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Modification of skin composition by conjugated linoleic acid alone or with combination of other fatty acids in mice

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The effects of conjugated linoleic acid (CLA), γ-linolenic acid (GLA), linoleic acid (LA), and their combinations, on skin composition in mice were investigated. Mice (8 weeks old) were orally administered with either LA, GLA, CLA, LA + GLA, LA + CLA, or CLA + GLA for 4 weeks. Then, the skin was analysed for triacylglycerol content, fatty acid composition and collagen content. Additionally, thicknesses of the dermis layer and subcutaneous tissue layer, and the size and number of adipocytes were measured histologically. The skin fatty acid composition was modified depending upon the fatty acid composition of supplemented oils. In each oil-alone group, skin triacylglycerol content was the highest in LA, followed by GLA and CLA treatments. Combinations with CLA had a similar triacylglycerol content compared with the CLA-alone group. No significant changes in collagen content were observed among any treatments. The effects on subcutaneous thickness were similar to the results obtained in the triacylglycerol contents, where groups supplemented with CLA alone or other fatty acids had significantly thinner subcutaneous tissue compared with the LA-alone group. However, no significant difference was detected in the thickness of the dermis layers. The number of adipocytes was highest in the LA + GLA group and tended to be reduced by CLA with or without the other fatty acids. These results suggest that CLA alone or in combination with other fatty acids strongly modifies skin composition in mice.

Conjugated linoleic acid: γ-Linolenic acid: Subcutaneous tissue

The skin has an important role in protecting the animal from the external influences including bacteria, UV radiation, etc. (Boelsma *et al.* 2001). The skin is divided into three layers including the epidermis, dermis, and subcutaneous tissue. The dermis includes collagen and elastin, and is associated with elasticity and flexibility of the skin. The subcutaneous tissue (hypodermis) accumulates fat as adipocytes. The subcutaneous blood flow is related to the thickness of the subcutaneous tissue layer or total body fat mass (Girolamo *et al.* 1971; Nielsen & Larsen, 1973). Obese subjects accumulate subcutaneous adipose tissues and have a higher subcutaneous blood pressure (Landin *et al.* 1989; Jansson *et al.* 1992), and have a greater risk for CVD (Lapidus *et al.* 1984; Larsson *et al.* 1984).

Skin function and attractiveness are greatly altered by nutritional conditions. For instance, ingested fatty acids influence skin composition (Boelsma *et al.* 2001). Long-chain *n*-6 and *n*-3 PUFA are considered essential fatty acids. These PUFA are metabolised by elongases and desaturases. In *n*-6 PUFA, linoleic acid (LA) is metabolised to γ-linolenic acid (GLA), dihomo-γ-linolenic acid (DGLA), and to prostaglandin (PG) E1 or arachidonic acid (AA). Eicosanoids derived from AA, such as PGE_2 and leucotriene B4, are involved in inflammation and can alter the allergic response in the skin (Horrobin, 2000; James *et al.* 2000). The importance of LA is confirmed by deficiency, since ingestion of a fat-free diet induced cornification of skin lesions in rats (Sinclair, 1990). This suggests that LA is an important factor in maintaining healthy skin. On the other hand, although LA is rich in the skin (Oikawa *et al.* 2003), excessive LA causes inflammation and allergic responses in various organs including the skin (Gleich & Kita, 1997; Fogh & Kragballe, 2000).

Evening primrose oil, blackcurrant-seed oil and borage oil are rich in GLA. Supplementation of these plant oils has been reported to increase GLA and DGLA levels in the epidermis and erythrocyte membrane because GLA is metabolised to DGLA by elongase (Brosche & Platt, 2000; Chung *et al.* 2002). These compositional changes alter skin barrier function and decrease transepidermal water loss in elderly individuals (Brosche & Platt, 2000). Additionally, GLA has anti-inflammatory and immunomodulating effects (Wu *et al.* 1999; Chung *et al.* 2002). PGE_1 or 15-hydroxyeicosatrienoic acid is produced from DGLA by cyclo-oxygenase or 15-lipoxygenase. PGE_1 and 15-hydroxyeicosatrienoic acid inhibit leucotrienes, which act as pro-inflammatory mediators (Ziboh *et al.* 2000).

Conjugated linoleic acid (CLA) is the generic term representing positional and geometrical isomers of LA, and is included mainly

Abbreviations: AA, arachidonic acid; CLA, conjugated linoleic acid; DGLA, dihomo-γ-linolenic acid; GLA, γ-linolenic acid; LA, linoleic acid; PG, prostaglandin.

