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Original article

Effect of specific amino acids on hepatic lipid metabolism in fructose-induced non-alcoholic fatty liver disease

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SUMMARY

Background & aim: Fructose diets have been shown to induce insulin resistance and to alter liver metabolism and gut barrier function, ultimately leading to non-alcoholic fatty liver disease. Citrulline, Glutamine and Arginine may improve insulin sensitivity and have beneficial effects on gut trophicity. Our aim was to evaluate their effects on liver and gut functions in a rat model of fructose-induced non-alcoholic fatty liver disease.

Methods: Male Sprague–Dawley rats (n = 58) received a 4-week fructose (60%) diet or standard chow with or without Citrulline (0.15 g/d) or an isomolar amount of Arginine or Glutamine. All diets were made isonitrogenous by addition of non-essential amino acids. At week 4, nutritional and metabolic status (plasma glucose, insulin, cholesterol, triglycerides and amino acids, net intestinal absorption) was determined; steatosis (hepatic triglycerides content, histological examination) and hepatic function (plasma aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin) were assessed; and gut barrier integrity (myeloperoxidase activity, portal endotoxemia, tight junction protein expression and localization) and intestinal and hepatic inflammation were evaluated. We also assessed diets effects on caecal microbiota.

Results: In these experimental isonitrogenous fructose diet conditions, fructose led to steatosis with dyslipidemia but without altering glucose homeostasis, liver function or gut permeability. Fructose significantly decreased *Bifidobacterium* and *Lactobacillus* and tended to increase endotoxemia. Arginine and Glutamine supplements were ineffective but Citrulline supplementation prevented hypertriglyceridemia and attenuated liver fat accumulation.

Conclusion: While nitrogen supply alone can attenuate fructose-induced non-alcoholic fatty liver disease, Citrulline appears to act directly on hepatic lipid metabolism by partially preventing hypertriglyceridemia and steatosis.

Abbreviations: NAFLD, non-alcoholic fatty liver disease; Cit, citrulline; Gln, glutamine; Arg, arginine; AA, amino acids; NEAA, non-essential amino acids; TG, triglycerides; HTG, hepatic triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; NASH, non-alcoholic steatohepatitis; IR, insulin resistance; TLR4, toll like receptor 4; HDNL, hepatic *de novo* lipogenesis; VLDL, very-low-density lipoprotein; ZO1, zonula occludens 1; NO, nitric oxide; BW, body weight.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a chronic obesity-associated liver disease and a frequent sign of metabolic syndrome [1]. Its pathophysiology is multifactorial and not completely understood. However, experimental and epidemiological studies suggest that fructose intake is associated with NAFLD onset and development [1,2]. Fructose intake has been shown to affect the gut–liver axis through changes in liver metabolism and gut barrier function. Indeed, both experimental and clinical studies have shown that increased fructose intake can result in hepatic steatosis together with insulin resistance (IR), elevated plasma triglycerides (TG), and oxidative stress in the liver [2]. In the gut, chronic fructose feeding has been demonstrated to induce increased intestinal permeability, through the loss of occludin expression, and increased endotoxin translocation [3]. This could activate Kupffer cells *via* Toll-like receptor 4 (TLR-4) on the cell membrane, leading to excessive TNF-α production and hepatic inflammation as demonstrated in mice [4]. Furthermore, evidence from clinical and experimental studies suggest gut microbiota may also play a role in NAFLD pathogenesis [5].

In this context, recent studies underline the possible interest of Arginine (Arg), Glutamine (Gln) and Citrulline (Cit) supplementation as a way to not only preserve intestinal trophicity but also support whole body metabolism in various pathophysiological situations [6].

Arg plays a key role in the regulation of epithelial barrier and the maintenance of junctions between cells [6]. It also modulates immune response and promotes tissue healing [7]. These effects of Arg may help decrease gut bacterial and endotoxin translocation [8]. Plasma Arg levels are decreased in IR settings [7] and Arg supplementation has been demonstrated to improve insulin sensitivity [7].

Gln, a preferential energy substrate for enterocytes and immune cells, helps preserve intestinal mucosa in inflammatory situations [9]. Besides its immunomodulatory properties, it preserves the intestinal barrier *via* stabilization of tight junction proteins [9,10]. In addition, Gln is a precursor for the synthesis of glutathione required for antioxidative defenses [11].

