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Randomized control trials

Amino acid supplementation is anabolic during the acute phase of endotoxin-induced inflammation: A human randomized crossover trial

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SUMMARY

Background & aims: Inflammation is catabolic and causes muscle loss. It is unknown if amino acid supplementation reverses these effects during the acute phase of inflammation. The aim was to test whether amino acid supplementation counteracts endotoxin-induced catabolism.

Methods: Eight young, healthy, lean males were investigated three times in randomized order: (i) normal conditions (Placebo), (ii) endotoxemia (LPS), and (iii) endotoxemia with amino acid supplementation (LPS + A). Protein kinetics were determined using phenylalanine, tyrosine, and urea tracers. Each study day consisted of a four-hour non-insulin stimulated period and a two-hour hyperinsulinemic euglycemic clamp period. Muscle biopsies were collected once each period.

Results: Endotoxin administration created a significant inflammatory response (cytokines, hormones, and vital parameters) without significant differences between LPS and LPS + A. *Whole body* protein breakdown was elevated during LPS compared with Placebo and LPS + A ($p < 0.05$). *Whole body* protein synthesis was higher during LPS + A than both Placebo and LPS ($p < 0.003$). Furthermore, protein synthesis was higher during LPS than during Placebo ($p < 0.02$). *Net muscle* phenylalanine release was markedly decreased during LPS + A ($p < 0.004$), even though muscle protein synthesis and breakdown rates did not differ significantly between interventions. LPS + A increased mammalian target of rapamycin (mTOR) phosphorylation ($p < 0.05$) and eukaryotic translation factor 4E-binding protein 1 (4EBP1) phosphorylation ($p = 0.007$) without activating AMPK or affecting insulin signaling through Akt. During insulin stimulation net muscle phenylalanine release and protein degradation were further reduced.

Conclusions: Amino acid supplementation in the acute phase of inflammation reduces whole body and muscle protein loss, and this effect is associated with activation of mTOR and downstream signaling to protein synthesis through mTORC1, suggesting a therapeutic role for intravenous amino acids in inflammatory states.

Clinical trial registry: The Central Denmark Region Ethics Commitee (1-10-71-410-12) www.clinicaltrials.gov (identification number NCT01705782).

1. Introduction

Inflammatory disease has serious physiological consequences. Thus, systemic inflammation causes loss of lean body mass and increased muscle breakdown [1]. Long-term immobilization due to illness or injury contributes to the wasting of skeletal muscle [2]. Furthermore, loss of appetite, nausea, and other gastrointestinal symptoms that accompany illness and systemic inflammation

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Abbreviations

LPS	lipopolysaccharide
LPS + A	lipopolysaccharide and amino acid supplementation
mTOR	mammalian target of rapamycin
mTORC1	mammalian target of rapamycin complex 1
mTORC2	mammalian target of rapamycin complex 2
4EBP1	eukaryotic translation factor 4E-binding protein 1
AMPK	adenosine monophosphate activated protein kinase
AS160	Akt substrate of 160
eIF2	elongation initiation factor 2
HMB	β-hydroxy-β-methylbutyrate
BHB	β-hydroxybutyrate
Raptor	regulatory-associated protein of mTOR
MLST8	mammalian lethal with SEC13 protein 8
BMI	body mass index
mRNA	messenger ribonucleic acids
GC–MS	gas chromatography mass spectrometry
Q_x	flux of x
i_x	infusionrate of x
C_x	concentration of x
BW	body weight
E_x	isotopic enrichment of x
NB	net balance
BF	blood flow
Ra	rate of appearance
Rd	rate of disappearance
ELISA	enzyme linked immunosorbent assay
HILIC-MS/MS	hydrophilic interaction liquid chromatography tandem mass spectrometry
TNF-α	tumor necrosis factor alpha
IL	interleukin
GH	growth hormone
HPLC	high-performance liquid chromatography
ANOVA	Analysis of variance
MAP	mean arterial pressure
GIR	glucose infusion rate

