Oral glutamine and amino acid supplementation inhibit whole-body protein degradation in children with Duchenne muscular dystrophy1–3

Elise Mok, Catherine Eléouet-Da Violante, Christel Daubrosse, Frédéric Gottrand, Odile Rigal, Jean-Eudes Fontan, Jean-Marie Cuisset, Joëlle Guilhot, and Régis Hankard

ABSTRACT
Background: Glutamine has been shown to acutely decrease whole-body protein degradation in Duchenne muscular dystrophy (DMD).

Objective: To improve nutritional support in DMD, we tested whether oral supplementation with glutamine for 10 d decreased whole-body protein degradation significantly more than did an isonitrogenous amino acid control mixture.

Design: Twenty-six boys with DMD were included in this randomized, double-blind parallel study; they received an oral supplement of either glutamine (0.5 g · kg FFM−1 · d−1) or an isonitrogenous, non-specific amino acid mixture (0.8 g · kg FFM−1 · d−1) for 10 d. The subjects in each group were not clinically different at entry. Leucine and glutamine metabolisms were estimated in the postabsorptive state by using a primed continuous intravenous infusion of [1-13C]leucine and [2,15N]glutamine before and 10 d after supplementation.

Results: A significant effect of time was observed on estimates of whole-body protein degradation. A significant (P < 0.05) decrease in the rate of leucine appearance (an index of whole-body protein degradation) was observed after both glutamine and isonitrogenous amino acid supplementation [5 ± SEM: 136 ± 9 to 124 ± 6 μmol · kg fat-free mass (FFM)−1 · h−1 for glutamine and 136 ± 6 to 131 ± 8 μmol · kg FFM−1 · h−1 for amino acids]. A significant (P < 0.05) decrease in endogenous glutamine due to protein breakdown was also observed (91 ± 6 to 83 ± 4 μmol · kg FFM−1 · h−1 for glutamine and 91 ± 4 to 88 ± 5 μmol · kg FFM−1 · h−1 for amino acids). The decrease in the estimates of whole-body protein degradation did not differ significantly between the 2 supplemental groups.

Conclusion: Oral glutamine or amino acid supplementation over 10 d equally inhibits whole-body protein degradation in DMD. Am J Clin Nutr 2006;83:823–8.

KEY WORDS Duchenne muscular dystrophy, children, randomized controlled clinical trial, supplement, oral administration, stable isotopes, protein metabolism, glutamine, leucine, amino acids

INTRODUCTION
Nutritional support was shown to improve outcome in several clinical conditions (1). Certain nutrients, such as glutamine, have distinct properties that are separate from their role of providing calories or nitrogen (2, 3), and they are called “pharmacconutrients.” Glutamine has a protein-sparing effect in cell cultures, animal models, and humans (4). This effect may be useful in catabolic situations, when cellular glutamine concentrations decline (5, 6).

Duchenne muscular dystrophy (DMD) is a genetic disease characterized by progressive muscle wasting and a reduced functional capacity. Protein metabolism in DMD, which has been studied by using stable isotope tracers and nitrogen balance, suggests that the cause of muscle wasting is a reduction in muscle protein synthesis or an increase in protein degradation (7–9). Furthermore, muscle wasting in DMD is associated with a decrease in glutamine turnover and a more negative whole-body leucine balance (10). Because glutamine is mainly produced by the muscle, and muscle mass is severely reduced in DMD, the need for glutamine may be increased in persons who have this disease. Moreover, in DMD, as in other protein-wasting situations, the intramuscular glutamine concentration is low (11). Thus, in DMD, as in situations of catabolic stress, glutamine may be considered a conditionally essential amino acid (10).

In a previous study using stable isotope methodology, we showed that acute (5 h) oral glutamine decreased whole-body protein degradation in DMD compared with placebo (12). The objectives of the present study were to test, in the same population, whether this effect persists when glutamine is administered...
over longer periods (10 d) and whether the effect is specific to glutamine.

