CASE STUDY

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Clinical outcomes of conjugated linoleic acid supplementation in the overweight and the obese: a study protocol

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ABSTRACT

This is the protocol of a study aiming to assess the impact of conjugated linoleic acid (CLA) on anthropometric parameters, body composition and densitometric parameters, carbohydrate and fat digestion and absorption, total energy expenditure, glucose and insulin homeostasis, lipid profile, adipokines concentrations, polyunsaturated fatty acids levels, markers of endothelial dysfunction and mineral status in humans. Seventy-four adult volunteers (BMI ⩾ 25 kg/m^2) will be randomized to receive 3.0 g of 80% CLA (50:50 cis-9, trans-11 and trans-10, cis-12 isomers) or 3.0 g of linoleic acid daily for 3 months. A range of parameters will be measured at baseline and after the intervention.

Keywords: obesity, randomized, digestion, absorption, body composition, lipid profile, adipokines, endothelial dysfunction, mineral status, breath test, 13C.

Introduction

Herein we present the protocol of a study aiming to assess the impact of conjugated linoleic acid (CLA) on anthropometric parameters, body composition and densitometric parameters, carbohydrate and fat digestion and absorption, total energy expenditure, glucose and insulin homeostasis, lipid profile, adipokines concentrations, polyunsaturated fatty acids (PUFA) levels, markers of endothelial dysfunction and mineral status in humans.

Basic Concept of the Project

Obesity-related morbidity and mortality burden societies worldwide [1]. Although many factors contribute to obesity, the dietary factors play a leading role. Among these, insufficient proportion of unsaturated fat seems to be of particular importance. CLA comprises of conjugated isomers of the 18-carbon polyunsaturated fatty acid. In many naturally occurring foods, such as dairy products and ruminant meats, cis-9, trans-10, and trans-10, cis-12-CLA can be found. On the other hand, CLA, present in small quantities in tissues, is not synthetized by the human organism. Although there are data that point towards the influence of CLA on adiposity, the mechanisms behind the observed effects remain unknown [2]. There is evidence supporting the influence of 50:50 mixture of cis-9, trans-10 and trans-10, cis-12-isomers in weight management [3]. The trans-10, cis-12-isomer seems to lead to body content of adipose tissue and increase insulin resistance at the same time [4]. However, the effect was not reported when the 50:50 mixture was investigated.

Research methodology

Study population

The study will comprise 74 adults with BMI ⩾ 25 kg/m^2 at the beginning of screening. Subjects will be instruct-

ed to maintain isocaloric diet and not to change their eating habits during the study period. Criteria for completion include consumption of 75% of the supplement provided. A summary of exclusion criteria is shown in **Table 1**.

Table 1. Exclusion criteria

Exclusion criteria
Subjects with: – history of chronic systemic disease (with the exception of hypertension) – celiac disease – type 2 diabetes – liver and/or pancreatic disease – current or recent (within the preceding month) treatment with CLA and agents interfering with fat digestion and/or absorption (chitosan, orlistat, green tea) – pregnancy.

Protocol

The subjects will be randomly assigned to receive CLA or placebo. Participants enrolled to the CLA group will be given capsules containing 3.0 g of 80% CLA (50:50 cis-9, trans-11 and trans-10, cis-12 isomers) daily for 3 months. Volunteers randomized to the placebo group will be given capsules containing 3.0 g of linoleic acid (LA) per day. Breath tests will be performed after an overnight fast with the use of Iris-Infrared Isotope Analyser (Wagner Analysen Technik, Bremen, Germany). Venous blood samples will be collected from each subject according to the standard already implemented in the institution after an overnight fast. The procedure will be performed before randomization and after 3 months of supplementation. The study protocol flowchart is shown in **Figure 1**. Statistical analyses will be performed to describe the studied populations; to determine the normality of distribution of parameter values in the groups; to compare the parameter values in the populations at baseline and after the study period; to compare observed changes of parameter values between the groups; to search for correlations between changes in parameter values. The level of significance will be set at $p < 0.05$.

