Original Communication

Conjugated Linoleic Acid Causes a Marked Increase in Liver α-Tocopherol and Liver α-Tocopherol Transfer Protein in C57BL/6 J Mice

Pei-Min Chao[1,2], Wan-Hsuan Chen[3], Chun-Huei Liao[1], and Huey-Mei Shaw[4]

[1]Institute of Nutrition, China Medical University, Taichung, Taiwan
[2]Department of Nutrition, China Medical University, Taichung, Taiwan
[3]Institute of Nutrition and Health Science, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan
[4]Department of Health and Nutrition, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan

Received for publication: December 29, 2008; Accepted for publication: June 3, 2009

Abstract: Conjugated linoleic acid (CLA) is a collective term for the positional and geometric isomers of a conjugated diene of linoleic acid (C18:2, n-6). The aims of the present study were to evaluate whether levels of hepatic α-tocopherol, α-tocopherol transfer protein (α-TTP), and antioxidant enzymes in mice were affected by a CLA-supplemented diet. C57BL/6 J mice were divided into the CLA and control groups, which were fed, respectively, a 5 % fat diet with or without 1 g/100 g of CLA (1:1 mixture of *cis*-9, *trans*-11 and *trans*-10, *cis*-12) for four weeks. α-Tocopherol levels in plasma and liver were significantly higher in the CLA group than in the control group. Liver α-TTP levels were also significantly increased in the CLA group, the α-TTP/β-actin ratio being 2.5-fold higher than that in control mice ($p<0.01$). Thiobarbituric acid-reactive substances were significantly decreased in the CLA group ($p<0.01$). There were no significant differences between the two groups in levels of three antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase). The accumulation of liver α-tocopherol seen with the CLA diet can be attributed to the antioxidant potential of CLA and the ability of α-TTP induction. The lack of changes in antioxidant enzyme protein levels and the reduced lipid peroxidation in the liver of CLA mice are due to α-tocopherol accumulation.

Key words: Conjugated linoleic acid; α-tocopherol; α-tocopherol transfer protein; mice; antioxidant enzyme

Introduction

Vitamin E is a well-known lipid-soluble antioxidant in the body and α-tocopherol is the form most active in preventing lipid peroxidation of biological membranes. The tissues preferentially retain α-tocopherol because of its specific affinity for α-tocopherol transfer protein (α-TTP) in the liver [1]. α-TTP is a cytosolic protein with a molecular weight of ~33 kDa and, in mammals, is predominantly expressed in the liver [2,3]. Humans with a defective α-TTP gene have extraordinarily low plasma levels of α-tocopherol [4–6]. α-TTP-null and heterozygous mice have lower α-tocopherol levels in the plasma and tissues compared to wild-type mice [7,8]. Since α-TTP is thought to be important in the homeostasis of plasma α-tocopherol, changes in its expression would affect plasma α-tocopherol levels, leading to a redistribution from the liver to the peripheral tissues. Studies of the regulation of hepatic α-TTP by dietary vitamin E have given inconsistent results. Animals fed a high-dose vitamin E-supplemented diet show a reduction [9], a slight increase [10], or no change [11] in hepatic α-TTP mRNA and protein levels. In rats fed a vitamin E-deficient diet, hepatic α-TTP mRNA is induced, but protein levels are unchanged [9]. In our previous study in vitamin E-deficient rats [11], no change was seen in hepatic α-TTP mRNA levels, but protein levels were reduced. Oxidative stress caused by hyperoxia is also reported to regulate α-TTP protein in rats [12].

