

ORIGINAL ARTICLE

The effect of 6 months supplementation with conjugated linoleic acid on insulin resistance in overweight and obese

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Background: Contradicting results have been published regarding the effect of conjugated linoleic acid (CLA) on insulin resistance. However, only a few studies have used the euglycemic hyperinsulinemic clamp method, which is considered the standard for measuring insulin resistance.

Objective: To evaluate if CLA as a mixture of the main isomers *trans*-10 *cis*-12 and *cis*-9 *trans*-11 affects the insulin resistance in healthy overweight and obese male and female adults.

Design: The main study was a randomized, double-blind, placebo-controlled trial with change in body composition as primary end point comprising 118 subjects receiving supplementation with either placebo (olive oil) or CLA (Clarinol) for 6 months. A sub-population of 49 subjects agreed additionally to participate in an euglycemic hyperinsulinemic clamp study at baseline and after 6 months of supplementation with study drug. The primary outcome was the change in glucose uptake (M) as measured by the hyperinsulinemic euglycemic glucose clamp method. Secondary outcomes were the correlates between insulin resistance and changes in body composition or blood chemistry parameters. Forty-one subjects completed the clamp test at both time points.

Results: The median M of the CLA group was 11.0 mg min⁻¹ lean body mass (lbm)⁻¹ (*n* = 24) at baseline, 10.3 mg min⁻¹ lbm⁻¹ (*n* = 24) after 6 months, and the median difference was +0.21 mg min⁻¹ lbm⁻¹ (*n* = 24). The median M of placebo group was 8.4 mg min⁻¹ lbm⁻¹ at baseline and 9.3 mg min⁻¹ lbm⁻¹ after 6 months and the median difference was -0.22 mg min⁻¹ lbm⁻¹ (*n* = 17). No significant (*P* < 0.05) differences were found within groups or between groups. Likewise, the glucose uptake insulin concentration ratio during clamp (M/I) was independent of treatment and time. Homeostasis model assessment (HOMA) and quantitative insulin sensitivity check index derived from fasting glucose and insulin were also independent of treatment and time, and HOMA for the clamp population (*n* = 49) corresponded well with HOMA for the per protocol population (*n* = 83). Correlation analysis showed that changes in M were inversely correlated to changes in glucohemoglobin (*P* = 0.002), but did not correlate with changes in either glucose, insulin, insulin c-peptide, leptin, adiponectin or percent body fat.

Conclusions: CLA does not affect glucose metabolism or insulin sensitivity in a population of overweight or obese volunteers. *International Journal of Obesity* advance online publication, 10 October 2006; doi:10.1038/sj.ijo.0803482

Keywords: conjugated linoleic acid; insulin resistance; euglycemic clamp; overweight; obese

Introduction

Commercially available preparations of conjugated linoleic acid (CLA) contain mainly a mixture of two main positional and geometric isomers (*trans*-10, *cis*-12 and *cis*-9, *trans*-11) and have been tested in several human studies with varying results regarding change in body composition,^{1–12} stimula-

tion of immune response,¹³ effects on lipid metabolism¹⁴ and glucose metabolism.^{15–18} CLA as a food supplement is widely promoted for reduction of body fat. Long-term studies have shown CLA to be safe.^{5,19}

Studies in men with the metabolic syndrome have, however, raised questions about the effect of CLA supplementation on glucose and fat metabolism.^{18,20–22} These data suggest that either of the bioactive isomers, *trans*-10, *cis*-12 and *cis*-9, *trans*-11, increased insulin resistance, whereas a mixture of both isomers did not.^{18,20} Furthermore, the supplementation of *trans*-10, *cis*-12 isomer was found to increase C-reactive protein, a marker of inflammation,

