Type and timing of protein feeding to optimize anabolism

Laurent Mosoni and Philippe Patureau Mirand

Purpose of review
The delivery rate of amino acids to an organism significantly affects protein anabolism. The rate can be controlled by the type and the timing of feeding. Our aim was to bring new insights to the way they may act.

Recent findings
During young and adult ages, when food supply is liberal, subjects can adapt to various modes of protein feeding. However, during food restriction, protein anabolism is favored when the delivery of amino acids is evenly distributed over the day, either with frequent meals, or through the use of slowly absorbed proteins like casein. In contrast, during aging, quickly absorbed protein sources become more efficient. During recovery after exercise, the timing of protein feeding after the end of exercise may or may not influence its anabolic effect, depending on the subject's age and the type of exercise.

Summary
The synchronization of variations in anabolic capability with amino acid supply partly explains the effects of the type and timing of protein feeding. This effect is modulated by the amount of amino acids required to increase whole-body proteins and by the signaling properties of some amino acids to stimulate protein synthesis. Indeed, the anabolic effect of amino acids is determined by their interaction with other anabolic factors (other nutrients or physiological factors, whose efficiency is mainly related to their effect on protein degradation). It is clear that benefits can be obtained from adapted protein feeding patterns.

Keywords
timing, protein feeding pattern, absorption rate, aging, exercise

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Protein Metabolism and Nutrition Unit, Inra, Theix, France

Correspondence to Laurent Mosoni, Protein Metabolism and Nutrition Unit, Inra, 63122 Theix, France
Tel: +33 473 62 48 27; fax: +33 473 62 47 55; e-mail: mosoni@clermont.inra.fr

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Introduction

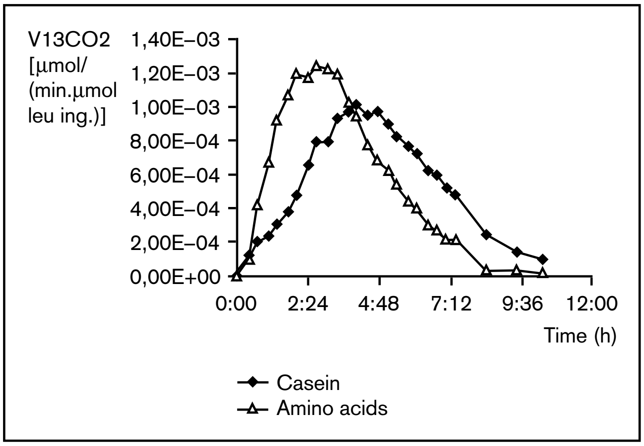
A recent review [1••] clearly demonstrates, 'the effectiveness of protein or amino acid intake [on protein anabolism] depends not only on the form in which the substrate is ingested but also on the pattern of ingestion and the interaction with other factors, such as exercise and nonprotein caloric intake.' The aim of the present review is to emphasize the importance of the chronological aspects of protein feeding in the control of protein anabolism. These aspects include two components: (1) the delivery rate of dietary amino acids to the metabolic pools after a protein meal; (2) the timing of protein feeding. Both have proved to be important because they participate in the adjustment between the amino acid supply and the time-related variations of metabolic demand driven by protein anabolic capabilities.

To understand, therefore, how protein anabolism is regulated by the type and the timing of protein feeding, it is necessary to take into account the factors which control amino acid entry rates into the metabolic pools. It is also necessary to consider the capacity of the organism to control amino acid homeostasis, in particular the mechanisms involving protein synthesis and degradation.

Molecular form of protein intake

The entry rate of amino acids, and the direct or indirect stimulation of protein metabolism by amino acids, depend on the molecular form of protein intake. Generally, the entry rate is the lowest with intact proteins and the highest with highly hydrolyzed proteins rich in short peptides; with amino acid mixtures, it is similar or slightly lower than with the highly hydrolyzed proteins [2]. However, there may be noticeable differences between native proteins, as shown by the well-documented differences between casein and whey proteins, which are respectively slowly and quickly digested. An example of how the molecular form of protein intake can affect the entry rate of amino acids is given in Figure 1. The anabolic effects of these nitrogen sources depend on their molecular absorption rate and also on other dietary factors. In growing animals fed restricted amounts of food [3–5], which limits potential anabolism, whole body protein deposition was higher with diets containing intact proteins than with amino acid mixtures [3–5]. This indicates that a massive flow of amino acids in conditions that are not favorable for protein synthesis stimulation is less effective than a reduced flow over a longer time period because oxidative catabolism is increased. In turn, when protein (or amino

Figure 1. Comparison of the evolution of ingested leucine oxidation rate after ingestion of casein or the corresponding amino acid mixture



