

## AminoBoostXXL

### Goal

To supply the proper combination of specific amino acids for the athletic/exercising population that has been shown in clinical trials to speed recovery from continuous training bouts and enhance exercise induced muscle protein synthesis (MPS) through specific pathways within a palatable, fast acting, and low-calorie delivery system. Through reduced recovery times and enhanced MPS, AminoBoostXXL (AB) would help athletes overcome "overtraining/reaching" syndromes or training plateaus. For the non-exercising adult population, AB would serve as a supplement to improve the otherwise declining normal net muscle protein balance that leads to the inevitable loss of muscle while aging. Proper use of AB delivers isolated essential amino acids (EAA) including high doses of leucine in amounts and ratios at precise times in relation to exercise in order to reduce normal muscle breakdown and stimulate synthesis through pathways that may be additive to all other muscle protein synthesis mechanisms such as, but not limited to: exercise, traditional pre/post exercise foods or intact protein feedings, meal timing, cell volumizing, creatine loading, etc. This formula, because of its very low-calorie contents and ability to preserve lean body mass (LBM) during calorie restriction, can serve as the essential (sole source if necessary) pre-and post exercise recovery and additive MPS stimulator supplement for athletes and exercisers involved in sports requiring extremely low body fat and/or prolonged dieting required to make weight classes. Additionally, AB's convenience and high palatability make it ideal for non-exercising adults during the onset of age-related muscle loss (beginning in the fourth decade known as sarcopenia) to help offset muscle loss with a relatively low nitrogen load (compared to whole proteins) which may be especially important in the later stages of aging when appetite and organ function begin diminishing.

### Rationale

#### Introduction

The maintenance of skeletal muscle (SM) tissue, like the mass of all tissues, is dependent on the ongoing processes of protein and cell turnover.<sup>1</sup> Therefore skeletal muscle mass is a result of the degradation (catabolism) of existing SM proteins and synthesis (anabolism) of new tissue throughout life. In the normal non-exercising, well fed human from birth and up through the third decade of life, SM protein balance is generally positive, meaning anabolism outpaces catabolism leading to SM growth and strength increases.<sup>2,3</sup> Exercise can stimulate natural human SM synthesis and muscle performance throughout life when compared to a non-exercise state and at a minimum, attenuate the obligatory age-related muscle loss when SM protein balance tilts to negative.<sup>4,5,6</sup>

However, the general goal of most athletes is to maintain a positive protein balance and in fact maximize the body's natural muscle protein synthesis processes while minimizing the necessary exercise induced muscle protein breakdown (MPB) in order to recover adequately from each training bout to increase performance and if desired, increase SM size. In other words, the goal of athletes is to continue to improve physically by making each training session build on the previous thus leading to continuous athletic/physical progress.<sup>7,8,9</sup> Eventually, as in all aspects of life, age will become a factor in abating progress but depending on age and level of training experience, this inevitable decline can be prolonged to a point where one has the greatest potential to not just extend their athletic success or "playing lifespan" but to also remain self-sufficient throughout life.<sup>10,11,12</sup>

#### Exercise and Amino Acids

Exercise and amino acids stimulate SM protein turnover independently, thus combined properly they have profound additive effects on training induced recovery, performance and muscle size.<sup>9</sup> Exercise is an event that can trigger a desired result, and although mechanical stresses from exercise activate their respective channels of MPS signaling, MPS remains negative without subsequent feeding of amino acids (AA), thus exercise alone would decrease muscle size and performance.<sup>8,13,14</sup>

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### Basic Mechanisms of Action

Exercise and amino acids stimulate skeletal muscle protein turnover by affecting the activity of intracellular signaling networks such as the mammalian target of rapamycin complex 1 (mTORC1) and the mitogen activated protein kinase (MAPK) cascades.<sup>15</sup>

**For a complete review of how exercise and AA activate MPS and breakdown and regulate SM balance the reader is referred to "A Brief Review of Critical Processes in Exercise Induced Muscular Hypertrophy," by Stuart M. Phillips<sup>8</sup> and "Exercise and Amino Acid Anabolic Cell Signaling and the Regulation of Skeletal Muscle Mass" by Stefan M. Pasiakos.<sup>9</sup>**

### Exercise

Studies show that various forms of mechanical loading initiates muscle protein turnover and related anabolic signaling, including mTORC1, and that the mode, intensity and volume of exercise differentially affect signaling thus long-term outcomes.<sup>16,17,18</sup> In response to exercise, identified mechanical sensors include cell membrane stretch-activated calcium channels,<sup>19</sup> phospholipase D (PLD), and the lipid second messenger phosphatidic acid (PA).<sup>20</sup> These influencers respond differently according to exercise protocols.<sup>21,22,23</sup> Although exercise itself activates channels to stimulate MPS which leads to a portion of the AA released from muscle during exercise to be lost, without protein (specifically AA) SM balance will always be negative,<sup>8</sup> and therefore the process of inward and outward AA transport are also direct signals that contribute to muscle protein synthesis.<sup>24</sup>

