# Dietary conjugated linoleic acid mixture affects the activity of intestinal acyl coenzyme A: cholesterol acyltransferase in hamsters

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The present study was designed to study the mechanisms by which dietary conjugated linoleic acids (CLA) decrease serum cholesterol. Hamsters were fed a semi-synthetic diet containing 1 g cholesterol/kg diet with or without supplementation with 20 g linoleic acid (LA) and 20 g CLA/kg diet. After 8 weeks, serum fasting total cholesterol (TC) and triacylglycerol (TG) were significantly lower in the LA-supplemented and CLA-supplemented groups compared with those of the control (CTL) hamsters. In contrast to LA, CLA significantly lowered hepatic cholesterol but it increased the level of adipose tissue cholesterol, suggesting that the hypocholesterolaemic mechanism of CLA is different from that of LA. CLA decreased the activity of intestinal acyl CoA:cholesterol acyltransferase (ACAT) whereas LA had no effect on this enzyme. Consequently, CLA supplementation increased the faecal excretion of total neutral sterols, but it had no or little effect on the faecal acidic sterols. If the ACAT is associated with cholesterol absorption, the part of mechanisms by which CLA decreases serum cholesterol may involve down-regulation of intestinal ACAT activity.

**Cholesterol: Conjugated linoleic acids: Linoleic acid: Triacylglycerol**

Conjugated linoleic acids (CLA) refers to a group of positional and geometric isomers of linoleic acid (LA). In contrast to those in LA, the double bonds in CLA are conjugated, instead of being in the typical methylene interrupted configuration. CLA is predominantly found in meat and dairy products (Ha *et al.* 1989; Chin *et al.* 1992). Low concentrations of CLA also occur in the lipids of human blood, tissue and milk (Cawood *et al.* 1983; Iversen *et al.* 1985). CLA can also be prepared chemically by biohydrogenation (Kepler & Tove, 1967; Hughes *et al.* 1982) and by alkali isomerization of LA (Nichols *et al.* 1951).

High plasma cholesterol has been ranked as one of the greatest risk factors in development of CHD (Neaton *et al.* 1984; Grundy, 1986). Supplementation with CLA could significantly lower serum total cholesterol (TC), LDL-cholesterol and triacylglycerol (TG) in rabbits (Lee *et al.* 1994) and hamsters (Nicolosi *et al.* 1997). Thus, the risk factors for atherosclerosis including the LDL-cholesterol: HDL-cholesterol ratio and the TC:HDL-cholesterol ratio were significantly reduced. However, the hypocholesterolaemic mechanism involved remains poorly understood. We have previously chosen Golden Syrian hamsters (*Mesocricetus auratus*) to study the potency of green tea catechins (Chan *et al.* 1999). Golden Syrian hamsters have been increasingly used as a model to estimate the efficacy of hypocholesterolaemic agents in human subjects (Sugiyama *et al.* 1995). Unlike rats, in which most of the serum cholesterol is in HDL, the major cholesterol carrier in hamsters is LDL as in man (Nistor *et al.* 1987; Lehmann *et al.* 1993). There are many similarities between hamsters and man in their intrinsically low rate of hepatic cholesterogenesis, in their response to different diet and drugs, and in the manner in which they handle biliary sterol secretion (Nistor *et al.* 1987). The present study was designed to test our hypothesis that dietary CLA decreases serum cholesterol, probably by its inhibition on cholesterol absorption, using hamsters as an animal model.

**Abbreviations:** ACAT, acyl CoA:cholesterol acyltransferase; CLA, conjugated linoleic acids; HMG, 3-hydroxy-3-methylglutaryl; LA, linoleic acid; TC, total cholesterol; TG, triacylglycerol.

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**Table 1.** Composition of experimental diets*

| Content | CTL (g/kg) | LA (g/kg) | CLA (g/kg) |
|---|---|---|---|
| Cornstarch | 488 | 478 | 478 |
| Casein | 200 | 200 | 200 |
| Lard | 100 | 100 | 100 |
| Sucrose | 150 | 140 | 140 |
| Mineral mix | 40 | 40 | 40 |
| Vitamin mix | 20 | 20 | 20 |
| DL-Methionine | 1 | 1 | 1 |
| Cholesterol | 1 | 1 | 1 |
| Linoleic acid | – | 20 | – |
| Conjugated linoleic acids | – | – | 20 |

CTL, control diet; LA, linoleic acid-supplemented diet; CLA, conjugated linoleic acids-supplemented diet.
* For details of preparation of the diets see p. 936.