Intrinsically ^{13}C leucine labelled casein (a gift from Y. Boirie) or the corresponding amino acid mixture (enriched with free ^{13}C leucine) was ingested by young healthy persons (n = 8 with each diet) during a mixed meal. VCO_2 and the enrichment of expired CO_2 was measured during 10h after meal ingestion. The ratio of the rate of $^{13}CO_2$ release to the amount of ingested ^{13}C leucine was plotted against time for each group. These data were never published before.

acid) intake was reduced, even if protein anabolism was limited, there was no longer any difference between the two molecular forms [3,4]. As would be anticipated, when protein anabolism potential was preserved and when animals were generously fed, there was no longer any difference between intact protein or hydrolyzed proteins or amino acid mixtures [6–8].

Studies in humans confirm that the anabolic effect of protein intake results from the synchronization of amino acid entry and the ability to maintain homeostasis via stimulation of protein anabolism. Indeed, in individuals whose protein anabolism capabilities were reduced because no non-protein energy was supplied, the massive entry of large amounts of amino acids resulting from a meal of ‘‘fast’’ (rapidly absorbed) proteins (whey protein or amino acid mixture) induced a large increase in free amino acid levels. This resulted in a marked increase of amino acid catabolism and in a temporary stimulation of whole body protein synthesis. By contrast, when amino acids were supplied in a more continuous way, either as casein or as frequent small meals, amino acid catabolism was not stimulated because the increase in free amino acid levels was moderate. Furthermore, there was lower whole body protein degradation [9,10] and higher retention of dietary amino acids in splanchnic tissues than with fast proteins [10]. This explains why leucine balance, which is an indicator of protein anabolism, was higher with slowly absorbed proteins than with fast proteins [9,10]. These experiments also suggest that a decrease of whole-body protein degradation in the post-prandial state requires a prolonged increase (even moderate) of blood free amino acid levels. This was the case when subjects were fed casein or frequent small meals [10]. The main target in this situation appears to be in the splanchnic area for both protein degradation and for protein synthesis. In humans, it is likely that the inhibition of whole body protein degradation occurs in splanchnic organs, as an amino acid infusion that resulted in a decrease in whole body protein breakdown did not affect leg muscle protein breakdown [11]. The higher rates of leucine incorporation into liver and plasma proteins in rats fed casein than in rats fed the corresponding amino acid mixture [5] demonstrated that casein feeding was more efficient at stimulating liver protein synthesis than a fast amino acid source.

Feeding non-protein energy simultaneously with protein induced a smaller difference between slow and fast proteins, either because digestion rates differed less or because additional energy reduced differences in amino acid catabolism between the two groups [12••].

Higher post-prandial leucine balances were also described when casein feeding was compared with casein hydrolysate (oligopeptides) [13] or with the corresponding amino acid mixture [14]. This appears to result from a lower leucine oxidation induced by lower plasma free leucine concentrations with casein than with the other two protein sources. However, this mechanism does not explain the discrepancies concerning protein synthesis and degradation rates between these two similar experiments (comparison of casein with quickly absorbed amino acid sources in a steady state, with non-protein energy supply). Both protein synthesis and degradation were higher in the casein fed volunteers than in the volunteers fed the other diet in the Metges study [14]. The opposite was true in the Collin-Vidal study [13]. Besides the difference between the amino acid mixture and the casein hydrolysate, the most likely explanation for this discrepancy is that the oral tracer was different: it was intrinsically labeled casein in the Metges study whereas it was free leucine in the Collin-Vidal study. Assuming that casein is digested more slowly than was supposed by authors of both studies, calculations show that whole-body protein synthesis and degradation rates could be overestimated in the Metges study due to an overestimation of 13C-leucine appearance flux. Similarly, whole-body protein degradation could be underestimated in the Collin-Vidal study due to an overestimation of dietary leucine input. Nevertheless, whatever the reason for leucine balance being higher with casein than with fast absorbed amino acid sources, the results of these experiments suggest that protein anabolism depends on the absorption rate even when energy is simultaneously fed. In both experiments, however, the effects of absorption rates were measured in a steady state and thus with a continuous feeding pattern. This method is questionable for a study of the effects of fast amino acid sources and depends on the reliability of the estimates of the tracer and dietary amino acid entry rate.

In conclusion, it appears that dietary proteins that are slowly absorbed promote overall protein deposition by reducing protein degradation, whether they are fed without or simultaneously with non-protein energy but, in the case that energy is also provided, quickly absorbed proteins can also be efficient.