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in food items produced from ruminant animals. CLA was reported to decrease epididymal white adipose tissue weight, mesenteric adipose depots, and adipocytes in the subcutaneous tissue (Nakanishi *et al.* 2001; Oikawa *et al.* 2003). CLA also blocks the metabolism from LA to GLA in *n*-6 PUFA (Bretillon *et al.* 1999; Tsuboyama-Kasaoka *et al.* 2000). As a result, AA and PGE_2 levels are reduced in some tissues (Belury, 2002). Furthermore, CLA inhibits the growth of cancer cells and induces apoptosis in the liver and adipose tissue (Belury, 2002). In addition, ingested CLA easily transfers to the skin (Oikawa *et al.* 2003), and was reported to affect allergic pruritus in mice (Ishiguro *et al.* 2002). CLA seems to be beneficial for skin composition. However, CLA increases the weight of the liver in mice (Tsuboyama-Kasaoka *et al.* 2000; Nakanishi *et al.* 2004). On the other hand, Nakanishi *et al.* (2004) reported that the combination of CLA and GLA prevented fatty liver induced by CLA. This combination, as well as CLA alone, had an anti-obesity effect on visceral fat (Nakanishi *et al.* 2004). So far, however, no available information on the mixture of CLA and GLA for skin composition has been reported. The purpose of the present study was to investigate the effect of the combination of CLA and LA or GLA on skin composition in mice compared with CLA, GLA or LA alone.

Materials and methods

Animals and treatments

Male mice (8 weeks old, Sea:ddY strain; Seac Yoshitomi, Ltd, Fukuoka, Japan), were kept at 24°C on a 12 h dark–12 h light cycle, housed individually, and had free access to a commercial diet (MF; Oriental Yeast Co. Ltd, Tokyo, Japan) and water. Food intake was monitored individually by using clean animal feeding equipment (Type M; Rodent Cafe, Oriental Yeast Co. Ltd, Tokyo, Japan). The chemical composition (%) of the commercial diet was moisture 6·9, crude protein 23·9, crude fat 5·4, crude ash 6·1, crude fibre 3·0 and N-free extract 54·7, respectively. The mice were divided into six groups of five mice each according to their body weights.

Mice were orally administered 250 μl of the LA, GLA, or CLA oil source per d for 4 weeks, respectively, by using 1 ml syringes attached with tubes. The remaining three groups were administered a combination of each oil source; these were the LA + GLA, LA + CLA and CLA + GLA groups. These groups receiving the combinations were given 250 μl of each oil (total 500 μl) every day for 4 weeks. The LA group served as the control, and was given high-LA safflower-seed oil (triacylglycerol-type; Rinoru Oil Mills Co. Ltd, Nagoya, Japan) which contained 71·8 % LA. The GLA group was given an oil containing 95·0 % GLA (free fatty acid-type; Idemitsu Technofine Co. Ltd, Tokyo, Japan). The CLA group was given an oil containing 70·8 % CLA (triacylglycerol-type; Rinoru Oil Mills Co. Ltd, Nagoya, Japan) that contained 32·4 % of 9*cis*, 11*trans*-CLA and 33·3 % of 10*trans*, 12*cis*-CLA. The fatty acid composition of these oils is shown in Table 1. The LA content of the safflower-seed oil was 158·2 mg in 250 μl. The GLA content of the ethyl GLA oil was 198·5 mg in 250 μl, and the CLA content was 143·7 mg in 250 μl. The amounts (mg) of CLA-isomers were: 9*cis*, 11*trans* (65·8); 10*trans*, 12*cis* (67·4); 9*cis*, 11*cis* (2·4); 10*cis*, 12*cis* (2·2); 9*trans*, 11*trans* + 10*trans*, 12*trans* (5·9), respectively. The experimental procedures followed the guidance for Animal Experiments in the Faculty of Agriculture and in the Graduate Course of Kyushu University and the Law (no. 105) and Notification (no. 6) of the Japanese Government.

Table 1. Compositions of experimental oils (% of total fatty acids)

	LA	GLA	CLA
16 : 0	6·5	–	6·6
18 : 0	2·5	–	2·4
18 : 1	17·1	–	16·6
18 : 2*n*-6	71·8	–	1·5
CLA	–	–	70·8
9cis, 11trans-	–	–	32·4
10trans, 12cis-	–	–	33·2
cis, cis-	–	–	2·3
trans, trans-	–	–	2·9
18 : 3*n*-6	–	95·0	–
Others	2·1	5·0	2·1

LA, linoleic acid; GLA, γ-linolenic acid; CLA, conjugated linoleic acid.