Conjugated linoleic acid (CLA) is a collective term for the positional and geometric isomers of a conjugated diene of linoleic acid (C18:2, n-6). Dietary CLA has diverse physiological functions, such as providing an anticarcinogenic effect, reducing the risk of atherosclerosis, and reducing body fat [13]. CLA has also been reported to have antioxidant or pro-oxidant properties [14]. A CLA-containing diet was found to reduce liver lipid peroxidation in hens [15] and rats [16,17] and increase liver total reduced glutathione in mice [18]. In rats fed a CLA-supplemented diet, liver thiobarbituric acid-reactive substances (TBARS), an index of lipid peroxidation, were found to be either unchanged [19] or increased [20]. However, little information is available on liver vitamin E levels in rats fed CLA. Since the plasma tocopherol/total cholesterol ratio in hamsters fed CLA was increased when no tocopherol was added to the diet, Nicolosi *et al.* [21] suggested that CLA has an α-tocopherol-sparing effect. Since plasma vitamin E is secreted with lipids in a lipoprotein fraction from the liver and since α-TTP mediates the transfer of α-tocopherol for very-low density lipoprotein (VLDL) packaging [22–24], we hypothesized that the hepatic vitamin E status and α-TTP levels would be affected by CLA. Mice are reported to be more sensitive than other rodents to the effect of CLA on lipid metabolism, insulin resistance, and induction of fatty liver [25]. Because the fatty liver is highly vulnerable to oxidative damage and shows higher lipid peroxidation and hepatic injury [26–28], we speculated that a CLA diet would also influence liver vitamin E levels and α-TTP. In our previous study, an increase of hepatic α-tocopherol concentration in CLA-fed mice was noticed [29]. Therefore, the CLA effect on α-tocopherol levels, α-TTP, and antioxidant enzymes in the liver were further examined in the present study.

Materials and Methods

Animals and diets

Seven-week-old male C57BL/6 J mice were purchased from the Laboratory Animal Center of the National Science Council (Taipei, Taiwan) and housed individually in stainless steel wire cages in a room maintained at 23±2 °C, with a controlled 12-hour light:dark cycle and free access to food and tap water. Body weight and food intake were recorded weekly. The mice were divided into two groups and fed either AIN-76 diet containing 5% lipid as soybean oil alone (control group) or 4% soybean oil supplemented with 1% CLA (CLA group). According to the manufacturer (Maypro, Purchase, NY, USA), the CLA used was 80% pure and contained equal amounts of the two isomers, *cis*-9, *trans*-11 and *trans*-10, *cis*-12. The protocols for animal care and handling were approved by the Institutional Animal Care and Use Committee (IACUC) of the China Medical University.

Tissue sampling and preparation

After 4 weeks of feeding, food was withheld overnight, then the mice were killed by carbon dioxide asphyxiation and the liver and adipose tissue (retroperitoneal fat) were excised and weighed. A small piece of fresh liver was homogenized in ice-cold 0.01 mol/L phosphate buffer, pH 7.4, containing 0.118 mol/L KCl using a Potter-Elvehjem-type homogenizer with a Teflon pestle. The homogenate was centrifuged at 100,000 × g at 4 °C for 1 hour. The

Table I: Initial and final body weights, food intake, feed efficiency, and liver weight of mice fed the 1 % CLA or control diet

	Initial weight (g)	Final weight (g)	Food intake (g/day)	Feed efficiency (g/g)	Liver weight (g)
Control	18.00±0.91	26.00±1.55	0.35±0.05	0.075±0.01	0.933±0.088
CLA	17.68±1.25	22.09±1.43*	0.19±0.09*	0.049±0.02*	1.497±0.353*

Each value is the mean±SD (n=8 for control group, n=11 for CLA group). * denotes $p<0.05$.

supernatant was stored at -70 °C for Western blot analysis.

Biochemical analysis

The concentration of α-tocopherol in plasma, liver, or adipose tissue homogenate was analyzed by high-pressure liquid chromatography (HPLC) as described previously [30]. TBARS levels measured in the liver homogenate were used as an index of lipid peroxidation [31] according to the method of Oteiza *et al.* [32]. Liver triglycerides were extracted using a 2:1 v/v mixture of chloroform/methanol containing 1 % Triton X-100 and measured by enzymatic methods using commercial kits (Randox Lab, Crumlin, Northland, UK).

