Decreased Whole-Body and Splanchnic Glutamate Metabolism in Healthy Elderly Men and Patients with Chronic Obstructive Pulmonary Disease in the Postabsorptive State and in Response to Feeding


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ABSTRACT Decreased plasma and muscle glutamate concentrations have been observed in patients with chronic obstructive pulmonary disease (COPD), suggesting disturbances in glutamate metabolism. The present study was conducted to further examine glutamate metabolism in 8 male COPD patients (68 ± 4 y) by measurement of whole-body (WB) glutamate production and splanchnic glutamate extraction in the postabsorptive state as well as in response to feeding. Because COPD is particularly prevalent in the elderly and aging per se may also affect glutamate metabolism, 2 male control groups were included: 8 healthy elderly (63 ± 3 y) and 8 young (22 ± 1 y) subjects. On 2 test days, the stable isotope L-15N-glutamate was infused i.v. or enterally according to a primed constant and continuous infusion protocol. After 90 min of infusion, subjects ingested a carbohydrate-protein drink (28% milk protein, 72% maltodextrin) every 20 min for 2 h. Arterialized-venous blood samples were taken at the end of the postabsorptive and feeding periods. Postabsorptive WB glutamate production and splanchnic glutamate extraction were significantly lower in the elderly and COPD patients than in the young (P < 0.01). Feeding further decreased WB endogenous glutamate production in the elderly and COPD patients, with COPD patients tending (P = 0.07) to have a greater decrease. Splanchnic glutamate extraction increased during feeding in the elderly (P < 0.05) but did not change in COPD patients. In conclusion, aging reduces postabsorptive WB endogenous glutamate production and splanchnic glutamate extraction. COPD does not affect postabsorptive WB glutamate metabolism but may influence splanchnic glutamate metabolism during feeding.

KEY WORDS: • glutamate • aging • chronic obstructive pulmonary disease • postabsorptive and fed states

Chronic obstructive pulmonary disease (COPD) is increasingly recognized as a chronic metabolic disorder, characterized by weight loss and abnormalities in body composition. Altered plasma and muscle amino acid profiles have been detected in COPD patients compared with healthy controls (1,2). Specifically, there is evidence for a disturbed glutamate metabolism because decreased skeletal muscle and plasma glutamate (Glu) concentrations are consistently found in clinically stable patients with COPD (3–5). Disturbances in glutamate metabolism could be the result of a chronic disease, but could also reflect age-related metabolic adaptations (6) because COPD is present predominantly in subjects ≥ 50 y old. To gain insight into the underlying mechanisms of the disturbed glutamate metabolism, it is essential to determine whether glutamate production is decreased or its consumption is increased. The present study focused on glutamate production and thus glutamate delivery to the organs via feeding or endogenous glutamate production.

Matthews et al. (7) showed that nearly all of an ingested glutamate tracer is absorbed by the splanchnic bed on its first pass in the postabsorptive state. However, plasma glutamate concentration increased after ingestion of 12.7 g monosodium glutamate (8), but ingestion of a protein meal did not raise plasma glutamate levels. No data are yet available concerning splanchnic glutamate extraction during feeding. Because nutritional protein comprises ~10% of glutamate, feeding can still be a source of glutamate for the body. Moreover, the study by Matthews et al. (7) was performed in healthy young volunteers, and there is no information available whether splanchnic glutamate extraction during feeding differs between the elderly and COPD patients. Because postabsorptive first-pass splanchnic glutamate extraction approaches its maximum in healthy young volunteers, we hypothesized that splanchnic glutamate extraction in the elderly and COPD patients, if different from the young, would be lower. Subsequently, a higher amount of glutamate would be released into...
the plasma, which suggests that glutamate delivery to the body is more dependent on an external (dietary) glutamate source.

The purposes of the present study were to first, to investigate whether whole-body and endogenous glutamate production and splanchnic glutamate extraction are different in COPD patients compared with healthy elderly people in the postabsorptive state and during feeding, and second, to examine whether aging per se influences glutamate metabolism in the postabsorptive state and in response to feeding.