### Amino Acids

It is well known that resistance exercise results in increased MPS in the post-exercise recovery period,<sup>25,26</sup> and depending on the protocol, the MPS rate can increase ~two to fivefold and persist for up to 48 hours in fed subjects.<sup>4</sup> As described above, SM balance will remain negative without the feeding of AA.<sup>8,9</sup> Therefore, it's clear that increasing plasma and muscle intracellular AA concentrations stimulate MPS with or without exercise.<sup>27</sup> Like exercise, AA ingestion alone also stimulates muscle mTOR signaling but independently through regulators of translation initiation (S6K1 and 4E-BP1) and elongation (eEF2), which strongly and rapidly stimulate muscle protein synthesis (i.e. within 1 hour).<sup>28,29</sup> It is also clear that the essential amino acids<sup>30</sup> are the primary drivers of the signals for protein synthesis, particularly leucine.<sup>31</sup>

### Exercise and Amino Acids

Increasing exogenous AA with exercise potentiates the MPS response, initiated through mechanical loading, by further enhancing mTORC1 activation as discussed, and other intracellular AA sensing mechanisms such as the human vacuolar protein sorting-34, (hVps34)<sup>32,33,34</sup> and also by increasing AA transporter expression.<sup>35,36,37</sup> In simple terms, exercise leads to muscle protein breakdown, which induces a heightened nutrient demand and their respective receptors sensitivity allowing exogenous AAs to be delivered timely in the right amounts (specifically EAA including a relatively high leucine content) to maximize an individual's MPS potential, leading to enhanced size and/or performance training induced results when all else is equal (e.g. training and overall diet).<sup>38,39,40</sup>

### Essential Amino Acids

The stimulation of MPS from AA is dependent on the EAA.<sup>41</sup> Although non-essential amino acids are necessary components of complete muscle tissue, they are not required to stimulate MPS.<sup>28,30,33,42</sup> Additionally, it's been shown that EAA feeding stimulates protein synthesis independently of all other mechanisms.<sup>9,43,44,45</sup>

### Basic Mechanisms of Action

Fujita et al. used a leucine enriched EAA mixture of .16 g/lb of fat free mass (FFM) containing histidine (8%), isoleucine (8%), leucine (35%), lysine (12%), methionine (3%), phenylalanine (14%), threonine (10%), and valine (10%), and demonstrated that EAA availability is the primary regulator of mTOR signaling and muscle protein synthesis as described above.<sup>28</sup> The study's conclusion was that EAA and glucose inhibit AMP-activated protein kinase, (AMPK) which plays critical roles in regulating growth and reprogramming metabolism, and activates mTOR signaling in SM

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leading to an increase in MPS due to enhanced translation initiation and the signaling that promotes elongation.<sup>28,46,47</sup> Further elucidating EAA mechanisms of actions, Carlin et al. used 13 g of EAA (2.4 g of leucine) and demonstrated an increase in SM Ras-related GTP Binding B (RAGB) mRNA (60%), mTOR phosphorylation (30%), leucine concentrations (130%) and protein abundance (100%), all leading to a 50% increase in protein synthesis.<sup>40</sup> They concluded that EAA ingestion increases RAGB expression, which may be an important target for maximizing protein synthesis. Drummond et al. demonstrated that increasing the availability of EAA, independently up-regulates skeletal muscle amino acid transporter expression.<sup>36</sup> And finally, EAA administered before exercise has also been shown to have an inhibitory effect on muscle breakdown,<sup>48,49</sup> which would be expected since exercise produces an increase in net amino acid efflux during activity due to the increase in proteolysis and probably from a greater inhibition of protein synthesis relative to breakdown if compared to a non-fed state.<sup>14,50,51</sup>

### Supplementation

Discovery of the EAA direct effects on MPS has given rise to the use of EAA supplementation as a successful strategy in maximizing MPS.<sup>9</sup> Plentiful evidence exists to support the use of specific blends of proteins and/or EAA combinations<sup>52,53,54</sup> for enhancing protein synthesis beyond what can be accomplished with normal feedings and exercise regimens.<sup>8,9,55,56</sup> Further evidence of EAA superiority in maximizing MPS was demonstrated when Bukhari et al. showed 3 g of EAA (40% leucine) to be as effective in MPS as 20 g of whey protein in older women after exercise.<sup>57</sup> Coker et al. added 6 g of EAA to a whey protein meal replacement and compared the weight loss effects of an equal amount of intact protein in a normal meal replacement.<sup>58</sup> They found a greater loss of adipose tissue and an increase in skeletal muscle protein fractional synthesis rate (FSR) in the EAA treated group. Churchward-Venne et al. found that adding leucine or a mixture of EAAs without leucine to 6.25 g of intact whey protein was as effective as 25 g of whey in stimulating rates of MPS but that the 25 g of whey is better suited to sustain increased rates MPS resistance exercise-induced muscle anabolism giving rationale for both EAA feedings and a pre/post exercise complete protein supplement.<sup>37</sup> It is evident that increasing levels of each EAA is required to maximize human MPS.<sup>27,59</sup> However, the branched-chain amino acid (BCAA) leucine stands out. Several *in vitro* and *in vivo* animal and human studies have demonstrated that leucine independently stimulates MPS<sup>60,61,62,63,64</sup> through, again, primarily the mTORC1 signal transduction cascade (integrating signals from insulin/insulin growth factor I (IGF1)).<sup>65,66,67</sup> Indeed, much of whey protein's documented added value over other sources may be due to its high leucine content as well as its rapid amino acid absorption rate.<sup>68,69,70</sup> Whey protein compared to other protein sources such as soy, red meat/steak, chicken, etc., has much more leucine.<sup>68</sup> Twenty grams of whey protein isolate contains 3 grams of leucine. Comparatively, soy has only 2.4 grams and most meats contain even less. Scientific data suggests that 2.5 grams and above of leucine may be that extra turning point for benefits when it comes to protein synthesis.<sup>68,37,71</sup> Although leucine enriched EAA clearly demonstrates added value in acute MPS, the other EAA are necessary (just as intact complete proteins) to sustaining MPS and resulting accretion throughout the day.<sup>9,72,73</sup> Since EAA are the primary drivers of the nutrition induced MPS actions and leucine appears to deliver the greatest AA impact, especially when combined with exercise,<sup>60-67,74,75</sup> including in the elderly,<sup>12,76</sup> among non-exercising populations,<sup>77</sup> and during weightlessness,<sup>78</sup> EAA formulas containing high doses of leucine have been studied extensively as shown above. The goal of these formulas is to maximize protein synthesis during each temporal opportunity, exercise induced or not, by utilizing every available mechanism of action up to its respective rate limiting effect. Data from studies are listed here:

- Robinson MM et al. found that EAA supplementation increased skeletal muscle mitochondria protein synthesis and oxidative enzyme activity in healthy humans, suggesting an exercise recovery benefit.<sup>39</sup>
- Churchward TA et al., demonstrating the power of leucine, found by adding 5 g of leucine to 6.25 g of protein that the supplement was just as effective as 25 g of whey protein in stimulating MPS.<sup>71</sup>
- Rowlands et al. compared the effects of 23 g of protein spiked with 5 g of leucine to 70 g of protein spiked with 15 g of leucine and a control formula on MPS after endurance exercise.<sup>79</sup> Both leucine groups reached near maximal fractional synthesis rates, which were 33% above controls. Of note is the 3 times higher leucine formula produced a negligible FSR increase compared to low dose (~13%) but mTORC1 (Ser2448)

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phosphorylation only increased significantly in the high dose, suggesting a threshold in leucine amounts and specific MPS target activity. Serum insulin was increased in the high leucine/protein but not with the 5 g.<sup>79</sup>

- Dreyer et al. found that enhanced activation of the mTOR signaling pathway is partially responsible for the greater synthesis of muscle proteins observed when resistance exercise is followed by EAA+carbohydrate (CHO) ingestion.<sup>73</sup> The formula used was a leucine enriched EAA+CHO solution consisting of histidine, 8%; isoleucine, 8%; leucine, 35%; lysine, 12%; methionine, 3%; phenylalanine, 14%; threonine, 10%; and valine, 10% at .16 g/lb.<sup>28</sup> The leucine-enriched EAA +CHO consumed after exercise induced mTOR activation associated with a 145% increase in mixed muscle protein synthesis compared with only a 41% increase in those performing exercise alone. They concluded that the formula ingestion following a single bout of resistance exercise significantly augments otherwise normal exercise-induced muscle protein synthesis, and may be partially explained by the increase in mTOR signaling.<sup>73</sup>
- Ukhari et al. showed 3 g of EAA (40% leucine) to be as effective at increasing MPS as 20 g of whey protein in older women post exercise.<sup>57</sup>
- Dickenson et al. found that ingesting a leucine enriched EAA formula after resistance exercise prolongs the anabolic response and sensitivity of SM to amino acids in older adults, which may be especially important to preserving aging muscle and establishing health preserving nutrition programs.<sup>12</sup>
- Xu ZR et al., in a systematic review and meta-analysis through 2013, determined leucine supplementation to be effective at increasing MPS, FSR, and LBM and thus deemed it to be a potential aid for addressing age-related muscle loss.<sup>76</sup>
- Komar et al. found leucine supplementation to illicit beneficial effects on body weight, BMI, and lean body mass in older persons.<sup>69</sup>
- Mobley et al. found that leucine,  $\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB) and creatine supplementation may independently reduce myostatin-induced muscle fiber atrophy by influencing Akirin-1/Mighty mRNA expression patterns, which may be very important to maximizing muscle hypertrophy, or at a minimum, reducing normal muscle losses.<sup>80</sup>
- Luiking et al. demonstrated that a low calorie leucine enriched (3 g/serving compared to 2 g in casein) whey protein source raised serum levels of total AA and EAA, including leucine, to a greater and faster extent than casein protein.<sup>81</sup> Based on the fact that MPS responds to extracellular EAA concentrations,<sup>53</sup> subsequent transport and intracellular AA rate of deposition,<sup>59</sup> and higher postprandial levels of EAA and leucine correlate to a greater protein synthesis rates, the authors suggest that the rapid postprandial profile of AA appearance in blood is especially important in older adults.<sup>81,82</sup> This study also supports the additional delivery of an EAA supplement without the presence of a caloric dense meal.
- Dreyer et al., using 20 g of EAA twice daily between meals for one week before and two weeks after total knee arthroplasty (TKA) showed three times less atrophy in the treated group versus placebo, and an accelerated return to functional mobility.<sup>83</sup>
- Aquilani R et al. analyzed three studies using EAA supplementation in subjects with chronic heart failure (CHF) and/or chronic obstructive pulmonary disease (COPD) and physical capacity.<sup>11</sup> The three studies consistently demonstrated CHF and COPD subjects improved exercise intolerance after 1-3 months of 8 g/d of EAA supplementation. In CHF subjects, exercise capacity increased 18.7% to 23% (watts; bicycle test), and 12% to 22% (meters) in a 6-minute walking test. Additionally, patients reduced their resting plasma lactate levels 25% and improved tissue insulin sensitivity by 16%. Results were similar in the COPD group. The authors attributed the benefits to the EAA's known effects on improving exercise intolerance through increases in muscle aerobic metabolism, mass and function (EAA supplementation increases myofibrils and mitochondria genesis in SM), and improvement of tissue insulin sensitivity (improved glucose control).<sup>11</sup>

