Non-fat milk solids attenuate hypercholesterolemic effect of conjugated linoleic acid

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Abstract The present study instigates the notion that non-fat milk has a beneficial effect on hypercholesterolemia caused by dietary conjugated linoleic acid (CLA). Two groups of mice (6 per group) were fed synthetic CLA (predominantly a mixture of t10, c12 and c9, t11 isomers) at 1 % level in the diet with or without skim milk powder (SMP). Another group (control) was fed a similar diet without CLA and SMP. The study was conducted for 60 days (d). Daily feed intake and weekly body weight were recorded. Plasma lipid profile was determined monthly. At the conclusion of experiment, animals were sacrificed and liver tissue was removed to assay cholesterol and triacylglycerols (TAG). Following 60 day feeding, the plasma total cholesterol content increased significantly in CLA group (218 mg/dl) than in control group (167 mg/dl), while in CLA + SMP group (fed non-fat milk solids along with CLA), it was similar to that in control group. The fractions of high density lipoproteins (HDL) and low density lipoprotein + very low density lipoprotein (LDL + VLDL) cholesterol as well as triacylglycerols (TAG) in plasma increased in CLA group, but in CLA + SMP group it remained similar to the control levels. Thus, it is concluded that dietary CLA increases plasma cholesterol and TAG contents in mice on normocholesterolemic diet and the inclusion of non-fat milk solids reverses this effect.

Keywords CLA · Skim milk powder · Normocholesterolemic diet · Plasma lipid profile · Hypercholesterolemia

Introduction

The discovery of conjugated linoleic acid (CLA) caused a scientific sensation years back. Still the hype of CLA as a

good fatty acid is sustained. Conjugated linoleic acid is a trans fatty acid, though researchers claim that it is not harmful in the same fashion as other trans fatty acids, but rather is beneficial. Conjugated linoleic acid is a naturally occurring group of dienoic derivatives of linoleic acid (18:2) formed by bacterial action in the forestomach of ruminants (Chin et al. 1994). Non-ruminants, such as humans, may be able to produce some isomers of CLA from vaccenic acid by delta-9-desaturase (Banni et al. 2001). Conjugated linoleic acid in diet comes predominantly from food derived from ruminant animals, especially dairy products. There are multiple potential isomers of CLA, but the cis-9, trans-11 and trans-10, cis-12 isomers are active as potential antioxidant, anticarcinogenic, immune modulating and antiobesity agents (Park et al. 1997, 1999; West et al. 1998; Rahman et al. 2001a, b). Keeping all these reports, substantial interest has been developed in the biochemical action of CLA and its potential application to foods, feeds and pharmaceuticals.

A couple of animal studies suggested that CLA supplementation decreased the development of early atherosclerotic lesions (Lee et al. 1994; Nicolosi et al. 1997). However, there was considerable variability within and among experimental model systems. Some studies reported a reduction in plasma cholesterol and triacylglycerols in rabbits and hamsters (Lee et al. 1994; Nicolosi et al. 1997) on hypercholesterolaemic dietary regimen. Another study in rabbits reported an increase in cholesterol and triacylglycerols contents in plasma (Kritchevsky et al. 2000). In a subsequent study in rabbits by same group of workers (Kritchevsky et al. 2002), CLA did not significantly change blood lipid profile. Further, a study (Wilson et al. 2000) in hamster showed an increase in triacylglycerols but decrease in cholesterol contents in plasma. One study on mice reported either no or even promotional role of CLA in atherosclerotic lesion development (Munday et al. 1999). The effect of CLA appears to be modified by composition of rest of the diet.

Milk as well as non-fat milk solids have been previously reported to reduce serum cholesterol and triacylglycerols

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(Oda and Hashiba 1994; Beena and Prasad 1997; Aggarwal and Kansal 1991; Buonopane et al. 1992; Yadav et al. 2006; Cichosz 2007). Thus, the present study investigated the synergistic effect of CLA and non-fat milk solids on blood lipid profile under normocholesterolaemic dietary regimen.

