



## Conference on ‘Nutrition and age-related muscle loss, sarcopenia and cachexia’ Symposium 3: Nutrition for prevention and interventions for sarcopenia and cachexia

### Nutritional interventions in sarcopenia: a critical review

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The aim of the present paper is to critically review the details of the published nutrition intervention trials, with and without exercise, targeting sarcopenia. Sarcopenia is the loss of muscle mass, strength and/or performance with age. Since amino acids and energy are required for muscle synthesis it is possible that nutritional intake influences sarcopenia. Nutritional studies are challenging to carry out because of the complexity of modulating dietary intake. It is very difficult to change one nutrient without influencing many others, which means that many of the published studies are problematic to interpret. The studies included evaluate whole protein, essential amino acids and  $\beta$ -hydroxyl  $\beta$ -methylbutyrate (HMB). Whole-protein supplementation failed to show a consistent effect on muscle mass, strength or function. This can be explained by the variations in study design, composition of the protein supplement and the failure to monitor voluntary food intake, adherence and baseline nutritional status. Essential amino-acid supplements showed an inconsistent effect but there are only two trials that have significant differences in methodology and the supplement used. The HMB studies are suggestive of a beneficial effect on older adults, but larger well-controlled studies are required that measure outcomes relevant to sarcopenia, ideally in sarcopenic populations. The issues of timing and distribution of protein intake, and increased splanchnic amino-acid sequestration are discussed, and recommendations for future trials are made.

**Sarcopenia: Vitamin D: Protein: Essential amino acid:  $\beta$ -Hydroxyl  $\beta$ -methylbutyrate**

Sarcopenia is the loss of muscle mass, strength and/or performance with age. There is as yet no universally agreed definition but a number of groups have published definitions which differ mainly around the exact cut-off points used for measures of mass, strength and performance<sup>(1–6)</sup>.

Since a consistent supply of amino acids and energy are required for muscle protein synthesis, as well as a number of other nutrients, it is possible that nutritional intake contributes to the process of sarcopenia<sup>(7)</sup>. Consequently modifying nutritional intake may also provide an opportunity to treat sarcopenia.

The process of muscle synthesis and breakdown is complex and influenced by various internal and external factors. A comprehensive review can be found at<sup>(8)</sup>. In

particular, from the nutritional perspective, it is accepted that amino acids have a stimulatory effect on muscle synthesis, especially leucine<sup>(9)</sup>. Thus, protein and amino acids have been among the first targets for interventions to combat sarcopenia, alone or in combination with exercise, and form the majority of nutritional intervention trials.

The aim of the present paper is to critically review the details of the published nutrition intervention trials, with and without exercise, targeting sarcopenia. Nutritional studies are challenging to carry out because of the complexity of modulating dietary intake. It is very difficult to change one nutrient without influencing many others, which means that many of the published studies are problematic to interpret. Studies were included if the

**Abbreviations:** EAA, essential amino acids; HMB,  $\beta$ -hydroxyl  $\beta$ -methylbutyrate.

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outcome measures reported for the interventions included muscle mass and at least one measure of muscle strength or physical performance, even when the population studied was not defined as sarcopenic, since these outcome measures form part of the definition of sarcopenia.

### Whole-protein studies

A recent systematic review<sup>(10)</sup> identified five protein supplement studies<sup>(11–15)</sup>, which are summarised in Table 1; Cruz-Jentoft *et al.* concluded that there was no consistent effect on muscle mass, strength or function. A closer examination of the studies reveal why this may be so. Table 1 shows that the studies are highly variable in a number of different aspects, including the population recruited, the duration, whether the intervention was combined with exercise, supplementing protein and energy or protein alone, the formulation and precise content of the supplements, and whether placebo controlled or not. In addition, the methodological quality as assessed by Cruz-Jentoft *et al.* varied from a PEDro score<sup>(16)</sup> of 4–10 (10 representing the highest quality score possible), outcomes measured all varied and all sample sizes were relatively small ranging from fifty-seven to ninety-eight people<sup>(10)</sup>.