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### Dosing

The goal for all humans (athletes, exercisers or not), would be to maintain a net positive protein balance as long as possible in order to maintain physical/performance improvements and desired active lifestyle into advancing years by delaying age-related loss of function or at a minimum, remain self-sufficient until end of days. Therefore, the aim of supplementation is to keep the MPS signaling/activity as strong as possible throughout the day by utilizing the LEAA MPS pathways. This includes reducing MPB during intense repeated exercise sessions to allow full recovery and ideal tissue adaptation before the next training bout. The ideal dosing would present proper amounts of LEAA at all time periods as the body could use them to maximize MPS throughout the day based on the individual (e.g. size and age) and their total activity. In other words, ingestion of EAA would maintain an extracellular level of LEAA that keeps MPS turned on while minimizing MPB beyond what normal feedings containing adequate levels of intact protein would accomplish. Although there is no known exact recipe to date that would accomplish this, based on all current data captured in this document, there is ample information to come very close.

### Composition

The composition of the formulas used with success closely mimic the EAA losses during MPB and subsequent incorporation or proportionate requirement into SM<sup>84</sup> with the exception of more recent formulas, including up to 40% leucine because of its unique and additive MPS signaling.<sup>28,57,60-67,69,71,73-81</sup>

### Amounts

Early studies set the stage for today's formulas by establishing a basic dose dependency up to the "muscle full" amount - i.e. more did not increase the MPS in that specific measured time period.<sup>42,84,85</sup> Borsheim et al. found no additional increase in MPS by increasing the EAA dose beyond 21 g (under these study conditions).<sup>84</sup> Tipton et al. observed equal MPS in response to the intake of 40 g of AAs composed of 18 g of EAA and 22 g of non-essential AAs, compared to 40 g of all EAAs.<sup>42</sup> In addition, the Borsheim study<sup>84</sup> neatly demonstrated that EAA ingestion following exercise stimulates MPS independently of all other mechanisms, (calories, insulin, exercise, etc.) and the authors calculated that approximately 26 g/d of muscle tissue (including muscle water content) was synthesized in response to the EAA supplementation. Two other long-term trials involving elite athletes using 6.6 g of EAA three times daily demonstrated significant improvements in physical condition and reduction in exercise induced muscle damage when compared to 2.2 and 4.4 g 3 times daily with everything else equal.<sup>86,87</sup> Because EAA are the only AA needed for MPS, you can extrapolate the same dosages from successful studies using timed ingestion of complete protein. Therefore, these early results<sup>30,42,84,85,88,89</sup> and those of more recent studies described above that have the added effect of increased leucine (~30-40% of total EAA content),<sup>28,57,60-67,69,71,73-81</sup> show that the total amount of EAA supplementation that might be required to maximize MPS throughout the day, (at least for the average-sized human), seems to lie between 6 and 18 g in one dose and ingested possibly 2-4 times daily depending on age, activities and goals.

### Timing

If the goal is to maximize MPS then the timing of the LEAA formula should be intuitive. Based on all data presented here, in order to achieve total tissue saturation of necessary materials for signaling and building muscle proteins, doses should be spread throughout the day between meals. Ideal protocol would also include: 1) a final dose before bedtime since sleep time is generally the longest lapse in which there would be a reduced extracellular EAA presence, and protein ingestion before sleep has demonstrated increases in MPS rates during overnight recovery from exercise bouts;<sup>90</sup> and 2) for exercisers/athletes before and after strenuous activity. Dosing in this manner would keep the extracellular EAA levels at concentrations necessary to deliver a maximum signaling/effect so that levels are appropriate at any opportune time when the body is most responsive including reducing MPB.<sup>4,8,9,27,91</sup> Between meal usage would be for two possible reasons: 1) regular frequent meals (3-5) should also contain protein components thus