## Materials and methods

### *Animals and diet*

The hypercholesterolaemic diet described by Sanders & Sandaradura (1992) was modified and used in the present study. The control diet was prepared by mixing casein, lard, cornstarch, sucrose, AIN-76 mineral mix, AIN-76A vitamin mix, DL-methionine and cholesterol as shown in Table 1. The ingredients were purchased from Harlan Teklad (Madison, WI, USA) except for lard, which was obtained from the local market, and DL-methionine and cholesterol, which were purchased from Sigma (St Louis, MO, USA). To achieve the high hypolipidaemic activity, CLA (purity 890 g/kg, Tonalin™, Natural Lipids Ltd, AS, Hovdebygda, Norway) and LA (Sigma) were added to the diet at a level of 20 g/kg diet. The diets (1 kg) were then mixed with 250 ml gelatin solution (40 g/l). Once the gelatin had set, the food was cut into cubed portions of about 20 g and stored frozen at −20°C.

Thirty-six male Syrian Golden hamsters (125–140 g; The Chinese University of Hong Kong, Shatin, Hong Kong) were randomly divided into three groups (*n* 12 per group) with approximately equal mean group bodyweights. The animals were housed (two hamsters per cage) in an animal room at 23°C with a 12 h light–dark cycle. The hamsters were fed one of the three diets for 8 weeks. Fresh diet was given to the animals daily, and uneaten food was discarded. Food intake was measured daily and body weight was recorded twice per week. The protocol was reviewed and approved by the Committee of Animal Ethics, The Chinese University of Hong Kong. The 4 d total faecal output was collected during feeding. All hamsters were killed at the end of experiment after food deprivation for 14 h. The blood was collected via the abdominal aorta. After clotting, the blood was centrifuged at 1300 ***g*** for 15 min, and serum was then collected. The liver, peri-renal adipose tissue, kidney, heart, brain and muscle (*Adductor longus*) were also retained.

At the same time, another thirty hamsters were divided randomly into three groups (*n* 10 per group) and were similarly maintained on one of the three diets for 8 weeks. The hamsters were then killed without overnight fasting (full stomach). The objective was to enhance the activity of intestinal acyl CoA:cholesterol acyltransferase (ACAT), which is believed to play an important role in esterification before absorption of dietary cholesterol. To be consistent, the first 10 cm of intestine from the stomach was discarded, and the next 30 cm was taken for the intestinal ACAT assay (Helgerud *et al.* 1981; Murakami *et al.* 1995). All the tissues were stored at −78°C.

### *Analysis of conjugated linoleic acids in the diet*

The pure CLA mixture (890 g/kg, 3 kg) was obtained as a gift from Natural Lipids Ltd, AS. The CLA from diet was converted to the corresponding fatty acid methyl esters with methanolic hydrogen chloride under $N_2$ at 90°C for 45 min. The CLA–fatty acid methyl esters were separated and quantified using capillary GLC method previously described by us (Chen *et al.* 1997) and $Ag^{2+}$ HPLC method described by Sehat *et al.* (1998). In the GLC method, the CLA–methyl esters were separated in a flexible silica capillary column (SP 2560, 100 m × 0·25 mm internal diameter; Supelco, Inc., Bellefonte, PA, USA) in a HP 5890 Series II GLC equipped with a flame-ionization detector (Hewlett-Packard, Palo Alto, CA, USA). In the $Ag^{2+}$ HPLC method, the analysis was performed on a Ag-impregnated column (4·6 mm internal diameter × 250 mm; Chrompack, Bridgewater, NJ, USA) with an Alltech Model 525 HPLC equipped with a ternary pump delivery system and a u.v. detector (Alltech, Deerfield, IL, USA). The fatty acid composition of the three diets is shown in Table 2.

### *Determination of blood cholesterol*

Enzymatic kits were purchased from Sigma to measure serum TG (catalogue number 336-20) and TC (catalogue number 352-20). HDL-cholesterol was isolated by precipitation of the apolipoprotein B-containing lipoproteins with sodium phosphotungstate–magnesium chloride using the commercial Sigma kit (catalogue number 352-4) as described by Lee *et al.* (1994) and Nicolosi *et al.* (1997).