Western blot analysis

The primary antibodies against α-TTP were prepared as previously described [11] except they were raised in a rabbit instead of BALB/c mice. Primary antibodies against enzymes were purchased from Abcam (UK), and HRP-Goat Anti-Rabbit IgG(H+L)Conjugate was used as the secondary antibody (Zymed Co.). Aliquots of the liver cytosolic fraction containing 10 μg of protein were separated by SDS-polyacrylamide gel electrophoresis and transferred to a PVDF membrane, which was then incubated overnight at 4 °C with blocking buffer (0.25 % gelatin, 0.15 M NaCl, 5 mM EDTA, 0.05 % Tween 20, 50 mM Tris, pH 8.0). The proteins on the membrane were immunostained for 1 hour at room temperature using antibodies against α-TTP, superoxide dismutase (SOD), glutathione peroxidase (GPx), or catalase. After 3 washes with washing buffer (PBS containing 0.05 % Tween 20, pH 7.0), the membranes were incubated with secondary antibody at room temperature for 1 hour, then washed 3 times, and the signal visualized by reaction with ECL-plus substrate (Amersham) for 20–40 seconds.

Statistical analysis

Data are expressed as the mean ±SD. Statistical analysis was carried out using Student's *t*-test and differences were considered significant at $p<0.05$.

Results

Animal growth

As shown in Table I, the final body weights, food intake, and feed efficiency were significantly reduced in mice fed with CLA diet. The liver weights in the CLA mice were significantly higher than those in the control group ($p<0.001$).

α–Tocopherol levels in dietary oils, plasma, and adipose tissue of animals

As shown in Table II, the α-tocopherol levels in soybean oil was about 2.1 times higher than that in CLA capsules. Since the main source of dietary vitamin E comes from Vitamin mixture, (all-rac-α-tocopherol acetate 5 IU/g mixture, 50 IU/kg diet), the α-tocopherol that comes from oil in the control diet (5 % soybean oil supplied about 1.86 mg/kg diet) was comparable with that from 4 % soybean oil plus 1 % CLA (about 1.66 mg/kg diet) in the CLA diet. Plasma α-tocopherol levels were significantly higher in the CLA group than in the control group (Figure 1 A). In the CLA group, the α-tocopherol concentration of adipose tissue was higher than that in the control group, but the difference was not statistically significant (Figure 1B).

Table II: Vitamin E levels in soybean oil and CLA

	α-Tocopherol	γ-Tocopherol
	ug/g oil	
Soybean oil	37.18	155.29
CLA	16.95	45.89

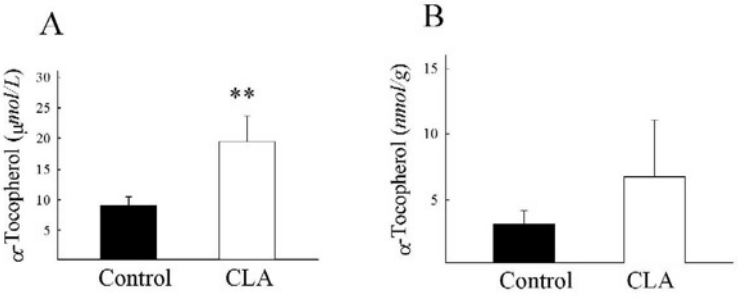

Figure 1: The concentration of α-tocopherol in (A) plasma and (B) adipose tissue in control and CLA-supplemented mice. (Values are mean±SD, n=6 for control group, n=5 for CLA group). ** $p<0.001$.

