Georgia State University
ScholarWorks @ Georgia State University

Nutrition Theses | Department of Nutrition

Summer 6-17-2013

Is Leucine Intake Associate with Enhanced Muscle Protein Synthesis and Attenuated Muscle Protein Breakdown?

Ashley D. Knight
Georgia State University

Follow this and additional works at: https://scholarworks.gsu.edu/nutrition_theses

Recommended Citation

Knight, Ashley D., "Is Leucine Intake Associate with Enhanced Muscle Protein Synthesis and Attenuated Muscle Protein Breakdown?." Thesis, Georgia State University, 2013.
doi: https://doi.org/10.57709/4326463

This Thesis is brought to you for free and open access by the Department of Nutrition at ScholarWorks @ Georgia State University. It has been accepted for inclusion in Nutrition Theses by an authorized administrator of ScholarWorks @ Georgia State University. For more information, please contact scholarworks@gsu.edu.

AUTHOR'S STATEMENT

In presenting this thesis as a partial fulfillment of the requirements for the advanced degree from Georgia State University, I agree that the library of Georgia State University shall make it available for inspection and circulation in accordance with its regulations governing materials of this type. I agree that permission to quote, to copy from, or to publish this thesis may be granted by the professor under whose direction it was written, by the Byrdine F. Lewis School of Nursing and Health Professions director of graduate studies and research, or by me. Such quoting, copying, or publishing must be solely for scholarly purposes and will not involve potential financial gain. It is understood that any copying from or publication of this thesis, which involves potential financial gain will not be allowed without my written permission.

_____

Signature of Author

NOTICE TO BORROWERS

All theses deposited in the Georgia State University library must be used in accordance with the stipulations prescribed by the author in the preceding statement. The author of this thesis is:

Ashley Knight
2465 Powers Ferry Drive
Marietta, GA 30067

The director of this thesis is:

Dan Benardot, PhD, RD, LD, FACSM
Department of Nutrition
Byrdine F. Lewis School of Nursing and Health Professions
Georgia State University
Atlanta, Georgia 30302

Vita

Ashley Knight

ADDRESS: 2465 Powers Ferry
Marietta, Georgia 30067

EDUCATION:

M.S.	2013	Georgia State University Health Sciences Coordinated Program
B.S.	2008	University of Georgia Dietetics

PROFESSIONAL EXPERIENCE:

- **Graduate Assistant** 2012-2013
  Dining Services, Georgia State University
  - Managed a team of student employees and staff workers in providing meals for 700+ students
  - Participated in manager meetings, resolved disputes, and assisted in human resources decision making
  - Educated freshman students individually on nutrition, healthy eating, and weight management

AWARDS AND SCHOLARSHIPS:

- **Hope Scholarship Recipient** 2004-2008
- **Dean's List** 2004-2007

PROFESSIONAL AND STUDENT ORGANIZATIONS:

- Greater Atlanta Dietetic Association, Student member 2012-Present

Abstract

Is Supplemental Leucine Intake Associated with Enhanced Post Exercise Muscle Protein Synthesis and Attenuated Muscle Protein Breakdown?

Knight AD, Benardot D, Thompson W, and Henes ST

Introduction: The role of individual amino acids on protein synthesis and their impact on physical performance is of high importance to athletes and to those studying the science of sports nutrition. Leucine, one of three branched-chain amino acids, is a frequently researched amino acid because of its potential stimulatory effect on muscle protein synthesis (MPS) following exercise in humans.

Purpose: Although there have been many studies conducted on leucine's muscle stimulatory effect, questions remain as to the efficacy and feasibility of leucine as an MPS catalyst. Contributing to these questions are the widely varied dosing and timing strategies that different researchers have employed. It is the purpose of this thesis, therefore, to assess the differences in study protocols and shed light on the potential effectiveness on leucine as a MPS stimulator. Central to this issue is whether supplemental leucine intake is associated with enhanced post exercise MPS and, if so, what associated factors, including timing and level of intake, are most likely to influence this effect.

Methods: A comprehensive review of the literature on leucine and its effect on MPS was performed. Studies were organized into similar topics, with an assessment and summary of effect produced for each topic area. A general conclusion was made that was based on the summary of each topic area.

Results: Leucine is involved in protein metabolism regulation through its role in stimulating the mammalian target of rapamycin (mTOR) signaling cascade and by indicating energy and amino acid availability. It functions to initiate MPS and decrease muscle protein breakdown by downregulating the ubiquitin-proteasome system, lysosomal activity, and/or increasing circulating insulin.

Conclusions: Supplementation with the amino acid leucine effectively enhances MPS and attenuates muscle protein degradation in humans following bouts of physical exertion. Leucine intake in amounts greater than that found in ~20g whole protein saturates MPS and increases leucine oxidation. For this reason, an upper limit of leucine intake should be established. While leucine successfully increases MPS, it remains unclear whether this translates to enhanced physical performance, an area that requires more studies to be conducted.

Key Words: Leucine, protein turnover, muscle protein synthesis, amino acid supplementation

Is Leucine Intake Associated with Enhanced Post-Exercise Muscle Protein Synthesis and Attenuated Muscle Protein Breakdown?

by

Ashley D. Knight

Presented to the Graduate Faculty of

Byrdine F. Lewis School of Nursing and Health Professions

at Georgia State University in

Partial Fulfillment of Requirements for the degree

Master of Science in Nutrition

Committee: Dan Benardot, PhD, DHC, RD, LD, FACSM (Chair)

Walter R. Thompson, PhD, FACSM, FAACVPR

Sarah Henes, PhD, RD, LDN

Acknowledgments

I extend my heartfelt gratitude to those whose assistance made this work possible. To Dr. Benardot, my thesis committee chair, thank you for sharing your excitement for this topic and your vision with me. The direction and constant encouragement you gave was crucial to the completion of this project. For their assistance as well, I thank my other committee members. Thank you, Dr. Henes, for the invaluable feedback and critiques; you made my thesis a much better project. Thank you, Dr. Thompson, for your time and encouragement as well. Finally, I want to thank those in my life who surrounded me with support, encouragement, and above all, the conviction that I would not only accomplish, but excel in my studies. I would not have been able to complete this thesis without you, and you are as much a part of this project as I am.

Table of Contents

Acknowledgments ii

Table of Contents iii

List of Tables iiv

Abbreviations v

Introduction 1

Hypotheses 8

Review of Literature 9

- Leucine and Muscle Protein Synthesis 9
- Leucine and Muscle Protein Degradation 18
- Dietary versus Supplemental Intake 24
- Timing, Dosing, and Long Term Effects 28
- Effects After Resistance and Endurance Exercise 33

Conclusion 43

References 46

List of Tables

Table Number	Title	Page
Table 1	Summary of studies related to effect of mixed AA supplementation on muscle protein synthesis	11
Table 2	Summary of studies related to effect of leucine supplementation on muscle protein synthesis	16
Table 3	Summary of studies related to effect of leucine supplementation on muscle protein breakdown	21
Table 4	Summary of studies related to dietary intake of leucine or supplementation	26
Table 5	Summary of studies related to timing, dosing, and long-term effects of leucine supplementation	31
Table 6	Summary of studies related to effect of leucine supplementation on resistance versus endurance exercise	40

Abbreviations

Abbreviation	Definition
BCAA	Branched Chain Amino Acids (Isoleucine, Leucine, and Valine)
CK	Creatine Kinase
DOMS	Delayed-Onset Muscle Soreness
EAA	Essential Amino Acid
FM	Fat Mass
FSR	Fractional Synthetic Rate
IGF-1	Insulin-like Growth Factor-1
LDH	Lactate Dehydrogenase
LTM	Lean Tissue Mass
MPB	Muscle Protein Breakdown
MPS	Muscle Protein Synthesis
mTOR	Mammalian Target of Rapamycin
RNA	Ribonucleic Acid
UPS	Ubiquitin-Proteasome System

Introduction

Improving physical performance is a goal for athletes and non-athletes alike. Thousands of research studies have been conducted with this specific purpose in mind. One noticeable contributor to enhanced performance is lean body mass (Reid et al., 2008). As a result of these studies, the effect of diet and specific substrate and nutrient consumption on muscle and lean body mass has become a highly researched field (Howald & Decombaz, 1983; Tipton & Wolfe, 2004; Burke et al. 2006; Lambert et al. 2004). In the course of this research, several possible ergogenic aids have been posited to enhance performance in athletes. The essential amino acid leucine has been identified as a key regulator in protein metabolism for over 30 years (Buse & Reid, 1975), but the biochemical basis for its effect, and its other prominent roles in vivo are still being examined at length (Norton & Layman, 2006; Roh et al. 2003; Li et al. 2011; Zanchi et al. 2012). This review focuses on the role of leucine in protein metabolism, especially synthesis, and its effects on several other contributors to physical performance.

Protein turnover occurs as muscle protein constantly changes and remodels by synthesizing new proteins and breaking down existing proteins (Norton & Layman, 2006). Throughout a typical day, the ratios of protein synthesis to breakdown vary significantly. Nutrients become available post-meal and protein synthesis and breakdown both increase, but protein degradation occurs to a lesser extent, resulting in a net positive

balance of protein turnover. During a prolonged period with no food consumption, protein synthesis decreases by 15 to 30%, resulting in a net catabolic period. That period of catabolism continues until adequate energy and amino acids are ingested to stimulate protein synthesis (Norton & Layman, 2006). Both exercise and nutrition have been found to strongly influence net muscle protein balance (Alvestrand et al., 1990; Morgan et al., 1971; Rannels et al., 1974; Tipton et al., 2009). Protein metabolism in skeletal muscle tissue is highly responsive to nutrient intake in healthy individuals and in addition to food ingestion, exercise can effectively alter protein turnover, as it stimulates both protein synthesis and protein breakdown (Biolo et al., 1995; Koopman et al., 2006). Protein gains occur only as a result of positive net muscle protein over a given time period. Positive net muscle protein balance results either from increased muscle protein synthesis and/or decreased muscle protein degradation. For hypertrophy of muscle to take place, muscle protein synthesis (MPS) must surpass muscle protein catabolism (Coburn et al., 2006). Consumption of amino acids through protein ingestion or intravenous injection is essential in accentuating post-exercise muscle protein synthesis. Contractile activity and availability of nutrients are strong modulators of exercise-induced adaptive responses (Moore et al., 2009; Wilkinson et al., 2008). Furthermore, lack of nutrient provision in timely proximity to high-intensity exercise bouts is possibly detrimental for maintenance or promotion of muscle mass (Coffey et al., 2011).

The metabolic roles for amino acids go beyond serving solely as building blocks for *de novo* protein synthesis. They serve in many roles, but for the purposes of this discussion, amino acids also stimulate secretion of insulin, glucagon, growth hormone and IGF-1

(Wilcox, 2005). Reports show that co-ingestion of a protein hydrolysate with or without additional leucine with carbohydrate effectively increases the plasma insulin secretion in several populations, including endurance-trained athletes (van Loon et al., 2000). The complex mechanisms by which amino acids promote and/or enhance insulin secretion are diverse and are not yet fully understood. Like glucose-mediated insulin secretion, intracellular catabolism of the metabolizable amino acids increases availability of substrate for the tricarboxylic acid cycle. In the absence of food intake, net muscle protein balance will stay negative (Coburn et al., 2006). Therefore, muscle hypertrophy only occurs when sufficient food is consumed during post-exercise recovery. Carbohydrate ingestion post-exercise, on the other hand, increases circulating insulin concentrations, thereby reducing protein breakdown, but does not increase muscle protein synthesis rates per se.