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Received 22 May 2006; revised 14 August 2006; accepted 15 August 2006

whereas the isomeric mixture did not. Also, F2-isoprostane, used as a marker of oxidative stress, was increased compared to placebo for both *trans*-10, *cis*-12 isomer and the mixture, although the increase was much larger for the pure isomer. On the other hand, it has recently been suggested that CLA may be a candidate nutraceutical for intervention against all components of the metabolic syndrome and as a therapy for type 2 diabetes-related cardiovascular disease.¹⁵

As only the mixture of both CLA isomers is available to consumers, and is mainly used for fat loss in overweight and obese subjects, further studies of the effect of CLA on insulin resistance are indicated. The aim of the present study was thus to assess insulin resistance in healthy overweight and moderately obese subjects during a 6-month CLA supplementation using the euglycemic hyperinsulinemic clamp method. The results regarding body composition and other clinical parameters for the whole population of 118 subjects are reported elsewhere (Gaullier J-M *et al.* – unpublished results).

Subjects and methods

Study design and subjects

The efficacy and safety of CLA (Clarinol) was tested in a randomized, double-blinded, placebo-controlled parallel study. A total of 118 healthy volunteers entered the main study. Twelve subject in the CLA and 13 in placebo group dropped out, whereas another 10 had <70% compliance, giving a population of 83 subjects with valid values at baseline and 6 months. A sub-population of 49 subjects entered a euglycemic hyperinsulinemic clamp study at visit 1 (baseline) of whom 41 completed a second clamp study after 6 months. Their selection based on the first subjects that accepted to perform the clamp procedure. The total study population comprised healthy male ($n=18$) and female ($n=65$) volunteers 18–65 years of age (mean 48 years), with body mass index (BMI) between 28 and 32 kg/m², recruited at two research centers (Diabetes and Overweight Medical Center, Oslo, Norway and the Hedmark Medical Center, Hamar, Norway). The participants at these centers were similar with regard to gender, age, BMI, smoking and alcohol consumption. The mean age of the 49 (24 and 25 subjects at centers 1 and 2, respectively) clamp study subjects was 47.6 years (range 27–64 years) and comprised 15 male and 34 female subjects.

Exclusion criteria included drug therapy, special diet or taking dietary substitutes for weight loss 2 weeks before study start, CLA consumption during the last 3 months before the study, pregnant and lactating women, type 1 diabetes or those with untreated type 2 diabetes, those with a history of hypertension, renal, liver, pancreatic or chronic inflammatory/infectious diseases, cardiac failure or malignant tumors, those with active thyroid disease or receiving thyroid hormone substitution, adrenergic agonist use, and

volunteers with known/suspected drug or alcohol abuse or with any clinical condition rendering them unfit to participate were also excluded from participation.

The subjects were randomized to either CLA 3.4 g/day (4.5 g Clarinol G80; Lipid Nutrition, Loders Croklaan, The Netherlands; $n=26$) or placebo (4.5 g olive oil, $n=23$) corresponding to six opaque soft capsules, all identical in taste and in appearance, for 6 months. The CLA oil was a mixture containing 37.5% *cis*-9 *trans*-11 and 38% *trans*-10 *cis*-12. The remaining mixture was made of other fatty acids (containing less than 2% of *trans*-*trans*, less than 7% of saturated fatty acids and less than 1% free fatty acids). No intervention in lifestyle or nutrition was implemented. Diet and exercise were assessed at baseline and at 6 month. Compliance was measured every 3 months by counting the returned unused capsules, and compared to the number of capsules prescribed. The study was approved by the regional Ethics Committee and conducted in agreement with the Declaration of Helsinki, and accordance with International Conference on Harmonisation/Good Clinical Practice (ICH/GCP) guidelines.

