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Basic nutritional investigation

Arginine-supplemented enteral nutrition in critically ill diabetic and obese rats: A dose-ranging study evaluating nutritional status and macrophage function

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ABSTRACT

Objective: Critically ill diabetic and obese patients are at high risk of complications. Arginine availability is lowered in diabetes and in stress situations, yet arginine is necessary for immune response, mainly by its action through nitric oxide (NO). These facts argue for arginine-supplemented diets in critically ill patients. However, studies have raised concerns about possible adverse effects of such diets in intensive-care patients. We therefore analyzed the metabolic and immunologic effects of an arginine-enriched diet in stressed diabetic-obese rats.

Methods: Zucker Diabetic Fatty rats (*fa/fa*) were made endotoxemic by an intraperitoneal injection of lipopolysaccharide and then fed 4-d enteral nutrition enriched with arginine (ARG group) or a non-essential amino acid mix (NEAA group). The two groups each were subdivided into three subgroups: the ARG subgroups received 0.5 g (ARG0.5), 2 g (ARG2), and 5 g (ARG5) of arginine per kilogram daily, and the NEAA groups were made isonitrogenous with the corresponding ARG subgroups (NEAA0.5, NEAA2, and NEAA5). Plasma and urinary biomarkers were measured. Cytokine and NO production levels and inducible NO synthase and arginase protein levels were determined from peritoneal macrophages.

Results: The survival rate was lower in the ARG5 and NEAA5 subgroups than in all the other subgroups. The nitrogen balance was higher in the ARG5 group than in the NEAA5 group. Plasma triacylglycerol levels were lower in the ARG2 group than in the NEAA2 group. Interleukin-6, tumor necrosis factor-α, and NO production in the macrophages decreased and arginase-1 was upregulated in the ARG-treated rats.

Conclusions: In this model, mortality was increased by the nitrogen burden rather than by arginine per se. Arginine improved nitrogen balance and had an anti-inflammatory action on macrophages by regulating NO production, probably through arginase-1 expression.

Introduction

Obesity and type 2 diabetes have increased in populations worldwide, and medical teams in hospital intensive care units (ICUs) are thus increasingly faced with such patients. In the ICU context, pre-existing diseases such as diabetes and obesity are associated with protein wasting, a higher risk of complications, a higher risk of infectious episodes [1,2], and ultimately increased mortality [3,4], although this last point is still debated [1]. Optimized nutritional therapy therefore may improve the outcome of these patients.

In a search for a new nutritional strategy, we considered the evidence that obesity is associated with a state of chronic low-grade inflammation that contributes to the development of insulin resistance and diabetes in addition to a depressed immune status and an impaired metabolic response to stress [5–7]. There is no pharmacologically based approach to nutrition

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in these patients. We therefore examined the utility of "immune-enhancing diets" (IEDs) in this specific context of type 2 diabetes and obesity associated with sepsis. We especially focused on the potential beneficial effects of the pharmaconutrient arginine.

Arginine is a conditionally essential amino acid in stress situations because its endogenous synthesis may not be sufficient in highly catabolic conditions such as sepsis [8]. Studies have found that plasma arginine concentration is decreased in insulin-resistant and obese Zucker rats [5,9] and in the general ICU population, which generally exhibits what is termed *arginine deficiency syndrome* [10] associated with various degrees of insulin resistance [11].

Arginine is at the crossroads of the inflammation pathway and the immune response by nitric oxide (NO) synthesis and has been shown to improve insulin sensitivity [12], to be essential for the immunologic response [13,14], and to exert anti-inflammatory effects [15]. Arginine is also associated with a decrease in infectious complication rates and a shorter hospital stay in surgical patients [16]. Nevertheless, because arginine-enriched diets have also been associated with harmful effects in critically ill septic patients [17,18], guidelines have recommended that IEDs, particularly those enriched with arginine, should not be used in ICU patients [19]. However, the conclusions are not based on evidence and have been criticized [20,21]. Furthermore, recent data [22] have suggested that arginine may not be the IED component implicated, and we underline that there is still no dose-ranging study available.

Macrophages are one of the main actors of innate immunity and have been found to present functional impairments in experimental models of diabetes and obesity [23]. Macrophage activity is largely controlled by the action of NO, whose sole precursor is arginine by the NO synthase (NOS) pathway. In these cells, arginine can be metabolized by NOS into NO and citrulline or by arginase into ornithine and urea [24]. Thus, it appears that two major keys to macrophage immune function are arginine availability and arginine metabolism.

Given the lower arginine availability observed in diabetes [5,9] and exacerbated in sepsis [15], our working hypothesis was that nutritional supplementation with arginine might improve systemic metabolism and innate immunity-related impairments in catabolic type 2 diabetic and obese rats. Clearly, diabetes associated with obesity and injury modifies protein metabolism and cell signaling in a way that renders the action of arginine-enriched diets unpredictable, thus justifying relevant experimentation. Our aim was to evaluate the effects of arginine-supplemented enteral nutrition compared with an isonitrogenous standard nutrition. We conducted a dose-ranging study including a high dosage of arginine to assess the potential adverse effects of excessive arginine intake [19]. For this purpose, we used a model of male Zucker Diabetic Fatty (ZDF) rats homozygous for non-functional leptin receptors (*fa*/*fa*) that develop type 2 diabetes and obesity [25].

