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Randomized control trials

Protein type and caloric density of protein supplements modulate postprandial amino acid profile through changes in gastrointestinal behaviour: A randomized trial☆

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SUMMARY

Background & aims: The requirement of leucine and essential amino acids (EAA) to stimulate muscle protein synthesis increases with age. To target muscle anabolism it is suggested that higher postprandial blood levels of leucine and EAA are needed in older people. The aim was to evaluate the impact of oral nutritional supplements with distinct protein source and energy density, resembling mixed meals, on serum amino acid profiles and on gastrointestinal behaviour.

Methods: Four iso-nitrogenous protein (21 g) supplements were studied containing leucine-enriched whey protein with 150/320 kcal (W150/W320) or casein protein with 150/320 kcal (C150/C320); all products contained carbohydrates (10 or 32 g) and fat (3 or 12 g). Postprandial serum AA profiles were evaluated in twelve healthy, older subjects who participated in a randomized, controlled, single blind, cross-over study. Gastrointestinal behaviour was studied *in vitro* by looking at gastric coagulation and cumulative intestinal protein digestion over time.

Results: The peak serum leucine concentration was twofold higher for W150 vs. C150 (521 ± 15 vs. 260 ± 15 μmol/L, $p < 0.001$), higher for W320 vs. C320 (406 ± 15 vs. 228 ± 15 μmol/L, $p < 0.001$), and higher for low-caloric vs. high-caloric products ($p < 0.001$ for pooled analyses; $p < 0.001$ for interaction protein source*caloric density). Similar effects were observed for the peak concentrations of EAA and total AA (TAA). *In vitro* gastric coagulation was observed only for the casein protein supplements. Intestinal digestion for 90 min resulted in higher levels of free TAA, EAA, and leucine for W150 vs. C150, for W150 vs. W320, and for C150 vs. C320 ($p < 0.0125$).

Conclusions: A low caloric leucine-enriched whey protein nutritional supplement provides a higher rise in serum levels of TAA, EAA and leucine compared to casein protein or high caloric products in healthy, elderly subjects. These differences appear to be mediated in part by the gastrointestinal behaviour of these products.

Clinical trial registration: ClinicalTrials.gov: NCT02013466.

1. Introduction

Aging often coincides with loss of muscle mass, strength and function, known as sarcopenia [1]. Sarcopenia, a geriatric syndrome closely linked to physical frailty, has a substantial impact on the quality of life of the individual and increases the risk of disability and hospitalization [1]. Sarcopenia has been linked to a decreased muscle anabolic response to insulin and dietary essential amino acids (EAA) [2–5].

☆ Conference presentation: the clinical data were presented at ESPEN 2010, Nice (France); the *in vitro* data were presented at ICFD 2014, Wageningen (The Netherlands).

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Muscle protein synthesis has been shown to respond to extracellular EAA concentration [6], subsequent transport, and intracellular amino acid rate of deposition in muscle [7,8]. A high amount of EAA and leucine in one single bolus has also been shown to enable stimulation of muscle protein synthesis in older adults [9–12]. Accordingly, higher postprandial blood levels of EAA and leucine correlate to a higher muscle protein synthesis rate [13,14]. This suggests that the postprandial profile by which amino acids and especially EAA and leucine appear in blood and become available for muscle, is relevant for subsequent stimulation of muscle protein synthesis. This appears to be even more important with advancing age, conditions of inflammation and insulin resistance. The threshold needed to trigger intracellular translation initiation pathways to activate protein synthesis is likely higher under these conditions [15].

The quantity and source of dietary proteins have been shown to impact postprandial blood levels of EAA and leucine [16]. As a result, the concept of "fast/slow" protein was implemented to indicate the postprandial profile of amino acids appearing in the systemic circulation [17,18]. As an example, the dairy proteins whey and casein contain a similar amount of EAA, but blood EAA levels increased faster and to a higher level after the intake of whey protein [14,19]. Therefore, whey is considered a "fast" protein, while casein is a "slow" protein. A difference in gastric emptying and digestion and absorption kinetics between casein and whey [14,20,21] are suggested as an underlying factor. Distinct factors have been shown to modulate gastric emptying and the gastrointestinal digestion of nutritional compositions. Gastric coagulation of casein with formation of solid particles [17], is an example of a physiological phenomenon that is known to delay gastric emptying of casein [21,22]. Another example is the difference in sequential release of casein and whey-derived peptides in the jejunum [23], that is indicative of distinct hydrolysis kinetics of casein and whey. Moreover, it is known that the higher the caloric density of nutrition the more it delays gastric emptying [24,25]. Supplementing sucrose to a casein or milk protein indeed delayed protein digestion and absorption kinetics [26] and increased the oro-ileal transit time [27]. However, the relevance of gastric coagulation and of intestinal digestion rate for postprandial amino acid profiles and systemic amino acid bioavailability of casein and whey protein-containing supplements, i.e. resembling mixed meals of different caloric densities, is less well known.