The duration of the interventions ranged from 6 to 18 months, but the results show no advantage to the longer duration, but this may be explained by other factors. Tieland *et al.* suggest that the lack of effect of protein supplementation with exercise on physical performance and strength may be due to duration, postulating that longer than 6 months intervention would be required<sup>(14)</sup>. Unfortunately the two available longer term studies<sup>(11,12)</sup> had significant design weaknesses making it difficult to deduce whether this hypothesis can be supported. To further confuse the issue, in another Tieland *et al.* study protein supplementation for 6 months without exercise did improve physical performance but did not show gains in muscle mass or strength<sup>(15)</sup>. The populations were also inconsistent, including older adults (mean age range 68–83 years) who were free living<sup>(12–15)</sup> or in residential care<sup>(11)</sup>. Three studies specifically identified frail or pre-frail people<sup>(11,14,15)</sup>. This means that the results cannot be combined and they can only be applied to these specific groups.

The ability to identify the singular effect of protein with or without exercise is only possible with two studies, where a four arm design was used (exercise, protein, both or none)<sup>(11,12)</sup>. Two other studies looked at the additive effect of protein on exercise<sup>(13,14)</sup>, and the final study examined protein supplementation excluding the effect of exercise<sup>(15)</sup>. Since there is widespread agreement that exercise can improve both muscle strength and physical performance, it would be useful to be able to examine how nutritional supplements enhance these effects or not. This requires four arm studies, until the optimally effective exercise intervention is described, when two arm studies could be justified looking at nutrition in addition to exercise. Nevertheless, two arm studies

examining nutrition only still have value given the difficulties in motivating people to take up exercise. This lack of consistency in study design means that comparing or combining results is difficult or impossible and explains to a certain extent the variability of the results to date.

Most importantly the protein interventions tested varied considerably. In two trials the protein was delivered with additional energy and micronutrients<sup>(11,12)</sup>, but these trials scored lowest in terms of quality on the PEDro scale. Bunout *et al.* do not describe the exact content of the supplement since it was apparently incorporated into either soup or porridge and it is not stated what additional nutrients these foods added. The protein content is relatively low at 13 g/d, but a range of vitamins and minerals are also included to provide a more complete supplement<sup>(12)</sup>. Bonnefoy *et al.* also aimed to provide a nutritionally complete supplement containing vitamins and minerals in addition to protein and energy. The quantities of energy and protein appear significant at 1686 kJ and 30 g protein/d, but this is difficult to assess without measuring usual food intake to enable an estimate of the overall energy and protein content of the diet<sup>(11)</sup>. The other three trials aimed to examine the effect of protein only, with no additional energy or micronutrients. Chale *et al.* specifically chose to use whey protein due to the high content of essential amino acids (EAA) in this protein<sup>(13)</sup>. Tieland *et al.* do not state the source of protein<sup>(14,15)</sup>. These three studies provide either 30 or 40 g protein/d, significantly increasing protein intake as shown by an estimation of dietary intake using 3 d dietary diaries.

The measurement of concurrent food intake is a key design issue in nutritional intervention trials, but is not always monitored. Since any nutritional supplement may have an impact on usual food intake it is important to measure macronutrient intakes. In the case of protein, an energy deficit will result in a reduction in protein synthesis even with apparent adequate supplies of amino acids<sup>(17)</sup>. Similarly, if protein intake from the diet is inadequate the supplement will simply improve intake rather than increasing intake above recommended levels. If positive benefits are found in the supplemented groups, then it is important to be able to identify the overall nutrient intake to enable accurate interpretation of the results. If food intake is not measured, then it is impossible to assess what has produced any positive effect, or explain why no effect is apparent. The two lower quality studies did not aim to measure usual food intake<sup>(11,12)</sup>, the other three studies used 3 d diet diaries at least at the start and end of the study<sup>(13–15)</sup>.

There are a number of recent articles from expert groups reviewing the recommended protein levels for older adults. These suggest that protein intake may need to increase with age (specifically over 65 years) to 1.0–1.2 g/kg body weight per d<sup>(18,19)</sup>. These levels are not universally accepted and there remains significant debate around this issue<sup>(20)</sup>. Therefore, it is vital that trials testing protein supplementation need to enable subjects to at least meet these newly proposed levels, rather than just providing a fixed amount in the hope protein intake increases appropriately.

