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PAPER

Conjugated linoleic acid (CLA) reduced abdominal adipose tissue in obese middle-aged men with signs of the metabolic syndrome: a randomised controlled trial

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BACKGROUND: Abdominal obesity is strongly related to metabolic disorders. Recent research suggests that dietary conjugated linoleic acid (CLA) reduces body fat and may improve metabolic variables in animals. The metabolic effects of CLA in abdominally obese humans have not yet been tested.
OBJECTIVE: To investigate the short-term effect of CLA on abdominal fat and cardiovascular risk factors in middle-aged men with metabolic disorders.
METHODS: Twenty-five abdominally obese men (waist-to-hip ratio (WHR), 1.05 ± 0.05; body mass index (BMI), $32 \pm 2.7\,kg/m^2$ (mean $\pm$ s.d.)) who were between 39 and 64-y-old participated in a double-blind randomised controlled trial for 4 weeks. Fourteen men received 4.2 g CLA/day and 10 men recieved a placebo. The main endpoints were differences between the two groups in sagittal abdominal diameter (SAD), serum cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, free fatty acids, glucose and insulin.
RESULTS: At baseline, there were no significant differences between groups in anthropometric or metabolic variables. After 4 weeks there was a significant decrease in SAD (cm) in the CLA group compared to placebo ($P = 0.04$, 95% CI; -1.12, -0.02). Other measurements of anthropometry or metabolism showed no significant differences between the groups.
CONCLUSIONS: These results indicate that CLA supplementation for 4 weeks in obese men with the metabolic syndrome may decrease abdominal fat, without concomitant effects on overall obesity or other cardiovascular risk factors. Because of the limited sample size, the effects of CLA in abdominal obesity need to be further investigated in larger trials with longer duration.
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Keywords: conjugated linoleic acid (CLA); abdominal obesity; abdominal sagittal diameter; metabolic syndrome; cardiovascular risk factors; randomised controlled trial (RCT)

Background

Conjugated linoleic acid (CLA) is an unsaturated fatty acid that has been shown to reduce body fat and increase lean body mass in animals,[1–5] by as yet unknown mechanisms. CLA comprises a group of positional and geometric isomers of conjugated octadecadienoic acid, derivates of linoleic acid (C18:2n-6) produced by bacterial biohydrogenation in the ruminant gut.[6] In humans, CLA is mainly derived from dairy and ruminant meat sources,[7] and has been found in serum[8] and in human adipose tissue.[9] Recent data have shown beneficial effects of CLA on several components of the metabolic syndrome, in Zucker diabetic fatty rats (ZDF), such as normalized glucose tolerance and reduced hyperinsulinaemia, and plasma levels of free fatty acids (FFA).[10] Furthermore, in CLA-fed rabbits and hamsters, the blood lipid profile was significantly improved[11] and signs of early atherosclerosis were decreased as compared to controls.[12,13]

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Abdominal visceral obesity, has been suggested to be a key factor in the metabolic syndrome[14] and the most prevalent cause of the atherogenic dyslipidaemic states associated with cardiovascular disease in Western populations.[15] Thus, men with visceral obesity are an appropriate risk group to test the putative beneficial metabolic effects of CLA treatment. Furthermore, epidemiological data from a population of elderly men showed an inverse correlation between estimated dietary intake of milk fat and abdominal obesity,[16] thus suggesting some indirect evidence for the postulated metabolic effects of CLA when consumed in high amounts. The sagittal abdominal diameter (SAD) has been suggested to be the best simple anthropometric measurement of visceral fat,[17–19] and is strongly associated with cardiovascular risk[20–24] and mortality among men.[25]

The antiobesity properties and metabolic effects of CLA are at present only described in animals, and should therefore be clinically tested in obese humans. The aim of the present trial was to investigate the short-term effects of CLA compared to placebo on abdominal adipose tissue and cardiovascular risk factors in men with signs of the metabolic syndrome. To the best of our knowledge, the present study is the first randomised controlled trial in humans, investigating the effects of CLA treatment in a cardiovascular high-risk group of abdominally obese middle-aged men.