SUBJECTS AND METHODS

Subjects. COPD patients, healthy elderly, and healthy young subjects \((n = 8/\text{group})\), all men, were studied. The healthy elderly were age-matched with the COPD patients. The patients were in clinically stable condition and suffered from moderate COPD (stage \(2 + 3\)) according to the recently established GOLD guidelines (9). Exclusion criteria for all groups were malignancy, cardiac failure, recent surgery, and endocrine, hepatic, or renal disorders. Also, subjects who were using systemic corticosteroids within 3 mo before the study were excluded. Written informed consent was obtained from all subjects, and the study was approved by the medical ethical committee of the University Hospital Maastricht.

Pulmonary function tests. Before the study, the healthy elderly and COPD patients underwent spirometry for determination of forced expiratory volume in 1 s (FEV\(_1\)), as a marker of disease severity, with the highest value from at least 3 technically acceptable maneuvers being used. The diffusion capacity for carbon-monoxide \((Dl_{co})\) as an indirect indicator of emphysema was measured using the single-breath method (Masterlab; Jaeger). All values obtained were related to a reference value and expressed as percentages of the predicted value (10). The COPD patients had lower values of FEV\(_1\) (COPD patients: 50 ± 4%pred; elderly: 110 ± 5%pred, \(P < 0.01\)) and \(Dl_{co}\) (COPD patients: 78 ± 7%pred; elderly: 104 ± 9%pred, \(P < 0.05\)) compared with the elderly.

Study design. On 2 test days and at least 4 d apart, subjects were invited to the metabolic ward of the University Hospital Maastricht after an overnight fast. All subjects were instructed to continue their habitual dietary intake for at least 3 d preceding the study. The food intake of the day before each test day was reported in a food questionnaire. From this, daily habitual protein intake was calculated.

Body composition measurements. Body weight was measured using an electronic beam scale with digital readout to the nearest 0.1 kg (model 708; Seca) with the subjects standing barefoot and wearing light indoor clothing. Body height was measured to the nearest 0.1 cm (model 220, Seca). Whole-body fat-free mass (FFM) was measured in each subject using bioelectrical impedance analyses (Xitron 4000B, Xitron Technologies) to express metabolic data per kilogram of FFM. FFM of the COPD patients was calculated using a specific regression equation (11), whereas FFM of the healthy elderly and young volunteers was calculated using a specific regression equation described by Dey et al. and Lohman et al., respectively (12).

On the 1st test day, a catheter was placed in an antecubital vein of the arm for infusion of the tracer (85 mL/h), according to a primed constant continuous infusion protocol (Fig. 1). \(1^5\)N-glutamate \((1^5\text{N-Glu})\) was used to measure WB Glu turnover. The following priming dose and infusion rate were used: 0.73 \(\mu\)mol/kg and 0.03 \(\mu\)mol/(kg FFM \(\times\) min), respectively. The tracer was obtained from Cambridge Isotopic Laboratories. Before i.v. administration of the priming dose, a venous blood sample was collected to measure baseline Glu enrichment. After administration of the priming dose, a constant continuous tracer infusion was administered until the end of the study day. A second catheter for arterialized venous blood sampling was placed in a superficial dorsal vein of the hand of the contralateral arm, which was placed in a thermostatically controlled hot box (internal temperature: 60°C), at least 20 min before the first blood sampling. The use of the hot box is a technique to mimic direct arterial sampling (13). Triple arterialized-venous blood samples were taken between 80 and 90 min after the start of the infusion. Subsequently, continuous nutrition was started via repeated ingestion (every 20 min) of a carbohydrate-protein drink for 2 h, when a tracer steady state was reached (14). At the end of the ingestion period, triple arterialized-venous blood samples were taken and the test day ended.

The study design of the 2nd test day was similar to the 1st test day, with the exception that \(1^5\)N-Glu was given enterally instead of i.v. After the priming dose was infused i.v. (priming dose = 0.73 \(\mu\)mol/kg), the glutamate tracer was ingested (25 mL/20 min) according to an infusion rate of 0.06 \(\mu\)mol/(kg FFM \(\times\) min). \(1^5\)N-Glu was ingested alone (first 90 min) or together with the carbohydrate-protein drink (last 2 h).

