roth, J, Muller, OA, Schopohl, J, and von Werder, VK. Arginine stimulates growth-hormone secretion by suppressing endogenous somatostatin secretion. *J Clin Endocrinol Meth* 67: 1186–1189, 1988.
3. Appleton, J. Arginine: clinical potential of semi-essential amino acid. *Altern Med Rev* 7: 512–522, 2002.
4. Ballard, TL, Clapper, JA, Specker, BL, Binkley, TL, and Vukovich, MD. Effect of protein supplementation during a 6-month strength and conditioning program on insulin-like growth factor I and markers of bone turnover in young adults. *Am J Clin Nutr* 81: 1442–1448, 2005.
5. Borst, SE, Hoyos, DV, Garzarella, L, Vincent, K, Pollock, BH, Lowenthal, DT, and Pollock, ML. Effects of resistance training on

- insulin-like growth factor and IGF binding proteins. *Med Sci Sports Exerc* 33: 648–653, 2001.
6. Buresh, R, Berg, K, and French, J. The effect of resistive exercise rest interval on hormonal response, strength, and hypertrophy with training. *J Strength Cond Res* 23: 62–71, 2009.
 7. Campbell, BI, La Bounty, PM, and Roberts, M. The ergogenic potential of arginine. *J Int Soc Sports Nutr* 1: 35–38, 2004.
 8. Castillo, L, Chapman, TE, Sanchez, M, Yu, YM, Burke, JF, Ajami, AM, Vogt, J, and Young, VR. Plasma arginine and citrulline kinetics in adults given adequate and arginine free diets. *Proc Natl Acad Sci USA* 90: 7749–7753, 1993.
 9. Chwalbinska-Moneta, J, Kruk, B, Nazar, K, Krzemiński, K, Kaciuba-Uściłko, H, and Ziemia, A. Early effects of short-term endurance training on hormonal responses to graded exercise. *J Physiol Pharmacol* 56: 87–99, 2005.
 10. Closs, EI, Simon, A, Vecony, N, and Rotmann, A. Plasma membrane transport for arginine. *J Nutr* 134: S2752–S2759, 2004.
 11. Collier, SR, Casey, DP, and Kanaley, JA. Growth hormone responses to varying doses of oral arginine. *Growth Horm IGF Res* 15: 136–139, 2005.
 12. Collier, SR, Collins, E, and Kanaley, JA. Oral arginine attenuates the growth hormone response to resistance exercise. *J Appl Physiol* 101: 848–852, 2006.
 13. De Castro Barbosa, T, Nicoletti de Carvalho, JE, Poyares, LL, Bordin, S, Fabres Machado, U, and Nunes, MT. Potential role of growth hormone in impairment of insulin signaling in skeletal muscle, adipose tissue, and liver of rats chronically treated with arginine. *Endocrinology* 150: 2080–2086, 2009.
 14. De Vries, WR, Abdesselam, SA, Schers, TJ, Maas, HC, Osman-Dualeh, M, Maitimu, I, and Koppeschaar, HP. Complete inhibition of hypothalamic somatostatin activity is only partially responsible for the growth hormone response to strenuous exercise. *Metabolism* 51: 1093–1096, 2002.
 15. Evain-Brion, D, Donnadieu, M, Roger, M, and Job, JC. Simultaneous study of somatotrophic and corticotrophic pituitary secretions during ornithine infusion test. *Clin Endocrinol* 17: 119–122, 1982.
 16. Filaire, E, Jouanel, P, Colombier, M, Bégue, RJ, and Lac, G. Effects of 16 weeks of training prior to a major competition on hormonal and biochemical parameters in young elite gymnasts. *J Pediatr Endocrinol Metab* 16: 741–750, 2003.
 17. Fischer, F, Schulte, H, Mohan, S, Tataru, M, Köhler, E, Assmann, G, and Eckardstein, A. Association of insulin-like growth factors, insulin growth factor binding proteins and acid-labile subunit with coronary heart disease. *Clin Endocrinol* 61: 595–602, 2004.
