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**Six months supplementation with conjugated linoleic acid (CLA) induces regional-specific fat mass decreases in overweight and obese.**

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**Running title:** CLA reduces region specific fat mass in human

**Keywords:** Conjugated linoleic acid, Body composition, Overweight, Obesity.

1 **Abstract:**

2 **Background:** Long-term supplementation with conjugated linoleic acid (CLA) reduces body  
3 fat mass (BFM) and increases or maintains lean body mass (LBM). However the regional  
4 effect of CLA was not studied. **Objective:** This study aimed to evaluate the effect of CLA per  
5 region and safety in healthy, overweight and obese adults. **Design:** 118 subjects (Body mass  
6 index (BMI): 28-32 kg/m<sup>2</sup>) were included in a double blind, placebo-controlled trial. Subjects  
7 were randomized into two groups supplemented with either 3.4 g/d CLA or placebo for 6  
8 months. **Results:** CLA decreased significantly BFM at month 3 ( $\Delta = -0.9\%$ ,  $P = 0.016$ ) and at  
9 month 6 ( $\Delta = -3.4\%$ ,  $P = 0.043$ ) compared to placebo. The reduction in fat mass was located  
10 mostly in the legs ( $\Delta = -0.8$  kg,  $P < 0.001$ ), and in women ( $\Delta = -1.3$  kg,  $P = 0.046$ ) with BMI  
11 over 30 kg/m<sup>2</sup> ( $\Delta = -1.9$  kg,  $P = 0.011$ ), compared to placebo. Waist/hip ratio decreased  
12 significantly ( $P = 0.043$ ) compared to placebo. LBM increased ( $\Delta = +0.5$  kg,  $P = 0.049$ )  
13 within the CLA group. Bone mineral content was not affected ( $P = 0.70$ ). All changes were  
14 independent of diet and physical exercise. Safety parameters including blood lipids,  
15 inflammatory and diabetogenic markers remained within normal range. Adverse events did  
16 not differ between the groups. **Conclusions:** Supplementation with CLA in healthy,  
17 overweight and obese adults decreases FM in specific regions and is well tolerated.

## 1 **Introduction:**

2 Conjugated linoleic acid (CLA), which refers to a mixture of linoleic acid isomers  
3 containing conjugated double bonds, has been shown in animal models to share several  
4 potential health benefits including positively influencing body composition, reducing  
5 atherosclerosis biomarkers, reducing cancer risk, diabetes management, reducing  
6 inflammation and positively influencing the immune response (Zu & Schut, 1992; Cook *et al.*,  
7 1993; Lee *et al.*, 1994; Ip *et al.*, 1994; Miller *et al.*, 1994; Liew *et al.*, 1995; Schonberg &  
8 Krokan, 1995; Wong *et al.*, 1997; Cunningham *et al.*, 1997; Nicolosi *et al.*, 1997; Dugan *et*  
9 *al.*, 1997; West *et al.*, 1998; Houseknecht *et al.*, 1998; Park *et al.*, 1999; DeLany *et al.*, 1999;  
10 Gavino *et al.*, 2000; Szymczyk *et al.*, 2001; Rahman *et al.*, 2001; Akahoshi *et al.*, 2002;  
11 Corino *et al.*, 2002; Ostrowska *et al.*, 2003; Bassaganya-Riera *et al.*, 2003; Bassaganya-Riera  
12 *et al.*, 2004; Wargent *et al.*, 2005; Bhattacharya *et al.*, 2005).

13 In humans, CLA has been tested extensively for its ability to modify body composition  
14 (Lowery *et al.*, 1998; Atkinson, 1999; Blankson *et al.*, 2000; Berven *et al.*, 2000; Thom *et al.*,  
15 2001; Riserus *et al.*, 2001; Kreider *et al.*, 2002; Gaullier *et al.*, 2004; Gaullier *et al.*, 2005).  
16 When the studies were performed with reliable body composition measurement methodology  
17 (*e.g.*, DXA scan) together with the mixture containing mainly the two bioactive isomers *cis*-9,  
18 *trans*-11 and *trans*-10, *cis*-12 CLA (Gaullier *et al.*, 2002), CLA reduced body fat mass in  
19 humans at the same time as lean body mass was preserved (Gaullier *et al.*, 2004; Gaullier *et*  
20 *al.*, 2005). However, it was not shown whether body fat mass was reduced equally in the  
21 whole body or if the reduction was restricted to some regions. This finding could be of  
22 importance since abdominal obesity has been associated to increased risk of cardiovascular  
23 disease (CVD) (Reaven, 2005).

24 Other studies with supplementation of the two bioactive CLA isomers in humans  
25 showed that CLA is able to stimulate immune response (Albers *et al.*, 2003; Song *et al.*,  
26 2005), improves insulin sensitivity in sedentary individuals (Eyjolfson *et al.*, 2004) and  
27 beneficially modifies lipid metabolism (Noone *et al.*, 2002). Human studies on type 2

1 diabetics showed conflicting results (Belury *et al.*, 2000; Belury *et al.*, 2003; Moloney *et al.*,  
2 2004). The reported negative effect on insulin sensitivity in one of the trials with type 2  
3 diabetic patients might however been due to the fact that there was a bias in glucose tolerance  
4 between placebo and the CLA group at the start of the trial and not due to CLA intake per se  
5 (Moloney *et al.* 2004). No acute toxic adverse events were reported in any of the trials. When  
6 reported, the adverse events were of mild character and were associated mostly with  
7 gastrointestinal disturbances (Blankson *et al.*, 2000; Berven *et al.*, 2000; Gaullier *et al.*, 2004;  
8 Gaullier *et al.*, 2005). In another controlled study, the incidence of adverse events was lower  
9 in the CLA treated group as compared to the control group (Whigham *et al.*, 2004). However,  
10 other studies showed that the mixture of the two bioactive isomers of CLA could increase  
11 markers of lipid peroxidation in healthy subjects (Basu *et al.*, 2000a), even though  
12 pathological alterations were not reported (Basu *et al.*, 2000b). When supplemented  
13 independently in high concentration of either the trans-10, cis-12 CLA or the cis-9, trans-11  
14 CLA these single isomers increased insulin resistance in subjects with the metabolic  
15 syndrome (Riserus *et al.*, 2002a; Riserus *et al.*, 2004a), whereas the mixture of both isomers  
16 did not affect insulin sensitivity of subjects with metabolic syndrome (Riserus *et al.*, 2002a),  
17 and improved in sedentary men (Eyjolfson *et al.*, 2004). To date only the mixture of both  
18 bioactive isomers, but not the high concentrated single isomers, is accessible commercially to  
19 all. It was therefore necessary to investigate how supplementation of a commercial mixture of  
20 CLA affects blood safety parameters including inflammation and diabetes markers in a long-  
21 term study to determine the safety of CLA in human.

22         The primary objective of the present study was thus to assess the localization of the  
23 body fat mass reduction in overweight and moderate obese subjects during a six-months CLA  
24 supplementation, and secondarily to determine if CLA supplementation was safe.

1 **Subjects and Experimental Methods:**

2 **Subjects.** 118 healthy male and female volunteers 18 to 65 years of age, with body mass index  
3 (BMI) of 28-32 kg/m<sup>2</sup>, were recruited by two research centers (Diabetes and Overweight  
4 Clinic, Specialist Medical Center, Oslo, Norway, n=50; and the Hedmark Medical Center,  
5 Hamar, Norway, n=68). All subjects signed an informed consent form before inclusion.

6 Exclusion criteria included drug therapy, special diet or taking dietary substitutes for  
7 weight loss two weeks prior to study start. CLA consumption in the form of supplements  
8 during the last three months prior to the study, pregnant and lactating women, Type 1  
9 diabetes or those with untreated Type 2 diabetes mellitus, those with a history of  
10 hypertension, renal, liver, pancreatic or chronic inflammatory/infectious diseases, cardiac  
11 failure or malignant tumors, those with active thyroid disease or receiving thyroid hormone  
12 substitution, adrenergic agonist use, and volunteers with known/suspected drug or alcohol  
13 abuse or with any clinical condition rendering them unfit to participate were also excluded  
14 from participation.

15  
16 **Ethics.** The regional Ethics Committee and the local authorities approved the study before it  
17 started. The study was conducted in agreement with the Declaration of Helsinki of 1975 as  
18 amended in 2000, and accordance with the International Conference on Harmonization (ICH)  
19 guidelines.

20  
21 **Study design.** This study had a parallel design with two arms, double blinded, randomized,  
22 and placebo-controlled. The subjects were randomized to either CLA 3.4g/day (4.5g  
23 Clarinol™, Lipid Nutrition, division of Loders Croklaan, The Netherlands, n=59) or placebo  
24 (4.5g olive oil, n=59) corresponding to 6 opaque soft gel capsules, all identical in taste and in  
25 appearance. The CLA oil was a mixture containing 37.5% cis-9, trans-11 and 38% trans-10,  
26 cis-12. The rest of the mixture was made of other fatty acids (containing less than 2% of trans,  
27 trans, less than 7% of saturated fatty acids and less than 1% in free fatty acids). The subjects

1 were on *ad-lib* diet and no restrictions in lifestyle or in caloric intake were implemented.  
2 However, on request, the study nurse gave the subject dietary advice and exercise  
3 recommendations of a general nature at the beginning of the study.