Composition of the carbohydrate-protein drink. The drink contained 28% milk protein (80% protein content, ~10% glutamate) and 72% maltodextrin, dissolved in ultrapure water. All intakes consisted of a fluid ingestion of 0.67 mL/kg body weight \(\times\) 20 min and contained 18 mg protein/kg body weight and 46 mg maltodextrin/kg body weight. In total, ~301 mL enteral nutrition (based on 6 ingestions and a 75-kg subject) was supplied during the study. The complete drink supplied ~8 g protein and 21 g maltodextrin, resulting in an ingestion of ~486 kJ in 2 h. The drink was prepared at 60°C, 1 h before the start of the experiment and kept at 4°C until use, to prevent bacterial growth. The absolute glutamate intake during the feeding period was 631 ± 8, 646 ± 10, and 768 ± 16 nmol/(kg FFM \(\times\) min) in the young, elderly, and COPD patients, respectively, and did not differ among the groups.

Biochemical analyses. Venous and arterialized venous blood was put in a heparinized tube, immediately put on ice, and centrifuged (4°C, 3120 \(\times\) g for 10 min) to obtain plasma. Subsequently, 250 \(\mu\)L plasma was deproteinized with 20 mg sulfosalicylic acid. Samples were frozen in liquid nitrogen and stored at ~80°C until analysis. Analysis of plasma Glu concentration was performed using a fully automated HPLC (Pharmacia) (15). Glu enrichment [tracer:tracee ratio (TTR)] was analyzed by LC-MS (Thermoquest) (16).

Calculations. As described by Darman et al. (17), the rate of glutamate appearance in plasma under steady-state conditions reflects interorgan transport. Therefore, whole-body glutamate metabolism into and out of plasma gives a reflection of whole-body glutamate production in and consumption from plasma, respectively. The following equations were used:

Whole-body glutamate production in the postabsorptive and the fed state:

\[
\text{WB Glu production} = \text{WB rate of appearance (Ra) of Glu} = \frac{\text{infusion rate/TTR in plasma}}{100}
\] (1)

Splanchnic extraction of Glu (SPEGlu) in the postabsorptive and fed states represents the fraction (%) of ingested glutamate, taken up by the gut and liver during its first pass (18):

\[
\text{SPEGlu} = \left[1 - \left(\frac{\text{Ra}_{\text{Glu}}}{\text{Ra}_{\text{Glu}} + \text{Ra}_{\text{Glu}}_{\text{ex}}}\right)\right] \times 100
\] (2)

where \(\text{Ra}_{\text{Glu}}\) and \(\text{Ra}_{\text{Glu}}_{\text{ex}}\) are WB Glu production calculated according to Eq. (1) with either the i.v. or enteral tracer, respectively. The WB rate of appearance of endogenous glutamate (\(\text{Ra}_{\text{Glu}}\)) in the fed state represents the amount of glutamate that is produced in plasma minus the amount of ingested glutamate that reaches plasma (corrected exogenous Glu intake):

![Figure 1](attachment:figure1.png)
WB Ra_{endo-Glu} = WB Ra_{Glu} - \text{corrected exogenous Glu intake} \quad (3)

Corrected exogenous Glu intake

\[ = \text{dietary Glu intake} \times \left[ 1 - \left( \frac{\text{SPE}_{\text{Glu}}}{100} \right) \right] \quad (4)\]

**Statistical analyses.** Results are expressed as means ± SEM. To minimize the variance among the triple measurements of glutamate turnover in the postabsorptive and the fed state, values that were >2 SD from the median were rejected. The mean values of the remaining data were used as whole-body glutamate turnover in the postabsorptive and fed states. ANOVA with the post-hoc Bonferroni test was performed to test whether there were significant differences in general characteristics among the groups. Repeated-measures ANOVA with within variable time (before and after 80 min ingestion) and between variable group (young, elderly, and COPD group) was performed to test effects for plasma glutamate concentration and whole-body glutamate turnover. If there was a group effect, the post-hoc Bonferroni test was used to compare the 3 groups. When there was a significant group × time effect, Student’s paired t test was used to evaluate the effect of the drinks within each group. Differences were considered significant at \( P < 0.05 \). The statistical package SPSS for Windows (Version 11.0; SPSS) was used for data analysis.

**RESULTS**

**General characteristics.** The young group was taller and had lower BMI and fat mass index (FMI) than the elderly and COPD groups [height and BMI (elderly vs. young): \( P < 0.05 \), FMI and BMI (COPD vs. young): \( P < 0.01 \), Table 1]. The BMI and FMI were higher in COPD patients compared with the elderly (\( P < 0.05 \)).