**Table 1.** Details of protein supplementation trials to treat sarcopenia

| Reference (population)                                                                   | Intervention (duration)                                                                | Total supplement/d                         | No./type of supplements                                                     | Volume/ supplements | Energy/ supplements                                         | Protein /supplements                           | Extra nutrients?                                       | Masking                                                                         | Placebo                                                                                                              | Adherence                                  | Dietary intake                                                                                                                                                     | Main results                                                                                                                 |
|------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|--------------------------------------------|-----------------------------------------------------------------------------|---------------------|-------------------------------------------------------------|------------------------------------------------|--------------------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|--------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|
| Bonnefoy <i>et al.</i> <sup>(11)</sup><br>(residents of retirement homes)                | (a) Ex + Supp<br>(b) Con + Supp<br>(c) Ex + Placebo<br>(d) Con + Placebo<br>(9 months) | 30 g protein<br>1686 kJ<br>(400 kcal)      | Two drinks<br>10:00 and<br>16:00                                            | 200 ml              | 843 kJ (200 kcal)                                           | 15 g (30 % of energy); g/kg bw per d not given | Yes – range of vits and mins providing 25 % RDA /supp  | Attempted same opaque packaging but products likely to taste and look different | Yes<br>No protein, energy, vits or mins.<br>Presumably was artificially sweetened and flavoured water but not stated | 54 % at 9 months counting unused units     | No measure made but BMI increased in the supp group and supp designed to increase energy intake by approximately 20 %                                              | Supp significantly increased MP at 3 months v. Con ( $P = 0.03$ ), but not at 9 months. Supp did not improve MM or PP v. Con |
| Bunout <i>et al.</i> <sup>(12)</sup> (free-living elderly attending out-patient clinics) | (a) Ex + Supp<br>(b) Supp<br>(c) Ex<br>(d) Con<br>(18 months)                          | 13 g protein<br>1746 kJ<br>(418 kcal)      | Two prepared as soup or porridge (probably powdered product but not stated) | 50 g                | 873 kJ (209 kcal) (excluding soup or porridge contribution) | 6.5 g; g/kg bw per d not given                 | Yes – range of vits and mins providing 25 % RDAs /supp | Not applicable                                                                  | No<br>Supp provision not randomised                                                                                  | 44–49 %<br>Self-reported                   | No assessment of dietary intake and no change in body weight or composition                                                                                        | Supp alone had no effect on MM, MS or PP. Supp did not show an additive effect over Ex outcome                               |
| Chale <i>et al.</i> <sup>(13)</sup> (free-living elderly)                                | (a) Supp + Ex<br>(b) Placebo + Ex<br>(6 months)                                        | 40 g whey protein<br>1582 kJ<br>(378 kcal) | Two powder sachets am and pm. If doing Ex taken after the session           | Not stated          | 791 kJ (189 kcal)                                           | 20 g; g/kg bw per d not given                  | No                                                     | Numerically coded packages                                                      | Yes<br>Iso-energetic 45 g maltodextrin 1 g fat; 791 kJ (189 kcal) PABA for compliance checking                       | 67 % Urinary PABA 72 % returned packages   | 3 d diet diary × 2<br>Protein intake greater in supp group and energy intake the same as baseline. Showed voluntary energy intake decreased but supp maintained it | Supp + Ex did not improve MM, MS or PP significantly v. Con + Ex                                                             |
| Tieland <i>et al.</i> <sup>(14)</sup> (Free living frail or pre-frail elderly)*          | (a) Supp + Ex<br>(b) Placebo + Ex<br>(6 months)                                        | 30 g protein                               | Two drinks after breakfast and lunch                                        | 250 ml              | Not stated                                                  | 15 g; 1.3 g/kg bw per d                        | 0.4 g Ca                                               | All drinks flavoured vanilla opaque packaging                                   | Yes<br>No protein same lactose and Ca                                                                                | 98 % Ticked calendars and returned cartons | 3 d diet diary × 3<br>No change in energy intake, protein increased in supp group                                                                                  | Supp + Ex significantly improved MM ( $P = 0.006$ ), but not MS or PP v. Placebo + Ex                                        |
| Tieland <i>et al.</i> <sup>(15)</sup> (free-living frail or prefrail elderly)*           | (a) Supp<br>(b) Placebo<br>(6 months)                                                  | 30 g protein                               | Two drinks after breakfast and lunch                                        | 250 ml              | Not stated                                                  | 15 g; 1.4 g/kg bw per d                        | 0.4 g Ca                                               | All drinks flavoured vanilla opaque packaging                                   | Yes<br>No protein same lactose and Ca                                                                                | 92 % Ticked calendars and returned cartons | 3 d diet diary × 3<br>Energy intake decreased at 24 months, protein increased in supp group                                                                        | PP improved significantly with supp ( $P = 0.02$ ), but not MM or MS v. Placebo                                              |

