Prostaglandins, Leukotrienes and Essential Fatty Acids 89 (2013) 115–119

Contents lists available at SciVerse ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Metabolism of c9,t11-conjugated linoleic acid (CLA) in humans

Maria Cristina Mele [a], Giorgio Cannelli [a], Gianfranca Carta [b], Lina Cordeddu [b], Maria Paola Melis [b], Elisabetta Murru [b], Catherine Stanton [c], Sebastiano Banni [b,*]

[a] Università Cattolica Sacro Cuore, Roma, Italy
[b] Dipartimento di Scienze Biomediche, Università degli Studi di Cagliari, Cagliari, Italy
[c] Teagasc, Moorepark Food Research Centre, Fermoy, Co. Cork, Ireland

ARTICLE INFO

Article history:
Received 6 October 2012
Received in revised form
29 May 2013
Accepted 30 May 2013

Keywords:
Conjugated linoleic acid (CLA)
Plasma concentration
Dose

ABSTRACT

The *c*9,*t*11 isomer of conjugated linoleic acid (CLA) is the most abundant CLA form present in the human diet, and is particularly prevalent in milk and dairy products, and is known to exert several health benefits in experimental animal models. A possible mechanism of action of *c*9,*t*11CLA relies on its metabolism via desaturases and elongases and partial beta oxidation in peroxisomes. In this study, we aimed to establish plasma incorporation of *c*9,*t*11CLA and its downstream metabolites in healthy volunteers after daily dietary intakes of 0.8 g, 1.6 g or 3.2 g of *c*9,*t*11CLA in capsule form for two months. Following supplementation, the plasma concentrations of *c*9,*t*11CLA and its metabolites conjugated dienes (CD) 18:3 and the beta oxidation product CD 16:2 were incorporated in a linear fashion, while on the other hand CD 20:3 reached a plateau following intakes of 1.6 g/d of dietary intake, and was not further increased following higher CLA intakes. We may conclude that supplementation of *c*9,*t*11 CLA levels result in linear responses of CLA and its main metabolites in plasma. In addition, only the highest concentration of CLA intake tested (3.2 g/d) yielded plasma concentrations of CLA and metabolites close to the range found sufficient to exert nutritional effects in experimental animal models.

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1. Introduction

Conjugated linoleic acid (CLA) is characterised by conjugated double bonds which can be present in several positional and geometrical configurations yielding 13 possible isomers [1]. Among them, just few have been tested for biological activities [2]. The *cis*-9,*trans*-11 CLA isomer (*c*9,*t*11-CLA) is naturally present in the lipid fraction of meat and dairy products from ruminants, because it is produced by the rumen intestinal microbiota during biohydrogenation [3]. Several studies using animal models have reported positive effects of dietary CLA, such as anti-obesity, anticarcinogenic activities and modulation of immune functions [2]. In particular, it has been proposed that CLA has potential antiatherogenic properties [4]. Kritchevsky et al. [5] showed a dose-response effect of CLA on the regression of atheroma of 30% in rabbits fed on an atherogenic diet. However, data from human studies are conflicting although CLA ingestion led to decreased plasma triacylglycerol and VLDL-cholesterol concentrations in one study [6], other human trials did not confirm these effects [7–9]. Such contrasting results may be due to differences in the composition of the CLA mixture used for intervention, because the two most common isomers used in intervention studies (*c*9,*t*11 and *t*10,*c*12) are known to exert distinct physiological effects and have distinct kinetics and metabolism, i.e. conversion to metabolites conjugated dienes (CD)18:3 or CD16:2 [10]. One study investigated the effects of ingestion of highly enriched preparations of either *c*9,*t*11 or *t*10,*c*12-CLA on blood lipid profiles in healthy men [11], and found that ingestion of the *t*10, *c*12-CLA isomer led to increased LDL:HDL-cholesterol ratio and total-HDL-cholesterol, whereas ingestion of the *c*9,*t*11-CLA caused the opposite effect, and decreased these parameters. These data suggest opposing effects of these two CLA isomers, with hyperlipidemic properties of *t*10,*c*12-CLA and hypolipidemic properties of *c*9, *t*11 CLA in humans. For this reason, research is focused on ruminant dietary strategies to enhance *c*9,*t*11 CLA in milk and dairy products for human consumption.