Methods

Euglycemic insulin clamp test was performed as described previously with minor modifications.²³ All subjects had two fasting blood samples drawn for glucose and insulin measurements. The mean values were used for analysis. Insulin was mixed with 0.9% NaCl to a concentration of 300 U l⁻¹ and infused at a constant rate of 1 mU kg⁻¹ min⁻¹. The plasma glucose concentration was checked every 5–10 min using the glucose dehydrogenase method (HemoCue AB, Angelholm, Sweden) and held constant at 5 mmol l⁻¹ by a variable glucose infusion (200 g l⁻¹). Blood for insulin measurements was drawn during the last 30 min of the clamp procedure. An electric blanket provided heating of the arm used for sampling. Under these steady-state conditions, the glucose infusion rate equals the glucose uptake by all body tissues and is therefore a measure of tissue sensitivity to exogenous insulin. Mean duration of test procedure was 2 h 57 min and the glucose uptake (M) (mg min⁻¹) was calculated from the amount of glucose (mg) infused over the last 30 min under euglycemic conditions. Both body weight (bw) and lean body mass (lbm) determined by dual-energy X-ray absorptiometry (DXA) were used to correct the values of M. Homeostasis model assessment (HOMA) was calculated from fasting insulin and fasting glucose data using the following formula: (fasting insulin μ U ml⁻¹) \times (fasting glucose mmol l⁻¹)/22.5. Quantitative insulin sensitivity check index (QUICKI) was calculated as 1/log(fasting insulin μ U ml⁻¹) + log(fasting glucose mg dl⁻¹).

Other clinical assessments

Weight, BMI, vital signs and adverse events were recorded every 3 months. Body composition was analyzed at baseline,

and at 3 and 6 months. Fasting blood samples were also analyzed for HbA1c, c-peptide, adiponectin and leptin in accredited laboratories (Furst Laboratory and Aker University Hospital, Oslo, Norway). Insulin was determined using radioimmunoassay kit with no cross-reaction to pro-insulin (LINCO Research, St Charles, MO, USA). Body composition was measured by DXA (Lunar Prodigy, Madison, WI, USA).

Statistical analysis

Subjects were randomized to CLA or placebo in blocks of six. Results are shown as medians and 95% confidence interval. Comparison within treatment groups regarding change from baseline was performed using paired Wilcoxon's signed rank test. Test for differences between groups was performed by Wilcoxon's rank sum test on the differences between 6 months and baseline. The differences were also analyzed by analysis of covariance (baseline as covariate) and repeated measure analysis of variance (ANOVA) to look for significant treatment, center or time effects. Correlation analysis was performed using Spearman rank test. A significance level of 5% was used in tests, and all tests were two-tailed. Statistics were computed using Number Cruncher Statistical System (Kaysville, UT, USA, 2001).

Results

In the clamp study population, six subjects from placebo group and two from the CLA group dropped out of the study. However, there was no significant difference in M between those placebo-treated subjects who completed two clamps ($n=17$, median M $5.1 \text{ mg min}^{-1} \text{ lbm}^{-1}$) and the total number of placebo subjects at baseline ($n=23$, median M $5.6 \text{ mg min}^{-1} \text{ lbm}^{-1}$; $P=0.87$). Reasons for drop-out were withdrawal of informed consent ($n=4$) and three were lost to follow-up. In one subject, a second clamp study could not be performed for technical reasons. Compliance was 95% in the placebo and CLA group ($n=39$), except for one with 65% and one with 17%, both in placebo group. These two latter subjects are included in all analysis.

For the clamp study participants, there were significant gender differences in median bw at baseline (98.0 kg (male) vs 84.2 kg (female), $P<0.001$), median percent body fat (31.9% (male) vs 44.6% (female), $P<0.001$) and median leptin concentrations (555 pmol l^{-1} (male) vs 1575 pmol l^{-1} (female), $P=0.001$). However, differences between 6 months data and baseline for these parameters were not significantly related to gender. Table 1 demonstrates that the only significant changes from baseline in the parameters listed were a decrease in waist circumference in the CLA group ($P=0.02$) and a decrease in glucohemoglobin (HbA1c) in the placebo group ($P=0.014$). There was a trend toward larger reduction in percent body fat and waist circumference after 6 months of supplementation in the CLA group compared to placebo ($P=0.10$ and 0.09 , respectively).

