Methionine kinetics are altered in the elderly both in the basal state and after vaccination\textsuperscript{1–3}

Sabine Mercier, Denis Breuillé, Caroline Buffière, Johan Gimonet, Isabelle Papet, Philippe Patureau Mirand, and Christiane Obled

ABSTRACT

Background: Inflammation is known to affect sulfur amino acid metabolism. Aging is associated with an increased prevalence of inflammatory conditions, but the metabolism of methionine has been poorly explored in the elderly.

Objectives: The aims of this study were to compare methionine kinetics between elderly and young subjects and to explore the effect of aging on the response to a mild inflammatory challenge induced by a vaccination.

Design: Seven elderly volunteers aged 66–76 y and 8 young volunteers aged 22–26 y were studied before and 2 d after a vaccination (diphtheria, tetanus, poliomyelitis, and typhoid vaccines). Methionine kinetics were measured by using an infusion of L-[1-\textsuperscript{13}C, methyl-\textsuperscript{3}H\textsubscript{3}]methionine in the postabsorptive and fed states.

Results: Before vaccination, the contribution of homocysteine remethylation to methionine-methyl flux (Q\textsubscript{H}) and the ratio of remethylation to homocysteine transsulfuration were significantly lower in the elderly subjects than in the young subjects (P < 0.05). In contrast, the contribution of transsulfuration to methionine transmethylation was higher in the elderly (P < 0.05). Vaccination significantly increased the ratio of transsulfuration to transmethylation and decreased the ratio of remethylation to Q\textsubscript{H} (P < 0.05).

Conclusions: The preferential methionine metabolism toward cysteine synthesis observed after vaccination suggests an increased requirement of sulfur amino acids even in mild inflammatory situations. The main finding of this study is a higher proportion of methionine entering the transsulfuration pathway in elderly subjects before vaccination. This finding suggests an increased cysteine demand during aging.


KEY WORDS Methionine kinetics, vaccination, elderly

INTRODUCTION

Aging is associated with increased concentrations of inflammatory components in the blood, including acute phase proteins and cytokines. Indeed, modest changes in acute phase proteins occur even among apparently healthy elderly individuals. Concentrations of C-reactive protein, \textalpha\textsubscript{1}-acid glycoprotein, or fibrinogen have been found to be slightly but significantly elevated in animals and humans (1–3). Moreover, concentrations of the negative acute phase protein albumin are low (2–4). Such changes are representative of subclinical inflammation. Indeed, a deregulation of the immune system occurs in the elderly (5). Elevated circulating concentrations of interleukin 6 and an imbalance between proinflammatory and antiinflammatory cytokines have been reported during aging (6, 7). However, the metabolic and nutritional implications of this low-grade inflammatory state are unclear.

The low-grade inflammation present in the elderly could affect the immune response to additional injury or diseases. An increased risk of death or of developing diseases has been reported in elderly persons with elevated concentrations of cytokines or acute phase proteins (8, 9). Several studies have suggested an altered acute phase response during infection or endotoxemia. Elderly patients with pneumonia had lower plasma cytokine concentrations and production by peripheral blood monocytes during the acute phase of the infection than did young subjects but had prolonged inflammatory activity (10, 11). Similar results were found in endotoxemia (12).

It is well established that the acute phase response leads to important metabolic changes in general and in protein and amino acid metabolism in particular (13–15), ie, the metabolism of individual amino acids, especially methionine and cysteine, is altered (14). Methionine is mainly metabolized in the liver through the transmethylation-transsulfuration pathway. The transmethylation pathway leads to homocysteine synthesis; homocysteine can then be remethylated to form methionine or catabolized via the transsulfuration pathway, which ultimately forms cysteine. Under normal circumstances, this pathway constitutes a significant source of cysteine (16, 17). In injury, the contribution of the transsulfuration pathway to methionine flux increases, which suggests an increased cysteine requirement in diseases (18, 19). Indeed, cysteine is required for the synthesis of taurine and mainly glutathione, which are important compounds for host defense against oxidative stress (15).

In humans, methionine kinetics has been widely studied in healthy young subjects in relation to the intakes of methionine, cysteine or folate, and vitamin B-6 (20–23). By contrast, to our knowledge, only one study has been devoted to methionine metabolism in the elderly (24), and the influence of inflammation...
has never been explored in elderly subjects. Therefore, the aims of this study were to compare methionine kinetics between elderly and young subjects and to explore the effect of aging on the response to a mild inflammatory challenge induced by a vaccination. The data on inflammatory markers and whole-blood cytokine production of these subjects were published previously (25).

