

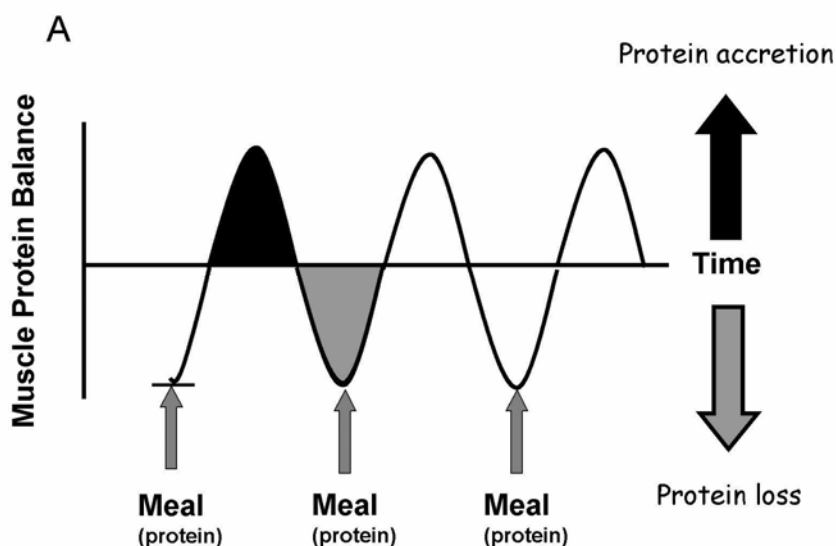
## **Role of Protein Absorption and Nutrient Timing on Muscle Mass Accretion**

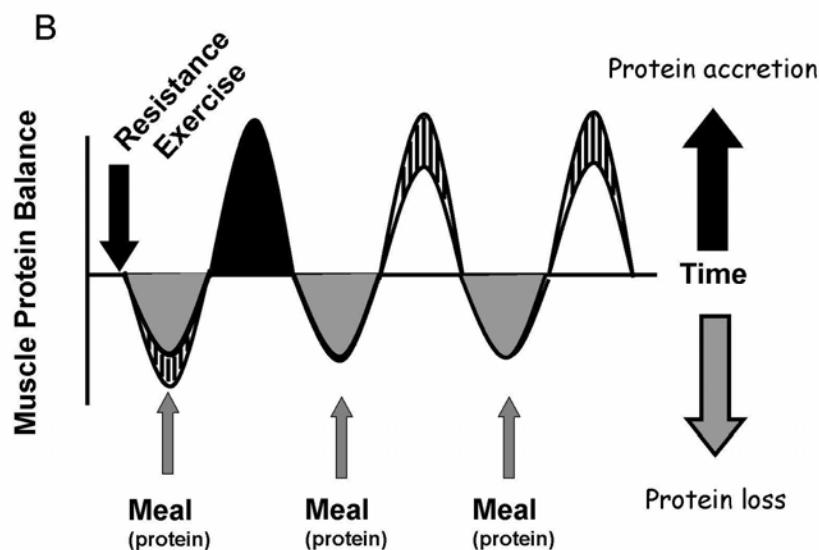
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In the years beyond those in which humans are growing there is normally no net new accretion of skeletal muscle mass. In fact, beginning in the 4<sup>th</sup> or 5<sup>th</sup> decade of life the mass of skeletal muscle begins to slowly decline, a condition referred to as sarcopenia.<sup>1-3</sup> Regardless of our age, however, muscle proteins are constantly and simultaneously being synthesized and degraded. Thus, maintenance of the mass of skeletal muscle is through the net balance between the processes of synthesis and breakdown of muscle proteins. This balance is maintained by ingestion of protein-containing meals, which results in a systemic increase in amino acids, which are stimulatory for the synthesis of new muscle proteins.<sup>4-7</sup>

Skeletal muscle is a plastic tissue with the ability to respond to a variety of external stimuli such as exercise. Regular performance of dynamic endurance-type exercise results in a muscle phenotype that is more fatigue resistant, highly oxidative, and has a high capacity for lipid oxidation.<sup>8-10</sup> By contrast, regular performance of resistance exercise induces an increase in muscle-fiber cross-sectional area, greater force generating capacity, and is less stimulatory for changes in oxidative capacity.<sup>11-15</sup> The potency of resistance exercise as a stimulator of muscle protein synthesis (MPS) is evident in the fact that the acute increase in MPS is of greater magnitude and, especially, of far longer duration than the change after feeding.<sup>16-18</sup>

