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Conjugated linoleic acid does not affect digestion and absorption of fat and starch—a randomized, double-blinded, placebo-controlled parallel study

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Keywords: ¹³C mixed triglyceride breath test, ¹³C starch breath test, ¹³C bicarbonate breath test, obesity, overweight

Abstract
Objective: Conjugated linoleic acid (CLA) is known as a potent agent for altering body weight and composition. However, its effect on the process of digestion is still unknown. The aim of this study has been to elucidate the effect of a 3-month supplementation with CLA on starch and fat digestion and absorption in humans. Approach: The study included 74 obese and overweight adults who were randomized to receive 3.0 g of CLA or sunflower oil as placebo daily for 3 months. Digestion and absorption of fat and starch was assessed using non-invasive breath tests with a stable ¹³C isotope (cumulative percentage dose recovery, CPDR) before and after the supplementation period. To exclude the effect of oxidation, in addition total energy expenditure (TTE) was measured by a ¹³C bicarbonate breath test. Results: The changes in CPDR values (ΔCPDR median (interquartile range)) were no different between subjects from the CLA group and the placebo group (fat: −0.2 (−9.1−4.1) versus 0.6 (−7.0−8.0), p < 0.4796; starch: −1.3 (−9.5−2.4) versus −1.0 (−5.1−1.7), p < 0.5520, respectively). The incidence of negative and positive values of ΔCPDR was no different between groups [for fat: 53.1% versus 46.7%, RR 1.138, (95% CI 0.689-1.882) and for starch: 67.7% versus 56.7%, RR 1.195, (95% CI 0.804-1.777)]. The changes in TTE did not differ between the CLA and the placebo group (respectively 1 (48; 267) versus 8 (−120;93) kcal; p < 0.2728). Conclusion: Supplementation with CLA for 3 months did not affect fat and starch digestion assessed by ¹³C mixed triglyceride breath test and ¹³C starch breath test.

Introduction
Excess weight and obesity is the consequence of a long-term positive energy balance that is frequently related with excessive intake of saturated fat or simple carbohydrates. The state of being overweight or obese leads to serious health implications such as type 2 diabetes mellitus and cardiovascular disease. Therefore, new therapeutic options for successful body mass reduction and maintenance of the obtained effects are needed. Conjugated linoleic acids (CLA) have recently gained much interest as a promising supplement for improving body weight and composition [1].

CLA, a polyunsaturated fatty acid, is known for its anti-carcinogenic and anti-atherogenic properties [1]. CLA is made by bacteria in the process of linoleic acid isomerization or desaturation of 11-trans octadecenoic acid [2]. CLA constitutes a mixture of geometric and positional isomers of linoleic acid, of which the most prevalent in the diet is cis-9, trans-11 octadecadienoic acid, and the less frequent is the trans-10, cis-12 isomer. These two isomers can be found in commercially available CLA products; however, the anti-obesity effect is attributed to the trans-10, cis-12 isomer [3].

Numerous animal studies indicate that CLA influences body composition. Most researchers found that
CLA decreases body fat; however, the results differ between species [4–6]. The evidence from randomized clinical trials proves that long-term CLA supplementation causes weight loss in humans, but of minor clinical relevance (at most 5% of baseline weight) [1, 7]. Other research shows that CLA supplementation reduces fat mass, although these studies have varied with regard to the dose, the type of isomer, and study duration [8, 9]. Therefore, as to the purported benefits of CLA in humans, the results are contradictory. Nevertheless, CLA has been reported to play a beneficial role in lipid metabolism via the activation of enzymes such as lipoprotein lipase and carnitine-palmitoyl-transferase-1. Its supplementation reduces lipogenesis and enhances the lipolysis and \( \beta \)-oxidation of fatty acids in animal models [10, 11]. CLA may also inhibit the differentiation of adipocytes and prevent fatty acid accumulation in adipose tissue [10, 12]. Studies on animals and humans report inconsistent results regarding CLA’s impact on glucose homeostasis [13]. CLA supplementation seems to have no effect on glucose or insulin levels, purportedly because of transient metabolic changes [14–16].