**Table 2.** Fatty acid composition of diets*

| Fatty acids | CTL (g/kg) | LA (g/kg) | CLA (g/kg) |
|---|---|---|---|
| Myristic | 2·05 | 1·93 | 1·96 |
| Palmitic | 26·65 | 25·99 | 26·31 |
| Palmitoleic | 1·91 | 1·90 | 1·87 |
| Stearic | 15·77 | 15·93 | 16·14 |
| Oleic | 31·94 | 32·46 | 33·00 |
| Vaccenic | 1·86 | 1·85 | 1·87 |
| Linoleic | 9·82 | 29·37 | 10·01 |
| Arachidic | 0·23 | 0·24 | 0·25 |
| Linolenic | 0·46 | 0·48 | 0·47 |
| Others | 4·3 | 4·84 | 5·11 |
| Total CLA | – | – | 17·97 |
| (t10,t012)-CLA | – | – | 0·92 |
| (t11,t13)-CLA | – | – | 0·98 |
| (c9,t11/t9,c11)-CLA | – | – | 7·92 |
| (c10,t12/t10,c12)-CLA | – | – | 7·82 |
| (c9,c11)-CLA | – | – | 0·16 |
| (c10,c12)-CLA | – | – | 0·17 |

CTL, control diet; LA, linoleic acid-supplemented diet; CLA, conjugated linoleic acids-supplemented diet; t, *trans*; c, *cis*.
* For details of analytic procedures see p. 936.

### *Determination of tissue cholesterol*

TC was determined as previously described by Chan *et al.* (1999). In brief, the total lipids were extracted, and the lipid extracts were then saponified. The non-saponified substances including cholesterol were converted to their trimethylsilyl-ether derivatives and subjected to the GLC analysis in a fused silica capillary column (SAC™-5, 30 m × 0·25 mm, internal diameter; Supelco, Inc.) using a Shimadzu GC-14 B GLC equipped with a flame ionization detector (Shimadzu, Tokyo, Japan).

### *Determination of faecal neutral and acidic sterols*

Faecal neutral and acidic sterols were determined as previously described by Chan *et al.* (1999). In brief, the faecal samples were saponified. The total neutral sterols were extracted using cyclohexane and were then converted to their corresponding trimethylsilyl-ether derivatives for GLC analysis. The remaining aqueous layer was treated with 10 M-NaOH and 3 M-HCl followed by twice-repeated extraction with diethyl ether. The acidic sterols were similarly converted to their trimethylsilyl-ether derivatives and subjected to GLC analysis.

### *Assays of 3-hydroxy-3-methylglutaryl-CoA reductase*

Liver microsomes were isolated according to Erickson *et al.* (1977). The activity of liver 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase was measured as previously described by Shapiro *et al.* (1969) and modified by Heller & Strewsbury (1976).

### *Acyl CoA:cholesterol acyltransferase assay*

The mucosa microsome was prepared according to the method previously described by Murakami *et al.* (1995). The ACAT activity was determined essentially using the method developed by Helgerud *et al.* (1981) with some modifications. Each assay contained 200 μg microsomal protein and 200 μg fatty acid-free bovine serum albumin in 190 μl potassium phosphate buffer (0·1 M, pH 7·4) containing 1 mM-EDTA. The mixture was incubated at 37°C for 6 min and the reaction was then initiated by adding 5 nmol [1-$^{14}$C]oleoyl-CoA (NEN™, MA, USA) followed by incubation at 37°C for 6 min. The reaction was stopped by adding 5 ml chloroform–methanol (2:1, v/v) and 1 ml 0·04 M-HCl. To the mixture, 10 μl [$^{3}$H]cholesteryl oleate was then added as an internal standard. After the mixture was vortexed and centrifuged, the organic layer was taken and dried under $N_2$ gas. The sample was then dissolved in 50 μl chloroform containing 0·1 mg cholesteryl oleate and spotted on a TLC plate (20 × 20 cm plate precoated with 250 μm silica gel 60A; Macherey-Nagel, Duren, Germany). The different lipid classes were separated in hexane–diethyl ether–acetic acid (80:20:1, by vol.) and then exposed to I vapour. The band containing cholesteryl oleate was scraped off into scintillation vials, and the radioactivity was counted.

