CALL FOR PAPERS | Endocrine and Metabolic Dysfunction during Aging and Senescence

The impact of delivery profile of essential amino acids upon skeletal muscle protein synthesis in older men: clinical efficacy of pulse vs. bolus supply


Department of Clinical, Metabolic, and Molecular Physiology, MRC-ARUK Centre of Excellence for Musculoskeletal Ageing Research, School of Medicine, University of Nottingham, Derby, United Kingdom

Submitted 9 March 2015; accepted in final form 3 July 2015

Mitchell WK, Phillips BE, Williams JP, Rankin D, Lund JN, Wilkinson DJ, Smith K, Atherton PJ. The impact of delivery profile of essential amino acids upon skeletal muscle protein synthesis in older men: clinical efficacy of pulse vs. bolus supply. Am J Physiol Endocrinol Metab 309: E450–E457, 2015. First published July 7, 2015; doi:10.1152/ajpendo.00112.2015.—Essential amino acids (EAA) are responsible for skeletal muscle anabolic effects after nutrient intake. The pattern of appearance of EAA in blood, e.g., after intake of “slow” or “fast” protein sources or in response to grazing vs. bolus feeding patterns, may impact anabolism. However, the influence of this on muscle anabolism is poorly understood, particularly in older individuals. We determined the effects of divergent feeding profiles of EAA on blood flow, anabolic signaling, and muscle protein synthesis (MPS) in older men. Sixteen men (≥70 yr) consumed EAA either as a single dose (bolus, 15 g; n = 8) or as small repeated fractions (pulse, 4 × 3.75 g every 45 min; n = 8) during 13C6 phenylalanine infusion. Repeated blood samples and muscle biopsies permitted measurement of fasting and postprandial plasma EAA, insulin, anabolic signaling, and MPS. Muscle blood flow was assessed by contrast-enhanced ultrasound (Sonovue). Bolus achieved rapid insulinemia (12.7 μIU/ml 25-min postfeed), essential aminoacidemia (~3,000 μM, 45–65 min postfeed), and mTORC1 activity; pulse achieved attenuated insulin responses, gradual low-amplitude aminoacidemia (~1,800 μM, 80–195 min after feeding), and undetectable mTORC1 signaling. Despite this, equivalent anabolic responses were observed: fasting FSRs of 0.051 and 0.047%/h, respectively. Moreover, pulse led to sustained MPS beyond 180 min, when bolus MPS had returned to basal rates. We detected no benefit of rapid aminoacidemia in this older population despite enhanced anabolic signaling and greater overall EAA exposure. Rather, apparent delayed onset of the “muscle-full” effect permitted identical MPS following low-amplitude-sustained EAA exposure.

protein synthesis; nutrition; amino acids; skeletal muscle; anabolic signaling; muscle full state

IN HEALTHY AND HABITUALLY ACTIVE ADULTS, muscle mass remains constant over early life to midlife, demonstrating effective proteostatic mechanisms balancing the synthesis and breakdown of muscle proteins. Beyond the sixth decade, however, breakdown of muscle proteins exceeds synthesis, and muscle mass is gradually lost. This results in muscle atrophy and frailty (36) referred to as sarcopenia, which is of great significance to an aging population, with decrements in muscle mass and strength being predictors of illness, loss of independence, and death (14, 31, 32, 41, 51).

One facet that may contribute to sarcopenia is anabolic resistance, a compromised anabolic response to protein/essential amino acid (EAA) consumption with decreased postprandial muscle protein synthesis (MPS) (15, 27, 52). Moreover, a preferential loss of lean tissue over fat in older age (24) and a tendency to consume less high-quality protein (39, 46) may exacerbate such anabolic resistance. The total anabolic value of one meal is limited, with a modest intake of high-quality protein maximally stimulating MPS and no additional benefit from excessive protein ingestion (48), although some have suggested that increases in protein load and/or EAA/leucine content of a meal may assist in promoting MPS in older adults (13, 43, 45). Sustained hyperaminoacidemia is associated with the onset of a “muscle-full” state, wherein MPS returns to fasting rates despite ongoing availability of circulating EAA (5, 8, 40). Together, these observations have stimulated interest in the potential for the delivery profile of a feed to impact upon its total “anabolic value.”