Methods

Subjects

Twenty-five abdominally obese men (all Caucasian), between the ages of 39 and 64 y, were recruited through an advertisment in the local newspaper of Uppsala, Sweden. Sixty men were invited to a screening test, which included anthropometry, serum lipids, plasma glucose, blood pressure, kidney, liver, thyroid function and a medical questionnaire. The men who fulfilled the inclusion criteria (described below) were informed about the study protocol and invited to participate. Twenty-five men fulfilled the inclusion criteria, agreed to be randomly assigned to study groups, and gave their informed consent.

At the clinical baseline examination one patient was found to be hypertensive and was, after a re-examination, treated with a β-receptor blocker (metoprolol). This patient wished to complete the trial and was included (data on blood pressure from this patient was excluded from statistical analyses).

One subject declined on the first day of the study and was excluded before taking any capsules. The reason for declining was gastrointestinal disorders prior to the baseline examination. This subject did not differ in characteristics in terms of age or biochemical or anthropometric data as compared to the men who completed the trial. The results and conclusions presented and discussed in this paper are based on the 24 subjects with complete data at 4 weeks. The protocol to this study was approved by the Ethics Commitee of the Faculty of Medicine of Uppsala University.

Inclusion criteria

The inclusion criteria were chosen to select obese men with signs of the metabolic syndrome (abdominal obesity, dyslipidemia, hypertension and impaired fasting glucose). The inclusion criteria were, as assessed by screening: age between 30 and 65 y, abdominal obesity (waist circumference >94 cm and waist-to-hip ratio (WHR) >0.95), BMI >27 and $<39\,kg/m^2$, and with stable body weight the preceeding 3 months.

Exclusion criteria

Subjects with a history of significant disease or with abnormal laboratory test results of clinical significance were excluded as well as those on medications or dietary supplements known to affect glucose and lipid metabolism, body composition or eicosanoid production (eg salicylates and fish-oils). Treatment with antihypertensive drugs were accepted if the subjects continued to take the same drug throughout the study period.

The primary outcome in the present trial was to investigate any statistical differences between CLA and placebo in SAD (representing visceral and total abdominal adipose tissue), serum cholesterol, fasting triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), FFA, plasma glucose and serum insulin. Secondary outcomes of interest were anthropometry and blood pressure.

Intervention and blinding

The current trial was performed between April and May 1999. All men were randomly assigned to treatment (CLA) or placebo. Each group received seven CLA or placebo capsules per day (divided in two doses). Compliance was monitored by counting any returned capsules and by follow-up interview. The blinding was obtained using capsules identical in taste, colour, size and odour, packed in identical bottles, with one bottle per subject. The daily dose of CLA comprised 4.2 g and the same amount of olive oil was used as placebo. The predominant isomers used in CLA preparations are *cis*9, *trans*11 CLA 18:2 and *trans*10, *cis*12 CLA.[1] The isomer content of the current CLA preparation was as follows: 37.0% *t*10*c*12 CLA, 36.9% *c*9*t*11 CLA, 1.4% *t, t*9, 11 + 10, 12 CLA, 1.3% *c*9*c*11 CLA, 0.9% *c*10*c*12 CLA, 13.9% 18:1*c*9, 4.3% 16:0, 1.4% 18:0. Both active (CLA 80) and placebo capsules were prepared by Natural Lipids Ltd, Hovebygda, Norway, which also was responsible for the individual randomisation and blinding of capsules. During the trial, the code was kept in sealed envelopes that were kept in a closed box located at the department of Geriatrics. None of the investigators, nurses, analysts or patients knew the identity of the treatments until the codes were revealed at the end of the study.