Clamp studies

Forty-five clamp tests were performed at each of the two centers. Baseline fasting glucose at center 1 was lower than at center 2 (median value 5.08 mmol l^{-1} ($n=24$) vs 5.35 mmol l^{-1} ($n=25$), $P=0.014$), although no other parameters differed significantly between centers. The median M at baseline for the centers was 10.6 and $10.0 \text{ mg min}^{-1} \text{ lbm}^{-1}$, respectively. After 6 months, no significant difference between centers for any of the parameters listed in Table 2 was found. Two insulin values of 83 and 86 pmol l^{-1} during the baseline clamp study were excluded from analysis. The corresponding values at 6 months were 627 and 556 pmol l^{-1} , indicating an error during the baseline study. There was a good correlation between fasting insulin and insulin during clamp at baseline ($R=0.57$, $P=0.00004$).

Clamp studies at baseline (15 male and 34 female subjects) revealed that median M corrected for bw was $7.0 \text{ mg min}^{-1} \text{ bw}^{-1}$ for male and $5.1 \text{ mg min}^{-1} \text{ bw}^{-1}$ for female subjects ($P=0.053$). If corrected for lbm as measured by DXA, the corresponding values were $10.5 \text{ mg min}^{-1} \text{ lbm}^{-1}$ for male and $9.7 \text{ mg min}^{-1} \text{ lbm}^{-1}$ for female subjects ($P=0.72$). Thus, M corrected for lbm was used to adjust for gender differences in body composition.

Effects of CLA on M

There was no statistical difference in either M, M corrected for bw or M corrected for lbm between the treatment groups at baseline. The range of M corrected for lbm at baseline was 4.0 – $27.5 \text{ mg min}^{-1} \text{ lbm}^{-1}$. Compared to baseline, none of the treatment groups had significant changes in M (relative to bw or lbm) after 6 months (Table 2). Also, no significant difference was found between treatment groups ($P=0.93$) when comparing the change from baseline to 6 months. When using published criteria regarding insulin resistance²⁴ for M corrected for lbm (by DXA), two subjects at baseline and one subject in each treatment group at 6 months were categorized as insulin resistant.

Effects of CLA on glucose uptake insulin concentration ratio during clamp (M/I)

The mean of the three insulin values measured at the last 30 min during clamp was used for calculation. The number of subjects is reduced compared to measurement of M because some samples of insulin were excluded owing to values similar to or lower than fasting insulin (hemolysis interference). The range of M/I adjusted for lbm was 1.9 – $75 \times 10^{-2} \text{ mg min}^{-1} \text{ lbm}^{-1} \mu\text{U}^{-1}$ at baseline. There was no significant change for M/I adjusted for lbm either within or between treatment groups in the sample that completed two clamps ($n=35$) (Table 2). Using published suggestions for insulin resistance based on M/I adjusted for lbm,²⁵ four subjects at baseline and three subjects receiving CLA and two receiving placebo at 6 months were categorized as insulin resistant.

Table 1 Characteristics of study population that completed two euglycemic clamps ($n=41$)

	CLA N = 24, 17 female and 7 male subjects			Placebo N = 17, 11 female and 6 male subjects		
	0 month	6 months	Change (CI)	0 month	6 months	Change (CI)
Weight (kg)	89.0	89.0	0.15 (-4.0, 1.6)	86.0	87.0	0 (-2.0, 1.0)
BMI (kg/m ²)	30.8	30.5	-0.06 (-1.1, 0.5)	30.8	29.9	0 (-0.5, 0.5)
Waist (cm)	100	99	-2 (-1, -6)	99	98	-1.5 (-2, 3)
Body fat mass (%)	43.3	41.2	-0.65 (-2.5, 0.8)	41.1	41.8	-0.40 (-1.2, 1.5)
HbA1c (%)	5.40	5.40	0 (-0.2, 0.1)	5.50	5.40	-0.2 (-0.3, 0.0)
c-Peptide (pmol l ⁻¹)	759	777	51 (-89, 140)	663	772	19 (-105, 141)
Adiponectin (mg l ⁻¹)	10.7	10.7	-0.6 (-2.2, 0.4)	9.8	9.6	0.27 (-1.5, 0.9)
Leptin (pmol l ⁻¹)	1223	1299	5.5 (-146, 231)	1221	1167	86 (-207, 322)

Abbreviations: BMI, body mass index; CI, confidence interval; CLA, conjugated linoleic acid; HbA1c, glycohemoglobin. Values are medians and 95% CI.

