Amino Acid Supplementation for Reversing Bed Rest and Steroid Myopathies[1,2]

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ABSTRACT Muscular inactivity is inherent in many circumstances, including convalescence from serious illness or injury, spaceflight, and the progression of aging. Inactivity in a healthy individual leads to a decrease in whole-body protein turnover composed primarily of a decrease in muscle protein synthesis. The decrease in muscle protein synthesis leads to a substantial loss of lean body mass. We have demonstrated that this loss of lean mass is greater when inactivity is accompanied by stress, specifically hypercortisolemia. During convalescence from trauma or injury, the anabolic stimulus provided by nutrient ingestion represents a primary means of ameliorating the loss of muscle protein. We have previously demonstrated that ingestion of essential amino acids (EAAs), formulated to mimic the proportion of EAAs in muscle, provides a potent anabolic stimulus for muscle protein. Recently, we demonstrated that EAA supplementation throughout 28 d of bed rest stimulated net muscle protein synthesis. The repeated stimulation translated to maintenance of lean body mass and an amelioration of functional decrement compared to a placebo treatment. We have also demonstrated that this EAA supplement stimulates net protein synthesis during acute hypercortisolemia and are currently testing the effects during prolonged inactivity. Although EAAs promote muscle anabolism during hypercortisolemia, it is unlikely that a nutritional intervention alone would be effective in maintaining lean body mass during severe stress. It may be necessary to concomitantly reduce the catabolic influence of cortisol or provide another anabolic stimulus. J. Nutr. 135: 1809S–1812S, 2005.

KEY WORDS: • *essential amino acids* • *hypercortisolemia* • *protein metabolism* • *muscle catabolism*

The loss of muscle mass and function are common and undesirable consequences of inactivity. Unfortunately, muscular inactivity is inherent in many circumstances, including convalescence from serious illness or injury, spaceflight, and the progression of aging. The loss of lean body mass with muscle inactivity alone is due to a chronic imbalance between muscle protein synthesis and breakdown. This imbalance can be exacerbated during periods of stress. Stress entails the inherent loss of homeostatic balance, including increases in circulating concentrations of the stress hormones cortisol, epinephrine, and glucogan (1–6). It is this stress hormone response, which includes hypercortisolemia, that represents the predominant event initiating muscle protein catabolism (3,7).

Our understanding of the effects of myopathies associated with inactivity or hypokinesia is primarily derived from the following sources: *i*) clinical studies that describe pathological conditions such as burn injury or orthopedic trauma; *ii*) flight studies conducted in microgravity (8–14); and *iii*) studies in healthy volunteers that mimic some aspect or consequence of inactivity, e.g., lower limb suspension (15,16) and bed-rest studies (4,17–22). Bed-rest studies in particular provide a unique means of isolating and examining many of the mechanisms contributing to muscle loss and can be used to demonstrate the efficacy of a number of potential countermeasures such as exercise, nutrition, and anabolic agents.

Inactivity and muscle protein metabolism

Net protein breakdown, and the subsequent loss of lean body mass, is characteristic of inactivity alone. Loss of nitrogen has been documented in bed rest through increased urea excretion (17,23) and negative nitrogen balance (17,24). Whole-body protein turnover (WBPT) measures represent an amalgamation of the metabolic processes in various tissues. The body's metabolic state determines the extent to which these measures reflect specific tissue metabolism. During inactivity alone, WBPT largely reflects muscle protein turnover (17). WBPT decreases with inactivity in as little as 9 h of bed rest (25). After 7 and 14 d of bed rest, WBPT decreases primarily as a result of decreased muscle protein synthesis.

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Over a 24-h period incorporating periods of nutrient ingestion, WBPT decreases by 15% after 14 d of bed rest as a result of decreased protein synthesis, whereas whole-body protein breakdown does not change. The 15% decrease in WBPT is essentially accounted for by a 50% decrease in skeletal muscle protein synthesis after 14 d (17).

Although inactivity alone results in a loss of muscle protein, the combination of inactivity and a stress response is particularly catabolic. Muscle protein metabolism is affected by many factors after insult; however, in terms of the stress response, a concomitant elevation in cortisol is most directly responsible for the breakdown of muscle protein (3). Thus, an investigation of muscle protein metabolism during the stress response of injury or surgery entails an investigation of the effects of hypercortisolemia on skeletal muscle.