Availability of amino acids has been shown to be an important factor in promoting net protein synthesis (Coburn et al. 2006). Administration of protein/amino acids (>15g) following resistance exercise effectively increases muscle protein rates (Koopman et al., 2007). Aside from their role in protein synthesis, amino acids play a key regulatory role as signaling molecules modulating muscle protein metabolism (Rennie, 2006). This is one reason why individuals who wish to enhance lean tissue mass must consume protein after exercising to gain a positive protein balance and capitalize on the muscle adaptive response.

Early studies showed that high concentrations of all amino acids stimulate protein synthesis and inhibit protein breakdown, particularly in skeletal muscle and specifically, the three branched-chain amino acids (BCAAs), leucine, valine, and isoleucine. One reason may be due to the fact that the BCAAs are the only essential amino acids oxidized primarily extrahepatically, and while most tissues can oxidize them, skeletal muscle is thought to be the principal site of branched-chain amino acid catabolism because of its mass (Buse & Reid, 1975; Ahlborg et al., 1974). Of the three BCAAs, leucine alone has been seen as the most potent stimulator of protein synthesis and it has an oxidation rate higher than that of isoleucine or valine (Buse & Reid, 1975; Garlick & Grant, 1988). Leucine is an essential amino acid, meaning it cannot be synthesized *de novo* by humans, and must be acquired in the diet or supplemented as in many of the studies examined here. These early studies began the continuing series of investigations into leucine's role in tissue protein mass, its mechanism of action, and its potential significance in enhancing muscle protein composition in healthy subjects or mediating muscle protein loss in states of catabolism (Garlick, 2005). Sufficient evidence points to leucine's functions as a protein synthesis initiator, as a modulator of the insulin signal cascade, and even as a donor of nitrogen for alanine and glutamine production in the muscle. (Odessey et al., 1974; Preedy & Garlick, 1986; Garlick & Grant, 1988; Norton & Layman, 2006).

In this thesis, the efficacy of leucine as a promoter of MPS will be examined, as well as its role in attenuating muscle protein breakdown (MPB). Leucine regulates protein synthesis in skeletal muscle, but also in other tissue as well, including cardiac and adipose tissue (Lynch et al., 2002; Roh et al., 2003; Suryawan et al., 2012). Whether or

not the increases seen in protein synthesis translate to improvements in physical performance or muscle hypertrophy remain to be seen.

The relationship between exercise and leucine intake is another point that has been extensively researched, but remains debatable. Resistance exercise produces increases in protein synthesis that manifest shortly after exercise and continue for up to 48 hours (Phillips, 2004). Does leucine intake, supplemental or dietary, enhance this already increased protein synthetic response?

Another question posed by many is in regard to the safety of individual amino acid intake. It has long been recommended that dosing of solo AAs is unnecessary and potentially harmful. If this is the case with leucine, should only dietary sources be recommended? The dose threshold of leucine and other EAAs is not clear, and neither is the efficacy of leucine in conjunction with other substrates, dietary or not. Studies have shown that elevations in serum EAAs were responsible for increases in muscle protein synthesis independent of insulin or IGF-1 changes (Cuthbertson et al., 2004). Larger doses of leucine have been shown to be significantly more effective at stimulating muscle protein responses than doses equivalent to the average dietary intake of leucine (Glynn, 2010). Also, leucine levels must be adequately high to function in signaling and metabolic roles since structural roles must be satisfied *a priori*, and leucine's capacity to signal and function as an oxidative substrate is based on sufficient intracellular concentration (Li et al., 2011). Most potential advocates for leucine supplementation will require a specific, numeric dose-response threshold.

Assuming an appropriate dose based on controlled therapeutic studies is reached, timing and length of supplementation is still necessary for initiation of therapy in any individual. Since it is well established that metabolic pathway thresholds are overwhelmed by bolus doses greater than ~20-25g protein and equivalent AA intake, leucine intake in bolus doses greater than these equivalents are not recommended (Moore et al., 2009; Churchward-Venne et al., 2012). Timing throughout a 24-hour period and in relationship to exercise and periods of inactivity are equally important (Adechian et al. 2012). Few studies have assessed the effects of long term supplementation, but initial results have indications for human consumption.

One major factor that influences physical performance is not muscle hypertrophy or physical endurance and strength, but mental stamina (Portier et al., 2008). It is apparent that causes of fatigue are multifactorial, influenced by operations occurring in the periphery and the central nervous system. This idea is posited in the central fatigue theory which suggests that the onset of fatigue in prolonged exercise can be attributed to changes in serotonin, dopamine, and noradrenaline concentrations. The synthesis of these chemicals is largely dependent on AAs that use the same transporter through the blood-brain barrier as the BCAAs, meaning that the plasma concentration ratio of these AAs is a significant indicator of neurotransmitter synthesis (Meeusen & Watson, 2007). Leucine in elevated concentrations may stymie these processes contributing to fatigue.

Many studies have been designed to study leucine and its potential attenuating effect on sarcopenic muscle have potential indications for older athletes and those in which muscle

wasting from disease are a concern. Data indicate that aging leads to a diminished muscle protein accretion after EAA ingestion (Katsanos et al., 2006). Aging is associated with a blunted response to EAA intake and thus, the effects of leucine on MPS. Other studies have shown leucine supplementation increases the potential for muscle protein synthesis in older adults and may supplement an otherwise insufficient or poor quality of dietary protein more biologically available for muscle tissue growth and repair (Casperson et al., 2012).

Despite its popularity over the years as a potential muscle enhancer and the multitude of studies performed, the efficacy of leucine is still controversial. One reason for the lack of consensus is the variability of methods utilized in these studies, and the inability to generalize research results across the board. Investigation methods vary on dose administered, additional substrates co-administered, timing prior to or following exercise, length of supplementation, length of exercise, type of exercise, subjects tested, route of administration, etc. Study results from different metabolites of leucine, like hydroxymethylbutyrate (HMB), cannot be generalized to leucine as they only account for only 5–10% of the metabolites produced from the degradation of leucine (Van Koevering & Nissen, 1992). For these reasons, the question of whether supplemental leucine intake is associated with enhanced post exercise muscle protein synthesis remains unclear.

Hypotheses

Hypothesis 1: Leucine stimulates muscle protein synthesis significantly more than other amino acids.

Null Hypothesis 1: Leucine does not stimulate muscle protein synthesis significantly more than other amino acids.

Hypothesis 2: Leucine attenuates muscle protein degradation following resistance exercise.

Null Hypothesis 2: Leucine does not attenuate muscle protein degradation following resistance exercise.

Hypothesis 3: Leucine intake enhances physical performance.

Null Hypothesis 3: Leucine does not enhance physical performance.

Review of Literature

Leucine and Muscle Protein Synthesis

One of the very first studies to specifically look at leucine as a possible muscle protein metabolism regulator was published in 1975. Buse and Reid (1975) hypothesized that at least one of the three branched chain amino acids (BCAAs) was rapidly oxidized and its stores in muscle cells depleted, serving as a catabolic signal. They surmised that leucine acts as a regulator of protein turnover through its depletion and/or through its presence.

The study utilized incorporation of radiolabeled precursors into muscle proteins. A compound of the BCAAs stimulated the incorporation of lysine into proteins. When tested independently, valine was ineffective, isoleucine was inhibitory, but leucine increased the specific activity of muscle proteins with or without the introduction of insulin. Preincubation with leucine improved the specific activity of muscle proteins and preincubation with other amino acids did not exhibit this same effect. The lysine concentration in the tissue water decreased during incubation with leucine, signifying that leucine also inhibited protein breakdown. During incubation with tyrosine the addition of leucine increased the specific activity of muscle proteins, while the specific activity of intracellular tyrosine remained constant and its concentration decreased, suggesting that leucine also promoted protein synthesis.

Researchers concluded that the muscle cell concentration of leucine may play a role in regulating muscle protein metabolism and influence the transition to negative nitrogen balance during fasting, uncontrolled diabetes, and the posttraumatic state. Leucine may play a pivotal role in the protein-sparing effect of amino acids. Despite the encouraging findings, authors of this study closed by adding the following caveat:

> "…studies of the potential protein anabolic effect of leucine…would have to take into account the possible deleterious effects that amino acid imbalance or antagonism may produce in vivo" (Buse & Reid, 1975).

This early warning is especially applicable today in our world of over-supplementation and amino acid mega-dosing.

Similar early studies demonstrated that high concentrations of amino acids stimulated protein synthesis and inhibited protein degradation, particularly in skeletal muscle, but also in cardiac muscle (May & Buse, 1989). Specifically, it was shown in heart muscle that the stimulation by amino acids could be reproduced with only the three BCAAs, and as seen in the isolated diaphragm muscle, leucine alone stimulated protein synthesis. They found that net uptake of BCAAs was observed suggesting that these AAs were utilized as an oxidative substrate and the increased rate of protein anabolism was due to a direct effect on the protein synthesis pathway rather than an indirect effect from simply increasing energy levels in the cell. Li and Jefferson (1978) found when they omitted BCAAs from an AA mixture infused in rat skeletal muscle, the remaining AAs, even at five times their normal plasma levels, had no effect on protein turnover. Another study demonstrated that the ratio of individual BCAAs to the amount of leucine was more

critical to nitrogen-sparing than the total percentage of BCAAs infused (Bonau et al., 1984). Leucine intravenously infused during a prolonged fasting state resulted in decreased plasma amino acid levels, nitrogen balance improvement and no difference in excretion of 3-methylhistidine, which was found to suggest muscle protein synthesis stimulation (Sherwin, 1978). This group of studies began a series of investigations into leucine's role in protein mass regulation and its mechanism of action (Morgan et al., 1971; Rannels et al., 1974; Li & Jefferson, 1978).

Table 1: Summary of studies related to effect of mixed AA supplementation on muscle protein synthesis

Reference	Population	Design	Findings
Morgan et al. (1971)	Rats	Protein synthesis was measured using incorporation of 14C-phenylalanine into heart protein.	Protein synthesis increased when AA levels increased from 1-5 times normal levels.
Buse & Reid (1975)	Rats	Muscle proteins were incubated in BCAAs and tracer incorporation was observed.	Leucine alone increased protein specific activity and increased MPS to the greatest extent.
Li & Jefferson (1978)	Rats	Fasted rats were infused with BCAAs.	MPS was stimulated to a greater degree than rats infused with an AA mixture without BCAAs.
Bonau et al. (1984)	25 post-operative males with bladder cancer.	Different concentrations of BCAA IV solutions were administered for 7 days.	The high leucine group had greater nitrogen balance and less muscle catabolism.
Preedy & Garlick (1986)	Rats	Fasted rats were refed by intragastric or IV infusion of an AA plus glucose mixture.	MPS was suppressed by anti-insulin injection.
Garlick & Grant (1988)	Rats	AA and insulin mixtures were given between meals.	BCAAs increased MPS over insulin infusion or non-BCAAs.
Louard et al.	10 humans	BCAAs were infused	MPB was suppressed, but

(1990)		intravenously.	MPS was not increased.
Schena et al. (1992)	16 humans	Participants trekked 21 days at high altitude and took either BCAA or placebo supplements.	BCAA supplementation increased MPS during chronic hypobaric hypoxia.
Blomstrand et al. (2006)	Humans	BCAAs were given to subjects after resistance exercise.	BCAAs stimulated the signaling pathways that control MPS.
Coffey et al. (2011)	8 healthy young males	Participants performed sprint cycling after ingesting nutrients or placebo.	Nutrient provision (including leucine) increased anabolic signaling and MPS.
AA: Amino Acids; BCAAs: Branched Chain Amino Acids; MPS: Muscle Protein Synthesis; IV: Intravenous; MPB: Muscle Protein Breakdown			

In the studies performed with mixed AAs or BCAAs (Table 1), there was consensus that MPS was stimulated. Most studies found that BCAAs increased MPS to a greater degree than mixed AAs, or by using less substrate. One study listed in Table 1 did not see increased MPS (Louard et al., 1990), but MPB was attenuated by BCAA infusion. Under many different types of conditions, and in both rat and human populations, MPS was stimulated by BCAA intake.