Table 2 Summary of M, M/I, fasting glucose, fasting insulin, HOMA and QUICKI for the 41 subjects that completed two clamps

Measure	Group	n	Month 0 Median (CI)	Month 6 Median (CI)	Difference 6-0 months Median (CI)	P-value within group ^a	P-value between groups ^b
M (mg min ⁻¹ lbm ⁻¹)	CLA	24	11.0 (7.7, 12.8)	10.3 (8.1, 12.4)	0.21 (-1.9, 1.1)	0.67	0.93
	Placebo	17	8.4 (7.0, 13.9)	9.3 (5.2, 11.8)	-0.22 (-1.5, 0.45)	0.91	
M (mg min ⁻¹ bw ⁻¹)	CLA	24	6.2 (4.4, 7.0)	6.1 (4.6, 7.9)	0.3 (-0.7, 1.0)	0.39	0.74
	Placebo	17	5.1 (4.0, 8.0)	6.0 (3.3, 7.6)	-0.1 (-0.9, 1.1)	0.86	
M/I (mg min ⁻¹ lbm ⁻¹ l μU ⁻¹) × 100	CLA	21	15.8 (8.6, 18.9)	15.4 (10.4, 19.4)	-0.5 (-4.8, 2.0)	0.57	0.95
	Placebo	14	12.6 (8.5, 17.6)	9.4 (6.8, 17.9)	-1.8 (-6.3, 2.4)	0.36	
Fasting glucose (mmol/l)	CLA	24	5.28 (5.10, 5.55)	5.13 (4.80, 5.25)	-0.075 (-0.55, 0.25)	0.38	0.60
	Placebo	17	5.35 (5.00, 5.55)	5.15 (4.95, 5.40)	-0.25 (-0.45, 0.10)	0.10	
Fasting insulin (pmol/l)	CLA	24	104 (76, 110)	93 (64, 118)	-0.75 (-26, 8.0)	0.52	0.39
	Placebo	17	98 (80, 117)	80 (65, 124)	-16 (-26, 5.0)	0.09	
HOMA	CLA	24	3.3 (2.7, 3.8)	2.9 (2.2, 3.7)	-0.08 (-0.88, 0.24)	0.42	0.35
	Placebo	17	3.3 (3.0, 3.8)	2.6 (2.1, 3.9)	-0.53 (-1.1, 0.18)	0.08	
QUICKI	CLA	24	0.320 (0.312, 0.327)	0.325 (0.315, 0.333)	0.0017 (-0.004, 0.01)	0.42	0.31
	Placebo	17	0.320 (0.314, 0.324)	0.330 (0.313, 0.342)	0.011 (-0.002, 0.02)	0.08	

Abbreviations: Bw, body weight; CI, confidence interval; CLA, conjugated linoleic acid; HOMA, homeostasis model assessment; lbm, lean body mass; M, glucose uptake; M/I, glucose uptake divided on insulin during clamp; QUICKI, quantitative insulin sensitivity check index. Values are medians and 95% CI. ^aPaired Wilcoxon's signed rank test. ^bWilcoxon's rank sum test on 6-0 months.

Effects of CLA on fasting insulin and fasting glucose levels

The range for mean fasting insulin was 32-537 pmol l⁻¹ at baseline. The maximum value was an extreme outlier (>3 times the interquartile range from the 75 percentile). For the whole population, the median value decreased from 104 pmol l⁻¹ ($n=49$) at baseline to 91 pmol l⁻¹ ($n=41$) after 6 months. Repeated measure ANOVA of insulin data showed a significant effect of time ($P=0.03$), but not for treatment or center.

