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Bolus Arginine Supplementation Affects neither Muscle Blood Flow nor Muscle Protein Synthesis in Young Men at Rest or After Resistance Exercise[1–3]

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Abstract

The aim of this study was to investigate the ergogenic potential of arginine on NO synthesis, muscle blood flow, and skeletal muscle protein synthesis (MPS). Eight healthy young men (22.1 ± 2.6 y, 1.79 ± 0.06 m, 76.6 ± 6.2 kg; mean ± SD) participated in 2 trials where they performed a bout of unilateral leg resistance exercise and ingested a drink containing either 10 g essential amino acids with 10 g L-arginine (ARG) or an isonitrogenous control (CON). Femoral artery blood flow of both the nonexercised and exercised leg was measured continuously using pulsed-wave Doppler ultrasound, while rates of mixed and myofibrillar MPS were determined using a primed continuous infusion of L-[ring-$^{13}C_6$] or L-[ring-$^{2}H_5$] phenylalanine. The plasma arginine concentration increased 300% during the ARG trial but not during the CON trial ($P <$ 0.001). Plasma nitrate, nitrite, and endothelin-1, all markers of NO synthesis, did not change during either the ARG or CON trial. Plasma growth hormone increased to a greater degree after exercise in the ARG trial than CON trial ($P < 0.05$). Femoral artery blood flow increased 270% above basal in the exercised leg ($P < 0.001$) but not in the nonexercised leg, with no differences between the ARG and CON trials. Mixed and myofibrillar MPS were both greater in the exercised leg compared with the nonexercised leg ($P < 0.001$), but did not differ between the ARG and CON treatments. We conclude that an oral bolus (10 g) of arginine does not increase NO synthesis or muscle blood flow. Furthermore, arginine does not enhance mixed or myofibrillar MPS either at rest or after resistance exercise beyond that achieved by feeding alone. J. Nutr. 141: 195–200, 2011.

Introduction

Muscle protein synthesis (MPS)[6] is intimately sensitive to changes in amino acid availability (1–3). Provision of amino acids, either i.v. or orally, stimulates a marked rise in MPS (4,5). These changes in MPS respond in a curvilinear manner that tracks closely with increasing extracellular amino acid concentrations (6). Moreover, it appears that only the essential amino acids (EAA) are required for this effect (7,8). The magnitude of the feeding-induced rise in MPS can be modulated by several factors, including the source of amino acids (i.e. type of protein consumed) (9,10), the quantity of amino acids (4,11–13), as well as coingestion of other nutrients (e.g. carbohydrates) (11,14). It appears, however, that only 8–10 g of EAA is sufficient to maximally stimulate MPS both at rest (15) and after resistance exercise (16).

The role of blood flow on amino acid delivery and its effects MPS are well known (5,17). NO, which is produced in the endothelial cells that line arterial walls, is a potent vasodilatory signaling molecule (18). The synthesis of NO occurs via the endothelial isoform of the enzyme NO synthase from the precursor amino acid arginine (19,20). Because arginine is a nonessential amino acid, it has no direct stimulatory effect on MPS (7,8); however, it has been postulated that bolus arginine ingestion may have a potential ergogenic effect on muscle anabolism by increasing NO synthesis and muscle blood flow (21). To date, this thesis has not been examined in healthy humans, although some support does exist in 1 experiment in lagomorphs (22).

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[3] Supplemental Figure 1 is available with the online posting of this paper at jn.nutrition.org.
[6] Abbreviations used: ARG, L-arginine treatment (10 g essential amino acids + 10 g arginine); CON, control treatment (10 g essential amino acids + 14.7 g glycine); EAA, essential amino acid; FSR, fractional synthetic rate; GH, growth hormone; MIF, muscle intracellular free; MPS, muscle protein synthesis.
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The purpose of this study, therefore, was to examine the effect of consuming a drink containing 10 g of EAA with or without 10 g of arginine on MPS both at rest and after resistance exercise. We chose to examine both mixed and myofibrillar MPS to determine whether arginine supplementation has a differential effect on muscle-specific protein subfractions. Furthermore, a crossover design and unilateral exercise model was employed to allow us to make comparisons within the same individual. EAA were provided because they stimulate MPS and therefore any increase in blood flow as a result of supplemental arginine would increase amino acid availability to the muscle. Our hypothesis was that consumption of an arginine-enriched amino acid drink would not affect muscle blood flow either at rest or after exercise and thus would not affect MPS in healthy young men.