SUBJECTS AND METHODS

Subjects and protocol

The subjects and the study protocol were previously described in detail (25). Briefly, 7 elderly volunteers (3 women and 4 men) aged 66–76 y were compared with 8 young volunteers (4 women and 4 men) aged 22–26 y (Table 1). The volunteers gave their informed consent to participate in the study, which was approved by the local ethical committee for biomedical research (CCPRB Auvergne). The subjects were studied at 2 time points: 6–8 d before vaccination and 2 d after vaccination (Figure 1). The vaccination consisted of an intramuscular injection of a combination of diphtheria, tetanus, and poliomyelitis vaccines and a typhoid vaccine (DT-Polio and Typhim Vi, respectively; Institut Merieux, Lyon, France).

Examples of the menus were furnished to each subject to standardize their diet to provide adequate energy intake on the basis of their estimated energy expenditure and adequate protein intake for 4 d before each infusion study. On the evening before each infusion study, the subjects consumed their meal in the Human Nutrition Unit (Clermont-Ferrand, France). At 0700 on the infusion day, an intravenous catheter was placed in a forearm vein for tracer infusion and in a dorsal vein of the hand for arterialized blood sampling after introduction of the hand into a ventilated box heated to 60 °C. At 0800, a priming dose of sodium [13C]bicarbonate (0.1 mg/kg; Eurisotop, Saint Aubin, France) and L-[1-13C, methyl-2H3]methionine (2.5 μmol/kg; Cambridge Isotope Laboratory, Andover, MA) was administered intravenously, and an infusion of L-[1-13C, methyl-2H3] methionine was begun and continued for 9 h (2.5 μmol·kg⁻¹·h⁻¹). After the first 4 h, the subjects were given small meals every 20 min for 5 h (Figure 1). The diet given as a drink (Clinutren 1.5, 1.5 mL·kg⁻¹·h⁻¹; Nestlé, Marne la Vallée, France) provided five-twelfths the total daily protein and energy intake (1 g·kg⁻¹·d⁻¹ and 27 kcal·kg⁻¹·d⁻¹). The methionine and cyst(e)ine supply was 25 and 7.8 mg·kg⁻¹·d⁻¹, respectively. Blood and breath samples were taken just before the start and at half-hourly intervals during the last 90 min of each metabolic phase.

### TABLE 1

Characteristics of the young and elderly subjects

<table>
<thead>
<tr>
<th></th>
<th>Young subjects (n = 8)</th>
<th>Elderly subjects (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>23 ± 1</td>
<td>70 ± 1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.3 ± 3.1</td>
<td>69.2 ± 2.2</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.73 ± 0.04</td>
<td>1.62 ± 0.03</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.3 ± 0.4</td>
<td>26.3 ± 0.5</td>
</tr>
<tr>
<td>Plasma folates (nmol/L)</td>
<td>13.5 ± 2.1</td>
<td>11.3 ± 2.9</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.4 ± 0.3</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>α1-Acid glycoprotein (g/L)</td>
<td>0.60 ± 0.04</td>
<td>0.95 ± 0.09²</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2.55 ± 0.17</td>
<td>3.76 ± 0.13²</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>43.4 ± 1.3</td>
<td>38.4 ± 1.3²</td>
</tr>
</tbody>
</table>

1 All values are x ± SE. CRP, C-reactive protein. Acute phase protein concentrations were published previously (25).

2 Significantly different from the young subjects, P < 0.05 (unpaired t test).

FIGURE 1. Study protocol. D, day.
(postabsorptive and fed states). Blood was collected in heparin- and EDTA-containing tubes. After centrifugation (2000 × g, 10 min, 4 °C), plasma was stored at −80 °C until analyzed. Breath samples were placed in evacuated tubes and stored at room temperature until measurements of $^{13}$CO$_2$ in the expired air were made by isotope ratio mass spectrometry. Carbon dioxide production was determined in the fasted and fed states by indirect calorimetry (Deltatrac; Datex, Geneva, Switzerland).

