The Effect of Dietary Arginine on Lean Body Mass

Amy Klash

ABSTRACT

The Arginine, a conditionally essential amino acid, has been shown to enhance nitrogen retention. This study sought to determine the effects of dietary arginine on preservation of body protein stores in mice post influenza challenge. Mice were fed an AIN93G diet (6.6g/kg arginine, n=4), the AIN93G diet with 2% arginine (20g/kg, n=5), or a diet made isonitrogenous to the arginine supplemented diet with the addition of 28g/kg alanine to AIN93G (n=5). After 33 days on the respective diets, mice were challenged with influenza and daily weights were obtained. Mice were sacrificed and the lungs, liver, heart, spleen, thymus, kidneys, intestine, and leg were harvested on day 16 post-challenge. Diet significantly contributed to the difference in mean weights post-challenge, with AIN93G fed mice losing less of their baseline weight. There was no difference among any of the diet groups in protein content of leg muscle or liver or in mean organ weights. The isonitrogenous (alanine) diet showed detrimental effects in that one of the five mice receiving the diet died and two had to be euthanized before the end of the experiment. These data suggest that arginine does not contribute to organ weights or protein content in the leg or liver and that alanine may not be a suitable isonitrogenous diet for comparison studies.

INTRODUCTION

Arginine is a non-essential amino acid because it can by synthesized by the human body. However during times of growth, injury, sepsis, or other stressors, the synthesis of this amino acid cannot keep pace with the body’s increased demands and a relative deficiency may occur [1]. Adding dietary arginine has beneficial effects on various metabolic responses during such deficiencies. Studies by Seifter and others have demonstrated that supplemental arginine enhances wound healing and immune function in injured rats [2, 3]. Arginine has also been shown to enhance nitrogen retention in stressed animals versus an isonitrogenous glycine and/or ornithine diet [4, 5]. Although it tends to accelerate the reversal of negative nitrogen balance, supplemental arginine has not been proven to significantly contribute to a positive nitrogen balance [4-8]. Sitren and Fisher found no differences in weights of the liver, spleen, or adrenals in those animals receiving additional arginine even though these animals retained significant amounts of nitrogen [9]. It has been suggested that this excess nitrogen may lie in muscle mass, which is addressed by this study.
Surgery or illness typically results in weight loss, negative nitrogen balance, and some degree of immune dysfunction. During a non-lethal influenza infection, a mouse may lose up to approximately 30% of its body weight [10]. This stressed state changes normal nutrient metabolism so that the body metabolizes protein from lean body stores. Because protein is not as calorically dense as fat, more protein mass must be broken down in order to provide adequate energy [11]. We propose that mice on an arginine supplemented diet will preserve more lean body mass and have more concentrated protein stores following influenza challenge than mice fed control diets.

**MATERIALS AND METHODS**

**Animals and Diets**

Fourteen female 3-4 week old BALB/c mice from Harlan Laboratories (Indianapolis, IN) were caged individually in a 22°C light controlled room (12 h light:dark cycle) and allowed to acclimate for 7 days prior to the experiments. Mice were randomly assigned to either an AIN93G diet (6.6 g/kg arginine), the AIN93G diet with 2% arginine (20g/kg), or a diet made isonitrogenous to the arginine diet with the addition of 28g/kg alanine to AIN93G (Table 1). The animals had access to diets and tap water ad libitum. Mice were subsequently moved to the University of Florida's infectious disease isolation unit for influenza challenge with three days for acclimation prior to infection. The University of Florida's Animal Care and Use Committee approved all methods for this experiment.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Composition of Diets [12]</th>
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</thead>
<tbody>
<tr>
<td>Ingredient (g/kg)</td>
<td>AIN-93G</td>
</tr>
<tr>
<td>Casein*</td>
<td>200</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>0</td>
</tr>
<tr>
<td>L-Arginine HCl</td>
<td>0</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>397.5</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>132</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>70</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
</tr>
<tr>
<td>AIN-93 Mineral Mix</td>
<td>35</td>
</tr>
<tr>
<td>AIN-93 Vitamin Mix</td>
<td>10</td>
</tr>
<tr>
<td>Choline Bitartate</td>
<td>2.5</td>
</tr>
<tr>
<td>Tert-Butylhydroquinone</td>
<td>0.014</td>
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</tbody>
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*Casein contains 3.3g/100g
Influenza Infection

On day 33 of the experiment, mice were infected with 2*10^5 TCID50 Influenza A/Port Chalmers/1/73 (H3N2). The mice were first anesthetized with intraperitoneal injection of 3:20 xylazine/ketamine diluted 1:4 in sterile phosphate buffered saline (PBS). Each mouse received 10 microliters of virus per nostril. Baseline weight was taken the day of influenza challenge.