Collection of skin tissues

At the conclusion of the experiment, mice were killed by diethyl ether anaesthesia. Then, their whole skin (with hair) was removed. Skin sheets (1 cm × 1 cm) were cut from the back of all mice for histology. Half of the remaining skin was used for collagen analysis and the other half was used for fatty acid analysis. After weighing, the skin was kept at −80°C until analysis.

Determination of collagen content

Skin samples were ground with a mill (Millser700G; Iwatani Co., Tokyo, Japan), and fragmentised in 10 ml 0·15 M-NaCl for 1 h with ultrasound. The collagen content was determined according to the modified method by Bergman & Loxley (1963). Briefly, 20 ml 6 M-HCl was added, and the suspension hydrolysed for 24 h at 20°C, and kept for 10 h at 120°C with a block heater (ALB-121; Asahi Technoglass Co. Ltd, Tokyo, Japan). The hydrolysed sample was filtered and adjusted to 100 ml with distilled water. The sample (1 ml) was evaporated with a centrifugal evaporator (CVE-3100; Eyela Co., Tokyo, Japan). Evaporated samples were diluted with distilled water. A 0·2 ml sample was mixed with 0·4 ml isopropanol, followed by mixing with 0·2 ml oxidant solution (75 % (w/v) chloramine T and acetate–citrate buffer (pH 6·0) were mixed at a ratio of 3:1). The mixed sample solution was kept for 4 min at room temperature (17 to 20°C). After adding 2·6 ml Ehrlich's reagent solution, the sample was incubated at 60°C for 25 min. After cooling with water for 2 to 3 min, the sample solution was diluted with 6·6 ml isopropanol. The hydroxyproline concentration in the sample solution was determined using a spectrophotometer at 560 nm.

Determination of triacylglycerol content

The skin was ground with a mill (Millser700G; Iwatani Co., Tokyo, Japan). Total lipid was extracted using a modified Folch method (Folch *et al.* 1957; Seya *et al.* 2000). A chloroform–methanol (2:1, v/v) solution was added to the skin sample, and homogenised in a centrifugal tube. The mixture was centrifuged

for 10 min at 850 ***g*** to separate into two distinct phases, and the lower phase was collected. The chloroform–methanol solution was again added to the upper phase followed by homogenisation and centrifugation. Both fractions were filtered and mixed with a KCl solution (0·9 %, w/v). After 5 min, the samples were centrifuged at 1250 ***g*** for 10 min. The lower phase was diluted to 20 ml with chloroform, and a 250 μl sample was placed in a microtube, evaporated, and then assayed using a triacylglycerol determination kit (Triglyceride-Wako; Wako Co., Osaka, Japan). The remaining diluted solution (5 ml) was placed in a screw-cap glass vial and evaporated. Air in the screw-cap glass vial was replaced by gaseous N_2, and the resulting extracts were stored at −80°C until fatty acid analysis.

Fatty acid analysis

Determination of fatty acid composition was done using GC (HP5890II; Hewlett Packard Co., Palo Alto, CA, USA) after transmethylation in 14 % methanolic BF_3 (Kamegai *et al.* 2001). The GC was equipped with a J&W DB-23 fused (50 %-Cyanopropyl)-methylpolysiloxane capillary column (30 m × 0·25 mm internal diameter, 0·25 μm film thickness; Agilent Technologies Inc., Palo Alto, CA, USA). The carrier gas used was He at a flow of 100 kPa. The oven was programmed from 160°C to 220°C at 2°C/min, and the injector and detector temperatures were set at 250°C.

Observation of skin histology

The skin samples were processed following a conventional procedure (Ohtani *et al.* 1988). Specimens were fixed in 3 % glutaraldehyde diluted with phosphate buffer (pH 7·5) at 4°C. The fixed samples were soaked in 10 % NaOH for 4 d and then in distilled water for 3 d. Samples were immersed in 1 % tannic acid for 2 h, and then soaked in a 2 % osmium tetroxide solution for 1 h. Skin samples were dehydrated through a graded series of ethanol washes. Specimens were placed in *t*-butyl alcohol and freeze-dried (TIS-U-DRY; FIS Systems, International Co., New York, NY, USA). Samples were laid on Al holders and coated with Pt-Pd (Eiko IB-3 Ion Coater; Eiko Co., Kobe, Japan) and examined under an ss-550 scanning electron microscope (Shimadzu Co., Kyoto, Japan) at the Center of Advanced Instrumental Analysis, Kyushu University. The thickness of the dermis layer and subcutaneous tissue layer between the dermis layer and fascia on the photomicrograph were measured.