Materials and methods

Animals, diet and experimental design Nu-Chek-Prep, Inc. (Elysian, MN, USA) was the source of conjugated linoleic acid (CLA) with the reported isomer contents: 39.1 % cis 9, trans 11 and trans 9, cis 11; 40.7 % trans 10, cis 12; 1.8 % cis 9, cis 11; 1.3 % cis 10, cis 12; 1.9 % trans 9, trans 11 and trans 10, trans 12; 1.1 % cis 9, cis 12 CLA and 14.1 % remainder. Autopak kits for cholesterol and TAG assays were procured from Bayer Diagnostics India Ltd., Baroda.

Male Swiss albino mice (5 weeks old), obtained from Small Animal House of the Institute, were maintained individually in polypropylene cages on basal diet for 1 week. Those were, then, randomly divided into three groups each consisted of 6 animals and were allowed respective diet and water ad libitum for 2 months. The control group received basal diet containing no CLA. The CLA group was fed diet containing CLA (1 %) substituting for equal amount of soybean oil in the basal diet. The third group (CLA + SMP) was fed diet containing skim milk powder along with CLA (1 %). The three diets were isocaloric, with calories derived 18.3 % from protein, 68.5 % from carbohydrate and 13.2 % from fat. The composition of diets (Table 1) was in accordance to AOAC (1975).

Daily food intake and weekly body weight were recorded for 60 days. Blood lipid profile was determined at 30 day intervals. The blood sample was drawn from orbital venous

plexus, after fasting the animal for 12 h, using a heparinized capillary, into microcentrifuge tube rinsed with heparin solution, and spun at $1,100 \times g$ for 10 min at 4 °C. The plasma was analyzed for total cholesterol, HDL-cholesterol and TAG using Autopak kits based on enzymatic oxidation of these molecules. At the end of the experimental period, the animals were sacrificed by cervical dislocation, and liver was removed for cholesterol and TAG estimation.

Statistical analysis All results are expressed as mean $\pm$ SE. Analysis of covariance (ANCOVA) (MedCalc statistical software version 10.4.0) was used to test for differences in body weight change. The initial body weight of the animals was used as the covariate to control for pre-existing differences. Homogeneity of regression assumptions were tested and met in the analysis. The mean values, adjusted for pretreatment difference, for the three experimental groups were further compared using the Bonferroni procedure. All other comparisons were made with the SAS general linear model procedure (SAS institute, Cary, NC). The differences were inspected again by Duncan's multiple range test. The critical level of statistical significance for all tests was $P < 0.05$.

Results & discussion

The body weight gain (g) in CLA (10.5 ± 1.26) and CLA + SMP (12.2 ± 1.69) fed mice was similar to those fed control (11.8 ± 1.54) diet despite significantly higher feed consumption (g/d) in the former groups (4.8 ± 0.38 and 4.7 ± 0.35 respectively) as compared to the latter (4.5 ± 0.18). This suggests reduced feed conversion efficiency caused by CLA, as opposed to the previous studies in rats (Chin et al. 1994) and pigs (Dugan et al. 1997; Ostrowska et al. 1999). Our results, however, are consistent with other studies with mice (Degraze et al. 2004), hamsters (Gavino et al. 2000) and broiler chickens (Badinga et al. 2003). The results vary, presumably depending upon the experimental conditions and the animal species used.

Following 30 day feeding, plasma total cholesterol concentration in CLA group was increased by 37 mg/dl, opposed to only 2 mg/dl rise observed in control group (Fig. 1). At 60 day, plasma total cholesterol in CLA group rose to 218 mg/dl, which was significantly higher than that (167 mg/dl) in control group. The CLA + SMP fed mice registered no significant rise in plasma total cholesterol content over the control diet fed counterparts. The increase in total plasma cholesterol content in CLA group was due to rise in both HDL- and VLDL + LDL-cholesterol, while in control and CLA + SMP groups the rise was due largely to HDL-cholesterol (Fig. 1). Previous studies (Lee et al. 1994; Nicolosi et al. 1997) showing cholesterol reducing effect of CLA in rabbits and hamster

Table 1 Composition of diets (g/kg)

Ingredients	Control	CLA ^a	CLA + SMP ^b
Skim milk powder	–	–	450
Starch	410	410	380
Casein	190	190	–
Lactose	230	230	–
Soybean oil	55	45	45
CLA	–	10	10
BHA ^c	0.01	–	–

In addition, each diet contained (g/kg) Cellulose (50), Mineral mixture (50), Vitamin mixture (10), DL-Methionine (3) and Choline chloride (2)

^a Conjugated linoleic acid

^b Skim milk powder

^c Butylated hydroxyanisole was added to CLA (100 mg/100 ml) as an antioxidant; Mineral and vitamin mixture contents confirmed to minimum requirements according to AOAC (1975)

employed hypercholesterolaemic basal diet, while the present study used normocholesterolaemic basal diet to compare the effect. Likewise, Sher et al. (2003) reported that CLA exerts cholesterol lowering effect in hamsters on cholesterol containing hypercholesterolaemic diet but not on normal diet.

