Reply to CP Unocc et al.

Dear Editor:

In their letter, Unocc et al. make a nice comment on the important influence of bone mineral density (BMD) on physical performance in older women. Unfortunately, we did not assess BMD very thoroughly in our trial (1) because we used dual-energy X-ray absorptiometry only to measure appendicular muscle mass and other variables relating to body composition. However, because this method does provide some information on bone (whole-body BMD), we would like to report this variable in an effort to answer the comments by Unocc et al.

We found no difference in whole-body BMD between our treated and control groups at baseline (0.98 ± 0.10 vs. 1.02 ± 0.09 g/cm²; P = 0.31), and these values did not change significantly over the course of 12 wk in either group (−0.04 ± 0.02 vs. −0.08 ± 0.03 g/cm²; P = 0.11). Nor did the changes in whole-body BMD significantly correlate with any of the physical performance or muscle strength measures investigated.

The lack of any effect of magnesium supplementation on BMD is probably due to several factors. The most important seems to be the length of follow-up in our trial, which may have been too short to see any changes in BMD. Unocc et al. rightly mention the findings of the COMB (Combination of Micronutrients for Bone) study (2), in which 12 mo of magnesium supplementation led to a significant increase in BMD; however, the study also involved supplementation with other minerals and vitamins, so the effect of magnesium could not be separated from that of other micronutrients. Another important point to consider is that whole-body BMD is not the best method for diagnosing osteoporosis, for which sites such as the spine and hip are more reliable (3). Furthermore, BMD is not the best method for diagnosing osteoporosis, for which sites such as the spine and hip are more reliable (3).

In conclusion, the lack of any effect of magnesium supplementation on BMD in our trial should be considered with caution and studies focusing on BMD are needed to arrive at a better understanding of the effect of magnesium supplementation on bone.

None of the authors had any conflicts of interest to declare.

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REFERENCES


Glutamine supplementation, citrulline production, and de novo arginine synthesis: is there a relation?

Dear Editor:

We would like to comment on the recent publication by Buijs et al. (1). The authors hypothesized that a parenteral supplement of glutamine stimulates citrulline formation and enhances de novo arginine synthesis. To test this hypothesis, they conducted an experiment with stable isotopes in patients undergoing abdominal surgery in the postprandial period. On the basis of an increase in plasma citrulline and arginine concentrations, the authors concluded that glutamine, in fact, increases citrulline and arginine production. We would like to explain in this letter why the article by Buijs et al. does not add any new information to their previous work (2) and omits crucial new insights in the glutamine-citrulline-arginine pathways that have emerged since.

Concentrations of plasma citrulline and arginine have previously been observed to increase with the provision of glutamine (3, 4) but are usually difficult to interpret because they are the product of the rates of entry into and removal from the circulating pool. For this reason, we find it puzzling that the authors decided to rely on the concentration data and not on the fluxes they reported on these amino acids. Fluxes, which unequivocally indicate the whole-body rate of appearance in the circulating pool, do not seem to support the conclusions reached by Buijs et al. Because the authors did not measure fluxes in patients before supplementation, for comparison we have to rely on the available data in humans. Although the flux of citrulline measured is comparable to those determined by other researchers in fasted healthy subjects, the flux of arginine was not only lower than in healthy controls but even lower than in subjects who consumed arginine-free diets (5–7). In addition, the de novo synthesis rate of arginine reported by Buijs et al. seems to be identical to the one determined in postprandial surgical patients (2) (Table 1). Therefore, the question of whether glutamine supplementation has an effect on citrulline production and de novo synthesis of arginine remains to be answered.

The second point the authors made was that glutamine is the precursor for citrulline and arginine synthesis, because they observed the incorporation of the infused [2-15N]glutamine label into citrulline and arginine, based on measurement of the total mass of complete molecules by liquid chromatography–mass spectrometry (LC-MS). We have already in 2010 shown the limitations of using a nitrogen label when the intent is to trace carbon (8). This is particularly true for the determination of a precursor-product relation between glutamine, citrulline, and arginine molecules with 2, 3, and 4 N atoms in their molecules, respectively. For [2-15N]glutamine to provide meaningful precursor-product information,
15N should not be reincorporated into the amido (or 5-N) group of glutamine and the 15N label should label only the amino (or 2-N) position of citrulline and arginine. However, this is not the case. For [2-15N]glutamine to contribute to the synthesis of citrulline, it needs to be deamidated first to [2-15N]glutamate. Glutamate, in turn, serves as both the precursor for glutamate semialdehyde and as the nitrogen donor for the δ (5-N) position of ornithine, the immediate precursor for citrulline synthesis. In addition, the role of glutamine as one of the

### Table 1

Whole-body rate of appearance of glutamine, citrulline, arginine, and de novo arginine synthesis

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Buijs et al. (1)²</th>
<th>Castillo et al. (6)³</th>
<th>Castillo et al. (5)³</th>
<th>Luiking et al. (7)³</th>
<th>Ligthart-Melis et al. (2)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrulline</td>
<td>9.5 ± 1.0</td>
<td>11.3 ± 3.3</td>
<td>13.3 ± 3.1</td>
<td>13.7 ± 4.1</td>
<td>6.2 ± 0.6</td>
</tr>
<tr>
<td>Arginine (fasted)</td>
<td>30.4 ± 2.2</td>
<td>60.2 ± 5.4</td>
<td>44.9 ± 5.4</td>
<td>64 ± 19</td>
<td>42 ± 2.9</td>
</tr>
<tr>
<td>Arginine (arginine-free diet)</td>
<td>47.5 ± 8.8</td>
<td>47.5 ± 10.2</td>
<td>37.5 ± 10.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine de novo²</td>
<td>4.5 ± 0.6</td>
<td>5.5 (fasted and fed)</td>
<td>—</td>
<td>11.9 ± 6.6</td>
<td>4.9 ± 0.9</td>
</tr>
</tbody>
</table>

¹ Values are presented as whole-body rates of appearance (μmol · kg⁻¹ · h⁻¹).
² Values are means ± SEMs.
³ Values are means ± SDs.
⁴ Whole-body flux of citrulline into arginine, which represents de novo arginine synthesis.