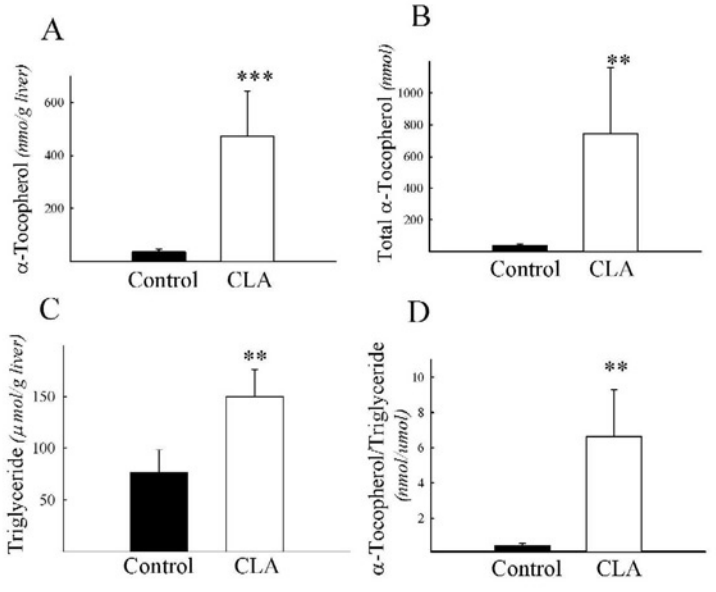

Figure 2: Hepatic tocopherol and triglyceride levels in control and CLA-supplemented mice. (A) Liver α-tocopherol levels expressed as nmol/g liver. (B) Total α-tocopherol amounts in the whole liver (n=8 for control group, n=11 for CLA group). (C) Liver triglyceride levels. (D) Molar ratio of α-tocopherol to triglyceride in the liver. (Values are means±SD, n=8 for control group, n=11 for CLA group in A and B; n=6 for CLA group in C and D). ** $p<0.001$, *** $p<0.0001$.

Liver α-tocopherol, triglyceride, and α-tocopherol transfer protein

Because of the higher liver weight of the CLA mice, liver α-tocopherol levels were expressed as per gram liver weight (Figure 2 A) and also as total liver α-tocopherol content (Figure 2B). Both were significantly higher in the CLA group by a factor of about 13-fold and 21-fold, respectively. The CLA mice showed liver steatosis, as evidenced by triglyceride accumulation (Figure 2C). Since α-tocopherol is an important lipid-soluble antioxidant, the α-tocopherol/triglyceride molar ratio was calculated. As shown in Figure 1D, the CLA diet caused an increase in this ratio of about 15-fold ($p<0.001$). Protein levels of liver α-TTP were analyzed by Western blotting. As shown in Figure 3, α-TTP levels were significantly increased in the CLA group, the intensity ratio for the α-TTP to β-actin bands being 2.5-fold higher in the CLA mice ($p<0.01$).

Liver TBARS and antioxidant enzyme proteins

As shown in Figure 4 A, liver TBARS levels in the CLA group were decreased significantly ($p<0.01$). Levels of the antioxidant enzymes SOD, GPx, and catalase that were analyzed by Western blotting did not differ significantly between the two groups (Figures 4B-D). This shows that the CLA mice had lower lipid peroxidation when expressed as TBARS without inducing expression of these antioxidant enzymes in the liver.

Discussion

A larger liver weight due to fatty liver was observed in the CLA mice, as in other reports [33,34]. Clément *et al.* [35] suggested that *t*10, *c*12 CLA was the major form of CLA which developed fatty liver by trigger-

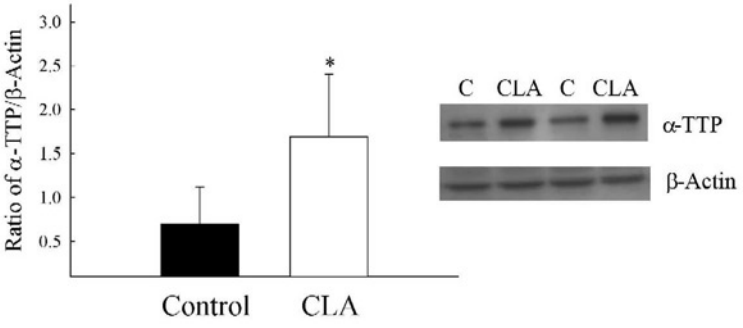

Figure 3: Liver α-tocopherol transfer protein levels analyzed by Western blotting in control (C) and CLA supplemented mice. The α-TTP/β-actin band intensity ratio is the means±SD. (n=6 for control group, n=7 for CLA group). * significantly different from the control group, $p<0.01$.

