ELSEVIER

Nutrition 23 (2007) 827–835

NUTRITION

www.elsevier.com/locate/nut

Basic nutritional investigation

Effects of CLA at different dietary fat levels on the nutritional status of rats during protein repletion

María Florencia Andreoli, B.Sc.[a,c], María Victoria Scalerandi, B.Sc.[a], Ileana Malan Borel, Ph.D.[b,c], and Claudio A. Bernal, Ph.D.[a,c,*]

[a] *Cátedra de Bromatología y Nutrición, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina*
[b] *Cátedra de Inmunología Básica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina*
[c] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Santa Fe, Argentina*

Manuscript received March 21, 2007; accepted August 2, 2007.

Abstract

Objective: Protein depletion is associated with decreased body weight gain, low nitrogen balance, intrahepatic lipid accumulation, and hypoalbuminemia. Because conjugated linoleic acid (CLA) can increase lean body mass, enhance feed efficiency, and modulate lipid metabolism, this study investigated the effects of CLA at two levels of dietary fat on energy efficiency, nitrogen retention, and plasmatic and hepatic lipid levels in rats during dietary protein repletion.

Methods: The animals were subjected to a moderate protein restriction for 14 d. After that, they were fed a protein repletion diet for 30 d, supplemented or not with CLA at recommended and high-fat levels. Energy efficiency, nitrogen balance, and nutritional parameters in serum and tissues were evaluated.

Results: Protein repletion improved most of the nutritional parameters evaluated independently of CLA supplementation at both fat levels. At recommended fat levels, CLA did not have any effect. At high-fat levels, energy efficiency increased more than 20% by fat accumulation in carcasses and epididymal pads, serum cholesterol increased (two-fold), and liver triacylglycerol accumulation remained elevated. However, at high-fat levels, CLA prevented lipid accumulation in liver and adipose tissue.

Conclusion: Protein repletion improved the nutritional status of protein-restricted rats with minor effects of CLA at both dietary fat levels. However, when high-fat diets were given, CLA-enriched oil showed preventive effects on liver and adipose tissue lipid accumulation and no deleterious effects were observed. Because there are no studies dealing with CLA effects on protein repletion, this experimental model could improve nutritional interventions to overcome the protein-deficit stage.

Keywords: Conjugated linoleic acid; Protein depletion; Protein repletion; Body composition

Introduction

Protein malnutrition is a worldwide problem affecting mainly pregnant women, infants, and children [1,2]. It has been largely documented that protein malnutrition with an adequate energy intake is characterized by body weight loss, protein-content depletion, intrahepatic lipid accumulation, edema, and hypoalbuminemia in animals and humans [1]. The consequences of protein depletion in the early stages of life or during infancy could compromise growth rate and lead to learning and behavioral alterations [3]. Therefore, an adequate nutrient selection and quick nutritional intervention might be crucial to overcome the protein-deficit stage.

Conjugated linoleic acid (CLA) is a generic term for a group of positional and geometrically conjugated dienoic isomers of linoleic acid. They are naturally found in dairy

This work was financially supported by the Agencia Nacional de Promoción Científica y Tecnológica Proyecto (ANPCyT) PICTO #05-13216 BID 1728/OC-AR.

* Corresponding author. Tel.: +54-342-457-5211; fax: +54-342-457-5221.

E-mail address: cbernal@fbcb.unl.edu.ar (C. A. Bernal).

0899-9007/07/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.nut.2007.08.003

and ruminant fats, the *c*9,*t*11-CLA being the main isomer (around 80% of total isomers) [4]. CLA is also synthetically prepared, where *c*9,*t*11- and *t*10,*c*12-CLA are equally abundant (30–40% of each isomer) [5]. The precise action of CLA is related to type [6,7] and level [8] of the specific isomer and to the animal model [7,9,10] used.

Conjugated linoleic acid is reported to have beneficial effects on human and experimental animals [11,12] even though there are certain detrimental effects that should be taken into account [11,13]. It has been demonstrated that increased lean body mass [14], enhanced feed efficiency [15], protection against the catabolic effects of endotoxins [16,17], and modulation of circulating lipids [18] are some of the beneficial effects of CLA in experimental animals.