SUBJECTS AND METHODS

The protocol was approved by the Paris-Bichat Ethics Committee (Comité Consultatif pour la Protection de la Personne dans la Recherche Biomédicale) and conducted according to the principles of the Declaration of Helsinki. All families gave their written informed consent after a thorough explanation of the study protocol by the investigator to both the parents and the children. The study was performed at the Robert Debré Hospital Clinical Investigation Center (CIC 9202 INSERM-Assistance Publique-Hôpitaux de Paris). Subjects were selected on the basis of the following eligibility criteria: male sex, aged 7–15 y, and diagnosis of DMD by clinical history, muscle biopsy sample, or molecular biology. Children with acute pathology who had either cardiac or respiratory insufficiency or those who were taking corticosteroids at the time were excluded from the study. Children had stable weights and clinical status before study entry.

The children were hospitalized for 24 h on 2 separate days, before and after a 10-d oral supplement of either l-glutamine or an isonitrogenous control made of a mixture of amino acids. The children were admitted the night before the tracer infusion. They were instructed to take the supplemental glutamine or control amino acid mixture once per day in the morning mixed with yogurt at breakfast. Breath samples were obtained si-multaneously with blood samples, and the carbon dioxide production rate (VCO2) was measured at baseline and over the last hour of the infusion. (1300 the following day). Whole-body glutamine exchange in plasma (RaGln,i n 13CO2) was measured with an infrared spectrometer (IRIS; Wagner Analysen Technik Vertrien GmbH, Bremen, Germany).

The rate of leucine appearance (RaLeu in mol/kg/h) and leucine oxidation (OxLeu) were calculated with plasma KIC enrichments (EiKIC). RaLeu was calculated as:

$$\text{RaLeu} = \frac{i_{13C}\text{Leu}}{(E\text{Leu}/E\text{KIC}) - 1}$$

where $i_{13C}\text{Leu}$ is the tracer infusion rate (in $\mu$mol $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$) and $E\text{Leu}$ is the leucine tracer enrichment in the intravenous infusate (16).

The leucine oxidation rate (OxLeu in $\mu$mol $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$) was calculated as:

$$\text{OxLeu} = \frac{(E^{13}\text{CO}_2 \times V\text{CO}_2 \times 60)}{(1/E\text{Leu} - 1/E\text{KIC})/k\text{CO}_2} \times 22.4 \times 1000 \times \text{weight}$$

where $E^{13}\text{CO}_2$ is the 13CO2 enrichment in expired air (mol % enrichment); VCO2 is the carbon dioxide production rate (in mL/min), as measured by indirect calorimetry (GEM; Europa scientific, Crewe, United Kingdom) (12): 60 converts min to h; kCO2 is the fractional recovery of carbon dioxide in expired air; 22.4 converts mL gas to mmol; 1000 converts mmol to $\mu$mol; and weight is in kg.

The nonoxidative leucine disposal rate (NOLD, in $\mu$mol/kg/h), an index of protein synthesis, was calculated as:

$$\text{NOLD} = \text{RaLeu} - \text{OxLeu}$$

Whole-body glutamine exchange in plasma (RaGln, in $\mu$mol $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$) was calculated as:

$$\text{RaGln} = i_{15N}\text{Gln} \times [(E\text{Gln}/E\text{Gln}) - 1]$$

where $E\text{Gln}$ and $E\text{Gln}$ are the glutamine enrichments (mol% enrichment) in the intravenous infusate and plasma, respectively, and $i_{15N}\text{Gln}$ is the glutamine tracer infusion rate (in $\mu$mol $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$).

In the postabsorptive state, by definition, the endogenous rate of appearance of glutamine (RaEndoGln) in plasma equals RaGln.

Endogenous (body) glutamine arising from protein degradation (BGl) was calculated as:

$$B\text{Gln} = \frac{\text{RaGln} \times (0.07 \times 146)}{(0.08 \times 131)}$$

where 0.07 and 0.08 are the assumed glutamine and leucine contents of body protein (g/g protein), respectively, and 146 and 131 g/mol are the glutamine and leucine molecular weights, respectively.

Glutamine de novo synthesis ($D\text{Gln}$) was calculated as:

$$D\text{Gln} = \text{RaEndoGln} - B\text{Gln}$$

Because fat-free mass (FFM) represents an index of metabolically active tissue, and because anthropometric measures, such as weight, can be misleading in DMD due to excess body fat (17), measures of whole-body leucine and glutamine metabolism were adjusted for FFM.