Ex, exercise component; con, control; supp, supplement; bw, body weight; PABA, para-aminobenzoic acid; MM, muscle mass; MP, muscle power; MS, muscle strength; PP, physical performance; vits, vitamins; mins, minerals.

\* Registered as two separate two arm studies.

Only two studies assessed this<sup>(14,15)</sup> and showed protein intake increased up to 1.3–1.4 g/kg body weight per d, but provided inconsistent results in terms of protein supplementation as mentioned previously.

Adherence is another critical factor in nutritional interventions. It is well known that nutritional supplements or changes to dietary intake are notoriously difficult for subjects to follow consistently<sup>(21)</sup>. All studies in this group reported adherence, but three had relatively low rates<sup>(11–13)</sup>, suggesting that a lack of adherence could be the reason for a lack of effect.

Producing a placebo supplement that enables single or double blinding to limit bias is also extremely difficult, particularly if a liquid supplement drink is employed. Some studies have attempted this; one aiming for an energy and protein-free placebo<sup>(11)</sup> and another aiming for an iso-energetic but protein-free placebo<sup>(13)</sup>. Both these placebos are likely to taste and feel very different to the supplement, resulting in unsuccessful masking, at least to the individuals taking it. Other studies either did not attempt to provide a placebo<sup>(12)</sup> or provided limited information regarding energy content<sup>(14,15)</sup>. None of the researchers have attempted to test whether masking was successful, but have simply obscured the liquids from view to avoid comparison or visual assessment of the drink. Chale *et al.* do not provide details of how the supplement powder is consumed; whether added to solid food or mixed with liquid to produce a drink. In all cases there is a significant risk that the placebo drink or powder will be readily identifiable to those consuming it, thus allowing potential bias to influence results.

Baseline nutrient status is similarly critical to assess. People who are deficient in any particular nutrient may respond differently to the given supplement. Vitamin D status provides a good example of how a deficient status may influence the effect of a protein and energy supplement. This is discussed later in the  $\beta$ -hydroxyl  $\beta$ -methylbutyrate (HMB) section.

Finally, recruitment in all the published trials appears to have been difficult. Recruitment rates ranged from 4 to 9 % of the initially screened population. One author comments ‘The greatest difficulty is to motivate elderly individuals to volunteer for such a study or to perform any effort to improve their health’<sup>(11)</sup>. This may introduce bias since it may be that only particularly health conscious individuals will participate in the studies and these people are not representative of older adult populations. It also illustrates the challenges faced by researchers in planning and funding such studies. They are likely to be resource intensive and expensive, and it may be necessary to conduct extensive preliminary work to establish interventions that are more attractive and acceptable to older adults.

In summary the trials testing whole-protein supplementation failed to show a consistent effect on muscle mass, strength or function. This can be explained by the variations in study design, composition of the protein supplement and the failure to monitor voluntary food intake, adherence and baseline nutritional status. Further large-scale clinical studies are warranted but will require significant funding and resources.

