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Dietary *trans* 10, *cis* 12-conjugated linoleic acid reduces the expression of fatty acid oxidation and drug detoxification enzymes in mouse liver

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Mice fed diets containing *trans* 10, *cis* 12 (t10, c12)-conjugated linoleic acid (CLA) develop fatty livers and the role of hepatic fatty acid oxidation enzymes in this development is not well defined. We examined the effects of dietary *cis* 9, *trans* 11-CLA (c9, t11-CLA) and t10, c12-CLA on the expression of hepatic genes for fatty acid metabolism. Female mice, 8 weeks old, (six animals per group) were fed either a control diet or diets supplemented with 0·5 % c9, t11- or t10, c12-CLA for 8 weeks. DNA microarray analysis showed that t10, c12-CLA increased the expression of 278 hepatic genes and decreased those of 121 genes (>2-fold); c9, t11-CLA increased expression of twenty-two genes and decreased those of nine. Real-time PCR confirmed that t10, c12-CLA reduced by the expression of fatty acid oxidation genes including flavin monooxygenase (FMO)-3 95 %, cytochrome P450 (cyt P450) 69 %, carnitine palmitoyl transferase 1a 77 %, acetyl CoA oxidase (ACOX) 50 % and PPARα 65 %; it increased the expression of fatty acid synthase by 3·5-fold ($P<0·05$ for all genes, except ACOX $P=0·08$). It also reduced the enzymatic activity of hepatic microsomal FMO by 40 % and the FMO3 specific protein by 67 %. c9, t11-CLA reduced FMO3 and cyt P450 expression by 61 % ($P=0·001$) and 38 % ($P=0·06$) and increased steoryl CoA desaturase transcription by 5·9-fold ($P=0·07$). Both decreased fatty acid oxidation and increased fatty acid synthesis seem to contribute to the CLA-induced fatty liver. Since FMO and cyt P450 are also involved in drug detoxification, suppression of the transcription of these genes by CLA may have other health consequences besides development of fatty liver.

Microarrays: Real-time PCR: Cytochrome P450: Flavin monooxygenase: Carnitine palmitoyl transferase: PPARα

Conjugated linoleic acid (CLA) is a collective term for a group of isomers of linoleic acid that have conjugated double bonds. Depending on the position and geometry of the double bonds, several isomers of CLA have been reported (Eulitz *et al.* 1999). Most of the published studies have used mixtures of CLA isomers, which comprised two major forms, *cis* 9, *trans* 11-CLA (c9, t11-CLA) and *trans* 10, *cis* 12-CLA (t10, c12-CLA), and a number of minor isomers. The major dietary sources of c9, t11-CLA are dairy products and ruminant meat, while that of t10, c12-CLA are partially hydrogenated vegetable oils from margarines and shortenings (McGuire *et al.* 1999).

Feeding a mixture of CLA isomers to animal models altered blood lipids, atherogenesis, diabetes, body composition, chemically induced carcinogenesis and immune cell functions (Belury, 2002). Diets containing CLA reduced the amount of adipose fat in several species including rat, pig, hamster, chicken and mouse (Kelley & Erickson, 2003; Tricon *et al.* 2005). The loss of adipose tissue in mice was associated with a several-fold increase in the amount of fat stored in the liver (Belury & Kempa-Steczko, 1997; Park *et al.* 1999; Tsuboyama-Kasaoka *et al.* 2000; Clement *et al.* 2002; Degrace *et al.* 2003; Kelley *et al.* 2004; Poirier *et al.* 2005). We, as well as others, have previously reported that t10, c12-CLA is the isomer that is responsible for the development of fatty liver in mice (Park *et al.* 1999; Clement *et al.* 2002; Kelley *et al.* 2004). Decreased expression of adipocytokines and up regulation of the expression and activity of the lipogenic enzymes acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), malic enzyme (ME), stearoyl CoA desaturase (SCD)-1 and δ 5 and 6 desaturases have been postulated to be the underlying mechanisms that lead to the development of fatty livers in mice fed diets containing CLA (Tsuboyama-Kasaoka *et al.* 2000; Degrace *et al.* 2003, 2004; Takahashi *et al.* 2003; Warren *et al.* 2003; Javadi *et al.* 2004; Ide, 2005; Poirier *et al.* 2005). Expression of mice hepatic fatty acid oxidation genes also increased in three studies with a mixture of CLA isomers (Takahashi *et al.* 2003; Javadi *et al.* 2004; Ide, 2005) and in one study with t10, c12-CLA (Degrace *et al.* 2004). Authors who supplemented the mouse diets with the purified t10, c12-CLA expected a reduction in hepatic fatty acid oxidation because CLA increased the hepatic concentration of malonyl CoA and the sensitivity of carnitine palmitoyl transferase (CPT)-1 to inhibition with malonyl CoA

Abbreviations: ACC, acetyl-CoA carboxylase; c9, t11-CLA, *cis* 9, *trans* 11-CLA; CLA, conjugated linoleic acid; CPT, carnitine palmitoyl transferase; cyt P450, cytochrome P450; FAS, fatty acid synthase; FMO, flavin-containing monooxygenase; ME, malic enzyme; SCD, stearoyl CoA desaturase; t10, c12-CLA, *trans* 10, *cis* 12-CLA.

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(Degrace *et al.* 2004). Development of fatty liver and increased hepatic fatty acid oxidation is a paradox that may be true, but normally we would expect hepatic fatty acid oxidation to be reduced if more fat is stored in the liver. None of the published reports has used the microarray technology to systematically examine all the hepatic genes involved in fatty acid metabolism, whose expression may be modulated by CLA-containing diets.

The purpose of the present study was to use a comprehensive approach to identify all the mouse liver genes involved in fatty acid oxidation (α,β and ω) and synthesis, whose expression may be altered by feeding diets containing CLA preparations enriched in one of the two common isomers (c9, t11-CLA and t10, c12-CLA). We used the Affymetrix Inc. (Santa Clara, CA, USA) microarray chips to identify the genes whose expression was altered by CLA. Changes in gene expressions detected by microarrays were confirmed by quantitative real-time PCR. The expressions of several genes involved in fatty acid oxidation and synthesis were altered by the feeding of t10, c12-CLA isomer. The largest reduction in gene expression was found for the two microsomal enzymes, cytochrome P450 (cyt P450) and flavin-containing monooxygenase (FMO)-3; these enzymes are involved in microsomal ω oxidation of fatty acids and the detoxification of a variety of xenobiotic compounds (White *et al.* 1978; Falls *et al.* 1997; Orellana *et al.* 1998; Reddy & Hashimoto, 2001; Krueger & Williams, 2005; Sanders *et al.* 2005; Weng *et al.* 2005). Under normal conditions microsomal fatty acid oxidation represents less than 10 % total fatty acid oxidation; however, during starvation and diabetes the contribution of this pathway in overall hepatic fatty acid oxidation is significantly increased (Orellana *et al.* 1998). Microsomal fatty acid oxidation may have a significant role in fatty acid oxidation in mice fed diets containing t10, c12-CLA, because this isomer produces diabetes-like symptoms of increased blood glucose and insulin resistance (Poirier *et al.* 2005). Expression of cyt P450 and FMO3 is altered by several compounds that induce non-alcoholic fatty liver (Krueger & Williams, 2005) and FMO3 is the major isoform of FMO found in human liver (Falls *et al.* 1997). The affect of CLA on the expression of these genes has not been previously published. We, therefore, also investigated the combined enzymatic activity of all the hepatic microsomal FMO and amount of the FMO3 specific protein. The present results show that diets containing the t10, c12-CLA reduced FMO activity, FMO3 specific protein and the transcription of several genes involved in fatty acid oxidation.

Materials and methods

Conjugated linoleic acid isomers and diets

Highly enriched c9, t11-CLA and t10, c12-CLA isomers in the form of NEFA were a kind gift from Natural ASA, Hovdebygda, Norway. The analytical data for these isomers were provided by the supplier and confirmed by GLC in our laboratory (Warren *et al.* 2003; Kelley *et al.* 2004). The preparation enriched in c9, t11-CLA contained (%): c9, t11-CLA 84·6; t10, c12-CLA 7·7; 18: 1*n*-9 3·8; t9, t11-CLA + t10, t12-CLA 2·0; other fatty acids 1·9. In the preparation enriched in t10, c12-CLA, this isomer was 88·1 %; c9, t11-CLA 6·6 %; t9, t11-CLA + t10, t12-CLA 2·5 %; 18 : 1*n*-9 1·1 %; other fatty acids 1·7 %.

The concentration of CLA used in the present study was 0·5 weight % of the diet, which is comparable to the concentrations used in previous studies with rodent models, which ranged from 0·1 to 1·5 weight % of a mixture of CLA isomers. AIN-93G, high carbohydrate, mouse diet was used as the basal diet. The nutrient and fatty acid composition of this diet has been previously reported (Warren *et al.* 2003; Kelley *et al.* 2004) and is shown in Table 1. For the two CLA-containing diets, CLA isomer-enriched oils were added by replacing 5 g/kg maize oil with an equivalent amount of the CLA source. Diets were constantly flushed with N gas while being gently mixed in a blender. Diets were packaged in 30 g aliquots, flushed with N gas and stored at −20°C. Fresh dietary packets were served each day. The animal protocol was approved by the Animal Use Committee at the University of California, Davis.

Animals, feeding and tissue collection

Eighteen, 8 week old, pathogen free C57BL/6N female mice were purchased from Charles River (Raleigh, NC, USA). Female mice were chosen because of their docility for housing in groups. They were maintained in a sterile air curtain isolator at the animal facility of the University of California, Medical School with controlled temperature (25°C) and light and dark cycle (12 h each). All animals were fed the laboratory chow diet for the first 7 d, then divided into three groups of six each and fed the experimental diets for 56 d. The dose of CLA and the duration of its feeding used in the present study are the same as we have used previously (Warren *et al.* 2003; Kelley *et al.* 2004), which are well within the ranges used by many other investigators. Details regarding animal handling, killing, tissue collection and storage have been published (Warren *et al.* 2003; Kelley *et al.* 2004).

Real-time PCR

Total RNA from approximately 100 mg liver slices was extracted by using Trizol reagent (Invitrogen, Carlsbad, CA, USA). This RNA (1 μg) was denatured and used to synthesize

Table 1. Composition of the basal diet*

Ingredient	g/kg
Maize starch	417·5
Casein	200
Dextrinized maize starch	132
Sucrose	100
Maize oil	50
Fibre source (cellulose)	50
Mineral mix (AIN-93G)	35
Vitamin mix (AIN-93)	10
Cystine	3
Choline bitartrate	2·5
c9,t11-CLA	0
t10,c12-CLA	0

c9,t11-CLA, *cis* 9, *trans* 11-conjugated linoleic acid; t10,c12-CLA, *trans* 10, *cis* 12-conjugated linoleic acid.
* For details of diets and procedures, see p. 59.

cDNA by using an Invitrogen pre-amplication kit. After the first strand cDNA synthesis the RNA templates were degraded by treatment with RNase H. Specific primers for different enzymes were designed based on published full-length cDNA sequences (Table 2). The PCR reactions were performed in a programmable thermal cycler (denaturation at 94°C for 3 min followed by 40 cycles, denaturation at 94°C for 30 s, annealing at 56°C for 30 s and extension at 72°C for 30 s followed by final extension of 72°C for 7 min). The PCR products were analysed by electrophoresis in 3 % agarose gels and stained with ethidium bromide. The amplicons were cloned into PCR2.1 plasmid vector (Invitrogen) and transformed into *Escherichia coli* competent cells by heat-shock. Cells were grown in Luria broth (LB) medium for 16 h. White colonies were picked and grown and plasmid DNA from these transformed colonies were isolated and analysed for presence of the genes inserted. The insertions were verified by digestion with EcoRI restriction enzyme. These plasmids were sequenced using M13 reverse and M13 forward primers. The purified plasmids were serially diluted and used to generate the standard curve.