Clinical protocol

The clinical examination was conducted in the morning between 07:15 and 09:00, after written and oral instructions to fast for 12 h, restrain from smoking, snuff or physical activity in the morning, and to avoid alcohol the day before the clinical examination. All men were encouraged to maintain their usual diet and exercise habits throughout the course of the study. After oral information, the same investigator measured anthropometry and blood pressure, followed by blood samples taken by a nurse. Blood pressure was measured on the right arm with the subject in the supine position after at least 5 min rest, by indirect auscultation and by a mercury sphygmomanometer. Systolic and diastolic blood pressure were defined as Korotkoff phases 1 and 5, respectively. Clinical examination at 4 weeks also included a systematic food frequency interview, for estimation of dietary CLA intake and to obtain the dietary history for the preceding month.[26] All men were asked about their habitual food consumption during the preceeding 4 weeks, with focus on sources of CLA. During the interview, various household measures were used to identify proper portion sizes. Dietary intake of CLA was calculated by use of food-composition data of the Swedish National Food Administration;[27] when necessary, the portion sizes of different food items were converted to weight on the basis of standard portions. Calculations of the CLA content in various dairy and meat products are based on the mean CLA content in dairy products (2.5 – 17.7 mg/g fat),[28] butter (6.2 mg/g fat) and ruminant meat (2.9 – 5.6 mg/g).[7]

All anthropometrics were measured by one trained investigator. Body weight was measured using an electronic scale to the nearest 0.1 kg, with the subjects wearing light clothing and no shoes. Height was measured without shoes to the nearest 0.5 cm. Bod mass index (BMI) was calculated as the weight (kg) divided by the square of the height (m). SAD (antero-posterior) was measured with patients in underwear and in a recumbent position with hips flexed, on a firm examination table. At the level of the iliac crest ($=L_{4\text{-}5}$), the perpendicular distance between the examination table up to the horizontal level was measured during a normal expiration to the nearest 0.1 cm.[17] Waist circumference was measured in standing position after a normal expiration, with a nonstretchable tape measure, at the level midway between the caudal part of the lateral costal arch and the iliac crest.[29] Hip circumference was measured at symphysis trochanter level.

Laboratory procedures

Blood was drawn from an antecubital vein, into vacuum tubes and centrifuged (20 min) at 2000 g after 2 h of coagulation at room temperature. Total cholesterol and TG levels in serum were assayed by enzymatic techniques, using a Monarch 2000 centrifugal analyser (Instrumentation Laboratories, Lexington, MA). HDL was isolated by centrifugation and precipitation with a sodium phosphotungstate and magnesium chloride solution.[30] LDL-cholesterol was calculated according to the formula of Friedwald. Sample for the determination of insulin and FFA were stored at -70°C. FFA was measured by an enzymatic colorimetric method (Wako Chemical GmbH, Germany). Insulin were measured in serum by an enzyme immunoassay, ELISA-kit (Mercodia AB, Uppsala, Sweden) in a Bio-Rad Coda automated EIA analyser (Bio-Rad Laboratories AB, California, USA). Insulin and FFA were analysed at one run. Plasma glucose was measured with an enzymatic method, using a Hitachi analysis system 717 (Boehringer Mannheim, Germany).

Statistical analysis

Values are expressed as means ± s.d. All data were continous and on an interval scale. Variables with skewed distributions (TG and insulin) were log transformed before analyses. A nonparametric test was used if data were not normally distributed after logarithmic transformation. The paired *t*-test was used to analyse the differences within the groups from baseline to 4 weeks. The mean differences between groups at baseline and after 4 weeks were assessed with an unpaired *t*-test, with 95% confidence intervals (CI) calculated according to standard procedures. For variables (waist) that were not normally distributed after logarithmic transformation, the Mann – Whitney nonparametric test was used for analyses. All tests were two-tailed and a P-value < 0.05 was regarded as significant. Statistical calculations were performed using the JMP software package (SAS Institute Inc., Cary, NC, USA).

Results

Baseline status and compliance

Twenty-four men completed the study after being randomly assigned to CLA treatment ($n=14$), or placebo ($n=10$). During the 4 weeks only one subject withdrew.

