Postprandial whole-body protein metabolism after a meat meal is influenced by chewing efficiency in elderly subjects

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ABSTRACT

Background: The rate of protein digestion affects protein utilization in elderly subjects. Although meat is a widely consumed protein source, little is known of its digestion rate and how it can be affected by the chewing capacity of elderly subjects.

Objectives: We used a [1-13C]leucine balance with a single-meal protocol to assess the absorption rate of meat protein and to estimate the utilization of meat protein in elderly subjects with different chewing efficiency.

Design: Twenty elderly volunteers aged 60–75 y were involved in the study. Ten of them had healthy natural dentition, and the other 10 were edentulous and wore complete dentures. Whole-body fluxes of leucine, before and after the meal (120 g beef meat), were measured with the use of a [1-13C]leucine intravenous infusion.

Results: A rapid increase in plasma aminoacidemia and plasma leucine entry rate was observed after meat intake in dentate subjects. In complete denture wearers the increase in leucine entry rate was delayed (P < 0.05), and the amount of leucine appearing in peripheral blood during the whole postprandial period was lower than in dentate subjects (P < 0.01). Postprandial whole-body protein synthesis was lower in denture wearers than in dentate subjects (30% compared with 48% of leucine intake, respectively; P < 0.05).

Conclusion: Meat proteins could be classified as fast digested proteins. However, this property depends on the chewing capacity of elderly subjects. This study showed that meat protein utilization for protein synthesis can be impaired by a decrease in the chewing efficiency of elderly subjects. Am J Clin Nutr 2007;85:1286–92.

KEY WORDS Leucine kinetics, protein metabolism, meat, chewing efficiency, elderly

INTRODUCTION

Aging is associated with a decline in body muscle mass, leading to a decrease in physical autonomy and an increase in vulnerability of elderly people. This loss of muscle mass must result from an imbalance between the constant synthesis and degradation of muscle proteins. An alteration of muscle protein anabolism in older subjects in the fed state was reported (1, 2), in relation with a decrease in muscle sensitivity to a postprandial increase of plasma free amino acids and in particular leucine (3–5). This phenomenon could explain the slow erosion of muscle mass during aging.

In response, dietary strategies aimed at optimizing a postprandial increase in plasma amino acids, without increasing total daily protein intake, were proposed. The use of rapidly digested proteins (6), concentration of daily protein supply in one meal (7, 8), and specific amino acid supplementation (9, 10) were all shown to improve protein retention, nitrogen balance, or muscle protein synthesis in elderly subjects.

A rapid and intense rise in postprandial aminoacidemia is therefore beneficial for protein anabolism in elderly subjects. However, although the amino acid composition and the total digestibility of all protein sources are now well known, the kinetics of their digestion and their impact on the postprandial rise of plasma amino acids are still poorly described. Although a lot of studies were done with milk proteins (6, 11), much less is known of meat proteins, a high-quality (12) and widely consumed protein source.

In addition, it seems possible that, for meat, this property could vary according to the subject and in particular to the person’s chewing efficiency (individual capability to transform a lump of food into a proper swallowable food bolus). Aging is often associated with a decrease in chewing efficiency, leading to a lower disruption of swallowed meat pieces (13). Chewing efficiency is even worse in subjects with impaired mastication such as denture wearers (14). Wearing dentures is known to lead to gastrointestinal discomfort (15), but it could also decrease protein utilization efficiency and modify the absorption kinetics of amino acids.

Therefore, this study was designed to determine the effect of a meat meal on the postprandial variations in plasma aminoacidemia and whole-body protein metabolism in elderly subjects. It was also designed to assess how chewing efficiency can modify these indicators, with 2 groups of subjects having different dentition characteristics, namely healthy dentate subjects and full-denture wearers.
and sodium [13C]bicarbonate (99 atom%, determined by the manufacturer) was administered. A continuous infusion of [1-13C]leucine (0.06 mol·kg⁻¹·min⁻¹) was then begun and continued for 580 min. Blood samples (∼5 mL) were collected and stored in 10-mL evacuated tubes (Becton-Dickinson, Grenoble, France). Production rates of carbon dioxide were measured during 20-min periods (starting at −80, 20, 80, 140, 200, 280, 400 min) by open-circuit indirect calorimetry (Deltatrac; Datex, Geneva, Switzerland).