Materials and methods

Chemicals, materials, and antibodies

All chemicals and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Sigma (Saint-Quentin Fallavier, France). Purina 5008 was from obtained from IPS (Wellingborough, UK). Sondalis HP was kindly provided by Nestlé Clinical Nutrition (Noisiel, France). Isoflurane was from Minerve (Esternay, France); the Linco Kit was from Labodia (Paris, France); and the Parameter Total NO/Nitrite/Nitrate, Quantikine Rat Tumor Necrosis Factor-α (TNF-α)/TNFSF1A, and Quantikine Interleukin-6 (IL-6) kits were from R&D Systems (Lille, France). Laemmli sample buffer was from Bio-Rad (Marne-La-Coquette, France); Hybond-C Extra membranes were from GE Healthcare (Orsay, France); the electrochemiluminescence (ECL) western blotting kit and ECL Hyperfilms were from Amersham GE Healthcare (Orsay, France); and anti-inducible NOS (iNOS) antibody was from BD Transduction Laboratories (Lexington, UK, USA). Anti–arginase-1 antibody was a gift from Dr. S. M. Morris; anti–arginase-2 antibody was a gift from Dr. O. Levillain; anti–α-actin antibody was from Prosci Incorporated (Montluçon, France); polyclonal goat anti-rabbit immunoglobulin/horseradish peroxidase antibody was from DakoCytomation (Glostrup, Denmark); and goat polyclonal antibody to chicken immunoglobulin Y (horseradish peroxidase) antibody was from Abcam (Paris, France).

Animals and study design

Animal care and experimentation complied with French regulatory requirements (French Ministry of Agriculture and Forestry authorization no. P2.CC.074.09), and one of the authors (L. C.) is authorized to perform experiments on rodents (government authorization no. 005226). The protocol used 11-wk-old male ZDF rats (Charles River, L'Arbresle, France). ZDF rats are characterized by type 2 diabetes, insulin resistance with hyperinsulinism, hyperphagia, and hyperlipidemia. They were housed at 21 ± 1°C with a 12-h light/dark cycle and had free access to water. During a 1-wk acclimatization period, they were fed ad libitum on a Purina 5008 diet (total calories were 27% proteins, 17% fat, and 56% carbohydrates).

Experimental groups and design

At the end of the acclimatization period, the rats were randomized into ARG and control groups. The ARG group was split into three subgroups, 0.5 g (ARG0.5; $n = 6$), 2 g (ARG2; $n = 6$), and 5 g (ARG5; $n = 6$) of arginine per kilogram of body weight per day, and placed under continuous enteral nutrition, which was infused at 25 mL/100 g of body weight per day using Sondalis HP (1.5 kcal/mL, protein 75 mg/mL). These three dosages were selected to be clinically relevant because nitrogen and energy requirements in adult rats are approximately 10 times higher than in humans. The rats received body weight–indexed extra arginine corresponding in humans to about 1.7, 6.7, and 17 g/d, respectively. The standard enteral nutrition control group, i.e., the non-essential amino acid (NEAA) group, was split into three subgroups, NEAA0.5 ($n = 6$), NEAA2 ($n = 6$), and NEAA5 ($n = 6$), and received Sondalis HP enteral nutrition enriched with a NEAA mix (alanine, asparagine, glycine, histidine, proline, and serine) made isonitrogenous to the ARG0.5, ARG2, and ARG5 subgroups, respectively. All groups had isovolumic and isocaloric intakes (320 kcal/kg of body weight per day).

The experiments were repeated seven times until six rats per subgroup were reached.

Because studies with arginine treatment have been conducted in lean endotoxemic rats and in non-endotoxemic diabetic obese rats [26,27], we focused on the effects of arginine on endotoxemic and diabetic obese rats.

On day −4, the rats underwent gastrostomy under anesthesia with 1 L of O_2/2.5% isoflurane, as previously described [28]. They were then placed in metabolism cages and fed ad libitum with Purina 5008 for 4 d. On day 0, the rats were given an intraperitoneal injection of *Escherichia coli* lipopolysaccharide (0127:B8 serotype) at the endotoxic dose of 5 mg/kg [29]. This model was chosen because it is well validated, provides reproducible results, and mimics several events associated with sepsis [30,31]. The rats were then placed under continuous enteral nutrition for 4 d. Body weight was measured daily. The 24-h urine samples were collected and stored at −20°C until analysis. Euthanasia was carried out by decapitation after anesthesia with isoflurane. Blood was sampled, and the macrophages were isolated from the peritoneal cavity (see below for details). Plasma samples were stored at −80°C until analysis.

Urinary parameter

Urinary creatinine was measured by the Jaffé reaction on an Olympus AU600 analyzer (Rungis, France) [32].

Nitrogen balance

Nitrogen balance corresponds to the difference between daily nitrogen intake and daily urinary nitrogen output (grams per 24 h). Urinary nitrogen (grams per liter) was quantified by pyrochemiluminescence [33] using an Antek 9000 apparatus (Antek, Houston, TX, USA).

Plasma insulin, glucose, creatinine, triacylglycerols, and amino acid measurements

Plasma insulin was determined by radioimmunoassay using a Linco Kit (RI 13K). Glycemia was measured by a hexokinase method using an Olympus AU600 analyzer [34]. Plasma creatinine and triacylglycerols were routinely measured by