**Table 3.** Changes in food intake, body-weight gain, and individual organ weights*

(Mean values and standard deviations for twelve hamsters per group)

| | CTL | | LA | | CLA | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Initial body weight (g) | 120·8 | 7·0 | 121·3 | 8·0 | 120·8 | 7·6 |
| Final body weight (g) | 166·7 | 10·5 | 167·5 | 16·7 | 161·3 | 12·3 |
| Food intake (g/d) | 14·0 | 2·4 | 14·0 | 2·4 | 13·9 | 2·6 |
| Organs (g) | | | | | | |
| Liver | 7·57 | 0·73 | 7·74 | 0·60 | 7·77 | 1·14 |
| Peri-renal adipose tissue | 2·19 | 0·82 | 2·23 | 0·69 | 1·83 | 0·39 |
| Kidney | 0·67 | 0·04 | 0·71 | 0·09 | 0·65 | 0·04 |
| Heart | 0·54 | 0·03 | 0·55 | 0·04 | 0·53 | 0·04 |
| Brain | 0·97 | 0·11 | 0·95 | 0·11 | 0·96 | 0·08 |

CTL, control diet; LA, linoleic acid-supplemented diet; CLA, conjugated linoleic acids-supplemented diet.
* For details of diets see Table 1, and for procedures see p. 936.

### *Statistics*

Data are expressed as mean values and standard deviations. ANOVA was used where applicable for statistical evaluation of significant differences among the control, the LA- and CLA-supplemented groups using Sigmastat (Jandel Scientific Software, San Rafael, CA, USA). Differences were considered significant when $P < 0·05$.

## Results

### *Body-weight gain, organ weight and food intake*

There were no significant differences in food intake and body-weight gain among the three groups throughout the study period (Table 3). No differences in organ weights among the three groups were observed.

### *Effect of conjugated linoleic acids supplementation on serum total cholesterol, HDL-cholesterol and triacylglycerol*

CLA and LA supplementation significantly reduced the fasting serum TC by 10 % and 12 % respectively, when compared with the control ($P < 0·01$, Table 4). Similarly,

**Table 4.** Effect of dietary conjugated linoleic acids as a mixture and linoleic acid on serum, liver and adipose tissue lipids*

(Mean values and standard deviations for twelve hamsters per group)

| | CTL | | LA | | CLA | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Serum total cholesterol (g/l) | 2·08$^{a}$ | 0·11 | 1·84$^{b}$ | 0·14 | 1·89$^{b}$ | 0·14 |
| Serum HDL-cholesterol (g/l) | 1·06 | 0·16 | 0·99 | 0·07 | 1·06 | 0·08 |
| Serum triacylglycerol (g/l) | 3·29$^{a}$ | 0·32 | 2·61$^{b}$ | 0·53 | 2·22$^{b}$ | 0·55 |
| Cholesterol | | | | | | |
| Liver (mg/g) | 20$^{a}$ | 3 | 22$^{a}$ | 5 | 8$^{b}$ | 2 |
| Perirenal adipose tissue (mg/g) | 1·1$^{b}$ | 0·1 | 1·1$^{b}$ | 0·2 | 1·6$^{a}$ | 0·2 |
| *Adductor longus* (mg/g) | 0·38$^{a}$ | 0·02 | 0·34$^{b}$ | 0·03 | 0·35$^{b}$ | 0·02 |

CTL, control diet; CA, linoleic acid-supplemented diet; CLA, conjugated linoleic acids-supplemented diet.
$^{a,b}$Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets see Table 1, and for procedures see p. 936.

fasting serum TG was markedly decreased by 34 % and 21 % respectively, in hamsters fed 20 g CLA and 20 g LA/kg diet in comparison with that in the control group ($P < 0{\cdot}01$, Table 4). However, no difference was observed in serum HDL-cholesterol among the three groups (Table 4).

### *Effect of conjugated linoleic acids supplementation on hepatic cholesterol and triacylglycerol*

As compared with that of the control and LA-supplemented group, the hepatic cholesterol level was significantly lower in CLA-supplemented hamsters (Table 4). However, all three groups had the similar level of hepatic TG.