At baseline, there were no significant differences between the two groups after randomisation, in age (Table 1), anthropometry or in any other characteristic (Table 2). Fourty-four percent of patients were hypertensive, defined as a mean systolic pressure $\geq$140 mmHg or mean diastolic pressure $\geq$90 mmHg or if the subject was receiving antihypertensive drugs. No patient in the study sample had a diagnosis of type 2 diabetes or history of cardiovascular events.

Table 1 Demographic and anthropometric characteristics at baseline after randomisation[a]

	CLA	*Placebo*
n	14	10
Age (y)	54 ± 5.7	52 ± 7.8
BMI (kg/m^2)	32.2 ± 3.4	31.7 ± 1.9
WHR	1.06 ± 0.07	1.04 ± 0.04

[a]There were no significant differences between groups using unpaired *t*-tests. Values are the mean ± s.d. BMI, body mass index, WHR, waist-to-hip ratio.

Table 2 Clinical characteristics of men at baseline and after 4 weeks of treatment with CLA ($n=14$) or placebo ($n=10$)

	Baseline		*4 weeks*		*Difference between groups*		
Variable	*CLA*	*Placebo*	*CLA*	*Placebo*	*Diff*	*95% CI*	P
Weight (kg)	106.9±12.3	103.0±12.7	106.6±12.1	102.6±12.7	0.13	(−0.87, 1.14)	0.13
BMI (kg/m^2)	32.2±3.4	31.7±1.9	32.1±3.4	31.6±2.0	0.05	(−0.24, 0.33)	0.79
SAD (cm)	29.5±2.0	29.2±1.8	28.9±1.8†	29.2±1.6	−0.57	(−1.12, −0.02)	0.04*
Waist (cm)	120.0±11.4	116.0±6.45	118.5±10.2†	115.3±6.91	−0.73	(−2.04, 0.59)	0.51
WHR	1.06±0.07	1.04±0.04	1.04±0.06†	1.04±0.04	−0.01	(−0.02, 0.59)	0.20
Chol (mmol/l)	6.27±1.25	6.31±1.01	6.35±1.02	6.31±1.01	0.05	(−0.4, 0.50)	0.82
LDL (mmol/l)	3.76±0.96	3.93±0.97	3.93±0.90	3.91±0.91	0.05	(−0.39, 0.48)	0.83
HDL (mmol/l)	1.12±0.17	1.13±0.18	1.18±0.18	1.26±0.11†	−0.07	(−0.16, 0.03)	0.17
TG (mmol/l)	2.78±1.49	2.81±1.20	3.0±1.31	2.99±1.36	0.02	(−0.68, 0.78)	0.70
FFA (mmol/l)	0.39±0.14	0.34±0.13	0.36±0.12	0.38±0.15	−0.07	(−0.17, 0.03)	0.22
Glucose (mmol/l)	5.44±0.83	5.28±0.73	5.69±0.73†	5.74±0.85‡	−0.20	(−0.54, 0.14)	0.22
Insulin (mU/l)	10.61±5.62	12.09±5.65	11.32±5.13	14.07±7.22	−1.27	(−4.71, 2.18)	0.82

BMI, body mass index; SAD, sagittal abdominal diameter; WHR, waist–hip ratio; Chol, serum cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglycerides. Values are the mean±s.d. and 95% confidence interval (CI).
Significant differences between the two groups using unpaired *t*-test: *$P<0.05$. Significant differences within groups using paired *t*-test: $^{\dagger}P<0.01$, $^{\ddagger}P<0.001$.

The supplements were well-tolerated in all patients, with no adverse events clinically detected or reported. Compliance did not differ between groups, with 96% of the capsules taken for both groups.