Real-time quantitative PCR was performed using a Light-Cycler rapid thermal cycler system (Roche Diagnostics Ltd, Palo Alto, CA, USA) according to the manufacturer's instructions. β Actin and hypoxanthine-guanine phosphoribosyl transferase 1 was used as the housekeeping gene and results for gene expression determined by real-time PCR are expressed as ratios between the RNA for the gene of interest and that for β actin and hypoxanthine-guanine phosphoribosyl transferase 1. Reactions were performed in a 20 μl volume with 0·5 nM primers and 4 nm-$MgCl_2$, dNTP, Taq DNA polymerase and buffer. The following programme was used to carry out the reaction: 30 s denaturation step (94°C) followed by 40 cycles with a 95°C denaturation for 1 s; annealing for 5 s at 56°C; extension at 72°C for 17 s. To confirm specific amplification, the PCR products from each primer pair were subjected to a melting curve. The melting curve was determined by holding the reaction at 55°C for 10 s and then heating slowly to 94°C with a linear rate of 0·2°C/s while the fluorescence emitted was measured. Melting curves were generated by plotting fluorescence against temperature. All assays were carried out in duplicate. Melting curve analysis demonstrated that each of the primer pair described amplified a single product with a distinct melting temperature. The predicted length of each product was confirmed by agarose gel electrophoresis.

DNA microarray analysis

Because of the high cost of the microarray chips, this analysis was performed only on two animals per group; however, the real-time PCR were performed on six animals per group. We performed DNA microarray analysis using Affymetrix Mouse GeneChips (430A 2.0 Array, representing 14 000 genes) to determine the liver genes whose expression was altered by the diets containing CLA. Total RNA was extracted from the livers of mice fed control and CLA-containing diets with the TRIzol reagent; its quality and integrity were determined utilizing an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and absorbance at A260/A280. Only high quality RNA, having a 28S/18S rRNA ratio of 1·5–2·0 and an A260/280 absorbance ratio of 2, was utilized for further experimentation. It was further purified with RNAeasy silica columns (Qiagen, Valencia, CA, USA). RNA was converted to double-stranded cDNA, which was then converted to biotin-labelled cRNA by *in vitro* transcription labelling with a HighYield™ BioArray™ RNA Transcript Labeling Kit (Enzo Diagnostics, Farmingdale, NY, USA). The quality of *in vitro* transcription and

Table 2. Sequence of primers used for quantitative real-time PCR analysis*

Target gene	Primer sequence	Affymetrix no.	Genbank accession no.	Primer location (from 5′ end of cDNA)
β Actin	5′-TCATGAAGTGTGACGTTGACATCCGT-3′	M12481_5_at	NM007393	925-950
	5′-CCTAGAAGCATTTGCGGTGCACGATG-3′			1209-1184
FMO3	5′-ACTTTGCCTTCTGTAAACGACATGA-3′	1449525_at	NM_008030	1229-1253
	5′-GAACTTTACTGACGACACGCGTCT-3′			1553-1530
Cyt P450	5′-GGAGAGACGCTGCTCTACTACGG-3′	1449316_at	NM_008898	1724-1746
	5′-ATACATCCAGCGAGTAGCGGC-3′			2081-2061
CPT1a	5′-GGGAGGACAGAGACTGTACGCTC-3′	1434866_x_at	BC054791	1865-1878
	5′-TGTAGGAAACACCATAGCCGTCAT-3′			2241-2218
ACOX	5′-GGTGGGTGGTATGGTGTCGTAC-3′	1416408_at	NM_015729	1682-1703
	5′-CAAAGACCTTAACGGTCACGTAGTG-3′			1959-1935
PPAR-α	5′-AGGCAGATGACCTGGAAAGTC-3′	1449051_a	NM_011144·2	490-510
	5′-ATGCGTGAACTCCGTAGTGG-3′			801-782
FAS	5′-CTGAAGAGCCTGGAAGATCGG-3′	1423828_at	NM_007988	7370-7390
	5′-CCCTCCCGTACACTCACTCGT-3′			7734-7714
ME	5′-AGCAGTGCTACAAGGTGACCAA-3′	1416632_at	NM00615	1273-1294
	5′-CTCCAGGGAACACGTAGGAATT-3′			1401-1380
Hprt	5′-GTTGGATACAGGCCAGACTTTGTTG -3′	1448736_at	NM_013556	601-625
	5′-GAGGGTAGGCTGGCCTATAGGCT -3′			952-930
ACC	5′-GAGGTGGATCAGAGATTTCATAGAGA -3′	1434185_at	AY451393	4024-4049
	5′-AATGCGGTCCTCCTCAAACTT -3′			4104-4084
SCD1	5′-TGTAAACAGCCTGTTCGTTAGCA-3′	1415964_at	BC007474·1	858-880
	5′-CCTTAGAAACTTTCTTCCGGTCGTAA -3′			1156-1131

FMO3, flavin monooxygenase 3; Cyt P450, cytochrome P450; CPT1, carnitine palmitoyl transferase 1; ACOX, acetyl CoA oxidase; ACC, acetyl CoA carboxylase; FAS, fatty acid synthase; ME, malic enzyme; SCD1, steoryl CoA desaturase. Hprt, hypoxanthine guanine phosphoribosyl transferase.

* For details of procedures, see pp. 59–60.

fragmentation products was assessed using the Agilent 2100 Bioanalyzer. Fragmented, biotin-labelled cRNA (15 fg) was hybridized at 45°C overnight as defined in the Affymetrix7 expression analysis protocol. The hybridization buffer contained 100 mM-2-(4-morpholino) ethanesulfonic acid (MES), 1 M-NaCl, 20 mM-EDTA, 0·01 % Tween 20, four eukaryotic hybridization controls (1·5 pM-BioB; 5 pM-BioC; 25 pM-BioD; 100 pM-cre), 0·1 mg/ml herring sperm DNA (Promega, Madison, WI, USA) and 0·5 mg/ml acetylated bovine serum albumin. After hybridization, the arrays were washed and stained with an Affymetrix® fluidics station following the Antibody Amplification Washing and Staining Protocol (Affymetrix Inc.). Hybridization was detected with streptavidin-phycoerythrin and a confocal laser scanner (Affymetrix Inc.).

Microarray Suite 5·0 (Affymetrix Inc.) was used to determine the probe intensities and to compare expression amongst different arrays. Scaling to a target median intensity value of 125 normalized average intensity for each array. The gene expression values were log transformed (log base 2). Genes were ranked on *t* test scores, *P*-values ($P<0·05$) and fold changes computed as actual expression values. A particular transcript was considered significantly differentially expressed between the control and CLA groups if it had a fold change >2, a *P*-value $<0·05$, and this was observed in both independent experiments (described earlier). The cross-reference of the differentially expressed genes was performed using information from the Affymetrix and National Center for Biotechnology Information EntrezGene websites.

Determination of flavin-containing monooxygenase activity

Liver tissues of experimental mice were collected, frozen in liquid N and stored at −80°C until used. The liver samples were homogenized in cold Tris/KCl buffer (0·05 M/0·15 M, pH 7·4), using glass/Teflon homogenizers, and microsomal fractions were purified (Chung & Buhler, 1994). Total catalytic activities of all the liver FMO were determined by oxidation of methyl p-tolyl sulfide to methyl p-tolyl sulfoxide (Cashman & Proudfoot, 1988). Reaction mixture contained 0·05 M-glycine buffer (pH 9·5), 50 μg microsomal protein, 0·065 mM-NADP +, 3·3 mM-glucose-6-phosphate, 0·4 unit/ml glucose-6-phosphate dehydrogenase, 3·3 mM-$MgCl_2$ and 2·0 mM-methyl p-tolyl sulfide in a total volume of 0·25 ml. After 10 min incubation at 37°C, the reaction was stopped with 75 μl acetonitrile and centrifuged (10 000 ***g***) for 5 min. A 50-μl aliquot of the supernatant was analysed with HPLC. Methyl p-tolyl sulfoxide was detected by measuring absorbance at 237 nm and its concentration was calculated by comparing the absorbance to a standard curve based on known concentrations. Enzyme activity is expressed as p mol sulfoxide/mg microsomal protein per 10 min.

Immunoprecipitation and Western blotting of flavin-containing monooxygenase 3 specific protein

Microsomes from mice liver were passed through a Qiashredder (Qiagen) by centrifugation for 10 min at 20 800 ***g*** at 4°C. These extracts were immunoprecipitated by incubating with primary polyclonal antibody that was originally made against human FMO3 amino acid sequence position 259–279. Since the immunogenic regions of this peptide exactly matched the amino acid sequence for mouse FMO3, we used it to detect mouse FMO3. These microsome extracts (100 μg) were immunoprecipitated by incubating with 2 μl primary polyclonal antibody for 1 h at 4°C, followed by additional 1-h incubation with 20 μl protein A/G PLUS-agarose. The pellet was collected by centrifugation at 2500 rpm for 5 min at 4°C and washed four times with PBS. After the final wash, electrophoresis sample buffer was added and the sample was heated at 95°C for 2 min. These extracts were separated by SDS polyacrylamide (10 % acrylamide) electrophoresis and transferred to nitrocellulose. The membrane was blocked with a solution of 5 % powdered non-fat milk, 25 mM-Tris (pH 7·5) and 150 mM-NaCl and then incubated with HRP-conjugated goat anti-rabbit Ig-G. The bound antibody was detected using ECL chemiluminescence detection kit (Amersham, Pharmacia Biotech, Inc. Piscataway, NJ, USA).

Statistical analysis

The SAS proc glm was used for a one-way ANOVA between treatments and Levene's test was used for heterogeneity of variance (Littell *et al.* 2002). The two treatment means were compared with the control using Dunnett's adjustment for multiplicity. When there was evidence of heterogeneity of variance, the SAS proc mixed was used to incorporate the heterogeneity in the model. In cases for which the control data is entirely zero, *t* tests were used to test for treatment means being significantly different from zero. Differences were considered statistically significant for $P<0·05$.

Results

Effect of conjugated linoleic acid isomers on body, liver and liver lipid weights

Body weights of animals in the three dietary groups did not differ at the start of the study. However, at the end of feeding experimental diets body weights of the t10, c12-CLA group was significantly lower than in the other two groups (control 25·4 (SEM 0·3) g; c9, t11-CLA 26·7 (SEM 0·5) g; t10, c12-CLA 23·2 (SEM 0·3) g; $P=0·02$). Weights of the livers in animals fed the diets containing t10, c12-CLA were significantly ($P<0·05$) greater than those in the control and c9, t11-CLA groups (mean 2·54 (SEM 0·07) g *v.* 1·28 (SEM 0·03) g and 1·47 (SEM 0·06) g respectively); similarly the weight of total liver lipids was approximately four times greater in the t10, c12-CLA than those in the control and c9, t11-CLA groups (775 (SEM 119) and 147 (SEM 18) and 175 (SEM 13) mg respectively).