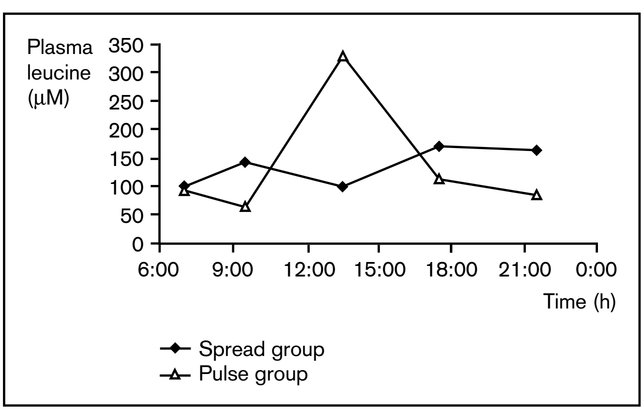
Influence of the timing of protein feeding

The timing of protein feeding determines whether protein intake is concentrated over a few meals or spread over frequent meals. This controls the entry rate of amino acids into the metabolic pools as illustrated in Figure 2. Timing also operates via synchronization between feeding and protein anabolism capacity at that time.

Meal pattern

The amount of protein consumed at each meal is the main factor which determines the tendencies of post-prandial protein metabolism. When daily protein intake is equal or higher than the recommended allowances and concentrated over one or two meals, it has the effects described earlier when amino acid entry rate is high. When it is spread over frequent meals, it has the effects described earlier with slowly digested proteins, at least for the whole body amino acid fluxes, as shown by Boirie's team [10]. In these conditions with moderate increase of free amino acid levels, net protein deposition is promoted by a slight increase of protein synthesis and a marked inhibition of protein degradation. It is possible that the stimulation of protein synthesis does not occur when the amounts of protein fed with small meals are low. In the early post-absorptive state, free amino acid levels decrease with time when the last meal becomes more and more distant; but then they tend to increase again during the late post-absorptive period [15,16]. Thus, in view of the results obtained with casein, daily protein intake spread over three meals or more should be more effective at stimulating protein anabolism than a one or two-meal pattern. This conclusion is supported by several experiments in non-restricted growing animals: pigs [17,18], rats [19] or trout [20]. By contrast, daily gains in body weight, lean body mass or whole body proteins did not differ with one meal compared with several meals, in restricted animals [21,22].

Figure 2. Plasma leucine level is affected by protein feeding pattern



Healthy old persons were fed during 15 days either with a spread protein feeding pattern (n = 9) during which proteins were given in equal proportion at each of the four meals of the day (at 8:00, 12:00, 16:00 and 20:00 h), or with a pulse protein feeding pattern (n = 10) during which 80% of daily proteins were consumed for lunch. The same amount of protein was consumed per day in each group. Leucine concentrations (μmoles/L) were measured in plasma and mean values were plotted against time. These data were never published before.

Similar results were obtained in adult humans. There was no difference in nitrogen balance between a two-protein-meals and a three-protein-meals pattern when protein intake was low (0.5 g/kg per day) [23]. By contrast, three protein meals allowed a better nitrogen balance than two meals for a protein intake between 0.87 and 1.2 g/kg per day [24]. There was a tendency for a higher nitrogen balance in young women fed four protein-containing meals a day compared with young women fed three meals a day, with lunch providing 80% of daily protein intake [25]. However, for adequate intakes of energy and protein, there was no difference in nitrogen balance between young women fed three or six meals per day [26]. In such conditions, it is not surprising that three meals distributed over 14 h induced a higher leucine balance than a single feeding period consisting of hourly meals which lasted 10 h [15,16]. The hourly meal-feeding pattern promoted protein anabolism only by reducing protein degradation, and was associated with higher leucine degradation during the post-absorptive period.

In summary, the results obtained in adult participants showed that the protein feeding pattern had either no effect or that spreading protein intake was favorable.

Timing of the protein meals

The consequences of a protein meal depend on the time it occurs [16,27]. In rats with free access to non-protein energy, feeding the protein meal during the dark period (feeding period) was more efficient than during the light period (rest period). That 80% of the non-protein energy was consumed during the dark period indicates the benefit of simultaneous energy supply to promote protein anabolism [27], as demonstrated in humans [28]. Other factors may also be involved. A higher gastric emptying rate has been shown to occur during the dark period in rats and it resulted in higher protein synthesis rates in small intestine and liver than during the light period [29]. In humans also, the response to identical meals has already been shown to differ depending on the time. The highest levels of protein degradation and of the two resulting parameters – free amino acids and their oxidative catabolism – were observed after the meal that followed the longest post-absorptive period. At that time, insulinemia was lower than after the following meal [16]. These results indicate that it would be necessary to adapt meal composition to the time at which it occurs.