This notion was first proposed after the observation of apparent enhancement of protein accretion after ingestion of “fast” (whey) vs. “slow” (casein) protein sources (9), although these sources also differ in constituent amino acid (AA) profiles, especially leucine content (18). Provision of rapidly absorbed casein hydrolysates, or “pulsed” feeding regimens with repeated ingestion of small fractions, can isolate the effects of digestion rate from EAA content (17). Insofar, work has focused on the importance of aminoacidemia in terms of 1) maximally stimulating MPS (49, 53), 2) the impact of splanchnic metabolism on systemic availability of AA (4, 11, 28), which is known to be higher in older people (38) and the poorly nourished (30), and 3) the potential for regimens that provide cyclical fluctuations in aminoacidemia to maximize anabolism (2, 12, 37), some (2, 4, 11, 53) but not all (3, 12, 28, 37) of which support profile of delivery as a key determinant of anabolism. Moreover, the majority of past work has focused on young populations, and after resistance exercise, the applicability of which to older populations (at rest) is questionable (15, 18, 29). We have shown previously that muscle anabolic responses in young healthy men are not perturbed by gradual/
low aminoacidemia (34). The aim of this follow-on study was to extend this work into an older population, determining the effects of rapid vs. gradual aminoacidemia in older men fed the same quantity of EAA provided as 1) a single (1 × 15 g) EAA bolus (bolus) or 2) small (4 × 3.75 g) pulses at ~45-min intervals (pulse).

MATERIALS AND METHODS

Study design. Ethical approval was obtained from the University of Nottingham Medical School Ethics Committee (United Kingdom), with all studies conducted in accordance with the Declaration of Helsinki and registered as clinical trials (clinicaltrials.gov registration no. NCT01735539). Healthy older men [n = 16, 70 ± 3 yr, BMI 26 ± 2 (means ± SD)] were studied after an overnight fast and were asked to refrain from heavy exercise for 48 h prior to the study. On the morning of the study (0800), subjects had an 18-g cannula inserted into the dorsum of the left hand for a constant infusion of L-[^13C6]phenylalanine (Cambridge isotopes, Cambridge, MA): 0.3 mg/kg primed and 0.6 mg·kg⁻¹·h⁻¹ continuous. Blood samples and muscle biopsies were taken as outlined in Fig. 1. Arterialized venous blood was sampled via a retrograde, 16-g intravenous cannula placed in the dorsum of the right hand, with the hand warmed to 35°C (1). Muscle biopsies were taken intermittently from m. vastus lateralis using the conchotome technique (20) after infiltration of 5 ml of 1% lignocaine. Muscle was washed in ice-cold phosphate-buffered saline, and visible fat and connective tissue were removed before being snap-frozen in liquid N₂ and stored at −80°C until analysis. Biopsies were taken 1 and 3 h after commencement of the tracer to permit assessment of basal (post-absorptive) MPS. Subjects were then provided with 15 g of mixed EAA (in g: 1.21 histidine, 1.73 isoleucine, 3.59 leucine, 3.07 lysine, 0.95 methionine, 0.91 phenylalanine, 1.13 tryptophan, 0.48 threonine, and 1.86 valine) in aqueous solution (250 ml) either as a single dose (bolus; n = 8) or in four equal fractions swallowed at 45-min intervals (pulse; n = 8). Feed composition was based on proportions reflective of skeletal muscle composition (50). Subsequent biopsies at 90, 180, and 240 min postcommencement of feeding allowed assessment of MPS in the intervening periods. After the study, cannulas were removed and the subjects fed and monitored for 30 min before departure. All laboratory analyses were performed blinded to feeding regimen. Subject characteristics are summarized (Table 1).

Plasma AA and insulin concentrations. Plasma insulin concentrations were measured using undiluted samples on a high-sensitivity human insulin enzyme-linked immunosorbent assay (DRG Instruments, Marburg, Germany). Area under the curve (AUC) analysis estimated the total insulin response to feeding and was calculated for each individual with a baseline equal to the mean of fasting, +155-min, and +195-min insulin concentrations. For AA analyses, equal volumes of plasma and 10% sulfosalicylic acid were mixed and cooled to 4°C for 30 min. Samples were centrifuged at 8,000 x g for 10 min, and the supernatant fluid was passed through a 0.22-μm filter before analysis (AA analyzer, Biochrom 30; Biochrom, Cambridge, UK). All 20 AA concentrations were measured by comparison with a standard AA mix, with norleucine as internal standard. AUC analysis estimating total EAA exposure was calculated for each individual without baseline correction.