Abdominal and total fat

The 4 week treatment with CLA induced a significant reduction of SAD, as compared to placebo ($P=0.041$, 95% CI; −1.12, −0.02; Table 2), with a significant decrease of SAD within the CLA group ($P=0.003$), while there was no measurable change in the placebo group ($P=1.00$). The mean decrease in SAD after CLA treatment was 0.6 cm. Waist circumference was not significantly different after 4 weeks compared to placebo ($P=0.51$). However, the CLA group experienced a significant mean decrease in waist circumference with 1.4 cm ($P<0.01$), while in the placebo group the mean decrease (0.7 cm) was not significant ($P=0.08$). WHR followed a similar pattern as waist circumference, with a significant decrease only in the CLA group ($P<0.01$), although without difference between groups ($P=0.20$). Mean body weights were slightly decreased (non-significant) to a similar degree after CLA and placebo with 0.3 and 0.4 kg, respectively (data not shown) with no significant difference between the groups ($P=0.79$). This pattern was also true for BMI without any significant mean difference between the two groups (Table 2).

Metabolic variables

There were no significant differences between groups in cardiovascular risk factors (Table 2). HDL was increased in both groups, significantly for the placebo group only ($P<0.01$). Plasma glucose was also significantly increased in both groups. No significant changes were detected in blood pressure (data not shown).

Dietary intake of CLA

The estimated mean CLA intake from dietary sources was, for the whole group, 328 mg CLA/day. The mean intake of CLA from dietary sources was 311 mg (range 123–470 mg) in the CLA group, and 346 mg (range 156–590 mg) in the placebo group, showing no statistical significance between groups ($P=0.24$). These data suggest that the CLA supplementation corresponded to an average daily dose, which was approximately 13-fold higher than what was usually obtained from the diet.

Discussion

The major finding in this randomised, controlled trial was that CLA supplementation appeared to reduce abdominal adipose tissue in obese middle-aged men as indicated by a significant decrease in SAD. It is possible that the decrease in SAD represents a preferential loss of visceral fat.[17–19,31,32] However, this assumption needs confirmation by computed tomography (CT) which is superior to SAD in the measurement of visceral fat. SAD is a valid measure of both intra- and extra-abdominal fat,[33] suggesting that CLA may have an effect on total abdominal adipose tissue. Within the CLA group the decrease in abdominal fat was significant for all measures of abdominal obesity, although there was no statistically significant difference in changes of waist circumference or WHR between the two groups (Table 2). The absensce of any statistical changes or differences in BMI suggests a reduction in abdominal fat rather independent of total obesity. The reduction of body fat as suggested by the decrease in SAD, is in concert with previous animal data that showed a reduced proportion of body fat after CLA treatment.[1–5] Moreover, the current finding is in agreement with a recent controlled trial in healthy normal weight men and women.[34] In that trial, the CLA-supplemented group experienced reduced body fat, as measured by bioelectric impedance analysis and skinfold techniques.

New data indicates that SAD has higher reliability (a lower intra-observer variation and a higher intra-class correlation) as compared to other anthropometrics, independent of BMI.[35] The high reliability and low degree of measurement error of SAD have been reported by others in a previous study.[36] To minimise measurement errors in the present trial, there was a strong emphasis put on accurate assessment of anthropometry, which was performed by one trained investigator in an identical fashion at both clinical examinations. Previous studies that used CT, suggest that visceral fat is highly correlated to metabolic risk factors as compared to other fat depots.[37–40] However, in contrast to animal data,[10,12,13] no differences between the groups in cardiovascular risk factors were detected in the present trial. If abdominal fat decreases after CLA, one would expect some parallel improvements in fasting glucose and insulin levels, which were not seen in the current study. In contrast, both plasma glucose and insulin tended to rise slightly within both groups. Because the increase in these variables occurred in both study groups it is difficult to evaluate the importance of these findings. DeLaney *et al* showed that CLA-fed mice experienced a rapid decrease in body fat accumulation (significant after only 2 weeks of treatment), with the visceral depots being the most sensitive to CLA, which was accompanied by an increase in fasting insulin levels as compared to controls.[41] They speculated that CLA may deteriorate pheripheral insulin dependent glucose disposal, as a consequence of increased lipolysis and fatty acid oxidation according to the Randle glucose-fatty acid cycle,[42] or by yet unknown mechanisms.