Feeding protein or amino acids stimulates MPS, an effect that appears to be due almost exclusively to the amino acids themselves.<sup>7,19-22</sup> It appears that only the indispensable amino acids are required to manifest this effect.<sup>22,23</sup> The amino acid leucine, in particular, occupies a position of prominence in that it alone can act as a stimulatory signal for MPS.<sup>24,25</sup> In humans, the ability of leucine alone to act as a signal for activating MPS has been tested only once<sup>26</sup>; however, many lines of evidence point to the ability of leucine to act in stimulatory manner for feeding-induced increases in MPS.<sup>27-29</sup> It should be noted, however, that even if leucine alone were to stimulate a rise in MPS by activating proteins in the mammalian target of rapamycin (mTOR) pathway, in the absence of substrate (ie, a full compliment of indispensable amino acids), MPS would ultimately slow and eventually revert to basal levels. Thus, complete mixtures of amino acids, both infused<sup>5,6,19</sup> and ingested,<sup>30</sup> or ingestion of intact proteins<sup>31-35</sup> have all reported increases in MPS. Fig 1 shows our general understanding of how muscle protein is accrued both with feeding, resistance exercise, and with the two stimuli together.



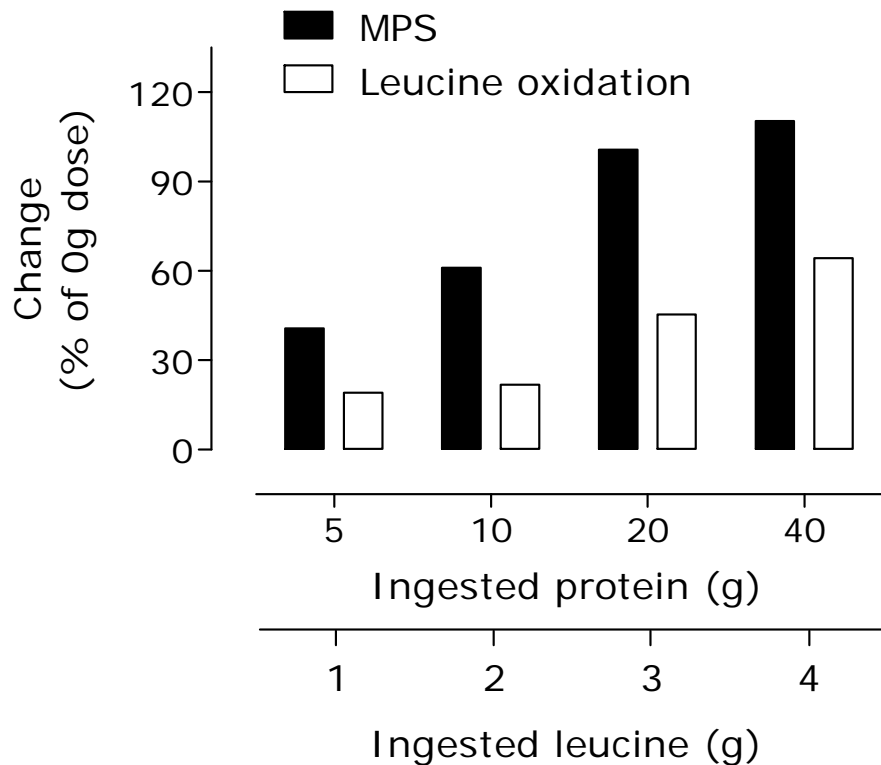


**Fig 1. (A) Normal fed-state gains and fasted-state losses in skeletal muscle protein balance (synthesis minus breakdown).** The area under the curve in the fed state (black area) would be equivalent to the fasted loss area under the curve (grey area); hence, skeletal muscle mass is maintained by feeding. **(B) Fed-state gains and fasted-state losses in skeletal muscle protein balance with performance of resistance exercise.** In this scenario, fasted-state gains are enhanced by an amount equivalent to the stimulation (striped area) of protein synthesis brought about by exercise (black area). In addition, fasted-state losses (striped area) appear to be less (grey area) due to persistent stimulation of protein synthesis in the fasted state.<sup>36</sup>