Measurements of adipocyte size and number

The skin samples were fixed in 3 % glutaraldehyde diluted with phosphate buffer (pH 7·5) at 4°C. The sample was immersed in 1 % tannic acid for 0·5 h and soaked in a 1 % osmium tetroxide solution for 1 h. Skin samples were dehydrated through a graded series of ethanol washes. Specimens were placed in *t*-butyl alcohol and freeze-dried (TIS-U-DRY; FIS Systems, International Co., New York, NY, USA). Samples were laid on Al holders and coated with Pt-Pd (Eiko IB-3 Ion Coater; Eiko Co., Kobe, Japan), and examined under an ss-550 scanning electron microscope (Shimadzu Co., Kyoto, Japan) at the Center of Advanced Instrumental Analysis, Kyushu University. The cell size and number of the adipocytes in the subcutaneous tissue layer were measured. The adipocyte size was determined by the average of major and minor axis of the adipocyte. The numbers of adipocytes with diameters more than 10 μm were counted in 1 mm^2 and divided into groups with 20 μm intervals.

Statistical methods

Data were statistically analysed by one-way ANOVA. When significant effects were found, the six dietary groups were compared by Fisher's Protected Least Significant Difference test and Tukey–Kramer's test. Statistical significant difference was set at $P<0·05$. The results are shown as means with their standard errors.

Results

Fig. 1 shows the effect of CLA, GLA or LA, and their combination, on skin triacylglycerol content in mice. Comparisons among the individual oil groups indicated that the triacylglycerol content was highest for LA treatment followed by GLA, then CLA. When combined with LA and GLA, the triacylglycerol content was similar to the GLA-alone group rather than the LA-alone group. Triacylglycerol contents in the LA + CLA or CLA + GLA groups were comparable with the CLA-alone group. The collagen content in the fresh skin (mg/g) was not significantly different among groups (LA 123 (SEM 9), GLA 122 (SEM 3), CLA 142 (SEM 10), LA + GLA 123 (SEM 9), LA + CLA 131 (SEM 5), CLA + GLA 149 (SEM 11)).

The effect of several oils and their combination on fatty acid composition of the skin is shown in Table 2. The amount of LA, GLA and CLA was modified by the administered oils. The LA content in the LA-alone, LA + GLA and LA + CLA groups was more than 33 %, and the remaining three groups contained less than 28 % LA. The GLA content was higher in the groups combining GLA with other fatty acids compared with the GLA-alone group. However, the CLA content was higher in the CLA-alone group than in the groups combining CLA with other fatty acids. In the CLA oil group, the isomer of 9*cis*,

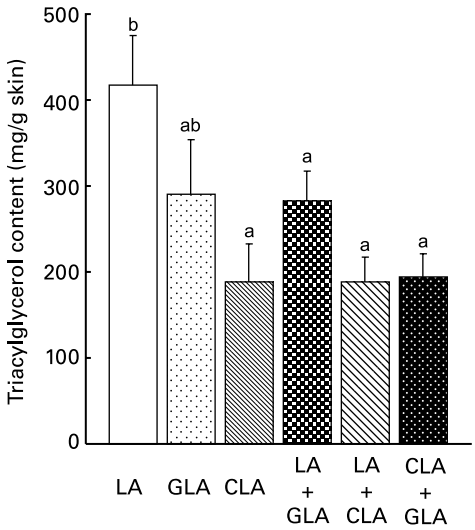

Fig. 1. Triacylglycerol content in the skin of mice administered linoleic acid (LA), γ-linolenic acid (GLA), conjugated linoleic acid (CLA) or their combinations. Values are means for five mice per group, with vertical bars representing the standard errors of the mean. [a,b] Mean values with unlike letters were significantly different ($P<0·05$). For details of diets and procedures, see Table 1 and p. 275.