Sample analysis

The 13C enrichments of leucine and α-ketoisocaprate (KIC) were measured by gas chromatography–mass spectrometry (model 5971A; Hewlett-Packard, Paris, France) with the use of tertiary-butyldimethylsilyl derivatives as described by Boirie et al (18). The 13C enrichments of carbon dioxide were measured on a gas chromatography combustion isotope ratio mass spectrometer (Fisons Instruments, VG Isotech, Middlewich, United Kingdom).

Leucine fluxes were calculated from the time-dependent evolution of the 13C enrichments of plasma leucine and KIC and from expired carbon dioxide. The experimental design allowed the determination of the rate of total leucine appearance (Tot Leu Ra) in the peripheral circulation and the rate of total leucine disappearance (Tot Leu Rd) from plasma, which is the sum of the fluxes of leucine, either oxidized (Leu Ox) or used for protein synthesis (nonoxidative leucine disposal or NOLD).

The equations used have been reported in detail by Boirie et al (18). Briefly, the equations include the following:

\[
\text{Tot Leu Ra} = (\text{IR} - (pV \times C_{\text{leu}(t)} \times \text{dE}_{\text{leu}(t)}/\text{dt}))/E_{\text{leu}(t)}
\]

(1)

\[
\text{Tot Leu Rd} = \text{Tot Leu Ra} - (pV \times \text{dC}_{\text{leu}(t)}/\text{dt})
\]

(2)

\[
\text{Leu Ox} = (V_{\text{CO}_2} \times E_{\text{CO}_2})/(k \times E_{\text{KIC}})
\]

(3)

\[
\text{NOLD} = \text{Tot Leu Rd} - \text{Leu Ox}
\]

(4)

where IR is [13C]leucine infusion rate (μmol·kg⁻¹·min⁻¹), p is the correction factor of the pool size for instant mixing.

### Subjects and Methods

**Materials**

Beef meat was obtained from semimembranous muscle kept 15 d postmortem at 4 °C. Then it was cut into slices, vacuum-packed, cooked at 65 °C, and stored at −18 °C. Before use, the meat was thawed by immersing packs in warm water and then grilled until the inner temperature reached 65 °C.

L-[1-13C]leucine (99 atom%, determined by the manufacturer) and sodium [13C]bicarbonate (99 atom%, determined by the manufacturer) were supplied by Eurisotop (Gif-sur-Yvette, France). Solutions of tracers were tested for sterility and pyrogenicity before use.

**Subjects**

Twenty healthy elderly subjects (aged 60–75 y) participated in the study. Ten of them were dentate (ND) with at least 8 pairs of natural postcanine teeth. The other 10 subjects were edentulous (DP) for ≥5 y. They wore full dentures with a correct interocclusal relation and good stability. The current dentures had been in place for ≥6 mo, and subjects felt comfortable with them. Chewing performance of these subjects is presented elsewhere (16, 17). Chewing efficiency can be evaluated from oral indicators (such as bite force and chewing duration), from the particle size distribution after a given number of strokes on a brittle material, or from bolus properties. It was shown that the denture wearers of the present study swallowed fewer disorganized boli than the dentate subjects (16). All subjects were in good general health without medical treatment and ate meat on a regular basis.

Volunteer characteristics [age, body mass, body mass index (in kg/m²)] in each group are given in Table 1. The study was approved by the local ethical committee (CCPPRB-Auvergne), and all subjects gave their informed consent.