DNA microarray analysis

Gene microarray analyses were performed to get clues regarding the genes whose expression may be altered by the dietary treatments. Fig. 1 shows an overview of the variation in hierarchical clustering of gene expression across liver tissue of mice fed diets containing c9, t11-CLA or t10, c12-CLA. The expression level of each gene (relative to its mean expression across all samples) is represented by different colours, and the colour intensities represent deviations from the mean. Mean expression is shown by the black colour, red colour indicates gene expression increased, while green

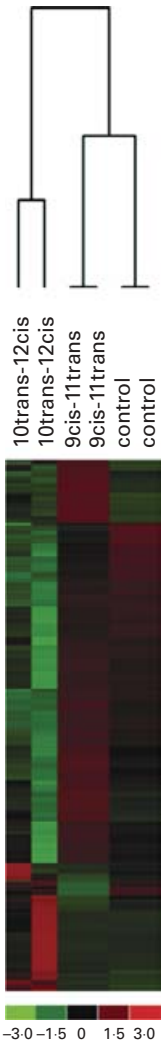

Fig. 1. Hierarchical clustering of gene expression profiles of liver tissue of mice fed diets with or without conjugated linoleic acid (CLA) isomers. Each column represents an individual mouse. Regional hierarchical clustering identified two major clusters; one representing *trans* 10, *cis* 12-CLA and the other control and *cis* 9, *trans* 11-CLA. Black colour represents the mean expression of all six animals, green represents lower expression than the mean and the red represents higher than the mean. The scale at the bottom represents 1·5 and 3 SD below and above the mean. Names of the genes altered are given online in Supplementary Table 1a–m). For details of diets and procedures, see pp. 59–61.

colour indicates gene expression decreased relative to the average. The observed pattern of gene expression identified two major clusters. The first cluster representing t10, c12-CLA-fed mice and the second cluster representing the control and c9, t11-CLA-fed mice. Names of individual genes whose expression was altered by 2-fold or more are listed in the online version in Supplementary Table 1a–m.

The focus of the current paper is only on the genes involved in fatty acid metabolism; hence we performed further studies only on the genes involved in fatty acid oxidation and synthesis. Feeding a diet containing c9, t11-CLA caused a 2-fold or greater change in the transcription of thirty-one hepatic genes (nine decreased and twenty-two increased) including nine genes involved in fatty acid metabolism (Supplementary Table 1a–m and Table 3). Expression of ME was significantly ($P<0·05$) increased (2·9-fold), while change in the expression of other enzymes involved in fatty acid metabolism did not attain statistical significance.

Feeding the diet containing t10, c12-CLA caused a 2-fold or greater change in the transcription 399 genes (121 increased, 278 decreased, Supplementary Table 1a–m). This isomer significantly ($P<0·05$) reduced the expression of FMO3 (93 %), cyt P450 (54 %), CPT1a (60 %) and PPARα (53 %) and increased the expression of ME by 6·3-fold ($P<0·05$). Expression of other lipogenic enzymes ACC, FAS and SCD1 increased by 3·2-, 2·6- and 1·9-folds respectively; however, these did not attain statistical significance (Table 2).

Confirmation of altered gene expression by real-time PCR

The afore-mentioned changes in the expression of fatty acid metabolism genes were confirmed by real-time PCR. Again, c9, t11-CLA diet did not alter the expression of most of the genes involved in fatty acid metabolism when compared with the control diet, with the exception of three genes. Expression of FMO3 and cyt P450 was reduced by 61 % and 38 % respectively, while that of SCD1 increased 5·9-fold (Table 3; $P<0·05$ for all three genes). Real-time PCR also confirmed that t10, c12-CLA reduced the expression of genes involved in fatty acid oxidation as indicated by the DNA microarray method (Table 3); it reduced the expression of FMO3 95 % ($P<0·0001$), cyt P450 61 % ($P=0·002$), CPT1a 77 % ($P=0·025$), acetyl CoA oxidase 50 % ($P=0·08$) and PPARα 65 % ($P=0·05$) when compared with the corresponding values in the control group. It also increased expressions of ACC by 18-fold ($P=0·01$) and FAS by 3·5-fold ($P=0·03$). Expressions of SCD1 and ME were increased by greater than 2-fold, but those did not attain statistical significance. Overall, the results from the microarray and real-time PCR data suggest that the t10, c12-CLA increased the expression of genes involved in fatty acid synthesis and reduced those of the genes involved in mitochondrial and peroxisomal β oxidation and microsomal ω oxidation; c9, t11-CLA did not alter the expression of any of these genes significantly except FMO3 ($P=0·001$), cyt P450 ($P=0·06$) and SCD1 ($P=0·07$) (Table 3).

Effect of conjugated linoleic acid isomers on the hepatic microsomal flavin-containing monooxygenase activity and flavin-containing monooxygenase 3 specific protein

We determined the effect of CLA isomers on the hepatic microsomal FMO activity because t10, c12-CLA reduced the expression of FMO3 by 95 % and c9, t11-CLA reduced it by 61 % as compared with the control group. We also determined the sum of enzymatic activity for all the hepatic isomers of FMO (FMO1, FMO3 and FMO5). Dietary c9, t11-CLA reduced the FMO activity by 15 % and FMO3 specific protein by 10 % (both non-significant; Fig. 2(A)(B). Diet containing t10, c12-CLA reduced the FMO activity by 40 % and FMO3 protein by 67 % ($P<0·05$; Fig. 2(A)(B)). Thus, the changes caused by the two CLA isomers in total FMO activity and FMO3 specific protein and mRNA are consistent.

Table 3. Effect of conjugated linoleic acid (CLA) isomers on the expression of genes involved in fatty acid oxidation and synthesis†
(Values are means with their standard errors)

	Control		c9, t11-CLA				t10, c12-CLA			
Gene‡	Mean	SEM	Mean	SEM	c9, t11-CLA/control	*P* value	Mean	SEM	t10, c12-CLA/control	*P* value
	Fatty acid oxidation enzymes									
FMO3	2573	122	1304	556	0·51	0·18	19*	13	0·007	0·01
	0·064	0·009	0·025*	0·008	0·39	0·001	0·003*	0·001	0·05	<0·0001
Cyt P450	1193	40	877	19	0·73	0·21	549*	186	0·46	0·02
	0·134	0·021	0·083	0·015	0·62	0·06	0·041*	0·004	0·31	0·002
CPT1a	722	55	503	46	0·70	0·23	292*	119	0·40	0·02
	0·058	0·011	0·040	0·014	0·69	0·27	0·013*	0·0030	0·23	0·025
ACOX	4726	347	4410	1256	0·93	0·49	4785	368	1·01	0·47
	0·006	0·001	0·004	0·001	0·67	0·22	0·003	0·001	0·50	0·08
PPARα	1400	266	1241	29	0·89	0·36	655*	76	0·47	0·04
	0·124	0·021	0·109	0·022	0·88	0·45	0·043	0·009	0·35	0·05
	Fatty acid synthesis enzymes									
ACC	490	169	1377	111	2·81	0·18	1542	728	3·15	0·14
	0·0025	0·0003	0·0161	0·0090	6·41	0·29	0·0456	0·0184	18·1	0·01
FAS	2067	1278	5621	204	2·72	0·17	5449	2574	2·64	0·17
	0·290	0·080	0·66	0·13	2·28	0·21	1·01	0·36	3·48	0·03
ME	699	152	2057*	357	2·94	0·05	4410*	1256	6·30	0·01
	0·0005	0·0001	0·0010	0·0005	2·23	0·34	0·0012	0·0004	2·60	0·24
SCD1	6160	3416	11 623	826	1·89	0·13	11 570	1140	1·88	0·13
	0·079	0·033	0·463	0·231	5·86	0·07	0·266	0·095	3·37	0·28

c9, t11-CLA, *cis* 9, *trans* 11-CLA; t10, c12-CLA, *trans* 10, *cis* 12-CLA; FMO3, flavin-containing monooxygenase; Cyt P450, cytochrome P450; CPT1a, carnitine palmitoyl transferase 1a; ACOX, acetyl CoA oxidase; ACC, acetyl CoA carboxylase; FAS, fatty acid synthase; ME, malic enzyme; SCD1, stearoyl CoA desaturase 1.
Mean values were significantly different from those in the control group: *$P<0{\cdot}05$ %.
† For details of diets and procedures, see pp. 59–60.
‡The top numbers for each gene listed were determined by microarrays (*n* 2); the bottom numbers (*n* 6) were determined by QRT-PCR.

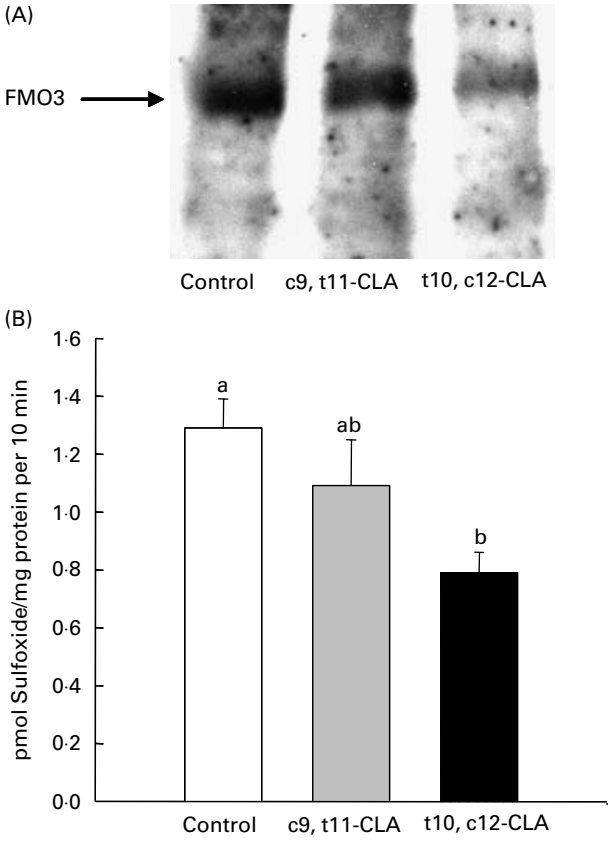

Fig. 2. Effect of dietary conjugated linoleic acid (CLA) isomers on mouse liver flavin-containing monooxygenase (FMO)-3 expression (A) and FMO activity (B). Data shown for FMO3 expression are representative of three experiments, while those for FMO activity are means with their standard errors represented by vertical bars (n 3). [a,b]Mean values with unlike superscript letters were significantly different ($P<0·05$). c9, t11-CLA, *cis* 9, *trans* 11-CLA; t10, c12-CLA, *trans* 10, *cis* 12-CLA. For details of diets and procedures, see pp. 59–61.

Discussion

We compared the effects of feeding two CLA preparations enriched in c9, t11-CLA and t10, c12-CLA isomers on the expression of hepatic enzymes involved in fatty acid metabolism. Transcription of CPT1a, FMO3, cyt P450 and PPARα genes was significantly reduced by the diet containing t10, c12-CLA as determined by both the microarray and real-time PCR methods (Table 3). CPT1 is the rate limiting enzyme for mitochondrial β oxidation, while PPARα regulates β oxidation in mitochondria and peroxisomes; cyt P450 and FMO3 are involved in the ω oxidation and drug detoxification in the microsomes. Expression of acetyl CoA oxidase, the rate limiting enzyme for peroxisomal fatty acid oxidation, was reduced by 50 % by the diet containing t10, c12-CLA, although it was not statistically significant ($P=0·08$; Table 3). The reduced expression of these five genes suggests that t10, c12-CLA may reduce fatty acid oxidation in the mitochondria, peroxisomes and the microsomes. Thus, reduced fatty oxidation in all these three organelles may contribute to the development of fatty liver in mice fed diets containing t10, c12-CLA. This diet also increased the expression of four lipogenic genes (ACC, FAS, ME and SCD1) by more than 2-fold; however, statistical significance was attained only for the expressions of ACC and FAS ($P<0·05$; Table 3). Neither of the CLA isomers significantly altered the expression of nuclear factors, liver X receptor α and regulatory element-binding protein-1c, that regulate fatty acid synthesis (Supplementary Table 1a–m). We cannot determine the specific contributions of reduced fatty acid oxidation and increased fatty acid synthesis to the development of fatty liver in animals fed a diet containing t10, c12-CLA, but it appears that both reduced fatty acid oxidation and increased fatty acid synthesis may contribute to the development of fatty liver. Other factors such as reduced transport of lipids from liver may also contribute to the development of the fatty liver; results from a published report (Poirier *et al.* 2005) and our own microarray data do not support this notion. Dietary c9, t11-CLA increased the liver lipid by only 15 %, which was not statistically significant. It did not reduce the expression of CPT1, acetyl CoA oxidase and PPARα genes and caused modest reductions in the expressions of FMO3 and cyt P450. These results suggest that c9, t11-CLA did not reduce the mitochondrial and peroxisomal fatty acid oxidation. The present study protocol cannot distinguish if the reductions in the expression of FMO3 and cyt P450 caused by c9, t11-CLA are specific to this isomer or because of its contamination with t10, c12-CLA. Completely pure CLA isomer preparations are needed to address this issue.