TABLE 1 Amino acid composition of ARG and CON drinks

L-Amino acid	ARG	CON
	g/400 mL	
Isoleucine	1.3	1.3
Leucine	1.9	1.9
Lysine	1.3	1.3
Histidine	0.5	0.5
Methionine	0.8	0.8
Phenylalanine	1.3	1.3
Threonine	1.0	1.0
Tryptophan	0.3	0.3
Valine	1.6	1.6
Arginine	10.0	—
Glycine	—	14.7

Methods

Participants. Eight recreationally active males (22.1 ± 2.6 y, 1.79 ± 0.06 m, 76.6 ± 6.2 kg; mean ± SD) were recruited for this study. Before participating in the study, all potential participants completed a medical screening questionnaire to identify any medical conditions that would prohibit their participation; all participants were deemed healthy and able to participate in the study. Participants were informed of all potential risks associated with the study and provided written informed consent before participating. This study was approved by the Hamilton Health Sciences and McMaster University Research Ethics Boards and was conducted in accordance with the Canadian Tri-Council Policy Statement on ethics in research with human participants (23).

Experimental protocol. Each participant completed 2 experimental trials, which were separated by a minimum of 1 wk. At least 1 wk before their first trial, participants participated in a familiarization session to become acquainted with the testing procedures and training equipment to be used. During the familiarization session, each participant's unilateral 10 repetition maximum was determined for the seated leg press and knee extension exercises (Universal Gym Equipment). To control for any influence of diet, each participant completed a dietary record for 2 d prior to their first trial and reproduced their diets for the 2 d leading up to their second trial. Participants were instructed to refrain from any strenuous activity or resistance exercise for the 3 d prior to each trial.

On the day of an experimental trial, participants arrived at the laboratory after an overnight fast (~10 h) at which point a 20-gauge i.v. catheter was inserted into an antecubital vein of 1 arm for blood sampling during the trial; the catheter was kept patent by a 0.9% saline drip. Resting measures of femoral artery blood flow were made in both legs using pulsed-wave Doppler ultrasonography (details below). After completing a unilateral bout of resistance exercise as described (24), the men consumed a drink containing EAA with or without additional arginine and another set of blood flow measurements were taken. At this time, a primed continuous infusion of L-[*ring*-$^{13}C_6$] or L-[ring-$^{2}H_5$] phenylalanine (0.05 μmol·kg^{-1}·min^{-1}, 2 μmol·kg^{-1} prime; Cambridge Isotope Laboratories) was initiated through a 0.2-μm filter into an antecubital vein catheter in the arm opposite that used for blood sampling to measure mixed and myofibrillar muscle protein fractional synthetic rate (FSR). Arterialized blood samples were obtained at 30, 60, 90, 120, and 180 min after consumption of the protein drink by warming the hand with a heating blanket (50°C) (**Supplemental Fig. 1**).

The drink order and exercised leg were determined in a randomized and counter-balanced manner. The postexercise drink contained 10 g of EAA (in proportion to that found in egg protein) (**Table 1**) dissolved in water (0.4 L) with an additional 10 g of arginine (ARG; Sigma Aldrich) or an isonitrogenous amount of glycine (14.7 g) as a control (CON; Sigma Aldrich). We chose to provide 10 g of arginine, because such a dose would be well tolerated when consumed orally, but to our knowledge this is the largest single oral bolus of arginine reported to date (25). Glycine was chosen as a control because it is a nonessential amino acid that does not stimulate MPS (7). A small amount of tracer was added to each protein drink (8% of phenylalanine content) to minimize changes in blood enrichment after consuming the drink. The drinks were identical in appearance and coded by 1 individual so that both the participants and the investigators did not know the content of the drinks during the experimental trials.