The minimum value for fasting glucose was 4.05 mmol l⁻¹ and the maximum value 6.7 mmol l⁻¹ at baseline. For the whole population, the median value decreased from 5.30 mmol l⁻¹ ($n=49$) at baseline to 5.15 mmol l⁻¹ ($n=41$) after 6 months. There was no significant difference within or between groups for either fasting insulin or fasting glucose concentration (Table 2). Repeated measure ANOVA of fasting glucose data showed a significant effect of time ($P=0.02$) and the interaction factor: center × time ($P<0.0001$).

HOMA and QUICKI for the clamp population

HOMA at baseline ranged from 1.05 to 21.5. The maximum value was an extreme outlier owing to a high fasting insulin value for one subject (537 pmol l⁻¹). After 6 months of supplementation, there was no significant difference either within or between groups for HOMA values (Table 2). Using a criterion for insulin resistance based on HOMA,²⁴ nine subjects at baseline and four subjects (three subjects receiving CLA) after 6 months were categorized as insulin resistant. QUICKI at baseline ranged from 0.2538 to 0.3805. After 6 months of supplementation, there was no significant difference either within or between groups for QUICKI (Table 2).

HOMA and QUICKI for the per protocol population ($n=83$)

HOMA and QUICKI values for the subjects that completed fasting glucose and insulin measurements at baseline and 6 months are listed in Table 3. After 6 months of

Table 3 Summary of HOMA and QUICKI for all the 83 subjects in the main study that completed measurements of fasting glucose and insulin at baseline and 6 months

Measure	Group	n	Month 0 Median (CI)	Month 6 Median (CI)	Difference 6–0 months Median (CI)	P-value within group ^a	P-value between groups ^b
HOMA	CLA	42	3.2 (2.8, 3.9)	3.0 (2.5, 3.5)	−0.25 (−0.65, 0.14)	0.16	0.58
	Placebo	41	3.2 (2.7, 3.8)	2.9 (2.5, 3.6)	−0.23 (−0.43, 0.04)	0.34	
QUICKI	CLA	42	0.321 (0.313, 0.327)	0.324 (0.317, 0.332)	0.0058 (−0.0017, 0.0097)	0.18	0.62
	Placebo	41	0.322 (0.314, 0.327)	0.326 (0.315, 0.333)	0.0027 (−0.0053, 0.0073)	0.32	

Abbreviations: CLA, conjugated linoleic acid; HOMA, homeostasis model assessment; QUICKI, quantitative insulin sensitivity check index. Values are medians and 95% CI. ^aPaired Wilcoxon’s signed rank test. ^bWilcoxon’s rank sum test on 6–0 months.

Table 4 Correlations that are significant (Spearman, $P < 0.05$) for a number of variables measured as change from baseline to 6 months ($n = 41$) for CLA and placebo groups combined

Variable	Correlate with (coefficient)
M ($\text{mg min}^{-1} \text{lbm}^{-1}$)	HbA1c (−0.47)
Fasting glucose	Fasting insulin (0.35), c-peptide (0.35)
Fasting insulin	c-Peptide (0.35)
Weight	HbA1c (0.36), leptin (0.48)
BMI	HbA1c (0.38), leptin (0.38)
Percent body fat	Leptin (0.31)
HbA1c	Adiponectin (−0.38)
c-Peptide	Leptin (0.45)

Abbreviations: BMI, body mass index; CLA, conjugated linoleic acid; HbA1c, glycohemoglobin; lbm, lean body mass; M, glucose uptake.

supplementation, there was no significant difference either within or between groups for either HOMA or QUICKI. Using the cutoff value of 4.65 for HOMA,²⁴ 13 subjects (seven subjects receiving CLA, five subjects placebo) (16%) were categorized as insulin resistant both at baseline and after 6 months.