**Plasma glutamate concentration.** Baseline plasma glutamate concentration did not differ among the groups (Table 2). Furthermore, in all 3 groups, plasma glutamate concentration did not change during ingestion of the meal.

**Whole-body rate of appearance of glutamate.** WB Ra_{Glu} was lower in the elderly and in the COPD group compared with the young group (\( P < 0.01 \), Table 2). WB Ra_{Glu} did not differ between the elderly and COPD groups.

**Splanchnic extraction of glutamate.** There was a significant group × time interaction for splanchnic glutamate extraction. Postabsorptive splanchnic extraction of glutamate was lower in the elderly and the COPD group compared with the young group (both \( P < 0.01 \), Table 2), but did not differ between the elderly and COPD group. Feeding did not alter splanchnic glutamate extraction in the young group and the COPD group, but increased splanchnic glutamate extraction in the elderly group (\( P < 0.05 \)).

**Ra of endogenous glutamate.** Because Ra_{endo-Glu} was derived using the corrected exogenous glutamate intake, it can be calculated only during feeding (Table 2). There was a significant group × time interaction for WB Ra_{endo-Glu}. In the young group, WB Ra_{endo-Glu} was higher compared with the elderly and COPD groups (both \( P < 0.01 \) and did not differ from the postabsorptive Ra_{Glu} in the young group. In the elderly and COPD groups, WB Ra_{endo-Glu} was lower than postabsorptive Ra_{Glu} (\( P < 0.01 \)). WB Ra_{endo-Glu} tended (\( P = 0.07 \)) to increase more in the COPD group than in the elderly group.

**DISCUSSION**

The present study shows that postabsorptive whole-body glutamate production and splanchnic glutamate extraction did not differ between healthy elderly and COPD patients but was lower compared with the healthy young group. This suggests that aging but not COPD influences whole-body glutamate production and splanchnic glutamate extraction in the postabsorptive state. Feeding lowered whole-body glutamate production in the elderly and COPD patients but not in the young, indicating that elderly and COPD patients are more dependent on external glutamate intake. Moreover, splanchnic glutamate extraction increased in the elderly during feeding but remained unchanged in the COPD patients, suggesting a COPD-related effect on splanchnic glutamate extraction in the prandial state.

**Whole-body glutamate production in the postabsorptive state.** This study is the first to measure whole-body Ra of glutamate in different population groups (healthy young, elderly, and COPD patients) under various conditions (postabsorptive and fed states). Whole-body glutamate production in the young was greater than in the elderly and COPD patients. This finding suggests that less glutamate is delivered to the organs in healthy elderly and COPD patients. However, because we did not measure glutamate consumption, conclusions about glutamate delivery are only speculative.

Matthews et al. (19) observed that postabsorptive whole-body glutamate production was lower after ingestion of a high-protein diet for 5 d before the test day compared with a normal- and low-protein diet. In the present study, mean daily habitual protein intake, calculated by a food questionnaire, was ~1 g/(kg · d) in all 3 groups, suggesting that the difference in postabsorptive whole-body glutamate production among the groups could not be explained by a difference in habitual protein intake.

**First-pass splanchnic glutamate extraction in the postabsorptive and fed states.** In this study, the percentage of first-pass glutamate extraction in the postabsorptive state in the young (~89%) agreed with the findings of Matthews et al. (7). Interestingly, we found that postabsorptive first-pass splanchnic glutamate extraction in the elderly and COPD group was lower than that in the young (Table 2).

Because the intestine may respond differently to a tracer incorporated into a meal than to ingestion of the tracer alone, we also measured splanchnic glutamate extraction during feeding. Although the 3 groups ingested the same amount of glutamate for 2 h, the effect on feeding differed. First-pass splanchnic glutamate extraction in the young group was not affected by feeding, whereas it increased in the elderly but was still lower than in the young. In the COPD group, first-pass splanchnic glutamate extraction remained stable during feeding. The lower splanchnic glutamate extraction during feeding in the elderly and COPD patients compared with the young may indicate that elderly and COPD patients are more dependent on external (dietary) glutamate delivery.