Table 2. Fatty acid compositions (%) of total skin (five rats per group)*
(Mean values with their standard errors)

Group...	LA		GLA		CLA		LA + GLA		LA + CLA		CLA + GLA	
Oil	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14:0	1·17[a]	0·07	1·71[b]	0·37	0·83[a]	0·13[a]	1·02[a]	0·06	0·74[a]	0·02	1·05[a]	0·08
16:0	18·78[b]	0·65	20·72[c]	0·80	20·56[c]	0·60[c]	17·54[a]	0·37	18·10[b,c]	0·40	19·41[a,b]	0·63
16:1	4·41[b,c]	0·40	5·84[c]	0·71	2·28[a]	0·59[a]	4·13[b]	0·44	2·45[a]	0·66	2·42[a]	0·16
18:0	2·60[a]	0·16	3·29[a,b]	0·44	4·31[c]	0·27[c]	2·84[a]	0·10	3·89[b,c]	0·14	3·81[b,c]	0·26
18:1	31·77[b]	0·64	31·10[a,b]	0·53	34·14[c]	0·56[c]	29·15[a]	0·54	30·86[a,b]	1·03	29·36[a]	0·69
18:2	36·31[d]	0·75	27·65[b]	0·79	23·71[a]	1·49[a]	35·15[c,d]	0·55	33·35[c]	0·99	25·16[a,b]	0·64
CLA	0·50[a]	0·07	0·49[a]	0·13	8·11[c]	0·63[c]	0·56[a]	0·14	5·29[b]	0·36	6·98[c]	0·80
9cis, 11trans	0·29[a]	0·04	0·27[a]	0·07	4·71[d]	0·37[d]	0·32[a]	0·08	3·02[b]	0·19	3·84[c]	0·42
10trans, 12cis	0·13[a]	0·02	0·13[a]	0·05	2·77[c]	0·25[c]	0·09[a]	0·04	1·78[b]	0·16	2·53[c]	0·33
cis, cis	0·01[a]	0·01	0·03[a,b]	0·01	0·28[d]	0·02[d]	0·08[b]	0·03	0·20[c]	0·01	0·27[d]	0·03
trans, trans	0·08[a]	0·01	0·05[a]	0·00	0·35[b]	0·01[b]	0·06[a]	0·01	0·29[b]	0·02	0·33[b]	0·04
18:3*n*-6	0·37[a]	0·17	2·99[b]	0·31	0·23[a]	0·09[a]	4·72[c]	0·56	0·42[a]	0·21	4·50[c]	0·52
18:3*n*-3	1·05[c]	0·06	1·14[c]	0·04	0·63[a]	0·10[a]	1·03[c]	0·08	0·57[a]	0·04	0·84[b]	0·06
20:3	0·23[a]	0·04	0·51[c,d]	0·05	0·42[b,c]	0·12[b,c]	0·67[d,e]	0·04	0·31[a,b]	0·05	0·83[e]	0·01
20:4	0·48	0·04	1·08	0·37	0·75	0·18	0·84	0·07	0·75	0·10	1·42	0·29
Others	11·60	0·17	3·49	1·00	4·03	0·61	2·30	0·14	3·25	0·20	4·25	0·61
Saturated	23·1	0·62	27·0	1·10	27·1	1·02	22·0	0·49	23·8	0·34	25·9	0·70
Unsaturated	76·9	0·62	73·0	1·10	72·9	1·02	78·0	0·49	76·2	0·34	74·1	0·70

LA, linoleic acid; GLA, γ-linolenic acid; CLA, conjugated linoleic acid.
[a,b,c,d,e] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 275.

11*trans*-CLA was higher than that of 10*trans*, 12*cis*-CLA. No significant effect was detected in AA content among groups.

Fig. 2 shows representative scanning electron microscopic photographs of the dermis and subcutaneous layers of mice administered LA, GLA, CLA, or their combinations. The thickness of the dermis layer was not significantly ($F(5,24)$ 0·245; $P>0·05$) different among the groups. The values (μm) of this layer were 399 (SEM 15) for the LA treatment, 419 (SEM 41) for GLA, 424 (SEM 25) for CLA, 424 (SEM 21) for LA + GLA, 435 (SEM 23) for LA + CLA and 431 (SEM 20) for CLA + GLA, respectively. On the other hand, the thickness of subcutaneous tissues was significantly ($F(5,24)$ 5·171; $P=0·002$) different among the groups (Fig. 3). The LA group was significantly thicker than the other groups. The subcutaneous layer of the LA + CLA group was the thinnest among treatment groups.