Many potential effects of CLA have been studied to date; however, no data regarding its influence on fat and starch digestion or absorption are so far available. Therefore, the aim of our study is to evaluate its impact in overweight and obese subjects using the reliable methods of \(^{13}\)C mixed triglyceride (MTG-BT) and starch \(^{13}\)C breath test (S-BT).

Methods

Study population

The study comprised 74 adults with BMI \( \geq 25 \) kg m\(^{-2}\). Volunteers were recruited in The Obesity and Overweight Treatment Clinic of Poznań University of Medical Sciences, Poznań, Poland. The eligibility criteria included females over 18 years old and BMI \( \geq 25 \) kg m\(^{-2}\). The exclusion criteria were as follows: chronic systemic disease (excluding hypertension), gastrointestinal diseases (e.g. celiac disease), type 2 diabetes mellitus, and pregnancy. Before the study commenced, subjects were examined by a physician. Subjects who used CLA and other dietary supplements (green tea, mulberry leaves, chitosan, phaseolamin, prebiotics and probiotics) and medications (e.g. orlistat, metformin, acarbose) interfering with fat and starch digestion and/or absorption within the preceding month were also excluded. Subjects were instructed to maintain habitual diets. Diets were recorded before and during the intervention period. Energy and macronutrient intake was calculated to assure the subjects did not change their eating habits.

Randomization and blinding

The protocol of the study was previously described by Mądry et al [17]. Subjects were assigned to receive placebo or CLA by a nurse unrelated to the study, according to a computer-generated randomization list (block size = 6) generated by an independent researcher. The study was conducted in parallel design with an allocation ratio of 1:1. No changes to methods were made after the trial’s commencement. To implement the random allocation sequentially numbered containers were used. All personnel (investigators, care givers, assessors and data analyst) involved in the study and all participants were unaware of the study group assignments until the end of the study.

Intervention

Each participant from the CLA subgroup was given 3.0 g of 80% CLA (50:50 trans-10, cis-12 isomers and cis-9, trans-11) daily for 3 months. Women were instructed to administer two capsules of the provided product three times a day with a meal. Likewise, volunteers from the placebo group consumed capsules containing 3.0 g of sunflower oil per day. Both intervention products were in identical transparent capsules packed in similar blisters. The intervention product was kindly provided by a pharmaceutical company (Olimp Laboratories, Dębica, Poland).

Outcome measures

Evaluation of fat and starch digestion as well as absorption was performed using a MTG-BT and S-BT, respectively. We assumed that the cumulative percentage dose recovery (CPDR) values reflected the process of digestion and absorption [18]. For the assessment of the changes in fat and carbohydrate digestion and absorption, the difference of CPDR after and before the supplementation period (ΔCPDR) was calculated. To evaluate total energy expenditure (TEE) a \(^{13}\)C bicarbonate breath test (B-BT) was performed [kcal/day].

MTG-BT procedure

The test was carried out after overnight fasting. Each of the study participants received a test meal containing 150 mg of \(^{13}\)C mixed triglyceride and 12.5 g of butter (fat content: 82%) mixed on a roll (50 g). Breath samples were obtained at baseline (fasting) and at half hour intervals (6 h) after ingestion of the test meal [19].

S-BT procedure

The baseline breath sample was obtained after an overnight fast. Afterwards, subjects received test meals containing naturally \(^{13}\)C-rich cornflakes (50 g) with low-fat milk (100 ml). Breath samples were obtained fasting (baseline) and every 30 min up to 4 h after the test meal [20, 21].
B-BT procedure
In the TEE assessment a dose of 50 mg of $^{13}$C bicarbonate was administrated orally after dissolution in about 125–150 ml of warm fruit tea. Breath samples were acquired from each subject over a total timespan of 3 h [22].

Breath tests were performed before and after the 3-month CLA or placebo supplementation period. The subjects did not receive either CLA or a placebo to the test meal. Both tests were performed one week apart. The subjects avoided eating food naturally abundant with $^{13}$C (kiwi fruit, pineapple, cane sugar,
maize) for 5 days before the examination. During the examination, subjects were instructed not to consume anything and to avoid physical activity. The isotope ratio $^{13}\text{CO}_2/\text{CO}_2$ was measured using isotope-selective nondispersive infrared spectrometry (IRIS Wagner Analysen Technik GmbH, Bremen, Germany).

