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FEBS Letters 340 (1994) 287–290

FEBS 13771

FEBS LETTERS

Endurance training alters alanine and glutamine release from muscle during contractions

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Received 20 January 1994

Abstract

The release of alanine and glutamine from perfused muscle of trained and control animals was investigated. Release rates did not differ between trained and control muscle at rest. During contractions in trained muscle, alanine release was higher than at rest, while glutamine release was transiently increased. Phenylalanine release did not differ between trained and control muscle, implying that protein degradation was not increased in trained muscle. The muscle cellular adaptations to training include a selective modification of amino acid output, which could potentially influence gluconeogenic flux and alter muscle ammonia levels during contractions.

Key words: Endurance training; Exercise; Alanine metabolism; Glutamine metabolism; Protein degradation; Muscle

1. Introduction

The amino acids glutamine and alanine are continuously released into the blood from resting skeletal muscle. These serve as the major carbon and nitrogen carriers from muscle to other tissues [1]. Their release occurs in excess of their content within muscle proteins [2]. Thus, glutamine and alanine must be formed as products of amino acid interconversions within the cell. The extent of these interconversions depends on the metabolic status of the muscle cell. For example, alanine and glutamine release are affected by nutritional and hormonal states [3,4], as well as exercise [5]. The contractions of muscular exercise increase rates of pyruvate metabolism [5], lactate production [6–8], amino acid transamination [6,9,10] and ammoniagenesis [8] which are also important determinants of glutamine and alanine formation [5,11–13]. However, the role of contractions per se on the magnitude and pattern of muscle amino acid release has received limited attention [14]. More importantly, endurance training induces muscle adaptations which lessen the change in cytosolic control signals [15], as well as shift substrate metabolism [16] within the trained muscle during contractions. Thus, the purpose of the present study was to examine the impact of endurance training on the pattern and magnitude of alanine and glutamine release from muscle, both at rest and during contractions.

2. Materials and methods

2.1. Animals

Adult, male Sprague–Dawley rats (fed ad libitum) were obtained (350–400 g; $n = 18$) from Taconic Farms (Germantown, NY). Animals were either sedentary controls, or were endurance trained on a treadmill for 9 weeks as described previously [6].

2.2. Perfused hindlimb protocol

Rats were anesthetized with sodium pentobarbital (60 mg/kg) and surgically prepared for single hindlimb perfusion. The high oxygen content Krebs–Heiseleit perfusion medium, identical to that described previously [6,9], was not recirculated. The experimental protocol consisted of a 40 min perfusion at rest, followed by 80 min of contractions via sciatic nerve stimulation using sequential frequencies of 15 and 45 tetani/min (6 V, 100 Hz, 100 ms) for 40 min periods each. This contraction sequence has been shown to result in 6- and 10-fold increases in oxygen consumption above rest, respectively [6,9]. To evaluate the effect of time, samples were taken from the arterial perfusate and venous effluent during steady-state rest (at 20–25 min) and at time intervals of 0–5, 20–25, 35–40, 40–45, 60–65 and 75–80 min of contractions. Amino acid concentrations were determined using HPLC as previously described [9]. Amino acid release was calculated as the product of the flow rate times the arterial-venous concentration difference. Following the perfusion protocol, the mixed-fibered plantaris muscle was clamp frozen in tongs pre-cooled in liquid nitrogen. Perchloric acid extracts of the plantaris were used for HPLC analysis of amino acids [9]. For comparison of amino acid concentrations between stimulated and resting plantaris muscle, measurements were also made in a subgroup of animals which perfused for 80 min ($n = 4$) but were not stimulated.

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SSDI 0014-5793(94)00156-P

2.3. Statistics

Comparisons between the trained and untrained groups were made using two-way analyses of variance with repeated measures on one factor (time). Differences between tissue amino acids levels were evaluated using Student's *t*-test.

3. Results

3.1. Amino acid efflux

Alanine output at rest was 5–6 nmol/min/g muscle in both trained and control animals (Fig. 1A). In the trained group, an increased ($P < 0.05$) release above that found at rest occurred with contractions at 15 and 45 tetani/min ($P < 0.05$). A significant increase was only evident in the control group between 40–80 min of contractions at 45 tetani/min. Analysis of variance revealed an effect of training ($0.05 < P < 0.10$) during the initial 20 min of contractions at 15 tetani/min.