Early work produced considerable advances in the comprehension of the signal transduction pathways involved in the control of muscle protein synthesis by amino acids and insulin. The physiological role of leucine is then to act in combination with insulin to activate the “switch” that stimulates muscle protein synthesis when availability of energy and amino acids from food is signaled (Preedy & Garlick, 1986). The advantage of this mode of regulation is that the switch calls for both amino acids, namely leucine, and insulin to be present concurrently, so is only activated in ideal conditions (Preedy & Garlick, 1986; Balage et al., 2011). Also contributing to the evidence for leucine’s

efficacy, when compared with glucose infusion, protein synthesis was stimulated equally by a complete mixture of AAs, by only EAAs, and by only the BCAAs (Garlick & Grant, 1988). This demonstrates that specifically the BCAAs enhance insulin sensitivity of muscle protein synthesis pathways.

An early study specifically demonstrated the ability of orally administered leucine to stimulate recovery of skeletal muscle protein synthesis after exercise (Anthony et al., 1999). Rather than simply providing post-exercise substrate, this study showed that leucine alone was effective since feeding carbohydrate alone did not increase protein synthesis rates above those of rats fasting post-exercise. Plasma insulin was elevated similarly in rats fed either the macronutrient-mixed meal or the carbohydrate meal, demonstrating that insulin acts together with another factor of the mixed meal to stimulate protein synthesis. The authors posited that the inability of researchers in previous animal studies to demonstrate that leucine stimulates skeletal muscle protein synthesis was due to an inadequate leucine quantity administered to the animals. A higher dose than previous was chosen in this study to maximize the effects of leucine. The findings here suggest leucine stimulation of muscle protein synthesis is dose dependent (Anthony et al., 1999).

Oral administration of a bolus amount of leucine equivalent to the daily intake (1.35g/kg body weight) has been found to enhance skeletal muscle protein synthesis in studies with food-deprived rats (Crozier et al., 2005). The dose of leucine used in previous studies is quite large, equal to what is consumed in a 24-hour period by rats when allowed free

access to standard lab chow. Since leucine is relatively insoluble, such a large dose may not be generalizable to human studies. But this study was able to demonstrate that smaller doses of leucine administered orally were successful in stimulating muscle protein synthesis as well (Crozier et al., 2005).

Plasma leucine concentrations significantly increased in rats fed one meal each day that contained proteins (i.e., β-lactoglobulin) high in leucine. As a result, MPS significantly improved in these rats fed a leucine-rich diet. And most notably, the increased MPS effect was maintained even after the 30-day supplementation. Additional studies are needed to determine the length of supplementation that would produce a significant gain in muscle protein (Rieu et al., 2007).

Another recent study showed that whey protein doses in insufficient quantities to maximally effect muscle protein synthesis (Moore et al., 2009), supplemented with leucine resulted in an increase in rates of MPS equal to those usually seen following 25g whey protein doses. However, contrary to the authors' hypothesis, supplementation of a low dose whey protein with an EAA mixture absent of leucine also resulted in stimulation of MPS no different from what was seen in the leucine-supplemented whey protein. These findings contradict the hypothesis that MPS stimulation is directly proportional only to the rise in leucine in the blood. The investigators postulated that while leucine is potently able to stimulate MPS, only a small amount (~0.75g) is

necessary to maximally stimulate MPS given that other EAAs are present in sufficient quantities (~8.5g) (Churchward-Venne et al., 2012).

One study that examined the physiological mechanism of leucine efficacy identified messenger RNA translation via the activation of a variety of intracellular signaling proteins, especially those of the mTOR signaling cascade as the primary level of regulation of acute muscle protein synthesis. Stimulation of muscle protein synthesis after food intake or resistance exercise is accompanied by increased phosphorylation, and activity, of the mTOR signaling pathway, and thus, protein synthesis. This biochemical pathway has been identified in several studies as a possible mechanism by which leucine increases MPS (Blomstrand et al., 2006; Casperson et al., 2012; Coffey et al., 2011; Dreyer et al., 2008; Li, 2011). This study examined the response of protein synthesis and total body leucine oxidation to increasing protein intake after an acute bout of resistance exercise. Increasing protein intake stimulated muscle and plasma albumin protein synthesis in a dose-dependent manner up to 20g dietary protein, after which there was a significant stimulation of whole-body leucine oxidation and no added increase in protein synthesis (Moore et al., 2009).

Other studies have not seen the efficacy of leucine as described by the aforementioned researchers. Some saw no difference between the effects of leucine alone and all EAAs (Bohé et al., 2003; Smith et al., 1992,1998; May & Buse, 1989); while others failed to see the purported protein synthetic effects of leucine administration in vivo (McNurlan et al., 1982; Louard et al., 1990).

Table 2: Summary of studies related to the effect of leucine supplementation on muscle protein synthesis

Reference	Population	Design	Findings
Buse & Reid (1975)	Rats	AA mixture added to media to stimulate incorporation of lysine into proteins.	Leucine stimulated protein synthesis, and mixtures without leucine did not.
Sherwin (1978)	Obese and Non-obese humans.	Leucine was given intravenously between meals and after fasting.	Leucine infusion stimulated MPS and attenuated MPB.
McNurlan et al. (1982)	Rats	Leucine was administered to rats that were either fed, starved, or protein-deprived.	Leucine had no effect on MPS in any tissues.
Smith et al. (1992)	7 males	Participants received a constant infusion of valine and a flooding dose of leucine intravenously.	Synthesis rates were higher after flooding, regardless of the precursor chosen.
Smith et al. (1998)	23 young males	Flooding doses of EAAs were given and effects on tracers were observed.	Flooding with EAAs increased tracer incorporation.
Anthony et al. (1999)	Rats	Rats were divided into groups by exercise and diet.	Leucine stimulated MPS following exercise, independent of plasma insulin.
Crozier et al. (2005)	Rats	Fasted rats were given bolus doses of oral leucine.	Leucine stimulated MPS.
Rieu et al. (2007)	Rats	Aging rats were fed diets with differing amounts of leucine content.	The leucine-rich diet stimulated MPS more than others & maintained the effect >30 days.
Dreyer et al. (2008)	16 males	Participants ingested either placebo or leucine-enriched solution after resistance exercise.	Enhanced signaling increased MPS after the leucine-enriched solution was consumed.
Moore et al. (2009)	6 young males	Participants consumed differing amounts of protein after resistance exercise.	20g protein was sufficient to maximally stimulate MPS. Excess intake led to oxidation.
Churchward-Venne et al. (2012)	24 males	Participants consumed differing amounts of whey with or without	Low doses of whey with added leucine stimulated MPS to the same degree

		added leucine after resistance exercise.	as larger whey doses.
Casperson et al. (2012)	Older humans	Meals were supplemented with leucine for 2 weeks.	Increased stimulation of the signaling pathways that control MPS was seen.
AA: Amino Acids; MPS: Muscle Protein Synthesis; MPB: Muscle Protein Breakdown; EAAs: Essential Amino Acids			

In these studies, performed to assess the effect of leucine intake on MPS, the consensus was that increased stimulation of MPS was seen after leucine supplementation. The majority of these studies found that leucine increased MPS to a greater degree than a control or a similar mixture containing less leucine. Three studies of those examined here did not see increased MPS, or saw the same effects induced by other EAAs. In conclusion, it has been shown that under many different types of conditions, and in both rat and human populations, MPS is stimulated by leucine intake or infusion.

Leucine and Muscle Protein Degradation

Carbohydrate and protein/amino acid ingestion during exercise recovery is part of an effective strategy to stimulate muscle protein synthesis, inhibit protein breakdown and, as a result, to augment net muscle protein accretion (Norton & Layman, 2006). Protein catabolism is stimulated to a greater extent than protein synthesis following exhaustive exercise, interestingly enough. Consequently, athletes need to consume sources of protein post-exercise to achieve a positive protein balance and maximize their skeletal muscle adaptive response (Koopman et al., 2007). While protein synthesis has been the focus of countless studies involving leucine (Casperson et al., 2012; Coffey et al., 2011; McNurlan et al., 1982; Pasiakos et al. 2011), protein degradation has not been a specific area of concentration. Nagasawa et al. (2002) found that postprandial protein degradation suppression was primarily due to amino acids, specifically leucine. Since muscle hypertrophy occurs as a result of either increased protein synthesis or decreased protein breakdown, leucine's effect on protein degradation is of great interest.

Several studies have examined the effect of leucine supplementation on muscle protein breakdown and post-exercise muscle recovery. One study evaluated the impact of BCAA supplementation in adult hikers during a 21-day trek at high altitudes. They concluded that BCAA supplementation may prevent muscle loss during the chronic hypobaric hypoxemic conditions (Schena et al., 1992). The anabolic effect of leucine has been

attributed to its inhibition of protein degradation by Nair et al. (1992), but interestingly, they found no evidence that leucine stimulated muscle or whole body protein synthesis.

Baptista et al. (2010) attempted to elucidate the mechanisms behind leucine's suppression of protein catabolism found leucine supplementation attenuates muscle wasting by downregulating the ubiquitin-proteasome system (UPS), which drives protein degradation. The authors noted, however, that leucine supplementation did not restore decreased protein synthesis that followed immobilization of rats in this study. This suggests the protective effect seen in leucine supplementation is mediated by downregulation of UPS gene expression, and not stimulation of protein synthesis. The authors posit that leucine may be utilized as a therapeutic supplement for those faced with long periods of immobilization following surgery, such as joint reconstruction.

In contrast, Sugawara et al. (2009) showed that leucine feeding in a protein deprived state minimized protein catabolism. They suggested that downregulation of activity of the lysosomal pathway, rather than UPS, is responsible for the muscle wasting attenuation seen in rats with a leucine-rich diet. In addition, the study showed protein degradation was significantly suppressed and muscle protein synthesis was not enhanced. This suggests that instead of protein synthesis, reduction in muscle is inhibited by leucine and regulated by protein degradation. When dietary protein is scarce, since amino acids are used as material to synthesize muscle protein, substrate for muscle synthesis is also deficient. The amount of amino acids supplied by muscle protein breakdown may not be sufficient for protein synthesis. In contrast, material is not necessary for the suppression

of protein degradation. According to the findings in these studies, muscle protein degradation is a key factor in regulation of muscle turnover (Sugawara et al., 2009).