4

5 ***Clinical assessments.*** Baseline characteristics and demographic data were recorded by the  
6 medical staff when entering the study to evaluate the eligibility of the participants. Smoking  
7 habits were divided into four categories (No, Yes: 1-10 cigarettes, 11-19 cigarettes and over  
8 or equal to 20 cigarettes per day), whereas alcohol drinking habits were divided into five  
9 categories (No, Yes: 1-7 units, 8-14 units, 15-25 units and over 25 units per day) with one unit  
10 of alcohol defined as 12g, or 1 shot (3 cl), 1 glass of wine (1.2 dl), or 1 glass of beer (3 dl).  
11 Weight, body mass index (BMI), vital signs were recorded every 3 months. Twelve hour-  
12 fasting blood samples were analysed for adiponectin, alkaline phosphatase (ALP), Alanine  
13 amino transferase (ALAT), Aspartate amino transferase (ASAT), C-reactive protein (CRP),  
14 creatinine, erythrocytes, gamma-glutamyltransferase ( $\gamma$ -GT), glucose, glucohemoglobin  
15 (HbA1c), high density lipoprotein-cholesterol (HDL), hemoglobin, leucocytes, low density  
16 lipoprotein-cholesterol (LDL), insulin, insulin c-peptide, leptin, lipoprotein a (Lp(a)),  
17 thrombocytes/platelets, total cholesterol, triglycerides, and white blood cells. All analyses  
18 were performed in accredited laboratories (Først Laboratory and Aker University Hospital,  
19 Oslo, Norway) at baseline and at 6 months. Cytokines analyses (interleukin-1 $\beta$  (IL-1 $\beta$ ),  
20 interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor alpha (TNF $\alpha$ )) were  
21 performed in a third laboratory (Bioceros, Utrecht, The Netherlands). Compliance was  
22 measured every 3 months by counting the returned unused capsules, and compared to the  
23 number of capsules that should have been used. A subject was considered compliant when  
24 taking  $\geq 70\%$  of administered supplement.

25

1 **Adverse events.** An adverse event (AE) was defined as any unfavourable, unintended event  
2 (or symptom) reported by a subject or observed by the investigator during the study. All AEs  
3 were recorded with information about the nature, severity, frequency, onset date, duration,  
4 and action taken regarding study products. The investigators classified also each AE as  
5 serious or non-serious. A serious adverse event (SAE) was defined as any untoward medical  
6 occurrence that resulted in death, was life-threatening, required subject hospitalization or  
7 prolongation of existing hospitalization, resulted in persistent or significant disability or  
8 incapacity. AEs were recorded every 3 months, while serious adverse events (SAE) were  
9 monitored continuously throughout the study. The AEs had not necessarily a causal  
10 relationship with the study product. It was up to the investigators to evaluate the relationship  
11 of the AE to the study product in a double blind manner (without code breaking of the  
12 randomization list). Their evaluation was based on previous studies as well as their general  
13 practice.

14  
15 **Diet and exercise.** Diet and exercise were assessed at baseline and at 6 month. During about 1  
16 hour prior to the visit at the medical center, each participant recorded diet and exercise forms  
17 covering for a period of 14 consecutive days according to a previously evaluated and  
18 validated method (Nes *et al.*, 1992). The method provides information on quantity and type  
19 of food consumed during the period for records as described before (Gaullier *et al.*, 2004),  
20 with using a specially designed software program BEREGN (Oslo University, Norway).  
21 Participation rate was high (81.7 %), and the non-responders included n=6 in the CLA group  
22 and n=14 in the placebo group. Exercise was assessed as the mean score of the number of  
23 training sessions per week with intensity (each subject was categorized according to the  
24 following scale: no exercise, score=0; exercise 1 time/week without sweat, score=1; exercise  
25 2-3 times/week without sweat, score=2, exercise >3 times/week without sweat, score=3;  
26 exercise 1 time/week with sweat, score=4; exercise 2-3 times/week with sweat, score=5,  
27 exercise >3 times/week with sweat, score=6).

1

2 **Measurement of body composition, body weight and anthropometric variables.** Dual-energy  
3 X-ray absorptiometry (DXA) was used to determine body composition (Lunar Prodigy,  
4 software version 6.0, Madison, WI). Measurements were providing masses for the whole  
5 body in fat (FBM), muscles and water together as lean body mass (LBM) and bone mineral  
6 content (BMC). In addition, regional masses in fat were provided in the arm (AFM), in the  
7 abdomen (TFM) and in the legs (LFM).

8 The Hologic spine phantom was used for daily quality control assessment (CV consistently  
9 around 0.5 %). For weekly assessment the Hologic whole body phantom 162 was used. This  
10 phantom has a total weight of 27.8 kg and consists of 5 sheets of polyethylene shaped in a  
11 pyramidal fashion. In the lower sheet an aluminium body shaped figure is embedded and  
12 sealed off by a polyvinyl sheet. CV for the surrogates of fat mass, LBM and BMC were 0.7;  
13 0.7 and 0.9 %, respectively.

14 The subjects were weighed on digital scales (TBF-305, Tanita, UK) in their underwear. No  
15 subtractions for clothes were performed. Height was measured using wall fixed Harpenden  
16 stadiometers (Holtain, UK) (CV less than 0.2 %). BMI was calculated as the ratio of the  
17 weight in kg by the square of the size expressed in meter. Waist and hip were measured as  
18 previously described (Hall & Young, 1989).

19

20 **Statistical analysis.** Results are shown as means  $\pm$  SD (see tables). The primary variable was  
21 the change of BFM as determined by DXA. A test power of 90% was planned, based on a  
22 relative difference in BFM reduction between each CLA group and placebo of at least 1 x SD.  
23 Testing between both groups to investigate comparability at baseline was applied using an  
24 overall F-test. Differences from baseline to month 6 within the groups were tested using a  
25 paired T-test for normally distributed variables. Categorical variables were analyzed using  
26 Fisher's exact test or a chi-square test. Comparisons between treatment groups regarding  
27 change from baseline in DXA and weight variable, were performed using ANCOVA

1 (treatment, center and gender as factors; baseline value, total energy intake, exercise and  
2 interaction treatment x energy intake and treatment x training score as covariates). A  
3 significance level of 5% was used in tests, and all tests were two-tailed. The software used to  
4 perform these analyses was the SAS for Windows (version 8.2).

5         Statistical analyses for efficacy and safety parameters were performed on all subjects  
6 with at least one post-baseline visit in a subpopulation (n=105) defined as the Intention to  
7 Treat population (ITT). The principle of last-value-carried-forward was applied on this  
8 population for efficacy variables only. In addition statistical analyses for efficacy on the main  
9 parameters were performed on the Per protocol (PP) population including subjects who  
10 completed all visits and with compliance  $\geq 70\%$  (n=83).

1 **Results:**

2 ***Study subjects.*** 118 subjects were randomized and 93 completed the study (78.8%) with  
3 similar rates of withdrawal in both groups (n=12 for CLA, and n=13 for placebo,  $P = 0.37$ )  
4 (**Figure 1**). Two subjects in the CLA group discontinued the study because of AEs, one left  
5 the trial after removal of a malignant breast cancer incidentally discovered, and the other  
6 subject suffered of constipation. In the whole population (n=118), both groups were similar at  
7 baseline in gender, age, alcohol use and physical exercise (data not shown).

8 Out of 118 subjects, 13 subjects discontinued the study prior to the second visit  
9 (month 3). Since no data were available for these subjects, they were excluded from all  
10 analyses. Both groups had similar baseline characteristics in the remaining population  
11 (n=105) (**Table 1**). Compliance at month 6 was  $96.7 \pm 16.7$  % and  $97.8 \pm 17.1$ % in the  
12 placebo group and in the CLA group, respectively.

13 Out of the 105 subjects, 12 subjects discontinued the study prior to the third visit  
14 (month 6), whereas 10 of the remaining subjects had a compliance lower than 70% as  
15 described in the protocol. Therefore 83 subjects completed the study according to the protocol  
16 and is defined here as the PP population. Both groups of the PP population (n=42 for CLA  
17 and n=41 for placebo) were similar at baseline and had compliance at month 6 of  $99.2 \pm 14.6$   
18 % in the placebo group and  $99.2 \pm 13.3$ % in the CLA group.

19

20 ***Effects of CLA on body composition.*** BFM that did not differ significantly between the  
21 groups at baseline ( $P = 0.69$ ) was significantly reduced 3.4 % after 6 months supplementation  
22 with CLA compared to placebo (n=105,  $\Delta = -1.2$  kg,  $P = 0.043$ ) (**Table 2**). This difference  
23 between the groups was also observed when the fat mass was measured as the percentage of  
24 the whole body weight (n=105,  $\Delta = -0.9$ %,  $P = 0.028$ ). The decrease in BFM and FM  
25 percentage were already significantly higher (n=105,  $\Delta = -0.3$  kg,  $P = 0.016$  and  $\Delta = -0.2$ %,  $P$   
26 = 0.040, respectively) in the CLA group as compared to the placebo group after 3 months  
27 supplementation. Compared to baseline, the CLA group reduced BFM significantly at month

1 6 (n=55,  $\Delta = -1.0$  kg,  $P = 0.036$ ), and BF percentage (n=55,  $\Delta = -1.0\%$ ,  $P = 0.009$ ), whereas  
2 the BFM and BF percentage in the placebo group remained unchanged compared to baseline  
3 (n=50,  $\Delta = +0.2$  kg,  $P = 0.64$ , and  $\Delta = -0.1\%$ ,  $P = 0.77$ , respectively) (Table 2). CLA was  
4 more efficient in women (n=84,  $\Delta = -1.3$  kg,  $P = 0.046$ ) than in men (n=21,  $\Delta = -0.7$  kg,  $P =$   
5  $0.61$ ) when compared to placebo, and more efficient for subjects with BMI over and equal to  
6  $30 \text{ kg/m}^2$  (n=63,  $\Delta = -1.3$  kg,  $P = 0.011$ ) than for subjects with BMI below  $30 \text{ kg/m}^2$  (n=42,  $\Delta$   
7  $= -0.3$  kg,  $P = 0.92$ ).

8         After 6 months supplementation, the difference in LBM between the CLA group and  
9 the placebo group was not statistically significant (n=105,  $\Delta = +0.4$  kg,  $P = 0.22$ ) (Table 2).  
10 However, within-group analyses revealed a significant increase in LBM in subjects  
11 supplemented with CLA (n=55,  $\Delta = +0.4$  kg,  $P = 0.049$ ), and slight but not significant change  
12 in the placebo group (n=50,  $\Delta = +0.1$  kg,  $P = 0.62$ ) (Table 2).

13         There was no difference in BMC between the groups (n=105,  $P = 0.70$ ) (Table 2), and  
14 no changes within both groups from baseline to month 6 (n=55, CLA:  $P = 0.36$ ; n=50,  
15 Placebo:  $P = 0.49$ ).