## Essential amino-acid trials

The rationale for supplementing with EAA is that they are an anabolic stimulus for muscle synthesis<sup>(9)</sup>, in particular leucine. However, there is no agreement on what mixture of EAA may provide the best stimulus. There are two studies which have used such a mixture (see Table 2), providing very limited evidence that there may be some effect on muscle mass and function through amino-acid supplementation<sup>(22,23)</sup>. The two studies used very different EAA mixtures with the main differences in threonine, phenylalanine and leucine, yet neither provided a clear rationale for the mixture in use. Furthermore, the method of delivery differed with one study providing capsules (20/d)<sup>(22)</sup> and other asking subjects to mix a powder with water or milk<sup>(23)</sup>. This is particularly problematic since using milk as an optional delivery vehicle for the EAA means that some subjects will also receive a supplement of energy, protein (including EAA) and other nutrients contained in the milk. Many amino acids have a bitter taste and it remains unclear how palatable this oral supplement was or whether other flavours or sweeteners were used within the powder to make its taste acceptable.

As with the whole-protein trials, these studies also suffer from a lack of adherence information, no assessment of baseline nutritional status and no assessment of voluntary food intake. They are also very different designs; two arm compared with four arm study, and with and without placebo.

Both these studies found an effect of the supplements; one on muscle mass only<sup>(22)</sup> and the other on mass, function and strength, but only in combination with exercise<sup>(23)</sup>. The EAA supplement alone did not result in significant change<sup>(23)</sup>. Because of the lack of rationale and different EAA supplements used, and the various design problems in both studies it is difficult to interpret whether further studies into the use of EAA are warranted. It may be that using HMB, a downstream metabolite of leucine, is a better approach, and there are five studies exploring this option.

## $\beta$ -Hydroxy $\beta$ -methylbutyrate trials

HMB has been shown to reduce protein degradation, up-regulate protein synthesis and increase muscle cell cholesterol production, leading to more stable cell membranes<sup>(24,25)</sup>. It is a downstream metabolite of leucine, with approximately 5–10 % ingested leucine being converted to HMB<sup>(26)</sup>. This means that in order to meet the dose of HMB generally given in trials (3 g/d)<sup>(27,28)</sup>, 60 g leucine/d would have to be consumed. To obtain this quantity of leucine through the diet would require an individual to eat impractically large amounts (>600 g) of high-quality protein sources (eggs, dairy and meat) daily<sup>(29)</sup>. The theory is that if only 10 % leucine is converted to HMB and HMB is what makes leucine a stimulator of muscle synthesis, a far larger effect could be elicited if the rate-limiting conversion is bypassed. A number of human studies have tested the theory of



**Table 2.** Details of amino-acid trials to treat sarcopenia

| Reference (population)                       | Intervention (duration) | Presentation                                 | Essential amino acid content/d g (%) |             |             |             |            |             |             |            |             |                        | Extra nutrients? | Placebo            | Adherence                                       | Dietary intake                  | Main results for treated group            |
|----------------------------------------------|-------------------------|----------------------------------------------|--------------------------------------|-------------|-------------|-------------|------------|-------------|-------------|------------|-------------|------------------------|------------------|--------------------|-------------------------------------------------|---------------------------------|-------------------------------------------|
|                                              |                         |                                              | His                                  | Ile         | Leu         | Lys         | Met        | Phe         | Thr         | Trp        | Val         |                        |                  |                    |                                                 |                                 |                                           |
| Dillon <i>et al.</i> (22)<br>(Healthy women) | (a) 15 g EAA            | Twenty capsules twice daily (forty in total) | 1.64 (10.9)                          | 1.56 (10.4) | 2.78 (18.6) | 2.34 (15.5) | 0.46 (3.1) | 2.34 (15.5) | 2.2 (14.7)  | 0 (0)      | 1.72 (11.5) | No                     | Yes              | Content not stated | Checked through empty capsules but not reported | Not assessed                    | Improved muscle mass and synthesis rate   |
|                                              | (b) Placebo (3 months)  |                                              |                                      |             |             |             |            |             |             |            |             |                        |                  |                    |                                                 |                                 |                                           |
| Kim <i>et al.</i> (23)<br>(Sarcopenic women) | (a) 6 g EAA + Ex        | 3 g powder twice daily                       | Not stated                           | 0.63 (10.5) | 2.52 (42)   | 0.84 (14)   | Not stated | 0.42 (7)    | 0.63 (10.5) | Not stated | 0.63 (10.5) | Added to water or milk | No               | No                 | Checked through record sheets but not reported  | Not assessed but BMI maintained | Improved muscle mass and strength with Ex |
|                                              | (b) Ex                  |                                              |                                      |             |             |             |            |             |             |            |             |                        |                  |                    |                                                 |                                 |                                           |
|                                              | (c) 6 g EAA             |                                              |                                      |             |             |             |            |             |             |            |             |                        |                  |                    |                                                 |                                 |                                           |
|                                              | (d) Con (3 months)      |                                              |                                      |             |             |             |            |             |             |            |             |                        |                  |                    |                                                 |                                 |                                           |