### *Effect of conjugated linoleic acids supplementation on levels of cholesterol in other tissues*

Unlike that of liver, the adipose tissue cholesterol level in the CLA-supplemented group was markedly increased by 45 % as compared with the control group ($P < 0{\cdot}01$, Table 4). Significantly lower muscle cholesterol levels in the CLA- and LA-supplemented groups were also observed as compared with that of the control (Table 4). However, no differences in the cholesterol level of brain, heart and kidney were observed (data not shown).

### *Effect of conjugated linoleic acids supplementation on hepatic 3-hydroxy-3-methylglutaryl-CoA reductase and intestinal acyl CoA:cholesterol acyltransferase*

No differences in hepatic HMG-CoA reductase were observed among the three groups. The HMG-CoA reductase activity for the control, LA and CLA groups was 20·3, 20·4 and 20·7 pmol/min per mg protein respectively. However, the intestinal ACAT activity of CLA-supplemented group was reduced by 58 % ($P < 0{\cdot}05$) as compared with that of control and LA-supplemented hamsters (Fig. 1). There was no difference in the intestinal ACAT activity between the control and LA-supplemented groups.

### *Effect of conjugated linoleic acids supplementation on faecal neutral and acidic sterols*

The neutral sterols refer to the sum of cholesterol, coprosterol, coprostanone, dihydrocholesterol, campesterol, β-sisterol and stigmastenol. The total faecal neutral sterols were significantly elevated in the CLA-supplemented groups as compared with the control and LA-supplemented groups (Fig. 2). The acidic sterols measured include lithocholic, deoxycholic, cholic acid and ursodeoxycholic acid (Chan *et al.* 1999). CLA supplementation did not affect the faecal excretion of acidic sterols as compared with the control and LA-supplemented groups throughout the study period except at day 36 and 44 (Fig. 3).

## Discussion

The present study confirmed that dietary CLA as a mixture possessed a favourable effect on serum lipids by significantly reducing fasting serum TC and TG with no effect on

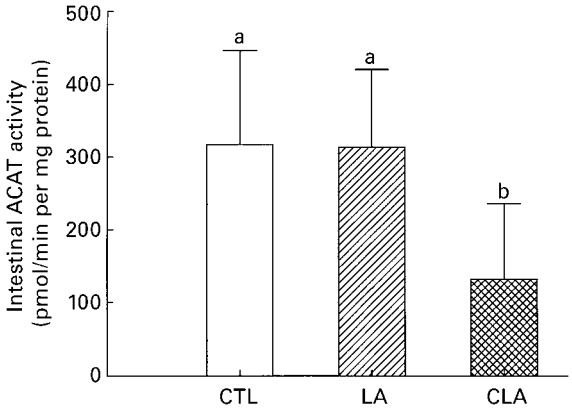


**Fig. 1.** Effects of conjugated linoleic acids (CLA) and linoleic acid (LA) on intestinal acyl CoA:cholesterol acyltransferase (ACAT) activity in hamsters. CTL, control. □, control; ▨, linoleic acid-supplemented; ▩ conjugated linoleic acids-supplemented group. For details of the diets see Table 1, and for procedures see p. 936. Values are means for ten hamsters per group with standard deviations represented by vertical bars. [a,b]Mean values with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

HDL-cholesterol. Thus, the TC:HDL-cholesterol ratio was significantly decreased. The result is in agreement with that of other studies using rabbits (Lee *et al.* 1994), mice (Munday *et al.* 1999) and hamsters (Nicolosi *et al*. 1997) as animal models. However, the present result is in disagreement with that reported by de Deckere *et al.* (1999), who found that CLA mixture decreased TC at week 4 but it had no significant effect on TC at week 8 in hamsters fed a diet containing 0·1 g cholesterol/kg.

The mechanisms by which dietary CLA lowered serum TC remains poorly understood. It is known that a diet higher in LA is associated with lower serum TC compared with a diet higher in saturated fatty acids. It is possible that CLA acts partially like LA. First, CLA, like LA, may occupy more space within lipoprotein particles, resulting in fewer cholesterol ester molecules residing in the core of LDL particles (Spritz & Mishkel, 1969), provided that CLA isomers significantly accumulate in LDL particle. Second, CLA, like LA, may reduce number of LDL particles by inhibition of hepatic synthesis of apolipoprotein B-containing lipoproteins (Vegas *et al.* 1982). In fact, it was recently found that *trans*-10, *cis*-12-CLA isomer could significantly reduce *in vitro* apolipoprotein B secretion in HepG2 cells (Yotsumoto *et al.* 1999). Third, dietary CLA like LA may increase the LDL receptor activity and thus enhance the fractional clearance rate of LDL in circulation (Grundy & Denke, 1990).