To determine whether the experimental diet altered breath $^{13}$CO$_2$ baseline enrichment during the fed state, 6 additional subjects were studied under the same conditions as in the experiment, except that no isotope was infused. The subjects drank the same meal as in the experimental trial, and breath samples were analyzed for $^{13}$CO$_2$ enrichment. Data for these 6 subjects were averaged for the last 90 min of the fed period, and this value was applied to the carbon-13 enrichment determined for each half-hourly period during the fed state.

**Analytic methods**

The free amino acids were isolated from a 1-mL plasma sample as previously described (26). However, 50 µL β-mercaptoethanol was added to the sample to preserve methionine. Plasma enrichment of free methionine was measured by using a tert-butylidimethylsilyl derivative and gas chromatography–mass spectrometry under electron impact ionization (Automass; Thermo Quest Finnigan, Paris, France). Methionine, [1-$^{13}$C]methionine, and [1-$^{13}$C, methyl-$^2$H$_3$]methionine were monitored at mass-to-charge ratios of 320, 321, and 324, respectively. Calibration graphs were prepared from standard mixtures of either [1-$^{13}$C]methionine or [1-$^{13}$C, methyl-$^2$H$_3$]methionine. $^{13}$CO$_2$ enrichment was measured by gas chromatography–isotope ratio mass spectrometry (Micromas; Micromass, Manchester, United Kingdom).

Total, free, and bound cysteine were measured in plasma according to the method of Gaitonde (27) adapted by Malloy et al (28). Briefly, total free cysteine was measured in plasma treated with dithiothreitol before deproteinization. Total free cysteine (free cysteine and cystine) was measured in plasma treated with dithiothreitol after deproteinization. Free cysteine was measured in deproteinized plasma without any reducing treatment. Cystine breakdown, $S_{\text{met}}$ and $T_{\text{met}}$ are the plateau plasma enrichments of [1-$^{13}$C]methionine $(M + 1)$ and [1-$^{13}$C, methyl-$^2$H$_3$]methionine $(M + 4)$, respectively.

**Experimental model**

Methionine kinetics were calculated according to the model of Storch et al (16) and Raguso et al (32) (Figure 2). Briefly, the whole-body methionine-methyl flux rate ($Q_m$) and the whole body methionine-carboxyl flux rate ($Q_c$) were calculated as follows:

$$Q_m = (I × E)/ (E_4 × R) \quad (1)$$

$$Q_c = (I × E_1)/(E_1 + E_4 × R) \quad (2)$$

where $I$ and $E$ are the infusion rate and the isotope enrichment, respectively, of [1-$^{13}$C, methyl-$^2$H$_3$]methionine, and $E_1$ and $E_4$ are the plateau plasma enrichments of [1-$^{13}$C]methionine $(M + 1)$ and [1-$^{13}$C, methyl-$^2$H$_3$]methionine $(M + 4)$, respectively.

The correction factor $R$ was used for the plasma intracellular gradient in methionine enrichment. The value used was 0.8 according to Storch et al (16) because we do not succeed in homocysteine enrichment determination.

In steady state conditions, the flux is the sum of inputs or the sum of outputs. Hence, in the post absorptive state

$$Q_c = B_{\text{met}} + I = S_{\text{met}} + TS \quad (3)$$

and in the fed state

$$Q_c = B_{\text{met}} + A = S_{\text{met}} + TS \quad (4)$$

where $B_{\text{met}}$ is the rate of methionine appearance from protein breakdown, $S_{\text{met}}$ is the rate of methionine disappearance via non oxidative metabolism (an index of the rate of protein synthesis), TS is the transsulfuration rate, and $A$ is the total methionine entry from the tracer and the alimentary input.

In steady state conditions, the whole-body methionine-methyl flux rate can also be related to its individual components as follows:

$$Q_m = I \text{ (or A)} + B_{\text{met}} + \text{RM} = S_{\text{met}} + \text{TM} \quad (5)$$

where $\text{RM}$ is the remethylation rate and $\text{TM}$ is the transmethylation rate.

Therefore

$$\text{RM} = Q_m - Q_c \text{ and } \text{TM} = \text{TS} + \text{RM} \quad (6)$$

TS was calculated as follows

$$\text{TS} = V^{13}\text{CO}_2/(E_1 + E_4 × R) \quad (7)$$

where $V^{13}\text{CO}_2$ is the rate of $^{13}$C output in expired air corrected for the retention of $^{13}$CO$_2$ according to Hoerr et al (33).