16         Analyses on the PP population confirmed analyses obtained on the main population  
17 but in general changes were more pronounced in the PP population (Table 2). Especially, the  
18 PP population demonstrated a significant reduction in BFM by  $-5.6\%$  (n=83,  $\Delta = -2.0$  kg,  $P =$   
19  $0.005$ ) for the CLA group when compared to the placebo group. Reduction of the BFM was  
20 already observed after 3 months (n=42,  $\Delta = -0.7$  kg,  $P = 0.034$ ) in the CLA supplemented  
21 group (data not shown). In the PP population CLA was also more efficient in women (n=65,  $\Delta$   
22  $= -2.1$  kg,  $P = 0.005$ ) than in men (n=18,  $\Delta = -1.1$  kg,  $P = 0.513$ ).

23

24 ***Localization of the decrease in body fat mass.*** FM did not differ significantly between the  
25 groups at baseline either in the arms (n=105, AFM,  $P = 0.83$ ), in the abdomen (n=105, TFM,  
26  $P = 0.25$ ) and in the legs (n=105, LFM,  $P = 0.77$ ) (Table 2). After 6 months of  
27 supplementation, the CLA reduced significantly FM in the legs compared to placebo (n=105,

1  $\Delta = -0.8$  kg,  $P < 0.001$ ), but not in the arms ( $n=105$ ,  $\Delta = +0.1$  kg  $P = 0.43$ ) nor in the abdomen  
2 ( $n=105$ ,  $\Delta = -0.5$  kg  $P = 0.18$ ) (Table 2). Compared to baseline CLA did not change AFM  
3 ( $n=55$ ,  $\Delta = -0.3$  kg,  $P = 0.12$ ) or TFM ( $n=55$ ,  $\Delta = -0.2$  kg,  $P = 0.47$ ), but reduced LFM ( $n=55$ ,  
4  $\Delta = -0.5$  kg,  $P = 0.005$ ) at month 6, while a reduction of AFM ( $n=50$ ,  $\Delta = -0.4$  kg,  $P = 0.027$ )  
5 only was observed in the placebo group (Table 2). The reduction of LFM in the CLA group  
6 was mainly seen in women ( $n=84$ ,  $\Delta = -1.0$  kg,  $P < 0.001$ ).

7 Analyses in the PP population showed also a reduction in LFM significantly higher in  
8 the CLA group as compared to the placebo group ( $n=83$ ,  $\Delta = -1.0$  kg,  $P = 0.003$ ), and a  
9 tendency of a higher reduction in the abdomen ( $n=83$ ,  $\Delta = -0.9$  kg  $P = 0.068$ ) (Table 2). In  
10 addition, CLA was more efficient in reducing LFM in women ( $n=84$ ,  $\Delta = -1.2$  kg,  $P < 0.001$ )  
11 and in adults with BMI  $\geq 30$  kg/m<sup>2</sup> ( $n=63$ ,  $\Delta = -1.4$  kg,  $P < 0.001$ ).

12

13 **Effects of CLA on weight and BMI.** There was no statistical difference between the study  
14 groups at baseline either for weight ( $n=105$ ,  $P = 0.63$ ) or BMI ( $n=105$ ,  $P = 0.25$ ) (Table 3).  
15 After 6 months of supplementation, there was no significant difference between the groups in  
16 weight ( $n=105$ ,  $\Delta = -0.9$  kg,  $P = 0.15$ ) and BMI ( $n=105$ ,  $\Delta = -0.3$ ,  $P = 0.19$ ) (Table 3).  
17 Compared to baseline, neither group had changes in body weight ( $n=105$ , CLA:  $\Delta = -0.9$  kg,  $P$   
18  $= 0.10$ ; Placebo:  $\Delta = 0.0$  kg,  $P = 0.096$ ) and BMI ( $n=105$ , CLA:  $\Delta = -0.3$ ,  $P = 0.07$ ; Placebo:  $\Delta$   
19  $= 0.0$ ,  $P = 0.86$ ) after 6 months of supplementation (Table 3). However, individuals with a  
20 BMI  $\geq 30$  kg/m<sup>2</sup> lost weight ( $n=63$ ,  $\Delta = -1.9$  kg,  $P = 0.020$ ) and their BMI decreases  
21 significantly ( $n=63$ ,  $\Delta = -0.6$ ,  $P = 0.031$ ) after CLA intake compared to placebo.

22 The PP analyses showed a tendency of a difference between the groups after 6 months  
23 of supplementation in weight ( $n=83$ ,  $\Delta = -1.5$  kg,  $P = 0.056$ ) and BMI ( $n=83$ ,  $\Delta = -0.6$ ,  $P =$   
24  $0.062$ ) (Table 3). Changes from baseline in the PP population showed a decrease in BMI in  
25 the CLA group ( $n=42$ ,  $\Delta = -0.5$ ,  $P = 0.040$ ), but not in the placebo group ( $n=41$ ,  $\Delta = +0.1$ ,  $P =$   
26  $0.71$ ).

27

1 **Effects of CLA on anthropometric parameters waist and hip.** There was no statistical  
2 difference between the study groups (n=105) at baseline either for waist ( $P = 0.54$ ), hip ( $P =$   
3  $0.54$ ) and waist/hip ratio ( $P = 0.80$ ) (Table 3). After 6 months supplementation, there was a  
4 higher reduction in waist/hip ratio in the CLA group as compared to the placebo group  
5 (n=105,  $\Delta = -0.01$ ,  $P = 0.043$ ) (Table 3). Compared to baseline the CLA group (n=55) had  
6 reduction in waist ( $\Delta = -2.6$  cm,  $P < 0.001$ ), hip ( $\Delta = -1.4$ ,  $P = 0.009$ ) and waist/hip ratio ( $\Delta =$   
7  $-0.02$ ,  $P = 0.042$ ), whereas there were no significant changes within the placebo group (n=50,  
8 waist:  $\Delta = -1.3$  cm,  $P = 0.065$ ; hip:  $\Delta = -0.6$  cm,  $P = 0.23$ ; waist/hip ratio:  $\Delta = -0.01$ ,  $P = 0.27$ )  
9 (Table 3).

10 The analyses in the PP population showed that CLA decreased significantly waist  
11 (n=42,  $\Delta = -3.1$  cm,  $P < 0.001$ ) and waist/hip ratio (n=42,  $\Delta = -0.02$ ,  $P = 0.018$ ), but not hip  
12 (n=42,  $\Delta = -1.1$  cm,  $P = 0.084$ ) from baseline to month 6 (Table 3). There was no difference  
13 between the groups at month 6 for any of the anthropometric parameters (data not shown).

14  
15 **Diet and exercise.** Caloric intake decreased significantly in the placebo group but not in the  
16 CLA group compared to baseline (Table 3). However, there were no differences between the  
17 groups either at baseline or after 6 months (n=105,  $\Delta = +910$  kJ/day,  $P = 0.22$ ). Physical  
18 exercise evaluations remained unchanged between baseline and month 6 within both group  
19 (Table 3), and there was no difference between the groups (n=105,  $\Delta = +0.29$  a.u.,  $P = 0.73$ ).

20 The same results were noted in the PP population (n=83) (Table 3).

21  
22 **Safety.** All laboratory parameters were similar between both groups at baseline (n=105). CLA  
23 did not alter clinically any of the following analyzed clinical chemistry variables: ALAT ( $\Delta =$   
24  $-0.20 \pm 13.64$  U/l,  $P = 0.92$ ), ALP ( $\Delta = +2.66 \pm 12.67$  U/l,  $P = 0.14$ ), ASAT ( $\Delta = -1.14 \pm 7.91$   
25 U/l,  $P = 0.32$ ), creatinin ( $\Delta = -14.88 \pm 9.18$   $\mu\text{mol/l}$ ,  $P < 0.001$ ; no difference with placebo  
26 group  $P = 0.99$ ), erythrocytes ( $\Delta = -0.06 \pm 0.18$   $10^{12}/\text{l}$ ,  $P = 0.04$ ; no difference with placebo

1 group  $P = 0.23$ ), hemoglobin ( $\Delta = -0.23 \pm 0.60$  g/100ml,  $P = 0.013$ ; no difference with  
2 placebo group  $P = 0.42$ ) and thrombocytes ( $\Delta = -3.85 \pm 38.09$   $10^9/l$ ,  $P = 0.27$ ).

3         Comparison of the diabetes markers and weight-associated hormones between groups  
4 demonstrated no significant differences in changes in fasting glucose ( $P = 0.40$ ), HbA1c ( $P =$   
5  $0.28$ ), fasting insulin ( $P = 0.93$ ), insulin c-peptide ( $P = 0.48$ ), leptin ( $P = 0.50$ ), and  
6 adiponectin ( $P = 0.72$ ) levels (**Table 4**). After 6 months supplementation with CLA there  
7 were marginal, but significant increase in insulin c-peptide levels ( $P = 0.017$ ). However, the  
8 increase in insulin c-peptide remained within the normal range. Only one subject in the CLA  
9 group and two in the placebo group had an increase in insulin c-peptide level above the  
10 normal range values from baseline to 6 months. Glucose ( $P < 0.001$ ) and HbA1c levels ( $P =$   
11  $0.047$ ) decreased compared to baseline. In the placebo group, only glucose levels decreased  
12 from baseline to month 6 ( $P < 0.001$ ) (Table 4).

13         Comparison of the blood lipids between the groups demonstrated no significant  
14 differences in Lp(a) ( $P = 0.97$ ), total cholesterol ( $P = 0.32$ ), HDL cholesterol ( $P = 0.28$ ), LDL  
15 cholesterol ( $P = 0.19$ ), and triglycerides ( $P = 0.22$ ) levels (**Table 5**). After 6 months with  
16 CLA supplementation there were also marginal, but significant increases in Lp(a) levels in  
17 both CLA group ( $P = 0.017$ ) and placebo group ( $P = 0.020$ ), whereas HDL cholesterol levels  
18 decreased in the CLA group ( $P = 0.030$ ) compared to baseline (Table 5), but these changes  
19 remained within the normal range. Twenty subjects with Lp(a) levels significantly above  
20 normal range at baseline ( $n=13$  and  $n=7$  in the CLA group and the placebo group,  
21 respectively) maintained or increased slightly their level in Lp(a) during the study. Finally one  
22 subject from the CLA group had a decrease in HDL cholesterol level to below the normal  
23 range at month 6.