The present results showing down regulation of the genes involved in fatty acid oxidation by the t10, c12-CLA are at variance with those showing an increased expression of the CPT1a in mice fed a mixture of CLA isomers (Takahashi *et al.* 2003; Javadi *et al.* 2004; Ide, 2005) or a purified t10, c12-CLA isomer (Degrace *et al.* 2004). We are not sure of the reasons for this discrepancy, but the design and methods used were considerably different between the present and other studies. We used female mice and fully quantitative real-time RT-PCR techniques, while those investigators used male mice and semi-quantitative RT-PCR methods. Furthermore, it is inappropriate to compare our results with those obtained with a mixture of CLA isomers (Takahashi *et al.* 2003; Javadi *et al.* 2004; Ide, 2005), since the two isomers have contrasting effects on fatty acid metabolism (Roche *et al.* 2003; Kelley *et al.* 2004). The amount of CLA and the duration of its feeding were also different between the present and other studies. Most significantly, the basal diet in these studies with a mixture of CLA isomers was a high-fat diet (total fat 15–19 weight %) rich in SFA (palm oil in Takahashi *et al.* 2003; Ide, 2005) or SFA and MUFA (coconut, olive, palm and high oleic sunflower oils in Javadi *et al.* 2004). In the present study, maize oil (5 weight %) was the source of fat and 0·5 % CLA was incorporated by replacing an equivalent amount of the maize oil. It is possible that CLA may increase hepatic fatty acid oxidation when fed with high-fat diets that are rich in SFA or MUFA, since the amount and type of dietary fatty acids regulate the expression of hepatic genes and the development of non-alcoholic steatosis and steatohepatitis (Demizieux *et al.* 2002). In the study with the purified t10, c12-CLA, the basal diet contained sunflower and linseed oils and 1 weight % oleic acid or CLA were added to the control and test diets, respectively (Degrace *et al.* 2004). In this study both the *in vitro* CPT1 activity and mRNA expression were significantly increased by t10, c12-CLA. Also increased were the liver malonyl CoA that inhibits CPT1 and the sensitivity of CPTI to malonyl CoA

(50 % inhibition, 2 *v.* 12 μmol/l). These authors recognize the inconsistency between their results and propose that *in vivo* hepatic fatty acid oxidation may actually be suppressed by this isomer. We found reduction in mRNA not only for CPT1, but also for four other genes involved in fatty acid oxidation. The present results are consistent with the proposal put forward by the investigators, which was discussed earlier (Degrace *et al.* 2004), the *in vitro* reduction of fatty acid oxidation by isolated mitochondria treated with CLA (Clarke, 2001) and the development of fatty liver observed in many studies with CLA. Increased expression of the lipogenic genes, ACC, FAS, ME and SCD1, found in the present study are consistent with those of published reports (Takahashi *et al.* 2003; Degrace *et al.* 2004; Javadi *et al.* 2004; Ide, 2005).

The most dramatic effect of t10, c12-CLA in the present study was the down regulation of the genes for cyt P450 and FMO3, which are involved in the ω hydroxylation of the fatty acids and the production of dicarboxylic fatty acids (White *et al.* 1978; Krueger & Williams, 2005; Sanders *et al.* 2005; Weng *et al.* 2005). Once formed, dicarboxylic fatty acids can be shortened from either end of the molecule by β oxidation. This pathway plays a significant role in overall fatty acid oxidation during starvation and diabetes (Orellana *et al.* 1998). It may play a significant role in the overall hepatic fatty acid oxidation in mice fed diet containing t10, c12-CLA, since these animals do develop symptoms of diabetes (Poirier *et al.* 2005). This interpretation is also consistent with a recent finding that mice deleted of the cyt P450 gene develop a fatty liver (Weng *et al.* 2005). In addition to their role in fatty acid metabolism, both these enzymes detoxify numerous foreign compounds and also limit the length of time during which different drugs may be effective (Krueger *et al.* 2006). Thus, the suppression of these detoxifying enzyme systems may have additional health risks in addition to the development of fatty liver. FMO3 is the major hepatic isomer in man and a mutation in this gene causes trimethyaminuria, a condition wherein individuals excrete trimethylamine rather than trimethylamine oxide; trimethylamine produces a fishy odour in urine, sweat, breath and other bodily excretions (Seibel & Walsh, 2002). It is difficult to extrapolate the results of this and many other mice studies to man because the amount of CLA used in most of the mice studies is equivalent to 30–60 g/60 kg person per d (Kelley & Erickson, 2003); however, the long-term consumption of even lesser amounts of t10, c12-CLA by man may have serious health consequences.

In summary, the results of the present study indicate that the development of the fatty liver in mice fed diets containing t10, c12-CLA may be due both to reduced fatty acid oxidation and increased fatty acid synthesis. Results in addition to FMO3 need to be confirmed at the level of enzyme specific proteins and activities. To the best of our knowledge this is the first *in vivo* report that shows reduced expression of fatty acid oxidation and drug detoxification genes by t10, c12-CLA. Further studies are needed to determine the health consequences of the reduced expression of these genes by t10, c12-CLA.

Note

Supplementary information accompanies this paper on the journal's website (http://www.nutritionsociety.org).

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Supplementary Table 1a. Effect of CLA isomers on mouse liver gene expression

Gene name	probe set (Affimetx)	Accession #	Biological function/Process	Control Mean[a]	t10-,c12-CLA		c9-,t11CLA	
					Mean[a]	% Control	Mean[a]	% Control
Jun-B oncogene	1415899_at	NM_008416	Regulation of transcription	259	99·9	38·5 %	134	51·5 %
Thioredoxin interacting protein	1415997_at	AF173681	Response to oxidative stress	241	99·0	41·1 %	282	117 %
Scavenger receptor class B, member 1	1416050_a_at	NM_016741	Cell adhesion	1610	584	36·3 %	1030	64·0 %
FK506 binding protein 5	1416125_at	U16959	Protein folding	474	213	44·8 %	403	85·0 %
Serine (or cysteine) proteinase inhibitor	1416318_at	AF426024	Regulation of protein catabolism	241	29·1	12·1 %	137	57·1 %
Calcium binding protein, intestinal	1416497_at	J05186	Electron transport	1400	630	44·9 %	886	63·2 %
Plexin B2	1416683_at	NM_138749	Positive regulation of axonogenesis	943	439	46·6 %	775	82·2 %
Kidney expressed gene 1	1416833_at	NM_029550	–-	1120	456	40·7 %	927	82·7 %
Lymphocyte antigen 6 complex, locus D	1416930_at	NM_010742	Defense response	88·5	528	597 %	93·6	106 %
P450 (cytochrome) oxidoreductase	1416933_at	NM_008898	Electron transport	1460	501	34·4 %	1040	71·5 %
Angiopoietin-like 4	1417130_s_at	NM_020581	Negative regulation of apoptosis	1030	433	41·9 %	737	71·4 %
EH-domain containing 3	1417235_at	BM234719	—	957	459	48·0 %	946	98·9 %
Olfactory receptor 56	1417292_at	NM_008330	Defense response	764	370	48·4 %	618	80·9 %
Sphingosine kinase 2	1417431_a_at	AF245448	Protein kinase C activation	252	90·1	35·8 %	195	77·6 %
Actin dependent regulator of chromatin	1417440_at	NM_033566	Maintenance of chromatin architecture	571	276	48·4 %	519	90·9 %
Serine (or cysteine) proteinase inhibitor,	1417498_at	NM_008878	Acute-phase response	5390	2510	46·7 %	4770	88·5 %
RIKEN cDNA 1300003D03 gene	1417566_at	AK007421	Lipid metabolism	158	328	208 %	267	169 %
Expressed sequence AI481100	1417793_at	NM_019440	—	466	220	47·3 %	413	88·7 %
Murinoglobulin 1	1417835_at	NM_008645	Transporter activity	4920	2150	43·6 %	3180	64·6 %
Glycosylphosphatidylinositol phospholipase D1	1418050_at	NM_008156	Cell-matrix adhesion	1950	896	46·1 %	1470	75·6 %
Neurogenic differentiation 4	1418055_at	NM_007501	Regulation of transcription	225	31·5	14·0 %	233	104 %
Arginine vasopressin receptor 1A	1418603_at	D49729	Signal transduction	373	151	40·5 %	345	92·4 %
Histidine ammonia lyase	1418645_at	L07645	Histidine metabolism	2140	913	42·6 %	2200	103 %
Cytochrome P450, family 2,	1418653_at	NM_134144	Electron transport	5020	2390	47·7 %	3410	68·0 %
Solute carrier family 38, member 3	1418706_at	NM_023805	Ion transport	5020	2450	48·9 %	5000	100 %
CD2 antigen	1418770_at	NM_013486	Cell adhesion	52·3	158	302 %	135	258 %
Alanine-glyoxylate aminotransferase	1418833_at	NM_016702	Metabolism	3090	1090	35·3 %	2650	85·9 %
Pleckstrin homology-like domain	1418835_at	NM_009344	FasL biosynthesis	1840	550	29·9 %	1060	57·8 %
RIKEN cDNA 1200011D03 gene	1418858_at	NM_023617	Electron transport	1180	499	42·4 %	720	61·2 %
Annexin A2	1419091_a_at	NM_007585	Angiogenesis / fibrinolysis	234	511	218 %	301	128 %
Cytochrome P450, family 2. subfamily c,	1419094_at	NM_010001	Electron transport	6250	3030	48·5 %	4990	79·9 %
N-acetyltransferase 6	1419213_at	NM_019750	Cell cycle	649	317	48·9 %	546	84·0 %
Serum amyloid A 4	1419318_at	NM_011316	Acute-phase response	1460	460	31·4 %	1000	68·4 %

[a] All means are from 2 animals per group.