**Experimental protocol**

Whole-body fluxes of leucine before and after the meal were measured with the use of [1-13C]leucine as an intravenous tracer. After an overnight fast, a catheter was inserted retrogradely into a dorsal vein of the hand for blood sampling after introduction of the hand in a 60 °C heated ventilated box. A second catheter was inserted into a vein of the contralateral arm for tracer infusion. After baseline collection of blood and breath samples, a priming dose of NaH13CO3 (0.1 mg/kg) and [1-13C]leucine (3.6 μmol/kg) was administered. A continuous infusion of [1-13C]leucine (0.06 μmol·kg⁻¹·min⁻¹) was then begun and continued for 580 min. After 150 min of infusion, the meat meal (a steak of 120 g) was served to the volunteers. They were allowed to add salt and to drink a glass of water. They had to ingest the whole meal within 20 min. Blood and breath samples were taken before the meal (−60, −40, −20, and 0 min) and after the meal at 20-min intervals for the first 300 min, and then at 340 and 420 min. Blood samples (∼5 mL) were collected in tubes containing lithium-heparin and immediately centrifuged at 1500 × g for 10 min at 4 °C. Supernatant fluid was subsampled, frozen in liquid nitrogen, and stored at −80 °C. Expired breath samples were collected and stored in 10-mL evacuated tubes (Becton-Dickinson, Grenoble, France). Production rates of carbon dioxide were measured during 20-min periods (starting at −80, 20, 80, 140, 200, 280, 400 min) by open-circuit indirect calorimetry (Deltatrac; Datex, Geneva, Switzerland).

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Leucine concentrations of amino acids were measured after deproteinization with sulfosalicylic acid by ion-exchange chromatography (Bio-Tek Instruments ARL, St Quentin Yvelines, France). Plasma urea concentrations were measured with the use of enzymatic reactions on an autoanalyzer (Cobas Mira; Roche Diagnostic Systems, Neuilly sur Seine, France).

**Calculations**

Leucine fluxes were calculated from the time-dependent evolution of the 13C enrichments of plasma leucine and KIC and from expired carbon dioxide. The experimental design allowed the determination of the rate of total leucine appearance (Tot Leu Ra) in the peripheral circulation and the rate of total leucine disappearance (Tot Leu Rd) from plasma, which is the sum of the fluxes of leucine, either oxidized (Leu Ox) or used for protein synthesis (nonoxidative leucine disposal or NOLD).

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where IR is [13C]leucine infusion rate (μmol·kg⁻¹·min⁻¹), p is the correction factor of the pool size for instant mixing.
Baseline \((n=10)\) & Dental prosthesis \((n=10)\) & \(P\) (ANOVA) \\
\[EAA\] & \[Leu\] & \[NOLD\] & \[Tot Leu Ra\] & \[Leu Ox\] & \\
Baseline (\(\mu\text{mol/L}\)) & 780 ± 24 & 790 ± 27 & 0.709 \\
\(\text{Y}^\text{max}\) (\(\mu\text{mol/L}\)) & 1852 ± 64 & 1766 ± 83 & 0.452 \\
\(t\text{Y}^\text{max}\) (min) & 134 ± 13 & 172 ± 20 & 0.153 \\
Baseline (\(\mu\text{mol/L}\)) & 122 ± 5 & 118 ± 6 & 0.467 \\
\(\text{Y}^\text{max}\) (\(\mu\text{mol/L}\)) & 355 ± 12 & 340 ± 20 & 0.542 \\
\(t\text{Y}^\text{max}\) (min) & 136 ± 11 & 178 ± 20 & 0.089 \\
\(\text{AUC}\) (\(\mu\text{mol/kg}\)) & 239 ± 11 & 185 ± 15 & 0.004 \\
Baseline (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)) & 1.41 ± 0.06 & 1.39 ± 0.08 & 0.914 \\
\(\text{Y}^\text{max}\) (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)) & 3.14 ± 0.15 & 2.68 ± 0.20 & 0.091 \\
\(t\text{Y}^\text{max}\) (min) & 98 ± 10 & 150 ± 18 & 0.012 \\
\(\text{AUC}\) (\(\mu\text{mol/kg}\)) & 239 ± 11 & 185 ± 15 & 0.004 \\
Baseline (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)) & 1.15 ± 0.05 & 1.14 ± 0.07 & 0.862 \\
\(\text{Y}^\text{max}\) (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)) & 2.47 ± 0.16 & 1.97 ± 0.08 & 0.047 \\
\(t\text{Y}^\text{max}\) (min) & 96 ± 7 & 164 ± 15 & 0.002 \\
\(\text{AUC}\) (\(\mu\text{mol/kg}\)) & 140 ± 20 & 89 ± 9 & 0.015 \\
Baseline (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)) & 0.25 ± 0.02 & 0.23 ± 0.02 & 0.576 \\
\(\text{Y}^\text{max}\) (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)) & 0.78 ± 0.04 & 0.70 ± 0.06 & 0.293 \\
\(t\text{Y}^\text{max}\) (min) & 178 ± 16 & 174 ± 16 & 0.941 \\
\(\text{AUC}\) (\(\mu\text{mol/kg}\)) & 100 ± 9 & 104 ± 11 & 0.862 \\
\(p=0.25\), \(V\) is the leucine pool size (\(V=0.5\ L/\text{kg body wt}\)). The choice of these factors was previously discussed (18). \(C_{\text{leu}}(t)\) is the mean plasma leucine concentration between 2 sampling times (in \(\mu\text{mol/L}\)), \(dE_{\text{leu}}/dt\) represents the time-dependent variations of \([^{13}\text{C}]\text{leucine enrichment (in mol % excess)}\), \(E_{\text{leu}}(t)\) is the mean \([^{13}\text{C}]\text{leucine enrichment between 2 sampling times (in mol % excess)}\), \(V_{\text{CO}2}\) is the carbon dioxide production rate (in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)), \(E_{\text{CO}2}\) and \(E_{\text{KIC}}\) are \([^{13}\text{C}]\text{CO}2\) and \([^{13}\text{C}]\text{KIC}\) enrichment (in mol % excess), respectively. The \(\bar{k}\) is a correcting factor for incomplete recovery of carbon dioxide in the breath; it was assumed to gradually increase from a fasted value of 0.76 to a maximum value of 0.91, 60 min after the beginning of the meal (19). Postabsorptive leucine fluxes were calculated by averaging sampling times from \(-60\) to \(0\) min. Leucine intake was calculated assuming 2.29 g leucine/100 g cooked meat (20).