Muscle microvascular blood volume and leg (femoral) blood flow. Changes in microvascular blood volume (MBV) with feeding were measured using contrast-enhanced ultrasound (CEUS) with an iU22 ultrasound scanner (Phillips Healthcare, Reigate, UK) and Sonovue microbubbles (Bracco, Milan, Italy), which were infused via an antecubital fossa vein, as described previously (35). Briefly, a 40-mm linear 3- to 9-MHz probe (Phillips L9–3) positioned on the left anterior thigh detected microbubble concentration within the quadriceps muscle. Intermittent high mechanical index “flashes” disrupted microbubbles while continuous low MI recording measured the rate of microbubble repletion. Infusion was at 2 ml/min for 1 min and 1 ml for 3 subsequent min. During the last 90 s of this protocol, 3 × 30 s flash/replenishment recordings were made. Offline region-of-interest analysis measured the peak linear acoustic intensity. This is proportional to microbubble concentration and therefore MBV. A 0.48-s window postflash was used to calculate background and noncontrast signal and to allow for rapid filling of larger nonexchange vessels. Leg blood flow was measured using Doppler ultrasound, with an identical probe positioned over the origin of the left common femoral artery. Leg flow was estimated as the product of vessel cross-sectional area and mean velocity over six cardiac cycles. Mean flow was calculated at a time corresponding to each CEUS recording episode based on three such recordings made on each of four occasions distributed across the study day, as indicated in Fig. 1 (i.e., flow during 72 cardiac cycles contributed to a mean leg flow measure). Each individual’s leg flow was standardized to that subject’s own fasting flow.

Muscle protein-bound and intramuscular free phenylalanine enrichment. Myofibrillar proteins were separated, hydrolyzed, and derivitized using our standard techniques (5, 23). Briefly, ~25 mg of muscle tissue was homogenized using ice-cold homogenization buffer, pH 7.5 (50 mM Tris-HCl, 50 mM NaF, 10 mM β-glycerophosphate disodium salt, 1 mM EDTA, 1 mM EGTA, and 1 mM Na₂VO₃; all from Sigma–Aldrich, Poole, UK), and a Complete Mini Protease inhibitor cocktail tablet (Roche Diagnostics) before centrifugation at 10,000 g to separate myofibrillar (pellet) and sarcoplasmic (supernatant) fractions. The myofibrillar pellet was washed in homogenization buffer, centrifuged to pellet the myofibrils, and then solubilized in 0.3 N NaOH. The insoluble collagen fraction was removed by centrifugation and the myofibrillar supernatant precipitated using 0.2 N perchloric acid; the resulting precipitate was pelleted by centrifuga-

Table 1. Subject characteristics for both bolus and pulse feeding groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bolus</th>
<th>Pulse</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>70.0 ± 0.8</td>
<td>70.3 ± 1.1</td>
<td>0.84</td>
</tr>
<tr>
<td>Height, cm</td>
<td>171 ± 2.1</td>
<td>174 ± 2.0</td>
<td>0.36</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75.4 ± 1.9</td>
<td>78.3 ± 3.3</td>
<td>0.48</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.5 ± 0.4</td>
<td>25.7 ± 1.0</td>
<td>0.90</td>
</tr>
<tr>
<td>ASMMI</td>
<td>8.16 ± 0.17</td>
<td>8.09 ± 0.30</td>
<td>0.83</td>
</tr>
<tr>
<td>HGS</td>
<td>40.7 ± 1.3</td>
<td>40.2 ± 2.7</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Values are means ± SE. ASMMI, appendicular skeletal muscle mass index [appendicular muscle mass/height^2 (kg/m²)]; HGS, hand grip strength (kg).
tion and washed twice with 70% ethanol. Protein-bound AAs were released by overnight acid hydrolysis at 110°C in Dowex H+ resin slurry (Sigma-Aldrich). AAs were eluted from the resin using 2 M NH4OH and derivatized as their N-acetyl-N-propyl esters were prepared. The labeling of t-[ring-13C6]phenylalanine in myofibrillar protein was determined by gas chromatography-combustion-isotope ratio mass spectrometry (Delta Plus XP; ThermoFisher Scientific, Hemel Hempstead, UK). Muscle intracellular phenylalanine enrichment was measured by gas chromatography-mass spectrometry (Trace DSQ; ThermoFisher Scientific) following precipitation of the sarcoplasmic fraction and purification of the aqueous supernatant using Dowex H+ resin as described above, with AAs converted to their tert-butylidimethylsilyl derivatives.