Finally, methionine balance, the difference between $S_{\text{met}}$ and $B_{\text{met}}$, was calculated from the difference between transsulfuration and $I$ or $A$. 

**FIGURE 2.** A schematic description of the methionine cycle with its components: transmethylation (TM), remethylation (RM), and transsulfuration (TS). If methionine is labeled on the methyl group and the carboxyl group, the methyl label will be lost during TM, and homocysteine RM will produce methionine labeled only on the carboxyl group. The carboxyl label will be lost during TS and will appear in carbon dioxide in the breath.
TABLE 2
Plasma concentrations of methionine, cysteine, and homocysteine and erythrocyte glutathione concentrations in the postabsorptive state before and after vaccination in the young and elderly subjects.

<table>
<thead>
<tr>
<th></th>
<th>Young subjects (n = 8)</th>
<th>Elderly subjects (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before vaccination</td>
<td>After vaccination</td>
</tr>
<tr>
<td>Methionine (μmol/L)</td>
<td>15.4 ± 1.0</td>
<td>15.6 ± 0.9</td>
</tr>
<tr>
<td>Total cysteine (μmol/L)</td>
<td>225 ± 5</td>
<td>219 ± 6</td>
</tr>
<tr>
<td>Total free cysteine (μmol/L)</td>
<td>130 ± 3</td>
<td>127 ± 4</td>
</tr>
<tr>
<td>Free cysteine (μmol/L)</td>
<td>51 ± 2</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>Free cysteine (μmol/L)</td>
<td>29 ± 1</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>Total homocysteine (μmol/L)</td>
<td>6.7 ± 0.7</td>
<td>6.6 ± 1.0</td>
</tr>
<tr>
<td>Erythrocyte glutathione (mM)</td>
<td>2.04 ± 0.10</td>
<td>2.07 ± 0.11</td>
</tr>
</tbody>
</table>

1 All values are ± SE. Before vaccination, significant differences were observed between the 2 groups for all variables (P < 0.05) except methionine and erythrocyte glutathione (unpaired t test). The effects of age and vaccination were analyzed by using a repeated-measures ANOVA (with age as the between-subject factor and vaccination as the within-subject factor).
2 Effect of age: P < 0.01; effect of vaccination: NS; age-by-vaccination interaction: NS.
3 Effect of age: P < 0.05; effect of vaccination: NS; age-by-vaccination interaction: NS.

Statistical methods and data evaluation
 Differences in plasma folates and acute phase protein concentrations, measured in the postabsorptive state before vaccination, between the young and old subjects were tested by unpaired t test. For amino acid concentrations obtained only in the postabsorptive state before the tracer infusion began, differences were tested by repeated-measures analysis of variance (STATVIEW; SAS Institute Inc, Cary, NC) for the effect of age (between-subject factor) and vaccination (within-subject factor). Otherwise, the effects of age, vaccination, and nutritional state (postabsorptive or fed) were analyzed by using a repeated-measures analysis of variance (with age as the between-subject factor and vaccination and nutritional state as the within-subject factors). Differences were considered to be significant when P < 0.05.

RESULTS

Subjects
 The elderly subjects included in our study were stringently selected for good health, and clinical and biological features matched the admission criteria of the SENIEUR protocol (25). However, as reported previously (25), these subjects had greater plasma concentrations of some acute phase proteins, such as α1-acid glycoprotein and fibrinogen and tended to have higher concentrations of C-reactive protein (P = 0.077) than did the young subjects, which suggested a low-grade inflammatory state. In contrast, the plasma concentration of folates was not significantly different between the 2 groups (Table 1).

There was no significant effect of age or of vaccination on plasma methionine and erythrocyte glutathione concentrations. Vaccination had no effect on plasma cysteine and homocysteine concentrations. In contrast, the plasma concentration of the various forms of cysteine and of total homocysteine were greater in the elderly than in the young subjects (Table 2).

Methionine fluxes
 The isotopic enrichments of plasma methionine and of 13C in expired air during the fasting and fed periods before and after vaccination are summarized in Table 3 for each group.

