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Short Communication

Supplementation with *trans*10*cis*12-conjugated linoleic acid induces hyperproinsulinaemia in obese men: close association with impaired insulin sensitivity

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Abstract

Aims/hypothesis. Hyperproinsulinaemia reflects both beta cell dysfunction and insulin resistance in cross-sectional studies, but it is not known whether changes in proinsulin concentrations are related to insulin resistance over time. As *trans*10*cis*12 (*t*10*c*12)-conjugated linoleic acid (CLA) supplementation induces insulin resistance in obese men, we used this fatty acid to investigate the effects on plasma proinsulin, insulin, C-peptide and adiponectin concentrations, including their associations with change in insulin sensitivity.
Methods. We randomised (double-blind) 57 non-diabetic abdominally obese men to receive either 3.4 g *t*10*c*12CLA, CLA-isomer mixture or control oil for 12 weeks. Insulin sensitivity (hyperinsulinaemic–euglycaemic clamp), intact proinsulin, insulin, the proinsulin : insulin ratio, C-peptide, glucose and adiponectin were assessed before and after supplementation.
Results. Supplementation with *t*10*c*12CLA increased proinsulin ($p<0.01$), the proinsulin : insulin ratio ($p<0.05$) and C-peptide concentrations ($p<0.001$) in comparison with control subjects. Adiponectin, however, did not change significantly. The change in proinsulin, but not the proinsulin : insulin ratio, was related to impaired insulin sensitivity ($r=-0.58$, $p<0.0001$), independently of changes in insulin, C-peptide, glucose, adiponectin and BMI. Conversely, the correlation between insulin sensitivity and specific insulin ($r=-0.46$, $p<0.001$) did not remain significant after adjustment for proinsulin. Induced hyperproinsulinaemia was also correlated to adiponectin concentrations ($r=-0.34$, $p<0.01$).
Conclusions/interpretation. In obese men, *t*10*c*12CLA induces hyperproinsulinaemia that is related to impaired insulin sensitivity, independently of changes in insulin concentrations. These results are of clinical interest, as hyperproinsulinaemia predicts diabetes and cardiovascular disease. The use of weight-loss supplements containing this fatty acid is worrying.

Keywords Adiponectin · Conjugated linoleic acid · Dietary supplements · Hyperproinsulinaemia · Insulin resistance · Insulin secretion · Proinsulin · Randomised controlled trial · *Trans* fatty acids

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Abbreviations: ANCOVA, analysis of covariance · CLA, conjugated linoleic acid · t10c12, *trans*10*cis*12

Introduction

Hyperproinsulinaemia independently predicts Type 2 diabetes [1] and cardiovascular disease [2]. Cross-sectionally, hyperproinsulinaemia is associated with insulin resistance [3, 4], and beta cell dysfunction [5]. However, it is not known whether changes in proinsulin concentrations are related to insulin resistance over time.

We recently reported that *trans*10*cis*12 (*t*10*c*12)-conjugated linoleic acid (CLA) causes isomer-specific insulin resistance in obese men [6]. The insulin resis-

Table 1. Baseline characteristics

	Control (*n*=19)	CLA (*n*=19)	*t*10*c*12CLA (*n*=19)
Age (years)[a]	53±10.1	51±7.1	55±7.1
BMI (kg/m^2)[a]	30.2±1.8	30.1±1.8	31.2±2.5
Plasma proinsulin (pmol/l)[a]	18.5±11.5	14.9±8.2	15.3±11.9
Plasma insulin (pmol/l)[a]	73.2±30	64.8±23.4	68.4±26.4
Proinsulin : insulin ratio	1.10±0.2	1.14±0.2	1.04±0.2
Plasma C-peptide (pmol/l)	984±329	781±243	881±315
Plasma glucose (mmol/l)[a]	5.7±0.6	5.9±0.7	5.6±0.6
Plasma adiponectin (μg/ml)	7.0±3.5	7.5±3.5	7.8±3.8
Insulin sensitivity (M, mg·kg^{-1}·min^{-1})[a]	3.7±1.6	4.5±1.5	3.9±1.5

Data are given as means ± SD. [a] These values were previously reported [6]. There were no significant differences between the groups in any variable (ANOVA). CLA, conjugated linoleic acid; *t*10*c*12CLA, *trans*10*cis*12CLA

tance thus induced by *t*10*c*12CLA gave us a unique possibility to study the longitudinal relationships between insulin resistance and changes in insulin-like peptides.