Rates of MPS. The fractional synthetic rate (FSR) of myofibrillar proteins was calculated from the increase in incorporation of t-[ring-13C6]phenylalanine between subsequent muscle biopsies, using muscle intracellular phenylalanine as a surrogate of the true precursor phenylalanyl-t-RNA (56). Muscle intracellular phenylalanine was used as a surrogate of the true precursor phenylalanyl-t-RNA (56). FSR was calculated using the standard precursor-product method fractional protein synthesis (%/h):

\[
FSR = \frac{E_{p2} - E_{p1}}{E_{m1}} \times 60 \times 100
\]

where \(E_{p1}\) and \(E_{p2}\) are the enrichments of bound t-[ring-13C6]phenylalanine in two sequential biopsies, \(t\) is the time interval between two biopsies, and \(E_{m}\) is the mean t-[ring-13C6] free phenylalanine enrichment in the intramuscular pool. To offset a potential drop in intramuscular pool. To offset a potential drop in labeling of L-[ring-13C6] phenylalanine in the feed was provided as L-[ring-13C6] phenylalanine as a surrogate of the true precursor phenylalanyl-t-RNA (56). FSR was calculated using the standard precursor-product method fractional protein synthesis (%/h):

\[
FSR = \frac{E_{p2} - E_{p1}}{E_{m1}} \times 60 \times 100
\]

RESULTS

Plasma aminoacidemia and insulinemia in bolus and pulse. Bolus plasma EAA concentrations peaked between 45 and 65 min postfeed (+250% at 65 min, \(P < 0.001\) vs. fasting), whereas pulse plasma EAA appeared maximal between 80 and 195 min post-commencement of feed (peak +150% at 115 min, \(P < 0.001\) vs. fasting). EAA concentrations were significantly higher in bolus at 15–80 min postfeed and higher in pulse at 195 min postfeed (\(P < 0.001\); Fig. 2A). Plasma nonessential amino acid concentrations did not differ with feeding strategy and modulated little, with a small (10–20%) decrement seen during the fed period (Fig. 2B). Plasma leucine concentrations modulated with the general EAA pool (Fig. 2C). AUC analyses showed that bolus feeding achieved 25% more total systemic EAA (bolus 489 ± 11 μmol·l⁻¹·min⁻¹, pulse 399 ± 10 μmol·l⁻¹·min⁻¹, \(P < 0.0001\)) and leucine (bolus 102 ± 2.7 μmol·l⁻¹·min⁻¹, pulse 81.1 ± 1.8 μmol·l⁻¹·min⁻¹, \(P < 0.0001\)) exposure during the study period (Fig. 2D).

Fasting plasma insulin concentrations were the same between groups (bolus 4.39 ± 1.02, pulse 3.38 ± 1.11 μU/ml, \(P = 0.60\)). With bolus feeding, plasma insulin was elevated (+190% at 25 min after feeding and +154% at 45 min, both \(P < 0.0001\) vs. fasting) and had returned to basal levels by 80 min after feeding. Pulse feeding stimulated an attenuated plasma insulin response (+109% at 25 min, \(P = 0.0045\)) that was short-lived, returning to baseline by 45 min (Fig. 3A). AUC analyses estimated bolus feeding achieved c.106% more insulin release than pulse (bolus 574 ± 79 μU·ml⁻¹·min⁻¹, pulse 279 ± 81 μU·ml⁻¹·min⁻¹, \(P = 0.022\); Fig. 3B).

Muscle and leg blood flow in response to bolus and pulse. Fasting muscle MBV was similar between groups (bolus 0.79 ± 0.07 U/mm⁻³, pulse 1.02 ± 0.14, \(P = 0.20\)). Subsequent calculations were based on each individual standardized to their own fasting MBV. In keeping with other studies that have addressed vascular responsiveness to feeding in older age, neither feeding strategy was found to impact upon MBV or lean mass-standardized, whole leg blood flow (Fig. 4).

Intracellular signaling in response to bolus and pulse. Bolus feeding induced phosphorylation at p70S6K Thr389 (2.6-fold increase at 90 min, \(P = 0.0023\), and 1.6-fold increase at 180 min, \(P < 0.024\) vs. fasting), an effect not seen with pulse feeding. Neither feeding regimen elicited detectable dephosphorylation at the inhibitory eEF2 Thr56 or a change in PKB activity as assessed by phosphorylation at Akt Ser473. No change was seen at the activating eNOS Ser1177 (Fig. 5).