Supplementary Table 1b. Effect of CLA isomers on mouse liver gene expression

Gene name	probe set (Affimetx)	Accession #	Biological function/Process	Control Mean[a]	t10-,c12-CLA		c9-,t11CLA	
					Mean[a]	% Control	Mean[a]	% Control
Serum amyloid A 4	1419319_at	NM_011316	Acute-phase response	389	115	29·6 %	321	82·5 %
Diacylglycerol O-acyltransferase 2-like 1	1419504_at	NM_026713	Diacylglycerol biosynthesis	25·5	177	694 %	42·3	166 %
Lipase, hepatic	1419560_at	NM_008280	Lipid metabolism	1760	716	40·8 %	1230	70·1 %
Lectin, galactose binding, soluble 1	1419573_a_at	NM_008495	Myoblast differentiation	407	1600	394 %	651	160 %
Nuclear protein 1	1419665_a_at	NM_019738	—	69·3	175	253 %	90·0	130 %
RIKEN cDNA 5630401H01 gene	1419683_at	BB400773	Amino acid phosphorylation	177	1060	598 %	353	199 %
RIKEN cDNA 1700029F12 gene	1419715_at	NM_025585	—	328	116	35·5 %	273	83·5 %
Similar to plectin (LOC381012), mRNA	1419835_s_at	AW123286	—	503	232	46·0 %	461	91·7 %
Spinocerebellar ataxia 2 homolog (human)	1419866_s_at	AW544490	—	391	178	45·5 %	329	84·1 %
MOUSE TYROSINE-PROTEIN KINASE JAK3	1420310_at	BG083989		3·65	286	7850 %	3·99	109 %
Organic anion transporter family, member 1a1	1420379_at	AB031813	Ion transport	266	108	40·6 %	150	56·4 %
Organic anion transporter family, member 1a4	1420405_at	NM_030687	Ion transport	1160	518	44·7 %	709	61·1 %
Nucleosome assembly protein 1-like 1	1420479_a_at	BG064031	Nucleosome assembly	148	372	252 %	265	180 %
Neuronal d4 domain family member	1420529_at	AW553317	Regulation of transcription	35·0	146	417 %	34·3	97·9 %
Retinal dehydrogenase 6	1420541_at	NM_009040	Metabolism	608	1230	202 %	1070	176 %
ATP-binding cassette, sub-family G, member 8	1420656_at	AF324495	Transport	805	382	47·5 %	633	78·7 %
C-type lectin, superfamily member 12	1420699_at	NM_020008	Phagocytosis/ signal transduction	211	548	259 %	168	79·6 %
RIKEN cDNA 4933433D23 gene	1420836_at	BB032012	Transport	1260	494	39·2 %	953	75·6 %
Protein tyrosine phosphatase, receptor type, F	1420841_at	BF235516	Amino acid dephosphorylation	891	412	46·2 %	693	77·7 %
Protein tyrosine phosphatase, receptor type, F	1420843_at	BF235516	Amino acid dephosphorylation	2420	1080	44·9 %	1980	82·0 %
Glutathione S-transferase, alpha 2 (Yc2)	1421040_a_at	NM_008182	Transferase activity	90·1	321	356 %	130	145 %
Glutathione S-transferase, alpha 2 (Yc2)	1421041_s_at	NM_008182	Glutathione metabolism	150	519	347 %	192	129 %
Cytochrome P450, family 7, subfamily b	1421074_at	NM_007825	Lipid metabolism	637	256	40·3 %	379	59·6 %
ATP-binding cassette, sub-family C, member 6	1421212_at	NM_018795	Transport	867	400	46·2 %	755	87·1 %
cmp-N-acetylneuraminic acidhydroxylase	1421214_at	NM_007717	Electron transport	231	557	241 %	342	148 %
Solute carrier family 6, member 6	1421346_a_at	NM_009320	Beta-alanine transport	534	247	46·2 %	442	82·7 %
Prolactin receptor	1421382_at	NM_008932	Regulation of cell adhesion	1220	601	49·1 %	1320	108 %
RAD51-like 1 (S. cerevisiae)	1421430_at	NM_009014	DNA repair	108	324	301 %	182	169 %
RIKEN cDNA 5730402K07 gene	1421622_a_at	NM_019688	Protein amino acid phosphorylation	312	124	39·9 %	250	80·1 %
Cyclin-dependent kinase 8	1421741_at	NM_007820	Electron transport	443	182	41·2 %	472	107 %
Mitochondrial ribosomal protein L19	1421913_at	BB041267	Protein biosynthesis	39·4	162	411 %	86·9	220 %
Basic transcription element binding protein 1	1422264_s_at	NM_010638	Regulation of transcription	383	156	40·7 %	271	70·8 %

[a] All means are from 2 animals per group.

Supplementary Table 1c. Effect of CLA isomers on mouse liver gene expression

Gene name	probe set (Affimetx)	Accession #	Biological function/Process	Control Mean[a]	t10-,c12-CLA Mean[a]	t10-,c12-CLA % Control	c9-,t11CLA Mean[a]	c9-,t11CLA % Control
Cdk5 and Abl enzyme substrate 1	1422477_at	AF328140	Regulation of cell cycle	220	102	46·5 %	150	68·2 %
Metallothionein 1	1422557_s_at	NM_013602	Metal ion homeostasis	2140	883	41·3 %	1570	73·4 %
Solute carrier family 7, member 2	1422648_at	BF533509	Amino acid transport	430	182	42·4 %	248	57·8 %
Forkhead box Q1	1422735_at	NM_008239	Regulation of transcription	366	170	46·4 %	259	70·8 %
Insulin-like growth factor binding protein	1422826_at	NM_008340	Cell adhesion	1310	405	31·0 %	1170	89·5 %
SMC6 structural maintenance of chromosomes 6	1422910_s_at	AU022584	—	29·9	133	445 %	95	317 %
Peroxisomal acyl-CoA thioesterase 2A	1422925_s_at	NM_134246	Acyl-CoA metabolism	946	357	37·7 %	1380	146 %
Methyltransferase-like 3	1423099_a_at	AW556332	RNA methylation	403	149	36·8 %	214	53·1 %
CD36 antigen	1423166_at	BB534670	Cell adhesion	697	1470	210 %	842	121 %
Natural killer tumor recognition sequence	1423249_at	BB317504	Protein folding	21·1	122	578 %	106	501 %
Hepatoma-derived growth factor, related protein 3	1423252_at	BB291880	Cell proliferation	98·3	202	205 %	102	104 %
Frizzled homolog 8 (Drosophila)	1423348_at	AV345166	Signal transduction	202	40·1	19·8 %	149	73·8 %
Phosphoenolpyruvate carboxykinase 1, cytosolic	1423439_at	AW106963	Gluconeogenesis / lipid metabolism	4390	1690	38·5 %	3080	70·2 %
Serine (or cysteine) proteinase inhibitor	1423867_at	BF234005	Endopeptidase inhibitor	318	129	40·6 %	167	52·4 %
Peroxisomal biogenesis factor 6	1424078_s_at	BC003424	Protein binding	612	283	46·2 %	566	92·5 %
HECT domain containing 1	1424141_at	BC010205	Protein binding	1050	508	48·5 %	820	78·3 %
Thyrotroph embryonic factor	1424175_at	BC017689	Regulation of transcription	403	201	49·9 %	202	50·1 %
Carboxylesterase 2	1424245_at	BC015290	Hydrolase activity	204	528	259 %	331	162 %
RIKEN cDNA 1600023A02 gene	1424351_at	AF334269	Endopeptidase inhibitor	224	695	310 %	303	135 %
LIM and senescent cell antigen like domains 2	1424408_at	BC010816	Metal ion binding	705	342	48·5 %	514	72·9 %
cDNA sequence BC011468	1424544_at	BC012437	Metal ion binding	271	131	48·4 %	208	76·8 %
cDNA sequence BC034834	1424576_s_at	BC025819	Monooxygenase activity	2340	600	25·7 %	1670	71·4 %
RIKEN cDNA 4432417N03 gene	1424585_at	BC024698	—	365	159	43·6 %	304	83·5 %
RIKEN cDNA 2700053F16 gene	1424650_at	BC009151	Electron transport	531	240	45·2 %	374	70·5 %
Laminin, alpha 4	1424807_at	BB053010	Blood vessel development	109	246	226 %	126	116 %
RIKEN cDNA A330049M08 gene	1424838_at	BC005730	—	171	69·7	40·8 %	136	79·4 %
Bromodomain containing 4	1424922_a_at	BC008532	—	310	147	47·4 %	309	100 %
Epidermal growth factor receptor	1424932_at	U03425	Signal transduction	597	162	27·1 %	347	58·1 %
RIKEN cDNA C730032N17 gene	1425034_at	BC018306	Transport	641	311	48·5 %	370	57·7 %
RIKEN cDNA 2610034N03 gene	1425050_at	AK010892	Metabolism	226	463	205 %	422	187 %
Sodium channel, type I, alpha polypeptide	1425088_at	AF112185	Ion transport	265	113	42·5 %	218	82·1 %
Leukemia inhibitory factor receptor	1425107_a_at	D17444	Cytokine receptor activity	1830	534	29·2 %	1060	57·7 %
Colony stimulating factor 1 (macrophage)	1425154_a_at	M21149	Regulation of cell proliferation	261	578	221 %	521	200 %

[a] All means are from 2 animals per group.

Supplementary Table 1d. Effect of CLA isomers on mouse liver gene expression

Gene name	probe set (Affimetx)	Accession #	Biological function/Process	Control Mean[a]	t10-,c12-CLA		c9-,t11CLA	
					Mean[a]	% Control	Mean[a]	% Control
A disintegrin and metalloproteinase domain 15 (metargidin)	1425170_a_at	BC009132	Proteolysis and peptidolysis	87·9	209	237 %	72·8	82·8 %
RIKEN cDNA 2810433K01 gene	1425184_at	BG069231	—	159	57·9	36·4 %	140	88·1 %
cDNA sequence BC021917	1425300_at	BC021917	—	1040	2120	204 %	1900	183 %
zinc finger protein 295	1425305_at	BC027135	Protein binding	557	211	37·9 %	290	52·1 %
RIKEN cDNA 1300007K12 gene	1425365_a_at	BC018344	Metabolism	445	212	47·6 %	359	80·7 %
RIKEN cDNA 5730402K07 gene	1425518_at	AK004874	Protein amino acid phosphorylation	988	419	42·4 %	580	58·7 %
RIKEN cDNA 9130022B02 gene	1425615_a_at	BC010318	Gluconeogenesis	276	96·7	35·0 %	154	55·6 %
Cystathionine beta-synthase	1425623_a_at	BC013480	Amino acid metabolism	2170	1070	49·4 %	2050	94·7 %
beta-1,3-glucuronyltransferase 1	1425691_at	BM945167	Glucuronosyltransferase activity	179	67·1	37·5 %	174	97·4 %
TGF beta 1 induced transcript 4	1425742_a_at	AF201285	Regulation of transcription	569	1380	242 %	1070	188 %
cDNA sequence BC014805	1425751_at	AJ132857	—	416	83	19·9 %	346	83·1 %
cDNA sequence BC014805	1425752_at	AJ132857	—	344	70·8	20·6 %	219	63·8 %
Pregnancy-specific glycoprotein 28	1425881_at	AF113598	Pregnancy	47·4	779	1640 %	62·4	132 %
Sema domain, TM, and cytoplasmic domain 6A	1425903_at	AF288666	Cell differentiation	721	284	39·3 %	466	64·6 %
RIKEN cDNA 4933433D23 gene	1425948_a_at	BC022676	Transport	225	90·5	40·2 %	177	78·7 %
Regulator of G-protein signaling 16	1426037_a_at	U94828	Signal transduction	944	371	39·3 %	1420	151 %
Cullin 4A	1426060_at	BC007159	Cell cycle	52·5	250	476 %	63·1	120 %
Cullin 4A	1426061_x_at	BC007159	Cell cycle	54·8	224	409 %	67·0	122 %
Cytochrome P450, family 3, subfamily a	1426064_at	AB039380	Monooxygenase activity	1960	904	46·1 %	1520	77·7 %
Ribosome binding protein 1	1426123_a_at	AF273691	Protein transport	1090	508	46·8 %	942	86·7 %
Cryptochrome 2 (photolyase-like)	1426383_at	BF303057	Circadian rhythm	277	127	45·6 %	215	77·4 %
Nuclear receptor subfamily 1, group D, member 1	1426464_at	W13191	Regulation of transcription	373	34·7	9·31 %	209	55·9 %
Signal transducer and activator of transcription 3	1426587_a_at	AI325183	Photoreceptor cell differentiation	937	464	49·5 %	844	90·0 %
Heterogeneous nuclear ribonucleoprotein M	1426698_a_at	AK011521	Nucleic acid binding	293	132	44·9 %	236	80·6 %
Death associated protein kinase 1	1426915_at	BC021490	Protein amino acid phosphorylation	1060	508	47·8 %	996	93·7 %
Cysteine-rich motor neuron 1	1426951_at	AK018666	Regulation of cell growth	180	67·7	37·7 %	130	72·3 %
RIKEN cDNA 4632417N05 gene	1427082_at	AK014586	Nucleic acid binding	925	435	47·1 %	866	93·7 %
RIKEN cDNA 1500031N24 gene	1427093_at	BC026404	Regulation of transcription	305	135	44·2 %	258	84·6 %
FYVE and coiled-coil domain containing 1	1427177_at	AJ428065	—	220	79·7	36·2 %	165	75·0 %
Natriuretic peptide receptor 2	1427191_at	AW558468	cGMP biosynthesis	558	225	40·3 %	352	63·1 %
RIKEN cDNA 2510002A14 gene	1427199_at	BM118442	—	519	196	37·7 %	276	53·1 %
Tripartite motif protein 24	1427258_at	BB611004	Regulation of transcription	430	191	44·5 %	402	93·6 %
Tripartite motif protein 24	1427259_at	BB611004	Regulation of transcription	309	144	46·5 %	278	89·8 %

[a] All means are from 2 animals per group.