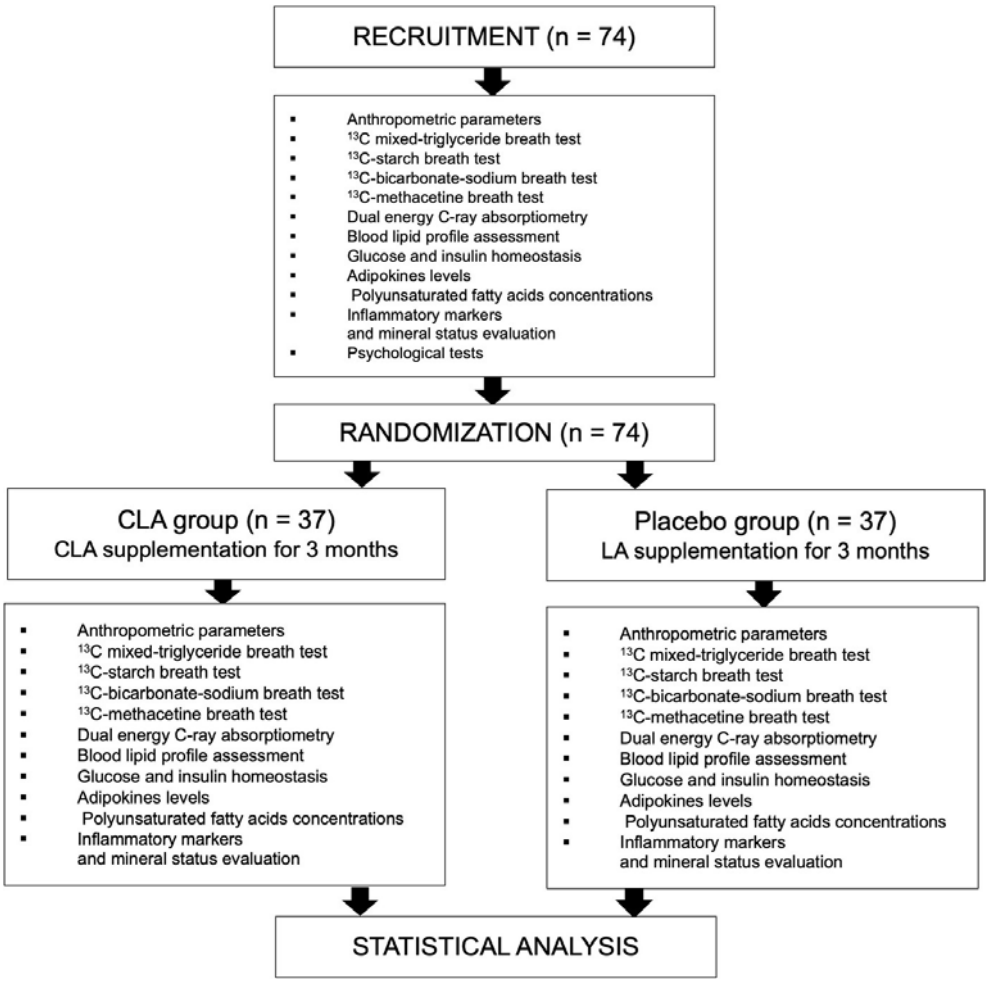

Figure 1. Study protocol

The following methodological approach will be consistently applied:

- Body composition and densitometric parameters will be measured by dual-energy X-ray absorptiometry (DXA).
- Fat digestion and absorption will be assessed by ^{13}C-mixed-triglyceride breath test. A dose of 150 mg of ^{13}C-mixed triglyceride will be administered with a standardized meal, that is 50 g roll with 12.5 g of butter (82% of fat). Breath samples will be collected before the test meal and every 30 minutes for 6 hours after ingestion.
- Total energy expenditure will be assessed by ^{13}C-bicarbonate sodium breath test. A capsule of ^{13}C-bicarbonate sodium will be administered orally after dissolution in 125 ml of warm fruit tea. Sixteen breath samples will be acquired from the patient over 3 hours.
- Liver metabolism will be assessed by ^{13}C-methacetine breath test (a dose of 75 mg ^{13}C-methacetin will be dissolved in 100 ml of fruit tea and administered orally. Breath samples will be collected before and every 10 minutes for 2 hours after ingestion.
- Carbohydrates digestion and absorption will be assessed by ^{13}C-starch breath test. A standard meal will consist of 50 g of cornflakes with 100 ml of low fat milk. The breath samples will be collected before and every 30 minutes for 4 hours after the test meal.
- Blood lipid profile will be determined by enzymatic methods (Olympus, Beckman Coulter, Pasadena, USA).
- Mineral status will be assessed in 24-hour urine collection and in hair samples.
- PUFA concentration in blood will be determined by gas chromatography-mass spectrometry (Agilent Technologies, Palo Alto, USA).
- Adipokines levels using an enzyme-linked immunosorbent assay.
- Blood analyses related to glucose and insulin homeostasis and endothelial dysfunction will be performed.

The study was approved by the Bioethical Committee at Poznan University of Medical Sciences and adheres to the revised Declaration of Helsinki. Written consent to participation in the study will be obtained from all volunteers after providing full information.

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Expected results

Reports provide contradictory accounts of CLA effects. No influence of CLA on body weight or composition was found by Joseph et al. [5]. On the other hand, Gaullier et al, Pfeuffer et al., and Chen et al. observed a reduction in fat mass that could be attributed to CLA provision [6–8]. Beneficial changes in lipid levels were attributed to CLA in animal models [9, 10]. This study will verify whether CLA supplementation promotes lipid profile normalization in humans.

It was suggested that CLA may increase the basal metabolic rate, thermogenesis, and lipid oxidation [11, 12]. CLA could also lead to reducing adiposity by inhibiting heparin-releasable lipoprotein lipase [13]. This would result in increased apoptosis of preadipocytes.

The authors are aware that CLA was linked to hepatitis [14, 15]. A meta-analysis which found that CLA at a daily dose of 3.2 g leads to reduction in adiposity in humans also suggested that more research into CLA safety is warranted [16]. Therefore, the proposed study will also gather information on CLA supplementation safety.

In conclusion, this study will aim to reproduce the many reported benefits of CLA supplementation in humans.

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Author contributions
Contributions to conception and design: KŁ, AG, JKN, MDŻ, KG, PB, EF, EM, JW. Article drafting: KŁ, AG, JKN, EF, JW. Final approval of the version to be published: KŁ, AG, JKN, MDŻ, KG, PB, EF, EM, JW.

Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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