Treatment With Dietary *trans*10*cis*12 Conjugated Linoleic Acid Causes Isomer-Specific Insulin Resistance in Obese Men With the Metabolic Syndrome

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OBJECTIVE — Conjugated linoleic acid (CLA) is a group of dietary fatty acids with antiobesity and antidiabetic effects in some animals. The *trans*10*cis*12 ($t10c12$) CLA isomer seems to cause these effects, including improved insulin sensitivity. Whether such isomer-specific effects occur in humans is unknown. The aim of this study was to investigate whether $t10c12$ CLA or a commercial CLA mixture could improve insulin sensitivity, lipid metabolism, or body composition in obese men with signs of the metabolic syndrome.

RESEARCH DESIGN AND METHODS — In a randomized, double-blind controlled trial, abdominally obese men ($n = 60$) were treated with 3.4 g/day CLA (isomer mixture), purified $t10c12$ CLA, or placebo. Euglycemic-hyperinsulinemic clamp, serum hormones, lipids, and anthropometry were assessed before and after 12 weeks of treatment.

RESULTS — Baseline metabolic status was similar between groups. Unexpectedly, $t10c12$ CLA increased insulin resistance (19%; $P < 0.01$) and glycemia (4%; $P < 0.001$) and reduced HDL cholesterol (-4%; $P < 0.01$) compared with placebo, whereas body fat, sagittal abdominal diameter, and weight decreased versus baseline, but the difference was not significantly different from placebo. The CLA mixture did not change glucose metabolism, body composition, or weight compared with placebo but lowered HDL cholesterol (-2%; $P < 0.05$).

CONCLUSIONS — These results reveal important isomer-specific metabolic actions of CLA in abdominally obese humans. A CLA-induced insulin resistance has previously been described only in lipodystrophic mice. Considering the use of CLA-supplements among obese individuals, it is important to clarify the clinical consequences of these results, but they also provide physiological insights into the role of specific dietary fatty acids as modulators of insulin resistance in humans.

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Conjugated linoleic acid (CLA) is a group of polyunsaturated fatty acids that has received considerable attention for its metabolic and antiobesity actions in animals (1). CLA is dienoic isomers of linoleic acid, naturally found in dairy and beef fat. In mice, CLA decreases body fat (2,3), and in male ZDF rats, CLA improves insulin sensitivity (4,5). In contrast, CLA-fed female mice developed marked lipodystrophic insulin resistance (6), indicating important species and sex differences. The effect of CLA on human insulin sensitivity is unknown.

Recently, we reported that CLA treatment decreased abdominal fat in obese men but without improving metabolism (7). The latter was surprising, because abdominal fat and insulin sensitivity are strongly related (8). Similarly, studies on healthy subjects that used a similar CLA mixture indicated decreased body fat after CLA treatment (9,10) without a reduction in insulin levels (9). However, a strictly controlled study (11,12) failed to show any change in body fat or fasting glucose metabolism in lean, healthy women. Thus, possible antiobesity actions of CLA are still unclear. In spite of lacking clinical and safety human data, dietary CLA supplements are widely used as weight-loss agents among obese subjects, a high-risk group for type 2 diabetes. Therefore, clinical studies are critically needed on such subjects.

Until now, most (7,9,10), but not all (11,12), human studies have used CLA mixtures containing mainly two isomers in approximately equal amounts: *trans*10*cis*12 ($t10c12$) CLA and $c9t11$ CLA. Both isomers are present in the diet, with the $c9t11$ isomer being the most prevalent (13). In rodents, different isomers have distinct effects, and it has been suggested that $t10c12$ CLA is responsible for the antiobesity (14) and insulin-sensitizing (5) properties of CLA. To address isomer specificity on glucose and lipid metabolism in humans, we evaluated the effects of purified $t10c12$ CLA and a commercial CLA mixture. In a randomized controlled trial, we investigated insulin action in abdominally obese men, a potential target group for the putative antidiabetic and antiobesity effects of CLA as reported in some animals.