Inactivity and hypercortisolemia

The physiological consequences of prolonged inactivity can be devastating, particularly when combined with traumatic insult such as a burn injury (1,2). One of the more overt and debilitating symptoms of injury and/or a prolonged period of bed rest is the loss of lean body mass. This imbalance between muscle protein synthesis and breakdown is likely facilitated and amplified by an increased circulating level of cortisol, which in turn is generally proportional to the severity of the injury (2,4,26). We previously demonstrated a catabolic interaction between inactivity and hypercortisolemia (4). Young subjects were challenged with 12 h of hypercortisolemia before and after 14 d of bed rest. Cortisol was infused over this period to mimic blood concentrations observed following severe trauma [≈33 μg/dL (0.33 mg/L)]. The hypercortisolemic challenge prior to inactivity did not produce any greater muscle catabolism than fasting alone. However, after 14 d of inactivity, the same hypercortisolemia resulted in a dramatic increase in protein breakdown such that the net muscle protein balance became substantially more negative. In other words, inactivity may prime skeletal muscle for a catabolic response to hypercortisolemia. The prevailing effect of this interaction may best be demonstrated in **Figure 1**. When these young, healthy subjects were challenged with cortisol after 14 d of inactivity, muscle protein kinetics were similar to those of severely burned patients (>70% total body surface area) 2 wk postinjury. These striking similarities in protein kinetics illustrate that the interaction of inactivity and hypercortisolemia may account for a substantial portion of lean body mass loss with trauma.

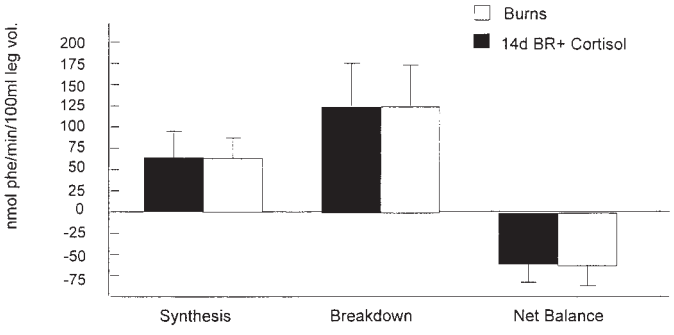

FIGURE 1 The combination of hypercortisolemia and bed rest in normal volunteers resulted in a catabolic state similar to that in patients with severe burns (n = 6; >70% total body surface area burns). Synthesis = skeletal muscle protein synthesis; breakdown = skeletal muscle protein breakdown; net balance = net balance between skeletal muscle protein synthesis and breakdown.

In healthy free-living adults, a 24-h integration of periods of protein breakdown (e.g., overnight fasting) and protein synthesis (e.g., protein/meal ingestion, physical activity) results in no net change in protein degradation/deposition. However, during bed rest or following trauma, the anabolic stimulus afforded by physical activity is lost and catabolism is favored. In such circumstances, the anabolic stimulus provided by nutrient ingestion represents one of the only means of preventing the unrelenting loss of body protein. Fortunately, recent evidence suggests that the acute anabolic response to amino acid ingestion is not impaired by concurrent hypercortisolemia (27). These data raise the possibility of successfully using amino acids as a countermeasure for muscle loss associated with longer-term periods of stress or inactivity.

Rationale for amino acid supplementation during inactivity

Although physical interventions such as exercise clearly provide a potent anabolic stimulus (28,29), exercise may not be a feasible countermeasure in all situations, particularly when inactivity is the result of injury or trauma. Consequently, there is a need to examine additional strategies, such as nutrition, that may arrest the catabolic process. Following severe injury or traumatic insult, the normal anabolic stimulus to feeding is disrupted. Even with elevated energy intakes, some severely injured individuals fail to maintain lean body mass (30,31). Further, although chronic outcome measurements such as changes in body weight, strength, and functional ability clearly demonstrate protein catabolism following stressful events as diverse as injury or microgravity, attempts to blunt or offset this disruption of physiological homeostasis via nutritional supplementation have been largely unsuccessful (30,31). The ineffectiveness of nutritional supplementation has also been documented in the elderly (32). This negative result was attributed to the observation that elderly subjects would voluntarily reduce their normal daily energy intake to account for the nutritional supplement. In such instances, the supplement simply acted as a energy replacement and did not confer any additional nutritional benefit (32). In light of the fact that supplementation may well entail substitution, it is desirable that the supplement elicit a greater anabolic response than normal feeding or corresponding energy intake. This is particularly important in situations where strict monitoring of nutritional intake is not possible.