Regardless of age and treatment, there was a significant effect of nutritional state on methionine fluxes, which were generally increased in the fed state (P < 0.001) (Table 4). Before vaccination, methionine-methyl flux was greater in the young than in the elderly subjects. There was a significant interaction between age and vaccination (P = 0.027), which indicated that the effects of vaccination on methionine-methyl flux differed between the young and elderly subjects. Indeed, no difference between the 2 groups was observed after vaccination. For the other methionine fluxes, there were no significant interactions between age, vaccination, and nutritional state, but there were significant main effects (Table 4). Without regard to vaccination and nutritional state, methionine-carboxyl flux (P = 0.036) was lower in the elderly than in the young subjects. A similar trend (P = 0.077)

TABLE 3
Plasma isotope enrichments, 13CO2 enrichment, and carbon dioxide production in the young and elderly subjects before and after vaccination in the fasted and fed states.

<table>
<thead>
<tr>
<th></th>
<th>Young subjects (n = 8)</th>
<th>Elderly subjects (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before vaccination</td>
<td>After vaccination</td>
</tr>
<tr>
<td>Fasted</td>
<td>Fed</td>
<td>Fasted</td>
</tr>
<tr>
<td>[1-13C, methyl-2H3]Methionine (MPE)</td>
<td>12.0 ± 0.5</td>
<td>9.2 ± 0.5</td>
</tr>
<tr>
<td>[1-13C]Methionine (MPE)</td>
<td>2.52 ± 0.11</td>
<td>1.98 ± 0.13</td>
</tr>
<tr>
<td>Breath 13CO2 enrichment (MPE × 103)</td>
<td>3.8 ± 0.3</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>Carbon dioxide production (mL/min)</td>
<td>185 ± 10</td>
<td>224 ± 10</td>
</tr>
</tbody>
</table>

1 All values are ± SE. MPE, mole percent excess.
and decreased methionine balance (4.30 by age (5.33 0.022). Homocysteine remethylation was significantly reduced methionine balance/L50512 vaccination (5.00 young and the elderly subjects, respectively; acid metabolism is regulated through homocysteine production and mild inflammation on methionine kinetics. Sulfur amino state was found for all variables, and a significant effect of age was found for /L1155 (fasted or fed) were analyzed by using a repeated-measures ANOVA (with age as the between-subject factor and vaccination and nutritional state as the within-subject factor). A significant effect of the nutritional state was found for all variables, and a significant effect of age was found for Qm, Qc, and RM (P < 0.05). The effects of age, vaccination, and nutritional state (fasted or fed) were analyzed by using a repeated-measures ANOVA (with age as the between-subject factor and vaccination and nutritional state as the within-subject factors).

was observed for methionine transmethylation. Whatever the age of the subject and the nutritional state, vaccination significantly increased transsulfuration (5.18 ± 0.17 and 5.73 ± 0.17 μmol · kg⁻¹ · h⁻¹ before and after vaccination, respectively; P = 0.035) and decreased methionine balance (4.30 ± 0.17 and 3.68 ± 0.17 μmol · kg⁻¹ · h⁻¹ before and after vaccination, respectively; P = 0.022). Homocysteine remethylation was significantly reduced by age (5.33 ± 0.27 and 4.00 ± 0.29 μmol · kg⁻¹ · h⁻¹ in the young and the elderly subjects, respectively; P = 0.006) and vaccination (5.00 ± 0.14 and 4.44 ± 0.14 μmol · kg⁻¹ · h⁻¹ before and after vaccination, respectively; P = 0.022).

Relative activities of various components of the methionine cycle

The proportion of methionine transmethylation that entered transsulfuration was significantly increased by age (P = 0.024), vaccination (P = 0.0006), and nutritional state (P = 0.0001) with no interaction between age, vaccination, and nutritional state (Figure 3). The ratio of remethylation to transsulfuration was decreased by all factors (age: P = 0.035; vaccination: P = 0.0005; and nutritional state: P = 0.0001). A significant interaction was found between vaccination and nutritional state (P = 0.006), which indicated that the effect of vaccination was less pronounced in the fed state than in the post absorptive state. The proportion of methionine-methyl flux provided by homocysteine remethylation (remethylation rate/methionine-methyl flux) was significantly reduced by age (P = 0.013) and vaccination (P = 0.013), but there was no interaction between age, vaccination, and nutritional state.