RESEARCH DESIGN AND METHODS

Subjects
Sixty Caucasian men (35–65 years old) with signs of the metabolic syndrome (abdominal obesity, insulin resistance, dys-

Table 1—*Baseline characteristics*

	Placebo	CLA	*t*10*c*12 CLA
n	19	19	19
Age (years)	53 ± 10.1	51 ± 7.1	55 ± 7.1
Heredity for diabetes (%)	8.8	10.5	7.0
Antihypertensive therapy (%)	9.0	7.2	6.1
Weight (kg)	97.8 ± 10.0	98.3 ± 10.1	100.2 ± 12.0
BMI (kg/m^2)	30.2 ± 1.8	30.1 ± 1.8	31.2 ± 2.5
Waist-to-hip ratio	1.01 ± 0.03	1.01 ± 0.02	1.01 ± 0.03
Waist girth (cm)	112.2 ± 5.0	112.5 ± 7.1	116.0 ± 9.6
SAD (cm)	28.2 ± 1.8	28.2 ± 1.9	28.9 ± 2.3
Body fat (%)	35.3 ± 4.6	36.4 ± 4.4	36.7 ± 3.0
Lean body mass (kg)	61.9 ± 6.9	61.1 ± 6.0	61.9 ± 7.3
Insulin sensitivity (M) (mg · kg^{-1} · min)	3.7 ± 1.6*	4.5 ± 1.5	3.9 ± 1.5
Plasma insulin (pmol/l)	73.2 ± 30	64.8 ± 23.4	68.4 ± 26.4
Plasma glucose (mmol/l)	5.7 ± 0.6	5.9 ± 0.7	5.6 ± 0.6
HbA$_{1c}$ (%)	4.4 ± 0.4	4.4 ± 0.4	4.4 ± 0.3
Serum cholesterol (mmol/l)	5.8 ± 1.2	5.5 ± 0.8	6.0 ± 1.3
LDL cholesterol (mmol/l)	4.0 ± 0.9	3.8 ± 0.7	4.0 ± 1.1
HDL cholesterol (mmol/l)	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.1
VLDL triglycerides (mmol/l)	1.5 ± 0.9	1.2 ± 0.4	1.9 ± 2.1
Serum TG (mmol/l)	2.0 ± 1.0	1.7 ± 0.5	2.4 ± 2.2
Serum FFAs (mmol/l)	0.56 ± 0.16	0.55 ± 0.18	0.61 ± 0.18
Serum free glycerol (mmol/l)	0.120 ± 0.02	0.122 ± 0.02	0.131 ± 0.03
Serum leptin (ng/ml)	10.4 ± 4.6	13.2 ± 10.2	11.6 ± 5.6

Data are means ± SD unless noted otherwise. There were no significant differences between the groups (ANOVA). *n = 18 in the placebo group.

lipidemia, and hypertension) (15) were recruited from the local community through media advertisements. Because subjects with combined abdominal obesity and dyslipidemia are nearly always insulin resistant (8,16), both of these disorders were included in the inclusion criteria: waist girth >102 cm, waist-to-hip ratio >0.95, BMI 27–39 kg/m^2, triglycerides (TG) >1.7 mmol/l, and/or HDL cholesterol <0.9 mmol/l and stable body weight for the preceding 3 months. No one had diagnosed heart, liver, or renal disease or diabetes. Six subjects had fasting plasma glucose ≥7.0 but ≤7.2 mmol/l, indicating mild diabetes; all men had HbA$_{1c}$ ≤5% (Table 1). All subjects gave written consent. The protocol was approved by the Ethics Commitee of the Medical Faculty, Uppsala University.

Protocol
The study was performed after a 4-week run-in screening period. Outcome measures included insulin sensitivity; fasting levels of glucose, lipids, and leptin; and body composition. Subjects were randomly assigned to 3.4 g/day of CLA (isomer mixture), purified *t*10*c*12 CLA, or placebo. Six capsules were taken daily

with morning and evening meals. The isomer content of the CLA preparation (80% free fatty acids [FFAs]) was 35.9% *t*10*c*12 CLA, 35.4% *c*9*t*11 CLA, 13.1% 18:1*c*9, 7.2% 16:0, 2.4% 18:0, and 1.3% 18: 2*c*9*c*12; that of the *t*10*c*12 CLA preparation (75% FFAs) was 76.5% *t*10*c*12 CLA, 11.4% 18:1*c*9, 3.4% 16:0, 2.9% *c*9*t*11 CLA, 2.0% 18:2*c*9*c*12, and 0.8% 18:0. All capsules (identical in appearance) were prepared by Natural Lipids (Hovebygda, Norway), which also assessed isomer separation using gas chromatography (17) (possible isomerization products from the *t*10*c*12 CLA preparation were very low as identified on the chromatogram) and generated the randomization numbers for patients and incorporation into double-blind labeling. The solution code was broken after the study was completed.

