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Enhancement of antibody synthesis in rats by feeding *cis*-9,*trans*-11 conjugated linoleic acid during early life☆

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Abstract

Previous studies have demonstrated that the intake of a 1% conjugated linoleic acid (CLA) diet in an 80:20 mixture of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 exerts age-specific effects on the immune system: immunoglobulin enhancement and proliferative down-modulation in neonatal and adult rats, respectively. The present study evaluates the influence of the same diet on antibody synthesis of early infant Wistar rats during suckling and/or after weaning. Dietary supplementation was performed during suckling and early infancy (4 weeks), only during suckling (3 weeks), or only in early infancy (1 week). CLA content in plasma and serum immunoglobulin (Ig) G, IgM and IgA concentration were determined. Proliferation, cytokines and Ig production were evaluated on isolated splenocytes. *Cis*-9,*trans*-11- and *trans*-10,*cis*-12-CLA isomers were detected in the plasma of all CLA-supplemented animals, and the highest content was quantified in those rats supplemented over the longest period. These rats also exhibited higher concentrations of serum IgG, IgM and IgA. Moreover, splenocytes from CLA-supplemented rats showed the highest IgM and IgG synthesis and interleukin (IL)-6 production, whereas their proliferative ability was lower. In summary, in infant rats, we observed both the enhance antibody synthesis previously reported in neonates, and the reduced lymphoproliferation previously reported in adults.

Keywords: Conjugated linoleic acid; Early life; Immunoglobulin synthesis; Lymphocyte proliferation

1. Introduction

Historically, considerable attention has been focused on neonatal nutrition, and within this field, the importance of polyunsaturated fatty acids (PUFAs) other than arachidonic acid and docosahexaenoic acid (DHA) has been highlighted [1]. Neonatal development, particularly neonatal immunity, is influenced after birth by consumption of breast milk [2,3]. Breast milk includes many bioactive components, such as antibodies, growth factors, cytokines, nucleotides, cells and lipids as conjugated linoleic acid (CLA), that influence maturation of the developing immune system [4,5]. In addition, it has been demonstrated in animal models that milk-derived immune factors can cross the neonatal intestine and influence beyond this compartment [3]; moreover, they can provide further protection in weaning [6].

CLA was first identified as a main component of fried ground beef with anti-carcinogenic properties [7], and it has also been found in human breast milk ranging from 2.23 to 5.43 mg/g fat [8]. Many effects have been attributed to CLA, such as reducing atherosclerosis severity [9,10] and body fat, while enhancing lean body mass [11]. The diversity of the biological activities of CLA is due to its variable composition, since it is a mixture of more than 20 geometric and positional isomers. However, the primary research is focused on the two biologically active isomers of CLA: *cis*-9,*trans*-11 (*c*9,*t*11) and *trans*-10,*cis*-12 (*t*10,*c*12) [12]. These two isomers have shown additive, independent, or even antagonistic effects. Both CLA isomers have anticarcinogenic effects and probably immunomodulatory properties, but the *t*10,*c*12 CLA isomer is better known to be responsible for body fat reduction [13]. The *c*9,*t*11 isomer constitutes more than 80% of CLA in breast milk and dairy products and, because of that, its influence in early age becomes of special interest [8,14,15].

Over the past 20 years, numerous studies have been conducted in rodents, pigs and chickens, using CLA at doses ranging from 0.5% to 1% of total dietary fat [16]; however, most of them have been performed

Abbreviations: CLA, conjugated linoleic acid; PPAR, peroxisome proliferator-activated receptor; IL, interleukin; Ig, immunoglobulin; IFN, interferon.

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in adult animals, and in humans, using 50:50 isomeric mixtures of *c*9, *t*11 and *t*10,*c*12 CLA. These mixtures and the pure isomers have reported different effects depending on the age of the animals used. CLA modulates lymphoproliferation in adult animals and in humans by decreasing polyclonal proliferative ability, like many other PUFAs, but increases specific proliferative response after challenge [17–20]. However, no effect on lymphoproliferation is observed in neonatal animals fed CLA [21]. On the other hand, although several studies in adult animals following CLA diets do not modify in vitro or in vivo immunoglobulin (Ig) production [19,20,22,23], some enhancing effects on immunoglobulin synthesis are described for neonatal and young rodents after CLA consumption [21,24,25].

In previous studies, we have demonstrated substantial immunomodulatory effects on neonatal and adult rats after a *cis*-9,*trans*-11 CLA-enriched diet. The present work evaluates the influence of the same CLA diet on early infant Wistar rats (4 weeks old). At this age, their immune system is still in maturation (i.e., antibody production); however, some immune functions are already mature and very similar to those of adult animals (i.e., lymphoproliferative ability). Moreover, as it has been suggested that CLA intake during early stages of development may have effects later in life [26,27], we have also studied whether CLA supplementation limited to suckling produces effects which can last until early infancy (1 week later).

2. Material and methods

2.1. Animals

Pregnant Wistar rats at 7 days of gestation were obtained from Harlan (Barcelona, Spain). The animals were housed in individual cages under controlled temperature and humidity conditions in a 12 h:12 h light:dark cycle and had access to food and water ad libitum. The rats were allowed to deliver at term. The delivery day was identified as Day 1 of life. Litters were randomized and unified to 10 pups per lactating dam; pups had free access to the nipples and rat diet. All daily handling was done in the same time range to avoid the influences of biological rhythms. Body weight and body length (nose–anus length) were used to determine the following morphometrical parameters: body mass index (BMI), calculated as body weight/length^2 (g/cm^2) and Lee index, calculated as $\sqrt[3]{}$weight/length ($\sqrt[3]{}$g/cm).