DISCUSSION

The aims of this study were to investigate the effects of aging and mild inflammation on methionine kinetics. Sulfur amino acid metabolism is regulated through homocysteine production from methionine (transmethylation) and the balance between the 2 pathways of homocysteine utilization (transsulfuration and remethylation). An understanding of the effect of aging on these metabolic pathways is essential to improving knowledge about sulfur amino acid requirements in the elderly.

The model used to calculate methionine kinetics has several limits. The main point is the uncertainty of the true intracellular tracer enrichment. The approach generally used is the measurement of the plasma enrichment of an intracellular-derived metabolite of the tracer, such as α-ketoisocaproate, when labeled leucine is used as tracer (34). The enrichment of plasma α-ketoisocaproate has been found about 20% lower than plasma leucine enrichment (34) and Storch et al (16) have proposed to use this correction for methionine. Plasma homocysteine or cystathionine enrichments can be used to estimate intracellular methionine enrichment and some data have been reported recently. Values of 40 to 84% have been found for the ratio of plasma homocysteine to methionine enrichments (20, 21, 35) and 54 to 66% for that of cystathionine to methionine (21, 35). These results suggest that methionine fluxes calculated with a correction factor of 0.8 would be underestimated. Moreover, it was found that the ratio of α-ketoisocaproate to leucine enrichment did not change in subjects and conditions similar to those explored in this study (26, 36, 37). We hypothesized that the same correction factor could be applied in all groups and conditions in our study. Therefore, we can assume that the corrections based on homocysteine enrichments would change the absolute values of methionine fluxes but not our conclusions. The second limit of the model is the indirect estimation of transsulfuration. A better estimate would be provided by cysteine synthesis. However, only sulfur from methionine appears in cysteine and such measurements requiring radioactive tracers are not possible in humans.

The methionine fluxes found in the group of young subjects were in the range of those previously reported with a similar correction factor for intracellular methionine enrichment (16, 17,

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**TABLE 4**

Methionine fluxes in the young and elderly subjects before and after vaccination in the fasted and fed states

<table>
<thead>
<tr>
<th></th>
<th>Young subjects (n = 8)</th>
<th>Elderly subjects (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before vaccination</td>
<td>After vaccination</td>
</tr>
<tr>
<td>Qm Methionine⁵</td>
<td>24.9 ± 1.1</td>
<td>32.6 ± 1.8</td>
</tr>
<tr>
<td>Qc Methionine⁴</td>
<td>19.2 ± 0.5</td>
<td>25.2 ± 1.1</td>
</tr>
<tr>
<td>TM⁴</td>
<td>8.2 ± 0.6</td>
<td>13.5 ± 0.8</td>
</tr>
<tr>
<td>TS⁴</td>
<td>3.3 ± 0.2</td>
<td>7.0 ± 0.5</td>
</tr>
<tr>
<td>RM⁵</td>
<td>5.0 ± 0.4</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>Methionine balance</td>
<td>−0.9 ± 0.9</td>
<td>9.5 ± 0.5</td>
</tr>
</tbody>
</table>

¹ All values are ± SE. Qm, methionine-methyl flux; Qc, methionine-carboxyl flux; TM, transmethylation; TS, transsulfuration; RM, remethylation; methionine balance = TS − intake (alimentation and tracer). Before vaccination, the effects of age and nutritional state (fasted or fed) were analyzed by using a repeated-measures ANOVA (with age as the between-subject factor and nutritional state as the within-subject factor). A significant effect of the nutritional state was found for all variables, and a significant effect of age was found for Qm, Qc, and RM (P < 0.05). The effects of age, vaccination, and nutritional state (fasted or fed) were analyzed by using a repeated-measures ANOVA (with age as the between-subject factor and vaccination and nutritional state as the within-subject factors).

² There was a significant effect of age and nutritional state and a significant age-by-vaccination interaction (P < 0.05 for all).

³ There was a significant effect of age and nutritional state (P < 0.05 for both).

⁴ There was a significant effect of nutritional state (P < 0.05).

⁵ There was a significant effect of vaccination and nutritional state (P < 0.05 for both).