Supplementary Table 1e. Effect of CLA isomers on mouse liver gene expression

Gene name	probe set (Affimetx)	Accession #	Biological function/Process	Control Mean[a]	t10-,c12-CLA		c9-,t11CLA	
					Mean[a]	% Control	Mean[a]	% Control
DNA segment, Chr 11, ERATO Doi 498	1427323_s_at	BG068076	—	243	604	249 %	273	113 %
CLIP associating protein 2	1427328_a_at	BM221361	Microtubule depolymerization	73·5	196	267 %	142	194 %
RIKEN cDNA 2810021G02 gene	1427349_x_at	AK012776	Regulation of transcription	93·0	202	217 %	79·6	86 %
Immunoglobulin heavy chain 6	1427351_s_at	BB226392	Activation of MAPK activity	113	233	207 %	160	142 %
NACHT, leucine rich repeat & PYD containing 6	1427369_at	BB071996	Nucleic acid binding	875	390	44·6 %	708	80·9 %
RIKEN cDNA 1300019J08 gene	1427370_at	AK005066	Imidazolonepropionase activity	1400	648	46·4 %	1200	85·8 %
ATP-binding cassette, sub-family A, member 8a	1427371_at	BC026496	Transport	1300	418	32·2 %	784	60·3 %
Integrin beta 4	1427387_a_at	L04678	Cell adhesion	143	14·8	10·3 %	151	105 %
Clone:D230030K09 product:unknown EST	1427410_at	BB812902	—	205	84·9	41·4 %	107	51·9 %
RIKEN cDNA 1300018K11 gene	1427459_at	BC025836	—	2000	888	44·3 %	1560	77·8 %
Glutathione S-transferase, mu 3	1427473_at	J03953	Metabolism	127	303	238 %	191	150 %
Protein tyrosine phosphatase, receptor type, B	1427486_at	AF157628	Protein amino acid dephosphorylation	410	204	49·6 %	293	71·3 %
Zinc finger protein 125	1427536_at	AI615965	Nucleic acid binding	100	240	239 %	93·3	93·1 %
CD80 antigen	1427717_at	X60958	Defense response	49·1	155	315 %	58·4	119 %
Splicing factor, arginine/serine-rich 2 (SC-35)	1427815_at	U14648	mRNA processing	146	309	211 %	206	140 %
Tubulin, beta 2	1427838_at	M28739	Microtubule-based process	131	301	230 %	221	169 %
Acylphosphatase 2, muscle type	1427943_at	BI730288	Acylphosphatase activity	42·9	167	390 %	59·8	139 %
Clone:9130009H04 product:unknown EST	1428083_at	AK018202	—	1840	547	29·8 %	1310	71·1 %
basic transcription element binding protein 1	1428289_at	AW488885	Transcription	1380	473	34·3 %	788	57·2 %
RIKEN cDNA 2610042L04 gene	1428301_at	BM195235	—	32·0	161	504 %	28·9	90·4 %
RIKEN cDNA 5830413E08 gene	1428306_at	AK017926	—	67·6	220	326 %	156	231 %
RIKEN cDNA 4121402D02 gene	1428872_at	AW495537	—	441	203	46·0 %	300	68·0 %
RIKEN cDNA 1810013D05 gene	1429523_a_at	AK008448		25·3	184	727 %	60·9	241 %
Protein geranylgeranyltransferase type I, b subunit	1429769_at	BI107300		43·1	149	345 %	121	280 %
Clone:5330406M23 product:unknown EST	1429900_at	BM241296		499	193	38·8 %	571	115 %
Spermatogenesis associated glutamate-rich prot	1429993_s_at	AK006975		17·8	118	663 %	28·3	159 %
RIKEN cDNA 4930432J16 gene	1430076_at	AK015290		223	103	46·1 %	208	93·0 %
RIKEN cDNA 4930539A06 gene	1430666_at	AK015993		306	1090	356 %	235	76·9 %
kidney-derived aspartic protease-like protein	1430744_at	AK007861		306	81·5	26·6 %	187	61·1 %
RIKEN cDNA 1700051I12 gene	1430855_at	AK006759		87·9	222	253 %	137	156 %
RIKEN cDNA 2410030K01 gene	1431087_at	BF577722		100	249	250 %	157	157 %
Clone:4921537I17 product:unknown EST	1431259_at	BE635076		59·3	225	380 %	62·1	105 %

[a] All means are from 2 animals per group.

Supplementary Table 1f. Effect of CLA isomers on mouse liver gene expression

Gene name	probe set (Affimetx)	Accession #	Biological function/Process	Control Mean[a]	t10-,c12-CLA Mean[a]	t10-,c12-CLA % Control	c9-,t11CLA Mean[a]	c9-,t11CLA % Control
Clone:9530046B11 product:unknown EST	1431493_at	AK020596		146	361	247 %	120	82·3 %
RIKEN cDNA 4933404G15 gene	1431509_at	AV278869		140	38·9	27·8 %	114	80·9 %
RIKEN cDNA 2610100K07 gene	1431571_at	AV283841		61·3	204	332 %	63·8	104 %
Hypothetical protein C330001M22	1431698_at	AK020009		103	439	428 %	93·0	90·5 %
Clone:4930422C21	1431842_at	AK015175		175	71·9	41·0 %	217	124 %
Clone:2700089I24 product:unclassifiable	1431912_at	AK012591		346	113	32·6 %	339	97·8 %
Acyloxyacyl hydrolase	1431989_at	AK015300		179	370	207 %	213	119 %
Endothelial cell growth factor 1 (platelet-derived)	1432181_s_at	AK013765	Pyrimidine base metabolism	1990	978	49·1 %	2030	102 %
Clone:2610012C04 product:unclassifiable	1432606_at	AI120134		55·2	172	311 %	107	194 %
CLONE = 4921527H02	1432694_at	AK014973		11·4	203	1780 %	7·13	62·4 %
gb:AK014604·1	1432885_at	AK014604		21·7	130	598 %	21·9	101 %
gb:AK017704·1	1433049_at	AK017704		88·4	277	313 %	87·3	98·8 %
Golgi autoantigen, golgin subfamily b,	1433135_at	AK015226		154	49·8	32·4 %	167	109 %
Cyclin-dependent kinase inhibitor 1A (P21)	1433359_at	AK006261		378	181	47·9 %	266	70·3 %
gb:AK005121·1	1433383_at	AK005121		64·7	250	387 %	88·0	136 %
Clone:B930037P14 product:	1433449_at	BM233059		83·0	213	256 %	147	178 %
RIKEN cDNA 5830434P21 gene	1433624_at	AV316216		530	264	49·8 %	405	76·4 %
RIKEN cDNA E130305N23 gene	1433632_at	BB183385		303	133	43·9 %	264	87·2 %
RIKEN cDNA E130305N23 gene	1433634_at	BB183385		1190	551	46·2 %	1150	96·0 %
Nischarin	1433757_a_at	BB025231	Negative regulation of cell migration	351	147	41·9 %	259	73·8 %
RIKEN cDNA F830029L24 gene	1433834_at	BQ176049	Metal ion binding	1390	682	49·1 %	1160	83·6 %
RIKEN cDNA 8430408G22 gene	1433837_at	AV365503		1520	431	28·3 %	880	57·9 %
RIKEN cDNA 4933433D23 gene	1433898_at	AV000840		586	245	41·8 %	537	91·8 %
RIKEN cDNA 5430413K10 (LOC329543)	1433967_at	AI413838		142	30·8	21·7 %	114	80·0 %
Hypothetical protein C130032F08	1434044_at	AV286809	Inflammatory response	517	204	39·5 %	422	81·6 %
Solute carrier family 4, member 4	1434096_at	BB283443		720	346	48·1 %	624	86·7 %
Endothelin converting enzyme 1	1434177_at	AI551117	Peptide hormone processing	1150	334	29·2 %	993	86·6 %
gb:BG076122	1434278_at	BG976607	Phospholipid dephosphorylation	54·8	273	498 %	59·7	109 %
gb:BG976607	1434280_at	BG976607	—	25·4	242	951 %	17·3	68·1 %
Transient immune abnormalities	1434283_at	BB079486		561	199	35·5 %	379	67·5 %
Insulin receptor	1434446_at	BM206023		679	317	46·7 %	524	77·2 %
RIKEN cDNA A130015N09 gene	1434473_at	AI647939		301	130	43·1 %	261	86·5 %
RIKEN cDNA 2610103N14 gene	1434592_at	BB735478		452	212	46·9 %	305	67·5 %
v-erb-b2 erythroblastic leukemia viral oncogene	1434606_at	BF140685	Regulation of cell cycle	1460	532	36·5 %	1040	71·2 %

[a] All means are from 2 animals per group.

Supplementary Table 1g. Effect of CLA isomers on mouse liver gene expression

Gene name	probe set (Affimetx)	Accession #	Biological function/Process	Control Mean[a]	t10-,c12-CLA Mean[a]	t10-,c12-CLA % Control	c9-,t11CLA Mean[a]	c9-,t11CLA % Control
Similar to Cdc42-binding protein kinase beta	1434652_at	BI154551		259	122	47·2 %	270	104 %
Forkhead box O3	1434831_a_at	BB364488	Regulation of transcription	263	128	48·5 %	198	75·4 %
Carnitine palmitoyltransferase 1a, liver	1434866_x_at	BB021753	Lipid metabolism	1070	435	40·7 %	827	77·4 %
Two pore channel 1	1434930_at	BI904914	—	480	222	46·3 %	426	88·6 %
DNA segment, Chr 4, Wayne State University 53	1435357_at	BE652553	—	334	167	49·8 %	273	81·6 %
Expressed sequence AI464131	1435417_at	BG063189	—	257	128	49·8 %	239	92·9 %
Mus musculus transcribed sequences	1435436_at	BI647951		1600	618	38·6 %	1590	99·2 %
Flavin containing monooxygenase 2	1435459_at	BM936480		290	58·7	20·3 %	225	77·7 %
Clone:C030039M13 product:RAN binding protein 16,	1435814_at	BB198104		15·2	126	831 %	10·7	70·1 %
RIKEN cDNA 1810063B05 gene	1435864_a_at	BG975168	—	371	820	221 %	409	110 %
Contrapsin-like protease inhibitor-related protein	1435887_at	BB806208		1270	607	47·9 %	941	74·2 %
RIKEN cDNA 5430400N05 gene	1435954_at	BG069330		444	922	208 %	456	103 %
Clone:6430557O09	1435961_at	BB702376		1·28	109	8480 %	0·480	37·5 %
Similarity to protein ref:NP_057365·1	1435964_a_at	BB194075	—	718	228	31·8 %	449	62·6 %
Clone:4930512I01	1436054_at	BM220028		422	146	34·5 %	284	67·3 %
Mus musculus transcribed sequences	1436093_at	BE981269		655	277	42·3 %	457	69·8 %
DNA Segment, Mouse Genome Informatics 8	1436128_at	AI324154		89·4	200	223 %	98·7	110 %
LISCH7-like	1436221_at	BG067625		208	93·2	44·8 %	159	76·3 %
Clone:B230214O09 product:unknown EST	1436240_at	BM211445		212	99·1	46·7 %	111	52·3 %
LISCH7-like	1436293_x_at	AI852300		444	196	44·1 %	296	66·8 %
Clone:B930092N05 product:unknown EST,	1436317_at	BM115569		186	83·8	45·1 %	89·7	48·3 %
Nuclear factor I/X	1436363_a_at	AW049660	DNA replication	1040	346	33·1 %	647	62·0 %
Nuclear factor I/X	1436364_x_at	AW049660	DNA replication	1000	333	33·2 %	642	64·1 %
RIKEN cDNA B830010L13 gene	1436731_at	BB333374		230	74·2	32·3 %	118	51·5 %
Angiotensin receptor 1	1436739_at	AI551199		1070	527	49·4 %	1090	102 %
aarF domain containing kinase 5	1436753_at	BB317588		275	135	49·1 %	220	80·2 %
Basic transcription element binding protein 1	1436763_a_at	AI267126	Transcription	282	122	43·1 %	166	58·8 %
Partitioning defective 3 homolog (C. elegans)	1436764_at	BE199556		329	144	43·9 %	247	75·2 %
RIKEN cDNA 4933433D23 gene	1437073_x_at	BB115446		911	409	44·9 %	800	87·9 %
Proviral integration site 3	1437100_x_at	BB206220	Protein amino acid phosphorylation	397	196	49·4 %	438	110 %
Fibronectin 1	1437218_at	BM234360	Acute-phase response	406	163	40·1 %	359	88·4 %
Scavenger receptor class B, member 1	1437378_x_at	BB224405	Cell adhesion	927	327	35·3 %	557	60·1 %
Expressed sequence AI987712	1437397_at	AW554594		1010	359	35·4 %	606	59·7 %
Clone:C130020M04:transcription factor	1437645_at	BE225843	Transcription	295	134	45·3 %	176	59·8 %

[a] All means are from 2 animals per group.