Fig. 4 (a) shows a representative sample of a scanning electron microscopic of the subcutaneous tissue layer. Fig. 4 (b) shows the total number of adipocytes in subcutaneous tissue with the number of adipocytes classified into 20 μm intervals. The number of adipocytes in the subcutaneous tissue was highest in the LA + GLA group.

Discussion

The CLA oil used in the present study consisted mainly of 9*cis*, 11*trans*-CLA (32·4 %) and 10*trans*, 12*cis*-CLA (33·2 %). However, the tissue content of 9*cis*, 11*trans*-CLA was 1·7 times higher than that of 10*trans*, 12*cis*-CLA in the CLA group (Table 2). This result was in agreement with the previous report (Oikawa *et al.* 2003). Even when combined with other fatty acids, the composition of 9*cis*, 11*trans*-CLA was 1·7 times and 1·5 times higher than that of 10*trans*, 12*cis*-CLA in the LA + CLA group and the CLA + GLA group, respectively. Without CLA administration, the mouse skin contained small amounts of several isomers of CLA, and the composition of 9*cis*, 11*trans*-CLA was 2·2–3·6 times higher than that of 10*trans*, 12*cis*-CLA. These results suggest that CLA is a naturally occurring fatty acid, and 9*cis*, 11*trans*-CLA is easily transferred into the skin, but the rate of transfer between 9*cis*, 11*trans*-CLA and 10*trans*, 12*cis*-CLA may be altered by the combination of specific fatty acids. Since individual CLA isomers were not given in the present study, it was not clear which isomers caused the reduction in triacylglycerol content and the decrease in the thickness of the subcutaneous layer in the skin. Brown *et al.* (2001) reported that the 10*trans*, 12*cis*-CLA isomer inhibited accumulation of fat in the body, since the isomer played a role in the inhibition of hepatic stearoyl-CoA desaturase activity (Park *et al.* 2000) and affected lipoprotein lipase activity (Lin *et al.* 2001). Taken together, the reduction in triacylglycerol content and the decrease in the thickness of the subcutaneous layer in the skin may be caused by 10*trans*, 12*cis*-CLA rather than 9*cis*, 11*trans*-CLA.

CLA has several beneficial effects; one of which is the inhibition of accumulation of visceral fat (Tsuboyama-Kasaoka *et al.* 2000; Nakanishi *et al.* 2001). In contrast, excessive CLA intake induces fatty liver in mice (Nakanishi *et al.* 2004). Nakanishi *et al.* (2004) reported that the combination of GLA and CLA ameliorated the fatty liver induced by CLA. In the present study, total doses of fatty acids in the combination groups were twice as much as those receiving single fatty acids. However, the CLA + GLA and CLA + LA groups had clearly reduced triacylglycerol content in total skin and thickness of the subcutaneous tissue compared with LA-alone group (Figs. 1 and 3). Additionally, since these results were similar to those obtained in the CLA-alone group, CLA was effective even when given in combination with other oils.

The composition of GLA was increased in the LA + GLA group and the CLA + GLA group compared with the GLA-alone group. In the LA + GLA group, the enhancement of GLA came from metabolism of LA to GLA, but the reason for the increase of GLA in the CLA + GLA group compared with the GLA-alone group was unclear. The metabolism of GLA may be modified by CLA; however, we are not aware of any