Body composition was estimated by using monofrequency (50 kHz) bioelectrical impedance analysis (101 Q; RJL systems, Clinton Township, MI) (18), because a bioelectrical impedance analysis provides estimates close to the labeled-water dilution...
GLUTAMINE AND AMINO ACID SUPPLEMENTATION IN DMD

TABLE 1
Baseline characteristics of children with Duchenne muscular dystrophy

<table>
<thead>
<tr>
<th></th>
<th>Glutamine group (n = 13)</th>
<th>Amino acid group (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>12.5 ± 0.5</td>
<td>11.0 ± 0.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>39.2 ± 3.0</td>
<td>37.9 ± 3.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>154 ± 4</td>
<td>144 ± 5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.7 ± 1.3</td>
<td>17.8 ± 1.0</td>
</tr>
<tr>
<td>BMI z score</td>
<td>−0.7 ± 0.9</td>
<td>0.3 ± 0.6</td>
</tr>
</tbody>
</table>

*All values are x ± SEM. The groups did not differ significantly at baseline (Mann-Whitney U test).*

The present study showed an effect on protein metabolism that was not measured during glutamine or amino acid administration but was measured 24 h after supplementation ceased while plasma amino acid concentrations were normal.

**RESULTS**

The baseline characteristics of the 26 children with DMD are presented in Table 1. No significant differences in age, weight, height, BMI, and BMI z scores were observed between the 2 supplemental groups at baseline (Table 1). No statistically significant differences were observed between the groups at baseline for all outcome measures except for plasma isoleucine concentrations, which were significantly lower in the glutamine group than in the amino acids group at baseline (56 ± 3 μmol/L for the glutamine group compared with 69 ± 3 μmol/L for the amino acids group, P < 0.05) (Table 2).

A significant effect of time on estimates of whole-body protein degradation was observed (Figure 1). After 10-d supplementation with either glutamine or isonitrogenous amino acids, a significant (P < 0.05) decrease in Ra_Gln, an index of whole-body protein degradation (x ± SEM: 136 ± 9 to 124 ± 6 μmol · kg FFM⁻¹ · h⁻¹ for the glutamine group and 136 ± 6 to 131 ± 8 μmol · kg FFM⁻¹ · h⁻¹ for the amino acids group), and in B_Gln (91 ± 6 to 83 ± 4 μmol · kg FFM⁻¹ · h⁻¹ for the glutamine group and 91 ± 4 to 88 ± 5 μmol · kg FFM⁻¹ · h⁻¹ for the amino acids group, P < 0.05) was observed (Figure 1). The decrease in whole-body leucine and glutamine turnover did not differ significantly between the 2 supplemental groups (Figure 1). Other estimates of leucine and glutamine turnover were not affected, namely Ox_Leu (30 ± 2 compared with 31 ± 4 μmol · kg FFM⁻¹ · h⁻¹ for the glutamine group and 29 ± 3 compared with 27 ± 4 μmol · kg FFM⁻¹ · h⁻¹ for the amino acids group), NOLD (105 ± 9 compared with 91 ± 6 μmol · kg FFM⁻¹ · h⁻¹ for the glutamine group and 105 ± 4 compared with 102 ± 6 μmol · kg FFM⁻¹ · h⁻¹ for the amino acids group), and D_Gln (308 ± 30 compared with 327 ± 23 μmol · kg FFM⁻¹ · h⁻¹ for the glutamine group and 352 ± 38 compared with 369 ± 25 μmol · kg FFM⁻¹ · h⁻¹ for the amino acids group). Furthermore, measures of body composition (muscle mass, weight, FFM, and percentage fat mass) were not significantly affected after glutamine or isonitrogenous amino acid administration for 10 d (Table 3).

Apart from plasma taurine concentrations, which showed a significant (P < 0.001) decrease over time in both supplemental groups (63 ± 4 to 47 ± 4 μmol/L for the glutamine group and 58 ± 4 to 50 ± 6 μmol/L for the amino acids group), plasma amino acid concentrations were not significantly affected after 10-d supplementation with either glutamine or amino acids (Table 2).