Supplementary Table 1h. Effect of CLA isomers on mouse liver gene expression

Gene name	probe set (Affimetx)	Accession #	Biological function/Process	Control Mean[a]	t10-,c12-CLA		c9-,t11CLA	
					Mean[a]	% Control	Mean[a]	% Control
Annexin A2	1437692_x_at	AW240637	Angiogenesis	187	492	263 %	229	122 %
Sal-like 1 (Drosophila)	1437983_at	BB739342		406	188	46·2 %	301	74·1 %
Spinocerebellar ataxia 2 homolog (human)	1438143_s_at	BB705334	—	686	299	43·7 %	609	88·8 %
Carnitine palmitoyltransferase 1a, liver	1438156_x_at	BB119196	Lipid metabolism	970	379	39·1 %	733	75·6 %
Solute carrier family 1, member 2	1438194_at	AW488243		542	247	45·6 %	360	66·4 %
Mus musculus transcribed sequences	1438245_at	BI664122	—	476	203	42·7 %	252	52·9 %
Cysteine conjugate-beta lyase	1438348_x_at	BB039821		330	160	48·3 %	245	74·0 %
Zinc finger protein 288	1438443_at	BB751546	DNA binding	625	298	47·7 %	428	68·5 %
Similar to PRAMEl4 (LOC384075), mRNA	1438468_at	BG070047	—	348	155	44·5 %	338	97·1 %
Hypothetical protein E130112L23	1438485_at	BB770854	—	203	84·5	41·7 %	175	86·5 %
RIKEN cDNA A630025O09 gene	1438596_at	AW114007		479	202	42·1 %	329	68·6 %
Mus musculus transcribed sequences	1438643_at	BB230839		424	158	37·3 %	274	64·6 %
Calmin	1439117_at	AU067755		409	184	44·9 %	241	58·9 %
Clone:B230308F23 product:unknown EST,	1439128_at	AI595815		561	270	48·0 %	346	61·6 %
Deltex 2 homolog (Drosophila)	1439429_x_at	BB518874	Notch signaling pathway	147	45·4	30·9 %	100	68·4 %
Phosphoenolpyruvate carboxykinase 1, cytosolic	1439617_s_at	AI265463		1260	305	24·1 %	772	61·1 %
CLONE = 9330161F22 /UG_TITLE = ESTs,	1439920_at	BB080140		38·7	387	999 %	38·7	100 %
Clone:C130042N08/ KIAA1276 protein	1440200_at	BB128528		202	37·3	18·5 %	105	52·1 %
CLONE = C630033O16 /FEA = EST	1440508_at	BB430574		52·1	178	342 %	56·9	109 %
Mus musculus transcribed sequences	1440730_at	BB284266		40	155	386 %	99·8	249 %
Mus musculus transcribed sequences	1440790_x_at	BB394466		256	103	40·3 %	159	62·0 %
CLONE = 9430066O17 /UG_TITLE = ESTs	1440815_x_at	BB099075		26·3	137	520 %	24·6	93·3 %
cDNA sequence BC035291	1440836_at	BB090304		324	155	47·7 %	293	90·4 %
METASTASIS SUPPRESSOR PROTEIN homolog	1440847_at	BB326749		302	101	33·5 %	177	58·4 %
RIKEN cDNA 2510049J12 gene	1440916_at	BE200006		136	408	301 %	193	142 %
Leucine-rich-repeat protein	1440921_at	AI527293		359	69·7	19·4 %	204	56·8 %
RAR-related orphan receptor alpha	1441085_at	AW494655		56·4	223	396 %	60·5	107 %
Expressed sequence AI987712	1441102_at	BB429201		199	86·1	43·2 %	148	74·0 %
Clone:5430414B12 product:unknown EST	1441109_at	BG070250		117	266	227 %	231	197 %
Mbt domain containing 1	1441287_at	BB369299		231	113	49·1 %	243	105 %
Mus musculus transcribed sequences	1441343_at	BG070780		251	101	40·2 %	237	94·5 %
Mus musculus transcribed sequences	1441392_at	BB307362		150	47·5	31·7 %	112	74·7 %
RIKEN cDNA 1110056A04 gene	1441551_at	BB196537		229	111	48·5 %	198	86·6 %

[a] All means are from 2 animals per group.

Supplementary Table 1i. Effect of CLA isomers on mouse liver gene expression

Gene name	probe set (Affimetx)	Accession #	Biological function/Process	Control Mean[a]	t10-,c12-CLA Mean[a]	t10-,c12-CLA % Control	c9-,t11CLA Mean[a]	c9-,t11CLA % Control
Mus musculus transcribed sequences	1441580_at	AW551517		113	230	203 %	176	155 %
Clone:9530006C21 product:unknown EST	1441779_at	BB750043		270	130	48·1 %	161	59·6 %
RIKEN cDNA 4930471K13 gene	1441790_at	AW489900		111	253	228 %	130	117 %
RIKEN cDNA 1500031N24 gene	1441842_s_at	AV031885		273	92·8	34·0 %	175	63·9 %
Clone:B230330B21 product:unknown EST	1442416_at	BB311940		92·5	207	223 %	89·7	96·9 %
Mus musculus transcribed sequences	1442593_at	AW553880		460	190	41·2 %	291	63·3 %
Mus musculus transcribed sequences	1442688_at	BG084733		14·6	144	993 %	—	—
Mus musculus transcribed sequences	1442694_at	BG066676	—	157	50·4	32·1 %	105	66·6 %
Phosphodiesterase 4B, cAMP specific	1442700_at	BG793493		256	126	49·4 %	231	90·3 %
Clone:A130024J23 product:unknown EST,	1442812_at	BB155332		19·9	130	653 %	14·9	74·9 %
Mus musculus transcribed sequences	1443147_at	BB505010		830	207	24·9 %	914	110 %
Mus musculus transcribed sequences	1443516_at	BE953583		189	83·6	44·2 %	127	67·4 %
Sorbin and SH3 domain containing 1	1443983_at	BB218653		180	68·7	38·1 %	120	66·6 %
RIKEN cDNA A130090K04 gene	1444298_at	BB703415		119	247	207 %	203	170 %
RIKEN cDNA A130039I20 gene	1444311_at	BB138395		151	46·6	30·9 %	175	116 %
APLT class I, type 8A, member 1	1444355_at	AW125445		33·5	135	401 %	112	334 %
Clone:D630017L16 product:EST	1444425_at	BE994902		0·160	134	83 500 %	60·6	37 900 %
M.musculus S12207 hypothetical protein	1444458_at	AI593288		228	103	45·2 %	147	64·3 %
Mus musculus transcribed sequences	1444518_at	BM240237		374	175	46·7 %	401	107 %
Clone:A830083P21 product:unknown EST	1444627_at	BB273517		119	292	245·0 %	158	133 %
Mus musculus transcribed sequences	1444700_at	BE985761		70·7	176	248·0 %	137	194 %
gb:BG069414	1444927_at	BG069414		30·0	177	591 %	22·7	75·7 %
gb:BG066639	1445196_at	BG066639		257	122	47·5 %	267	104 %
Killer cell lectin-like receptor subfamily B	1445399_at	AV294178		677	231	34·1 %	505	74·6 %
Clone:6430400O22 product:unknown EST	1445402_at	AV337434		268	112	41·7 %	175	65·5 %
cDNA sequence BC031575	1445862_at	BB123487		307	142	46·2 %	169	55·1 %
RIKEN cDNA C730034F03 gene	1446063_at	BB667700		188	67·1	35·6 %	124	65·6 %
Clone:7030419G12 product:unknown EST	1446270_at	BB535337		32·3	144	446 %	121	375 %
gb:BG068594 /UG_TITLE = ESTs	1446602_at	BG068594		96·6	205	212 %	108	111 %
Mus musculus transcribed sequences	1446848_at	C77955		196	83·7	42·8 %	130	66·2 %
M.musculus S12207 hypothetical protein	1446850_at	BM234464		225	83·4	37·1 %	175	77·9 %
Mus musculus transcribed sequences	1447121_at	BM224713		264	102	38·5 %	142	53·9 %

[a] All means are from 2 animals per group.

Supplementary Table 1j. Effect of CLA isomers on mouse liver gene expression

Gene name	probe set (Affimetx)	Accession #	Biological function/Process	Control Mean[a]	t10-,c12-CLA		c9-,t11CLA	
					Mean[a]	% Control	Mean[a]	% Control
gb:AI647619 /UG_TITLE = ESTs	1447172_at	AI647619		275	137	49·9 %	207	75·3 %
gb:AI506532 / /UG_TITLE = ESTs,	1447329_at	AI506532		11·1	140	1260 %	9·47	85·7 %
gb:BE954474 /UG_TITLE = ESTs	1447458_at	BE954474		1080	334	31·1 %	676	62·8 %
Clone:2900006A08 product:unknown EST	1448019_at	BE849316	—	322	152	47·4 %	266	82·7 %
Mus musculus transcribed sequences	1448080_at	AI256288		695	236	34·0 %	478	68·8 %
Kruppel-like factor 15	1448181_at	BC013486	Transcription	822	390	47·5 %	640	77·9 %
Histone H1-like protein in spermatids 1	1448512_at	NM_018792	Transcription	92·0	215	234 %	145	157 %
Lipopolysaccharide binding protein	1448550_at	NM_008489	Lipid transport	1640	764	46·6 %	1160	70·5 %
Prolactin receptor	1448556_at	BC005555	Regulation of cell adhesion	774	213	27·6 %	436	56·4 %
RIKEN cDNA 2310066P17 gene	1448626_at	NM_025876	Metal ion binding	258	105	40·7 %	309	120 %
Low density lipoprotein receptor-related protein 1	1448655_at	NM_008512	Lipid metabolism	1560	760	48·9 %	1400	89·7 %
Occludin	1448873_at	NM_008756	Protein binding	243	83·3	34·3 %	143	58·9 %
Lactoperoxidase	1448998_at	NM_080420	Peroxidase activity	1150	510	44·4 %	820	71·3 %
Kallikrein B, plasma 1	1449034_at	BC026555	Inflammatory response	2910	1380	47·5 %	2110	72·5 %
Peroxisome proliferator activated receptor alpha	1449051_at	BC016892	Glucose / lipid metabolism	1700	795	46·8 %	1390	81·8 %
Dihydrolipoamide branched chain transacylase E2	1449118_at	NM_010022	Metabolism	577	288	49·9 %	396	68·6 %
Midnolin	1449188_at	NM_021565	—	346	173	49·8 %	320	92·3 %
Ribosome binding protein 1	1449221_a_at	NM_133626	Protein targeting	1150	542	46·9 %	981	84·9 %
Kelch-like 1 (Drosophila)	1449241_at	NM_053105	Actin binding	19·4	138	710 %	15·7	80·9 %
Cytochrome P450, family 4, subfamily f	1449316_at	NM_134127	Electron transport	1690	641	37·8 %	1200	71·1 %
RIKEN cDNA 9130231C15 gene	1449375_at	NM_133960	Carboxylesterase activity	1220	557	45·5 %	997	81·5 %
Hydroxysteroid (17-beta) dehydrogenase 9	1449385_at	NM_013786	Metabolism	2280	793	34·8 %	1260	55·4 %
Flavin containing monooxygenase 3	1449525_at	NM_008030	Electron transport	1820	30	1·65 %	1010	55·6 %
Fetuin beta	1449555_a_at	NM_021564	Cysteine protease inhibitor activity	3680	1510	41·0 %	3030	82·1 %
Nuclear receptor subfamily 0, group B, member 2	1449854_at	BC019540	Transcription	509	188	36·9 %	566	111 %
Hyaluronidase 1	1449954_at	NM_008317	Cell cycle	238	112	46·9 %	186	78·1 %
Polymeric immunoglobulin receptor	1450060_at	NM_011082	Receptor activity	3390	1340	39·5 %	2850	84·2 %
Growth arrest specific 2	1450112_a_at	NM_008087	Apoptosis	280	582	208 %	314	112 %
Clone:E330039I02 product:weakly similar FRAGMENT	1450192_at	NM_013582	Signal transduction	47·2	151	320 %	53·1	113 %
RIKEN cDNA C730049F20 gene	1450717_at	NM_007447	Cell differentiation	3070	1160	37·9 %	1890	61·7 %
CD36 antigen	1450883_a_at	BB534670	Transport / cell adhesion	570	1480	259 %	848	149 %
CD36 antigen	1450884_at	BB534670	Transport / cell adhesion	92·0	212	231 %	97·9	106 %