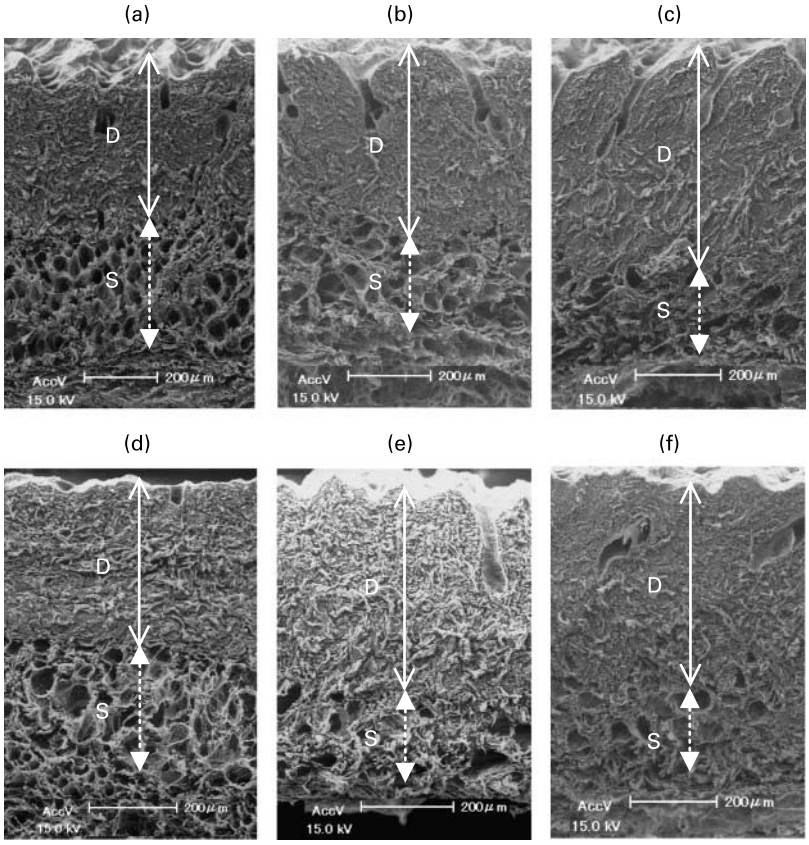

Fig. 2. Representative scanning electron microscopic photographs of the dermis (D) and the subcutaneous (S) tissue layers of mice administered linoleic acid (LA; (a)), γ-linolenic acid (GLA; (b)), conjugated linoleic acid (CLA; (c)), LA + GLA (d), LA + CLA (e) or CLA + GLA (f). Bars indicate 200 μm. For details of diets and procedures, see Table 1 and p. 275.

work in this area. The reduction in triacylglycerol content reflects GLA composition in the CLA + GLA group (Fig. 1 and Table 2). The triacylglycerol content of the total skin tended to be lower in the GLA than LA group (Fig. 1). Furthermore, the thickness of the subcutaneous tissue was significantly thinner in the GLA than LA group (Fig. 3). GLA-enriched oil reduced white adipose tissue compared with LA-enriched oil because carnitine palmitoyltransferase and peroxisomal β-oxidation activities were increased by the GLA treatments (Takada *et al.* 1994; Takahashi *et al.* 2000).

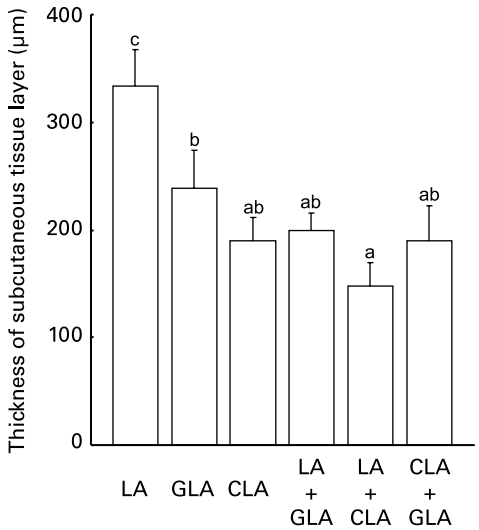

Fig. 3. Thickness of the subcutaneous layer of mice administered linoleic acid (LA), γ-linolenic acid (GLA), conjugated linoleic acid (CLA) or their combinations. Values are means for five mice per group, with vertical bars representing the standard errors of the mean. [a,b,c] Mean values with unlike letters were significantly different ($P<0{\cdot}05$). For details of diets and procedures, see Table 1 and p. 275.

The number of adipocytes in the LA + GLA group was about twice that of the LA or GLA groups (Fig. 4 (b)). The triacylglycerol content of the LA + GLA group tended to be higher than other mixture-oil groups. This suggests that higher triacylglycerol content was related to an increase in the number of adipocytes.

It was reported that PGE_2 enhanced collagen synthesis (Levi-Schaffer *et al.* 1995), and PGE_1 inhibited collagenase gene expression in human cultured fibroblasts (Salvatori *et al.* 1992). Although the amounts of prostaglandins were not analysed in the present study, CLA appears to decrease their contents based on a previous report (Belury, 2002). However, the thickness of the dermis layer and collagen content were not significantly different among the six groups. These results were supported by our previous report (Oikawa *et al.* 2003). The difference may be explained by the method used; the present study was done *in vivo*, oils were administered orally and doses of the oils used did not induce excessive production of collagen (for example, keloid).