All measurements were obtained in the morning, after instructions to fast (12 h) and refrain from smoking, taking snuff (nicotine-containing moist), or engaging in physical activity in the morning and to avoid alcohol and exercise the day before visits. Subjects completed a questionnaire concerning diabetes heredity (having at least one first-degree relative with type 2 diabetes) and the use of medication or di-

etary supplements. All men were encouraged to maintain their usual diet and exercise habits during the study. To assess possible changes in dietary intake during the study, a 3-day weighed-food record was completed during weeks 1 and 8.

Anthropometrics
The sagittal abdominal diameter (SAD) was measured at L4–5 level, and waist and hip girth were measured as previously described in detail (7). Anthropometrics were measured by a single investigator. Bioelectrical impedance analysis (BIA) was done with a multifrequency analyzer (Xitron Technologies, San Diego, CA). From the estimation of body water, body fat content was calculated based on the assumption that fat-free mass contains 73.2% water (18). Lean body mass was calculated with the formula provided by the manufacturer.

Euglycemic-hyperinsulinemic clamp
A 120-min hyperinsulinemic clamp was conducted to determine insulin sensitivity, as described by Defronzo et al. (19) with slight modification previously described in detail (20). Insulin (Actrapid Human; Novo, Copenhagen, Denmark)

Table 2—*Change in body composition from baseline to 12 weeks*

	Placebo	CLA	$t10c12$ CLA
n	19	19	19
Weight (kg)	0.14 ± 1.5	-0.46 ± 1.8	-0.88 ± 1.2*
BMI (kg/m²)	-0.05 ± 0.5	-0.15 ± 0.6	-0.27 ± 0.4*
Waist girth (cm)	-0.63 ± 1.8	-0.92 ± 2.1	-1.57 ± 1.6*
SAD (cm)	-0.43 ± 0.7*	-0.89 ± 0.8†	-0.88 ± 0.5†
Body fat (%)	-0.08 ± 3.0	-0.94 ± 1.6‡	-1.02 ± 2.0‡
Lean body mass (kg)	-0.02 ± 3.1	0.57 ± 2.2	0.46 ± 1.9

Data are means ± SD. There were no differences between the groups. All P values indicate within-group differences; *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

was infused (336 pmol/l · m⁻² · min⁻¹) resulting in a mean steady-state insulin level of 624 pmol/l, a level shown to almost completely suppress hepatic glucose production in insulin-resistant and type 2 diabetic subjects (21). Venous blood sampling was obtained in the left hand, which was kept warmed to provide arterialized blood. Insulin and glucose were infused in the right antecubital vein. Plasma glucose was assayed in duplicate in a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA), using an enzymatic method. Glucose disposal (M) was calculated as the glucose infusion rate (mg·kg⁻¹·min⁻¹) during the last 60 min of the clamp.

Laboratory measurements
Venous blood was drawn into vacuum tubes, coagulated, and centrifuged at room temperature and then frozen at –20°C. Lipoproteins were isolated from fresh serum by a combination of preparative ultracentrifugation (22) and precipitation with a sodium phosphotungstate and magnesium chloride solution (23). Serum lipoproteins and TG were assayed by enzymatic techniques using a Monarch 2000 centrifugal analyzer (Instrumentation Laboratories, Lexington, MA). Serum samples were stored at –70°C. Serum free glycerol and FFAs were measured using an enzymatic colorimetric method (Boehringer Mannheim and Wako Chemical, respectively) in the Monarch centrifuge. Samples from each subject were analyzed within the same run. Plasma insulin was measured by an enzyme immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala, Sweden) in a Bio-Rad Coda automated EIA analyzer (Bio-Rad Laboratories, Hercules, CA). Serum leptin was measured using a human leptin radioimmunoassay kit (Linco Research, St. Charles, MO).