CLA and effects on adipose tissue—possible mechanisms

The mechanisms behind a possible fat reducing effect of CLA are currently unknown, although there are some clues from *in-vitro* data and experimental animal models. Locally synthesized prostaglandins (PGs) may have antilipolytic effects in human adipose tissue.[43] *In vitro* evidence have indicated an inhibitory effect of CLA on PGE2 production,[44] a prostaglandin with anti-lipolytic effects *in vitro*.[45,46] Thus, the modulation of PG production by CLA supplementation may contribute to increased lipolysis of adipose tissue. Park *et al* suggested an enhanced noradrenaline-induced lipolysis and hormone-sensitive lipase activity, with an accompanying fatty acid oxidation in skeletal muscle, due to increased activity of carnitine palmitoyltransferase after CLA treatment.[1] More recent data confirm these *in vitro* effects of CLA on lipolysis, lipoprotein lipase (LPL) activity and intracellular accretion of triglycerides and glycerol.[47] It has been described that visceral fat cells in abdominally obese men seem to have a greater ability to mobilise fatty acids than subcutaneous fat cells, in response to cathecholamines.[14,48] One might postulate that dietary CLA, as a possible inducer of a cathecolamine-related lipolysis as shown *in vitro* by Park *et al*,[1] could cause a selective reduction of visceral fat, indirectly measured as SAD in the present trial. The *in vitro* results on CLA and lipolysis are in agreement with the finding that CLA increased energy expenditure and fat oxidation in mice.[2]

Regarding, a possible thermogenic effect of CLA, skeletal tissue analyses of uncoupling protein (UCP) gene expression in prediabetic ZDF-rats have showed an increased brown adipose tissue UCP-2mRNA expression in CLA fed rats compared to controls.[49] This finding may also be related to the loss of body fat in animals. Interestingly, possible PPAR-γ ligands such as CLA and thiazolidinediones have recently been reported to induce gene expression of UCP2 isoforms in both skeletal muscle and adipose tissue.[50] Moreover, there are also relevant data on the molecular level that give support to CLA as being a ligand of two isoforms of PPARs, both having important function in energy and adipose tissue metabolism. First, CLA is, in contrast to linoleic acid, a potent ligand and activator of peroxisome proliferator activating receptor alpha (PPAR-α),[51,52] which pivotal role in obesity and lipid metabolism has been described in transgenic mice,[53] although the effects of PPAR-α activators on human adipose tissue are still unknown.

Second, it was previously reported that CLA activates PPAR-γ response elements *in vitro*.[10] Since PPAR-γ genes seem to regulate both metabolic functions and adipose tissue development,[54] it is interesting to note that the mRNA levels of PPAR-γ are subjected to regional variation in adipose tissue,[55] with different sensitivity to the fat-cell differentiation in response to PPAR-activators.[56] In clinical trials, the PPAR-γ agonist troglitazone was found to markedly decrease visceral fat in type 2 diabetic patients, without affecting subcutaneous fat or BMI.[57,58] Thus, it is tempting to speculate that the reduced SAD in the current trial might be related to an effect of CLA on PPARs.

Some limitations of this study need to be adressed. First, the limited sample size does not exclude the possibility that the observed decrease in SAD occurred by chance, although we did calculate confidence intervals to provide some complementary information about the power of the study, therefore the findings need to be confirmed by CT measurements in future studies. If CLA indeed has an affect on abdominal fat, it would be interesting to further study glucose and insulin metabolism after CLA administration. We are at present investigating the effect of CLA on obesity-related insulin resistance in a larger trial which will provide information about the possible effect of CLA on glucose metabolism.

Conclusion

In conclusion, in men with marked abdominal visceral obesity, CLA supplementation for 4 weeks may reduce abdominal fat as indicated by a significant reduction in SAD. However, many questions remain unanswered. The fact that the decrease in SAD occurred without concomitant improvements in related metabolic variables also indicates that the results of the current trial must be interpreted with caution. Nevertheless, because abdominal obesity is a strong

risk factor these results are interesting, and further trials with a larger number of subjects and performed during longer time periods are needed to determine if CLA may be a safe and effective antiobesity agent in subjects with abdominal obesity and the metabolic syndrome.

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