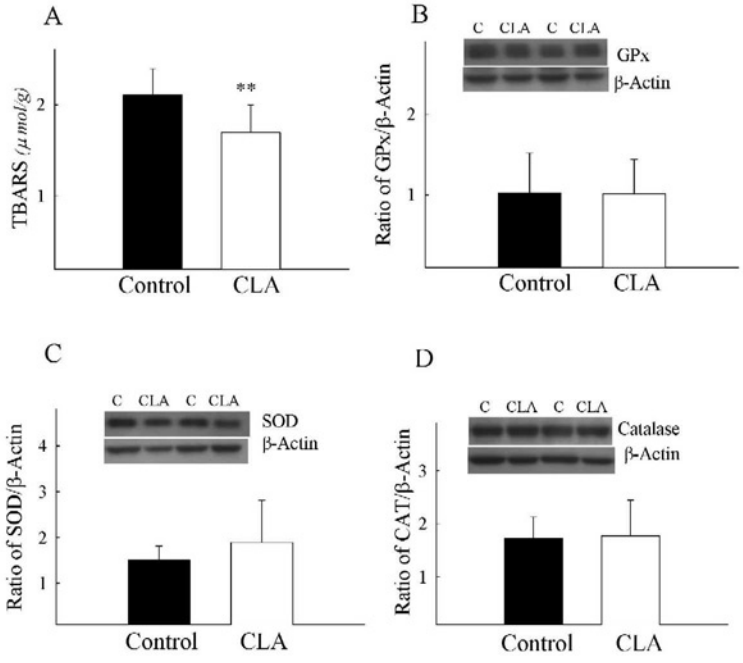

Figure 4: Liver TBARS levels and Western blots for GPx, SOD, and catalase protein levels in control and CLA-supplemented mice. (A) TBARS levels in the liver. (B-D) Representative Western blot results and summarized results for GPx (B), SOD (C), or catalase (D). The ratios for the enzyme/β-actin band intensities are the means±SD. (n=6 for control group, n=7 for CLA group). ** significantly different from the control group, $p<0.001$.

ing the peroxisome proliferator-activated receptor-gamma (PPARγ)-activation and inducing the expression of the fatty acid synthase genes. In our previous study [29], the liver lipogenesis was found to be enhanced by the increased sterol regulatory element binding protein-1c (SREBP-1c) and TNF receptor superfamily member 6 (FAS) mRNA levels in CLA-fed mice and thus leading to liver steatosis.

Fatty livers are generally highly vulnerable to oxidative damage, resulting in high lipid peroxidation and hepatic injury [26–28]. In contrast, in this study, lower liver TBARS levels and higher liver α-tocopherol levels were observed with fatty liver in the CLA mice. Hepatic vitamin E levels are thought to correlate with hepatic lipid levels. Since increased liver α-tocopherol levels are seen in obese mice with fatty liver [36], the α-tocopherol/triglyceride ratio was calculated and was found to be almost 15-fold higher in the CLA mice than in the control group, whereas liver triglyceride levels in the CLA mice were only about 2-fold higher than in the control group, showing a much greater increase in α-tocopherol than triglyceride. This suggests that the accumulation of high levels of vitamin E in the CLA mice cannot be fully explained by the close correlation between vitamin E and triglyceride in the liver. Liver α-tocopherol levels were increased 13-fold in CLA mice. The food intake of the CLA group was only half that of the control group, so this could not be the reason for the higher liver vitamin E levels in CLA mice.

Vitamin E is a lipid-soluble antioxidant and there are several reports suggesting that dietary antioxidants can spare liver α-tocopherol due to the