One explanation for leucine's observed ability to abate MPB is in its stimulation of plasma insulin. A higher leucine concentration than that typical of high-quality proteins was found to produce transient decreases in MPB and enhance mTORC1 signaling, which may induce protein synthesis in muscle (Dreyer, 2008; Li et al., 2011). However, the observed changes did not translate into significant improvements in overall muscle protein balance. Leucine in bolus doses, like many nutrients, causes an increase in circulating insulin. Since insulin decreases MPB (Louard et al., 1992), the insulin response in the leucine supplemented group may have contributed to the observed decreases in MPB (Glynn et al., 2010). Another study found administration of leucine suppressed protein degradation even without insulin action, to which this effect can often be attributed (Nagasawa et al., 2002).

Another study found that high doses of supplemental leucine (>20g) did not to prevent an increase in muscle damage biochemical markers following eccentric-based resistance exercise, but that supplementation may have aided in the maintenance of force output (Kirby et al., 2012). Another study researched the isolated effects of leucine added to a carbohydrate beverage on recovery from resistance exercise. Biochemical markers of muscle damage like creatine kinase (CK) and LDH, as well as athletes' perception of delayed onset muscle soreness (DOMS) and leucine's possible attenuation of these was examined. Researchers found that there were no significant differences in CK, LDH,

DOMS, and number of squat repetitions in subjects who consumed pre- and post-exercise carbohydrate beverages with or without leucine. The addition of 45 mg/kg leucine to carbohydrate beverages did not attenuate blood markers of exercise-induced muscle damage or participants' perception of DOMS (Stock et al., 2010).

Decreases in protein synthesis and/or increases in protein degradation also occur during tumor development. These changes induce skeletal muscle loss, fatigue, weakness, and atrophy. While physical exercise can be an effective part of the rehabilitation of cancer patients, any compounded effects from leucine on decreased MPB are significant. The promising effects of leucine supplementation give rise to interest in leucine's use for individuals in catabolic disease states. Results in rats show that exercise and/or leucine-supplementation led to an improvement in lean body mass due to attenuation of protein breakdown, maintenance of total muscle protein content, and increase in the muscle myosin content (Salomão et al., 2010).

Table 3: Summary of studies related to effect of leucine supplementation on muscle protein breakdown

Reference	Population	Design	Findings
Schena et al. (1992)	16 humans	Participants trekked 21 days at high altitude and took either BCAA or placebo supplements.	BCAA supplementation decreased muscle loss during chronic hypobaric hypoxia.
Nair et al. (1992)	6 healthy males	Leucine or saline was infused intravenously.	Leucine decreased MPB across several muscle sites.
Nagasawa et al. (2002)	Rats	Rats were fed diets with differing protein compositions or a leucine diet.	Leucine was able to decrease the rate of MPB.
Koopman et al. (2007)	8 elderly males	Crossover design where participants consumed a	Coingestion of leucine did not attenuate MPB.

		control diet or one supplemented with leucine after exercise.	
Sugawara et al. (2009)	Rats	Rats were fed a protein-free diet for 7 days and supplemented with leucine.	Leucine supplementation inhibited MPB.
Baptista et al.(2010)	Rats	Rats were supplemented with leucine during hind limb immobilization.	Leucine supplementation inhibited muscle wasting.
Glynn et al. (2010)	14 humans	Participants consumed EAAs with normal or high leucine content.	Leucine supplementation showed a modest decrease in MPB.
Stock et al. (2010)	20 trained humans	Participants performed resistance exercise and consumed a leucine supplemented beverage before and after.	Leucine supplementation did not attenuate MPB.
Salomão et al. (2010)	Tumor-bearing rats	Rats were divided into groups and performed exercise or did not, and consumed a leucine-rich diet or did not.	Leucine, in coordination with exercise, decreased MPB.
Kirby et al. (2012)	27 males	Participants performed drop jumps and consumed a placebo, leucine, or nothing.	Leucine supplementation did not attenuate MPB.
Nelson et al. (2012)	12 males	Participants performed high intensity endurance exercise and subsequently ingested leucine/protein supplement or control.	The high leucine dose in the supplement saturated BCAA metabolism, increased leucine oxidation and attenuated MPB.
BCAA: Branched Chain Amino Acids; MPB: Muscle Protein Breakdown; EAAs: Essential Amino Acids			

In the studies (see Table 3) performed to assess the effect of leucine supplementation on MPB, the consensus was that attenuation of MPB was observed. Most studies found that leucine ingestion inhibited MPB to a greater degree than a control or a similar mixture containing less leucine. The studies where leucine supplementation was seen to be effective utilized long-term ingestion, or were consumed in conjunction with exercise.

Three studies (Koopman et al., 2007; Stock et al., 2010; Kirby et al., 2012) of those examined here (Table 3) did not see decreased MPB. Interestingly, all three of those studies focused on the ability of leucine to decrease MPB through ingestion post-exercise. In conclusion, it has been shown that under many different types of conditions, and in both rat and human populations, MPB can be attenuated by leucine intake or infusion.

Dietary versus Supplemental Intake

In the world of nutrition and exercise, the notion that "if a little is good, a lot is great" has been long held. For this reason, the use of supplements and megadoses of many nutrients has skyrocketed in recent years. Many individuals consider their diets healthy, but want to enhance muscle synthesis and think supplementation may be the correct route. Previous studies have utilized both dietary and supplemental sources of leucine, but none have directly compared the two sources. Beyond the obvious question of safety, the question begs, is this practice necessary to achieve desired results?

The concept of ingesting an isolated AA to stimulate MPS in the absence of an abundance of other AAs is not new. However, since protein is comprised of multiple AAs, a homogenous AA substrate would be unable to build muscle in the absence of another AA source. If leucine were able to independently stimulate MPS, substrate would be drawn from the intracellular AA pool. If there is sufficient delivery of AAs, synthesis should continue. However, *in vivo*, intramuscular AA concentrations fall as seen in studies on the effects of leucine infusion on human muscle (Alvestrand et al., 1990). After initial stimulation of anabolism, the concentration of all other AAs fell and leucine oxidation rose, likely because complete proteins could no longer be synthesized (Alvestrand et al.,1990). Leucine-rich amino acid mixtures are more effective than leucine alone at improving muscle mass and performance, suggesting the efficacy of leucine is dependent on the presence of other amino acids (Balage et al., 2011; Preedy &

Garlick, 1986). Likewise, Glynn et al. (2010) found no differences in MPS despite significantly higher extracellular concentrations of leucine in one study.

If the biggest factor affecting MPS is AA availability, then dietary intakes of protein should serve to provide adequate substrate for ongoing muscle synthesis. One study specifically assessed the impact of leucine derived from complete meals on MPS stimulation. Rats were fed diets of differing protein content from either wheat or whey sources. Given equal protein intakes, whey protein increased MPS by as much as 52% more than wheat protein. The authors concluded that postprandial mTOR signaling and MPS stimulation is primarily directed by leucine, but the response duration is influenced by additional factors (Norton et al., 2009).

Other studies have shown MPS becomes saturated when blood EAAs rise above 50-80% greater than the fasted state (Alvestrand et al., 1990). Observers found that the concentration of serum urea increased dramatically at high rates of AA infusion, due to the increase of hepatic catabolism of AAs delivered in excess of bodily ability to utilize them for muscle (Bohé et al., 2003). Others have agreed that there is little evidence that moderate physical activity increases protein requirements beyond those achieved by eating a normal diet that satisfies daily nutrient requirements (Rennie et al., 2006; Glynn et al., 2010). Provision of exogenous BCAAs may stimulate MPS but the effect is likely to be transitory given the “muscle-full” phenomenon already mentioned (Rennie et al., 2006).

In contrast, some studies suggest leucine supplementation may improve muscle protein synthesis in response to lower protein meals (Casperson et al., 2012; Churchward-Venne et al., 2012). Following exercise, a suboptimal dose of protein high in leucine content or a mixed nutrient meal supplemented with leucine enhances MPS (Rieu et al., 2003; Churchward-Venne et al., 2012). Casperson et al. (2012) found that supplementing the daily meals of older adults for two weeks with 4g leucine increased FSR and anabolic signaling.

The evidence from these studies suggests consumption of free leucine versus a dietary protein leads to an imbalance between leucine signaling and AA availability. This discrepancy in the initiation of MPS signaling pathways and delayed, or deficient, availability of AAs as substrates may even explain why MPS is stimulated for a short duration in many of these studies and did not convert to significant muscle accretion (Dardevet et al., 2012)

Table 4: Summary of studies related to dietary intake of leucine or supplementation

Reference	Population	Design	Findings
Alvestrand et al. (1990)	12 females	Participants were infused with leucine intravenously.	Plasma and intracellular levels of AAs decreased, and 40% of excess leucine was oxidized.
Bohe et al. (2003)	21 humans	Participants were infused with mixed AAs at a rate 240% above basal levels.	MPS became saturated at high concentrations of intramuscular EAAs.
Rennie et al. (2006)	Humans	Participants were infused with mixed AAs or leucine.	Leucine stimulated MPS to the same extent as complete meals.
Norton et al. (2009)	Rats	Rats consumed diets with differing leucine content.	Higher leucine content of meals correlated to peak

			activation of MPS.
Glynn et al. (2010)	14 humans	Participants consumed EAAs or EAAs with increased leucine content.	Leucine content in 10g EAAs is sufficient to maximize MPS.
Casperson et al. (2012)	8 older humans	Meals were supplemented with leucine for 2 weeks.	Leucine improved MPS in response to lower protein meals.
Churchward-Venne et al. (2012)	24 males	Participants completed resistance exercise and consumed varying doses of whey supplemented with leucine or EAAs without leucine.	Low doses of whey supplemented with leucine or EAAs stimulated MPS to the same degree as larger doses.
Nelson et al. (2012)	12 males	Participants performed high intensity endurance exercise and subsequently ingested leucine/protein supplement or control.	The high leucine dose in the supplement saturated BCAA metabolism, increased leucine oxidation and attenuated MPB.

AAs: Amino Acids; MPS: Muscle Protein Synthesis; EAAs: Essential Amino Acids; BCAA: Branched Chain Amino Acid; MPB: Muscle Protein Breakdown

In these studies, performed to assess the effect of leucine supplementation and/or dietary intake, the general consensus was that leucine intake in excess of the amount found in adequate whole protein is unnecessary. Most studies found that leucine supplementation in addition to protein intake saturated metabolism and leucine oxidation resulted. The studies where leucine supplementation was seen to be additive were in conjunction with lower protein doses, or supplementation was seen to have the same effect as complete meals. In summary, research has shown that excessive intake of leucine in addition to adequate protein ingestion is unnecessary to stimulate MPS.

Timing, Dosing, and Long Term Effects

Researchers have speculated on the specific intake of leucine that constitutes an adequate dose to promote MPS. Churchward-Venne et al. (2012) reported that, leucine ingestion in larger amounts than that found in a saturating dose (20-25g whey protein containing 2.5-3g leucine), would not be likely to promote a further increase in MPS magnitude or duration. The authors cautioned that as the data were taken from young healthy men (weighing ~86 kg), the dose of protein necessary to be maximally effective could be far different in a ~50 kg female gymnast or a 120 kg bodybuilder.