Tissue Preparation and Protein Assay

The animals were sacrificed and the lungs, liver, heart, spleen, thymus, kidneys, intestine, and leg were harvested from the animals after approximately 50% had reached their baseline weight and maintained this weight for 3 days (16 days post-infection). The mice were anesthetized and killed via cardiac puncture and exsanguinations. The organs of each mouse were frozen in liquid nitrogen, and stored at -86°C until needed.

All organs were weighed. The leg muscles (between the patella and ankle) and the liver were homogenized separately in 20% weight/volume PBS with 1 mM ethylene diamine tetraacetic acid and protease inhibitor cocktail (100 mM AEBSF, 0.08 mM Aprotonin, 2.2 mM Leupeptin, 4.0 mM Bestatin, 1.5 mM Pepstatin A, and 1.4 mM E-64) obtained from Sigma (St. Louis MO). Protein content of the leg muscles and liver was determined using BioRad Laboratories' Detergent Compatible Protein Assay Kit (Richmond, CA) following the instructions provided by the manufacturer.

Statistical Analysis

Baseline weights, organ weights, and protein content were analyzed using a one-way analysis of variance (ANOVA) with Duncan's a posteriori comparisons. Post viral challenge mouse weights were compared using a univariate repeated measures ANOVA. Data are expressed as means +/- standard error of the mean, and a P value of Œ 0.05 denotes significance.

RESULTS

On the first day of infection (33 days on the diets), mean body weights were not significantly different among diet groups. However, after 17 days of infection, mean weights for each diet group differed significantly (P £ 0.05). The group receiving the AIN93G diet (n=4) lost less average weight post-challenge than the mice receiving the arginine supplemented diet (n=5) or the isonitrogenous control (n=5). Weight change for individual mice is shown in Figure 1. Three of the mice on the isonitrogenous control (alanine) diet lost a striking amount of weight and had severe, non-improving symptoms of influenza. One died of natural causes and the other two were euthanized since recovery seemed remote. There was no difference among any of the diet groups in protein content in the liver or leg (Figure 2) nor was there a significant difference in mean organ weight among diet groups (Figure 3).
Figure 1. Weights as a Percent of Baseline.

Figure 2. Protein Content in Whole Organs
DISCUSSION

This study sought to determine the effects of arginine supplementation on mice that had lost a significant amount of weight due to infection with influenza virus. It was proposed that mice on the arginine-supplemented diet would have greater protein content and organ weights and lose less overall body weight than those receiving the isonitrogenous control or AIN93G diets. The arginine-supplemented mice did not show any significant benefits from the diet in this study. Average organ weights and average protein content in the leg or liver did not differ among any of the groups and arginine-supplemented mice lost more body weight on average than the group receiving AIN93G.

Several mechanisms have been proposed to account for the beneficial effect of arginine seen in previous studies. Its action as a secretagogue of growth hormone and insulin is thought to be a major contributor to its varied actions in the body [13, 14]. Growth hormone increases lean body mass as well as promotes lipolysis and nitrogen retention [15]. Intravenous infusion of arginine has been shown to increase plasma insulin levels in protein-depleted rats [6]. Protein synthesis can be stimulated by insulin in vitro and in the body, however the amount of insulin needed to stimulate synthesis in vivo was twice as much as the concentration normally found in the blood of fed animals [16].

It may not be appropriate to make comparisons to those studies that infuse arginine directly into the bloodstream. Oral arginine may not go into the bloodstream at levels consistent with the supplementation. Since there was no difference in the muscle and protein stores of the mice, it is difficult to determine at which point arginine failed to evoke a response. An amino acid profile would have been helpful in this study to determine how much of the supplemented amino acid actually reaches the bloodstream.

Alanine has been shown to be non-contributory to the variability of tissue weights or tissue protein content and is not detrimental to animals after stress [17]. The deaths of the three mice in the isonitrogenous group may have skewed the results by the data collected not being representative of this group as a whole. Supplementation with alanine in the absence of added arginine, a conditionally essential urea cycle intermediate, may be harmful to young, stressed animals because of the added metabolic stress of excreting excess nitrogen.

This study did not show that arginine provides any beneficial effects toward recovery of body or organ weight or of tissue protein content post-influenza challenge. Furthermore, because mice on the isonitrogenous control diet suffered such severe illness, alanine may not be appropriate for supplementation in this experimental model.
REFERENCES