Study oversight
The protocol of the study was conducted according to the Declaration of Helsinki and approved by the Ethical Committee of the Poznań University of Medical Sciences, Poland (decisions 606/12 and 453/13). Every subject signed an informed written consent form to participate in the study. The study was financed by the Nutricia Foundation (grant number 504-06-01103115-000-15-07588). The nutritional intervention was carried out independently of any commercial entities. The study design was performed accordingly to the standards of CONSORT [23]. The trial was registered on 4 May 2016 in the Deutsches Register Klinischer Studien (approved WHO Primary Register in Germany) with the number DRKS00010462.

Statistical analysis
For calculation of the sample size, we assumed that the mean difference in starch and fat digestion and absorption was supposed to reach 75% of those obtained in our previous studies [19, 21]. Given a statistical power of 80%, a significance level of 5%, and a potential drop-out rate of 20%, 37 subjects were assigned to each group. The subjects’ randomization codes were concealed until the time of the final analysis. Consumption of 75% of the provided supplement every week of the study was the criterion for completion of the study [22]. The level of significance was set at $p < 0.05$. The comparison between the groups (CLA versus placebo) was performed using the Mann-Whitney U test. Within-group analyses (baseline versus 3 months) were done using the Wilcoxon test. Statistical analysis was carried out with the use of STATISTICA 10.0 (StatSoft Inc., Tulsa, USA).

Results
Volunteers were recruited from July 2014 through May 2015 with the follow up until August 2015 when the last participant finished the supplementation. Of 187 registered subjects, 81 met the inclusion criteria, of which seven were excluded due to personal problems (1), suspicion of an ovarian tumor (1), shortage of time (3), difficulties in cooperation (1), stomach pain, and diarrhea (1) (figure 1). Consequently, 74 women were randomized and included in the current data analyses. After the 3-month supplementation period, 12 subjects did not accomplish the study (three participants from the CLA subgroup and four participants from the placebo subgroup did not appear for the final visit; two participants from the placebo subgroup reported nausea; one woman from the placebo subgroup reported a rash; one subject from the CLA group had become pregnant). Additionally, one subject from the CLA group did not arrive for the S-BT. At the end of the intervention, data from 62 (83.8%) subjects were available for analysis. The drop-out rates were comparable in both arms of the study—13.5% for MTG-BT and 16.2% for S-BT in the CLA arm, and 18.9% for both measurements in the placebo group. The baseline characteristics of the group studied is presented in table 1. No other changes to trial outcomes were implemented after the trial had commenced. No significant adverse events were reported by participants. The study was concluded as planned. The mean compliance rate was $\geq 90\%$ in every subject studied.

MTG-BT
The values of CPDR before and after supplementation in the groups receiving the CLA intervention and placebo did not differ significantly (table 2). The changes in CPDR values ($\Delta$CPDR median (interquartile range)) were no different between subjects from the CLA and placebo groups (table 3). The incidence of negative and positive values of $\Delta$CPDR was no

<table>
<thead>
<tr>
<th>Table 2. Values of cumulative percentage dose recovery (CPDR) after mixed triglyceride breath test for placebo and conjugated linoleic acid (CLA) intervention before (pre) and after 3 months of supplementation (post).</th>
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</thead>
<tbody>
<tr>
<td><strong>CLA</strong></td>
</tr>
<tr>
<td>Median (1st–3rd quartile)</td>
</tr>
<tr>
<td>Pre</td>
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<tr>
<td>Post</td>
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</table>

<table>
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<tr>
<th>Table 3. The increment values of cumulative percentage dose recovery (CPDR) after mixed triglyceride breath tests for conjugated linoleic acid (CLA) and placebo.</th>
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</thead>
<tbody>
<tr>
<td>$\Delta$CPDR</td>
</tr>
<tr>
<td><strong>CLA</strong></td>
</tr>
<tr>
<td>Median (1st–3rd quartile)</td>
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<tr>
<td>Mean ± SEM</td>
</tr>
</tbody>
</table>

*The level of the statistical significance before and after the supplementation period.
Table 4. The number of subjects with positive and negative values of changes in cumulative percentage dose recovery (CPDR) after the mixed triglyceride breath test for conjugated linoleic acid (CLA) and placebo group.