Several studies analyzed the timing of protein or amino acid feeding in relation with recovery after exercise [1••]. In young persons, a drink made of a mixture of essential amino acids taken 2 or 3 h after the end of a 40–45 min resistance exercise bout was shown to have the same effect on leg protein anabolism as the same drink taken 1 h after the exercise [30,31•]. However, in elderly people, it was only when the protein–carbohydrate–fat supplement was given immediately after resistance exercise (as opposed to 2 h after the end of exercise) that an increase in muscle cross-sectional and fiber area was obtained after 12 weeks [32]. This may be a consequence of the age-related decrease of the response to feeding of muscle protein anabolism [33,34] and suggests that the maximal response would persist only immediately after the end of exercise. In the case of endurance training, this effect seems also to occur immediately after the end of exercise, even in young subjects [35•].

Timing of feeding and aging

The consequences of the time of feeding on protein anabolism depend on the physiological state, as exemplified by aging. In old individuals, a marked increase in plasma free amino acids is necessary to promote stimulation of muscle protein synthesis [36,37]. This stimulation appears to be mediated through the variations of plasma free leucine levels [38••]. A marked increase in blood free amino acids may result from digestion of fast proteins or of a high protein meal. Both were proved to be effective at promoting protein anabolism. Preliminary results indicate that whey protein feeding induced a higher post-prandial leucine balance in the elderly than casein feeding [12••], which is the opposite of what was described in young adults [9].

Furthermore, it was shown that nitrogen balance and fat free mass gain were significantly higher in elderly women fed three meals per day, with lunch providing 80% of daily protein intake (pulse pattern) than in women fed four protein-containing meals (spread pattern) per day [39]. This result was significantly different from results obtained in young women in the same experiment [25]. It was related to a stimulation of the 24-h whole-body protein synthesis rate in old women fed by the pulse pattern compared with old women fed by the spread pattern. By contrast in young women, 24-h whole-body protein synthesis was unchanged by protein feeding pattern, but 24-h whole-body protein degradation was higher with the pulse pattern [25]. However, in the post-absorptive state, there was no age difference in the response of leucine flux to protein feeding pattern: the pulse pattern induced a lower rate of whole-body leucine oxidation and a lower rate of whole body protein degradation, with no difference regarding protein synthesis [40]. Thus, it is likely that the higher 24-h whole-body protein synthesis rates observed in the pulse pattern fed old women were related to a higher stimulation of protein synthesis rates during the post-prandial state. Similarly, the higher 24-h protein degradation rates observed in pulse pattern fed young women should be related to a lower inhibition of protein degradation in the fed state, as shown when fast proteins were compared with slow proteins.

The effects of these protein feeding patterns (pulse or spread) were also studied at the tissue level in rats [41•]. The pulse pattern restored normal stimulation of post-prandial muscle protein synthesis in old rats and, whatever the age, induced stimulation of liver protein synthesis rates in the post-prandial state.

In summary, the pulse pattern results in a higher nitrogen balance in the elderly because of a lower leucine oxidation and whole-body protein degradation during the post-absorptive state, and due to higher stimulation of protein synthesis in whole-body, liver and muscle during the fed state. In young subjects, the differences in protein anabolism were minor between the spread and the pulse patterns because the response of muscle protein synthesis to feeding was similar whatever the feeding pattern.

Conclusion

Amino acid availability is a key factor for protein anabolism due to its effects on extracellular free amino acid levels. However, other factors have to be taken into account because they are able to modulate the response to the variations of the free amino acid levels, such as energy intakes, exercise or aging.

Thus protein anabolism is usually promoted (1) when protein feeding is evenly distributed throughout the day and when the rate of absorption of ingested amino acids induces a moderate rise in peripheral amino acid concentrations; (2) when sharper rises in peripheral amino acid concentrations are induced either through the ingestion of rapidly absorbed nitrogen sources, or through modulation of the protein feeding pattern leading to ingestion of high protein meals during aging; (3) when nitrogen ingestion coincides with sensitive physiological states like recovery after exercise.

Most of the effects of the timing of protein feeding on protein anabolism appear to be understandable in the light of the response of protein anabolism to the availability of amino acids and non-protein energy and to stimulatory signals connected with variations of extracellular free amino acid concentrations or with specific physiological states. A better understanding of the effect of the timing of protein feeding would contribute to the promotion of good health. The improvement of dietary protein efficiency would minimize the risk related to excessive protein intake in people undertaking resistance training. It would also be of help in the prevention of sarcopenia in the elderly and thus preservation of the capacity to restore a healthy status.

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