Blood flow measurement. Longitudinal images of the femoral artery and blood velocity measurements were made as previously described (26) using a 10-MHz linear array pulse Doppler ultrasound probe (System FiVe; GE Medical Systems) placed on the skin surface 2–3 cm proximal to bifurcation of the femoral artery into the superficial and profundus segments. Femoral artery blood flow was calculated as the product of cross-sectional area and mean blood velocity at the same location on the vessel.

Muscle needle biopsy. A percutaneous needle biopsy was taken, under local anesthetic, from the vastus lateralis muscle of both the exercised and nonexercised legs 180 min following the consumption of the postexercise drink. Because participants had not previously been infused with either tracer, the baseline enrichment of the muscle was estimated from plasma protein enrichment in the preinfusion blood samples (27,28).

Blood analyses. Blood amino acid concentrations were determined by HPLC as previously described (9). Plasma nitrate and nitrite were analyzed using a Griess reagent method after deproteinization of the plasma with absolute ethanol (29); CV on quadruplicate samples were <5.7% for nitrate and <6.5% for nitrite, respectively. Endothelin-1 was analyzed by using a commercially available endothelin-1 (human) enzyme immunoassay kit (Assay Design); the CV on triplicate samples was never >3.8%. Growth hormone (GH) was measured using an Immulite 2000 chemiluminescent assay resident in the core clinical chemistry laboratory of the McMaster University Medical Centre, which has a repeat CV < 2%.

GC-MS. Amino acids from plasma, the muscle intracellular free (MIF) space, bound mixed muscle protein, and myofibrillar muscle proteins were isolated and prepared for GC-MS analysis as previously described (24). Plasma and MIF enrichment were determined by making the heptafluorobutyryl isobutyl derivative of phenylalanine (30). Plasma and MIF enrichments were measured by electron impact ionization GC-MS (Hewlett-Packard 5980/5989B) with ions selectively monitored at *m/z* ratios of 316, 321, and 322 and where appropriate a skewed abundance distribution correction was applied (31). Bound mixed and myofibrillar muscle protein enrichments were determined by the standard curve method using negative chemical ionization GC-MS (Hewlett-Packard 6890/5973) and monitoring ions at *m/z* 407, 409, and 410 (30).

Calculations and statistics. Plasma enrichments were analyzed over time using linear regression. Mixed and myofibrillar muscle protein FSR

was calculated using the precursor product equation as previously described (10,30). Blood flow data were analyzed using a 3-factor ANOVA. All other data were analyzed using a 2-factor repeated-measure ANOVA followed by Bonferroni's multiple comparison tests as appropriate. Statistical analyses were performed using SigmaStat 3.10.0 (www.systat.com, Systat Software) and significance was accepted at $P \leq 0.05$. All data are presented as means ± SD.

Results

Blood arginine, glycine, and EAA concentrations. The blood concentration of arginine remained constant in the CON trial but increased significantly throughout the ARG trail ($P < 0.001$) (**Fig. 1***A*). The plasma glycine concentration was elevated in the CON trial ($P < 0.001$) but constant in the ARG trial (Fig. 1*B*). The pattern of change in EAA following drink consumption was similar in both trials (Fig. 1C). There was a significant increase in EAA by 30 min after ingestion of the beverage ($P < 0.001$). Although the increase in EAA at 30 min was slightly greater in the ARG trial ($P < 0.05$), EAA concentrations returned to baseline sooner compared with that in the CON trial (120 vs. 180 min; both $P < 0.001$). The EAA area under the curve after ARG and CON treatments did not differ (data not shown).