At rest, glutamine efflux did not differ between trained and control muscle and averaged approximately 22 nmol/min/g (Fig. 1B). Glutamine efflux was increased transiently above rest ($P < 0.05$) during 0–5 min of contractions in the trained group. This increase was followed by a diminished efflux toward rest by 20–25 min of contractions. This pattern of glutamine output was not apparent in the control group. Glutamine output was higher in trained muscle compared to control muscle ($P < 0.05$) during the initial 5 min interval (40–45 min of contractions) at 45 tetani/min. An attenuation of glutamine efflux below that found at rest occured between 35–65 min of contractions in control muscle ($P < 0.05$).

In contrast to the work of others in vitro [14], glutamate efflux was not significantly different from zero either at rest or during contractions, averaging 2.28 ± 0.80 and 0.13 ± 0.84 nmol/min/g in control and trained muscle, respectively. No effect of training was observed. Contractions induced an increase in phenylalanine release from approximately 0.80 nmol/min/g muscle at rest to 3–4 nmol/min/g during 40 min at 15 tetani/min, and to 6–7 nmol/min/g muscle ($P < 0.05$) by 75–80 min of contractions (45 tetani/min) in both groups (Fig. 2). There was no effect of training on phenylalanine release.

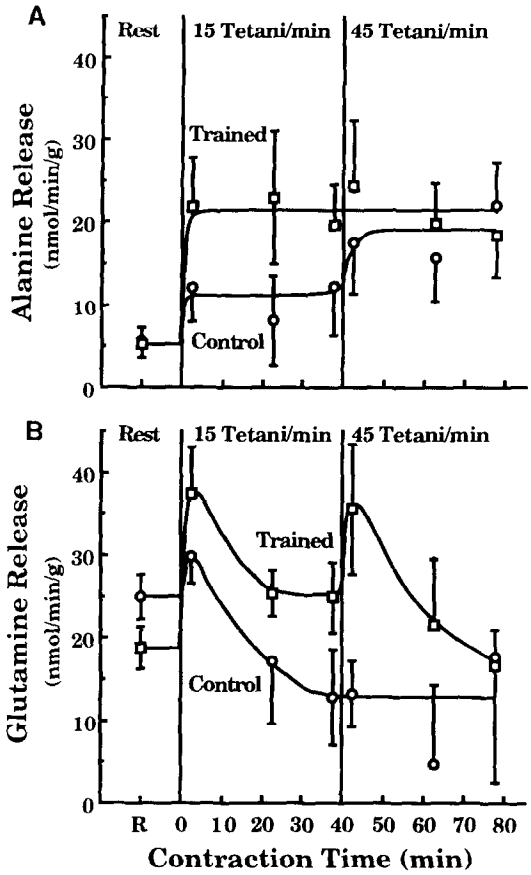

Fig. 1. Alanine (A) and glutamine (B) release from perfused muscle at rest and during contractions at 15 and 45 tetani/min. Squares, trained muscle; circles, control muscle; R, rest. Values are means ± S.E.M. ($n = 7$ animals/group).

3.2. Muscle amino acid concentrations

Contractions decreased ($P < 0.05$) muscle concentrations of leucine, alanine and glutamine, but not aspartate and glutamate (Table 1). Similar reductions in leucine, alanine and glutamine concentrations were also found in trained muscle. However, aspartate and glutamate concentrations were increased ($P < 0.05$) by contractions in trained muscle. The leucine concentation was reduced ($P < 0.05$) to a greater extent by contractions in trained muscle, compared to control muscle.

Table 1
Amino acid content in trained and control stimulated and control non-stimulated plantaris muscle

Group	Amino acid content (μmol/g wet weight)				
	Leucine	Aspartate	Glutamate	Glutamine	Alanine
Trained	0.060 ± 0.004	0.136 ± 0.020	0.362 ± 0.036	0.846 ± 0.097	0.413 ± 0.034
Control	0.079 ± 0.006*	0.070 ± 0.014*	0.254 ± 0.037	0.767 ± 0.080	0.359 ± 0.042
Non-stimulated control	0.114 ± 0.008**,†	0.056 ± 0.008†	0.231 ± 0.017†	1.590 ± 0.170**,†	0.657 ± 0.073**,†

Values are means ± S.E.M.; $n = 9$ per group, except in the non-stimulated muscle ($n = 4$). *$P < 0.05$, trained vs. control; **$P < 0.05$, non-stimulated control vs. control; †$P < 0.05$, non-stimulated control vs. trained.