A significant effect of time was observed on blood urea nitrogen. Blood urea nitrogen significantly (P < 0.001) increased (but remained within the normal range) after 10-d supplementation with either glutamine or the amino acid mixture (3.7 ± 0.2 compared with 1.9 ± 0.2 for the amino acids group). Glutamine and amino acid administration both decreased plasma insulin (5.1 ± 0.7 compared with 7.4 ± 0.1 g · kg⁻¹ · d⁻¹ for the amino acids group). Glutamine and amino acid suplementations were safe and well tolerated at the doses prescribed.

**DISCUSSION**

Oral glutamine or isonitrogenous amino acid supplementation for 10 d equally inhibit whole-body protein degradation in children with DMD. The present study showed an effect on protein metabolism that was not measured during glutamine or amino acid administration but was measured 24 h after supplementation ceased while plasma amino acid concentrations were normal.
Similarly, a protein feeding pattern induced chronic regulation of protein turnover that persisted 1 d after the end of dietary treatment (22). The response of protein metabolism to supplemental protein intake can also be compared with the studies by Motil et al (23, 24). These previous studies used a similar methodology to the present work and showed an effect of increased protein intake on whole-body amino acid metabolism after a 12 h fast. To date, mechanisms of the “chrono-biological” regulation of whole-body protein metabolism are not fully understood.

In the present study, plasma glutamine and amino acid concentrations did not increase after supplementation. Hence, the effect of glutamine on whole-body protein metabolism cannot be attributed to increased plasma glutamine concentrations, as shown in previous studies (25). We used the same glutamine dose as that used in previous studies (4, 12, 26), which was selected to double the plasma glutamine concentration and concurrently explore whole-body protein metabolism. The present study suggests that the effects of glutamine are not solely driven by substrate availability. Alternatively, glutamine uptake by the intestine might be sparing other amino acids and substrates. Previous studies of enterally administered glutamine in very-low-birthweight infants showed similar findings; that is, there was no change in plasma glutamine concentration after glutamine administration, thereby suggesting uptake of glutamine by the gut (27, 28).

The significant decrease in plasma taurine concentrations after supplementation with either glutamine or amino acids may reflect an improvement in muscle cellular status, because the plasma taurine concentration was shown to be elevated in the mdx mouse model of DMD (29). Moreover, mdx mice fed a high-protein diet showed a decrease in the elevated plasma taurine concentrations, which was associated with decreased muscle protein catabolism due to decreased protein degradation (29).

Although the decrease in whole-body protein degradation did not significantly differ between the supplemental groups, the glutamine group showed a larger decrease in RaLeu than did the amino acid group (9% compared with 4%). Moreover, the decrease in plasma taurine after 10 d glutamine and isonitrogenous amino acid supplementation was significant, P < 0.01.

### Table 2

<table>
<thead>
<tr>
<th>Glutamine group</th>
<th>Amino acid group</th>
</tr>
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<tbody>
<tr>
<td>(α = 13)</td>
<td>(α = 12)</td>
</tr>
<tr>
<td><strong>Before</strong></td>
<td><strong>After</strong></td>
</tr>
<tr>
<td>Leucine (μmol/L)</td>
<td>128 ± 5</td>
</tr>
<tr>
<td>Isoleucine (μmol/L)</td>
<td>56 ± 3</td>
</tr>
<tr>
<td>Valine (μmol/L)</td>
<td>288 ± 13</td>
</tr>
<tr>
<td>Methionine (μmol/L)</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>Phenylalanine (μmol/L)</td>
<td>51 ± 1</td>
</tr>
<tr>
<td>Threonine (μmol/L)</td>
<td>138 ± 10</td>
</tr>
<tr>
<td>Glutamine (μmol/L)</td>
<td>166 ± 12</td>
</tr>
<tr>
<td>Glutamate (μmol/L)</td>
<td>511 ± 17</td>
</tr>
<tr>
<td>Aspartate (μmol/L)</td>
<td>47 ± 3</td>
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<tr>
<td>Cysteine (μmol/L)</td>
<td>233 ± 13</td>
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<tr>
<td>Serine (μmol/L)</td>
<td>296 ± 37</td>
</tr>
<tr>
<td>Arginine (μmol/L)</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>Tyrosine (μmol/L)</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>Taurine (μmol/L)</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>Asparagine (μmol/L)</td>
<td>139 ± 4</td>
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<tr>
<td>Citrulline (μmol/L)</td>
<td>66 ± 6</td>
</tr>
<tr>
<td>Ornithine (μmol/L)</td>
<td>63 ± 4</td>
</tr>
<tr>
<td>Histidine (μmol/L)</td>
<td>59 ± 3</td>
</tr>
<tr>
<td>Hydroxyproline (μmol/L)</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Proline (μmol/L)</td>
<td>53 ± 3</td>
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<tr>
<td></td>
<td>72 ± 2</td>
</tr>
<tr>
<td></td>
<td>18 ± 1</td>
</tr>
<tr>
<td></td>
<td>160 ± 9</td>
</tr>
</tbody>
</table>