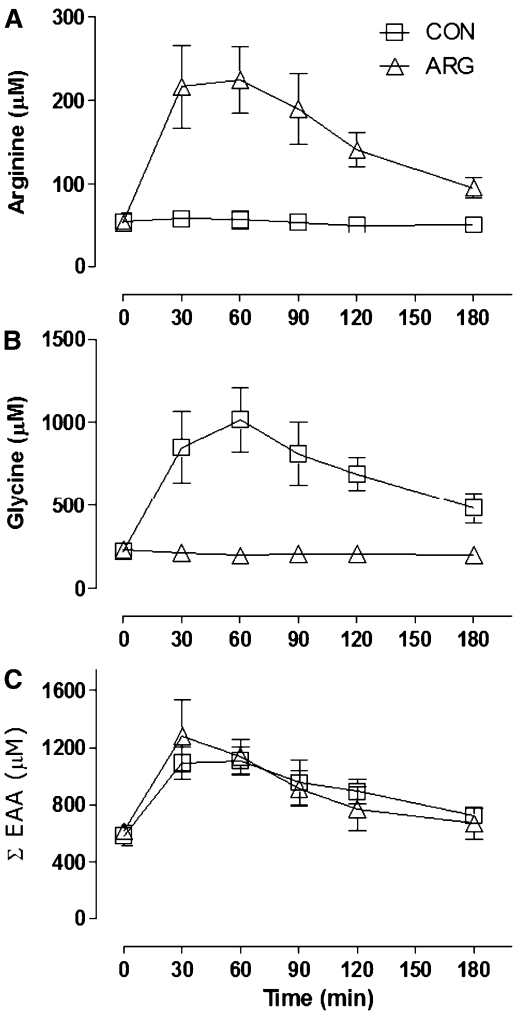

FIGURE 1 Blood concentrations of arginine (*A*), glycine (*B*), and EAA (*C*) in young men during the ARG and CON trials. All values are means ± SD, $n = 8$. *Different from preexercise for a given treatment, $P < 0.05$. †Different from CON, $P < 0.05$.

Plasma endothelin-1, nitrate, nitrite, and GH concentrations. Plasma endothlein-1, nitrate, and nitrite concentrations were unchanged over time, with no difference between the ARG or CON treatments (**Table 2**). At 30 min, GH was elevated above basal ($P < 0.001$), with a greater increase in the ARG trial than the CON trial ($P < 0.05$) (Table 2). At 60 min, the increase in GH returned to baseline in the CON trial but remained elevated in the ARG trial ($P < 0.05$).

Femoral artery blood flow. Rates of femoral artery blood flow were similar in both legs before exercise in both the ARG and CON trials (**Table 3**). Following the bout of exercise, femoral artery blood flow increased ~270% above basal in the exercised leg but not in the nonexercised leg ($P < 0.001$) (Table 3), with no difference between the ARG and CON treatments. The increase in blood flow in the exercised leg was transient and returned to baseline by 15 min in both the ARG and CON trials.

MPS. Linear regression analysis (not shown) indicated that the slopes of the plasma phenylalanine enrichment over time were not significantly different from zero (**Table 4**), suggesting that plasma enrichments had reached a plateau and participants were at isotopic steady state over the incorporation period. Mixed and myofibrillar muscle protein FSR were greater in the exercised leg than in the nonexercised leg (both $P < 0.001$) (**Fig. 2***A*,*B*), with no difference between the ARG and CON treatments.

Discussion

To our knowledge, this is the first study to examine the effect of acute oral arginine supplementation on MPS after resistance exercise in humans. One commonly cited rationale for the consumption of arginine (or its analogs) after resistance exercise is that it stimulates blood flow via NO-mediated mechanisms, because arginine is a precursor for NO synthesis. Theoretically, this would increase nutrient delivery (e.g. substrates such as amino acids) to the muscle (21), which is known to be important in stimulating MPS (32). Despite marked differences in plasma arginine levels following ingestion of 10 g of arginine, there were no differences in femoral artery blood flow in either the rested or exercised leg and no difference in markers of vasodilation such a plasma nitrate, nitrite, or endothelin-1 concentration. Ultimately, there was no effect of arginine supplementation on fed-

TABLE 2 Plasma concentrations of endothelin-1, nitrate, nitrite, and GH in young men during ARG and CON trials[1]