A review by Rennie et al. (2006) similarly stated that there is little evidence that high rates of physical activity increase protein requirements beyond those achieved by eating a normal diet that fulfills energy requirements. They acknowledged that providing supplemental leucine will stimulate MPS, but the effect will likely be transitory given the "muscle-full" phenomenon. The researchers posited that amino acid administration that mimics meal feeding would allow the muscle fullness set point to be modified to increase anabolism. Another reason to restrain dosing to modest levels is that amino acids have effects on protein synthesis and breakdown within a narrow range of concentration as the daily variation in the blood is only ~ ±50 % of the daily mean (Bergstrom et al., 1990).

More recent evidence points to saturation of phosphorylation of initiation factors and, as stated previously, decreased sensitivity of signaling as the mechanism behind leucine's

seeming decrease in efficacy. Researchers examined rat liver and skeletal muscle after administration of leucine, ranging from 0.169 to 1.35 g/kg of body weight. In skeletal muscle, phosphorylation reached a plateau at 0.675 g/kg of body weight. The mTOR signaling sensitivity to leucine in the skeletal muscle appeared to be higher than that in the liver (Yoshizawa et al., 2013).

Aside from leucine, there may be other EAAs that can stimulate MPS. Valine, phenylalanine and threonine have each been shown to increase human MPS when given in a flooding dose (Smith et al., 1998). While the proposed effects of mega-doses of other EAAs may hold true, the relative efficiency of much smaller doses of leucine indicates greater efficacy and potency.

Despite possible benefits of supplementation with leucine, some adverse effects have been seen related to doses administered. Effects reported include reduced food intake (Blouet et al., 2009), imbalance of other BCAAs (Allen & Baker 1972), and decreased growth and immune response in pigs (Gatnau et al., 1995). In contrast, Nguema et al. (2007), found no changes in food intake associated with leucine supplementation. Since the leucine dose necessary for increased plasma leucine concentration is not supraphysiological, it is unlikely to suppress appetite and reduce whole food intake.

The increase in blood AA concentrations postmeal lasts ~3 hours, (Bergstrom et al., 1990) and the increased AA supply is available for up to six hours, however, MPS appears to become unresponsive after 2.5 hours (Bohé et al., 2001). Another study

suggested this decrease in efficacy over time is due to desensitization of the signaling mechanism that senses and transmits information about AA availability. The authors further stated that long-term parenteral infusions of AAs will not maintain anabolism longer than a couple of hours and excess AAs are simply catabolized to urea. They warned that if studies are to obtain accurate values of human MPS, they need to be confined to periods of <3 hours, with 2.5 hours being optimal (Rennie et al., 2006).

Since studies show stimulation of MPS is time limited (Bohé et al., 2001), it appears that only modest amounts of dietary amino acids would be needed to achieve maximal stimulation of the muscle anabolic processes (i.e., for adults of average weight, 30–40g of protein). The amount of protein necessary to build and maintain muscle can be easily obtained from a normal diet which is adequate to satisfy total energy needs for the individual since most diets contain at least 10-15% protein. Moreover, dietary protein consumed after exercise in excess of the amount which can be incorporated into muscle protein stimulates oxidation (Moore et al., 2009).

Recent evidence suggests that physical training increases protein metabolism efficiency, whether moderate endurance exercise or resistance training. Endurance training does not increase leucine oxidation and protein metabolism becomes more efficient- increased protein balance is achieved at a lower rate of oxidation (Gaine et al., 2005).

Table 5: Summary of studies related to timing, dosing, and long-term effects of leucine supplementation

Reference	Population	Design	Findings
Allen & Baker (1972)	Chickens	Chickens were fed varying amounts of excess leucine in their diets.	The availability of the other BCAAs decreased as excess dietary leucine increased.
Gatnau et al. (1995)	Pigs	Pigs were supplemented with varying levels of dietary leucine.	High dietary leucine intake contributed to poor growth and immunity.
Smith et al. (1998)	23 young males	Participants were given flooding doses of individual EAAs and non-EAAs.	Flooding doses of EAAs stimulated increased incorporation of tracers.
Bohé et al. (2001)	6 humans	Mixed AAs were infused by IV.	MPS rates declined rapidly after 2 hours.
Gaine et al. (2005)	7 humans	Participants consumed a controlled diet and performed aerobic exercise for 4 weeks.	Leucine oxidation decreased as protein utilization improved.
Nguema et al. (2007)	Rats	Rats were fed long term diets with differing sources of protein.	Diets composed of casein and supplemented with EAAs sustained appetite.
Blouet et al. (2009)	Rats	Rats consumed a diet supplemented with leucine or a high protein diet.	Increased leucine availability in mediobasal hypothalamus led to decreased food intake.
Moore et al. (2009)	6 young males	Participants performed resistance exercise then consumed differing amounts of protein.	Leucine oxidation increased after 20g protein as MPS was maximally stimulated.
Churchward-Venne et al. (2012)	24 males	Participants completed resistance exercise and consumed varying doses of whey supplemented with leucine or EAAs without leucine.	Low doses of whey supplemented with leucine or EAAs stimulated MPS to the same degree as larger doses.
Nelson et al. (2012)	12 males	Participants performed high intensity endurance exercise and subsequently ingested leucine/protein supplement or control.	Supplemental leucine dose saturated BCAA metabolism, increased leucine oxidation, attenuated MPB.
Yoshizawa et	Rats	Rats were supplemented	Muscle phosphorylation

al. (2013)		with oral leucine in varying doses.	plateaued at 0.675 g/kg of body weight.
BCAAs: Branched Chain Amino Acids; EAAs: Essential Amino Acids; AAs: Amino Acids; MPS: Muscle Protein Synthesis; MPB: Muscle Protein Breakdown			

In the studies compiled in Table 5, performed to assess the optimal timing and dosing of leucine supplementation and the long-term effects, the consensus was that leucine intake in excess of that found in ~20g whole dietary protein or ~0.75mg/kg free leucine is unnecessary. Other studies found the window to evaluate MPS post-exercise should be contained to 2.5 hours. There was little consensus and inadequate data to postulate on the effects of long-term leucine supplementation.

Effects After Resistance and Endurance Exercise

Do the previously seen effects of leucine supplementation on MPS after exercise have consistent results despite varying types of exercise? Resistance exercise and training can have a remarkable effect on body composition by increasing muscular strength and hypertrophy. For hypertrophy to occur MPS must surpass muscle protein catabolism and as previously stated, availability of AAs is a chief factor in promoting protein synthesis (Norton & Layman, 2006; Rannels et al., 1974; Garlick & Grant, 1988). Significantly lower levels of serum AAs, predominantly leucine and isoleucine, have been noted following resistance training. Supplementing the diet with EAAs circumvents this decline and increases AA availability to the muscles.

Studies that have examined the physiological mechanism of leucine efficacy identify messenger RNA translation via the activation of a variety of intracellular signaling proteins, especially those of the mTOR signaling cascade as the primary level of regulation of acute MPS. Stimulation of MPS after food intake or exercise is accompanied by increased phosphorylation, and activity, of the mTOR signaling pathway (Karlsson et al., 2004; Kimball & Jefferson, 2004, 2006; Wilkinson et al., 2013; Coffey et al., 2011).

Moore et al. (2009) examined the response of protein synthesis and total body leucine oxidation to increasing protein intake after an acute bout of resistance exercise.

Increasing protein intake stimulated muscle and plasma albumin protein synthesis in a dose-dependent manner up to 20g dietary protein, after which there was a significant stimulation of whole-body leucine oxidation and no added increase in protein synthesis.

Given that energy needs are met, the findings by Moore et al. have implications for protein recommendations for athletes participating in resistance exercise in terms of the quantity of dietary protein to maximize muscle growth. If a 20g protein dose maximally stimulates MPS after exercise and resistance exercise enhances the synthesis of muscle protein for at least 24 hours, the question becomes: How often in a day should an athlete consume such a dose to stimulate muscle anabolism and maximize muscle growth? As MPS becomes refractory to continual aminoacidemia and excessive AAs are lost to oxidation, the authors speculated that 20g of high quality dietary protein could be ingested 5–6 times and MPS could be expected to be maximally stimulated. Consumption of protein in excess of this dose would just lead to oxidative loss. More importantly, given that AAs oxidation capacity adapts to diet and acts as a key regulator of protein stores, chronic excessive protein may actually lead to weakening of the protein synthetic response to protein doses less than the optimal 20g (Moore et al., 2009).

The resistance exercise and EAA supplementation combination is an effective stimulator of protein synthesis (Biolo et al., 1997; Preedy & Garlick, 1986). Whey protein is a rich source of EAAs, including the BCAAs, and especially leucine. As a source of AAs, whey has been examined for potential ergogenic effects in combination with resistance training Burke et al., 2001). The purpose of a study by Coburn et al. (2006) was to examine the

effects of resistance training in conjunction with a leucine (6.2g) and whey protein (20g) supplement. Significantly greater strength increases were seen in the group consuming the leucine and whey supplement over those consuming a carbohydrate placebo. The two groups were not matched for protein intake, however, and these effects may be contributed solely to the addition of protein to leucine and whey supplemented group.

In order to elucidate the efficacy of supplemental leucine and resistance exercise on muscle anabolism, Tipton et al. (2009) investigated the response of net muscle protein balance to ingestion of additional leucine with protein in association with resistance exercise. Subjects performed resistance exercise following ingestion of 1 of 2 drinks: placebo or 16.6g of whey protein + 3.4g of leucine. Whey protein plus leucine ingestion resulted in an elevated anabolic response in muscle, but not one that is greater than the response to whey protein alone.

Researchers surmised that since protein synthesis results not only from provision of substrate for synthetic processes, but also from stimulation of signaling pathways in muscle cells, bolus doses cannot further stimulate protein synthesis once the signaling pathway thresholds are met. The anabolic pathways are likely fully stimulated by the amino acid provision of the whey protein and/or resistance exercise, so the additional leucine presents no further advantage (Tipton et al., 2009).

Another study sought to investigate the effects of daily oral leucine ingestion on strength, lean tissue mass (LTM), and fat mass (FM) of subjects during a 12-week resistance-

training program (Ispoglou et al., 2011). Participants ingested either 4g per day leucine or a corresponding amount of lactose and trained under supervision twice per week. Strength on each exercise was assessed by five repetition maximum, and body composition was assessed by DEXA. The leucine group demonstrated significantly higher strength gains, but there were no significant differences between groups in either total percentage LTM gains or FM losses. This research suggests that 4g per day leucine ingestion could be used as a supplement to enhance strength performance during resistance training.

Koopman et al. (2007) also agreed that resistance exercise is a potent stimulus to augment muscle protein anabolism, but that the intake of food during post-exercise recovery is required for hypertrophy to take place. Interestingly, protein catabolism is stimulated to a greater extent than protein synthesis following exercise. In consequence, athletes need to consume sources of protein post-exercise to achieve a positive protein balance and maximize their skeletal muscle adaptive response. For this reason, carbohydrate and protein/AA ingestion during exercise recovery is part of an effective strategy to stimulate MPS, inhibit protein breakdown and, as a result, augment net muscle protein accretion.