The concentrations of triacylglycerols (TAG) in plasma at 60 day significantly increased in all the three groups, and the rise in CLA group was 115 % over its 0 day level. Also, the content of plasma TAG at 60 day in CLA group was significantly greater than in control or CLA + SMP groups (Fig. 1). This suggests that the inclusion of SMP in CLA diet reversed the rise in plasma cholesterol and TAG concentrations caused by CLA. The cholesterol lowering effect of milk and non-fat milk solids has been reported earlier also in rats (Oda and Hashiba 1994; Beena and Prasad 1997; Yadav et al. 2006) and rabbits (Aggarwal and Kansal 1991). Therefore, the study implies that the effect of CLA on serum cholesterol is modified by other dietary ingredients, and milk is a better medium for delivery of CLA.

The atherogenic index, a ratio of VLDL + LDL-cholesterol to HDL-cholesterol, decreased transiently in CLA group at 30 day owing to rise in HDL-cholesterol; however, there was no significant difference among three dietary groups (Fig. 1).

Deposition of fat in liver was increased by 47.2 % and 42.5 % on CLA and CLA + SMP diets, respectively, when compared with control diet fed mice. The deposition of cholesterol in liver was significantly greater in CLA (0.8 mg/g tissue $\pm$ 0.10) and CLA + SMP (0.9 mg/g tissue $\pm$ 0.10) groups than in control (0.5 mg/g tissue $\pm$ 0.06) group, the difference between CLA and CLA + SMP groups was statistically not significant. TAG content was also greater on CLA (2.4 mg/g tissue $\pm$ 0.26) and CLA + SMP (2.1 mg/g tissue $\pm$ 0.18) diets than on control (1.4 mg/g tissue $\pm$ 0.21) diet. The proportion of cholesterol and TAG in liver fat was, however, similar in three dietary groups. The increase in liver cholesterol and TAG content, thus, could be due to an increased fat accumulation in liver of the two groups which may in turn be the result of decreased β -oxidation in CLA and CLA + SMP groups as compared to the control group (data published) (Sagwal and Kansal 2010). Takahashi et al. (2003) also reported similar observation with a 10-fold increase in the liver TAG content in mice fed linoleic acid. Wilson et al. (2006) observed that t10, c12 isomer of CLA is effective in reducing blood cholesterol levels but not aortic cholesterol accumulation in hamsters. Our study showed that non-fat milk is effective in averting the CLA induced increase in blood cholesterol, but the fat accumulation in liver could not be prevented. One of the mechanisms behind an increase in liver lipids may involve the reduction of leptin

Fig. 1 Effect of dietary CLA/CLA + SMP on blood lipid profile in mice. Values (mean $\pm$ SE for $n=6$) at given day with different letters are significantly different ($P<0.05$); *Significantly higher from the corresponding value at 0 day ($P<0.05$); CLA, Conjugated linoleic acid; SMP, Skim milk powder; HDL, High density lipoprotein; VLDL, very low density lipoprotein; LDL, Low density lipoprotein; TAG, Triacylglycerols

and adiponectin mRNA by t10, c12-CLA (Warren et al. 2003). Also, the accumulation of fat in liver may be due to the hyperinsulinemia caused by CLA (Clément et al. 2002) which SMP could not prevent as milk proteins are also insulinotropic (Nilsson et al. 2004). Insulin activates fatty acid uptake in the liver possibly causing high fat deposition in liver of CLA and CLA + SMP groups.

Conclusions

Various ingredients of a particular diet influence the effect of CLA on blood lipid profile of an individual. The present study reports hypercholesterolaemic effect of CLA on normal calorie diet, which is attenuated by non-fat milk solids. Thus, milk system is suggested to be a better medium for delivery of CLA so as to counteract the side effects. Further studies on intervention of milk components and other nutrients are needed to understand the mechanism of action of CLA in biological systems.

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