24         Comparison of the laboratory inflammation markers between groups demonstrated no  
25 differences in IL-6 ( $P = 0.65$ ), IL-8 ( $P = 0.47$ ), TNF $\alpha$  ( $P = 0.61$ ) and leucocytes ( $P = 0.07$ )  
26 levels from baseline to month 6, whereas differences in CRP levels were significantly higher  
27 but remained within normal limits in the CLA group compared to the placebo group at month

1 6 ( $P = 0.011$ ) (**Table 6**). IL-1 $\beta$  levels were too low to be detected both at baseline and month  
2 6. Six months supplementation with CLA increased within the normal range CRP levels ( $P =$   
3 0.004) as compared to baseline but not the other inflammation markers (Table 6). Three  
4 subjects in the CLA group and one subject in the placebo group increased their CRP levels  
5 above normal range at month 6.

6  
7 **Adverse events.** AEs were reported by 37% of all randomized subjects with similar  
8 frequencies in both study groups ( $P = 0.85$ ). Of 58 single events, the investigators considered  
9 five of them related to supplementation (n=4 in the CLA group and n=1 in the placebo group)  
10 when the study code was still not broken. Of these one event (diarrhea) was classified as  
11 “Probable” and the rest as “Possible”. All AEs were rated as either “mild” or “moderate”, and  
12 were most likely to occur within the gastrointestinal and musculoskeletal systems. The  
13 registration of infections reflected a well-known seasonal pattern.

14 As reported elsewhere two subjects (1.7 % of the total) left the trial due to adverse  
15 events, one case of incidentally discovered breast cancer considered unrelated to  
16 supplementation, and one case of constipation ‘possibly’ related to CLA supplementation.

17 Study supplementation was temporary interrupted in another three subjects. One male  
18 subject suffered an acute myocardial infarction of moderate severity. The serious adverse  
19 event was considered as ‘possibly’ related to supplementation without breaking the study  
20 code. However the subject came back in the study a short time after recovery and completed it  
21 accordingly to the protocol. After unblinding the study, it was found that the subject was  
22 supplemented with CLA. The second subject suffered a rash that was not judged related to  
23 supplementation. The third subject supplemented with placebo was suffering a hernia. The  
24 investigators chose to reintroduce the three subjects once recovery was established and all  
25 three continued until scheduled end of study.

1 **Discussion:**

2 In the present study with overweight and moderately obese subjects, we observed an  
3 approximately 3.4% decrease in BFM after 6 months of supplementation in the main  
4 population, and an approximately 5.6% decrease in BFM in the PP population compared to  
5 placebo. These reductions were thus similar to the reductions observed at 6 months in the  
6 previous study performed with CLA containing the same amount of both active isomers trans-  
7 10, cis-12 and cis-9, trans-11 (Gaullier *et al.*, 2004). Fat mass might even further decrease up  
8 to about 9% after 1 to 2 years as observed previously in overweight subjects (Gaullier *et al.*,  
9 2004; Gaullier *et al.*, 2005). In addition, the current study confirmed previous observation  
10 (Blankson *et al.*, 2000) indicating that reduction in BFM was already significant after 3  
11 months of supplementation. In both studies, the best responders were women, and subjects  
12 with the highest BMI at study start. However, the efficacy of CLA by gender should be  
13 interpreted with care since only one man for four women participated in these studies, thus  
14 reducing the probability to observe small but significant body composition changes in men.

15  
16 The current study is the first to report that the reduction of body fat mass occurred  
17 mostly in the legs and that CLA had a tendency to decrease the amount of abdominal fat mass  
18 which is in line with the observed significant decrease in waist/hip ratio. The decrease in fat  
19 mass in the legs was observed especially in women, probably due to their gynoid fat  
20 distribution (Kirchengast *et al.*, 1998). On the other hand, men (Mueller & Joos, 1985) and  
21 women with several weight loss/regain cycles (Wallner *et al.*, 2004) mostly accumulate fat in  
22 the abdomen. In the current study, CLA had the tendency to reduce abdominal fat as  
23 supported by a concomitant reduction in waist/hip ratio. Whereas the clinical importance of  
24 the reduction in leg fat mass is unclear and does probably not influence risks for developing  
25 CVD in the future (Hara *et al.*, 2004), the reduction in abdominal fat and in waist/hip ratio  
26 may represent both independent indicators of a possible reduction in risks for CVD (Reaven,  
27 2005; Yusuf *et al.*, 2005).

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The reduction in body fat mass was accompanied with maintenance or increase in lean body mass for a 6 months period in the current study when compared between the groups or within the CLA group, respectively. This lean body mass may even increase further with time as seen previously (Gaullier *et al.*, 2004). Loss of FM without loss of LBM confers to CLA a unique feature as compared to other weight reducing agents or physical exercise (Zachwieja *et al.*, 2001; Cox *et al.*, 2003). Maintenance of LBM may help in maintaining energy expenditure thus preventing regain of body weight as suggested in our previous study (Gaullier *et al.*, 2005). Lack of effect on body mineral mass does not necessarily mean that CLA does not affect it at all, but it might be that the period of investigation was too short (even though 2 years did not affect BMC either (Gaullier *et al.*, 2005)) or that variations are under the detection limits of the DXA. In addition susceptible populations with decreased bone mineral content were not investigated in this study.

The present study is the third confirmation in the published literature that CLA supplementation changes body composition in healthy, overweight and/or obese subjects on unrestricted diets and without specific life style restrictions. The reasons why previous studies did not manage to document the efficiency of CLA in body composition maybe due either to the use of other and possibly less reliable technologies than the sensitive DXA technology (Tylavsky *et al.*, 2003), to the use of blends of multiple CLA isomers that are not representative of the 50:50 mixture of the two main isomers used in this study, or/and the studies were carried out with not enough power (Zambell *et al.*, 2000; Berven *et al.*, 2000; Noone *et al.*, 2002). Although measurement of regional body composition by DXA technology is commonly used, questions has been raised concerning the accuracy of measuring changes in abdominal fat mass (Salamone *et al.*, 2000). However, recent publications suggest that this error is very small (Glickman *et al.* 2004; Woodhouse *et al.*, 2004) and may partly be due to experimental conditions as well as differences in DXA

1 hardware and software between manufacturers to the advantage of Lunar Prodigy (Soriano *et*  
2 *al.* 2004; Aasen *et al.*, under press). In the present study a high number of participants with a  
3 relative narrow BMI range and measurement under similar conditions on the same apparatus  
4 would tend to minimize the importance of this error.

5  
6 Physical exercise and daily caloric intake, both possible confounders, were not  
7 different between groups either at baseline or after 6 months. In accordance with previous  
8 studies (Gaullier *et al.*, 2004; Gaullier *et al.*, 2005), a modest reduction in caloric intake in  
9 both groups (but not significant in the CLA group) was observed during the study. The  
10 amount of physical exercise was also slightly reduced, but non-significantly, suggesting that  
11 changes in diet or exercise did not play a role in the body composition changes observed  
12 during CLA supplementation.

13 The results of this study confirmed and expanded on the findings of the previous  
14 studies: CLA reduces BFM in specific regions of the body and maintains or increases LBM  
15 (Berven *et al.*, 2000).

16  
17 The mechanism(s) by which CLA decreases BFM and maintains or increases LBM is  
18 still an active area of research. It is known that CLA is readily metabolized in tissues of  
19 animals and humans where it accumulates and may thus (a) induce adipocyte apoptosis  
20 (Evans *et al.*, 2000), (b) inhibit the lipoprotein lipase and increase the carnitine  
21 palmitoyltransferase resulting in a reduced accumulation of fatty acids in adipocytes (Park &  
22 Pariza, 2001), (c) bind to PPAR- $\gamma$  present in fat tissue and down regulate the expression of  
23 leptin (Kallen & Lazar, 1996) and prevent the accumulation of triglyceride in adipocytes  
24 (Granlund *et al.*, 2003), or (d) modify energy expenditure and the metabolic rate (West *et al.*,  
25 1998; Terpstra, 2001). In previous studies, leptin levels were shown to reduce accordingly to  
26 the body fat mass reduction (Gaullier *et al.*, 2004; Gaullier *et al.*, 2005), whereas the leptin  
27 levels in the current study decreased not significantly in the CLA group. However, one

1 subject from the CLA group had, similarly to an increase of weight by 5 kg, an increase in  
2 leptin level that might have altered the mean result of leptin in the CLA group. Therefore, the  
3 third mechanistic hypothesis involving PPAR $\gamma$  may still be valid.

4  
5 The current study also corroborated the previous long-term studies with high  
6 compliance and low drop out rates indicating that CLA supplementation was well tolerated  
7 (Gaullier *et al.*, 2004; Gaullier *et al.*, 2005). A very low percentage of the AEs were related to  
8 CLA supplementation. These AEs were mostly gastro-intestinal and musculoskeletal as  
9 observed previously (Vessby & Smedman, 1999; Blankson *et al.*, 2000; Berven *et al.*, 2000;  
10 Thom *et al.*, 2001; Gaullier *et al.*, 2004; Gaullier *et al.*, 2005). The absence of difference  
11 between the groups indicated that CLA was as safe as olive oil.

12  
13 None of the blood lipids or inflammatory blood chemical markers were affected  
14 except HDL and CRP. A small reduction in HDL and an increase in CRP were observed  
15 within the CLA group. However, both changes were within normal ranges. This is in line with  
16 earlier findings showing that the CLA 50:50 mixture did not affect CRP levels in healthy  
17 subjects and in patients with metabolic syndrome and type-2 diabetes (Riserus *et al.*, 2002b;  
18 Moloney *et al.*, 2004; Tricon *et al.*, 2004; Song *et al.*, 2005). Lp(a) and leucocytes levels were  
19 not significantly affected in the current study when compared to placebo which corroborates  
20 the findings of three other studies (Blankson *et al.*, 2000; Berven *et al.*, 2000; Moloney *et al.*,  
21 2004). In contrast two other studies showed an increase in Lp(a) (Gaullier *et al.*, 2005).  
22 Cytokines IL-6, IL-8, and TNF $\alpha$  were not modified as observed in two other studies (Riserus  
23 *et al.*, 2002b; Ramakers *et al.*, 2005). Furthermore, a previous study has shown that CLA  
24 decreases fibrinogen levels in diabetics (Moloney *et al.*, 2004). Thus taken together the results  
25 suggest that the CLA mixture used in this study does not seem to change the risk for CVD.