Kirby et al. (2012) focused on leucine's ability to attenuate muscle damage, more so than its specific effect on MPS, but as previously discussed, muscle hypertrophy is a result of decreased muscle breakdown as well. Kirby et al. (2012) attempted to determine the effect of leucine supplementation on indices of muscle damage following eccentric-based

resistance exercise. Muscle function was determined by peak force during an isometric squat and by jump height. The leucine supplemented group experienced an attenuated drop in mean peak force compared to the placebo group. The principal discovery was that high-dose leucine supplementation was not able to attenuate the increase in biochemical markers of muscle damage that follows eccentric-based resistance exercise. It may, however, aid in maintaining isometric force output.

Although some previously mentioned studies have seen positive results by combining leucine supplementation with carbohydrate ingestion (Dreyer et al., 2007; Coffey et al., 2011), the following study utilized EAAs, which would theoretically further enhance MPS. This study evaluated whether EAA and carbohydrate ingestion before resistance exercise can improve direct measures of post-exercise MPS like fractional synthetic rate (FSR). Researchers found that muscle FSR was enhanced in the EAA + CHO group immediately following EAA + CHO ingestion, declined to basal values during exercise, and remained unchanged at 1-hour post-exercise. Muscle FSR decreased in the fasting group during exercise and increased at 1-hour post-exercise. The researchers concluded that EAA + CHO ingestion before resistance exercise did not enhance post-exercise FSR compared with exercise in a fasting state (Fujita et al., 2009).

The effects of endurance exercise differ from those of resistance exercise, as endurance training generates lower muscle contractile forces and characteristically does not induce muscle hypertrophy (Phillips et al., 1996). Sustained endurance exercise is largely

catabolic and reduces MPS and plasma leucine concentrations during exercise due to the metabolic demand for BCAAs in training skeletal muscle (Norton & Layman, 2006).

Provision of supplemental leucine during endurance exercise could stimulate greater post-exercise MPS by diverting protein utilization form endogenous stores (Pasiakos et al. 2011). In this study the effects of consuming an optimal EAA dose (consistent with high-quality proteins) supplemented with leucine during endurance exercise on MPS, intracellular signaling, and protein metabolism during recovery was assessed. Participants completed 2 bouts of cycle ergometry, and one of two isonitrogenous drinks with differing leucine contents [leucine+EAA, 3.5g leucine; EAA, 1.87g leucine] was consumed during exercise. MPS and protein turnover were determined by continuous tracer infusions. This trial found the supplement enhanced post-exercise MPS by 33% when compared with a control. These findings signify that increasing leucine content in an already optimal EAA dose results in further MPS increases following endurance exercise. Additional leucine availability may have spared endogenous protein stores to a greater extent than EAA alone, however, the EAA supplement plus leucine concentration did not improve mTORC1 intracellular signaling (Pasiakos et al., 2011).

Thomson et al. (2011) attempted to establish how consuming leucine-rich protein and high carbohydrate post-exercise would affect recovery, as measured by subsequent cycling and mechanistic markers. Cyclists performed 2- to 2.5-hour interval training bouts, then ingesting leucine-enriched protein (0.1 & 0.4g/kg/hr), high carbohydrate (1.2g/kg/hr) nutrition or isocaloric control nutrition. Post-exercise leucine ingestion

improved mean sprint power by 2.5% and a reduction in creatine kinase (CK) activity was suggestive of attenuated disruption to skeletal muscle integrity and faster muscle repair. The findings are relevant to real life practice as the supplement was provided along with regular food items, ingested following exercise in a realistic time frame, and provided with an appropriate quantity of carbohydrate to optimize glycogen repletion (Thomson et al., 2011).

While not directly researching leucine and MPS, a study by Greer et al. (2007) found BCAA ingestion prior to and during endurance exercise attenuated indices of muscle damage, which may have a limited effect on physical performance. Participants ingested a carbohydrate or BCAA beverage before, during, and after a 90 minute cycling bout. Lactate dehydrogenase (LDH) and CK measures of muscle damage were lower in the BCAA-supplemented group compared to placebo and carbohydrate groups, and leg-flexion torque was higher at the 48-hour mark, possibly due to decreased muscle damage. If this exercise-induced muscle damage can be reduced by supplementation, then logically speaking, subsequent performance should improve or be maintained.

One more study (Nelson et al., 2012) looking at leucine's effects following endurance exercise used a leucine plus carbohydrate, fat, and protein supplement (7.5/20/89/22 g/hour, respectively) and an isocaloric fat and carbohydrate control. Subjects ingested one of the supplements following high-intensity cycling and researchers measured repeat performance and muscle indices of damage and turnover. The leucine supplemented group saw greater plasma BCAA levels and leucine oxidation indicating the 10g leucine

dosage exceeded BCAA metabolism capacity. CK levels were lower in the experimental group, but the apparent effect on subsequent performance was "trivial" per the study authors.

Table 6: Summary of studies related to effect of leucine supplementation on resistance versus endurance exercise

Reference	Population	Design	Findings
Karlsson et al. (2004)	7 males; age 25 ± 1 yr	Participants consumed a BCAA or placebo before and after performing resistance exercise.	BCAA ingestion increased phosphorylation of signaling proteins in skeletal muscle.
Coburn et al. (2006)	33 males; age 24.4 ± 2.4 yrs	Participants consumed a leucine/whey or control supplement and performed resistance exercise.	The supplement group saw greater strength increases.
Dreyer et al. (2007)	16 young males	Participants consumed an EAA+CHO or control supplement 1 hour after resistance exercise.	Enhanced mTOR signaling and FSR were seen in the EAA+CHO group.
Greer et al. (2007)	9 males; age 21.6 ± 3.2 yrs	Participants consumed BCAA, CHO, or placebo beverage before and during endurance cycling.	BCAA supplementation attenuated CK and LDH concentrations.
Koopman et al. (2008)	8 elderly males; age 73 ± 1 yr	Participants consumed a supplement with leucine or a control 30 min after resistance exercise.	Whole body protein balance was greater in the leucine group, but FSR was not affected.
Moore et al. (2009)	6 males; age 22 ± 2 yrs	Participants consumed differing doses of whole egg protein after resistance exercise.	MPS displayed a dose response to protein ingestion and was maximally stimulated at 20g.
Tipton et al. (2009)	15 humans	Participants performed resistance exercise after consuming placebo or leucine supplement	Leucine stimulated MPS to a greater extent than placebo.
Fujita et al. (2009)	22 humans	Participants either fasted or consumed EAA+ CHO prior to performing resistance exercise.	EAA+CHO did not increase FSR postexercise compared to fasting.
Coffey et al.	8 males; age	Participants performed	MPS and anabolic

(2011)	21.4 ± 2.6 yrs	sprint cycling after consuming either whey+leucine+CHO or placebo.	signaling was greater following the whey+leucine+CHO provision.
Ispoglou et al. (2011)	26 males	Participants consumed either leucine or lactose daily for 12 wks and performed resistance exercise 2x/wk.	The leucine group had greater strength gains, but not LTM or FM losses.
Pasiakos et al. (2011)	8 humans; age 24 ± 2 yrs	Participants consumed either a low-leucine or high-leucine EAA supplement during endurance exercise.	The leucine-enriched supplement induced greater MPS, less MPB, and greater oxidation.
Thomson et al. (2011)	10 males cyclists	Participants performed endurance cycling and consumed leucine-enriched supplement or isocaloric control post-exercise.	Post-exercise leucine ingestion improved sprint power and reduced perceived overall tiredness during sprints.
Howatson et al. (2012)	12 males; age 23 ± 2 yrs	Participants consumed a BCAA supplement or placebo and performed drop jumps.	BCAA supplementation reduced indices of muscle damage and improved recovery time.
Kirby et al. (2012)	27 males; age 21.3 ± 1.6 yrs	Participants consumed leucine or placebo before, during, after performing depth jumps.	The leucine group had an attenuated drop in mean peak force compared to placebo.
Nelson et al. (2012)	12 male cyclists	Participants consumed a leucine-enriched supplement or isocaloric control after endurance exercise.	Leucine ingestion increased BCAA oxidation, decreased CK concentration, and had trivial effect on sprint power.

BCAA: Branched Chain Amino Acid; EAA: Essential Amino Acid; CHO: Carbohydrate; mTOR: Mammalian Target of Rapamycin; FSR: Fractional Synthetic Rate; CK: Creatine Kinase; LDH: Lactate Dehydrogenase; MPS: Muscle Protein Synthesis; LTM: Lean tissue mass; FM: Fat mass; MPB: Muscle Protein Breakdown

These studies, compiled in Table 6, performed to assess the effect of leucine supplementation on resistance or endurance exercise, found supplementation in addition to exercise increased MPS in conjunction with resistance exercise and attenuated MPB in

conjunction with endurance exercise. The two studies (Fujita et al. 2009; Koopman et al. 2008) in which leucine supplementation was not seen to have a greater effect than the control had no similar characteristics from which to draw conclusions as to the type of situation where leucine may be most effective. In summary, the studies shown in Table 6 indicated leucine may improve subsequent performance in relation to both resistance and endurance exercises.

Conclusion

Leucine has been shown throughout numerous studies over the last 30+ years to be an effective stimulator of MPS in varied populations and in different training scenarios (Buse & Reid, 1975; Casperson et al., 2012; Coburn et al., 2006; Dreyer, 2008; Katsanos et al., 2006; Pasiakos et al., 2011). This essential amino acid has been established as a potent regulator of metabolism, a signal in the mTOR transduction pathway, and of key importance in its ability to help spare endogenous protein stores (Alvestrand et al., 1990; Balage et al., 2011; Drummond & Rasmussen, 2008; Garlick, 2005; Kimball & Jefferson, 2004; Louard et al., 1990). Many studies mentioned previously examined its role in protein metabolism and identified leucine as the most effective amino acid in activating MPS, preventing muscle breakdown, and maintaining positive net muscle protein. Leucine's efficacy is not clear cut however, as there are many qualifications to consider.

Some may assume since leucine is ingested on a daily basis and is found in many common foods, its isolated consumption would be beneficial and harbor no dangers. However, intake of isolated amino acids often leads to imbalances in these nutrients and can instigate deleterious effects in vivo. Normally, amino acids are consumed as a part of an entire meal, and that intake is accompanied by a resultant rise in blood glucose and insulin release, which have been found to facilitate leucine's mechanism of action (Preedy & Garlick, 1986; Garlick & Grant, 1988; Li et al., 2013). This may be due to an

increase in the insulin sensitivity in cells post-meal caused by amino acid consumption. Researchers have surmised that as protein synthesis results not only from availability of substrates for synthetic processes, but also from activation of signaling pathways in cells, bolus doses cannot further increase protein synthesis once the signaling pathway thresholds are met (Drummond & Rasmussen, 2008). Bolus dosing of any amino acid, in particular leucine, could potentially lead to insulin resistance due to prolonged stimulation of the insulin response (Li et al., 2013). Also, as cells' amino acids oxidation capacity adapts to dietary intake, chronic excessive intake could lead to blunting of the protein synthetic response to protein doses less than bolus intakes.

Provided that total body energy requirements are met, leucine supplementation has a dose dependent protein synthesis response, a greater activation of synthetic signaling pathways, as well as the ability to spare body protein stores. MPS is stimulated immediately following intake while the amino acid pool is elevated, so studies have seen greater effects on muscle hypertrophy when leucine is consumed post-exercise than when it is consumed prior to activity. This is also because leucine appears to be more effective in stimulating MPS when muscle is in an anabolic state, as it is post-exercise (Anthony et al., 1999; Dreyer et al., 2008).