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Interestingly, the process of MPS is saturable and appears to be a function of the extracellular amino acid concentrations rather than the intracellular.<sup>5</sup> The response of MPS in both young and older people is curvilinear, with an approximate plateau at 10 g of indispensable amino acids.<sup>37</sup> Recently, we reported on the dose-response of MPS following resistance exercise using intact isolated egg protein as a dietary source.<sup>38</sup> What we observed, similar to what was reported previously,<sup>37</sup> was that at 20 g of ingested protein (~8.5 g of essential amino acids) MPS plateaued. Fig 2 shows a plot of the response of MPS as well as a corresponding plot of leucine oxidation as an index of

amino acid catabolism, as percentage of the basal (ie, 0 g) protein dose.



**Fig 2. Percentage increases (from basal or 0g) in muscle protein synthesis (MPS) and leucine oxidation after resistance exercise in young men as a function of ingested protein and leucine dose.**<sup>38</sup> The ingested protein was isolated egg protein.

Data adapted from Moore et al. “Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *American Journal of Clinical Nutrition* 2009;89:161.

What becomes evident is that as MPS plateaus the extra amino acids are burned for fuel. Thus, the consumption of massive quantities of amino acids and/or protein by many resistance-trained athletes is clearly unnecessary when a mere 20 g of high-quality protein will suffice to maximally stimulate the process (MPS) that underpins changes in muscle mass.

Resistance-exercise stimulation of muscle protein synthesis lasts at least 48 hours.<sup>17</sup> Hence, resistance exercise and protein ingestion should interact synergistically to stimulate protein synthesis at any time within 48 hours following exercise cessation, and ultimately lead to protein accretion. However, evidence exists to support the contention that consumption of protein (or amino acids), and not simply energy as carbohydrate, in close temporal proximity, both before and/or after, to resistance exercise is important to support greater hypertrophy.<sup>39-43</sup> These chronic training studies suggest that, in younger people, the window during which consumption of protein or amino acids should be consumed is likely 30-45 minutes before and/or less than 2 hours after exercise to support greater increases in lean body mass and muscle hypertrophy. In the elderly, it is possible that the window for nutrition may be as little as 1 hour after exercise.<sup>41</sup> Notably, one acute study in young people has shown that a full anabolic response can be mounted by skeletal muscle at both 1 hour and 3 hours post-exercise with crystalline amino acid consumption<sup>44</sup>; however, it has not been investigated whether this feeding pattern would translate into similar increases in muscle hypertrophy with training. Therefore, to support greater hypertrophy with resistance training at any age, consuming a source of protein within 1 hour after exercise cessation would be beneficial.

Researchers who study the phenomenon of resistance-training-induced hypertrophy have long held that a contraction intensity threshold exists that induces hypertrophy. This belief dates back to the classic work of DeLorme,<sup>45</sup> who in 1945 made the following conclusions, "...Low-repetition, high-resistance exercises produces power. High-repetition, low-resistance exercises produce endurance. Each of these two types of

exercise is incapable of producing results obtained by the other. In order to obtain rapid hypertrophy in weakened, atrophied muscle, the muscle should be subjected to strenuous exercise and, at regular intervals, to the point of maximum exertion.” In fact, advocating the practice of lifting heavier loads to induce hypertrophy and strength still is inherent in even the most up-to-date reviews on this topic.<sup>46</sup> Acute studies appear to support the “lift heavier” paradigm, at least in part,<sup>47</sup> inasmuch as a rise in MPS is seen only when intensities of load lifted exceed 60% of the single repetition maximum (1RM). Of note, however, is that at intensities beyond 60% and up to 90% of 1RM stimulation of MPS is similar. This finding may indicate that chronic performance of resistance exercise at intensities greater than 60% of 1RM has little additional value for stimulating MPS and possibly hypertrophy. This supposition is predicated on the assumption that acute changes are meaningful in terms of predicting long-term hypertrophic changes, which is a concept that does have support.<sup>35,42</sup>