It was reported previously that first-pass splanchnic extraction of other amino acids such as phenylalanine and leucine is

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**TABLE 1**

| General characteristics of the healthy young and elderly men, and male COPD patients† |
|----------------------|----------------------|----------------------|
|                       | Young                | Elderly              | COPD                 |
| Age, y                | 22 ± 1c              | 63 ± 3a              | 68 ± 4a              |
| Height, m             | 1.82 ± 0.01a         | 1.74 ± 0.02b         | 1.74 ± 0.03b         |
| Weight, kg            | 73.4 ± 2.1           | 77.5 ± 3.7           | 81.8 ± 3.5           |
| BMI, kg/m²            | 22.3 ± 0.7c          | 25.4 ± 0.9b          | 27.2 ± 0.8a          |
| FFMI, kg/m²           | 18.4 ± 0.4           | 19.2 ± 0.9           | 18.9 ± 0.2           |
| FMI, kg/m²            | 3.9 ± 0.4c           | 6.3 ± 0.5b           | 8.4 ± 0.8a           |

† Values are means ± SEM, \( n = 8 \)/group. Within a row, means with superscripts without a common letter differ, \( P < 0.05 \).
higher in the elderly than in the young (18,20). Glutamate is the main energy substrate for the intestine, whereas phenylalanine and leucine are incorporated predominantly into protein. These findings indicate differences in splanchnic extraction between amino acids with aging.

Whole-body endogenous glutamate production in the fed state. There was no acute effect of feeding on whole-body endogenous glutamate production in the young group, indicating that it is relatively independent of external dietary glutamate intake. On the other hand, in the healthy elderly and COPD groups, whole-body endogenous glutamate production decreased during feeding. This decrease is noteworthy because some glutamate from the drink also entered the circulation.

Various routes could cause the decreased whole-body endogenous glutamate production. First, feeding decreases whole-body protein breakdown in both the young and elderly (20,21). Consequently, endogenous amino acid (including glutamate) production and its release in plasma may decrease. However, because glutamate is very compartmentalized (17), the contribution of this route to whole-body glutamate production might be quite small. Second, skeletal muscle extracts large amounts of glutamate from the circulation. However, muscle glutamate can also be formed by the transamination reaction of BCAAs (leucine, isoleucine, and valine) (22). The test meal of the present study contained 20.5 g BCAA/100 g protein, suggesting that these amino acids can serve as an endogenous source for glutamate in muscle. Consequently, endogenous glutamate production from liver and kidney may decrease. However, more research is warranted to test these hypotheses.

Whole-body glutamate production in the healthy elderly vs. COPD patients. There were no baseline differences in whole-body glutamate production or splanchnic glutamate extraction between COPD patients and the elderly. However, there were some differences during feeding. In the elderly, feeding resulted in increased splanchnic glutamate extraction, whereas prandial splanchnic glutamate extraction was not affected in COPD patients. Furthermore, the decrease in endogenous glutamate turnover after feeding tended to be smaller (P = 0.07) in the elderly than in the COPD patients. These differences may indicate a disturbed adaptation of the intestinal glutamate metabolism in response to a meal, whereas endogenous glutamate production may be more dependent on external glutamate intake in COPD patients compared with the healthy elderly.

The fact that the difference in endogenous glutamate production was not significant may be due to several reasons. First, the number of study subjects may have been too small to detect significant differences in response to feeding. Because this is the first study that examined glutamate turnover in the fed state, the power calculation was based on differences in whole-body protein turnover (20). Second, the COPD patients in this study were characterized by moderate airflow obstruction with no or only a mild level of emphysema. A previous study (23) showed that these patients had lower muscle glutamate concentration compared with healthy elderly, but that their concentration was higher than that of emphysema patients. This suggests that disturbances in glutamate metabolism are more pronounced in patients with emphysema. It would be interesting in future studies to compare the present data with data obtained in COPD subgroups such as emphysema patients.

The present study focused on disturbed whole-body and endogenous glutamate production in COPD patients compared with healthy elderly and young subjects. However, to further clarify the underlying mechanisms for the disturbed glutamate metabolism, it will be essential in future studies to investigate whether specific disturbances are present in glutamate consumption.

To summarize, we showed that aging is associated with changes in whole-body and splanchnic glutamate metabolism in the postabsorptive state. Moreover, we suggest that the elderly, and COPD patients in particular, are more dependent on external glutamate intake than the young.
LITERATURE CITED