EAA, essential amino acids; Ex, exercise component; Con, control (health education sessions); His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Trp, tryptophan; Val, valine.

supplementing HMB rather than leucine,<sup>(29,30)</sup> but only five examine outcomes relevant to sarcopenia<sup>(31–35)</sup> (see Table 3).

There is some interesting work in ageing rats which supports the hypothesis that HMB modulates negative age-related changes in body composition and function<sup>(36)</sup>. This group supplemented young (44 weeks) and old (86 weeks) rats with Ca-HMB for 16 weeks to produce middle-aged (60 weeks) and very old (102 weeks) rats, which were compared with baseline, middle-aged control and very old control data. The results showed a leaner, stronger body phenotype for those rats supplemented with HMB at both middle and very old age. Animal data are particularly useful in modelling ageing since longitudinal human studies are so expensive, difficult and time-consuming. Nevertheless, human intervention trials to demonstrate efficacy of HMB supplementation are required.

The results of the five studies examining sarcopenia relevant outcomes are more consistent than either EAA or whole protein, with three studies showing an effect on muscle mass<sup>(31,33,35)</sup> and the other two studies showing an effect for strength<sup>(32,34)</sup>. The details of the studies also show a more consistent approach. All included free-living older adults with exclusions for major illness, although two studies recruited from assisted living and care facilities<sup>(32,35)</sup>. All provided 2–3 g HMB/d, although two studies included additional amino acids, arginine and lysine, and vitamin C<sup>(32,35)</sup>. The rationale for this addition was that lysine is one of the three indispensable amino acids<sup>(37)</sup> and its requirement is potentially higher than previously thought; thus it was added to avoid an inadequate supply<sup>(38)</sup>. Both lysine and arginine, such as HMB, are also thought to be stimulants of muscle protein synthesis<sup>(9,39)</sup>. The format of the supplement however varied between studies; either as capsules or a powder, and the powder either mixed with water or other liquid. All studies used a placebo, which appeared adequately masked, although testing this was not done. Only one study used an iso-energetic and iso-nitrogenous placebo<sup>(35)</sup>; the others all using what appear to be iso-energetic placebos, although this is not clearly stated in the papers. Adherence was generally checked through measurement of urinary HMB, with expected increases in urinary excretion to demonstrate adherence, and in some studies this was backed up with logs or returned unused capsules. Only one study rigorously controlled voluntary dietary intake, including the protein intake to 0.8 g/kg body weight per d<sup>(33)</sup>, but this study looked specifically at prevention of muscle loss during bed rest, rather than muscle gain in free-living populations. A second study monitored dietary intake with 3 d diet diaries<sup>(34)</sup>.

The importance of assessing baseline nutritional status is effectively illustrated in a re-analysis of the Baier *et al.* study<sup>(35)</sup>. The symptoms of vitamin D deficiency are known to include muscle pain and weakness<sup>(40)</sup>. There is some evidence that there is a vitamin D receptor on muscle<sup>(41)</sup>, and observational studies indicate a positive association between vitamin D and muscle function<sup>(42)</sup>. A recent systematic review confirmed that vitamin D supplementation can improve muscle