26 It has previously been reported that CLA could induce lipid peroxidation (Basu *et al.*,  
27 2000a,b), and that each of both bioactive CLA isomers could contribute to the increase insulin

1 resistance in subjects with the metabolic syndrome due to increased lipid peroxidation and  
2 inflammation (Riserus & Vessby, 2001; Riserus *et al.*, 2002a,b; Riserus *et al.*, 2004a,b). In the  
3 present study, and with the exception of a small increase in insulin c-peptide level within  
4 normal range, all indices of glucose metabolism including fasting glucose and insulin levels  
5 were reduced rather than increased, indicating that CLA supplementation was not  
6 diabetogenic in this population of overweight and moderately obese at high risk of developing  
7 metabolic syndrome or diabetes.

8  
9       Only appropriately designed long-term studies will unravel the potential benefits of  
10 CLA supplementation in obese subjects. We conclude however that CLA seems to be safe  
11 and well tolerated. The regionalized reduction in fat mass is encouraging and may represent  
12 an attractive dietary supplement, especially but not exclusively, for women with high BMI.

1 **Abbreviations:**

2 AE: Adverse Event, AFM: Fat mass in arms, ANCOVA: Analysis of Covariance, ANOVA:  
3 Analysis of Variance, ALAT: Alanine Amino Transferase, ALP: Alkaline Phosphatase,  
4 ASAT: Aspartate Amino Transferase, BF: Body Fat, BFM: Body Fat Mass, BMI: Body Mass  
5 Index, BMC: Body Mineral Content, CLA: Conjugated Linoleic Acid, DXA: Dual Energy X-  
6 ray Absorptiometry,  $\gamma$ GT: gamma-glutamyltransferase, GI: Gastro Intestinal, HbA1c:  
7 Glucohemoglobin, HDL: High Density Lipoprotein, ICH: International Conference on  
8 Harmonization, ITT: Intention-To-Treat, LBM: Lean Body Mass, LDL: Low Density  
9 Lipoprotein, LFM; Fat mass in legs, Lp(a): Lipoprotein a, PP: Per Protocol, PPAR- $\gamma$ :  
10 Peroxisome proliferator activated receptor-gamma; SAE: Serious Adverse Event, TFM: Fat  
11 mass in abdomen (trunk), TG: Triglycerides, v-LDL: Very Low Density Lipoprotein.

12  
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1 **References:**

2

3 Aasen G, Fagertun H & Halse J. Body composition analysis by dual X-ray absorptiometry: in  
4 vivo and in vitro comparison of three different fan-beam systems. *Scand J Clin Lab*  
5 *Invest.* Under press.

6 Akahoshi A, Goto Y, Murao K, Miyazaki T, Yamasaki M, Nonaka M, Yamada K & Sugano  
7 M (2002) Conjugated linoleic acid reduces body fats and cytokine levels of mice.  
8 *Biosci Biotech Biochem* **66**, 916-920.

9 Albers R., van der Wielen R., Brink E., Hendriks H., Dorovska-Taran V. & Mohede I. (2003)  
10 Effects of cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid (CLA) isomers  
11 on immune function in healthy men. *Eur J Clin Nutr* **57**, 595-603.

12 Atkinson R. (1999) Conjugated linoleic acid for altering body composition and treating  
13 obesity. pp. 348-353 [M. Yurawecz, M. Mossoba, J. Kramer, M. Pariza, and G.  
14 Nelson, editors]. Champaign, Illinois.

15 Bassaganya-Riera J, Pogramichniy RM, Jobgen SC, Halbur PG, Yoon KJ, O'Shea M, Mohede  
16 I & Hontecillas R (2003) Conjugated linoleic acid ameliorates viral infectivity in a pig  
17 model of virally induced immunosuppression. *J Nutr* **133**, 3204-3214.

18 Bassaganya-Riera J, Reynolds K, Martino-Catt S, Cui YZ, Hennighausen L, Gonzalez F,  
19 Rohrer J, Benninghoff AU & Hontecillas R (2004) Activation of PPAR gamma and  
20 delta by conjugated linoleic acid mediates protection from experimental inflammatory  
21 bowel disease. *Gastroenterology* **127**, 777-791.

22 Basu S., Riserus U., Turpeinen A. & Vessby B. (2000a) Conjugated linoleic acid induces lipid  
23 peroxidation in men with abdominal obesity. *Clin Sci (Lond)* **99**, 511-516.

- 1 Basu S., Smedman A. & Vessby B. (2000b) Conjugated linoleic acid induces lipid  
2 peroxidation in humans. *FEBS Lett* **468**, 33-36.
- 3 Belury M., Mahon A. & Banni S. (2003) The conjugated linoleic acid (CLA) isomer, t10c12-  
4 CLA, is inversely associated with changes in body weight and serum leptin in subjects  
5 with type 2 diabetes mellitus. *J Nutr* **133**, 257S-260S.
- 6 Belury M., Mahon A. & Lingling S (2000) Role of conjugated linoleic acid (CLA) in the  
7 management of type 2 diabetes: evidence from Zucker diabetic (fa/fa) rats and human  
8 subjects.
- 9 Berven G., Bye A., Hals O., Blankson H., Fagertun H., Thom E., Wadstein J. & Gudmundsen  
10 O. (2000) Safety of conjugated linoleic acid (CLA) in overweight or obese human  
11 volunteers. *Eur J Lipid Sci technol* **102**, 455-462.
- 12 Bhattacharya A, Rahman MM, Sun DX, Lawrence R, Mejia W, McCarter R, O'Shea M &  
13 Fernandes G (2005) The combination of dietary conjugated linoleic acid and treadmill  
14 exercise lowers gain in body fat mass and enhances lean body mass in high fat-fed  
15 male Balb/C mice. *J Nutr* **135**, 1124-1130.
- 16 Blankson H., Stakkestad J., Fagertun H., Thom E., Wadstein J. & Gudmundsen O. (2000)  
17 Conjugated linoleic acid reduces body fat mass in overweight and obese humans. *J*  
18 *Nutr* **130**, 2943-2948.
- 19 Cook M., Miller C., Park Y. & Pariza M. (1993) Immune modulation by altered nutrient  
20 metabolism: nutritional control of immune-induced growth depression. *Poult Sci* **72**,  
21 1301-1305.
- 22 Corino C, Mourot J, Magni S, Pastorelli G & Rosi F (2002) Influence of dietary conjugated  
23 linoleic acid on growth, meat quality, lipogenesis, plasma leptin and physiological  
24 variables of lipid metabolism in rabbits. *J Anim Sci* **80**, 1020-1028.

- 1 Cox KL, Burke V, Morton AR, Beilin LJ & Pudley IB (2003) The independent and combined  
2 effect of 16 weeks of vigorous exercise and energy restriction on body mass and  
3 composition in free-living overweight men-a randomized controlled trial. *Metabolism*  
4 **52**, 107-115.
- 5 Cunningham D., Harrison L. & Shultz T. (1997) Proliferative responses of normal human  
6 mammary and MCF-7 breast cancer cells to linoleic acid, conjugated linoleic acid and  
7 eicosanoid synthesis inhibitors in culture. *Anticancer Res* **17**, 197-203.
- 8 DeLany J., Blohm F., Truett A., Scimeca J. & West D. (1999) Conjugated linoleic acid  
9 rapidly reduces body fat content in mice without affecting energy intake. *Am J Physiol*  
10 **276**, R1172-R1179.
- 11 Dugan M., Aalhus J., Schaefer A. & Kramer J. (1997) The effects of conjugated linoleic acid  
12 on fat to lean repartitioning and feed conversion in pigs.
- 13 Evans M., Geigerman C., Cook J., Curtis L., Kuebler B. & McIntosh M. (2000) Conjugated  
14 linoleic acid suppresses triglyceride accumulation and induces apoptosis in 3T3-L1  
15 preadipocytes. *Lipids* **35**, 899-910.
- 16 Eyjolfson V, Spriet LL & Dyck DJ (2004) Conjugated linoleic acid improves insulin  
17 sensitivity in young, sedentary humans. *Med Sci Sport Exercise* **36**, 814-820.
- 18 Gaullier J-M., Berven G., Blankson H. & Gudmundsen O. (2002) Clinical trial results support  
19 a preference for using CLA preparations enriched with two isomers rather than four  
20 isomers in human studies. *Lipids* **37**, 1019-1025.
- 21 Gaullier JM, Halse J, Høye K, Kristiansen K, Fagertun H, Vik H & Gudmundsen O (2005)  
22 Supplementation with conjugated linoleic acid for 24 months is well tolerated by and  
23 reduces body fat mass in healthy, overweight humans. *J Nutr* **135**, 778-784.

- 1   Gaullier JM., Halse J., Høyе K., Kristiansen K., Fagertun H., Vik H. & Gudmundsen O.  
2           (2004) Conjugated linoleic acid (CLA) supplementation for one year reduces body fat  
3           mass in healthy, overweight humans. *Am J Clin Nutr* **79**, 1118-1125.
- 4   Gavino V., Gavino G., Leblanc M. & Tuchweber B. (2000) An isomeric mixture of  
5           conjugated linoleic acids but not pure cis-9, trans-11-octadecadienoic acid affects  
6           body weight gain and plasma lipids in hamsters. *J Nutr* **130**, 27-29.
- 7   Glickman SG, Marn CS, Supiano MA & Dengel DR (2004) Validity and reliability of dual-  
8           energy X-ray absorptiometry for the assessment of abdominal adiposity. *J Appl*  
9           *Physiol* **97**, 509-514.
- 10   Granlund L, Juvet L, Pedersen J & Nebb H (2003) Trans 10, cis 12 conjugated linoleic acid  
11           prevents triacylglycerol accumulation in adipocytes by acting as a PPARgamma  
12           modulator. *J Lip Res* **44**, 1441-1452.
- 13   Hall TR & Young TB (1989) A validation study of body fat distribution as determined by  
14           self-measurement of waist and hip circumference. *Int J Obes* **13**, 801-807.
- 15   Hara M, Saikawa T, Kurokawa M, Sakata T & Yoshimatsu H (2004) Leg fat percentage  
16           correlates negatively with coronary atherosclerosis. *Circ J* **68**, 1173-1178.
- 17   Houseknecht K., Vanden Heuvel J., Moya-Camarena S., Portocarrero C., Peck L., Nickel K.  
18           & Belury M. (1998) Dietary conjugated linoleic acid normalizes impaired glucose  
19           tolerance in the Zucker diabetic fatty fa/fa rat. *Biochem Biophys Res Commun* **244**,  
20           678-682.
- 21   Ip C., Singh M., Thompson H. & Scimeca J. (1994) Conjugated linoleic acid suppresses  
22           mammary carcinogenesis and proliferative activity of the mammary gland in the rat.  
23           *Cancer Res* **54**, 1212-1215.