⁶ There was a significant effect of age, vaccination, and nutritional state (P < 0.05 for all).
Regardless of the nutritional state, methionine-methyl and methionine-carboxyl fluxes were lower in the elderly than in the young subjects when expressed per kilogram body weight. However, because the percentage of lean body mass decreases with aging, the difference between the 2 groups could be explained by a difference in lean body mass. In a large number of men and women across the adult age span (between 19 and 87 y) with controlled diets and physical exercise, Short et al (40) showed that leucine and phenylalanine kinetics decline with age, even after correction for fat-free mass. Until now, the effect of aging on the methionine cycle was not clear because data on young and old subjects were reported in separate studies (16, 17, 24, 38). Of the components of the methionine cycle, transsulfuration was better preserved than was transmethylation because the ratio of transsulfuration to transmethylation was greater in the elderly than in the young subjects. Moreover, the ratio of remethylation to transsulfuration and the proportion of the methionine-methyl flux provided by homocysteine remethylation decreased in the elderly subjects, despite normal folate status. Indeed, it is well known that homocysteine remethylation is impaired in folate deficiencies (20). Therefore, the lower remethylation observed in the elderly group was not due to folate deficiency, which suggests another explanation. Taken together, these results indicate that methionine metabolism was preferentially directed toward transsulfuration and, therefore, toward cysteine synthesis in the elderly. Inflammation and oxidative stress were found to activate the methionine cycle and transsulfuration, which allowed an increased cysteine availability for glutathione synthesis (19, 29, 41). Indeed, glutathione is the most important intracellular antioxidant of the body, and the maintenance of glutathione pools is essential for the defense of the organism (15). Blood glutathione concentrations were not low in the elderly group, in contrast with previous studies (42, 43). The concentration of some acute phase proteins, such as fibrinogen, was higher in the group of elderly subjects than in the young subjects in the present study. This observation indicated a moderate basal inflammatory state in this group of elderly subjects (aged 66–76 y), who were selected as healthy (25). Data on acute inflammation (18, 19, 44) allow us to hypothesize that the preferential orientation of methionine metabolism toward transsulfuration in elderly persons may be related to their low-grade inflammatory state.

Vaccination, used as a model of moderate inflammatory stress, was also associated with changes in the methionine cycle in favor of transsulfuration and, therefore, toward cysteine synthesis.
of a predominance of homocysteine transsulfuration over remethylation with no change in the transmethylation rate. These results are in general agreement with the perturbation of sulfur amino acid metabolism found in acute diseases. The contribution of the transsulfuration pathway to methionine flux was shown to be greater in burn patients than in control subjects, and an increased cysteine synthesis from methionine was found in septic rats (18, 19). In addition, cysteine catabolism was reduced, whereas its utilization for glutathione synthesis was increased (29, 44–46). All these data strongly suggest increased cysteine utilization, even under a mild inflammatory stress such as vaccination.

Homocysteine metabolism was oriented in favor of cysteine synthesis after vaccination in young and elderly subjects. However, this change seemed to be less pronounced in the elderly than in the young subjects. For example, the ratio of transsulfuration to transmethylation increased by 21% and 11% after vaccination in the postabsorptive and fed states, respectively, in the young subjects instead of 11% and 8%, respectively, in the elderly subjects. These results suggest that methionine utilization may be preserved in the elderly subjects after vaccination so that homocysteine remethylation is better maintained than in young subjects. Therefore, the competition between homocysteine remethylation and transsulfuration seems to be more severe in the elderly, which leads to a trend for a decrease in blood glutathione. Another explanation could be defective metabolic adaptation to an inflammatory challenge, as already established for the immune system with advancing age (5). In the same subjects, we found lower increases in acute phase proteins and cytokine production in blood cultures in response to vaccination in the elderly subjects than in the young subjects (25). It can be hypothesized that the age-related differences in the metabolic response to vaccination could be linked to alterations in the inflammatory response, and studies in more severe inflammatory states could help to test this hypothesis.

In conclusion, methionine metabolism was affected after vaccination in agreement with previous data obtained in acute diseases. The preferential methionine metabolism toward cysteine synthesis confirms an increased requirement of sulfur amino acids in these situations. The main finding of this study was a higher proportion of methionine entering the transsulfuration pathway in elderly subjects before vaccination, probably because of a low-grade inflammatory state in these subjects. These data suggest that healthy aging may be associated with an increased cysteine requirement related to a low-grade inflammatory state. Moreover, in elderly subjects, the increased cysteine requirement in response to any inflammatory stress occurs in a situation in which the cysteine requirement is already elevated. The consequences of these findings relative to sulfur amino acid requirements during aging warrant further study.

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