reduction in vitamin E consumption. Butylated hydroxytoluene (BHT) and catechin [37], quercetin [38], and other flavonoids [39] are all well known antioxidants that increase α-tocopherol levels in the liver due to their free radical scavenging capacity. The antioxidant potential of CLA is well documented. CLA can protect paraoxonase 1, an antioxidant enzyme which prevents the formation of oxidized low-density lipoprotein (LDL) [40]. CLA can be incorporated into high-density lipoprotein (HDL) membranes and decrease the oxidative susceptibility of lipoprotein [21,41]. It also increases the saturated fatty acid/monounsaturated fatty acid ratio in membranes [15,16]. CLA increases liver reduced glutathione levels by inducing expression of γ-glutamylcysteine ligase in the liver [18]. Together, these data suggest that CLA has antioxidant potential, which contributes to the increase in α-tocopherol levels in the liver. The *cis*-9, *trans*-11 (c9, t11) and *trans*-10, *cis*-12 (t10, c12) isomers of CLA are usually used in commercial CLA supplements. Although the c9, t11 CLA isomer has been reported to show more antioxidant effects than the t10, c12 isomer [42, 43], it is hard to confirm this result in the present study because the two CLA isomers were equally mixed.

Changes in liver α-tocopherol can also result from the degradation of vitamin E. Cytochromes P450 3 A [44] and 4F2 [45], which have ω-hydroxylase activity, have been reported to be involved in shortening the phytyl side chain of vitamin E to carboxyethyl hydroxychrome (CEHC), which is excreted into the urine. Dietary factors, such as sesamin, the major nonsaponifiable lipid in sesame seed oil [46], can increase γ-tocopherol levels in rat liver by inhibiting CYP3 A [47]. γ-Tocopherol was found to be a better substrate of CYP 4F2 than α-tocopherol in the rat liver microsome system [45]. CLA has been suggested to be a peroxisome proliferator [48] and to increase mRNA or protein levels of PPARα targets, such as the CYP4 family, in mice [48,49]. One might speculate that a CLA diet activates PPARα and induces cytochrome P450 (CYP) expression, then increases tocopherol metabolism in the liver. PPARα stimulates β-oxidation and fatty acid clearance by the liver [50], whereas, in our study, fatty liver was observed with no induction of hepatic PPARα target gene expression [29]. Thus, the enhancement in vitamin E metabolism mediated by PPAR activation seems not to be involved in the α-tocopherol accumulation in the livers of the CLA mice.

α-TTP has been reported to regulate plasma vitamin E levels [4–8] and also to play a role in the trafficking of vitamin E in the liver [51]. Thus, the high levels of α-tocopherol in liver, adipose tissue, and plasma in the CLA mice could possibly be partly explained by the induction of liver α-TTP. However, only about a 2-fold increase in α-TTP levels was seen in the liver in the CLA group, whereas the increase in α-tocopherol was >13-fold. Bella *et al.* [10] reported that, in mice fed a high vitamin E diet, levels of α-TTP protein were increased only by 20%, whereas liver α-tocopherol levels were increased 16-fold. In our previous study [11], a vitamin E-supplemented diet caused a 14.4-fold and 1.8-fold increase in liver and plasma α-tocopherol levels, respectively. These results show that α-TTP seems to be one of the causes of an increase in the α-tocopherol levels in mice fed a CLA-containing diet.

The mechanism of regulation of α-TTP expression is still unclear. Many studies have suggested that α-TTP is down-regulated by oxidative stress. When rats are exposed to hyperoxia for 48 hours [12] or are fed a low-protein diet [11], α-TTP mRNA levels are reduced and lipid peroxidation is increased. α-TTP mRNA and protein levels are also reduced in rat hepatoma [52] and galactosamine-induced liver injury [53]. Reports of induction of α-TTP by dietary factors are rare. Expression of hepatic α-TTP mRNA [54] and its protein [10] are induced when rats are fed a vitamin E-supplemented diet. The present study is the first to report that CLA causes induction of hepatic α-TTP and an increase in α-tocopherol levels. This data show that the regulation of α-TTP could be mediated by oxidative stress or CLA itself.