Other studies offer credence to the concept that it may not be the intensity of the lift that is the active variable in determining the response of MPS. Fujita et al<sup>48</sup> reported that even low intensity exercise (20% of 1RM) stimulated MPS when blood flow was occluded. This acute finding is perhaps not surprising when one considers that a number of training studies have shown that this practice of blood flow occlusion can result, when practiced chronically and even when lifting at low intensities (30%-40% of 1RM), in substantial hypertrophy and strength gains equivalent to those seen at 80% of 1RM.<sup>49</sup> Why occlusion has this effect is not known, but the most likely explanation is that it induces a local fatigue that forces recruitment of type II muscle fibers, which would not normally be

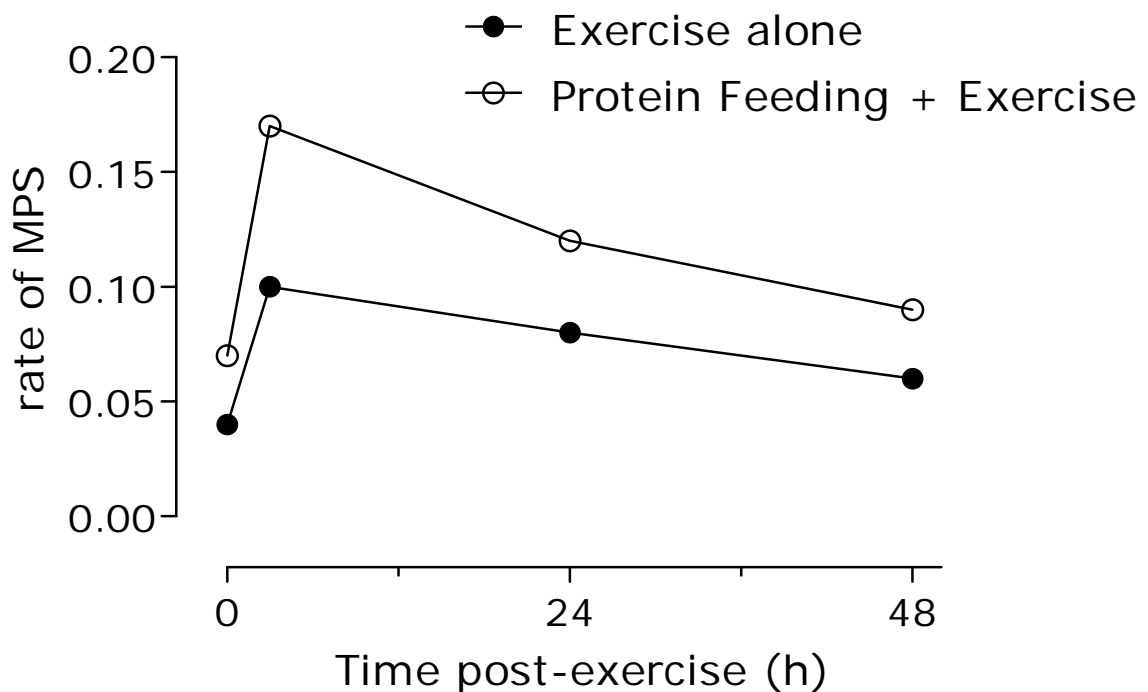
recruited at such low intensities. An alternative explanation is that the acute rise in growth hormone is somehow responsible for increases in skeletal muscle growth. Evidence to support that growth hormone is in any way anabolic for skeletal muscle and even affects MPS is lacking, however, with a lot of evidence to support the contrary position.<sup>50-52</sup> Thus, the possibility that the recruitment of type II fibers per se, independent of the exercise intensity, is the prime variable affecting the stimulation of MPS and ultimately training-induced hypertrophy provides an interesting avenue for future study.

We have known for some time that in response to resistance training the larger type II fibers display a greater degree of hypertrophy than the smaller type I.<sup>13,53,54</sup> Moreover, recent evidence demonstrates that following resistance exercise, primarily type II fibers activate many of the critical signaling proteins involved in the regulation of MPS.<sup>55</sup> This is notable because the phosphorylation of this protein is reported to predict hypertrophy in rats,<sup>56</sup> humans,<sup>57</sup> and, at least in young people, the intensity-dependent rise in MPS.<sup>47</sup> Collectively, therefore, these data suggest that, regardless of exercise intensity, a prerequisite condition to maximizing the anabolic effect of resistance exercise may be the activation of these highly trainable type II muscle fibers. This effectively means that one needs to work to fatigue, even at a low load, to see effective gains in muscle mass.