We recently demonstrated that ingestion or infusion of essential amino acids (EAAs) provides a potent anabolic stimulus in healthy young and elderly subjects (27,33,34). Furthermore, we have preliminary data demonstrating that this combination of EAAs, formulated to mimic the proportion of EAAs present in muscle protein, stimulates muscle protein anabolism to a greater degree than an intact protein (e.g., whey protein) or a mixed meal (e.g., liquid meal replacement).

Based on these positive metabolic study results, dietary amino acid supplementation appears to be an attractive interventional strategy to ameliorate muscle loss associated with bed rest. EAA administration stimulates muscle protein synthesis, the primary defect in muscle protein metabolism during bed rest. Further, it is easy to administer, it is relatively inexpensive, it can be used in most populations, and it can also be used in conjunction with other therapies such as exercise and androgens.

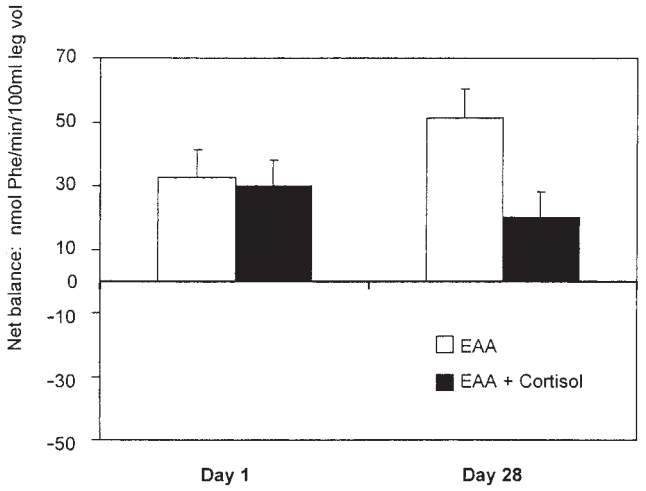

FIGURE 2 Net phenylalanine balance (3 h post-EAA ingestion), a reflection of net protein balance, in response to a cortisol challenge before and after 28 d of bed rest. EAA, essential amino acids; EAA + cortisol, essential amino acids + cortisol infusion to elevate blood levels to ~25 μg/dL (0.25 mg/L).

Amino acid supplementation during bed rest

We recently tested the hypothesis that the stimulation of muscle protein synthesis with EAA supplementation would preserve lean body mass, and in turn maintain muscle function during prolonged inactivity. Subjects were randomly assigned to receive either a placebo ($n = 6$) or an EAA plus carbohydrate supplement ($n = 7$) 3 times/d throughout 28 d of bed rest. Each group was "stressed" by cortisol challenge/infusion on the first and last days of bed rest. The results indicated that the response of muscle protein to EAA supplementation remained anabolic throughout bed rest, although the magnitude was diminished after 28 d. In contrast, the anabolic response of muscle protein to a standard meal essentially dissipated with inactivity. The repeated stimulation of net muscle protein synthesis translated to maintenance of lean body mass after 28 d of inactivity, whereas the loss in the placebo group was substantial. Further, the maintenance of lean body mass ameliorated the functional decrement seen in the placebo group, although a loss of muscle function was still present. We believe that the effect of EAA supplementation on muscle protein was a direct anabolic effect and not due simply to additional energy intake. Compared to the anabolic response following mixed meal ingestion in the same study, the acute anabolic effect of EAA was ~10-fold greater. Further, previous investigations in our laboratory indicate that EAA supplementation provides a much greater anabolic response than either a standard meal or an isocaloric carbohydrate equivalent.