Statistics
Values are expressed as means ± SD. Variables with skewed distributions were logarithmically transformed before analysis. A nonparametric test was used if data were not normally distributed after logarithmic transformation. Paired t test was used for within-group effects from baseline. Differences between groups from baseline to 12 weeks were assessed using overall test (ANOVA or nonparametric test). In case of a significant overall test result, ANOVA or Mann Whitney's nonparametric test was used for differences between two groups. Pearson's correlation coefficient was determined. It was estimated that $n = 20$/group would be needed to detect a 20% change in cholesterol levels with a power of 0.80 at a significance level of 0.05. All tests were two-tailed. $P < 0.05$ was regarded as significant. JMP software (SAS Institute, Cary, NC) was used.

RESULTS

Baseline status and compliance
Baseline characteristics and diabetes heredity were similar in all groups (Table 1). Of 60 men, 58 completed the study. Reasons for withdrawal were gastrointestinal symptoms (CLA group) and weight gain ($t10c12$ CLA group). One patient (placebo) was hypertensive and treated (candesartan) during the trial. This patient completed the trial, but his data were excluded from statistics; results were not influenced if his data were included. Data are based on 57 subjects with complete follow-up data. For M, $n = 56$ because in one subject only insulin values were available. Supplements were generally well tolerated, with only minor transient gastrointestinal problems reported. No adverse events occurred, and no changes in liver enzymes occurred (data not shown).

Compliance (capsule count) did not significantly differ between groups (89.5%, $n = 57$). No significant changes in dietary intake occurred during the study (Table 4).

Body composition
There were no significant differences between groups in body weight, BMI, body fat, lean body mass, or waist girth at 12 weeks (Table 2). CLA preparations did not decrease weight or change body fat, BMI, or waist girth compared with placebo, although these variables significantly decreased within the $t10c12$ CLA group, whereas SAD and body fat decreased within the CLA group (paired t test). SAD tended to decrease more with both CLA treatments than with placebo ($P = 0.07$) (Table 2).

Insulin sensitivity
Insulin sensitivity (M) decreased significantly in the $t10c12$ CLA group compared with placebo but not compared with CLA (Table 3). Also, when correcting M for the mean plasma insulin level during the clamp ($M/I = M$/pmol/l × 100) (19), insulin sensitivity decreased (-19%; $P < 0.01$) after $t10c12$ CLA treatment (data not shown). Fasting insulin increased in the $t10c12$ CLA group ($P < 0.05$) but not differently from placebo (Table 3). The significant reduction in M after $t10c12$ CLA treatment was not affected by adjustment for age or changes in glucose levels, body fat, BMI, or abdominal fat. Baseline adjustments for HbA$_{1c}$, diabetes, or diabetes heredity did not affect significance, nor did exclusion of six subjects with plasma glucose ≥7.0 mmol/l. Of all variables, only adjustment for changes in VLDL TG abolished the significant change in M after $t10c12$ CLA treatment. Fasting glucose increased after $t10c12$ CLA compared with placebo ($P = 0.0009$) (Table 3), and HbA$_{1c}$ decreased after $t10c12$ CLA treatment versus baseline ($P = 0.01$) but was not significantly different from placebo.

Lipoproteins and leptin
The changes in serum lipids or leptin did not differ, except for HDL cholesterol, which decreased with both CLA and $t10c12$ CLA treatment ($P = 0.03$ and $P = 0.006$, respectively) versus placebo (Table 3). VLDL TG tended to increase with $t10c12$ CLA versus placebo and CLA ($P = 0.06$).

Table 3—*Absolute and relative changes in glucose and lipid metabolism from baseline to 12 weeks*