 18. Fogelholm, GM, Näveri, HK, Kiilavuori, KT, and Härkönen, MH. Low-dose amino acid supplementation: No effects on serum human growth hormone and insulin in male weightlifters. *Int J Sport Nutr* 3: 290–297, 1993.
 19. Fry, AC, Kraemer, WJ, Stone, MH, Warren, BJ, Kearney, JT, Maresch, CM, Weseman, CA, and Fleck, SJ. Endocrine and performance responses to high volume training and amino acid supplementation in elite junior weightlifters. *Int J Sport Nutr* 3: 306–22, 1993.
 20. Fryburg, DA. Insulin-like growth factor I exerts growth hormone- and insulin-like actions on human muscle protein metabolism. *Am J Physiol* 267: E331–E336, 1994.
 21. Gentili, A, Muligan, T, Godschalk, M, Clore, J, Patrie, J, Iranmanesh, A, and Veldhuis, JD. Unequal impact of short-term testosterone repletion on the somatotrophic axis of young and older men. *J Clin Endocrinol Meth* 87: 825–834, 2002.
 22. Gibney, J, Wolthers, T, Johannsson, G, Umpleby, AM, and Ho, KKY. Growth hormone and testosterone interact positively to enhance protein and energy metabolism in hypopituitary men. *Am J Physiol Endocrinol Metab* 289: E266–E271, 2005.
 23. Häkkinen, K and Pakarinen, A. Acute hormonal responses to two different fatiguing heavy-resistance protocols in male athletes. *J Appl Physiol* 74: 882–887, 1993.
 24. Hameed, M, Lange, KH, Andersen, JL, Schjerling, P, Kjaer, M, Harridge, SD, and Goldspink, G. The effect of recombinant human growth hormone and resistance training on IGF-1 mRNA expression in the muscle of elderly men. *J Physiol* 555: 231–240, 2004.
 25. Hameed, M, Orrell, RW, Cobbold, M, Goldspink, G, and Harridge, SD. Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. *J Physiol* 547: 247–254, 2003.
 26. Harridge, SDR. Plasticity of human skeletal muscle: gene expression in vivo function. *Exp Physiol* 92: 783–797, 2007.
 27. Heinemeier, KM, Olesen, JL, Schjerling, P, Haddad, F, Langberg, H, Baldwin, KM, and Kjaer, M. Short-term strength training and the expression of myostatin and IGF-I isoforms in rat muscle and tendon: differential effects of specific contractions types. *J Appl Physiol* 102: 573–581, 2007.
 28. Jones, JI and Clemmoms, DR. Insulin-like growth factors and their binding proteins: biological actions. *Endocr Rev* 16: 3–34, 1995.
 29. Kanalay, JA. Growth hormone, arginine and exercise. *Curr Opin Clin Nutr Metab Care* 11: 50–54, 2008.
 30. Koziris, LP, Hickson, RC, Chatterton, RT Jr, Groseth, RT, Christie, JM, Goldflies, DG, and Unterman, TG. Serum levels of total and free IGF-I and IGFBP-3 are increased and maintained in long-term training. *J Appl Physiol* 86: 1436–1442, 1999.
 31. Kraemer, WJ, Aguilera, BA, Terada, M, Newton, RU, Lynch, JM, Rosendaal, G, McBride, JM, Gordon, SE, and Häkkinen, K. Responses of IGF-I to endogenous increases in growth hormones after heavy-resistance exercise. *J Appl Physiol* 79: 1310–1305, 1995.
 32. Kraemer, WJ, Harman, FS, Vos, NH, Gordon, SE, Nindl, BC, Marx, JO, Gómez, AL, Volek, JS, Ratamess, NA, Mazzetti, SA, Bush, JA, Dohi, K, Newton, RU, and Häkkinen, K. Effects of exercise and alkalosis on serum insulin-like growth factor I and IGF-binding protein-3. *Can J Appl Physiol* 35: 127–138, 2000.