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delivering a constant daily AA release,<sup>83,92</sup> albeit not necessarily in the proportions or amounts ideal to MPS at given times (especially around exercise) as demonstrated in studies, which timely isolated LEAA supplementation can accomplish, and 2) co-ingestion with normal meals may result in insulin-stimulated removal of a portion of the LEAA supplement by the splanchnic bed, thus reducing its unique potential time period effect on MPS<sup>93</sup> (this may be only important in the post exercise window).<sup>93,94</sup> All this said, since LEAA stimulates MPS through insulin-dependent and independent mechanisms, consuming a fast acting pre or post workout formula, containing protein and carbohydrates, would be appropriate and may have an additive benefit (if calories allow) when separated 30-40 minutes from the LEAA supplement pre, during, and post workout ingestion.<sup>9</sup> Before and after exercise supplementation of complete fast acting protein and/or EAA have clearly shown an incremental MPS benefit when everything else (diet and exercise) was equal.<sup>38,85,89,91,95,96</sup> The two major opportunities that present themselves in this exercise induced "metabolic window" are 1) EAA supplementation can reduce MPB by also being used before and during activity<sup>9,97</sup> allowing less time and material needed for repair and maximum remodeling,<sup>98</sup> and 2) MPS potential is at its highest point immediately post exercise but also wanes quickly,<sup>9,29,73,99</sup> thus immediate delivery of specific and isolated optimal amounts (described above) can maximize this short but incremental MPS window.<sup>38,73,85,89,91,95,96</sup> As mentioned, post exercise ingestion should probably take place independently of foods including whole proteins in order to avoid any potential EAA clearance by the splanchnic bed and perhaps more importantly, to avoid slower gastric emptying by accompanying foods since the goal is rapid hyper aminoacidemia during this critical period.<sup>81,93,100</sup> On that note, West et al. found that a rapid rise in extracellular EAA led to a greater MPS response in the immediate post exercise period than a slower administered equal amount of EAA.<sup>100</sup> Inactivity would likely reduce the need for more frequent dosing, allowing larger doses to achieve the desired daily "muscle full" effect.<sup>101</sup> And finally, MacKenzie et al.<sup>102</sup> demonstrated that contractile-induced (muscle breakdown) elevations of leucine can activate hVps34 (nutrient sensing target that helps regulate MPS<sup>32</sup>) and a sustained provision of leucine can accomplish the same, which the authors suggest may prolong mTORC1 activity crucial to maximizing MPS and contribute to long-term skeletal muscle hypertrophy, thus providing another rationale for continuous feedings of LEAA and especially before exercise to reduce leucine losses.<sup>103</sup>

### Summary of AminoBoostXXL Dose and Timing Which Suggest Benefits

#### Exercisers

Considering all data to date, including managing the interplay between normal feedings and insulin's ability to also promote muscle protein synthesis, we can establish a potential dosage recommendation range including timing and amounts that may "cover most bases" (i.e., compensate for individuality including age, activity type, intensity, volume, size, etc.). The protocol would be designed to create a superior, more continuous exercise-induced anabolic environment compared to a chronic non-supplemented state. Maintaining frequent feedings with adequate protein (~0.8-1.0 g/lb of body weight daily, not including the grams of the AminoBoostXXL™)<sup>104</sup> and energy intake no less than a 10-20% calorie deficit,<sup>105</sup> below is a safe and potentially effective recommendation for most healthy athletes seeking continuous physical improvement. Ranges\* are based on size using data extrapolated from above LEAA studies and information regarding complete protein's "muscle full effect" per serving (~0.14 g/lb of body weight):<sup>5,8,106</sup>

#### Dose

- For those weighing less than 150 lbs use 12 g per dose (1 scoop). One before training and repeat immediately post activity
- Persons weighing more than 150 lbs add ~5% for each 10 lbs of body weight (0.6 g for each 10 lbs of weight). Example: 200 lb person would add 3 g (15 g per dose); 250 lb person would use 18 g per dose
- Aging (>50 years old) may also increase dosage needs based on the body's resistance to the anabolic effects of exercise, amino acids, insulin and other related protein synthesis mechanisms in advancing years.<sup>12,107</sup>

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Therefore, even if exercise is halted, maintaining the dosing may be appropriate (see below for non-exercisers).<sup>108,109</sup>

\* Amounts within all ranges should be sufficient under most training circumstances including but not limited to size or greater stresses such as prolonged dieting, extreme training conditions, etc.

### Timing

- Approximately 10 minutes before workout, begin ingestion and may continue to consume throughout workout
- Consume same dose immediately post workout
- If calories permit, use a dotFIT pre-workout formula (e.g. WheySmooth, FirstString, favorite bar, etc.,) approximately 40 minutes before workout and 30-40 minutes after post workout AB dose
- Consume normal post-workout whole food meal at ~ 1-2 hours post-training
- Repeat dose before bedtime to potentially maximize results (optional)

### Non-exercisers 30 years and Older

#### Dose & timing

- 15 g per dose (~1.25 scoop) and take three times daily between meals with one dose before bedtime
- Aging (> 50 years old) may increase dosage needs 20% for each decade (ex: at age 60 years dosage may be 18 g) based on the body's resistance to the anabolic effects of amino acids<sup>76,107</sup> and related protein synthesis mechanisms in advancing years.<sup>108,109</sup>

### Data Summary

The goal for humans that desire progressive physical improvement or simply to remain functionally mobile throughout life would be to keep the daily muscle protein synthesis signals as strong as possible, thus favoring a positive muscle protein balance as long as possible, making leucine-enriched essential amino acid supplementation a valuable adult sport and primary prevention health practice. This practice would offer additive benefits as described above to all sport and lifestyle programs when everything else is equal (diet, supplements and exercise).