**Table 3.** Details of  $\beta$ -hydroxyl  $\beta$ -methylbutyrate trials to treat sarcopenia

| Reference (population)                                                           | Intervention (duration)                                               | Dose                                           | HMB supplement                                                                                                               | Placebo                                                                                                                                                  | Adherence                                                                                                                           | Dietary intake                                                                                                           | Main result                                                                 |
|----------------------------------------------------------------------------------|-----------------------------------------------------------------------|------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Vukovich <i>et al.</i> <sup>(31)</sup><br>(free-living older adults)             | (a) HMB and Ex<br>(b) Placebo + Ex (2 months)                         | Twelve capsules/d (four caps $\times$ 3/d)     | 3 g/d One capsule: 250 mg Ca-HMB<br>50 mg potassium phosphate                                                                | Yes<br>Rice flour                                                                                                                                        | Returned caps every 10 d<br>Elevated plasma HMB during study period 1.69 v. 67.73 $\mu$ mol/l                                       | Not controlled but weight stable                                                                                         | Muscle mass improved for HMB group                                          |
| Flakoll <i>et al.</i> <sup>(32)</sup><br>(free- and assisted-living older women) | (a) HMB, Arg + Lys<br>(b) Placebo (No Ex) (3 months)                  | One 8 oz drink taken at breakfast              | 2 g/d Orange drink containing:<br>2 g Ca-HMB;<br>5 g Arg; 1.5 g Lys HCl; 0.5 g ascorbic acid                                 | Yes<br>Same vol, same flavour, iso-energetic.<br>Maltodextrin; 0.5 g ascorbic acid $\pm$ 1.8 g non-EAA protein                                           | None reported                                                                                                                       | Not monitored but weight stable                                                                                          | Performance and muscle strength improved for HMB group, but not muscle mass |
| Baier <i>et al.</i> <sup>(35)</sup><br>(free- and assisted-living older women)   | (a) HMB, Arg + Lys<br>(b) Placebo (No Ex) (12 months)                 | One sachet/d orange flavoured mixed with water | 2 or 3 g/d depending on body weight >or <68 kg<br>2/3 g Ca-HMB;<br>5/7.5 g L-Arg;<br>1.5/2.25 g Lys HCl; 0.1 g ascorbic acid | Yes<br>Iso-nitrogenous/energetic. 11.8 g non-essential amino acids (alanine 5.6 g, glutamic acid 0.9 g, glycine 3.1 g, serine 2.2 g) 0.1 g ascorbic acid | Monthly questionnaires and daily supplementation records<br>Approximately 95 % over 12-month.<br>Urinary HMB increased in HMB group | Not monitored but weight stable or increased                                                                             | Muscle mass improved for HMB group but no changes for strength or function  |
| Deutz <i>et al.</i> <sup>(33)</sup><br>(healthy older adults)                    | (a) HMB<br>(b) Placebo (No Ex) (10 d)                                 | Two sachets/d mixed with water                 | 3 g/d One sachet contained:<br>1.5 g Ca-HMB; 4 g maltodextrin;<br>200 mg Ca; additional sweetener flavouring agents          | Yes<br>One sachet contained: 4 g maltodextrin;<br>200 mg Ca; additional sweetener flavouring agents                                                      | Urinary HMB increased in HMB group                                                                                                  | Metabolically controlled 0.8 g/kg per d protein. Harris-Benedict for energy with different activity factors for bed rest | Preserved muscle mass during bed rest for HMB group                         |
| Stout <i>et al.</i> <sup>(34)</sup><br>(free-living older adults)                | (a) Placebo<br>(b) HMB<br>(c) Placebo + Ex<br>(d) HMB + Ex (6 months) | Two sachets/d mixed with water, milk or juice  | 3 g/d one sachet contained:<br>1.5 g Ca-HMB;<br>4 g carbohydrate                                                             | Yes<br>200 mg Ca; 4 g carbohydrate                                                                                                                       | Urinary HMB increased during study, Plus logs                                                                                       | Dietary recall $\times$ 3 d $\times$ 3                                                                                   | Improved muscle strength for HMB group                                      |

Ex, exercise component to the intervention; EAA, essential amino acid; HMB,  $\beta$ -hydroxyl  $\beta$ -methylbutyrate; Arg, arginine; Lys, lysine.

strength, in particular in those most deficient and those whose levels increase most<sup>(40)</sup>. Thus, it is possible that vitamin D status will influence the response to tests of muscle strength and performance. Fuller *et al.* re-analysed data to account for baseline vitamin D status<sup>(43)</sup>. They found that subjects who had a low vitamin D status (<30 ng 25OH-vitaminD<sub>3</sub>/ml serum) did not respond to the HMB/Arginine/Lysine supplement to the same extent as the higher status subjects ( $\geq$ 30 ng/ml). Lean mass was increased in both vitamin D groups but muscle strength was only increased in the high vitamin D group, suggesting a synergistic effect of vitamin D and the supplement. Clearly, in future

studies it will be critical to assess vitamin D status at baseline and supplement those who are insufficient.