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1 All values are ± SEM.

2 Essential amino acid.

3 The groups differed significantly at baseline, P < 0.05 (Mann-Whitney U test). The effects on plasma isoleucine change at 10 d were not significant (analysis of covariance with baseline plasma isoleucine as the covariate and group as the factor).

4 The effect of time on the plasma taurine concentration was significant, P < 0.01 (2-factor repeated-measures ANOVA). The decrease in plasma taurine after 10 d glutamine and isonitrogenous amino acid supplementation was significant, P < 0.01.
given enteral glutamine showed less dramatic anabolic effects on protein metabolism (27, 31, 32) than when patients without DMD were given intravenous glutamine (2, 33). Also, glutamine treatment in DMD may have different effects depending on the stage of the disease (34). However, we could not stratify by age in the present study because of the sample size. Larger age-stratified studies are needed to test this hypothesis.

The present study showed no significant effect of glutamine or isonitrogenous amino acids on the RaGln measured 24 h after the last dose (ie, while the plasma glutamine concentration was normal). In our previous study (12), oral glutamine administration was associated with a decrease in RaGln due to a decrease in both glutamine protein degradation (Bglu) and glutamine de novo synthesis (Dglu). In contrast with the previous study, we observed a decrease only in Bglu without any effect on Dglu. Experimental conditions could account for the difference in findings. In the previous study, glutamine kinetic variables were measured during glutamine administration, and plasma glutamine concentrations doubled. In the present study, the glutamine concentration remained within the normal range.

The present study compares the effects of oral glutamine and isonitrogenous supplementation over 10 d on whole-body protein metabolism in patients with DMD. The results suggest that nitrogen supplementation might have a protein-sparing effect in DMD that results from a decrease in protein degradation. This short-term approach provides a mechanism to explain the recent results from a randomized, placebo-controlled trial that showed less deterioration in measures of function with long-term (6 mo) nitrogen supplementation (glutamine and creatine) in younger children with DMD (34). Decreased body protein breakdown after short-term nitrogen supplementation has implications for present disease therapies and could be a possible route for therapeutic modulation in DMD as well as in other protein-wasting situations.

EM contributed to the analysis of data and the writing of the manuscript. CE-DV contributed to the collection and analysis of data. CD, FG, OR, J-EF, and EM contributed to the collection of data. JG contributed to the analysis of data. RH contributed to the design of the experiment, collection and analysis of data, and the writing of the manuscript. The authors had no conflicts of interest to report.

**TABLE 3**

<table>
<thead>
<tr>
<th>Glutamine group (n = 13)</th>
<th>Amino acid group (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MM (kg)</strong></td>
<td><strong>Weight (kg)</strong></td>
</tr>
<tr>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>5.6 ± 0.6</td>
<td>5.6 ± 0.8</td>
</tr>
<tr>
<td>39.2 ± 3.0</td>
<td>39.4 ± 3.0</td>
</tr>
<tr>
<td>27.4 ± 1.7</td>
<td>27.4 ± 1.7</td>
</tr>
<tr>
<td>28.3 ± 4.1</td>
<td>26.5 ± 4.4</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM. MM, muscle mass; FFM, fat-free mass; FM, fat mass. The differences between groups at baseline were not significant (Mann-Whitney U test). The effects were not significant (2-factor repeated-measures ANOVA).

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**REFERENCES**

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