Even though considerable research efforts have resulted in advances in the field of CLA effects on energy, protein, and lipid regulation in experimental animal models, to the best of our knowledge, no studies have been published thus far showing the effect of CLA on nutritional status during protein repletion. Therefore, this study investigated the effects of CLA at two levels of dietary fat diet on energy efficiency, nitrogen retention, and plasmatic and hepatic lipid levels in rats during dietary protein repletion.

Because the presence of CLA and the level of dietary fat may play an important role in the recovery from malnutrition status [1], it is expected that this animal model could contribute to the knowledge of nutritional interventions for potential protein malnutrition recovery.

Materials and methods

Materials

Most nutrient compounds, including vitamins and minerals, for diet preparations were chemical grade or better, with the exception of corn oil (Mazola, Buenos Aires, Argentina), sucrose, cellulose, and corn starch, which were obtained from local sources. The CLA mixture oil was kindly provided by La Serenísima Co. (General Rodriguez, Buenos Aires, Argentina). Standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All solvents and reagents used for the fatty acid (FA) quantification were chromatographic grade and all other chemicals used were at least American Chemical Society degree.

Preparation of diets

The composition of the diets is presented in Table 1 and was based on the American Institute of Nutrition Ad Hoc Committee recommendation (AIN-93G) [19]. The control (C) diet contained 20% of casein as a protein source and 7% of corn oil (20% of energy) as a dietary fat source. The low-protein (LP) diet contained 5% of casein, balancing isocalorically the protein with carbohydrates. Because slightly high energy-dense diets could be useful for recovery after protein malnutrition [1], high-fat (HF) diets with and without CLA were used. High-energy diets were achieved by increasing 2.86-fold the total dietary fat content. Therefore, in the HF diet, 13% of the oil was replaced with an identical amount of carbohydrates, obtaining a hypercaloric diet. Because CLA competition with linoleic acid may affect the action of CLA [20], CLA supplementation was increased three-fold, maintaining 15% of CLA in the normal and HF diets. Hence, CLA supplementation was achieved by replacing 1% (C + CLA) or 3% (HF + CLA) of corn oil by a CLA mix oil. Corn oil was used as a source of *cis* unsaturated FA. The CLA mix oil was formed by an equimolecular mixture of *c*9,*t*11-CLA and *t*10,*c*12-CLA as the main sources of isomeric fat. All diets exceeded the essential FA recommendations. The FA composition of the experimental oil, as methyl esters, was determined by gas chromatography with a Hewlett-Packard 5890 chromatograph (Hewlett-Packard, USA) equipped a flame ionization detector. The FA methyl esters were identified by comparison of their retention times relative to those of commercial standards. The FA composition of the fats used is presented in Table 2. Each diet was freshly prepared every 3 d throughout the experimental period.

Table 1
Composition of the experimental diets (grams per kilogram of diet)

Ingredient	LP	C	C + CLA	HF	HF + CLA
Corn starch	679.5	529.5	529.5	399.5	399.5
Casein	50	200	200	200	200
Sucrose	100	100	100	100	100
Corn oil	70	70	60	200	170
CLA-rich oil	—	—	10	—	30
Fibre	50	50	50	50	50
Mineral mixture*	35	35	35	35	35
Vitamin mixture*	10	10	10	10	10
L-cystine + L-methionine	3.0	3.0	3.0	3.0	3.0
Choline	2.5	2.5	2.5	2.5	2.5
Energy (kJ/100 g)	1656.9	1656.9	1656.9	1928.8	1928.8

C + CLA, control diet supplemented with conjugated linoleic acid; C, control diet; HF + CLA, high-fat diet supplemented with conjugated linoleic acid; HF, high-fat diet; LP, low-protein diet

* Vitamin and mineral mixtures were formulated according to Reeves et al. [19].

Animals and general protocol

All studies were conducted in agreement with the regulations of the School of Biochemistry and the Guide to the Care and Use of Experimental Animals in the Laboratory [21]. Experiments were carried out on male Wistar rats provided by the Comisión Nacional de Energía Atómica (Buenos Aires, Argentina), which were housed in the animal quarter. During the experimental period, they were kept under controlled conditions (23 ± 2°C and 12-h light/dark cycle) in individual stainless-