**Statistical analysis**

Time and time \(\times\) group effects on arterial concentrations and leucine fluxes were tested with the use of the repeated option of the PROC MIXED procedure of SAS (SAS/STAT Users Guide, release 8.1, 2000; SAS Institute Inc, Cary, NC), with subjects as random effect and with time, group, and time \(\times\) group as factors. When significant time \(\times\) group interaction was found, the LS-MEANS procedure was used to test differences at specific times, between groups, and within groups compared with baseline.

Each postprandial curve was characterized by its zenith \(\text{Y}^\text{max}\), by the time at which \(\text{Y}^\text{max}\) was observed \((t\text{Y}^\text{max})\), by baseline value (postabsorptive value), and postprandial area under the curve (AUC; calculated by integrating the difference between postabsorptive flux and observed flux, trapezoidal method). Data were analyzed by analysis of variance with the use of the GLM procedure of SAS, with dentition and sex as factors.

**RESULTS**

Postabsorptive concentrations in measured essential amino acids (EAAs) were not different between groups (Table 2). They were unchanged within the 20 min after the beginning of the meal. Then they sharply increased in both groups (Figure 1). From 100 to 140 min, plasma EAA concentrations were greater for the ND group than for the DP group. From 180 to 420 min, the concentrations decreased in both groups. At 420 min, concentrations were still higher than postabsorptive values. \(\text{Y}^\text{max}\) and \(t\text{Y}^\text{max}\) for EAAs were not affected by chewing efficiency (Table 2). In both groups, plasma leucine kinetics was similar to EAA kinetics (Figure 1). In the DP group, \(t\text{Y}^\text{max}\) tended to be 40 min later than in the ND group. The increase in plasma leucine concentrations as a result of meal intake in the ND group averaged 230 \(\mu\text{mol/L}\).

The kinetics of plasma urea concentrations were not different between groups (Figure 2). Increase in the carbon dioxide production rate after the meal tended to be greater \((P=0.080)\) in the ND group than in the DP group (Figure 2). However, total postprandial increase in carbon dioxide production was not affected by chewing efficiency.
The variations in $^{13}$C enrichment of plasma leucine and KIC throughout the sampling period are presented in Figure 3. Before the meal, $[^{13}C]$leucine and $[^{13}C]$KIC enrichments reached a plateau (4.21 $\pm$ 0.07 and 3.91 $\pm$ 0.08 mol % excess, respectively), and no significant differences were observed between premeal means ($P < 0.01$), and lines at the bottom of the graph indicate a significant difference ($P < 0.05$) from baseline for each curve.