GPx, SOD, and catalase are well-known cellular antioxidant enzymes and changes in enzyme activity show their adaptation to oxidative stress. Cantwell *et al.* [55] found that CLA had an antioxidant enzyme-sparing effect in hepatocytes by decreasing the activities of GPx, SOD, and catalase. GPx activity was reduced and SOD and catalase activities unchanged in the liver of rats fed a CLA diet [16]. Our results are similar, as we found that the protein levels of the three enzymes in the liver were unchanged in the CLA-fed group, though the activity of the enzymes was not measured. In our study, vitamin E accumulation in the liver was also seen in the CLA mice. Eder *et al.* [56] found that high vitamin E supplementation not only increased red blood cell (RBC) α-tocopherol levels, but also reduced RBC GPx, SOD, and catalase activity without affecting hemolysis. This suggests that high vitamin E levels in tissues protects them from lipid peroxidation and spares the antioxidant enzymes, and this could also explain our observations. Ban [12] reported that exposure to hyperoxia reduced α-TTP mRNA levels,

but had no effect on SOD protein and mRNA levels, suggesting that SOD is more stable than α-TTP. In α-TTP null mice, levels of SOD and GPx were again unchanged, although expression of the glutathione-S-transferase gene was induced [57]. These results suggest that the regulation of antioxidant enzymes is α-TTP independent.

In conclusion, a CLA diet for mice resulted in induction of α-TTP expression, accumulation of α-tocopherol in the liver, and a decrease in liver lipid peroxidation expressed as TBARS, but had no effect on antioxidant enzyme protein levels. The liver α-tocopherol accumulation effect seen with the CLA diet can be attributed to the antioxidant potential of CLA and the induction of α-TTP protein. It is hypothesized that the lack of change in antioxidant enzymes and reduced lipid peroxidation in CLA mice were due to α-tocopherol accumulation in the liver.

References

1. Kayden, H.J. and Traber, M.G. (1993) Absorption, lipoprotein transport, and regulation of plasma concentration of vitamin E in human. J. Lipid Res. 34, 343–358.

2. Sato, Y., Arai, H., Miyata, A., Tokita, S., Yamamoto, K. and Tanabe, T. (1993) Primary structure of α-tocopherol transfer protein from rat liver. Homology with cellular retinaldehyde-binding protein. J. Biol. Chem. 268,17705–17710.

3. Yoshida, H., Yusin, M., Ren, I., Kuhlenkamp, J., Hirano, T., Stolz, A. and Kaplowitz, N. (1992) Identification, purification, and immunochemical characterization of a tocopherol-binding protein in rat liver cytosol. J. Lipid Res. 33,343–350.

4. Traber, M.G., Sokol, R.J., Burton, G.W., Ingold, K.U., Papas, A.M., Huffaker, J.E. and Kayden, H.J. (1990) Impaired ability of patients with familial isolated vitamin E deficiency to incorporate alpha-tocopherol into lipoproteins secreted by the liver. J. Clin. Invest. 85,397–407.

5. Traber, M.G. (1997) Regulation of human plasma vitamin E. Adv. Pharmacol. 38,49–63.

6. Ben Hamida, M., Belal, S., Sirugo, G., Ben Hamida, C., Panayides, K., Ionannou, P., Beckmann, J., Mandel, J.L., Hentati, F. and Koenig, M. (1993) Friedreich's ataxia phenotype not linked to chromosome 9 and associated with selective autosomal recessive vitamin E deficiency in two inbred Tunisian families. Neurology 43,2179–2183.

7. Terasawa, Y., Ladha, Z., Leonard, S.W., Morrow, J.D., Newland, D., Sanan, D., Packer, L., Traber, M.G. and Farese, R.V. Jr. (2000) Increased atherosclerosis in hyperlipidemic mice deficient in alpha -tocopherol transfer protein and vitamin E. Proc. Natl. Acad. Sci. U S A. 97,13830–13834.

8. Leonard, S.W., Terasawa, Y., Farese, R.V.J. and Traber, M.G. (2002) Incorporation of deuterated RRR- or all rac α-tocopherol into plasma and tissues of α-tocopherol transfer protein null mice. Am. J. Nutr. 75,555–560.

9. Kim, H.S., Arai, H., Arita, M., Sato, Y., Ogihara, T., Inoue, K., Mino, M. and Tamai, H. (1998) Effect of α-tocopherol status on α-tocopherol protein expression and its messenger RNA level in rat liver. Free Radical Res. 28, 87–92.