It was also noticed that the mechanism for the cholesterol-lowering effect of dietary CLA, on the other hand, might be different from that of dietary LA. First, dietary CLA decreased not only serum TC, but also liver cholesterol (Table 4) in comparison with LA, which had no effect on hepatic cholesterol level. Second, dietary CLA led to a higher level of adipose tissue cholesterol whereas dietary LA did not influence the level of adipose tissue cholesterol as compared with the control hamsters. We have no explanation for the cholesterol-raising effect of

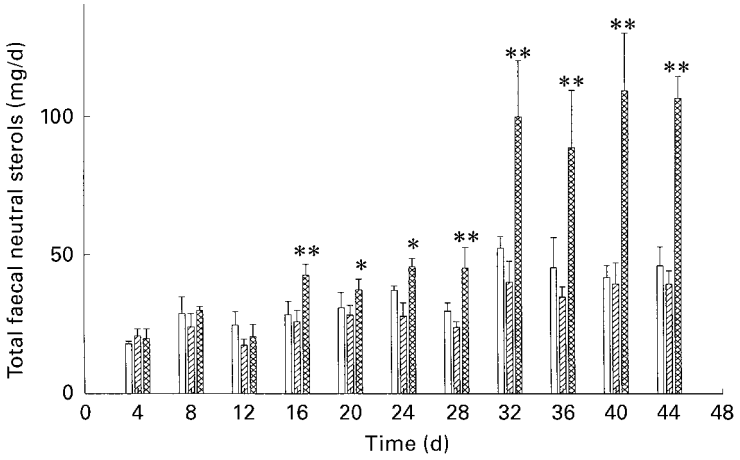


**Fig. 2.** Effects of conjugated linoleic acids (CLA) and linoleic acid (LA) on faecal output of total neutral sterols in hamsters. For details of the diets see Table 1, and for procedures see p. 936. Values are means for 10–12 hamsters per group with standard deviations represented by vertical bars. □, control; ▨, linoleic acid-supplemented, ▩, conjugated linoleic acids-supplemented group. Mean values were significantly different from those of the control group: $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$.

CLA in adipose tissue at the present time. However, CLA supplementation appeared to reduce the adipose tissue fat pads although it was not significant when compared with the control and LA-supplemented groups (Table 3). Similar observations were also made in the studies of Belury & Kempa-Steczko (1997), DeLany *et al.* (1999) and Park *et al.* (1999). It is possible that CLA may reduce the fat deposition and increase lipolysis in adipocytes coupled with enhanced fatty acid oxidation in both muscle cells and adipocytes as described previously in mice by Park *et al.* (1997). Therefore, the level of cholesterol per unit mass increased as the total adipose tissue weight and TG was decreased. The present results clearly suggest that CLA may cause 'redistribution' of cholesterol between serum and tissues with a lower level of hepatic cholesterol but a higher level of adipose tissue cholesterol.

One of the other possible mechanisms by which dietary CLA decreased serum cholesterol was explored in the present study. Dietary CLA was associated with increased faecal excretion of neutral sterols. Some of neutral sterols are the products of bacterial modification of cholesterol in large intestine (Kellog, 1974). To the best of our knowledge, the effect of dietary CLA on the faecal excretion of sterols has not previously been reported. Whereas ACAT functions mainly to esterify cholesterol and store it as cholesteryl ester, it may be involved in the intestinal absorption of cholesterol (Heider *et al.* 1983; Largis *et al.* 1989). There is evidence that the majority of dietary

