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Original Study

Protein Redistribution From Skeletal Muscle to Splanchnic Tissue on Fasting and Refeeding in Young and Older Healthy Individuals

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A B S T R A C T

Background: During aging, a shift of protein metabolism from muscle to splanchnic tissue contributes to increased muscle protein loss after a period of metabolic stress (eg, fasting).
Objective: To study the adaptation of protein metabolism in the whole body and tissue (ie, skeletal muscle and splanchnic area) to metabolic stress, such as short-term fasting and refeeding, in aged people.
Design and participants: We studied splanchnic and muscle protein metabolism after 38 hours of fasting and refeeding in 7 young (5 men/2 women, 24.4 ± 2.0 years) and 8 elderly individuals (6 men/2 women, 70.6 ± 3.1 years).
Measurements: We used intravenous (IV) L-[$^{13}C_6$]phenylalanine, IV L-[$^{2}H_3$]leucine, and oral L-[$^{13}C_1$]leucine to obtain (1) whole-body protein kinetics, (2) muscle and albumin fractional synthesis rate (FSR, %/d; $^{13}C_6$-Phe, and $^{13}C_1$-Leu), and (3) splanchnic extraction during fasting and refeeding (%, $^{2}H_3$- and $^{13}C_1$-Leu).
Results: Whole-body protein breakdown was activated during fasting in young and older individuals ($P < .01$ vs fasted state). Muscle FSR remained unchanged in both groups and not stimulated by refeeding in either group with either IV ^{13}C Phe or oral ^{13}C Leu, probably because of high plasma levels of essential amino acids (EAAs) and branched-chain amino acids (BCAAs). Splanchnic extraction of leucine was 42% higher in the elderly individuals ($P = .03$ vs young) and was associated with an increased albumin synthesis rate in elderly individuals in the fed state ($P < .05$ vs young).
Conclusion: Splanchnic protein metabolism is modified by age, but this metabolic change is not associated with a lower synthesis rate of muscle protein, provided high plasma levels of essential EAAs are maintained. Our data also suggest that splanchnic protein synthesis is a metabolic priority during recovery after metabolic stress in healthy elderly persons and that it might be even more affected in polymedicated older individuals having chronic diseases.

Sarcopenia is a common feature of aging, but the pathophysiological mechanisms leading to this progressive loss of muscle protein with age are still not well defined.[1,2] For instance, although the effect of age on whole-body protein turnover has been addressed in previous studies, no consensus has been reached. As attested by several studies,[3–7] whole-body protein turnover in elderly persons is not different from that in young people in the postabsorptive state when expressed per kilogram of lean body mass. Conversely, in other investigations, the decline with age in whole-body protein kinetics is evident in both men and women and persists even after adjustment for differences in fat-free mass.[8] In vivo isotopic studies of muscle protein fractional synthesis rate (FSR) have also yielded conflicting results in the basal state: in the postabsorptive state, mixed muscle protein or specific muscle protein FSR values have generally been found to be lower in the elderly,[7,9–12] but not by some authors.[13] Whatever the changes in muscle FSR, the absolute synthesis rate of muscle is reduced, because muscle protein mass is lower. However, the postabsorptive metabolic state does not reflect the changes occurring throughout the day, as circadian rhythms, such as food

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intake, are known to strongly influence muscle protein synthesis. Thus, another hypothesis that may help to explain the age-associated decline in muscle mass could be a decreased response to anabolic stimuli also called the "anabolic resistance." The stimulating action of nutrients, such as amino acids, is preserved when large amounts of amino acids are supplied intravenously[14] or given alone orally in the form of free amino acid mixtures.[15] However, when amino acids are administered with glucose or in a complete mixed meal, their stimulating effect on muscle protein synthesis is blunted in both animals[16,17] and humans.[18,19] During the administration of a mixed meal, amino acids are first absorbed via the splanchnic tissues, that is, mainly the gut and liver, before being delivered to the peripheral tissues.[20] As the relative weight of these organs compared with the muscle increases during aging, their contribution to whole-body protein metabolism may be greater in the elderly. Protein metabolism in the splanchnic area has been specifically investigated during feeding in elderly individuals.[3,21] These studies indicate that leucine or phenylalanine splanchnic extraction is increased in elderly individuals,[22,23] implying that it could to some extent limit dietary amino acid availability for muscle protein synthesis. This observation suggests that either dietary amino acid availability is limited for muscle after food intake or that anabolic hormones, such as insulin, may be less active with aging.[19,24–26] Possible resistance to the anabolic action of nutrients or hormones with aging is of particular relevance in a situation of injury or sepsis, as it could prevent recovery of lean body mass through a higher amino acid demand from splanchnic tissues for acute phase protein synthesis. Thus, muscle protein loss in the elderly could occur after a metabolic stress situation not only owing to a reduction in recovery capacities, but also because of an increased sensitivity of muscle protein to catabolic factors. However, the determination of protein turnover rate in elderly undernourished patients is hampered by complex environmental influences, such as illness. We hypothesized that the adaptation of protein metabolism in the whole body and tissue (ie, skeletal muscle and splanchnic area) to metabolic stress, such as short-term fasting and refeeding, was impaired in aged people. Given the regulatory action of essential amino acids (EAAs), especially branched-chain AAs (BCAAs) on protein metabolism, we also analyzed the effect of changes in plasma AAs induced by fasting and refeeding in young and elderly healthy individuals. We went on to compare the fasting and refeeding responses between the 2 groups for protein metabolism, with special emphasis on mixed muscle protein synthesis, first-pass splanchnic extraction of leucine, and splanchnic metabolic activity, as reflected by albumin synthesis rate. This work demonstrates that adaptation of muscle protein synthesis does occur in non-malnourished healthy elderly individuals despite specific changes in splanchnic protein metabolism.