FIGURE 1. Mean (± SEM) plasma essential amino acids (EAAs) and leucine concentrations during the last 60 min of the postabsorptive period and the first 420 min after a meat meal in 2 groups of volunteers having either healthy natural dentition (○; n = 10) or complete dental prosthesis (●; n = 10). Data were analyzed by a mixed-model ANOVA, and time × group interaction was significant for both measures ($P < 0.01$). Statistical differences between the 2 groups: *$P < 0.05$. Time effect was significant for both measures ($P < 0.001$), and lines at the bottom of the graph indicate a significant difference ($P < 0.05$) from baseline for each curve.

FIGURE 2. Mean (± SEM) plasma urea concentrations and carbon dioxide (CO$_2$) productions during the last 60 min of the postabsorptive period and the first 420 min after a meat meal in 2 groups of volunteers having either healthy natural dentition (○; n = 10) or complete dental prosthesis (●; n = 10). Data were analyzed by a mixed-model ANOVA, and time × group interaction was significant for carbon dioxide production ($P < 0.01$) but not for urea ($P = 0.501$). Time effect was significant for both measures ($P < 0.001$), and lines at the bottom of the graph indicate a significant difference ($P < 0.05$) from baseline for each curve.

The present study was designed to characterize the modifications of whole-body protein metabolism in elderly subjects after a meat meal and to determine how it is affected by chewing efficiency. For this purpose, the leucine appearance rate and the NOLD rate were compared in 2 groups of elderly subjects: one group of subjects with a good natural dentition and another group of complete denture wearers known to have a poor chewing efficiency (21). Postprandial increase in the leucine appearance rate was used as a surrogate of meat leucine entry rate because leucine meat was not labeled and because postprandial variations in endogenous leucine appearance rates were reported to be similar in slowly and fast digested proteins (6). However, it could underestimate meat leucine entry rate because of the transitory
postprandial decrease in whole-body protein degradation but in a similar way in both groups.

Postprandial increase in the plasma amino acid concentrations observed in the present study was in agreement with data reported for young adults after a beef meat meal (22–24), with the highest concentrations being generally recorded 90–180 min after the beginning of the meal. In the present work, a close relation was observed between the variations in plasma leucine concentrations and plasma leucine entry rate.

In reference to their absorption rate, proteins have been classified as “fast” or “slow” proteins (11). The present observations indicate that meat proteins could be categorized as fast proteins; variations in aminoacidemia and leucine appearance rate are closer to what is observed with whey proteins (fast proteins) than with casein (slow proteins) (6, 11). Together with its high-protein content, the rapid digestion of meat makes it a good source of protein to produce a rapid and intense rise in postprandial aminoacidemia in elderly subjects.

Both the plasma leucine concentration and the total whole-body rate of appearance of leucine were lower in denture wearers than in dentate subjects 60–140 min after the meal, and the greatest values were reached in denture wearers ≈60 min later than in dentate subjects. Together with the pattern of carbon...
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dioxide production, these observations indicate a delayed absorption of meat proteins in denture wearers. To flow out of the stomach, solid food has to be reduced to small-sized particles, and it was observed in young subjects that incomplete comminution of ham cubes during mastication decreased the gastric emptying rate (25). With the use of the same subjects and meat preparation as in the present study, a parallel study was conducted to analyze the effect of chewing impairment on ready-to-swallow meat bolus properties (16). It was shown that denture wearers swallow fewer disrupted bolus than dentate subjects. Thus, a decrease in the gastric emptying rate could explain the lag in meat protein absorption observed in the present study. These observations clearly show that ranking protein according to digestion rate not only depends on the intrinsic quality of the protein but also on the physiologic characteristics of the consumer, mainly chewing efficiency and gastric emptying rate. Both of these characteristics can be altered in the elderly (21, 26).