There is one other study in bed-ridden older adults receiving artificial tube feeding for 6 months or more<sup>(44)</sup>, which also shows an effect for HMB on nitrogen excretion and body weight and body circumference. The results are suggestive of a positive effect of HMB on muscle mass but this was not measured, neither were measures of strength or function.

Overall, these data are suggestive of a beneficial effect of HMB on older adults, but larger well-controlled studies are required that measure outcomes relevant to sarcopenia, ideally in sarcopenic populations.

### Discussion of the evidence

The available evidence for protein does not allow us to make anything more than quite tentative suppositions. The most promising avenue does appear to be HMB supplementation but in the context of an otherwise adequate diet. However, the use of EAA is far from well-investigated and further work may offer other treatment options. It may also be that subjects with clearly defined sarcopenia respond differently to a generally healthy older population.

One other related issue is the timing and distribution of the dietary protein. Some researchers have suggested that there is a ceiling effect on the amount of protein used for synthesis at a given meal, and suggested an optimum protein intake of 20–30 g per meal<sup>(45,46)</sup>. Others have shown that feeding protein spread evenly throughout meals gives greater fractional synthesis rate compared with a skewed distribution<sup>(47)</sup>, and that in free-living older subjects protein intake can be skewed towards a higher lunch intake, with less at breakfast and other meals<sup>(48)</sup>. However, other investigators refute the view that there is a maximal effect of protein at a meal, proposing a large bolus of protein should be as useful as providing the same amount spread over the day<sup>(49)</sup>. Others have provided evidence that pulse feeding protein (72–80 % provided at lunch) increases whole-body protein retention, lean muscle index (lean soft-tissue mass/height m<sup>2</sup>) and appendicular skeletal muscle mass index (sum of lean soft-tissue in the four limbs/height m<sup>2</sup>) compared with evenly spread protein<sup>(50,51)</sup>. Further research is evidently needed to better understand how protein distribution can affect optimum health in older people.

There is also the issue of increased splanchnic amino acid sequestration. The splanchnic bed includes the liver, stomach, intestines, pancreas and spleen (or portal-drained viscera). Because the portal-drained viscera receive nutrients from dietary digestion first, their requirements are satisfied first, potentially limiting the availability of nutrients, including amino acids, to peripheral tissues<sup>(52)</sup>. There is some evidence that splanchnic amino-acid sequestration is increased with age<sup>(53)</sup>, and this could potentially complicate the supplementation of EAA to stimulate muscle protein synthesis and protein distribution throughout the day.

Protein may be the most obvious target for a treatment to prevent muscle loss but there are several other dietary strategies that have been explored, but require further investigation, such as vitamin D, alcohol, dietary acid–base load, fatty acids and antioxidants. These have all been recently reviewed by Welch<sup>(7)</sup>.

### Recommendations for the design of future trials

Cruz-Jentoft *et al.* make a series of recommendations in their review paper for the design of future nutritional studies. These include: clear well-defined populations, four arm studies nutrition with or without exercise, standardised outcome measures, attention to the timing of the nutritional supplement particularly in respect of exercise,

and consideration of the baseline nutritional status and frailty of the study population<sup>(10)</sup>.

In addition to these recommendations I would like to add that adherence to the nutritional intervention must be assessed as well as its effect on voluntary food intake, and if protein or amino acids are supplemented they are considered in the context of the subject's or group's present intake, with efforts made to improve poor intakes, as well as including an additional supplement. This of course is far more complex and is not as appealing as a simple daily drink, bar or capsule, yet if we are to successfully address the problem of sarcopenia and improve the health of the older population, it may be that a whole diet approach is required.

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### Authorship

The author was solely responsible for all aspects of preparation of this paper.

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