Correlation analysis

Non-parametric Spearman correlation for a number of variables is shown in Table 4. Baseline values of M adjusted for lbm ($\text{mg min}^{-1} \text{lbm}^{-1}$) were significantly correlated with fasting glucose ($R = -0.38$), fasting insulin ($R = -0.55$), HbA1c ($R = -0.50$), adiponectin ($R = 0.39$) and c-peptide ($R = -0.63$). Also, uncorrected M (mg min^{-1}) was significantly correlated to lbm ($R = 0.41$) and trunk fat mass ($R = -0.37$), but not to total fat mass ($R = -0.25$). M adjusted for lbm ($\text{mg min}^{-1} \text{lbm}^{-1}$) showed high correlation with HOMA and QUICKI at baseline ($R = -0.58$ for HOMA and 0.65 for QUICKI, respectively). Changes in M adjusted for lbm ($\text{mg min}^{-1} \text{lbm}^{-1}$) were inversely correlated to changes in HbA1c. No correlation was found between M (mg min^{-1}) and either changes in lbm (kg), total fat mass (kg) or trunk fat mass (kg) as measured by DXA, or waist circumference.

Discussion

Euglycemic hyperinsulinemic clamp method is considered a reference to measure insulin resistance or sensitivity. The

values and variations of M or M/I seen in this experiment are comparable to published values for healthy subjects^{23,24,26,27} and obese and non-obese subjects with and without type 2 diabetes.²⁸ The only other study on CLA isomer mixture using the euglycemic hyperinsulinemic clamp technique reported M to be 3.7–4.5 $\text{mg min}^{-1} \text{bw}^{-1}$ (range of means) in obese men with the metabolic syndrome,¹⁸ which is lower than M values of 5.1–6.2 $\text{mg min}^{-1} \text{bw}^{-1}$ observed in the present study comprising male and female subjects. In our study, healthy overweight and obese subjects had a median M/I of 7.6–8.2 $\text{M} \mu\text{U}^{-1} \text{l}$ (corrected for bw), which is similar to type 2 diabetics with mean BMI of 23.1 kg/m^2 but without the metabolic syndrome.²⁹ In one study on the effect of *cis*-9, *trans*-11 isomer of CLA in obese men with metabolic syndrome mean values of M/I of 4.2–4.3 $\text{M} \mu\text{U}^{-1} \text{l}$ (corrected for bw) were measured,²⁰ which is lower than corresponding values in the present study.

Consequently, few subjects were categorized as being insulin resistant according to cutoff values, which have been suggested for M ($M < 28 \mu\text{mol min}^{-1} \text{lbm}^{-1}$; Stern *et al.*²⁴), for M/I ($M/I \leq 6.3 \text{M} \mu\text{U}^{-1} \text{l}$; McAuley *et al.*²⁵) and for HOMA ($\text{HOMA} > 4.65$; Stern *et al.*²⁴). The distribution of M, M/I or HOMA did not change significantly during the 6 months of treatment and no pattern of change in insulin resistance status for CLA- or placebo-supplemented subject was seen. Furthermore, the prevalence of insulin resistance was not consistent between the different methods used and no single subject was categorized as being insulin resistant by all three methods either at baseline or at study completion. One subject from the CLA and one from the placebo group changed to insulin resistant as measured by both M and M/I. However, none of these were insulin resistant according to HOMA at 6 months.

There was good consistency between values for HOMA and QUICKI in the clamp sub-population ($n = 41$) and the whole population ($n = 83$) that completed baseline and 6 months measurements. The similar values obtained with the two populations suggest that the clamp study subjects are representative of the main population for measures of insulin resistance. In both cases, there is a slight improvement in both parameters with time, for both groups, but the differences are not significant.