 33. Kraemer, WJ, Marchitelli, L, Gordon, SE, Harman, E, Dziados, JM, Mello, R, Frykman, P, McCurry, D, and Fleck, SJ. Hormonal and growth factor responses to heavy resistance exercise protocols. *J Appl Physiol* 69: 1442–1450, 1990.
 34. Kraemer, WJ and Ratamess, NA. Hormonal responses and adaptations to resistance exercise and training. *Sports Med* 35: 339–361, 2005.
 35. Le Boucher, J and Cynober, LA. Ornithine α -ketoglutarate: The puzzle. *Nutrition* 14: 870–873, 1998.
 36. Lee, S, Barton, ER, Sweeney, HL, and Farrar, RP. Viral expression of insulin-like growth factor-I enhances muscle hypertrophy in resistance-trained rats. *J Appl Physiol* 96: 1097–1104, 2004.
 37. Le Roith, D, Bondy, C, Yakar, S, Liu, J, and Butler, A. The somatomedin hypothesis. *Endocr Rev* 22: 53–74, 2001.
 38. Lind, DS. Arginine and cancer. *J Nutr* 134: S2837–S2841, 2004.
 39. Loche, S, Colao, A, Cappa, M, Bellone, J, Aimeretti, G, Farello, A, Faedda, Lombardi, G, Deghenghi, R, and Ghigo, E. The growth hormone response to hexarelin in children: reproducibility and effect of sex steroids. *J Clin Endocrinol Meth* 82: 87–91, 1997.
 40. Maas, HCM, de Vries, WR, Maitimu, I, Bol, E, Bowers, CY, and Koppeschaar, HP. Growth hormone responses during strenuous exercise: The role of GH-releasing hormone and GH-releasing peptide-2. *Med Sci Sports Exerc* 32: 1226–1232, 2000.
 41. Manetta, J, Brun, JF, Fedou, C, Maimoun, L, Prefaut, C, and Mercier, J. Serum levels of insulin-like growth factor-I (IGF-I), and IGF-binding proteins-1 and-3 in middle-aged and young athletes versus sedentary men: Relationship with glucose disposal. *Metabolism* 52: 821–826, 2003.
 42. Manetta, J, Brun, JF, Maimoun, L, Callis, A, Prefaut, C, and Mercier, J. Effect of training on the GH/IGF-I axis during exercise in middle-aged men: Relationship to glucose homeostasis. *Am J Physiol Endocrinol* 283: 929–936, 2002.

43. May, PE, Barber, A, D'Olimpio, JT, Hourihane, A, and Abumrad, NN. Reversal of cancer-related wasting using oral supplementation with a combination of beta-hydroxy-beta-methylbutyrate, arginine, and glutamine. *Am J Surg* 183: 471–479, 2002.
44. Nemet, D, Connolly, PH, Pontello-Pescatello, AM, Rose-Gottron, CH, Larson, JK, Galassetti, P, and Cooper, DM. Negative energy balance plays a major role in the IGF-I response to exercise training. *J Appl Physiol* 96: 276–282, 2004.
45. Nindl, BC, Kraemer, WJ, and Hymer, WC. Immunofunctional vs immunoreactive growth hormone responses after resistance exercise in men and women. *Growth Horm IGF Res* 10: 99–103, 2000.
46. Nindl, BC, Kraemer, WJ, Marx, JO, Arciero, PJ, Dohi, K, Kellogg MD, and Loomis, GA. Overnight responses of the circulating IGF-I system after acute, heavy-resistance exercise. *J Appl Physiol* 90: 1319–1326, 2001.
47. Penalva, A, Pombo, M, Carballo, A, Barreiro, J, Casanueva, FF, and Dieguez, C. Influence of sex and adrenergic pathways on the growth hormone response to GHRP-6. *Clin Endocrinol* 38: 87–91, 1993.