Dietary LA is easily transferred into the skin (Table 2; Oikawa *et al.* 2003). Excessive LA intake induced inflammation of the skin (Gleich & Kita, 1997; Fogh & Kragballe, 2000), because

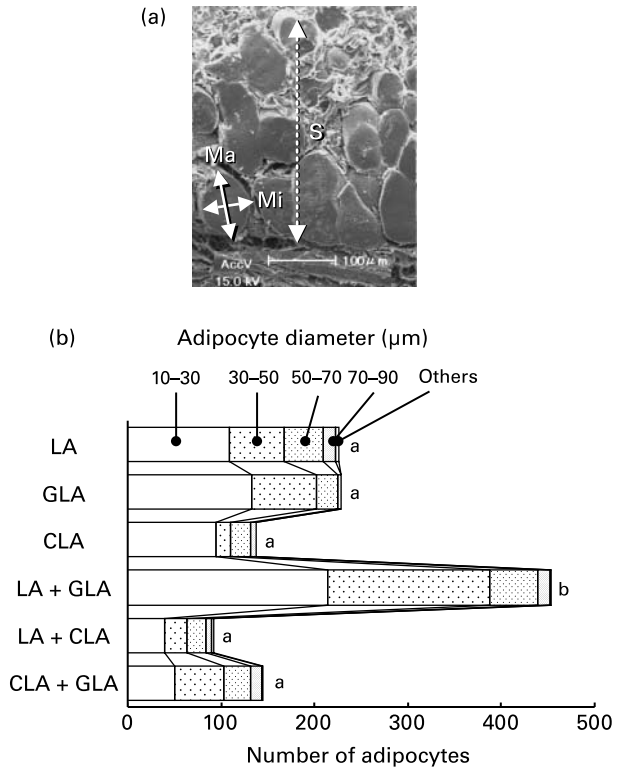

Fig. 4. (a) Representative scanning electron microscopic photographs of the subcutaneous tissue layer (S) of mice. Ma, major axis, Mi, minor axis. Bars indicate 100 μm. (b) Total number of adipocytes with different sizes in the subcutaneous tissue of mice administered linoleic acid (LA), γ-linolenic acid (GLA), conjugated linoleic acid (CLA) or their combinations. Values are means for five mice per group. [a,b] Mean values with unlike letters were significantly different ($P < 0{\cdot}05$). For details of diets and procedures, see Table 1 and p. 275.

LA was a primary source of chemical mediators such as PGE_2 and leucotriene B4 via AA. It was reported that these eicosanoids stimulated pain or itch responses in the skin (Andoh & Kuraishi, 1998; Kabashima *et al.* 2003). The amounts of fatty acids or lipids in the skin may have an important role for dermatitis and dehydration. Horrobin (2000) described that atopic eczema is caused by not only deficiency of essential fatty acids, but also by administration of excessive LA. The skin lesions of dietary essential fatty acids deficiency in human patients can be readily corrected by daily LA doses in the 0·5–3·0 g range, while excessive LA is required to reduce intake of LA and correct the ratio of LA to the downstream PUFA (Horrobin, 2000). On the other hand, increased GLA and DGLA composition in the epidermis improved skin barrier function and decrease transepidermal water loss in elderly individuals (Brosche & Platt, 2000). The combination of CLA and other fatty acids may have a beneficial effect on the skin health. Although amounts of eicosanoids in the skin were not analysed in the present study, CLA (5–8 %) were included in the skin of the CLA-alone group and CLA with other fatty acids groups. Dietary CLA may reduce PGE_2 or leucotriene B4 in the skin, since CLA blocks the desaturation of LA (Kavanaugh *et al.* 1999; Belury, 2002), release of AA from phospholipids, and production of eicosanoids from AA (Urquhart *et al.* 2002). However, since CLA modulates production of eicosanoids histotypically (Belury, 2002), the eicosanoid contents remain to be studied in another experiment.

In conclusion, dietary CLA altered the fatty acid composition of the skin, triacylglycerol content, thickness of subcutaneous tissue, and the number and size of adipocytes. Additionally, these effects of CLA can be similarly seen when CLA was co-administered with other fatty acids. Therefore, it is suggested that CLA alone, or a mixture of CLA with GLA or LA, serve to improve the condition of the skin. For instance, CLA may reduce the subcutaneous adipose tissue in obese subjects, and then may induce a lower subcutaneous blood pressure. The best supplementation among the six groups may be dependent upon the skin and body conditions. For instance, CLA + GLA may regulate skin composition (present study) and prevent fatty liver (Nakanishi *et al.* 2004) in the obese.

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