MP5 in response to bolus and pulse. Fasting FSR was similar between groups (bolus 0.051 ± 0.004%/h, pulse 0.047 ± 0.007%/h, \(P = 0.60\)). Neither bolus nor pulse feeding altered FSR during the period of 0–90 min postfeed. During the period of 90–180 min postfeed, bolus feeding significantly increased FSR (to 0.084 ± 0.005%/h, +65%, \(P = 0.0025\) vs. fasting), as did pulse feeding (to 0.073 ± 0.005%/h, +55%, \(P = 0.0285\) vs. fasting). In the period of 180–240 min post-commencement of feeding, bolus FSR returned to fasting (0.054 ± 0.012%/h, \(P = 0.43\) vs. fasting), whereas pulse FSR remained elevated (0.071 ± 0.017%/h, \(P = 0.0377\) vs. fasting) during this period (Fig. 6).

Total synthetic activity during the fed period was the same between groups (bolus 0.220 ± 0.016% vs. pulse 0.233 ± 0.017%, both in 4 h, \(P = 0.62\)).
DISCUSSION

To our knowledge, this study provides the first comparison of the anabolic impact of bolus vs. pulse oral EAA feeding regimens, that is to say rapid vs. gradual aminoacidemia, in older individuals. In terms of major findings, we have demonstrated 1) a latency of ~90 min after commencement of both bolus and pulse feeding before any detectable increase in MPS, and this latency was evident despite plasma EAA and leucine concentrations peaking ~45 min after bolus feeding; 2) that after this ~90 min latency, MPS was elevated to a similar extent in both groups despite the weaker, more gradual onset of aminoacidemia with pulse feeding and the failure of pulse (vs. bolus) to stimulate mammalian target of rapamycin complex 1 (mTORC1), as assessed by the phosphorylation of its cognate substrate p70S6K; 3) that pulse but not bolus feeding exhibited a persistence in elevation of MPS (vs. basal) over the last study period (180–240 min), whereas MPS had returned to fasted rates despite plasma EAA concentrations remaining elevated in both pulse and bolus; and 4) that the net synthetic effect of bolus vs. pulse feeding, e.g., area under the curve, was indistinguishable.

The fact that distinct profiles of aminoacidemia/leucinemia (of a fixed quantity) could achieve equivalent MPS queries the notion of any benefit being afforded by promoting rapid aminoacidemia in older populations. Previously, it was observed in young men after resistance exercise training that bolus feeding achieved significantly enhanced MPS vs. a pulse feeding strat-
egy that provided the same systemic EAA exposure (53). However, in older men fed either intact protein or a more rapidly absorbed hydrolysate, no significant difference in overall MPS could be detected despite the gradual onset of amino-acidemia, with intact protein feeding also being associated with lower total EAA exposure due to greater splanchnic EAA extraction (28). Indeed, the area under the arterialized plasma EAA and leucine curves in our present study shows more EAA exposure with bolus feeding, which we attribute to greater splanchnic extraction of EAA. The overnight fast used to ensure uniform postabsorptive conditions also results in glycogen depletion, perhaps promoting gluconeogenesis and splanchnic EAA metabolism (30). Moreover, because there was little difference in MPS when older men were fed 10 vs. 20 g EAA (15), we may have expected the 25% lower systemic EAA exposure with pulse to have had little impact on anabolism. Finally, our observation that MPS remained above fasting rates beyond 180 min after the commencement of pulse feeding would support the hypothesis that dose rather than time-dependent mechanisms underlie the onset of the “muscle full” state (this is a state describing the point at which stimulation of MPS becomes refractory to the ongoing availability of EAA).

Bolus-fed individuals exhibited a trend for MPS rates being below fasting values in the first 90 min after feeding ($P = 0.08$). Transient suppression of MPS on delivery of a large EAA bolus may explain why bolus feeding did not enhance MPS over pulse despite achieving increased EAA delivery to peripheral tissue and robust anabolic signaling. Mechanisms

![Fig. 4. Change in leg blood flow (LBF; A) and muscle microvascular blood volume (MBV; B) in older men after consumption of 15 g of mixed EAA doses by bolus or pulse treatment. Values are means ± SE; $n = 8$. Black arrows represent ingestion of 15 g of EAA; gray arrows represent 3.75 g of EAA.](image)