	PRE	0 min	30 min	60 min
Endothelin-1, $ng \cdot L^{-1}$				
ARG	1.41 ± 0.13	1.49 ± 0.10	1.49 ± 0.07	1.51 ± 0.09
CON	1.44 ± 0.22	1.47 ± 0.11	1.49 ± 0.05	1.53 ± 0.12
Nitrate, $\mu mol \cdot L^{-1}$				
ARG	15.3 ± 1.91	15.5 ± 1.42	15.3 ± 1.28	15.5 ± 1.35
CON	15.1 ± 2.21	15.6 ± 0.74	15.2 ± 1.21	15.1 ± 1.00
Nitrite, $\mu mol \cdot L^{-1}$				
ARG	3.64 ± 0.48	3.74 ± 0.55	3.50 ± 0.51	3.70 ± 0.50
CON	3.84 ± 0.52	4.09 ± 0.42	3.63 ± 0.53	3.56 ± 0.44
GH, $g \cdot L^{-1}$				
ARG	3.20 ± 1.13	4.54 ± 2.50	16.1 ± 4.15*†	7.74 ± 3.74*
CON	3.06 ± 1.87	3.50 ± 1.80	9.45 ± 2.49*	5.69 ± 1.37

[1] Values are means ± SD, $n = 8$. *Different from preexercise (PRE) for a given treatment, $P < 0.05$. † Different from CON at that time, $P < 0.05$.

TABLE 3 Femoral artery blood flow in the nonexercised and resistance-exercised legs of young men during ARG and CON trials[1]

	Femoral artery blood flow						
	PRE	0 min	15 min	30 min	60 min	90 min	120 min
REST[2]				*mL·min*$^{-1}$			
ARG	0.272 ± 0.129	0.304 ± 0.097	0.267 ± 0.100	0.273 ± 0.113	0.276 ± 0.117	0.274 ± 0.106	0.264 ± 0.062
CON	0.309 ± 0.068	0.278 ± 0.057	0.294 ± 0.076	0.255 ± 0.101	0.257 ± 0.138	0.268 ± 0.067	0.294 ± 0.165
EX[2]							
ARG	0.279 ± 0.102	1.09 ± 0.412*	0.415 ± 0.190	0.388 ± 0.172	0.372 ± 0.150	0.374 ± 0.165	0.359 ± 0.144
CON	0.302 ± 0.115	1.07 ± 0.371*	0.434 ± 0.170	0.380 ± 0.161	0.366 ± 0.147	0.362 ± 0.160	0.355 ± 0.139

[1] Values are means ± SD, $n = 8$. *Different from preexercise (PRE) for given treatment, $P < 0.05$.
[2] REST, nonexercised; EX, resistance-exercised.

state mixed or myofibrillar protein synthesis either at rest or after resistance exercise in young men.

It is possible that any potential effect of arginine on bulk muscle blood flow was masked by the increase in blood flow expected with exercise (33); however, we did not observe any difference in muscle blood flow in the rested leg with arginine ingestion. In contrast to our findings, several studies have previously reported that i.v. infusion of arginine increases vasodilation and arterial blood flow in healthy individuals (34–36). This effect, however, was only seen at high doses (e.g. 30 g) and not when arginine was provided orally (e.g. 6 g). Arginine bioavailability following oral administration has been found to be ~70% (34). Participants in our study orally consumed 10 g of arginine during the ARG trials, which resulted in a 300% increase in plasma arginine levels that reached a peak of 225 ± 14 μmol·L^{-1}. This is considerably lower than the peak plasma arginine levels reported to induce vasodilation following 30 g of i.v. arginine administration (6223 ± 407 μmol·L^{-1}) (34); thus, it appears that achieving a certain level of arginine in the plasma may be necessary to exert a vasodilatory effect.

Only 1 study has previously examined the effect of arginine on muscle anabolism, albeit in a catabolic rabbit model. Zhang et al. (22) reported an increase in muscle protein net balance due to an increase in protein synthesis following administration of an i.v. mixture of amino acids and arginine; however, this anabolic effect was found to be independent of any change in NO synthesis or bulk muscle blood flow. Although the mechanism behind the reported increase in MPS is unclear, exceeding or sustaining a certain threshold concentration of plasma arginine appears to be required to achieve this anabolic effect; a 14-fold increase in arginine above postabsorptive levels increased MPS, but a 4-fold increase as seen in the current study had no effect on MPS. Assuming 70% bioavailability, our participants would have had to orally consume ~43 g of arginine to achieve a similar plasma concentration of arginine as that previously reported to result in a vasodilatory response (34–36). It is possible that individuals in states of catabolism may benefit from supplemental arginine as an adjunct to other strategies for maintaining lean mass (22).