<table>
<thead>
<tr>
<th></th>
<th>Negative ΔCPDR (% of subjects with negative ΔCPDR)</th>
<th>Positive ΔCPDR (% of subjects with negative ΔCPDR)</th>
<th>Relative risk (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA group (n = 32)</td>
<td>17 (53.1)</td>
<td>15 (46.9)</td>
<td>1.1384 (0.6889–1.8822)</td>
<td>0.6130</td>
</tr>
<tr>
<td>Placebo group (n = 30)</td>
<td>14 (46.7)</td>
<td>16 (53.3)</td>
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</table>

*CI denotes confidence interval.

Table 5. The values of cumulative percentage dose recovery (CPDR) in starch breath test for placebo and conjugated linoleic acid (CLA) intervention before and after 3 months of supplementation.

<table>
<thead>
<tr>
<th></th>
<th>CLA</th>
<th>Placebo</th>
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<tbody>
<tr>
<td></td>
<td>Median (1st–3rd quartile)</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Pre</td>
<td>14.2 (12.1–18.5)</td>
<td>15.2 ± 1.0</td>
</tr>
<tr>
<td>Post</td>
<td>11.5 (9.3–17.6)</td>
<td>12.5 ± 1.2</td>
</tr>
<tr>
<td>p value</td>
<td>0.0919</td>
<td></td>
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</tbody>
</table>

Table 6. The increment values of cumulative percentage dose recovery (CPDR) in the starch breath test for conjugated linoleic acid (CLA) and placebo group.

<table>
<thead>
<tr>
<th></th>
<th>CLA</th>
<th>Placebo</th>
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<tbody>
<tr>
<td></td>
<td>Δ CPDR</td>
<td>p value</td>
</tr>
<tr>
<td></td>
<td>Median (1st–3rd quartile)</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Pre</td>
<td>−1.3 (−9.5–2.4)</td>
<td>−1.0 (−5.1–1.7)</td>
</tr>
<tr>
<td>Post</td>
<td>−2.7 ± 1.6</td>
<td>−1.0 ± 1.1</td>
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</tbody>
</table>

*The level of the statistical significance before and after the supplementation period.

different between the subgroups studied (table 4). The incidence of decreased digestion and absorption was no different in subjects in the CLA subgroup compared with those of the placebo subgroup (53.12% versus 46.67%, RR 1.1384, 95% CI 0.6889 to 1.8822).

S-BT
The level of starch digestion and absorption was reflected by the CPDR values. The values of CPDR before and after supplementation in groups receiving CLA intervention and placebo did not differ significantly (table 5). The changes in CPDR values (ΔCPDR median (interquartile range)) were no different between subjects from the CLA and the placebo groups (table 6). The incidence of negative and positive values of ΔCPDR was no different between the subgroups studied (table 7). The incidence of decreased digestion and absorption was no different between participants from the CLA subgroup and participants from the placebo subgroup (67.74% versus 56.67%, RR 1.1954, 95% CI 0.8044 to 1.7765).

B-BT
The TEE before and after supplementation in the CLA and placebo groups did not differ (table 8). No changes in TEE (∆) were observed between subjects from CLA and placebo groups (table 9).

Discussion
The effect of CLA in humans is still widely disputed worldwide. Herein, we attempt to elucidate for the first time the impact of a 3-month intake of CLA on metabolism of fat and starch. This study indicates that CLA does not exert any significant effect in this respect.

Previous investigations differ widely in their designs. The dosage, composition as well as duration of the CLA treatment, as well as subjects’ health conditions may considerably affect the study outcomes in humans. A meta-analysis by Onakpoya et al revealed that long-term CLA intake significantly reduced body weight and, to a lesser extent, body fat, as compared to placebo [7]. Similarly, Whigham et al showed a modestly significant fat loss in 15 eligible trials [1].