The effect of the timing of amino acid delivery after resistance exercise has been examined acutely<sup>44,58,59</sup> and long term.<sup>40-43,60</sup> It appears to make little difference whether a protein-plus-carbohydrate supplement (6 g of amino acids plus 35 g of sucrose) is consumed 1 hour or 3 hours post-exercise because the same positive net protein balance



results at both times.<sup>44</sup> In another investigation by Tipton et al,<sup>59</sup> pre-exercise consumption of the same protein plus carbohydrate supplement used previously<sup>44</sup> did augment muscle protein balance. However, this finding was not reproduced with pre- and post-exercise ingestion of whey protein.<sup>58</sup> In longer-term training studies, the effects of immediate (or at least temporally close) provision of nutrition appears to enhance muscle mass gains.<sup>40-43,60</sup> As Fig 3 summarizes, it appears that consuming protein sometime following the performance of resistance exercise, say 1-2 hours following, does appear to additively stimulate MPS and enhance gains in muscle and, presumably, strength.



**Fig 3. Schematic figure illustrating the effect of an isolated bout of resistance exercise alone on the rate of muscle protein synthesis (MPS) from rest (0h) and at 4h, 24h, and 48h post-exercise.** The additive effect of feeding is shown at the same times. Note that the additive effect of feeding is greatest at 4h post-exercise, which is proposed to occur after ingesting protein immediately post-exercise<sup>37</sup> and is diminished with time post-exercise. Thus, it appears that consuming protein in close temporal proximity to exercise (ie, at least within 1-2h after) is advantageous from the standpoint of promoting gains in muscle mass.

In summary, we are now beginning to understand the factors that regulate gains in muscle mass, and MPS is the primary locus of control. As such, studies examining acute and chronic changes in MPS are of primary importance to understanding what factors regulate gains in muscle mass with feeding and resistance exercise. Perhaps more important are studies of situations in which muscle mass is lost, such as with aging. With aging, it is also likely important that declines in the feeding-induced sensitivity to protein feeding, and as such the declines in the feeding-induced rise in protein balance, are also underpinning the decline in muscle mass. Clearly, more work needs to be done and acute mechanistic studies will ultimately inform, at least qualitatively, how chronic interventions may be able to help athletes gain the muscle they need to compete or help the elderly reclaim the muscle they lose with aging. Regardless of the situation, an enormous capacity for good exercise planning, good overall nutrition, and specialized nutritional products exists that can enhance these processes and provide benefit to athletes and the elderly alike.

#### Acknowledgements

The author is a recipient of grants from the US National Dairy Council, The Dairy Farmers of Canada, The Canadian National Science and Engineering Research Council (NSERC), and the Canadian Institutes for Health Research (CIHR) and would like to acknowledge those sources of funding.

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## Q&A

**Dr Keith Wheeler [Abbott Nutrition]:** Dr Phillips, did you use essential amino acids throughout your studies or essential and nonessential amino acids? If both kinds, what ratio did you target? Did you vary that ratio or was it constant?

**Dr Phillips:** All the studies I have shown you are using high-quality whole proteins, either whey protein or milk protein. All I have reported has been the rise in blood leucine concentration or the rise in essential amino acid concentration. At no point have we



manipulated using crystalline amino acids or anything like that—the essential amino acid composition.

**Dr Schols:** You performed a nice series of experiments. As far as I remember, they were all performed in males. Do you expect any gender-specific anabolic responses?

**Dr Phillips:** We are about to submit a paper describing research using the same resistance-exercise protocol—milk drinking vs control—in women. The women who drank the milk responded similarly to the men; acutely, of course, we see no rise in testosterone in those women. We do see a small rise in growth hormone, but it is entirely consistent between groups, indicating that the differential gain in muscle mass has nothing to do with hormones. Of course, women have the capacity to hypertrophy, but it has very little to do with a change in testosterone. It is all driven internally within the muscle itself.