As the hormone adiponectin is inversely associated with insulin resistance [7], the effect of *t*10*c*12CLA on adiponectin and its possible relationship with hyperproinsulinaemia is also of interest. Another reason why it is important to investigate the effects of CLA on proinsulin, C-peptide and adiponectin concentrations is that *t*10*c*12CLA is found in hydrogenated vegetable oils and also contained in dietary CLA supplements.

Subjects and methods

Subjects. This study is a re-analysis of a controlled study. As previously described [6], non-diabetic, obese Swedish men were included (Table 1). Subjects on antidiabetic and/or lipid-lowering drugs or with diagnosed metabolic diseases were excluded. All subjects gave their written consent and the protocol was approved by the Ethics Committee of Uppsala University.

Protocol. Insulin sensitivity, intact proinsulin, specific insulin, glucose, C-peptide and plasma adiponectin concentrations were determined before and after the intervention. The data on insulin sensitivity and the protocol have been described [6]. A total of 60 men were randomly assigned (double-blind) to a 3-month-long supplementation of 3.4 g of purified *t*10*c*12CLA, 3.4 g CLA-isomer mixture or 3.4 g olive oil (control). Capsules were prepared by Natural Lipids (Hovebygda, Norway). All men had fasted for 12 h, and restrained from smoking, alcohol and exercise for 24 h before their visits. The subjects were encouraged to maintain their usual lifestyle during the study.

Metabolic measurements. Insulin sensitivity was determined by euglycaemic–hyperinsulinaemic clamp as previously described [6]. Insulin sensitivity (M) was the glucose infusion rate (mg·kg bw^{-1}·min^{-1}). Plasma glucose was assayed in a Beckman analyser (Beckman Instruments, Fullerton, Calif., USA).

Venous blood was drawn into vacuum tubes, centrifuged and stored at –70 °C. Plasma adiponectin (intra-assay CV 3.6%, interassay CV 9.3%) was measured using a human RIA kit (Linco Research, St. Charles, Mo., USA). Specific plasma insulin (intra-assay CV 2.8%, interassay CV 2.8%) was measured using an ELISA kit (Mercodia, Uppsala, Sweden). Plasma intact proinsulin (intra-assay CV 3.2%, interassay CV 5.2%) was measured with an enzyme immunosorbent assay (ELISA) kit (Mercodia). The cross-reactivity with insulin and C-peptide was less than 0.03 and 0.006% respectively. Plasma C-peptide (intra-assay CV 3.1%, interassay CV 4.4%) was measured using a specific enzyme immunoassay ELISA kit (Mercodia). Cross-reactivity with 32–33 split-proinsulin was 2%. All insulin-like peptides were measured in an automated enzyme immunoassay analyser (Bio-Rad Laboratories, Hercules, Calif., USA).

Statistics. Proinsulin, insulin and adiponectin had skewed distributions and were logarithmically transformed. Differences between the three groups over time were assessed with an overall-test (ANOVA). If an overall-test yielded significant results, an unpaired *t* test was used to test differences between groups. ANCOVA (analysis of covariance) was used to compare changes between groups after adjustment for baseline or delta (Δ)-values. Pearson's correlation coefficients were determined and partial correlation analysis for Δ-values was assessed using baseline and Δ-values as covariates. Correlations are calculated on the basis of *n*=57. Twenty subjects per group would be needed to detect a 15% change in proinsulin with a power of 0.80 (*p* level of 0.05). A two-tailed *p* value of less than 0.05 was significant. The JMP software statistics package was used (SAS Institute, Cary, N.C., USA).