The present study demonstrates that CLA as a mixture of the two dominating isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12 has no effect on insulin sensitivity and glucose metabolism assessed by change in distribution of M, M/I, HOMA or QUICKI in overweight and obese subjects. This is in accordance with the data on insulin resistance in obese men with metabolic syndrome.¹⁸ In that study, CLA was supplemented for 12 weeks to 19 men without significant changes in M, fasting insulin, fasting glucose, HbA1c or leptin, whereas the pure CLA isomer *trans*-10, *cis*-12 caused a significant decrease in M (14%) and an increase in fasting glucose (3.8%). In another study, the pure CLA isomer *cis*-9, *trans*-11 supplemented to 13 obese men for 12 weeks caused a significant decrease in insulin sensitivity (M/I) compared to placebo (9.9% decrease in CLA group and 3.1% increase in placebo).²⁰ The change in M/I was reported to be 0.13 ± 1.48 for placebo ($n=12$) and -0.62 ± 0.72 for *cis*-9, *trans*-11 ($n=13$) (mean \pm s.d.). Estimating the confidence interval (95%) for the difference between groups (-0.17 to 1.67) indicates that the change was not significant. The 19 subjects receiving the *trans*-10, *cis*-12 isomer showed increased fasting insulin and glucose (significant), whereas in the 13 subjects receiving the *cis*-9, *trans*-11 isomer fasting glucose and insulin decreased from baseline (although the decrease was less than for placebo and not significant). Thus, it seems that different effects on glucose metabolism are obtained with the pure isomers, whereas the non-effect result of the isomer mixture of CLA is consistently found in separate studies.

The present study corroborates previous ones using either the CLA mixture^{2-5,7,9,10,13,14,19,30-32} or either one of the two main isomers^{33,34} demonstrating that fasting insulin and glucose do not change during supplementation. The CLA doses in these studies ranged from 0.6 to 6.8 g/day for time periods ranging from 5 days to 2 years in healthy, overweight or obese individuals. One study using an oral glucose tolerance test (OGTT) reported in improved insulin sensitivity in healthy subjects after intake of the CLA mixture.¹⁶ On the other hand, in a study of type 2 diabetics,¹⁷ 16 subjects receiving a mixture of CLA isomers showed changes in fasting glucose, HOMA, insulin sensitivity index and oral glucose sensitivity suggestive of increased insulin resistance compared to placebo-treated subjects. In the same study, however, no changes in HbA1c, QUICKI, fasting insulin or glucose and insulin response during OGTT, were observed.

Correlation analysis revealed as expected a positive correlation between M (mg min^{-1}) and lbm ($R=0.41$) and an inverse correlation between M and trunk fat mass ($R=-0.37$), whereas the respective correlation with total fat mass was not significant. The importance of abdominal fat mass for insulin resistance is well established. Indeed, Virtanen *et al.*²⁸ have shown that mild, recent diabetes, adds little to the insulin resistance compared to abdominal obesity. However, we were unable to correlate individual changes in either lbm, total fat mass or trunk fat mass, which in some cases were quite large (e.g. loss of 12.6 kg fat mass) to changes in any of the indices of insulin resistance. This is in

accordance with a controlled trial reported by McAuley *et al.*³⁴ in which normoglycemic insulin-resistant subjects were randomized either to a control group, a group with moderate dietary and exercise program or a third group with an intensive dietary and exercise program. Subjects in both intervention groups reduced total and truncal fat masses as well as waist circumference, whereas lbm was maintained. However, only the intensive program induced a significant improvement in insulin resistance (16–23%), indicating that intensive physical exercise is of more importance to insulin resistance than diet or fat loss.

Conclusion

The present long-term study using the euglycemic hyperinsulinemic clamp method suggests that CLA as a commercial preparation containing a mixture of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers has no deleterious effect on either glucose metabolism or insulin resistance in healthy overweight and obese subjects.

Acknowledgements

We are very thankful to the clinical nurses Bente Ryen, Lill Johannessen and Sissel Paulsen Sundeng for their active contribution in the success of this study. Lipid Nutrition, a division of Loders Croklaan has supported this study.

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