The amount of leucine appearing in peripheral blood during the whole postprandial period was lower in denture wearers than in dentate subjects (63% compared with 82% of leucine intake). This difference is surprising; we were expecting a delay in leucine appearance but not a difference in the total amount of leucine absorption. For both groups, the appearance rate returned to baseline values 2 h before the last sampling time, indicating that no more detectable amounts of dietary leucine entered the peripheral blood. Thus, the decrease in total entry rate does not seem to be attributable to an insufficiently long sampling time. It could theoretically be due to a higher inhibition of whole-body proteolysis in denture wearers than in dentate subjects. However, it seems unlikely because it was shown that postprandial inhibition of whole-body proteolysis in the elderly is not affected by the digestion rate (6). In addition, proteolysis inhibition is greater when leucinemia is higher (4, 27), which is the opposite of what is observed in denture wearers.

Thus, the lower amount of leucine appearing in plasma of denture wearers is more likely due to a higher retention of ingested leucine in splanchnic organs. Whether it is due to a longer retention of amino acids in the digestive lumen (incomplete digestion) or to a higher extraction by splanchnic tissues (digestive tract, liver) remains undetermined. A lower gastric emptying rate is likely to occur together with prolonged intestinal digestion because of fewer disrupted pieces of meat. Whether this results in a lower efficiency in the digestion of meat is unclear because an increase in transit time could compensate more or less for the differences in initial buccal disintegration of meat. Few investigators have studied the impact of chewing on digestive processes and more particularly on meat digestion. Farrell (28) observed that the degree of mastication required for maximum digestion is low and that there is no reason to suppose that full denture wearers are less capable of digesting their food. This study addressed whole-gut digestibility and did not provide information on the digestion in the small intestine, where amino acid absorption takes place. It was shown in subjects with ileostomies that beef meat proteins (from fried rump steak) have a high (94%) true ileal digestibility (29). However, swallowing of poorly disrupted bolus could produce a decrease in digestion in the small intestine, as a consequence of stomach outflow of larger pieces of food, which may limit protein accessibility for digestive enzymes. This could lead to a shift in the site of meat digestion, from the small intestine to the large intestine.

A greater utilization of dietary leucine in splanchnic tissues in denture wearers than in dentate subjects also has to be considered. A high variability in splanchnic extraction of dietary leucine exists in elderly subjects; its range was 26–88% in 6 elderly men (11) and 17.5–62.6% in 14 elderly women (30). In addition, a greater splanchnic extraction was reported with slowly digested proteins when compared with fast digested proteins (6). Although in the present study the magnitude of the difference in absorption rate was much smaller than between slowly and fast digested proteins (6), the fact that denture wearers are chronically exposed to slower absorption rates may induce adaptation in the long term and lead to an increase in amino acid splanchnic extraction.

Finally, because of the delayed absorption of meat proteins and to the lower total amount of amino acids entering peripheral blood in the postprandial period, whole-body protein synthesis was lower in denture wearers than in dentate subjects (30% compared with 48% of leucine intake was used for protein synthesis during the whole postprandial period in dentate subjects and denture wearers, respectively). Muscle protein metabolism was not investigated in the present study, which would have needed muscle biopsy sampling to determine the protein fractional synthesis rate. Nevertheless, the result on whole-body protein synthesis is consistent with previous experiments showing that muscle protein synthesis sensitivity to feeding is altered during aging (1, 2). Higher amounts of blood free amino acids are necessary to stimulate muscle protein synthesis (4, 5, 31, 32). The increase in postprandial aminoacidemia observed in the present study in elderly denture wearers was inadequate to reach maximal stimulation of whole-body protein synthesis.

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The authors’ responsibilities were as follows—DR: planned and implemented the study, analyzed statistics, interpreted data, and wrote the manuscript; MM: volunteer recruitment volunteers and sample collection and analysis; CY: volunteer recruitment and sample collection and analysis; CB: volunteer recruitment and sample collection and analysis; L Mioche: study design and critical revision of the manuscript; L Mosoni: study design and critical revision of the manuscript. None of the authors had a conflict of interest.

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