Some of the biological effects of CLA could be attributed to its metabolism in vivo [10,12]. In previous studies, we detected CLA metabolites, CD18:3 and CD20:3 in human plasma and adipose tissue [13]. However, one of the most critical issues is to establish the CLA dose in humans that is comparable to the dose previously shown to be sufficient in experimental animals to exert beneficial activity in vivo, usually 0.5–1% of the animal diet [2]. Plasma concentration and allometric measurements, which take into consideration the different metabolic rates, as recently proposed [14], should be reliable markers to compare efficacy levels of dietary *c*9,

* Correspondence to: Università degli Studi di Cagliari, Dipartimento di Scienze Biomediche, Cittadella Universitaria, S.S. 554, km. 4,500 09042 Monserrato, Cagliari, Italy. Tel.: +39 070 6754128; fax: +39 070 6754191.
E-mail address: banni@unica.it (S. Banni).

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http://dx.doi.org/10.1016/j.plefa.2013.05.005

*t*11 CLA. Therefore, the primary aim of the present study was to verify whether the pure isomer *c*9,11-CLA and its metabolites are incorporated in a linear fashion in plasma lipids of healthy humans following daily dietary supplementation at three different doses (0.8, 1.6 and 3.2 g/d CLA) over a period of two months.

2. Materials and methods

2.1. Subjects and study design

All protocols involving human subjects were performed according to the Good Clinical Practice rule, and each study was submitted to the appropriate Ethical Committee. The study was carried out at The Catholic University Sacred Heart in Rome, Italy. Thirty six volunteers were recruited, within the personnel of the Policlinico Universitario "A. Gemelli" of the Catholic University Sacred Heart, Rome, with the following inclusion criteria: age between 30 and 40 years; BMI = 20–26 kg/m^2; systolic blood pressure (SBP) = < 150 mmHg and diastolic blood pressure (DBP) < 95 mmHg; total cholesterol concentration in serum of ≤6 mM; glucose level in serum of ≤4.2–6 mM. Exclusion criteria included subjects with BMI out of the normal range, with systemic diseases such as diabetes mellitus, liver disorders, neoplasms, collagen-vascular diseases, smokers, plasma total cholesterol or triglycerides higher than 250 mg/dl, blood transfusions within six months prior to the study and history of recent acute illness. Furthermore, the use of alcohol, tobacco and medicines that can influence the absorption or the metabolism of lipids were not permitted. The participants gave their informed consent after carefully reading the protocol. It was made clear that the participants could withdraw at any stage of the study. The participants were apparently healthy volunteers, male and female, free of prescribed drugs and without dietary restrictions. Prior to enrolment in the trial, the health status of each subject was checked with a questionnaire and screening tests (blood pressure, urinary protein and glucose, serum cholesterol, triglycerides and gamma-glutamyltransferase). Throughout the study, subjects registered side effects, intercurrent illnesses and use of medicines. Except for diet, they were advised to keep their customary living habits unchanged. Blood samples were taken from the antecubital veins after an overnight fast.

2.2. Ingestion of synthetic CLA by healthy human volunteers

Thirty-six healthy subjects were randomly assigned to one of the following four groups, for the dose–response study with the synthetic CLA as triglyceride form (Lipid Nutrition B.V., Wormerveer, Netherlands. Each capsule which contained 1 g of 80% CLA (63% *c*9*t*11, 13% *t*10,*c*12, 3% *c*11,*t*13), 14% oleic acid, 3% linoleic acid and 5% palmitic acid) was given daily for two months.

CLA capsules were supplied as follows:

- *Group 1* was a control with no CLA supplement.
- *Group 2* was assigned to a dietary intake of 0.8 g/d CLA.
- *Group 3* was assigned to a dietary intake of 1.6 g/d CLA.
- *Group 4* was assigned to a dietary intake of 3.2 g/d CLA.