Following either resistance or endurance exercise, leucine has been shown to be effective in stimulating muscle protein synthesis. It has been seen to be more effective as a post-exercise supplement than water, an isocaloric carbohydrate supplement, lactose, an

isonitrogenous EAA supplement, or fasting (Churchward-Venne et al., 2012; Dreyer et al., 2008; Stock et al., 2010; Ispoglou et al., 2011).

While hundreds of studies have been performed on leucine in various situations, a couple holes in the literature may still exist. To prevent some of the poor outcomes discussed here, an upper limit of leucine intake should be established or, preferably, a widely accepted recommendation for appropriate doses of high-quality protein as leucine is more effective when consumed as part of meal. The long-term effects of chronic leucine supplementation would be a study worth noting as well. An investigation into the effects of leucine intake prior to and following exercise may find a consistently elevated amino acid pool and continued FSR maintenance.

In conclusion, adding high leucine foods, including whey, eggs, poultry, fish, and kidney beans, to meals during post-exercise recovery may aid in increasing muscle hypertrophy, attenuating muscle breakdown, and maintaining net positive muscle protein. Some studies have found that bolus doses of leucine are not more effective than doses that could be ingested from a healthy diet, and may induce detrimental health effects, including insulin resistance (Zanchi et al., 2012; McCormack et al., 2013). Others have found that increasing dietary leucine has a positive effect on insulin sensitivity (Li et al., 2013; Zhang et al., 2012). Despite the lingering questions regarding a potentially negative health impact with high dose intakes, consumption of high leucine foods and leucine-enhanced foods and supplements (e.g., whey protein food bars) post-exercise have been found to enhance muscle protein synthesis.

References

Adechian S, Rémond D, Gaudichon C, Pouyet C, Dardevet D, Mosoni L. Spreading intake of a leucine-rich fast protein in energy-restricted overweight rats does not improve protein mass. *Nutrition*. 2012;28(5):566–571.

Ahlborg G, Felig P, Hagenfeldt L, Hendler R, Wahren J. Substrate turnover during prolonged exercise in man. *Journal of Clinical Investigation*. 1974; 53: 1080-90

Allen NK, Baker DH. Quantitative efficacy of dietary isoleucine and valine for chick growth as influenced by variable quantities of excess dietary leucine. *Poultry Science.* 1972;51(4):1292–1298.

Alvestrand A, Hagenfeldt L, Merli M, Oureshi A, Eriksson LS. Influence of leucine infusion on intracellular amino acids in humans. *European Journal of Clinical Investigation.* 1990;20(3):293–298.

Anthony JC, Anthony TG, Layman DK. Leucine supplementation enhances skeletal muscle recovery in rats following exercise. *Journal of Nutrition.*1999;129(6):1102–1106.

Balage M, Dupont J, Mothe-Satney I, Tesseraud S, Mosoni L, Dardevet D. Leucine supplementation in rats induced a delay in muscle IR/PI3K signaling pathway associated with overall impaired glucose tolerance. *Journal of Nutritional Biochemistry.* 2011;22(3):219–226.

Baptista I, Leal M, Artioli G, Aoki MS, Fiamoncini J, Turri AO, Curi R, Miyabara EH, Moriscot AS. Leucine attenuates skeletal muscle wasting via inhibition of ubiquitin ligases. *Muscle Nerve*. 2010;41(6):800–808.

Bergström J, Fürst P, Vinnars E. Effect of a test meal, without and with protein, on muscle and plasma free amino acids. *Clinical Science.* 1990;79(4):331–337.

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. *American Journal of Physiology.* 1995;268(3 Pt 1):E514–520.

Blomstrand E, Eliasson J, Karlsson HKR, Köhnke R. Branched-chain amino acids activate key enzymes in protein synthesis after physical exercise. *Journal of Nutrition.* 2006;136(1 Suppl):269S–73S.

Blouet C, Jo Y-H, Li X, Schwartz GJ. Mediobasal hypothalamic leucine sensing regulates food intake through activation of a hypothalamus-brainstem circuit. *Journal of Neuroscience.* 2009;29(26):8302–8311.

Bohé J, Low A, Wolfe RR, Rennie MJ. Human Muscle Protein Synthesis is Modulated by Extracellular, Not Intramuscular Amino Acid Availability: A Dose-Response Study. *Journal of Physiology*. 2003;552(1):315–324.

Bonau RA, Jeevanandam M, Daly JM. High-branched chain amino acid solutions: relationship of composition to efficacy. *Journal of Parenteral and Enteral Nutrition*. 1984;8(6):622–627.

Burke DG, Chilibeck PD, Davidson KS, Candow DG, Farthing J, Smith-Palmer T. The effect of whey protein supplementation with and without creatine monohydrate combined with resistance training on lean tissue mass and muscle strength. *International Journal of Sport Nutrition, Exercise & Metabolism*. 2001;11(3):349–364.

Burke LM, Loucks AB, Broad N. Energy and carbohydrate for training and recovery. *Journal of Sports Science*. 2006;24(7):675–685.

Buse MG, Reid SS. Leucine. A possible regulator of protein turnover in muscle. *Journal of Clinical Investigation*. 1975;56(5):1250–1261.

Casperson SL, Sheffield-Moore M, Hewlings SJ, Paddon-Jones D. Leucine supplementation chronically improves muscle protein synthesis in older adults consuming the RDA for protein. *Clinical Nutrition*. 2012;31(4):512–519.

Churchward-Venne TA, Burd NA, Mitchell CJ, West DW, Philp A, Marcotte GR, Baker SK, Baar K, Phillips SM. Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men. *Journal of Physiology. (Lond.)*. 2012;590(Pt 11):2751–2765.

Coburn J, Housh D, Housh T, Malek M, Beck T, Cramer J, Johnson G, Donlin P. Effects of leucine and whey protein supplementation during eight weeks of unilateral resistance training. *Journal of Strength & Conditioning Research*. 2006;20(2):284–291.

Coffey VG, Moore DR, Burd NA, Rerecich T, Stellingwerff T, Garnham AP, Phillips SM, Hawley JA. Nutrient provision increases signalling and protein synthesis in human skeletal muscle after repeated sprints. *European Journal of Applied Physiology.* 2011;111(7):1473–1483.

Crozier SJ, Kimball SR, Emmert SW, Anthony JC, Jefferson LS. Oral Leucine Administration Stimulates Protein Synthesis in Rat Skeletal Muscle. *Journal of Nutrition.* 2005;135(3):376–382.

Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor P, Rennie MJ. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB Journal*. 2004;19(3):422-4.

Dardevet D, Rémond D, Peyron M-A, Papet I, Savary-Auzeloux I, Mosoni L. Muscle Wasting and Resistance of Muscle Anabolism: The "Anabolic Threshold Concept" for Adapted Nutritional Strategies during Sarcopenia. *The Scientific World Journal*. 2012;2012:1–6.

Dreyer H. Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. *American Journal of Physiology, Endocrinology & Metabolism*. 2008 ;294:E392–E400.

Drummond M, Rasmussen B. Leucine-enriched nutrients and the regulation of mammalian target of rapamycin signalling and human skeletal muscle protein synthesis. *Current Opinion in Clinical Nutrition & Metabolic Care*. 2008;11(3):222–226.

Fujita S, Dreyer HC, Drummond MJ, Glynn EL, Volpi E, Rasmussen BB. Essential amino acid and carbohydrate ingestion before resistance exercise does not enhance post-exercise muscle protein synthesis. *Journal of Applied Physiology*. 2009;106(5):1730–1739.

Gaine PC, Pikosky MA, Bolster DR, Martin WF, Maresh CM, Rodriguez NR. Postexercise Whole-Body Protein Turnover Response to Three Levels of Protein Intake. *Medicine & Science in Sports & Exercise*. 2007;39(3):480–486.

Garlick PJ, Grant I. Amino acid infusion increases the sensitivity of muscle protein synthesis in vivo to insulin. Effect of branched-chain amino acids. *Biochemical Journal*. 1988;254(2):579–584.

Garlick PJ. The Role of Leucine in the Regulation of Protein Metabolism. *Journal of Nutrition.* 2005;135(6):1553S–1556S.

Gatnau R, Zimmerman DR, Nissen SL, Wannemuehler M, Ewan RC. Effects of excess dietary leucine and leucine catabolites on growth and immune responses in weanling pigs. *J Anim Sci*. 1995;73(1):159–165.

Glynn EL, Fry CS, Drummond MJ, Timmerman KL, Dhanani S, Volpi E, Rasmussen BB. Excess Leucine Intake Enhances Muscle Anabolic Signaling but Not Net Protein Anabolism in Young Men and Women. *Journal of Nutrition*. 2010;140(11):1970–1976.

Greer BK, Woodard JL, White JP, Arguello EM, Haymes EM. Branched-chain amino acid supplementation and indicators of muscle damage after endurance exercise. *International Journal of Sport Nutrition, Exercise & Metabolism*. 2007;17(6):595–607.

Howald H, Decombaz J. Nutrient intake and energy regulation in physical exercise. *Experientia Supplementum*. 1983;44:77–88.

Ispoqlou T, King RFGJ, Polman RCJ, Zanker C. Daily L-Leucine Supplementation in Novice Trainees During a 12-Week Weight Training Program. *International Journal of Sports Physiology & Performance*. 2011;6(1):38–50.

Karlsson HKR, Nilsson P-A, Nilsson J, Chibalin AV, Zierath JR, Blomstrand E. Branched-chain amino acids increase p70S6k phosphorylation in human skeletal muscle after resistance exercise. *American Journal of Physiology, Endocrinology & Metabolism.* 2004;287(1):E1–7.

Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *American Journal of Physiology, Endocrinology & Metabolism*. 2006;291(2):E381–E387.

Kimball SR, Jefferson LS. Regulation of global and specific mRNA translation by oral administration of branched-chain amino acids. *Biochemical & Biophysical Research Communications.* 2004;313(2):423–427.

Kimball SR, Jefferson LS. Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. *Journal of Nutrition.* 2006;136(1 Suppl):227S–31S.

Kirby TJ, Triplett NT, Haines TL, Skinner JW, Fairbrother KR, McBride JM. Effect of leucine supplementation on indices of muscle damage following drop jumps and resistance exercise. *Amino Acids*. 2012;42(5):1987–1996.

Koopman R, Verdijk L, Manders R, Gijsen AP, Gorselink M, Pijpers E, Wagenmakers A, van Loon LJ. Co-ingestion of protein and leucine stimulates muscle protein synthesis rates to the same extent in young and elderly lean men. *American Journal of Clinical Nutrition*. 2006;84(3):623–632.

Koopman R, Saris W, Wagenmakers A, van Loon LJ. Nutritional interventions to promote post-exercise muscle protein synthesis. *Sports Medicine*. 2007;37(10):895–906.

Koopman R, Verdijk LB, Beelen M, Gorselink M, Kruseman AN, Wagenmakers AJ, Kuipers H, Loon LJ. Co-ingestion of leucine with protein does not further augment post-exercise muscle protein synthesis rates in elderly men. *British Journal of Nutrition*. 2007;99(03).

Lambert CP, Frank LL, Evans WJ. Macronutrient considerations for the sport of bodybuilding. *Sports Medicine*. 2004;34(5):317–327.