	Placebo	CLA	$t10c12$ CLA
n	19	19	19
Insulin sensitivity (M) (mg $\cdot$ kg^{-1} $\cdot$ min^{-1})	0.44 ± 1.02 (12)	-0.05 ± 0.97 (-1)	-0.55 ± 0.95 (-15)*
Plasma insulin (pmol/l)	5.52 ± 23.3 (8)	4.8 ± 19.2 (7)	14.4 ± 23.7 (21)†
Plasma glucose (mmol/l)	-0.14 ± 0.24† (-2)	0.01 ± 0.30 (0)	0.21 ± 0.33 (4)†‡
HbA$_{1c}$ (%)	0.04 ± 0.11 (1)	0.04 ± 0.12 (1)	0.10 ± 0.16 (2)†
Serum cholesterol (mmol/l)	-0.01 ± 0.09 (0)	-0.03 ± 0.1 (0)	-0.01 ± 0.1 (0)
LDL cholesterol (mmol/l)	-0.05 ± 0.4 (-1)	-0.08 ± 0.5 (-2)	-0.03 ± 0.5 (-1)
VLDL TG (mmol/l)	-0.15 ± 0.4 (-10)	-0.05 ± 0.4 (-4)	0.21 ± 0.6 (11)§
HDL cholesterol (mmo/l)	0.07 ± 0.1 (7)	-0.02 ± 0.1 (-2)‖	-0.04 ± 0.08 (-4)*
TG (mmol/l)	-0.2 ± 0.6 (-10)	-0.13 ± 0.5 (-8)	0.02 ± 1.3 (1)
FFAs (mmol/l)	0.022 ± 0.18 (4)	0.005 ± 0.10 (1)	0.040 ± 0.12 (7)
Glycerol (mmol/l)	-0.004 ± 0.02 (-3)	-0.003 ± 0.02 (-2)	0.006 ± 0.02 (5)
Serum leptin (ng/ml)	0.5 ± 3.6 (5)	-1.1 ± 4.5 (-8)§	-0.7 ± 2.7 (-6)

Data are means $\pm$ SD (%). Values for M, $n = 18$. *$P < 0.01$ vs. placebo; †$P < 0.05$ within the group; ‡$P < 0.001$ vs. placebo; §$P < 0.01$ within the group; ‖$P < 0.05$ vs. placebo.

Correlations

The changes in M in the $t10c12$ CLA group correlated with the changes of VLDL TG ($r = -0.74$, $P = 0.0003$) but not with other variables. The decrease in HDL cholesterol in both CLA groups was correlated only to a change in leptin ($r = 31$, $P = 0.02$), which remained significant when including all subjects.

CONCLUSIONS — This randomized placebo-controlled trial has revealed unexpected metabolic actions by conjugated fatty acids in humans—actions that seem isomer-specific. The $t10c12$ CLA isomer, but not a CLA mixture, significantly increased insulin resistance, fasting glucose, and dyslipidemia in abdominally obese men. Such men are prone to develop type 2 diabetes and are considered a possible target group for the putative beneficial effect of CLA. Commercial CLA had no metabolic benefit, which is consistent with previous data (7,9,12). Instead, $t10c12$ CLA might be diabetogenic in the metabolic syndrome.

Previous human studies have used CLA mixtures, which do not provide separate information regarding $t10c12$ CLA, although in a study on healthy women, insulin levels tended to increase after treatment with a CLA mixture containing 23% $t10c12$ CLA (12), indicating impaired insulin action. However, unlike other studies, diet and physical activity were controlled using metabolic suites in that study, and the relative amount of $c11t13$ CLA was higher and $c9t11$ CLA was lower, which should be taken into account when comparing that study with our data.

The current results are dissimilar from the antidiabetic effects of CLA in male ZDF rats (4,5) but are in agreement with the effects in female C57BL/6J mice that became severely insulin resistant and lipodystrophic after receiving a CLA mixture containing 36% $t10c12$ CLA (6). Similarly, male AKR/J mice showed signs of insulin resistance after CLA treatment (24). Whether insulin resistance in CLA-fed mice shares a common mechanism with the $t10c12$ CLA–induced insulin resistance is unclear. Thus, the mechanism of current insulin resistance is unknown, but some animal data are worth considering. An intriguing speculation is that CLA induces adipocyte apoptosis as shown in mice (6) and in vitro (6,25,26), and by the $t10c12$ isomer in particular (26). $t10c12$ CLA might also inhibit the formation of new, small, and insulin-sensitive fat cells (27), possibly via downregulated peroxisome proliferator–activated receptor (PPAR)-γ (6). Other mechanisms for a $t10c12$ CLA–induced insulin resistance might involve impaired cell membrane function (28), possibly via increasing intramuscular fat content (29). Insulin resistance appeared to occur mainly in the peripheral tissues rather than in the liver, as serum IGF binding protein (IGFBP)-1, a marker of hepatic insulin sensitivity (30), did not change (unpublished data). To elucidate the mechanism, it is probably necessary to investigate insulin-sensitive tissue directly, which is more complicated in humans than in mice. Our primary goal, however, was to investigate the clinical effects of CLA in an insulin-resistant phenotype.