4. Discussion

This study has shown that muscle adaptations induced by endurance training modify the release of specific amino acids from muscle. While differences between trained and control muscle were not evident during resting conditions, specific differences became apparent during contractions when muscle V_{O_2} was markedly elevated. During contractions, both glycolytic flux and amino acid transamination are increased in proportion to the work intensity [9]. Alanine formation should increase in the muscle, since this is regulated primarily by the availability of pyruvate [1,5], and involves the transamination of amino groups from glutamate. The branched-chain amino acids (BCAA) are particularly important in this transamination [11] as their oxidation is increased during contractions [6,9,17]. An increase in alanine release from muscle [5], as well as an augmented nitrogen transfer from the BCAA to alanine [10] have previously been demonstrated during exercise in humans, but modifications due to endurance training have not been reported. The lower NADH/NAD and lactate/pyruvate ratios found during contractions in trained muscle [18] should favor the formation and subsequent release of alanine via a mass action effect on flux through the glutamate-pyruvate transaminase reaction. The V_{max} of this enzyme is also increased following training [19]. Thus, metabolic conditions found during contractions of trained muscle serve to divert pyruvate away from lactate formation toward the production of alanine. The increased alanine release observed during contractions, and the augmented release following training, could provide a greater amount of substrate for gluconeogenesis. Gluconeogenic flux appears to be dependent, in part, on the availability of amino acids derived from muscle [20].

Skeletal muscle is a major source of circulating glutamine at rest [2]. The de novo synthesis of glutamine, catalyzed by glutamine synthase, depends mainly on the availability of ammonia, since the addition of NH_4Cl stimulates the formation of glutamine in perfused rat muscle [12]. The transient increase in glutamine at the onset of contractions is likely due to ammonia production derived from AMP deamination to IMP. AMP deamination is prompted during contractions by an energy imbalance [8], which is determined by the energy demands of the contraction effort relative to the capacity for ATP provision within the muscle fiber [7]. While ammonia production from AMP deamination is less in trained muscle during moderate energy demands [7], the release of glutamine was greatest (cf. Fig. 1B). Thus, it is likely that trained muscle diverted a greater fraction of its ammonia accumulation through glutamine synthase to form glutamine. While we did not assay blood ammonia in this experiment, we have previously demonstrated a lesser ammonia production in trained muscle during similar stimulation conditions [7]. The reasons

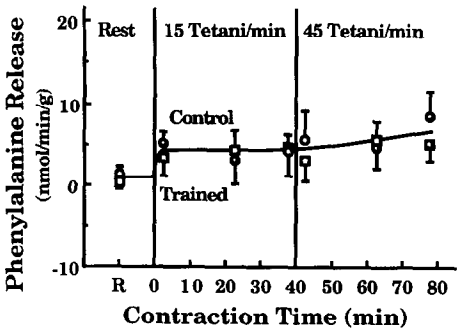

Fig. 2. Phenylalanine release from perfused muscle at rest and during contractions at 15 and 45 tetani/min. Squares, trained muscle; circles, control muscle; R, rest. Values are means ± S.E.M. ($n = 7$ animals/group).

why a smaller fraction of the ammonia produced is used in glutamine formation during the excessive energy demands of the higher contraction intensity remains unclear. However, the better retained adenine nucleotide content of the trained muscle [6] during the initial contraction sequence would permit a greater subsequent extent of AMP deamination during the second, more intense contraction sequence. Thus, ammonia produced during AMP deamination appears to be an important factor leading to glutamine efflux at the onset of contractions.

Phenylalanine is commonly used as an indicator of muscle protein degradation [14,21]. The observed increase in phenylalanine release supports the concept that muscle protein degradation is augmented by contractions, regardless of training status. These data agree with some [22], but not all [14] studies, which suggest that protein degradation as measured using this method is increased in muscle during exercise. Our data do not provide evidence for an exaggerated protein degradation in trained muscle, in contrast to others [23]. Similar rates of phenylalanine release, coupled with the tendency of trained muscle to exhibit a lesser decrement in protein synthesis during contractions [6], suggest a greater anabolic state within trained muscle during contractions.

Apart from its utility as a nitrogen donor to glutamate via transamination, aspartate is used in the purine nucleotide cycle for the reamination of IMP during [24,25], or subsequent to [8], stimulation. The 2-fold higher aspartate level within trained muscle could serve to accelerate the rate of IMP reamination during stimulation [6,7]. Alternatively, it could represent a greater driving force for the transport of reducing equivalents from the cytosol to the mitochondria via the malate-aspartate shuttle.

The data obtained illustrate that the cellular adaptations to training include a modification of the release of specific amino acids from muscle during contractions. An altered amino acid output may be important in in-

creasing gluconeogenic flux, and likely modify ammonia levels in trained muscle during contractions.

Acknowledgements: The authors acknowledge the expert technical assistance of Judy Freshour, Jean Clark and Rick McAllister. This study was supported by NIH Grant AR-21617.

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