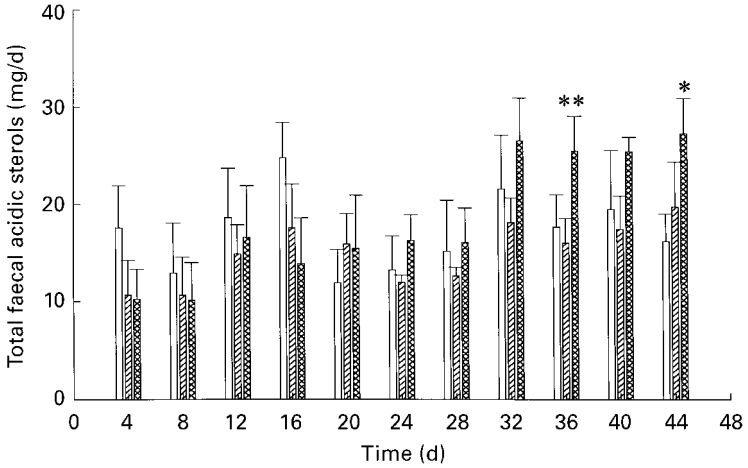


**Fig. 3.** Effects of conjugated linoleic acids (CLA) and linoleic acid (LA) on faecal output of total acidic sterols in hamsters. For details of the diets see Table 1, and for procedures see p. 936. Values are means for 10–12 hamsters per group, with standard deviations represented by vertical bars. □, control, ▨, linoleic acid-supplemented, ▩, conjugated linoleic acids-supplemented group. Mean values were significantly different from those of the control group: $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$.

cholesterol is esterified before it is assembled in the chylomicron and secreted into the lymphatic system (Wrenn *et al.* 1995). We hypothesize that dietary CLA may interfere with the absorption of cholesterol by inhibiting the ACAT activity. In fact, the present study clearly showed that the intestinal ACAT was down-regulated by CLA supplementation. This was in agreement with the observation that dietary CLA increased only the faecal excretion of neutral sterols, but it had no or little effect on faecal acidic sterols (Figs 2 and 3). It is known that a high-cholesterol and -lard diet would produce an increase in both liver cholesterol and TG of hamsters (Sessions & Salter, 1994). Thus, if CLA inhibits cholesterol absorption, CLA supplementation will lead to a decrease in both serum TC and TG, and hepatic cholesterol. In addition, dietary supplementation exhibited neutral effect on HMG-CoA reductase. The reduction in serum cholesterol by dietary supplementation is therefore not associated with the inhibition of hepatic HMG-CoA reductase activity, but is most likely to be mediated, at least in part, by its inhibitory effect on cholesterol absorption via the down-regulation of intestinal ACAT activity.

It remains unclear whether dietary CLA reduces serum lipids in man. To the best of our knowledge, there has been no study to date that examines the hypolipidaemic activity of dietary CLA in human subjects. There were, however, only four animal studies to date which examined the hypolipidaemic potency of CLA. When rabbits were given a high-cholesterol diet and 0·5 g CLA/d, both serum TC and TG concentrations were significantly lower than the control group (Lee *et al.* 1994). Examination of the aortas of CLA-fed rabbits showed less atherosclerosis. When hamsters were used as an animal model and fed a high-cholesterol diet with three levels of CLA (0·06, 0·11 and 1·1 % energy), they had significantly reduced levels of plasma TC, non-HDL-cholesterol and TG as compared with the control hamsters (Nicolosi *et al.* 1997). Morphometric analysis of aortas revealed less early atherosclerosis in the CLA-fed hamsters compared with the control. In contrast, another study in hamsters fed a high-cholesterol diet containing 6 g CLA/kg diet showed that CLA decreased total cholesterol at week 4 but it had no effect at week 8 (de Deckere *et al.* 1999). When mice were fed a high-cholesterol diet supplemented with 5 g CLA/kg diet, they developed a significantly higher serum HDL-cholesterol:TC ratio and a significantly lower serum TG concentration than the control (Munday *et al.* 1999). However, the CLA-fed mice showed an increased development of aortic fatty streak. The present study is the first to explore the mechanism by which dietary CLA favourably alters serum lipoprotein profile. The results demonstrated that CLA decreased intestinal ACAT activity and thereby increased the neutral sterol excretion in hamsters. Although most animal experiments have demonstrated the beneficial effect of dietary CLA, it is premature to extrapolate the data to man because the doses of CLA used in the animal studies, including the present study, would be difficult to achieve by human subjects. It should be pointed out that meat and dairy products contain a certain amount of CLA but they are also rich in saturated fatty acids.

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