Results

Changes observed. Baseline characteristics are shown in Table 1. Of the 60 men, 57 completed the intervention. Reasons for dropping out have been reported elsewhere [6]. Insulin sensitivity decreased and fasting glucose increased, but insulin concentrations did not significantly change after *t*10*c*12CLA when compared with control subjects (Table 2) [6]. The novel data obtained were for proinsulin, C-peptide and adiponectin. Treatment with *t*10*c*12CLA elevated proinsulin (p=0.016; ANOVA) and C-peptide levels (p<0.001; ANOVA) versus control subjects (p<0.01 and p<0.001; unpaired *t* test, Table 2). This remained significant after adjustment for age, BMI and baseline

Table 2. Absolute and relative (%) changes in glucose metabolism from baseline to 12 weeks

	Control (n=19)	CLA (n=19)	$t10c12$ CLA (n=19)
BMI (kg/m^2)[a]	–0.05±0.5 (0.1%)	–0.15±0.6 (0.5%)	–0.27±0.4 (0.9%)
Plasma proinsulin (pmol/l)	–0.86±4.7 (–5%)	1.90±9.4 (13%)	2.84±7.3 (19%)[c]
Plasma insulin (pmol/l)[a]	5.5±23.3 (8%)	4.8±19.2 (7%)	14.4±23.7 (21%)
Proinsulin : insulin ratio	–0.06±0.09 (–5%)	–0.02±0.10 (–2%)	0.02±0.11 (2%)[b]
Plasma C-peptide (pmol/l)	–96±181 (–10%)	47±127 (6%)	88±122 (10%)[d]
Plasma glucose (mmol/l)[a]	–0.14±0.24 (–2%)	0.01±0.30 (0%)	0.21±0.33 (4%)[d]
Plasma adiponectin (µg/ml)	0.49±1.5 (14%)	0.26±1.5 (3%)	0.01±2.5 (0.1%)
Insulin sensitivity (M, mg·kg^{-1}·min^{-1})[a]	0.44±1.02 (12%)	–0.05±0.97 (–1%)	–0.55±0.95 (–15%)[c]

Data are means ± SD. [a] These values were previously reported [6]. [b] p<0.05 vs controls; [c] p<0.01 vs controls, [d] p<0.001 vs controls (unpaired t tests). Unpaired t tests were performed in the case of a significant ANOVA test (p<0.05). CLA, conjugated linoleic acid; $t10c12$CLA, *trans*10*cis*12CLA

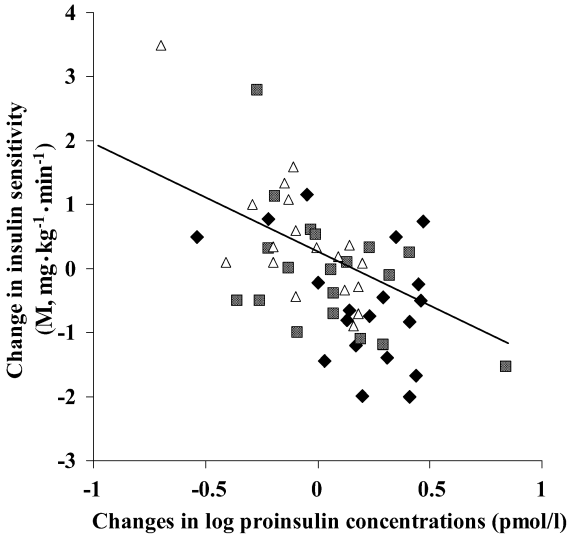

Fig. 1. Correlation between changes in log proinsulin concentrations and changes in insulin sensitivity from baseline to 12 weeks (r=–0.58, p<0.0001, n=57). Black diamonds: treatment with *trans*10*cis*12-conjugated linoleic acid (CLA); shaded squares: treatment with CLA; white triangles: placebo

values (p<0.01; ANCOVA). The difference between groups in proinsulin also remained significant after adjusting for changes in insulin concentrations (p=0.03; ANCOVA). The proinsulin : insulin ratio also increased after treatment with $t10c12$CLA (p=0.04; ANOVA) versus control subjects (p<0.05; unpaired t test, Table 2), but did not remain significant after further adjustments (p=0.17; ANCOVA). There was no difference in adiponectin concentrations between groups after treatment (p=0.40; ANOVA).