48. Reyes, AA, Karl, IE, and Klahr, S. Role of arginine in health and in renal disease. *Am J Physiol* 267: F331–F346, 1994.
49. Rosenfeld, RG, Wilson, DM, Dollar, LA, Bennett, A, and Hintz, RL. Both human pituitary growth hormone and recombinant DNA-derived human growth hormone cause insulin resistance at a postreceptor site. *J Clin Endocrinol Metab* 54: 1033–1038, 1982.
50. Schaefer, A, Piquard, F, Geny, B, Doutreleau, S, Lampert, E, Mettauer, B, and Lonsdorfer, J. L-Arginine reduces exercise-induced increase in plasma lactate and ammonia. *Int J Sports Med* 23: 403–407, 2002.
51. Schwarz, AJ, Brasel, JA, Hintz, RL, Mohan, S, and Cooper, DM. Acute effect of brief low- and high-intensity exercise on circulating insulin-like growth factor (IGF) I, II, and IGF-binding protein-3 and its proteolysis in young healthy men. *J Clin Endocrinol Metab* 81: 3492–3497, 1996.
52. Sheffield-Moore, M and Urban, J. An overview of the endocrinology of skeletal muscle. *Trends Endocrinol Metab* 15: 110–115, 2004.
53. Thissen, JP, Ketelslegers, JM, and Underwood, LE. Nutritional regulation of the insulin-like growth factors. *Endocr Rev* 15: 80–101, 1994.
54. Tong, BC and Barbul, A. Cellular and physiological effects of arginine. *Mini Rev Med Chem* 4: 823–832, 2004.
55. Valverde, I, Penalva, A, and Dieguez, C. Influence of different serotonin receptor subtypes on growth hormone secretion. *Neuroendocrinology* 71: 145–153, 2000.
56. Visek, WJ. Dietary protein and experimental carcinogenesis. *Adv Exp Med Biol* 206: 163–186, 1986.
57. Vorwerk, P, Yamanaka, Y, Spagnolli, A, Oh, Y, and Rosenfeld, RG. Insulin IGF binding by IGFBP-3 fragments derived from proteolysis, baculovirus expression and normal urine. *J Clin Endocrinol Metab* 83: 1392–1395, 1998.
58. Weltman, A, Weltman, JY, Womack, CJ, Davis, SE, Blumer, JL, Gaesser, GA, and Hartman, ML. Exercise training decreases the growth hormone (GH) to acute constant-load exercise. *Med Sci Sports Exerc* 29: 669–676, 1997.
59. Wideman, L, Weltman, JY, Hartman, ML, Veldhuis, JD, and Weltman, A. Growth Hormone release during acute and chronic aerobic and resistance exercise. *Sports Med* 32: 987–1004, 2002.
60. Wideman, L, Weltman, JY, Patrie, JT, Bowers, CY, Shah, N, Story, S, Veldhuis, JD, and Weltman, A. Synergy of l-arginine and GHRp-2 stimulation of growth hormone in men and women: modulation by exercise. *Am J Physiol Regul Integr Comp Physiol* 279: R1467–R1477, 2000.
61. Willoughby, DS, Taylor, M, and Taylor, L. Glucocorticoid receptor and ibiquitin expression after repeated eccentric exercise. *Med Sci Sports Exerc* 35: 2023–2031, 2003.
62. Wilmore, D. Enteral and parenteral arginine supplementation to improve medical outcomes in hospitalized patients. *J Nutr* 134: S2863–S2867, 2004.
63. Witte, MB and Barbul, A. Arginine physiology and its implication for wound healing. *Wound Repair Regen* 11: 419–423, 2003.
64. Zebrowska, A, Gąsior, Z, and Langfort, J. Serum IGF-I and hormonal responses to incremental exercise in athletes with and without left ventricular hypertrophy. *J Sports Sci Med* 8: 67–76, 2009.