- 1 Kallen CB & Lazar MA (1996) Antidiabetic thiazolidinediones inhibit leptin ob gene  
2 expression in 3T3-L1 adipocytes. *Proc Natl Acad Sci USA* **93**, 5793-5796.
- 3 Kirchengast S, Gruber D, Sator M, Knogler W & Huber J (1998) The impact of nutritional  
4 status on body fat distribution patterns in pre- and postmenopausal females. *J Biosoc*  
5 *Sci* **30**, 145-154.
- 6 Kreider R., Ferreira M., Greenwood M., Wilson M. & Almada A. (2002) Effects of  
7 conjugated linoleic acid supplementation during resistance-training on body  
8 composition, bone density, strength, and selected hematological markers. *J Strength*  
9 *Cond Res* **3**, 325-334.
- 10 Lee K., Kritchevsky D. & Pariza M. (1994) Conjugated linoleic acid and atherosclerosis in  
11 rabbits. *Atherosclerosis* **108**, 19-25.
- 12 Liew C., Schut H., Chin S., Pariza M. & Dashwood R. (1995) Protection of conjugated  
13 linoleic acids against 2-amino-3- methylimidazo[4,5-f]quinoline-induced colon  
14 carcinogenesis in the F344 rat: a study of inhibitory mechanisms. *Carcinogenesis* **16**,  
15 3037-3043.
- 16 Lowery L., Appicelli P. & Lemon P. (1998) Conjugated linoleic acid enhances muscle size  
17 and strength gains in novice bodybuilders. p. 182.
- 18 Miller C., Park Y., Pariza M. & Cook M. (1994) Feeding conjugated linoleic acid. *Biochem*  
19 *Biophys Res Commun* **198**, 1107-1112.
- 20 Moloney F, Yeow TP, Mullen A, Nolan JJ & Roche HM (2004) Conjugated linoleic acid  
21 supplementation, insulin sensitivity, and lipoprotein metabolism in patients with type  
22 2 diabetes mellitus. *Am J Clin Nutr* **80**, 887-895.

- 1 Mueller WH & Joos SK (1985) Android (centralized) obesity and somatotypes in men:  
2 association with mesomorphy. *Ann Hum Biol* **12**, 377-381.
- 3 Nes M., Andersen L., Solovoll K., Sandstad B., Hustvedt B., Løvø A. & Drevon C. (1992)  
4 Accuracy of a quantitative food frequency questionnaire applied in elderly Norwegian  
5 women. *Eur J Clin Nutr* **46**, 809-821.
- 6 Nicolosi R., Rogers E., Kritchevsky D., Scimeca J. & Huth P. (1997) Dietary conjugated  
7 linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in  
8 hypercholesterolemic hamsters. *Artery* **22**, 266-277.
- 9 Noone E., Roche H., Nugent A. & Gibney M. (2002) The effect of dietary supplementation  
10 using isomeric blends of conjugated linoleic acid on lipid metabolism in healthy  
11 human subjects. *Br J Nutr* **88**, 243-251.
- 12 Ostrowska E, Suster D, Muralitharan M, Cross RF, Leury BJ, Bauman DE & Dunshea FR  
13 (2003) Conjugated linoleic acid decreases fat accretion in pigs: evaluation by dual-  
14 energy X-ray absorptiometry Conjugated linoleic acid decreases fat accretion in pigs:  
15 evaluation by dual-energy X-ray absorptiometry. *Brit J Nutr* **89**, 219-229.
- 16 Park Y., Albright K., Storkson J., Liu W., Cook M. & Pariza M. (1999) Changes in body  
17 composition in mice during feeding and withdrawal of conjugated linoleic acid. *Lipids*  
18 **34**, 243-248.
- 19 Park Y. & Pariza M. (2001) Lipoxygenase inhibitors inhibit heparin-releasable lipoprotein  
20 lipase activity in 3T3-L1 adipocytes and enhance body fat reduction in mice by  
21 conjugated linoleic acid. *Biochim Biophys Acta* **1534**, 27-33.
- 22 Rahman S., Wang Y., Han S., Cha J., Fukuda N., Yotsumoto H. & Yanagita T. (2001) Effects  
23 of short-term administration of conjugated linoleic acid on lipid metabolism in white

- 1 and brown adipose tissues of starved/refed Otsuka Long-Evans Tokushima Fatty rats.  
2 *Food Res Int* **34**, 515-520.
- 3 Ramakers JD, Plat J, Sebedio JL & Mensink RP (2005) Effects of the individual isomers cis-  
4 9, trans-11 vs. trans-10,cis-12 of conjugated linoleic acid (CLA) on inflammation  
5 parameters in moderately overweight subjects with LDL-phenotype B. *Lipids* **40**, 909-  
6 918.
- 7 Reaven G (2005) All obese individuals are not created equal: insulin resistance is the major  
8 determinant of cardiovascular disease in overweight/obese individuals. *Diab Vasc Dis*  
9 *Res* **2**, 105-112.
- 10 Riserus U, Vessby B, Arner P & Zethelius B (2004b) Supplementation with trans10cis12-  
11 conjugated linoleic acid induces hyperproinsulinaemia in obese men: close association  
12 with impaired insulin sensitivity. *Diabetologia* **47**, 1016-1019.
- 13 Riserus U, Vessby B, Arnlov J & Basu S (2004a) Effects of cis-9,trans-11 conjugated linoleic  
14 acid supplementation on insulin sensitivity, lipid peroxidation, and proinflammatory  
15 markers in obese men. *Am J Clin Nutr* **80**, 279-283.
- 16 Riserus U., Arner P., Brismar K. & Vessby B. (2002a) Treatment with dietary trans10cis12  
17 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with  
18 the metabolic syndrome. *Diabetes care* **25**, 1516-1521.
- 19 Riserus U., Basu S., Jovinge S., Fredrikson G., Arnlov J. & Vessby B. (2002b)  
20 Supplementation with conjugated linoleic acid causes isomer-dependent oxidative  
21 stress and elevated C-reactive protein: a potential link to fatty acid-induced insulin  
22 resistance. *Circulation* **106**, 1925-1929.
- 23 Riserus U., Berglund L. & Vessby B. (2001) Conjugated linoleic acid (CLA) reduced  
24 abdominal adipose tissue in obese middle-aged men with signs of the metabolic

1 syndrome: a randomised controlled trial. *Int J Obes Relat Metab Disord* **25**, 1129-  
2 1135.

3 Riserus U. & Vessby B. (2001) Diverging effects of CLA isomers on insulin resistance and  
4 lipid metabolism in obese men with the metabolic syndrome. p. 2.

5 Salamone LM, Fuerst T, Visser M, Kern M, langT, Dockrell M, Cauley JA, Nevitt M,  
6 Tylavsky F & Lohman T (2000) Measurement of fat mass using DEXA: a validation  
7 study in elderly adults. *J Appl Physiol* **89**, 345-352.

8 Schonberg S. & Krokan H. (1995) The inhibitory effect of conjugated dienoic derivatives  
9 (CLA) of linoleic acid on the growth of human tumor cell lines is in part due to  
10 increased lipid peroxidation. *Anticancer Res* **15**, 1241-1246.

11 Song HJ, Grant I, Rotondo D, Mohede I, Sattar N, Heys SD & Wahle KW (2005) Effect of  
12 CLA supplementation on immune function in young healthy volunteers. *Eur J Clin*  
13 *Nutr* **59**, 508-517.

14 Soriano JM, Ioannidou E, Wang J, Thornton JC, Horlick MN, Gallagher D, Heymsfield SB,  
15 Pierson RN (2004) Pencil-beam vs fan-beam dual-energy X-ray absorptiometry  
16 comparison across four systems: body composition and bone mineral. *J Clin Densitom*  
17 **7**, 281-9.

18 Szymczyk B., Pisulewski P., Szczurek W. & Hanczakowski P. (2001) Effects of conjugated  
19 linoleic acid on growth performance, feed conversion efficiency, and subsequent  
20 carcass quality in broiler chickens. *Br J Nutr* **85**, 465-473.

21 Terpstra A. (2001) Differences between humans and mice in efficacy of the body fat lowering  
22 effect of conjugated linoleic acid: role of metabolic rate. *J Nutr* **131**, 2067-2068.

- 1 Thom E., Wadstein J. & Gudmundsen O. (2001) Conjugated linoleic acid reduces body fat in  
2 healthy exercising humans. *J Int Med Res* **29**, 392-396.
- 3 Tricon S, Burdge GC, Kew S, Banerjee T, Russell JJ, Grimble RF, Williams CM, Calder PC  
4 & Yaqoob P (2004) Effects of cis-9,trans-11 and trans-10,cis-12 conjugated linoleic  
5 acid on immune cell function in healthy humans. *Am J Clin Nutr* **80**, 1626-1633.
- 6 Tylavsky FA, Lohman TG, Dockrell M, Lang T, Schoeller DA, Wan JY, Fuerst T, Cauley JA,  
7 Nevitt M & Harris TB (2003) Comparison of the effectiveness of 2 dual-energy X-ray  
8 absorptiometers with that of total body water and computed tomography in assessing  
9 changes in body composition during weight change. *Am J Clin Nutr* **77**, 356-363.
- 10 Vessby B. & Smedman A. (1999) Conjugated linoleic acid (CLA) reduces the body fat  
11 content in humans. pp. T2-01.
- 12 Wallner SJ, Luschnigg N, Schnedl WJ, Lahousen T, Sudi K, Crailsheim K, Möller R, Taifet E  
13 & Horejsi R (2004) Body fat distribution of overweight females with a history of  
14 weight cycling. *Int J Obes Relat Metab Disord* **28**, 1143-1148.
- 15 Wargent E, Sennit MV, Stocker C, *et al.* (2005) Prolonged treatment of genetically obese  
16 mice with conjugated linoleic acid improves glucose tolerance and lowers plasma  
17 insulin concentration: possible involvement of PPAR activation. *Lipids Health Dis* **4**,  
18 3.
- 19 West D., DeLany J., Camet P., Blohm F., Truett A. & Scimeca J. (1998) Effects of conjugated  
20 linoleic acid on body fat and energy metabolism in the mouse. *Am J Physiol* **275**,  
21 R667-R672.
- 22 Whigham LD, O'Shea M, Mohede ICM, Walaski HP & Atkinson RL (2004) Safety profile of  
23 conjugated linoleic acid in a 12-month trial in obese humans. *Food Chem Toxicol* **42**,  
24 1701-1709.