Neither CLA preparation decreased weight or changed body composition compared with placebo. SAD tended to decrease after CLA treatment, but the difference was not significant versus placebo. The standard deviations for changes in anthropometrics were large, and the measurement errors for SAD, BIA, and waist girth have to be considered when interpreting the data.

Adjustment for several variables did not affect the $t10c12$ CLA–induced insulin resistance, with one exception. Adjusting for VLDL TG abolished statistical significance, reflecting the well-known tight relation between these two variables (31). It is likely that impaired insulin action after $t10c12$ CLA treatment preceded dyslipidemia (31), but this remains to be proven.

HDL cholesterol decreased after treatment with both CLA forms, although the effect was more pronounced with $t10c12$ CLA. This was also observed in overweight, normolipidemic subjects using a CLA mixture (10), but not in normal-weight subjects (9,32). Because low HDL cholesterol is an independent cardiovascular risk factor (33), the current reduction of 0.04 mmol/l (-4%) with $t10c12$ CLA is of clinical concern, as a change of 0.026 mmol/l has been inversely related to coronary risk (33). Interestingly, the current changes in HDL cholesterol were not related to insulin resistance, but were positively related to leptin levels.

Table 4—Changes in dietary intake during the study period

Dietary intake	Placebo	CLA	*t10c12* CLA
n	15	15	13
Energy (kcal)			
Baseline	2,386 ± 832	2,018 ± 538	2,178 ± 593
8 weeks	2,405 ± 991	1,947 ± 296	1,905 ± 573
P	0.92	0.71	0.20
Fat (g)			
Baseline	70 ± 29	56 ± 17	61 ± 16
8 weeks	72 ± 31	59 ± 12	54 ± 15
P	0.82	0.74	0.36
Carbohydrates (g)			
Baseline	296 ± 127	246 ± 67	259 ± 64
8 weeks	299 ± 64	230 ± 37	232 ± 69
P	0.80	0.49	0.30
Protein (g)			
Baseline	137 ± 49	130 ± 12	143 ± 56
8 weeks	135 ± 63	120 ± 6	119 ± 53
P	0.97	0.60	0.24
P/S*			
Baseline	0.36	0.42	0.44
8 weeks	0.39	0.49	0.47
P	0.34	0.25	0.85

Data are means ± SD. *P* values represent paired *t* tests. Data are based on subjects with two completed food records (*n* = 42). There were no differences between groups (all *P* values for unpaired *t* tests >0.2; data not shown). *Amount of total polyunsaturated fats divided by saturated fats.

Further, the *t10c12* CLA–induced insulin resistance is impressive. Whereas the concurrent increase in fasting glucose was clinically irrelevant, the 19% reduction in insulin sensitivity was large enough to have clinical relevance (34), especially considering the prediabetic high-risk group studied. Estimated from dose-response clamp studies in obese and lean control subjects, the relative decrease in insulin sensitivity after *t10c12* CLA treatment was equivalent to having an excess body weight of ~15 kg at a current mean weight of ~100 kg (35), indicating a rather powerful effect of this isomer. The amounts of *t10c12* CLA in the diet (where the major isomer is *c9t11* CLA) are very low, but commercial CLA mixtures do contain ~20–45% *t10c12* CLA, indicating that a long-term use might be of concern. Considering that nonprescription "weight loss agents" such as CLA are used by a significant number of people (36), the present results should be of interest for health care professionals treating obese patients. However, more clinical trials are needed to make firm conclusions regarding the clinical safety of CLA isomers and mixtures in obese patients.

In summary, *t10c12* CLA increased insulin resistance and dyslipidemia in men with the metabolic syndrome, in agreement with data in CLA-fed mice (6,24) but unlike data in ZDF rats (4), suggesting important isomer-specific metabolic effects of CLA in humans.

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