Assessing all data points: 1) all amino acids (complete proteins) are necessary to continually replace and build skeletal muscle; 2) Essential amino acids are the primary protein components that drive muscle protein synthesis; 3) EAA deliver unique MPS signals to unique targets - i.e. additive to all others; 4) EAA supplementation enriched with higher doses of leucine (3-5 g/dose) are most effective in optimizing MPS because of leucine's independent actions on MPS signaling; 5) The ingestion of leucine enriched EAA supplementation and its effect on MPS can be amplified throughout the day in all situations by supplementing protocols that maintain a specific rate and frequency of LEAA appearance in the extracellular space thus maximizing intracellular transport at given opportune MPS times; 6) LEAA effects can be further amplified by exercise and especially related to proper timing of ingestion around the activity in order to reduce muscle protein breakdown and increase MPS within the most active "metabolic windows," yielding a greater net synthesis in this timeframe that may not be compensated for at a later period - i.e. a significant percentage of gains from this period may be lost no matter the daily total protein or food intake; 7) co-ingestion with foods may not be ideal for efficacy of LEAA based on insulin's nutrient clearance properties or slower gastric emptying; 8) threshold of efficacy range or maximum dose (rate limiting substance amounts) that still impacts individual targets, thus total additive increase contribution to MPS appears to be 6-21 g of EAA with 3-5 g of leucine in proportion to skeletal muscle requirements during favorable adaptation. The range is probably a function of age, body size, exercise type, volume and intensity, all which may drive a different threshold; 9) 2-4 dosages administered daily before and after exercise and between meals for non-exercisers may keep the MPS signaling activated throughout the day including an optional bedtime dosage to "hedge all bets."

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### Formula Components in a 12 g Dose

The majority of the essential amino acids, with the exception of leucine, have not been studied individually as ergogenic aids but only as a group as referenced in this document and contained in the formula. This formula's composition is proportional to the skeletal muscle's requirements during favorable remodeling.

**For more information on all amino acids, please see the comprehensive review by Neal Spruce and Dr. Alan Titchenal in the publication *Sports Nutrition, Fats and Proteins*<sup>24</sup>**

**BCAA** (See above and dotFIT Recover&Build™)

- **Leucine (4,000 mg)**
- **Isoleucine (900 mg)**
- **Valine (1,100 mg)**

**Phenylalanine (1,670.5 mg):** Phenylalanine (P) is an essential amino acid that participates in protein synthesis. It is converted to tyrosine via hydroxylation. Phenylalanine is both glucogenic and ketogenic.<sup>110</sup> Sports drinks that contain a mixture of carbohydrate and free-form amino acids, including phenylalanine, can result in a greater insulin response than carbohydrate by itself.<sup>111,112</sup>

**Lysine (1669.5 mg):** L-lysine is an indispensable dibasic amino acid (L-2,6-diaminohexanoic acid) required for human growth and for maintaining nitrogen balance in adults. Lysine cannot be synthesized by the body, and therefore must be supplied through diet.<sup>113</sup> Lysine, like most other amino acids, is a building block of body proteins. Among the indispensable AA, lysine is present in the greatest amounts, at 93.0 mmol/dl and 38 mmol/dl in tissues and serum respectively. Lysine is also required for collagen synthesis, and may be central to bone health.<sup>114,115</sup>

**Threonine (1300 mg):** Threonine is an essential amino acid often low in vegetarian diets. Aminotransferases exist for all amino acids except threonine and lysine. Its main routes of catabolism lead to both ketogenic and glucogenic metabolites.<sup>116</sup> The human requirement for threonine set by FAO/WHO/UNU at 7 mg/kg/day<sup>117</sup> has been challenged by more recent data suggesting a level more than twice this amount to maintain AA homeostasis<sup>118,119</sup> in healthy adults. The Institute of Medicine recently established a threonine RDA for adults at 27 mg/kg/day.<sup>120</sup>

**Histidine (900 mg):** The end product of histidine catabolism is glutamate, making histidine one of the glucogenic amino acids. Kriengsinyos, et al. investigated histidine's essentiality in healthy adult humans consuming a histidine-free diet for 48 days. They discovered a gradual decrease in protein turnover and a substantial decrease in plasma protein concentrations, including albumin, hemoglobin, and transferrin. Although histidine deficiency may not affect nitrogen equilibrium, it can impact other important health parameters.<sup>121</sup> Histidine, like cysteine, also may have antioxidant properties.<sup>122</sup> In regards to sports/fitness applications, histidine alone has not been studied as a supplement for improving athletic outcomes. Carnosine is related metabolically to histidine and histamine. It is a naturally occurring histidine-containing dipeptide present in muscle tissue. Being immuno-protective, carnosine has been shown to detoxify free-radical species, protect cell membranes, and act as a buffer against lactic acid and hydrogen ions.<sup>123</sup> This is especially important in athletic events where lactic acid buildup (metabolic acidosis) can affect performance by causing fatigue.<sup>124</sup> Intracellular buffering agents such as phosphates and histidine-containing peptides may help delay fatigue by buffering hydrogen ions, reducing oxidative damage, and maintaining cell membrane integrity.<sup>125,126,127</sup>