Univariate correlations. The change (Δ) in proinsulin was inversely correlated to Δinsulin sensitivity (Fig. 1) and Δadiponectin (r=–0.34, p<0.01) and positively correlated to Δinsulin (r=0.64, p<0.0001) and C-peptide (r=0.71, p<0.0001). The Δproinsulin : insulin ratio and Δadiponectin were not significantly correlated to Δinsulin sensitivity (r=0.22, p=0.09 and r=0.10, p=0.46 respectively).

Multivariate correlations. The correlation between Δproinsulin and Δinsulin sensitivity remained significant when adjusted separately for each of the following: Δinsulin, ΔC-peptide, Δproinsulin : insulin ratio, Δglucose, ΔBMI, Δadiponectin, age (all p values <0.04). The correlation between specific Δinsulin and Δinsulin sensitivity (–0.46, p<0.001) remained unchanged after adjustment for changes in Δglucose, ΔBMI, Δadiponectin or ΔC-peptide, but was statistically insignificant when adjusting for Δproinsulin (r=–0.19, p=0.30).

Discussion

This randomised controlled study in obese men demonstrates that treatment with $t10c12$CLA, which induces insulin resistance, also increases plasma proinsulin concentrations, independently of changes in insulin concentrations. Treatment with $t10c12$CLA caused a marked increase in proinsulin (~20%). This isomer-specific, fatty-acid-induced hyperproinsulinaemia was closely related to insulin resistance, and also independent of changes in plasma concentrations of specific insulin, C-peptide, glucose, adiponectin and obesity. Thus, proinsulin seems to be independently related to changes in insulin sensitivity, when measured prospectively in a controlled fashion. This association has previously only been described in observational cross-sectional studies [3, 4].

Interestingly, the association between insulin and insulin resistance did not remain significant after adjusting for proinsulin concentrations.

It is possible that hyperproinsulinaemia occurred due to a compensatory increase in beta cell function to match insulin resistance [8]. The increased C-peptide concentrations following treatment with $t10c12$CLA may support such an idea, but it is unclear why insulin was not also significantly increased. As we did not measure insulin secretion directly, we cannot draw firm conclusions regarding the effects of the treatment on insulin secretion. The proinsulin : insulin ratio, a

reflection of beta cell function [5], also increased after *t*10*c*12CLA, but was not related to insulin resistance.

Interestingly, adiponectin was not related to insulin sensitivity, but to proinsulin (inverse correlation). However, this latter relationship should be interpreted cautiously as adiponectin did not change significantly after *t*10*c*12CLA.

Different CVs among insulin-like peptides may affect results in statistical analyses in favour of proinsulin over insulin. The current CV, in fact, was similar among the peptides. However, it cannot be ruled out that the stronger correlation to insulin resistance in multivariate analyses could be explained by the fact that proinsulin has a longer half-life than insulin.

This is the first study to provide human data on the effects of CLA on proinsulin, adiponectin and C-peptide concentrations. As hyperproinsulinaemia predicts Type 2 diabetes [1, 9] and cardiovascular death [2], our results are clinically relevant, especially as *t*10*c*12CLA is a constituent of commercial weight-loss supplements. Supporting previous data [6, 10], our results suggest that CLA supplementation might increase cardiovascular risk.

In summary, *t*10*c*12CLA elevates markers of insulin secretion in obese men, but the hyperproinsulinaemia induced seems to mainly reflect impaired insulin sensitivity.

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