According to various research studies, the safe CLA dose ranges from 3 to 6 g per day. In our study, we used 3 g of CLA, which is comparable to most studies [24–26]. Blankson et al proved that a higher amount (>3.4 g CLA per day) does not imply better weight reduction results [27]. The study evaluated the effect of a 12-week CLA intake on a similar study group using four various doses of CLA (1.7, 3.4, 5.1 or 6.8 g) and with olive oil as a placebo (9 g). Significant differences in the reduction of body mass were obtained only in the groups assigned to receive 3.4 g and 6.8 g of CLA, but the study included additional physical activity concomitant to the study; thus, it is difficult to discern the real influence of CLA.

The results of animal studies assessing different stages of fat metabolism are inconclusive [28, 29]. In vivo fatty acids β-oxidation could be evaluated by carnitine-palmitoyltransferase activity (CPT). CPT is a
Median (dependent diabetes mellitus model) 4-week intake of CLA increased CPT in a non-insulin-liver, brown adipose tissue of visceral-adipose tissue, red gastrocnemius muscle, mitochondrial rate limiting enzyme controlling the oxidation process. Rahman et al indicated that a 4-week intake of CLA increased CPT in a non-insulin-dependent diabetes mellitus model (perirenal fraction of visceral-adipose tissue, red gastrocnemius muscle, liver, brown adipose tissue) [28]. The study used a homogenate fraction of different tissues to represent overall fatty acid oxidation. Martin et al, however, documented that a 6-week diet containing 1% of CLA did not affect β-oxidation in skeletal and cardiac muscles [29]. Based on another mice model it was suggested that CLA (in the form of free fatty acids as well as triacylglycerols) may promote a mild increase in energy expenditure, while increased energy losses in excreta reflect decreased nutrient absorption [30, 31]. Assuming that both effects are present in humans, one could expect unchanged breath test results in this study. However, in that case it should be accompanied by weight losses in the CLA group, which were not observed in our trial [17]. To exclude the effect of oxidation, in this study we performed B-BT, which is a non-invasive and reliable method providing results in accordance with indirect calorimetry as the gold standard [32, 33]. We did not observe any differences in TEE changes in the course of CLA supplementation. Reports on different animal models raise a question as to which species mimic the human model best and what is the appropriate CLA dose per kg of body weight [30, 31, 34], which in animal models is far higher. Our results suggest that the impact of CLA on metabolic processes in humans remains unclear.

The present study attempts to assess the impact of 3-month CLA intake on starch and fat digestion and the subsequent absorption using a valid method of MTG-BT and S-BT. The method uses molecules of two stearic acids and 13C octanoic acid to serve as a labelled tracer through the process of hepatic β-oxidation and formation of 13CO2. During the 6 h of the test procedure, the dose of 13C recovered in the exhaled air is registered as a measure of intestinal lipolysis [35]. The other method, S-BT, measures CO2 in breath that comes from metabolized glucose previously hydrolyzed from starch [36]. Our study used cornflakes as a test meal. Starch from corn is digested in the small intestine, absorbed, then metabolized in the liver and transported to the lungs. No data are available referring to the impact of long-term CLA supplementation on carbohydrate digestion and absorption in humans so far. Some studies indicate that CLA can improve glucose intolerance and balance hyperinsulinemia in the animal model (obese-diabetic insulin resistant, prediabetic animals on a high-fat diet) [37, 38]. The study by Fariña et al documented that CLA increases glucose oxidation and also enhances glucose uptake as well as incorporation into the rat muscle [39]. On the other hand, serum glucose levels have not changed in any of the animal studies [40–42].

The limitations of the study include the potential lack of full patients’ compliance, which we tried to minimize. The participants were called on the phone three times during the 3 months to provide motivation and ensure the subjects follow the regime. Additionally, women were asked to bring empty packages and to maintain a 90 day calendar to monitor the supplementation. Other limitations involve the fact that gut
Although this subject needs further investigation.

Acknowledgments

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Author contributions

JW, EM & AL designed the experiment; KM, AG, PB, EFW, MS, ICS, AMC & AL performed the research; JW supervised the study; JW, KM, AG & AL created database and analyzed data; JW, AG, KM & AL wrote the manuscript; EM & PB provided revisions. All authors reviewed and approved the manuscript.

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