**Methionine (360 mg):** Methionine is a major source of sulfur in human diets, and is an essential amino acid for normal growth and development. It is considered glucogenic, due to its conversion to pyruvic acid via succinyl CoA. It



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is a major methyl-donor, and important in the metabolism of phospholipids. It is also prominent in methylation reactions, and as a precursor for cysteine, which is the rate-limiting AA for glutathione synthesis. High levels of methionine are associated with hyperhomocysteinemia and endothelial dysfunction, which are risk factors for cardiovascular disease.<sup>128</sup> Deficiency of methionine produces hepatic steatosis similar to that seen with ethanol,<sup>129</sup> and supplementation with this lipotrope can prevent ethanol-induced fatty liver.<sup>129</sup> Besides methionine's role in methyl-group metabolism, and in serving as a substrate for protein synthesis, its other functions include participation in the synthesis of polyamines, catecholamines, nucleic acids, carnitine, and creatine.<sup>130,131,132</sup> Because of its many functions, methionine has a high intracellular turnover.<sup>133,134</sup> It may be the amino acid that is most rate-limiting for the building of body proteins, including maintaining nitrogen balance and the effective reutilization of the other amino acids.<sup>135,136</sup> Therefore, the requirement for methionine increases significantly during times of high protein turnover, as seen in burn and trauma patients.<sup>137,138</sup>

### Typical Use

- All exercisers/athletes seeking continuous physical and performance progress
- Especially important recovery aid for older exercisers/athletes
- Because of its low-calorie content and high anabolic potential, AB may be used as the sole pre and post activity supplement for athletes requiring low body fat, prolonged restricted calorie dieting and/or weight restrictions (weight classes)
- Ideal for recovery on days of multiple training sessions or tournament play before and after each event
- Can be used with NO7Rage™ and CreatineXXL™ as part of the dotFIT™ "Super Stack," providing enhanced progressive exercise-induced results
- All non-exercisers more than 30 years of age

### Precautions

Presently, insufficient data exists to use the risk assessment model for determining an upper limit (UL) for any of the amino acids. Furthermore, chronic excessive use of individual amino acids is likely to be highly unusual in athletes (no perceived value at levels that may lead to danger) and potentially uncomfortable (e.g., stomach distress). Consequently, collecting data on amino acid toxicity is difficult and possibly unnecessary. Reported adverse events from acute and chronic high-level intake of amino acids are extremely rare.<sup>139</sup> Amino acid supplementation safety appears to have survived the "test of time" as it relates to use by athletes. Despite the lack of adverse events reported by athletes who use amino acid products and the lack of UL values for amino acids, the safety of chronic high intakes of amino acids is unknown. However, the risk/benefit ratio appears to be extremely low. And the amounts present in the AminoBoostXXL™ do not approach any level of amino acid intake that may lead to adverse events. Phenylketonuria (PKU) is a rare disease (generally diagnosed at birth) caused by an inborn error in the ability to metabolize phenylalanine (lacking the enzyme phenylalanine hydroxylase).<sup>140</sup> In affected people, if the diet is not controlled by severe restriction of phenylalanine intake, PKU can lead to serious irreversible neurological disorders, such as mental retardation. Because homocysteinemia is linked with cardiovascular disease, long-term use of methionine supplements may be of concern.<sup>141</sup>

### Contraindications

This product, as with any protein or creatine-containing supplement, is contraindicated for users with kidney or liver disease. This product is contraindicated for phenylketonurics because it contains phenylalanine. This product is also contraindicated for pregnant or lactating females because it has not been tested in these groups and because protein can be adequately supplied by the diet for fetal growth or lactation needs.

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### Adverse Reactions

**BCAA** have been used in studies in doses of at least 12 g/day with no side effects, making the dose in AminoBoostXXL quite safe for healthy users.<sup>142</sup>

**Lysine** is often used for herpes simplex at an oral dose of 1 to 3 g/day. It has been used in doses from 400 mg to 6 g/day without adverse events. Above 8 g/day, however, can cause profuse watery diarrhea in those with lysinuric protein intolerance.<sup>143,144</sup> These studies suggest the lysine in AminoBoostXXL should be well tolerated in healthy users.

**Methionine:** The average American consumes at least 2 grams (2,000 mg) of methionine each day.<sup>145</sup> 2.5 grams every four hours for 16 hours has been used for acetaminophen poisoning and for liver disorders.<sup>146</sup> Methionine is frequently used in doses of 100 mg/kg to test individuals with various diseases and levels of homocysteine in their blood.<sup>145,147</sup> The 100 mg/kg dose is generally considered a safe dose during short-term use for medical testing with mild side effects reported.<sup>148</sup> 100 mg/kg is a much larger dose than users will receive in AminoBoostXXL™. Coincidentally, both methionine deficiency and an excessive acute intake (>100 mg/kg) are associated with liver diseases and other adverse events.<sup>149,150</sup> These studies suggest the methionine in AminoBoostXXL should be well tolerated in healthy users.