The measurement of nitrate and nitrite (stable metabolites of NO) as a proxy for quantifying endogenous NO production has been well established (29,37). Consistent with a lack of change in femoral artery blood flow, we found no effect of arginine on levels of nitrate or nitrite. In addition, we also observed no change in plasma levels of the potent vasoconstrictor endothelin-1. The release of endothelin-1 is strongly inhibited by NO (38), which taken together with the lack of change in nitrate and nitrite suggests that enzyme NO synthase activity was unchanged in response to a bolus of arginine. All of the studies that have previously observed increased blood flow with acute arginine provision have been conducted in the postabsorptive state (34–36). Amino acids themselves may increase blood flow above basal rates (5); however, we did not observe an effect of feeding on muscle blood flow in the present study that may have masked any nominal effect of arginine. In the present study, mixed and myofibrillar protein synthesis were similarly increased in the rested and exercised leg when we provided participants with 10 g of EAA with or without 10 g of supplemental arginine. This dose of EAA has been shown to be sufficient to maximally stimulate MPS at rest (15) as well as after resistance exercise (16). These data suggest that blood flow is not limiting for MPS either at rest or after resistance exercise, provided there is an adequate supply of EAA available to support anabolism.

Resistance exercise is known to transiently increase circulating levels of GH (39). In the present study, arginine supplementation stimulated a greater GH response than control. GH is widely accepted to be an important signal regulating muscle

TABLE 4 Plasma L-[*ring*-$^{13}C_6$] and L-[ring-$^{2}H_5$]phenylalanine enrichment in young men during ARG and CON trials[1]

	30 min	60 min	90 min	120 min	180 min
L-[*ring*-$^{13}C_6$]phenylalanine			*Tracer:tracee ratio*		
ARG	0.073 ± 0.008	0.074 ± 0.004	0.079 ± 0.005	0.077 ± 0.005	0.078 ± 0.005
CON	0.069 ± 0.006	0.071 ± 0.008	0.074 ± 0.009	0.077 ± 0.006	0.075 ± 0.007
L-[ring-$^{2}H_5$]phenylalanine					
ARG	0.063 ± 0.009	0.066 ± 0.006	0.070 ± 0.007	0.065 ± 0.004	0.069 ± 0.003
CON	0.063 ± 0.002	0.069 ± 0.002	0.067 ± 0.002	0.066 ± 0.003	0.067 ± 0.003

[1] Values are means ± SD, $n = 8$.

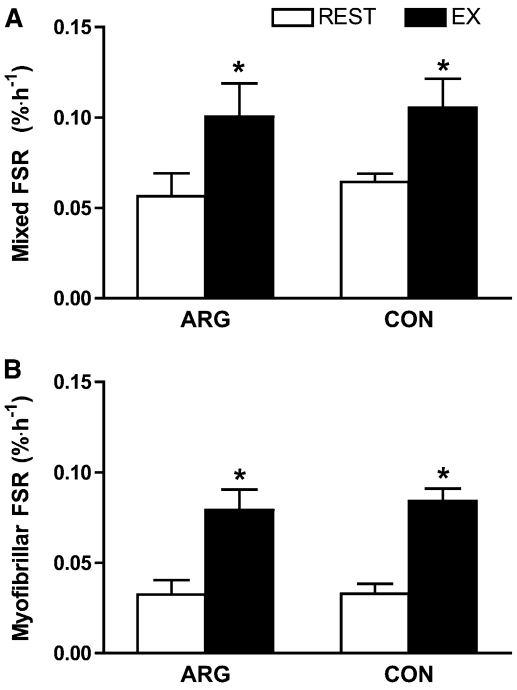

FIGURE 2 FSR of mixed (*A*) and myofibrillar (*B*) skeletal muscle protein in the nonexercised (REST) and resistance-exercised (EX) legs of young men following ingestion of ARG and CON drinks. All values are means ± SD, $n = 8$. *Different from REST for given treatment, $P < 0.05$.

growth during periods of development (40); however, the effect of exercise-induced increases in GH on MPS in healthy adults appears to be restricted to collagen synthesis and not myofibrillar protein (41,42). At this time, the importance of the greater increase in GH following arginine supplementation is unclear.