Subjects and Methods

Participants

Seven young (Y, 5 men/2 women, 24.4 ± 2.0 years) and 8 older individuals (E, 6 men/2 women, 70.6 ± 3.1 years) took part in the study. Each person gave a normal physical examination result with no medical history of renal, cardiovascular, endocrine, or currently evolving disease. The individuals took no medication or dietary supplements during either the month before the study started or the study period itself. All the individuals maintained their usual physical activity before the study. The physical characteristics of the individuals are listed in Table 1. The purpose and the potential risks of the study were fully explained to the participants, and written informed consent was obtained from each participant. The experimental protocol was approved by the Ethical Committee of Auvergne. Of note, this protocol had already been successfully used as a model to study the effect of nutritional stress on different physiological functions in elderly people.[27]

Table 1
Characteristics of the Participants

	Young Participants	Older Participants
Age, y	24.4 ± 2.0	70.6 ± 3.1
Weight, kg	71.5 ± 5.4	68.8 ± 8.5
Height, cm	173 ± 0.0	166 ± 0.0
Waist circumference, cm	80.9 ± 1.7	84.9 ± 4.1
Body mass index, kg/m^2	23.8 ± 1.9	25.4 ± 2.0
Lean body mass, kg	57.2 ± 6.2	50.5 ± 8.8
Muscle mass	33.7 ± 17.5*	28.8 ± 18.4†
Fat mass, %	20.3 ± 6.0*	27.6 ± 7.0†

Data are mean ± SEM; n = 7 (Young) and n = 8 (Older), respectively; * ≠ † with $P < .05$. Lean body mass, muscle mass, and fat mass were determined by bioelectrical impedance and dual-energy x-ray absorptiometry (DEXA) analyses. Muscle mass was determined from appendicular estimation (DEXA).

Materials

Tracers L-[$^{13}C_6$]phenylalanine (95 MPE), L-[$^2H_3$]leucine (96 MPE) and L-[$^{13}C_1$]leucine (98 MPE) were purchased from Cambridge Isotope Laboratory (Andover, MA). Their isotopic and chemical purity were checked by gas chromatography-mass spectrometry (GC-MS). Solutions of the tracers were tested for sterility and pyrogenicity before use and were prepared in sterile nonpyrogenic saline. In each experiment, the tracers were filtered through 0.22-μm filters.

Experimental Protocol

The isotopic study was preceded by a 7-day period (days 0–6) of controlled diet. Energy intake was based on measured resting energy expenditure (REE) multiplied by an activity factor of 1.6. REE was determined for each participant before the study by open-circuit indirect calorimetry (Deltatrac; Datex, Geneva, Switzerland). During this period, protein intake provided 16% of total energy. The individuals' usual and controlled intakes were assessed by dietary inquiries.

On the morning of day 7, the individuals were admitted to the laboratory for 2 days (days 7 and 8) for the fasting and refeeding study (Figure 1). The last meal was ingested at 8:00 PM on day 6 and during the 38-hour fasted period (day 7 and part of day 8). The individuals were allowed to drink water with 4 g of NaCl.[28] This period was followed by a 6-hour fed state (day 8). Blood glucose, free fatty acids and insulin, together with whole-body leucine and phenylalanine kinetics, and muscle and albumin synthesis rates were measured at baseline (H11) (ie, at 7:00 AM on day 7 after a fasted night) and during the fasted and fed periods. Urine was collected for 3 days (days 6, 7, and 8) for the measurement of total urinary cortisol during fasting and refeeding. A 38-hour fasting period was chosen, as this duration is not uncommon in elderly hospitalized patients, in particular when surgery is planned. It would have been ethically unjustifiable to design a longer fasting period, particularly in older persons.

After an overnight fast on day 7 and day 8, samples for blood glucose, insulin, albumin, amino acids, hematocrit, and natural isotopic abundance were withdrawn, and body composition was estimated by bioelectrical impedancemetry. After 35 hours of fasting (7:00 AM on day 8), a catheter was inserted retrogradely into a dorsal vein for arterialized blood sampling after introduction of the hand into a 70°C heated, ventilated box. Another catheter was inserted into a contralateral dorsal vein for tracer infusion. Continuous infusions of