[a] All means are from 2 animals per group.

Supplementary Table 1k. Effect of CLA isomers on mouse liver gene expression

Gene name	probe set (Affimetx)	Accession #	Biological function/Process	Control Mean[a]	t10-,c12-CLA		c9-,t11CLA	
					Mean[a]	% Control	Mean[a]	% Control
Fusion, derived from t(12;16) malignant liposarcoma	1451285_at	AF224264	Regulation of transcription	564	233	41·4 %	699	124 %
Expressed sequence R75183	1451348_at	BC004774	Rntracellular signaling cascade	1350	597	44·2 %	882	65·4 %
Regulator of G-protein signaling 16	1451452_a_at	U72881	Regulation of signal transduction	218	107	49·2 %	402	184 %
Solute carrier family 22, member 7	1451460_a_at	BC026598	Ion transport /	430	176	41·0 %	281	65·3 %
RIKEN cDNA 1110028A07 gene	1451488_at	AB054000	—	112	396	353 %	199	177 %
RIKEN cDNA 4930438M06 gene	1451543_at	BC021871	Ubiquitin cycle	337	161	47·8 %	231	68·7 %
Solute carrier family 1, member 2	1451627_a_at	U75372	Dicarboxylic acid transport	504	231	45·8 %	399	79·1 %
Hypothetical protein D630002G06	1451635_at	AB056443	Protein targeting	98	246	251 %	126	128 %
C/EBP, gamma	1451639_at	AB012273	Regulation of transcription	143	299	208 %	230	160 %
cis-retinol/3alpha hydroxysterol short-chain dehydrogenase-like	1451681_at	BC018263	—	2840	1370	48·1 %	2540	89·4 %
v-maf musculoaponeurotic fibrosarcoma oncogene	1451716_at	AW412521	Transcription	213	50·7	23·8 %	261	123 %
Two pore channel 1	1451772_at	AF217002	—	304	144	47·2 %	228	75·0 %
Coagulation factor XI	1451788_at	AF356627	Proteolysis and peptidolysis	465	226	48·6 %	355	76·4 %
RIKEN cDNA 9130422G05 gene	1452008_at	AK018685	—	208	90·8	43·7 %	126	60·8 %
RIKEN cDNA 2010005A06 gene	1452294_at	AK008111	Homophilic cell adhesion	554	220	39·7 %	500	90·4 %
DNA segment, Chr 14,	1452406_x_at	AJ007909	—	230	114	49·5 %	289	126 %
gb:BB662083	1452433_at	BB662083	—	120	425	355 %	119	99·6 %
gb:X14678·1 /UG_TITLE = zinc finger protein 36	1452519_a_at	X14678	mRNA catabolism	1190	484	40·7 %	1350	114 %
Cysteine conjugate-beta lyase	1452678_a_at	AK008165		420	183	43·5 %	297	70·8 %
Cullin 5	1452722_a_at	BB702110		25·9	153	589 %	126	485 %
RIKEN cDNA 4930438D12 gene	1452943_at	BF383782		40·3	264	654 %	76·7	190 %
Sestrin 3	1453313_at	AK017464		381	189	49·5 %	271	71·1 %
RIKEN cDNA 3830408G10 gene	1453345_at	AK014427		425	188	44·3 %	274	64·6 %
RIKEN cDNA 1500005J14 gene	1453369_a_at	AK007686	—	167	336	201 %	215	128 %
Flavin containing monooxygenase 2	1453435_a_at	AK009753		249	73·3	29·4 %	203	81·6 %
Insulin-like growth factor binding protein 2	1454159_a_at	AK011784	Regulation of cell growth	3820	1070	28·1 %	2660	69·7 %
Expressed sequence AI450344	1454617_at	BG072824		1830	792	43·2 %	1260	68·9 %
RIKEN cDNA E430026E19 gene	1454646_at	BM245221		276	134	48·6 %	161	58·4 %
Spermatogenesis associated 13	1454656_at	AV271736	—	311	147	47·4 %	201	64·9 %
TGF beta 1 induced transcript 4	1454758_a_at	AU016382	Regulation of transcription,	388	852	220 %	691	178 %
Retinoid X receptor alpha	1454773_at	BQ175050	—	1270	619	48·9 %	1070	84·7 %
Fibrillin 2	1454830_at	AV010392		209	61·9	29·6 %	127	60·9 %
gb:BG066923 / = ectodermal-neural cortex 1	1454904_at	BG976607	Phospholipid dephosphorylation	50·6	200	394 %	56·4	111 %

[a] All means are from 2 animals per group.

Supplementary Table 1l. Effect of CLA isomers on mouse liver gene expression

Gene name	probe set (Affimetx)	Accession #	Biological function/Process	Control Mean[a]	t10-,c12-CLA		c9-,t11CLA	
					Mean[a]	% Control	Mean[a]	% Control
Mus musculus transcribed sequences	1454971_x_at	BB357514	Regulation of transcription	680	1610	237 %	1160	171 %
Clone:D330042I16 product:unknown EST	1454984_at	AV246615	—	1720	548	31·8 %	1290	75·2 %
Regulator of G-protein signaling 16	1455265_a_at	BB100249	Regulation of signal transduction	691	306	44·2 %	926	134 %
Preferred translocation partner in lipoma	1455314_at	BM236111		805	379	47·1 %	612	76·1 %
Clone:D930035P11 product:unknown EST	1455324_at	BQ176176		803	345	43·0 %	538	67·0 %
Angiopoietin 4	1455427_at	AV269710		98·8	250	253 %	21·6	21·9 %
Lectin, galactose binding, soluble 1	1455439_a_at	AI642438	Myoblast differentiation	324	1510	465 %	507	157 %
Polymeric immunoglobulin receptor	1455490_at	AV027632	Regulation of transcription	5020	2280	45·4 %	4370	87·0 %
H.sapiens STE20-like kinase	1455733_at	AW208927	Threonine kinase activity	541	183	33·8 %	395	73·0 %
Endothelin converting enzyme 1	1455741_a_at	AW553715	Peptide hormone processing	895	396	44·2 %	865	96·5 %
Scavenger receptor class B, member 1	1455820_x_at	BB138434	Cell adhesion	555	167	30·1 %	303	54·7 %
Beta-site APP cleaving enzyme	1455826_a_at	BB114336	Proteolysis and peptidolysis	191	82·6	43·2 %	135	70·8 %
Synaptonemal complex protein 3	1455901_at	AI642069	Transferase activity	213	474	222 %	165	77·5 %
Expressed sequence C77892	1456112_at	AW554765	—	163	340	208 %	322	197 %
RIKEN cDNA 2610205E22 gene	1456340_at	AV309800	—	66·6	284	427 %	69·8	105 %
Basic transcription element binding protein 1	1456341_a_at	AV354744	Transcription	1290	470	36·4 %	754	58·4 %
Cartilage homeo protein 1	1456454_at	BB759122		170	36·5	21·5 %	84·4	49·6 %
cDNA sequence BC038313	1456610_at	AW763746		196	75·1	38·2 %	154	78·2 %
RIKEN cDNA 4933430A16 gene	1456614_at	BF122715		126	290	230 %	119	94·4 %
RIKEN cDNA E130016E03 gene	1456674_at	BB772877		500	223	44·6 %	475	95·0 %
Mus musculus transcribed sequences	1456710_at	BB481932		822	383	46·6 %	498	60·6 %
mKIAA0881 protein	1456826_at	BB313996		254	98·9	39·0 %	187	73·8 %
Dehydrogenase E1 and transketolase	1457027_at	BB667395		336	163	48·6 %	215	64·1 %
Mus musculus transcribed sequences	1457110_at	BB440150		180	69·2	38·3 %	91·1	50·5 %
Mus musculus transcribed sequences	1457132_at	BF456117		31·2	144	462 %	90·0	289 %
Mus musculus transcribed sequences	1457380_at	C85504		62·1	184	297 %	75·5	122 %
Similar to fatty acid desaturase (LOC240957)	1457403_at	AV378018		88·1	194	220 %	76·1	86·3 %
Mus musculus transcribed sequences	1457520_at	C76369	Regulation of transcription	32·1	141	440 %	63·3	197 %
Expressed sequence AV047578	1457627_x_at	AV210805		82·8	183	221 %	173	209 %
Clone:B130004P22 product:unclassifiable	1458099_at	BB291417		504	176	34·8 %	298	59·1 %
Hypothetical protein 1190030G24	1458128_at	BB363084		192	436	227 %	304	159 %
Mus musculus transcribed sequences	1458304_at	BE985725		121	249	206 %	110	90·7 %
Weak similarity to protein ref:NP_038603·1	1459015_at	BG079315		1410	677	48·1 %	947	67·3 %

[a] All means are from 2 animals per group.

Supplementary Table 1m. Effect of CLA isomers on mouse liver gene expression

Gene name	probe set (Affimetx)	Accession #	Biological function/Process	Control Mean[a]	t10-,c12-CLA		c9-,t11CLA	
					Mean[a]	% Control	Mean[a]	% Control
gb:BG074885 /UG_TITLE = ESTs	1459407_at	BG074885		392	955	244 %	331	84·6 %
gb:C79743 /	1459468_at	C79743		553	264	47·8 %	423	76·4 %
Carnitine palmitoyltransferase 1a, liver	1460409_at	AI987925	Lipid metabolism	2480	1010	40·9 %	2260	91·3 %
RIKEN cDNA 2610042L04 gene	1460500_at	AK017182		77·9	188	241 %	102	131 %
cDNA sequence BC010245	1460559_at	BB038765		319	155	48·4 %	325	102 %
Fas-activated serine/threonine kinase	1460635_at	NM_023229	Apoptosis	482	236	48·9 %	379	78·6 %
CEA-related cell adhesion molecule 2	1460682_s_at	BC024320	—	1470	727	49·5 %	1530	104 %

[a] All means are from 2 animals per group.