EAA supplementation stimulated net protein synthesis during mild hypercortisolemia, although again, the effect diminished with inactivity (**Fig. 2**). Although it is tempting to speculate that this simple nutritional intervention can ameliorate the loss of lean body mass during stress, it is most likely not a singular solution. The short duration of the anabolic effects combined with the inability of meals alone to stimulate net muscle protein synthesis argues against translated effects over a 24-h period of hypercortisolemia. Further, we have demonstrated that the pattern of muscle anabolism is altered when hypercortisolemia and EAA are combined, such that peak anabolism is blunted (27). Thus, it is likely that the stimulatory effects of EAA on skeletal muscle may diminish over a period of prolonged stress or hypercortisolemia.

The amelioration of catabolism

The effects of hypercortisolemia on skeletal muscle can be investigated by inducing hypercortisolemia in a normal subject population or by ameliorating hypercortisolemia in a clinical population. The infusion of cortisol in healthy subjects offers many advantages in safety and experimental control. However, the amelioration of hypercortisolemia in a clinical setting provides a more realistic and clinically relevant investigation, because the stress of trauma and severe injury is clearly multifactorial (2).

We are currently investigating the effects of ameliorating hypercortisolemia on skeletal muscle in severely burned patients. These patients are treated with a common antifungal agent, ketoconazole, which has secondary properties of blocking adrenal cortisol synthesis. Patients are studied before and after 7 d of ketoconazole administration (200 mg PO BID). Treatment with ketoconazole for 7 d consistently decreases 24-h urinary cortisol excretion (**Fig. 3**). Although 24-h urinary cortisol levels are still above normal after 7 d, preliminary results indicate that skeletal muscle protein turnover is substantially reduced and net protein balance is improved. Although sufficient placebo patients will be studied at similar time periods postinjury to control for the effects of healing, these data indicate that hypercortisolemia directly affects skeletal muscle. These data also corroborate our previous work demonstrating the interactive effects of inactivity and hypercortisolemia on muscle catabolism.

SUMMARY

Although bed rest alone reduces muscle protein synthesis and induces a loss of lean body mass, this loss is most likely exacerbated when coupled with the persistent hypercortisolemia that accompanies trauma. Despite the catabolic influence of elevated cortisol concentrations, EAA supplementation stimulated net muscle protein synthesis in healthy volunteers. Further, the ameliorative effect of EAA supplementation on muscle loss during bed rest is only partly compromised with continued inactivity. Continued stimulation of muscle anabolism positively affects the preservation of lean body mass and the amelioration of functional decrement throughout inactivity. The stimulation of muscle protein synthesis with EAAs is greater than that achieved by meals, intact proteins, or similar energy intake. Although EAAs promote muscle anabolism during hypercortisolemia, it is unlikely that a nutritional intervention alone would be effective in maintaining lean body

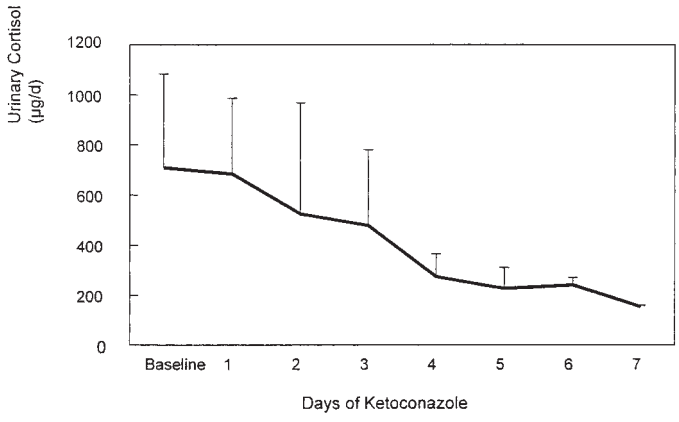

FIGURE 3 Ketoconazole treatment (200 mg PO BID) consistently decreases 24-h urinary cortisol levels.

mass during severe stress or prolonged hypercortisolemia. In these circumstances, it may be necessary to concomitantly reduce the catabolic influence of cortisol on skeletal muscle or provide another anabolic stimulus.

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