**Histidine** supplementation in doses of 4 g/day has shown no side effects,<sup>151</sup> whereas doses of 24 to 64 g have caused anorexia and increased urinary zinc excretion.<sup>152</sup> These studies suggest the histidine in AminoBoostXXL should be well tolerated in healthy users.

**Threonine** has been used for spinal spasticity and amyotrophic lateral sclerosis (Lou Gehrig's disease) in doses from 2 to 7.5 g/day.<sup>150,153</sup> Threonine in doses up to 4 g/day is associated with mild adverse events ranging from nothing to slight GI discomfort. One study of spasticity at 7.5 g/day showed no adverse events.<sup>153</sup> These studies suggest the threonine in AminoBoostXXL should be well tolerated in healthy users.

**Phenylalanine** has been used for a depigmentation disorder called vitiligo in doses of up to 100 mg/kg with minimal to no side effects.<sup>154</sup> A typical 154-pound athlete would be consuming 7 g/day at that dose, making the amount in AminoBoostXXL safe for users without PKU.

Overall, essential amino acid supplementation in combined doses from 12 to 60 g/day and as described here is well tolerated in individuals without PKU, kidney or liver disease.<sup>155,156</sup>

### Upper Limit/Toxicity

- Amino acid blends and protein supplements have been studied for use in numerous disease states and to improve sports performance for decades with a large margin of safety between the typical doses and those needed for toxic effects in healthy users.<sup>155,156,157</sup>
- A proposed upper limit for leucine (only based on plasma and urinary variables, not an adverse reaction) is ~227 mg/lb (150 lb person would need to consume ~34 g of leucine to approach this level)<sup>158</sup> We should be mindful that even at this proposed level there are no known side effects. It was simply not making a positive contribution in protein metabolism/synthesis in the subjects under the conditions of the study.
- In addition, the amino acids in AminoBoostXXL all appear on the Generally Regarded As Safe (GRAS) list and are in forms which may be safely used when added to foods<sup>159</sup>

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**Table 6:** Toxicity in animals\*

AA	NOAEL/LOAEL <sup>†</sup>	LD <sub>50</sub> Mouse	LD <sub>50</sub> Rat
Histidine	>4.5 g/d	15,000 mg/kg orally	15,000 mg/kg orally
Isoleucine	14.4 g BCAA/d		6822 mg/kg*
Leucine	>6 g/d		5379 mg/kg*
Lysine	3 – 40 g/d		10,000 mg/kg <sup>^</sup>
Methionine	5 g/d	9500 mg/kg*	36,000 mg/kg orally
Phenylalanine	>4 g/d	1322 mg/kg*	5287 mg/kg*
Threonine	>6 g/d		3098 mg/kg*
Valine	14.4 g BCAA/d		5390 mg/kg*

\* Intraperitoneal

\*\*Data from TOXNET; ChemIDplus Lite.

<sup>^</sup> Data from MSDS sheets.

<sup>†</sup>Derived from Garlick et al.<sup>141</sup> Doses given are levels used in studies that showed mild to no adverse events and are clearly below toxic levels.

### Summary

#### Purpose

- Deliver a specialized leucine enriched EAA formula that can enhance muscle protein synthesis throughout the day especially when combined with exercise
- Extremely low calorie and high anabolic pre-and post workout supplement for athletes to maximize muscle protein synthesis during restricted calorie dieting to help maintain MPS during fat/weight loss (e.g. physique competitors, fighters/wrestlers, weight lifters, etc.)
- Especially important for older athletes seeking physical improvements as the body becomes more resistant to anabolic effects of food and exercise
- All exercisers and athletes seeking continuous physical progress - ideal for breaking through or avoiding fitness, size or performance plateaus
- Ideal for multiple daily training sessions or tournament play before and after each event
- All non-exercisers over 30 years of age to help stave off age-related loss of muscle

#### Unique Features

- The formula uses a leucine enriched EAA blend that has been shown to significantly increase muscle protein synthesis in clinical trials with both adult athletes and non-athletes
- The proprietary EAA composition is designed to increase the availability of the EAA in proportion to their requirement for muscle protein synthesis (MPS) and muscle deposition
- Extremely high anabolic formula delivered in a low calorie, relatively low nitrogen and very palatable drink/solution
- Used with any program, this unique formula provides an incremental effect to muscle/performance gains, thus additive to all other protocols including pre/post protein and carbohydrate feedings
- Can be used with NO7Rage™ and CreatineXXL™ as part of the dotFIT™ "Super Stack" providing enhanced progressive exercise-induced results

Supplement Facts Panel

<b>Supplement Facts</b>		
Serving Size: 1 Scoop (16 g)		
Servings Per Container: 37		
	Amount Per Serving	% Daily Value
Calories	5	
Total Carbohydrate	2 g	<1%*
Sodium (as Sodium Chloride)	90 mg	4%
<b>AminoBoost XXL Complex</b>		
L-Leucine	4g	**
L-Phenylalanine	1.67 g	**
L-Lysine HCl	1.67 g	**
L-Threonine	1.3 g	**
L-Valine	1.1 g	**
L-Histidine Base	900 mg	**
L-Isoleucine	900 mg	**
L-Methionine	360 mg	**
* Percent Daily Value Based on a 2,000 Calorie Diet		
** Daily Value Not Established		

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