Twenty-eight-day-old rats were anaesthetized with ketamine/xylazine to obtain spleens and blood for plasma and serum samples, which were immediately frozen at −80°C until processing. Studies were performed in accordance with the institutional guidelines for the care and use of laboratory animals established by the Ethical Committee for Animal Experimentation at the University of Barcelona and the Catalonian Government (CEEA 303/05 UB/DMA 3242).

2.2. Diets

The standard diet corresponded to the American Institute of Nutrition (AIN)-93G formulation [28], containing 7% soybean oil. A 1% CLA diet was obtained from modified standard flour AIN-513 (Harlan) containing 10 g CLA/kg [27,28]. Thus, the supplemented diet contained 6% soybean oil plus 1% CLA oil. The CLA isomer mixture was approximately 80% *c*9,*t*11 and 20% *t*10,*c*12 from the total CLA isomers in oil. This proportion has been chosen due to its resemblance to that one present in breast milk [8]. The CLA mixture had 0.69% free fatty acids as oleic acid, a peroxide value of 0.2 mEq/kg, 5.6% saturated fatty acids and less than 5% of minor CLA isomers. CLA oil was kindly supplied by Loders Croklaan, Lipid Nutrition, Wormerveer, The Netherlands.

As suckling pups did not eat pelleted diet until weaning, daily administration of 1.5 mg CLA/g per rat from Day 1 to 21 corresponds to a 1% CLA diet in suckling animals as previously described [21]. This data is based on the daily intake of rats from 21 to 28 days old (10–15 g chow/100 g of rat body weight). Low-capacity syringes (Hamilton Bonaduz, Bonaduz, Switzerland) adapted to oral 25- or 23-gauge gavage tubes, 27 mm in length (ASICO, Westmont, IL, USA), were used for oral administration before and after Day 5, respectively. To allow gastric emptying, litters were separated from dams 1 h before oral supplementation.

2.3. Study design

On the delivery day, animals were distributed into four experimental groups (two dams with 10 pups each, n=20/group), according to the total period of CLA supplementation (Fig. 1):

Four-week group: During suckling, pups received CLA daily by oral gavage; after weaning, animals were fed 1% CLA pelleted diet from Day 21 to 28. Total period of supplementation, 4 weeks.
Three-week group: During suckling, pups received CLA daily by oral gavage; after weaning, animals were fed standard diet until day 28. Total period of supplementation, 3 weeks.
One-week group: Rats received 1% CLA-pelleted diet exclusively for one week after weaning (Days 21–28). Total period of supplementation, 1 week.
Control group: these animals constitute the control diet group. Total period of supplementation, 0 week.

All animals from the four dietary groups were sacrificed at the age of 28 days.

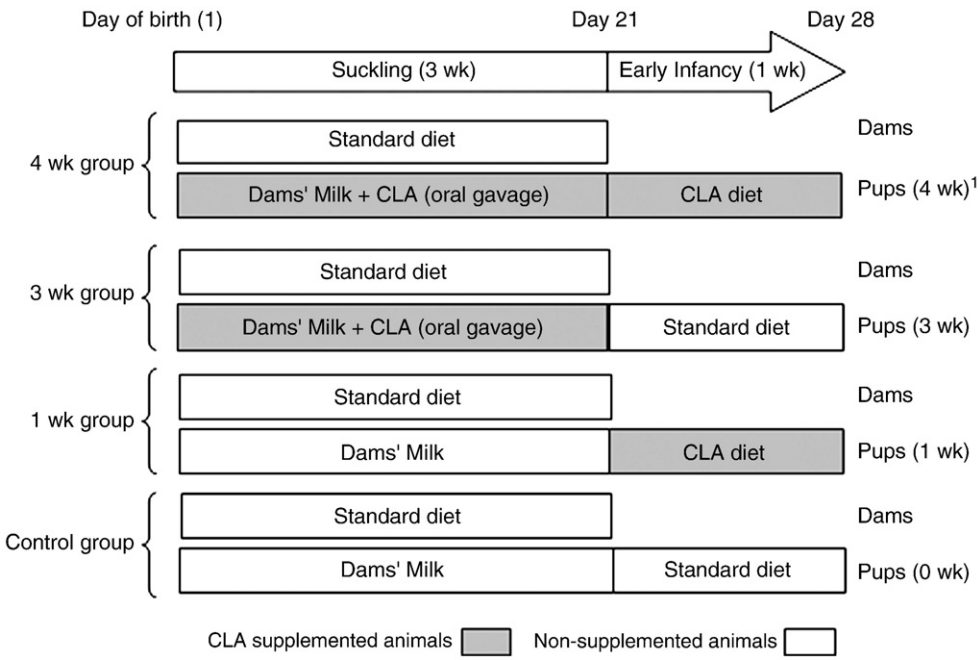

Fig. 1. Diagram of the experimental design beginning on the day of birth until day 28 of life. [1]Total period of CLA supplementation from the day of birth until 1 week after weaning.