Our primary study aim was to evaluate postprandial blood amino acids profiles in healthy older people after the intake of a leucine-enriched whey protein nutritional supplement compared to an iso-caloric and iso-nitrogenous control product containing casein protein. While distinct effects of intact whey and casein protein on blood amino acid profiles in older people have been reported, the use of carbohydrates and fat besides protein is less well known. This is relevant as it represents conventional products or a 'mixed meal'. Secondly, we aimed to study the impact of caloric density of the whey and casein products on postprandial amino acid profiles and systemic amino acid bioavailability, which was not studied like this before. Third, we aimed to understand the relevance of distinct gastrointestinal behaviour of the nutritional supplements for postprandial amino acid profiles. While *in vivo* digestion studies with jejunal sampling and/or intrinsically labelled proteins are preferred, these measures are also invasive and require that nutritionally supplements are produced with intrinsically labelled proteins. Therefore, we applied *in vitro* models to compare the coagulation behaviour and protein digestion rate (initial and overall cumulative digestion over time) of the nutritional supplements, under conditions closest to those found in a healthy, elderly population.

2. Materials and methods

2.1. Subjects in clinical study

Fifteen healthy adults that were 65 years or older were screened. A total of 12 subjects (5 male) were enrolled in the study. Subjects who signed the informed consent had a Body Mass Index (BMI) between 21 and 30 kg/m^2, and were willing and able to comply with the protocol. The protocol included adhering to a fasting state from 22:00 h the day prior to the study visits, refraining from alcohol consumption (24 h) and intense physical activities (24 h) before the study visits and not changing dietary habits for the duration of the study. Subjects with a (history of) gastrointestinal disease, or those that had been diagnosed with, or were suspected of having, diabetes mellitus (fasting glucose $\geq$ 7.0 mmol/L) were excluded from participation. Other exclusion criteria were: infection or fever in the past 7 days, medication use (antibiotics within 3 weeks of study entry, current use of corticosteroids or hormones, and the use of antacids or any medication influencing gastric acid production), known allergy to milk or milk products, lactose intolerance and known galactosaemia. Moreover, subjects were excluded when they currently participated in a weight loss or muscle strengthening program or used nutritional supplements that contained proteins or amino acids within one week of study entry, as well as those who had smoked for the past 3 months or abused alcohol or drugs. The Modified Baecke Questionnaire for Older People [28] was completed to measure the normal physical activity level. Body weight and height were measured. Supplementary Fig. 1 shows the Consort flow diagram.

All subjects were informed of the study procedures and possible risks before signing informed consent. The subject enrolment and study conduction was done by Ampha B.V. (clinical research unit, Nijmegen, the Netherlands) according to ICH-GCP principles, and in complied with the principles of the 'Declaration of Helsinki' (59th WMA General Assembly, Seoul, October 2008) and the local laws and regulations. The Independent Review Board Nijmegen (IRBN), the Netherlands, approved the study. This trial is registered at the ClinicalTrials.gov Trial Register under number NCT02013466.

2.2. Study products

Four study products, differing in protein source and caloric density (Table 1), were tested *in vivo* and *in vitro*. Two products composed a leucine-enriched whey protein nutritional supplement (20 g whey protein (from whey protein isolate), 3 g total leucine) with 150 kcal (W150) or 320 kcal (W320), respectively. The other products contained an iso-nitrogenous amount of casein (21 g

Table 1
Composition of the study products.

Nutrients	W150	C150	W320	C320
Energy (kcal)	150	150	320	320
Protein (En%)	56	56	26	26
Carbohydrates (En%)	26	26	40	40
Fat (En%)	18	18	34	34
Total protein (g)	21	21	21	21
Whey protein (g)	20	–	20	–
Casein protein (g)	–	21	–	21
Free BCAA (g)	1	0	1	0
Total leucine (g)[a]	3	2	3	2
Total EAA (g)[a]	10	9	10	9
Carbohydrates (g)	11[b]	10	33[b]	32
Fat (g)	3	3	12	12

BCAA, branched chain amino acids; EAA, essential amino acids.
[a] Provided by protein and free BCAA.
[b] Includes citric acid to obtain an acidic product.

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