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**FIGURE 1**

The metabolic pathways by which the amino-N of glutamine contributes to ≥1 nitrogen atoms of citrulline and arginine. Isotopomers cannot be distinguished by LC-MS. However, the LC-MS/MS analytic approach enables the measurement of the positional isomers of glutamine, citrulline, and arginine. CIT, citrulline; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; ORN, ornithine; TCA, tricarboxylic acid.
main fuels for the gut is well established (9); and the ammonia thus generated can be incorporated into the ureido group of citrulline and subsequently the guanidino group of arginine, thus labeling the third nitrogen group of these compounds. Furthermore, [2,15N]glutamine can contribute the other nitrogen in the guanidino group of arginine via aspartate through transamination. These pathways are shown in Figure 1. In addition, the infusion of [2,15N]glutamine also generates [5-15N]glutamine (8). By using LC–tandem MS (LC-MS/MS) and looking at 1-aspartate through transamination. These pathways are shown in

can contribute the other nitrogen in the guanidino group of arginine via aspartate through transamination. These pathways are shown in Figure 1. In addition, the infusion of [2,15N]glutamine also generates [5-15N]glutamine (8). By using LC–tandem MS (LC-MS/MS) and looking at different fragments we have shown this scrambling of the amino nitrogen of glutamine and the labeling of the 3 nitrogen groups of citrulline in mouse models (8). We recently confirmed using the same analytic approach these findings in postprandial surgical patients who received alanyl-[2-15N]glutamine as a stable isotope to investigate the precursor relation between glutamine, citrulline, and arginine (GC Ligthart-Melis GC, unpublished results, 2014). Had Buijs et al. used a similar LC-MS/MS analytic approach that allows for the measurement of the positional isomers of glutamine and citrulline (10), they would have observed this same phenomenon. For this reason and because every single assumption on the use of [2-15N]glutamine to determine precursor-product relations is violated, the conversion data generated by Buijs et al. cannot be interpreted.

We agree with Buijs et al. that “the development of optimal nutrition strategies relies on distinct insights into the effect of a supplemental component on metabolic pathways.” For these reasons, we believe that the correct approach to the interpretation of a precursor-mineral supplementation in surgical patients has to be done on the basis of fluxes and that the precursor-product relation between glutamine and citrulline can only be determined utilizing tracers that follow the carbon skeleton of these compounds.

None of the authors declared a conflict of interest related to this letter.

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Reply to GC Ligthart-Melis et al.

Dear Editor:

We thank Ligthart-Melis et al. for their insightful comments on our stable isotope study that was recently published in the Journal. Hereby, we would like to make some remarks on their comments.

First, Ligthart-Melis et al. state that we concluded that glutamine increases citrulline and arginine production on the basis of an increase in plasma citrulline and arginine concentrations. Instead, as described throughout the article, we concluded that renal arginine production from citrulline is increased in patients receiving glutamine on the basis of our renal arginine flux data compared with our previously published renal arginine flux (on which Ligthart-Melis was the first author) in patients without a supplement of glutamine (1, 2). As outlined, renal arginine production from citrulline was 3.0 ± 0.7 mmol · kg⁻¹ · h⁻¹ in surgical patients receiving a glutamine supplement compared with 1.5 ± 0.7 mmol · kg⁻¹ · h⁻¹ in the surgical patients without glutamine administration. Thus, the renal de novo arginine production was doubled. Our concentration data only substantiate this quantitative renal flux observation [Figure 2 in reference 1]. This is the first study to show a quantitative increase in renal arginine production in patients receiving 0.5 g · kg⁻¹ · d⁻¹ alanyl-glutamine intravenously by using a stable isotope method, and therefore we feel this study provides new interesting information for clinical nutritional research.

Our study clearly shows an increase in renal arginine flux in patients receiving extra glutamine compared with the patients who did not in our previous study (2). Furthermore, the whole-body rate of appearance of citrulline in the glutamine-supplemented group was 50% higher than in patients without glutamine supplementation (9.5 ± 1.0 vs. 6.2 ± 0.6 mmol · kg⁻¹ · h⁻¹, respectively) (1, 2). We agree with Ligthart-Melis et al. that de novo arginine synthesis on the whole-body level was similar to that in the postprandial surgical patients and whether glutamine supplementation has an effect on de novo synthesis of arginine on the whole-body level remains indefinite. Also, Table 1 in the letter by Ligthart-Melis et al. provides flux data on healthy volunteers in a greatly different (clinical) setting, which are not comparable to our data conducted in patients during major abdominal surgery (1, 3–5).

We studied whole-body and renal metabolism of glutamine, citrulline, and arginine in surgical patients receiving 0.5 g · kg⁻¹ · d⁻¹ alanyl-glutamine intravenously by using the stable isotopes [2-15N]glutamine, [5-13C,2H₄]citrulline, and [15N₂]arginine and...