Li JB, Jefferson LS. Influence of amino acid availability on protein turnover in perfused skeletal muscle. *Biochimica et Biophysica Acta*. 1978;544(2):351–359.

Li F, Yin Y, Tan B, Kong X, Wu G. Leucine nutrition in animals and humans: mTOR signaling and beyond. *Amino Acids*. 2011;41(5):1185–1193.

Li X, Wang X, Liu R, et al. Chronic leucine supplementation increases body weight and insulin sensitivity in rats on high-fat diet likely by promoting insulin signaling in insulin-target tissues. *Molecular Nutrition & Food Research*. 2013.

Louard RJ, Barrett EJ, Gelfand RA. Effect of infused branched-chain amino acids on muscle and whole-body amino acid metabolism in man. *Clinical Science.* 1990;79(5):457–466.

Lynch CJ, Patson BJ, Anthony J, Vaval A, Jefferson LS, Vary TC. Leucine is a direct-acting nutrient signal that regulates protein synthesis in adipose tissue. *American Journal of Physiology, Endocrinology & Metabolism.* 2002;283(3):E503–513.

May ME, Buse MG. Effects of branched-chain amino acids on protein turnover. *Diabetes Metabolism Review*. 1989;5(3):227–245.

McCormack SE, Shaham O, McCarthy MA, Deik AA, Wang TJ, Gerszten RE, Clish CB, Mootha VK, Grinspoon SK, Fleischman A. Circulating branched-chain amino acid concentrations are associated with obesity and future insulin resistance in children and adolescents. *Pediatric Obesity*. 2013;8(1):52–61.

McNurlan MA, Fern EB, Garlick PJ. Failure of leucine to stimulate protein synthesis in vivo. *Biochemical Jouurnal.* 1982;204(3):831–838.

Meeusen R, Watson P. Amino acids and the brain: do they play a role in “central fatigue”? *International Journal of Sport Nutrition and Exercise Metabolism*. 2007;17 Suppl:S37–46.

Moore D, Robinson M, Fry J, Tang J, Glover E, Wilkinson S, Prior T, Tarnopolsky M. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *American Journal of Clinical Nutrition*. 2009;89(1):161–168.

Morgan HE, Earl DCN, Broadus A, Wolpert EB, Giger KE, Jefferson LS. Regulation of Protein Synthesis in Heart Muscle I. Effect of Amino Acid Levels on Protein Synthesis. *Journal of Biological Chemistry.* 1971;246(7):2152–2162.

Nagasawa T, Kido T, Yoshizawa F, Ito Y, Nishizawa N. Rapid suppression of protein degradation in skeletal muscle after oral feeding of leucine in rats. *The Journal of Nutritional Biochemistry*. 2002;13(2):121–127.

Nair KS, Schwartz RG, Welle S. Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *American Journal of Physiology.*1992;263(5 Pt 1):E928–934.

Nelson AR, Phillips SM, Stellingwerff T, Rezzi S, Bruce SJ, Breton I, Thorimbert A, Guy PA, Clarke J, Broadbent S, Rowlands DS. A protein-leucine supplement increases branched-chain amino acid and nitrogen turnover but not performance. *Medicin & Science in Sports & Exercise*. 2012;44(1):57–68.

Nguema GN, Debras E, Grizard J, Alliot J. Amino acid supplementation prevents the loss of appetite for casein in old Lou/Cjall rats. *Experimental Gerontology.* 2007;42(7):652–661.

Norton LE, Layman DK. Leucine regulates translation initiation of protein synthesis in skeletal muscle after exercise. *Journal of Nutrition.* 2006;136(2):533S–537S.

Norton LE, Layman DK, Bunpo P, Anthony TG, Brana DV, Garlick PJ. The leucine content of a complete meal directs peak activation but not duration of skeletal muscle protein synthesis and mammalian target of rapamycin signaling in rats. *Journal of Nutrition*. 2009;139(6):1103–1109.

Odessey R, Khairallah EA, Goldberg AL. Origin and possible significance of alanine production by skeletal muscle. *Journal of Biological Chemistry.* 1974;249(23):7623–7629.

Pasiakos S, McClung H, McClung J, Margolis L, Andersen N, Cloutier G, Pikosky M, Rood J, Fielding R, Young A. Leucine-enriched essential amino acid supplementation during moderate steady state exercise enhances post-exercise muscle protein synthesis. *American Journal of Clinical Nutrition*. 2011;94(3):809–818.

Phillips SM, Green HJ, Tarnopolsky MA, Heigenhauser GJ, Grant SM. Progressive effect of endurance training on metabolic adaptations in working skeletal muscle. *American Journal of Physiology*. 1996;270(2 Pt 1):E265–272.

Phillips SM. Protein requirements and supplementation in strength sports. *Nutrition*. 2004;20(7-8):689–695.

Portier H, Chatard JC, Filaire E, Jaunet-Devienne MF, Robert A, Guezennec CY. Effects of branched-chain amino acids supplementation on physiological and psychological performance during an offshore sailing race. *European Journal of Applied Physiology*. 2008;104(5):787–794.

Preedy VR, Garlick PJ. The response of muscle protein synthesis to nutrient intake in postabsorptive rats: the role of insulin and amino acids. *Bioscience Reports.* 1986;6(2):177–183.

Rannels DE, Hjalmarson AC, Morgan HE. Effects of noncarbohydrate substrates on protein synthesis in muscle. *American Journal of Physiology.* 1974;226(3):528–539.

Reid KF, Naumova EN, Carabello RJ, Phillips EM, Fielding RA. Lower extremity muscle mass predicts functional performance in mobility-limited elders. *Journal of Nutrition, Health, and Aging*. 2008;12(7):493–498.

Rennie MJ, Bohé J, Smith K, Wackerhage H, Greenhaff P. Branched-chain amino acids as fuels and anabolic signals in human muscle. *Journal of Nutrition.* 2006;136(1 Suppl):264S–8S.

Rieu I, Balage M, Sornet C, Debras E, Ripes S, Rochon-Bonhomme C, Pouyet C, Grizard J, Dardevet D. Increased availability of leucine with leucine-rich whey proteins improves postprandial muscle protein synthesis in aging rats. *Nutrition*. 2007;23(4):323–331.

Roh C, Han J, Tzatsos A, Kandror KV. Nutrient-sensing mTOR-mediated pathway regulates leptin production in isolated rat adipocytes. *American Journal of Physiology, Endocrinology & Metabolism.* 2003;284(2):E322–330.

Salomão E, Toneto A, Silva G, Gomes-Marcondes M. Physical exercise and a leucine-rich diet modulate the muscle protein metabolism in Walker tumor-bearing rats. *Nutrition & Cancer*. 2010;62(8):1095–1104.

Schena F, Guerrini F, Tregnaghi P, Kayser B. Branched-chain amino acid supplementation during trekking at high altitude. The effects on loss of body mass, body composition, and muscle power. *European Journal of Applied Physiology and Occupational Physiology*. 1992;65(5):394–398.

Sherwin RS. Effect of Starvation on the Turnover and Metabolic Response to Leucine. *Journal of Clinical Investigation*. 1978;61(6):1471–1481.

Smith K, Barua JM, Watt PW, Scrimgeour CM, Rennie MJ. Flooding with L-[1-13C]leucine stimulates human muscle protein incorporation of continuously infused L-[1-13C]valine. *American Journal of Physiology.* 1992;262(3 Pt 1):E372–376.

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. *American Journal of Physiology, Endocrinology & Metabolism*. 1998;275(1):E73–E78.

Stock MS, Young JC, Golding LA, Kruskall LJ, Tandy RD, Conway-Klaassen JM, Beck TW. The Effects of Adding Leucine to Pre and Postexercise Carbohydrate Beverages on Acute Muscle Recovery From Resistance Training. *Journal of Strength and Conditioning Research*. 2010;24(8):2211–2219.

Sugawara T, Ito Y, Nishizawa N, Nagasawa T. Regulation of muscle protein degradation, not synthesis, by dietary leucine in rats fed a protein-deficient diet. *Amino Acids*. 2009;37(4):609–616.

Suryawan A, Torrazza RM, Gazzaneo MC, Orellana RA, Fiorotto ML, El-Kadi SW, Srivastava N, Nguyen HV, Davis TA.. Enteral leucine supplementation increases protein synthesis in skeletal and cardiac muscles and visceral tissues of neonatal pigs through mTORC1-dependent pathways. *Pediatric Research.* 2012;71(4 Pt 1):324–331.

Thomson JS. Leucine-protein supplemented recovery feeding enhances subsequent cycling performance in well-trained men. *Applied Physiology, Nutrition & Metabolism*. 2011;36(2):242–253.

Tipton KD, Elliott TA, Ferrando AA, Aarsland AA, Wolfe RR. Stimulation of muscle anabolism by resistance exercise and ingestion of leucine plus protein. *Applied Physiology, Nutrition & Metabolism*. 2009;34(2):151–161.

Tipton KD, Wolfe RR. Protein and amino acids for athletes. *Journal of Sports Science*. 2004;22(1):65–79.

Van Koevering M, Nissen S. Oxidation of leucine and alpha-ketoisocaproate to beta-hydroxy-beta-methylbutyrate in vivo. *American Journal of Physiology.* 1992;262(1 Pt 1):E27–31.

Van Loon LJ, Kruijshoop M, Verhagen H, Saris WH, Wagenmakers AJ. Ingestion of protein hydrolysate and amino acid-carbohydrate mixtures increases postexercise plasma insulin responses in men. *Journal of Nutrition*. 2000;130(10):2508–2513.

Wilcox G. Insulin and Insulin Resistance. *Clinical Biochemist Reviews*. 2005;26(2):19–39.

Wilkinson DJ, Hossain T, Hill DS, Phillips BE, Crossland H, Williams J, Loughna P, Churchward-Venne TA, Breen L, Phillips SM, Etheridge T, Rathmacher JA, Smith K, Szewczyk NJ, Atherton PJ. Effects of Leucine and its metabolite, β-hydroxy-β-methylbutyrate (HMB) on human skeletal muscle protein metabolism. *Journal of Physiology. (Lond.)*. 2013.

Wilkinson SB, Phillips SM, Atherton PJ, Patel R, Yarasheski KE, Tarnopolsky MA, Rennie MJ. Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *Journal of Physiology*. 2008;586(15):3701–3717.

Yoshizawa F, Mochizuki S, Sugahara K. Differential dose response of mTOR signaling to oral administration of leucine in skeletal muscle and liver of rats. *Biosci Biotechnol Biochem*. 2013;77(4):839–842.

Zanchi NE, Guimarães-Ferreira L, de Siqueira-Filho MA, Felitti V, Nicastro H, Bueno Jr. C, Lira F, Naimo M, Campos-Ferraz P, Nunes M, Seelaender M, Carvalho C, Blachier F, Lancha Jr. A. Dose and latency effects of leucine supplementation in modulating glucose homeostasis: opposite effects in healthy and glucocorticoid-induced insulin-resistance States. *Nutrients*. 2012;4(12):1851–1867.

Zhang Y, Guo K, LeBlanc RE, Loh D, Schwartz GJ, Yu Y-H. Increasing dietary leucine intake reduces diet-induced obesity and improves glucose and cholesterol metabolism in mice via multimechanisms. *Diabetes*. 2007;56(6):1647–1654.