generally limit the exogenous nutrient supply. Muscle breakdown provides amino acids for gluconeogenesis, ketogenesis and subsequent oxidation. This adaptive response may eventually become harmful if muscle loss progresses to cachexia and sarcopenia [3,4]. Despite a growing interest in nutritional research, only few clinical trials have yet examined the effects of anabolic interventions in the course of inflammatory disease [5,6]. The lack of clinical evidence is related to the heterogeneous and diverse nature of inflammatory disease in clinical practice, which renders controlled clinical trials cumbersome and subject to wide between-subject variations. To circumvent this problem, endotoxin (lipopolysaccharide, LPS) administration has been used as a model for achieving a controlled inflammatory response in homogenous populations [7,8]. LPS binds to Toll-like receptor 4 and initiates signaling events, including the release of cytokines which are involved in acute and chronic inflammation, including gram-negative sepsis [9]. LPS administration in human models of the initial phase of sepsis has been reported to increase whole body protein breakdown, to release amino acids from skeletal muscle, to lower plasma amino acid concentrations, and to decrease protein synthesis [10,11].

Amino acids have been shown to induce protein synthesis and to inhibit protein breakdown in both the young and the elderly, independently of the route of administration (enteral vs. parenteral) [12]. Essential and branched chain amino acids are currently some of the most promising nutritional supplements. Among such supplements, particularly leucine and its keto-metabolite, β-hydroxy-β-methylbutyrate (HMB), have demonstrated anabolic effects on muscle metabolism under various conditions [13–15]. These effects are most likely mediated by the mammalian target of rapamycin (mTOR), which in complex with the regulatory-associated protein of mTOR (Raptor), mammalian lethal with SEC13 protein 8 (MLST8), and the non-core components PRAS40 and DEPTOR form the mTOR Complex 1 (mTORC1). This complex is a key regulator of protein synthesis in muscle tissue [13,16] which by signaling through S6 kinase 1 (S6K1) and eukaryotic translation factor 4E-binding protein 1 (4EBP1) regulates mRNA translation, thus controlling the rate of protein synthesis [17].

The purpose of this study was to (i) define the metabolic effects of amino acid supplementation during the acute phase of inflammation using the human endotoxin model, (ii) to test whether amino acids exhibit protein-anabolic effects both in muscle and at the whole body level during human endotoxemia, and (iii) to identify intracellular muscle signaling pathways involved in these processes.

2. Materials and methods

2.1. Subjects

Subjects were eligible for inclusion in the study if they were of male gender, had a body mass index (BMI) between 20 and 30 kg/m^2, were between 25 and 40 years, and were healthy without regular intake of medication. All subjects were screened using a medical interview, physical examination, an electrocardiography, and a blood test screen. Subjects were included in the study after an oral and written informed consent had been obtained. The study day was postponed in the event of febrile illness during the preceding week. Each subject was instructed to avoid physical exercise 48 h before each study day and to eat in accordance with general national recommendations (energy-distribution; 30% fat, 50–60% carbohydrates, and 10–20% protein) 24 h before each study day. Every subject arrived by car to our laboratory at 07:00 AM after an overnight fast. The Central Denmark Region Ethics Commitee approved the study in accordance with the Declaration of Helsinki (1-10-71-410-12) and the study was reported at www.clinicaltrials.gov (identification number NCT01705782).

2.2. Study design and protocol

The study was designed as a randomized crossover-trial with three different study days. Each study day was separated with a minimum of 21 days. The primary investigator enrolled subjects and assigned them to the interventions using a computerized randomizer program. The three days were categorized as *placebo day* (Placebo), *LPS day* (LPS), and *LPS + amino acid day* (LPS + A). Each study day was similar except for interventions; Placebo only received a continuous infusion of isotonic saline, LPS received a continuous infusion of saline and a bolus of LPS (*Escherichia coli* endotoxin, 1 ng/kg body weight), and LPS + A received the same as LPS together with a continuous infusion of amino acids (2.5 gram amino acids/kg body weight/day). All subjects were blinded in regards to interventions but could distinguish the Placebo day from the two days with LPS due to symptoms following LPS administrations.

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