- 1 Wong M., Chew B., Wong T., Hosick H., Boylston T. & Shultz T. (1997) Effects of dietary  
2 conjugated linoleic acid on lymphocyte function and growth of mammary tumors in  
3 mice. *Anticancer Res* **17**, 987-993.
- 4 Woodhouse LJ, Gupta N, Bhasin M, Singh AB, Ross R, Phillips J & Bhasin S (2004) Dose-  
5 dependent effects of testosterone on regional adipose tissue distribution in healthy  
6 young men. *J Clin Endocrinol Metab* **89**, 718-726.
- 7 Yusuf S, Hawken S, Ounpuu S, *et al.* (2005) Obesity and the risk of myocardial infarction in  
8 27,000 participants from 52 countries: a case-control study. *Lancet* **366**, 1640-1649.
- 9 Zachwieja JJ, Ezell DM, Cline AD, Ricketts JC, Vicknair PC, Schorle SM & Ryan DH (2001)  
10 Short-term dietary energy restriction reduces lean body mass but not performance in  
11 physically active men and women. *Int J Sports Med* **22**, 310-316.
- 12 Zambell K., Keim N., Van Loan M., Gale B., Benito P., Kelley D. & Nelson G. (2000)  
13 Conjugated linoleic acid supplementation in humans: effects on body composition and  
14 energy expenditure. *Lipids* **35**, 777-782.
- 15 Zu H. & Schut H. (1992) Inhibition of 2-amino-3-methylimidazo[4,5-f]quinoline-DNA  
16 adduct formation in CDF1 mice by heat-altered derivatives of linoleic acid. *Food*  
17 *Chem Toxicol* **30**, 9-16.

1 **Table 1:** Baseline characteristics of study population (n=105)<sup>†</sup>.

2

|                              | Placebo     | CLA         |         |
|------------------------------|-------------|-------------|---------|
|                              | (n=50)      | (n=55)      | p-value |
| Gender                       |             |             | 1.0     |
| Male                         | 10          | 11          |         |
| Female                       | 40          | 44          |         |
| Age (years) <sup>†</sup>     | 48.7 ± 9.2  | 45.8 ± 10.0 | 0.12    |
| Height (cm) <sup>†</sup>     | 169.7 ± 8.1 | 170.0 ± 8.8 | 0.25    |
| Alcohol use (%) <sup>‡</sup> | 68.0        | 63.6        | 0.64    |
| Tobacco use (%) <sup>‡</sup> | 10.0        | 14.5        | 0.48    |
| Exercise (%) <sup>§</sup>    |             |             | 0.54    |
| No exercise                  | 26.0        | 23.6        |         |
| Exercise without sweat       | 20.0        | 23.6        |         |
| Exercise with sweat          | 54.0        | 52.7        |         |

3

4 <sup>†</sup>Values for age and height are given as means ± SD and were recorded within 2 weeks of  
5 subjects inclusion in the study.

6 <sup>‡</sup>Alcohol and tobacco use are expressed as the percent of the subjects who answered  
7 positively to these questions (%Yes).

8 <sup>§</sup>Exercise is expressed as the percent of subjects no training or training at least once a week at  
9 low intensity (% without sweating) and high intensity (% with sweating).

10

1 **Table 2:** Body composition, total and regional fat mass<sup>†</sup>.

|                    |         | n = 105    | n = 105    | n = 105    | n = 105                 | n = 83                  |
|--------------------|---------|------------|------------|------------|-------------------------|-------------------------|
| Group <sup>‡</sup> |         | Month 0    | Month 3    | Month 6    | Δ 6-0                   | Δ 6-0                   |
| BFM (%)            | CLA     | 42.3 ± 6.1 | 42.0 ± 6.1 | 41.3 ± 6.2 | -1.0 ± 2.7*             | -1.4 ± 2.8*             |
|                    | Placebo | 42.2 ± 5.6 | 42.1 ± 5.7 | 42.1 ± 6.1 | -0.1 ± 2.2 <sup>#</sup> | +0.2 ± 2.0 <sup>#</sup> |
| BFM (kg)           | CLA     | 35.9 ± 4.7 | 35.5 ± 5.1 | 34.9 ± 5.7 | -1.0 ± 3.4*             | -1.5 ± 3.6*             |
|                    | Placebo | 35.5 ± 5.4 | 35.4 ± 5.5 | 35.7 ± 5.9 | +0.2 ± 2.3 <sup>#</sup> | +0.5 ± 2.2 <sup>#</sup> |
| AFM (kg)           | CLA     | 5.1 ± 1.4  | 5.1 ± 1.6  | 4.9 ± 1.6  | -0.3 ± 1.2              | -0.3 ± 1.2              |
|                    | Placebo | 5.2 ± 1.7  | 5.0 ± 1.3  | 4.9 ± 1.4  | -0.4 ± 1.1*             | -0.3 ± 1.0              |
| LFM (kg)           | CLA     | 11.2 ± 2.8 | 10.9 ± 2.6 | 10.7 ± 2.7 | -0.5 ± 1.2*             | -0.7 ± 1.3*             |
|                    | Placebo | 11.3 ± 2.4 | 11.4 ± 2.4 | 11.6 ± 2.7 | +0.3 ± 1.0 <sup>#</sup> | +0.3 ± 1.0 <sup>#</sup> |
| TFM (kg)           | CLA     | 18.6 ± 2.4 | 18.6 ± 2.5 | 18.4 ± 2.9 | -0.2 ± 2.0              | -0.4 ± 2.2              |
|                    | Placebo | 18.1 ± 2.7 | 18.0 ± 2.6 | 18.3 ± 2.9 | +0.3 ± 1.6              | +0.5 ± 1.6              |
| LBM (kg)           | CLA     | 49.6 ± 9.9 | 49.7 ± 9.7 | 50.1 ± 9.5 | +0.5 ± 1.9*             | +0.6 ± 1.9              |
|                    | Placebo | 49.1 ± 8.4 | 49.0 ± 9.0 | 49.2 ± 8.3 | +0.1 ± 1.4              | +0.2 ± 1.5              |
| BMC (kg)           | CLA     | 2.9 ± 0.5  | 2.9 ± 0.5  | 2.9 ± 0.5  | 0.0 ± 0.1               | 0.0 ± 0.1               |
|                    | Placebo | 2.8 ± 0.5  | 2.8 ± 0.5  | 2.8 ± 0.5  | 0.0 ± 0.1               | 0.0 ± 0.1               |

2  
3 <sup>†</sup>All values are expressed as mean ± SD.

4 <sup>‡</sup>n=55 and n=50 in the CLA group and placebo group, respectively in the main population.

5 n=42 and n=41 in the CLA group and placebo group, respectively in the PP population.

6 \* Difference within the groups between Month 6 and Month 0 (ANOVA paired T-test,  $P <$   
7 0.05).

8 <sup>#</sup> Difference between the groups (ANCOVA test,  $P <$  0.05).

9 Abbreviations: body fat mass (BFM), fat mass in the arms (AFM), fat mass in the legs (LFM),  
10 fat mass in the abdomen (TFM), lean body mass (LBM), and bone mineral content (BMC).

1 **Table 3:** Body weight, anthropometric measurements, daily caloric intake, and exercise  
 2 measurements<sup>†</sup>.

|                                  |         | n = 105     | n = 105     | n = 105     | n = 105                   | n = 83        |
|----------------------------------|---------|-------------|-------------|-------------|---------------------------|---------------|
| Group <sup>‡</sup>               |         | Month 0     | Month 3     | Month 6     | Δ 6-0                     | Δ 6-0         |
| Body weight<br>(kg)              | CLA     | 88.2 ± 9.7  | 87.8 ± 10.0 | 87.3 ± 10.4 | -0.9 ± 3.9                | -1.2 ± 4.2    |
|                                  | Placebo | 87.4 ± 9.8  | 87.7 ± 10.3 | 87.4 ± 10.1 | 0.0 ± 3.3                 | +0.3 ± 2.3    |
| BMI<br>(kg/m <sup>2</sup> )      | CLA     | 30.5 ± 1.4  | 30.4 ± 1.7  | 30.2 ± 2.0  | -0.3 ± 1.3                | -0.5 ± 1.5*   |
|                                  | Placebo | 30.2 ± 1.4  | 30.4 ± 1.6  | 30.2 ± 1.7  | 0.0 ± 0.9                 | +0.1 ± 0.9    |
| Waist<br>(cm)                    | CLA     | 99.3 ± 7.5  | 97.4 ± 7.5  | 96.6 ± 7.4  | -2.6 ± 5.0*               | -3.1 ± 5.4*   |
|                                  | Placebo | 98.5 ± 7.1  | 98.0 ± 7.2  | 97.2 ± 6.1  | -1.3 ± 4.9                | -0.9 ± 4.4    |
| Hip<br>(cm)                      | CLA     | 111.1 ± 5.0 | 110.1 ± 6.0 | 109.7 ± 6.2 | -1.4 ± 3.9*               | -1.1 ± 4.1    |
|                                  | Placebo | 110.5 ± 5.5 | 109.6 ± 4.8 | 109.8 ± 6.3 | -0.6 ± 3.8                | -0.6 ± 3.8    |
| Waist/Hip<br>ratio               | CLA     | 0.9 ± 0.1   | 0.9 ± 0.1   | 0.9 ± 0.1   | -0.02 ± 0.06*             | -0.02 ± 0.06* |
|                                  | Placebo | 0.9 ± 0.1   | 0.9 ± 0.1   | 0.9 ± 0.1   | -0.01 ± 0.05 <sup>#</sup> | -0.01 ± 0.05  |
| Diet <sup>§</sup><br>(kJ/day)    | CLA     | 8825 ± 2953 | ND          | 7883 ± 2940 | -526 ± 224 2              | -516 ± 2273   |
|                                  | Placebo | 8893 ± 2604 | ND          | 7719 ± 2204 | -1436 ± 2145*             | -1421 ± 2159* |
| Exercise <sup>  </sup><br>(a.u.) | CLA     | 3.27 ± 2.24 | ND          | 2.66 ± 2.39 | -0.62 ± 2.45              | -0.48 ± 2.36  |
|                                  | Placebo | 3.30 ± 2.42 | ND          | 2.94 ± 2.36 | -0.33 ± 2.25              | -0.35 ± 2.23  |

3  
 4 <sup>†</sup>All values are expressed as mean ± SD.