In summary, we report that despite a 4-fold increase in plasma levels, arginine supplementation does not stimulate an increase in NO synthesis or muscle blood flow in young men at rest or after resistance exercise. In addition, arginine supplementation does not enhance mixed or myofibrillar MPS above and beyond that stimulated by an oral dose of 10 g of EAA. These results bring into question the ergogenic potential of arginine in healthy young men and suggest that neither NO synthesis nor muscle blood flow are limiting to muscle anabolism when an adequate amount of EAA is provided.

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Literature Cited

1. Rennie MJ, Tipton KD. Protein and amino acid metabolism during and after exercise and the effects of nutrition. Annu Rev Nutr. 2000;20:457–83.
2. Rennie MJ, Wackerhage H, Spangenburg EE, Booth FW. Control of the size of the human muscle mass. Annu Rev Physiol. 2004;66:799–828.
3. Phillips SM. Protein requirements and supplementation in strength sports. Nutrition. 2004;20:689–95.
4. Tipton KD, Ferrando AA, Phillips SM, Doyle D Jr, Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. Am J Physiol. 1999;276:E628–34.
5. Biolo G, Tipton KD, Klein S, Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. Am J Physiol. 1997;273:E122–9.
6. Bohe J, Low A, Wolfe RR, Rennie MJ. Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose-response study. J Physiol. 2003;552:315–24.
7. Smith K, Reynolds N, Downie S, Patel A, Rennie MJ. Effects of flooding amino acids on incorporation of labeled amino acids into human muscle protein. Am J Physiol. 1998;275:E73–8.
8. Tipton KD, Gurkin BE, Matin S, Wolfe RR. Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. J Nutr Biochem. 1999;10:89–95.
9. Wilkinson SB, Tarnopolsky MA, MacDonald MJ, Macdonald JR, Armstrong D, Phillips SM. Consumption of fluid skim milk promotes greater muscle protein accretion following resistance exercise than an isonitrogenous and isoenergetic soy protein beverage. Am J Clin Nutr. 2007;85:1031–40.
10. Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J Appl Physiol. 2009;107:987–92.
11. Miller SL, Tipton KD, Chinkes DL, Wolf SE, Wolfe RR. Independent and combined effects of amino acids and glucose after resistance exercise. Med Sci Sports Exerc. 2003;35:449–55.
12. Borsheim E, Tipton KD, Wolf SE, Wolfe RR. Essential amino acids and muscle protein recovery from resistance exercise. Am J Physiol Endocrinol Metab. 2002;283:E648–57.
13. Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am J Clin Nutr. 2003;78:250–8.
14. Rasmussen BB, Tipton KD, Miller SL, Wolf SE, Wolfe RR. An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. J Appl Physiol. 2000;88:386–92.
15. Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor PM, Rennie MJ. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. FASEB J. 2005;19:422–4.
16. Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, Prior T, Tarnopolsky MA, Phillips SM. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. Am J Clin Nutr. 2009;89:161–8.
17. Fujita S, Rasmussen BB, Cadenas JG, Grady JJ, Volpi E. Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. Am J Physiol Endocrinol Metab. 2006;291:E745–54.
18. Fleming I, Busse RNO. The primary EDRF. J Mol Cell Cardiol. 1999;31:5–14.
19. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med. 1993;329:2002–12.
20. Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature. 1988;333:664–6.
21. Paddon-Jones D, Borsheim E, Wolfe RR. Potential ergogenic effects of arginine and creatine supplementation. J Nutr. 2004;134:S2888–94.
22. Zhang XJ, Chinkes DL, Wolfe RR. The anabolic effect of arginine on proteins in skin wound and muscle is independent of nitric oxide production. Clin Nutr. 2008;27:649–56.
23. Canadian Institutes of Health Research, Social Sciences and Humanities Research Council of Canada. Tri-Council policy statement: ethical conduct for research involving humans. Available from: http://pre.ethics.gc.ca/pdf/eng/tcps2/TCPS_2_FINAL_Web.pdf as of Dec, 2010.
24. Moore DR, Tang JE, Burd NA, Rerecich T, Tarnopolsky MA, Phillips SM. Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. J Physiol. 2009;597:897–904.
25. Bode-Böger S. Effect of L-arginine supplementation on NO production in man. Eur J Clin Pharmacol. 2006;62:91–9.
26. Rakobowchuk M, McGowan CL, de Groot PC, Hartman JW, Phillips SM, MacDonald MJ. Endothelial function of young healthy males following whole body resistance training. J Appl Physiol. 2005;98:2185–90.
27. Mittendorfer B, Andersen JL, Plomgaard P, Saltin B, Babraj JA, Smith K, Rennie MJ. Protein synthesis rates in human muscles: neither anatomical