![Fig. 5. Phosphorylation of p70S6K Thr$^{389}$ (A), eEF2 (eukaryotic elongation factor 2) Thr$^{56}$ (B), Akt Ser$^{473}$ (C), and p-eNOS (endothelial nitric oxide synthase) Ser$^{1177}$ (D) in older men after consumption of 15 g of mixed EAA doses by bolus or pulse treatment, all corrected to Coomassie total protein bands. Representative blots are shown. Values are means ± SE; $n = 8$. *$P < 0.05$ and **$P < 0.01$, differences from fasting.](image)
underlying this apparent transient early inhibition of MPS could include the active transport of EAA into myocytes (25, 26) impinging upon energetically demanding aminocacylation of tRNA (19) or the rapid increase in flush through plasma membrane AA exchangers on feeding causing temporary deficiency in specific AA either for tRNA charging or use in tertiary active transport. Alternatively, given impaired mitochondrial function in aging (6, 16) and the link between energy provision and anabolic responses to feeding (33, 55), it is possible a transient depression in MPS is due to energy deficits (i.e., ATP cost of mRNA translation) may underlie anabolic resistance. Further (crossover) studies may substantiate these hypotheses.

Certain EAAs, including leucine, are also moderate insulin secretogogues, and the plasma insulin profile after bolus feeding reflects these previous observations (22). The gradual onset aminoacidemia with PULSE feeding elicited an attenuated insulin response. Neither feeding profile was sufficient to achieve detectable stimulation of established proximal insulin signaling (Akt phosphorylation). This supports the observation that maximal MPS can be observed in response to EAA/protein provision despite fasting insulin concentrations and with nominal Akt phosphorylation (7, 21, 23, 42). Our observation of differential phosphorylation of an mTORC1 substrate between regimens confirms previous observations that feeding pattern impacts upon anabolic signaling (2, 53), whereas the similarity in MPS between the groups points toward activity in established signaling pathways being poor proxies of postprandial MPS (23) and not obligate in the maintenance of normal muscle mass. Our observation that pulse feeding effectively stimulated MPS without a detectable insulin/mTORC signaling response suggests that basal elements necessary for MPS are in place within the fasted myocyte and that EAA availability is a rate-limiting factor. In addition to this, we observed no significant vascular response to feeding and no evidence of capillary recruitment or increased leg blood flow. This supports previous demonstrations of diminished vascular responsiveness to feeding with age, which may contribute to a blunted anabolic response with aging (35, 44, 47). Finally, although we have demonstrated a significant persistence of elevated MPS with pulse but not bolus feeding, net anabolism over the 4-h postprandial period was only 6% greater and not significantly different. However, the sustainment of MPS to the end of the experimental period does raise the prospect that longer studies may yet demonstrate a benefit from pulse-type feeding, with an early postprandial MPS suppression and the onset of the muscle-full state together compromising anabolism achieved by provision of a single bolus. Conversely, because this study did extended to beyond the period of stimulated above-basal MPS with bolus feeding, we think it does challenge the hypothesis that rapid aminoacidemia affords benefits in this group.

In terms of potential limitations, the invasive nature of this study with repeated biopsy of leg muscle, prevented us employing a “crossover” study design. Furthermore, the duration of the study period was constrained for reasons of volunteer acceptability, preventing assessment of MPS beyond 4 h post commencement of feeding. Finally, although performing studies after an overnight fast and with provision of an EAA-only feed reduces potential confounding factors and thus variability between subjects, it may also compromise the direct applicability of these findings in understanding the true diurnal impact of dietary or nutritional supplement intake profiles in free-living individuals. In particular, hepatic glycogen depletion after overnight fasting may serve to increase splanchnic metabolism of EAA, especially in older people and the poorly nourished, and with “distributed” feeding (4, 10, 30, 38). Thus the study design may have led to overestimation of benefits afforded by bolus in terms of increasing tissue EAA exposure, consequentially overlooking the benefits of pulse feeding.

In conclusion, we have demonstrated that, in older men, pulse feeding can achieve equivalent MPS to bolus feeding despite the likelihood of greater splanchnic extraction, lower and more gradual aminoacidemia (and leucinemia), and attenuated anabolic signaling. Despite equal net anabolism, delayed onset of the muscle-full state associated with pulse feeding leaves open the possibility that MPS may remain above fasting levels for more than 4 h after commencement of feeding, which perhaps warrants further exploration for potential clinical benefits of pulse-type feeding in older populations.

ACKNOWLEDGMENTS

We thank Amanda Gates and Margaret Baker for expert clinical technical assistance, Libin Ashkir and Sabba Nasar for laboratory support, and Tanya Fletcher and Karen Kirkland for administrative support.

GRANTS

This work was supported by an Ajinomoto 3ARP New Investigator grant to P.J. Atherton.
DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