The subjects were instructed to take the assigned level of CLA for two months. During the intervention period, volunteers were also instructed to follow ad libitum diet.

2.3. Samples and lipid extraction

Blood samples (5 mL) were taken from the anticubital veins of volunteers at the end of the first month of CLA ingestion, at the end of the second month (i.e. at the end of intervention period) and after the wash-out period, that lasted two further months. EDTA (10IU/mL) was added to the blood samples and immediately centrifuged at 2000*g* for 10 min, and the resulting plasma was stored at −80 °C until analysis.

Total lipids were extracted by the method of Folch [15]. An aliquot of the lipid fraction was mildly saponified using a procedure described by Banni et al. [16] in order to obtain free fatty acids (FFA) for HPLC analysis. Briefly, lipid extracts were dissolved in 5 ml of ethanol, 100 μl of desferal (25 mg/ml H2O), 1 ml of a 25% ascorbic acid solution in water, 0.5 ml of 10 N KOH, and left in the dark at room temperature for 14 h, and then10 ml of *n*-hexane and 7 ml of H_2O were added and the mixture then acidified with 0.35 ml of 37% HCl, to a pH 3–4. Samples were centrifuged for 1 h at 900 × *g*, and the hexane phase was collected, the solvent was evaporated, and the residue was dissolved in 0.5 ml of CH_3CN/0.14% of CH_3COOH (v/v).

Separation of CLA and its metabolites was performed with Agilent1100 HPLC system (Agilent, Palo Alto, CA) equipped with a diode array detector. A C-18 Inertsil 5 ODS-2 Chrompack column (Chrompack International BV, Middleburg, The Netherlands), 5 μm particle size, 150 × 4.6 mm, was used with a mobile phase of CH_3CN/H_2O/CH_3COOH (70/30/0.12, v/v/v) at a flow rate of 1.5 mL/min [17]. Conjugated dienes unsaturated fatty acids were detected at 234 nm. Spectra (195–315 nm) of the eluate were obtained every 1.28 s and were electronically stored. Second-derivate UV spectra of the conjugated diene fatty acids were generated using Phoenix 3D HP Chemstation software (Agilent, Palo Alto, CA). These spectra were taken to confirm the identification of the HPLC peaks [18]. Details of the methodology regarding the characterization of conjugated dienes unsaturated fatty acids in both reference and biological samples have been published previously [17].

2.4. Statistical analyses

One-way ANOVA with the Tukey test for post-hoc analyses was applied to evaluate statistical differences among groups. Dose response curves were assessed by Pearson simple correlation analysis. *P* values < 0.01 were considered significant.

3. Results

Human plasma was analysed for CLA and its metabolites content following dietary supplementation for two months with synthetic CLA at three levels of supplementation, i.e. either 0.8 g/d, 1.6 g/d and 3.2 g/d, compared with control group, which received no CLA supplement. The data indicate that ingestion of 0.8 g/day of CLA for two months was sufficient to cause a significant difference in plasma CLA content with respect to unsupplemented controls.

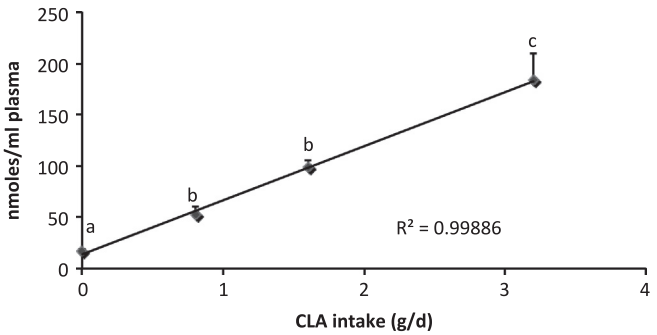

Fig. 1. Dose response curve of CLA plasma concentrations in function to dietary CLA intake. (n = 9 for each point). R^2 = 0.99886, $p < 0.01$. Different letters denote significant differences ($p < 0.05$).

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