Cit is involved in peripheral Arg availability and helps regulate protein and energy metabolism [12]. Plasma Cit is mainly the result of its production by enterocytes and its use by the kidney for Arg synthesis [12]. It is closely correlated to functional enterocytic mass [12]. Cit activates muscle protein synthesis [13], improves insulin sensitivity, has antioxidative properties [12], and, in a model of massive (80%) small bowel resection, was shown to improve gut adaptation [14].

Given the effects of these three specific amino acids (AA) on insulin sensitivity and intestinal trophicity, we hypothesized that Arg, Cit or Gln supplementation may be able to limit the fructose-induced alterations that lead to the development of NAFLD.

Thus, the aim of this study was to investigate the effects of Arg, Gln or Cit supplementation on intestinal and hepatic functions in a rat model of fructose-induced NAFLD.

2. Materials and methods

2.1. Animals

Fifty-eight male SpragueDawley rats (Charles River, Villemoisson-sur-Orge, France) weighing 190–220 g were housed individually in a temperature-controlled room under a 12/12-h light–dark cycle for one week. They were given *ad libitum* access to water and standard rodent chow (UAR A04, SAFE, Augy, France).

Animal care and experimentation complied with both French and EC regulations governing animal care and experimentation. All procedures were conducted in accordance with the guidelines issued by the Comité Régional d'Ile-de-France animal care committee, which also approved the study protocol (registration number: CEEA34.CM.015.11).

2.2. Experimental design

Rats were randomly allocated to 8 groups (n = 7–8 rats per group) to receive either a standard rodent chow (C) or a fructose-enriched (F, 60%) diet supplemented or not with either Cit 0.15 g/d (CCit or FCit) or an isomolar amount of Arg (CArg or FArg) or Gln (CGln or FGln) for 4 weeks. All diets were made isonitrogenous to the Arg-containing diet by addition of a mixture of non-essential amino acids (NEAA: alanine, glycine, proline, glutamate, aspartate and serine in isomolar amounts). The dose of Cit was chosen on the basis of our previous studies [15]. Diet compositions are given in Table 1.

Animals were euthanatized at the end of the 4-week feeding period for blood and tissue sampling and body composition assessment.

2.3. Nutritional assessment

During the 4-week feeding period, food intake and body weight gain were monitored daily. During the 2nd and the 4th week, 24-h urine was collected and nitrogen excretion was measured by pyrochemiluminescence (Antek 9000, Antek, Houston, TX). Net intestinal absorption of macronutrients was assessed on 24-h stools during the same periods. Nitrogen, fat and total energy content were determined by nitrogen elemental analysis (N analyser Flash EA1112, Thermo Scientific, Waltham, MA), by the van de Kamer method [16], and by bomb calorimetry (C200 bomb calorimeter, IKA, Staufen, Germany) respectively. Energy derived from carbohydrate was calculated as the difference between total energy and nitrogen and fat-derived energy. Net intestinal absorption was expressed as the percentage of total energy ingested from the 3 main energy sources (fat, nitrogen, and carbohydrates). Body composition, and in particular the localization and size of fat depots, was determined by dissection and weighing.

2.4. Blood and tissue sampling

On day 28, the rats in the fasted state were anesthetized by isoflurane inhalation. After shaving, disinfection and laparotomy, portal blood samples were taken on endotoxin-free material to measure endotoxin levels. Arterial blood was collected from abdominal aorta into heparin-containing tubes and immediately centrifuged (10 min, 2500 *g*, +4 °C). An aliquot of plasma was immediately deproteinized with a 30% (w/v) sulfosalicylic acid solution for plasma AA analysis. Another aliquot was frozen at −80 °C until analysis. Rats were then euthanized by exsanguination, liver samples were taken and either frozen in liquid

Table 1
Compositions of the diets.

Ingredients (% total weight)	Control diet (UAR A04, SAFE)	Fructose diet (U8960, SAFE)
Lipids (%)	3	5
Proteins (%)	16	22
Carbohydrates (%)	60	65
Fibers, vitamins, minerals… (%)	21	8
Energy content (kcal/100 g)	389	404