location nor fibre-type composition are major determinants. J Physiol. 2005;563:203–11.
28. Miller BF, Olesen JL, Hansen M, Dossing S, Crameri RM, Welling RJ, Langberg H, Flyvbjerg A, Kjaer M, et al. Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. J Physiol. 2005;567:1021–33.
29. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide. 2001;5:62–71.
30. Phillips SM, Tipton KD, Aarsland A, Wolf SE, Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. Am J Physiol. 1997;273:E99–107.
31. Wolfe RR, Chinkes D. Isotope tracers in metabolic research. New York: John Wiley and Sons Ltd.; 2005.
32. Biolo G, Maggi SP, Williams BD, Tipton KD, Wolfe RR. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. Am J Physiol. 1995;268:E514–20.
33. Andersen P, Saltin B. Maximal perfusion of skeletal muscle in man. J Physiol. 1985;366:233–49.
34. Bode-Boger SM, Boger RH, Galland A, Tsikas D, Frolich JC. L-arginine-induced vasodilation in healthy humans: pharmacokinetic-pharmacodynamic relationship. Br J Clin Pharmacol. 1998;46:489–97.
35. Bode-Boger SM, Boger RH, Creutzig A, Tsikas D, Gutzki FM, Alexander K, Frolich JC. L-arginine infusion decreases peripheral arterial resistance and inhibits platelet aggregation in healthy subjects. Clin Sci (Lond). 1994;87:303–10.
36. Giugliano D, Marfella R, Verrazzo G, Acampora R, Nappo F, Ziccardi P, Coppola L, D'Onofrio F. L-arginine for testing endothelium-dependent vascular functions in health and disease. Am J Physiol. 1997;273:E606–12.
37. Granger DL, Taintor RR, Boockvar KS, Hibbs JB Jr. Measurement of nitrate and nitrite in biological samples using nitrate reductase and Griess reaction. Methods Enzymol. 1996;268:142–51.
38. Thorin E, Webb DJ. Endothelium-derived endothelin-1. Pflugers Arch. 2010;459:951–8.
39. Kraemer WJ, Ratamess NA. Hormonal responses and adaptations to resistance exercise and training. Sports Med. 2005;35:339–61.
40. Mauras N. Growth hormone and sex steroids. Interactions in puberty. Endocrinol Metab Clin North Am. 2001;30:529–44.
41. Doessing S, Heinemeier KM, Holm L, Mackey AL, Schjerling P, Rennie M, Smith K, Reitelseder S, Kappelgaard AM, et al. Growth hormone stimulates the collagen synthesis in human tendon and skeletal muscle without affecting myofibrillar protein synthesis. J Physiol. 2010;588:341–51.
42. West DW, Kujbida GW, Moore DR, Atherton P, Burd NA, Padzik JP, De Lisio M, Tang JE, Parise G, et al. Resistance exercise-induced increases in putative anabolic hormones do not enhance muscle protein synthesis or intracellular signalling in young men. J Physiol. 2009;587:5239–47.