5 <sup>‡</sup>n=55 and n=50 in the CLA group and placebo group, respectively in the main population.

6 n=42 and n=41 in the CLA group and placebo group, respectively in the PP population.

7 <sup>§</sup>Diet is expressed as the total energy intake per day.

8 <sup>||</sup>Exercise was assessed as the product of the number of training sessions per week with  
 9 intensity (with or without sweat) and was expressed with arbitrary units (a.u.).

10 \* Difference within the groups between Month 6 and Month 0 (ANOVA paired T-test, *P* <  
 11 0.05).

12 <sup>#</sup> Difference between the groups (ANCOVA test, *P* < 0.05).

1 **Table 4:** Laboratory glucose, HbA1c and hormones analyses (n=105)<sup>†</sup>.

|                            | Group <sup>‡</sup> | Month 0       | Month 6       | Δ 6-0          |
|----------------------------|--------------------|---------------|---------------|----------------|
| Glucose (mmol/l)           | CLA                | 5.56 ± 0.72   | 5.11 ± 0.57   | -0.48 ± 0.81*  |
|                            | Placebo            | 5.48 ± 0.72   | 5.08 ± 0.68   | -0.40 ± 0.74*  |
| HbA1c (%)                  | CLA                | 5.43 ± 0.25   | 5.37 ± 0.29   | -0.06 ± 0.20*  |
|                            | Placebo            | 5.45 ± 0.29   | 5.34 ± 0.27   | -0.10 ± 0.21*  |
| Insulin (pmol/l)           | CLA                | 108.6 ± 98.3  | 96.9 ± 43.2   | -3.2 ± 33.6    |
|                            | Placebo            | 93.6 ± 35.7   | 103.3 ± 72.2  | +8.7 ± 72.3    |
| Insulin c-peptide (pmol/l) | CLA                | 726.9 ± 380.3 | 764.6 ± 262.0 | +67.9 ± 187.4* |
|                            | Placebo            | 690.4 ± 230.4 | 781.5 ± 363.1 | +78.4 ± 377.4  |
| Leptin (pmol/l)            | CLA                | 1549 ± 778    | 1386 ± 693    | -82 ± 664      |
|                            | Placebo            | 1528 ± 739    | 1473 ± 718    | -3.5 ± 490     |
| Adiponectin (mg/l)         | CLA                | 11.7 ± 4.6    | 11.7 ± 4.9    | -0.17 ± 2.3    |
|                            | Placebo            | 11.1 ± 5.3    | 10.5 ± 4.9    | -0.32 ± 2.1    |

2  
3 <sup>†</sup>All values are expressed as mean ± SD. Normal range levels are the following: Glucose 3.5  
4 to 6.0 mmol/l; HbA1c 5.0 to 6.0%; Insulin < 200 pmol/l; Insulin c-peptid 220 to 1400 pmol/l;  
5 leptin (not defined for these BMI); Adiponectin 4.5 to 22.4 mg/l.

6 <sup>‡</sup>In the main population, n=55 and n=50 in the CLA group and placebo group at month 0,  
7 respectively; n=50 and n=48 in the CLA group and placebo group at month 6, respectively,  
8 for glucose. n=55 and n=50 in the CLA group and placebo group at month 0, respectively;  
9 and n=49 for both groups at month 6, for HbA1c. n=55 and n=50 in the CLA group and  
10 placebo group at month 0, respectively; and n=47 for both groups at month 6, for insulin,  
11 insulin c-peptide, leptin and adiponectin.

12 \* Difference within the groups between Month 6 and Month 0 (ANOVA paired T-test, *P* <  
13 0.05).

14 <sup>#</sup> Difference between the groups (ANCOVA test or Wilcoxon test, *P* < 0.05).

1 **Table 5:** Laboratory blood lipid analyses (n=105)<sup>†</sup>.

|                            | Group <sup>‡</sup> | Month 0       | Month 6       | Δ 6-0         |
|----------------------------|--------------------|---------------|---------------|---------------|
| Lp (a) (mg)                | CLA                | 364.3 ± 541.9 | 386.0 ± 561.2 | +29.6 ± 85.4* |
|                            | Placebo            | 257.1 ± 281.0 | 280.0 ± 316.3 | +23.6 ± 68.6* |
| Total cholesterol (mmol/l) | CLA                | 5.51 ± 1.12   | 5.31 ± 1.21   | -0.18 ± 0.80  |
|                            | Placebo            | 5.65 ± 1.00   | 5.58 ± 1.00   | -0.06 ± 0.71  |
| HDL cholesterol (mmol/l)   | CLA                | 1.50 ± 0.32   | 1.43 ± 0.33   | -0.06 ± 0.18* |
|                            | Placebo            | 1.58 ± 0.38   | 1.55 ± 0.35   | -0.03 ± 0.18  |
| LDL cholesterol (mmol/l)   | CLA                | 3.60 ± 0.97   | 3.40 ± 1.00   | -0.15 ± 0.65  |
|                            | Placebo            | 3.72 ± 0.95   | 3.69 ± 0.89   | -0.03 ± 0.63  |
| Triglyceride (mmol/l)      | CLA                | 1.35 ± 0.61   | 1.34 ± 0.71   | -0.02 ± 0.55  |
|                            | Placebo            | 1.16 ± 0.51   | 1.20 ± 0.56   | +0.03 ± 0.36  |

2  
3 <sup>†</sup>All values are expressed as mean ± SD. Normal range levels are the following: Lp(a) <500  
4 mg/l; Total cholesterol 3.1 to 8.5 mmol/l; HDL cholesterol 1.0 to 2.2 mmol/l for women and  
5 0.8-2.0 mmol/l for men; LDL cholesterol 1.6 to 5.7 mmol/l; Triglyceride <2.10 mmol/l.

6 <sup>‡</sup>In the main population, n=55 and n=50 in the CLA group and placebo group at month 0,  
7 respectively; n=50 and n=49 in the CLA group and placebo group at month 6, respectively,  
8 for all parameters.

9 \* Difference within the groups between Month 6 and Month 0 (ANOVA paired T-test, *P* <  
10 0.05).

11 <sup>#</sup> Difference between the groups (ANCOVA test or Wilcoxon test, *P* < 0.05).

1 **Table 6:** Laboratory inflammation analyses (n=105)<sup>†</sup>.

|                                 | Group <sup>‡</sup> | Month 0     | Month 6     | Δ 6-0                     |
|---------------------------------|--------------------|-------------|-------------|---------------------------|
| IL-6 (ng/l)                     | CLA                | 0.31 ± 0.15 | 0.43 ± 0.41 | +0.14 ± 0.41              |
|                                 | Placebo            | 0.42 ± 0.48 | 0.43 ± 0.43 | +0.05 ± 0.45              |
| IL-8 (ng/l)                     | CLA                | 3.65 ± 1.42 | 4.57 ± 4.03 | +0.92 ± 3.97              |
|                                 | Placebo            | 5.10 ± 3.41 | 5.99 ± 3.02 | +0.94 ± 3.63              |
| TNFα (ng/l)                     | CLA                | 5.96 ± 1.68 | 5.96 ± 1.41 | 0.00 ± 1.62               |
|                                 | Placebo            | 6.21 ± 1.54 | 5.93 ± 1.52 | -0.25 ± 1.49              |
| CRP (mg/l)                      | CLA                | 2.93 ± 2.87 | 4.46 ± 4.81 | +1.46 ± 3.43*             |
|                                 | Placebo            | 3.22 ± 3.29 | 3.14 ± 2.71 | -0.06 ± 3.20 <sup>#</sup> |
| Leucocytes (10 <sup>9</sup> /l) | CLA                | 5.95 ± 1.62 | 6.16 ± 1.55 | +0.21 ± 1.11              |
|                                 | Placebo            | 5.50 ± 1.82 | 5.47 ± 1.55 | -0.04 ± 1.39              |

2  
3 <sup>†</sup>All values are expressed as mean ± SD. Normal range levels are the following: IL-6 0.1 to  
4 10.73 pg/ml; IL-8 2.5 to 7.8 pg/ml; TNFα 0 to 2.1 pg/ml; CRP <10mg/l; Leucocytes 3.3 to  
5 11.0 10<sup>9</sup>/l.

6 <sup>‡</sup> In the main population, n=35 and n=32 in the CLA group and placebo group at month 0,  
7 respectively; n=33 and n=29 in the CLA group and placebo group at month 6, respectively,  
8 for IL-6. n=47 and n=45 in the CLA group and placebo group, respectively, at months 0 and 6  
9 for IL-8 and TNFα. n=55 and n=50 in the CLA group and placebo group at month 0,  
10 respectively, whereas n=50 and n=49 in the CLA group and the placebo group at month 6,  
11 respectively, for CRP. n=55 and n=50 in the CLA group and placebo group at month 0,  
12 respectively, whereas n=48 and n=49 in the CLA group and the placebo group at month 6,  
13 respectively, for leucocytes.

14 \* Difference within the groups between Month 6 and Month 0 (ANOVA paired T-test, *P* <  
15 0.05).

16 <sup>#</sup> Difference between the groups (ANCOVA test or Wilcoxon test, *P* < 0.05).

17

18

1 **Figure 1:** Study population

2

3