10. Bella, D.L., Schock, B.C., Lim, Y., Leonard, S.W., Berry, C., Cross, C.E. and Traber, M.G. (2006) Regulation of the α-tocopherol transfer protein in mice, lack of response to dietary vitamin E or oxidative stress. Lipids 41,105–112.

11. Shaw, H.M. and Huang, C.J. (1998) Liver α-tocopherol transfer protein and its mRNA are differentially altered by vitamin E deficiency and protein insufficiency in rats. J. Nutr. 128,2348–2354.

12. Ban, R., Takitani, K., Kim, H.S., Murata, T., Morinobu, T. and Ogihara, T. (2002) α-Tocopherol transfer protein expression in rat liver exposed to hyperoxia. Free Radical Res. 36,933–938.

13. Pariza, M.W. (2004) Perspective on the safety and effectiveness of conjugated linoleic acid. Am. J. Clin. Nutr. 79,1132S-1136S.

14. Devery, R., Miller, A. and Stanton, C. (2001) Conjugated linoleic acid and oxidative behaviour in cancer cells. Biochem. Soc. Trans. 29,341–344.

15. Du, M., Ahn, D.U., Nam, K.C. and Sell, J.L. (2001) Volatile profiles and lipid oxidation of irradiated cooked chicken meat from laying hens fed diets containing conjugated linoleic acid. Poultry Sci. 80,235–241.

16. Kim, H.Y., Kim, S.R., Ahn, J.Y., Cho, I.J., Yoon, C.S. and Ha, T.Y. (2003) Dietary conjugated linoleic acid reduces lipid peroxidation by increasing oxidative stability in rats. J. Nutr. Sci. Vitaminol. 51,8–15.

17. Livisay, S.A., Zhou, S., Ip, C. and Decker, E.A. (2000) Impact of dietary conjugated linoleic acid on the oxidative stability of rat liver microsomes and skeletal muscle homogenates. J. Agric. Food Chem. 48, 4162–4167.

18. Bergamo, P., Luongo, D., Maurano, F., Mazzarella, G., Stefanile, R. and Rossi, M. (2006) Conjugated linoleic acid enhances glutathione synthesis and attenuates pathological signs in MRL/MpJ-Fas^{lpr} mice. J. Lipid Res. 47,2382–2391.

19. Santos-Zago, L.F., Botelho, A.P. and Olivera, A.C. (2007) Supplementation with commercial mixtures of conjugated linoleic acid in association with vitamin E and the process of lipid autoxidation in rats. Lipids 42,845–854.

20. Yamasaki, M., Mansho, K., Mishima, H., Kimura, G., Sasaki, M., Kasal, M., Tachibana, H. and Yamada, K. (2000) Effect of dietary conjugated linoleic acid on lipid peroxidation and histological change in rat liver tissues. J. Agric. Food Chem. 48, 6367–6371.

21. Nicolosi, R.J., Rogers, E.J., Kritchevsky, D., Scimeca, J.A. and Huth, P.J. (1997) Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery 22, 266–277.

22. Bjorneboe, A., Bjorneboe, G.E.A., Hagen, B..F, Nossen, J.O. and Drevon, C.A. (1987) Secretion of α-tocopherol from cultured rat hepatocytes. Biochim. Biophys. Acta 922,199–205.

23. Cohn, W., Loechleiter, F. and Weber, F. (1988) α-Tocopherol is secreted from rat liver in very low density lipoproteins. J. Lipid Res. 29,1359–1366.

24. Traber, M.G., Ingold, K.U., Burton, G.W. and Kayden, H.J. (1988) Absorption and transport of deuterium-substituted 2R, 4'R, 8R'-α-tocopherol in human lipoproteins. Lipids 23,791–797.

25. Pariza, M.W., Park, Y. and Cook, M.E. (2001) The biologically-active isomers of conjugated linoleic acid. Prog. Lipid Res. 40,283–298.

26. Canbakan, B., Senturk, H., Tahan, V., Hatemi, I., Balci, H., Toptas, T., Sonsuz, A., Velet, M., Aydin, S., Dirican, A., Ozgulle, S. and Ozbay, G. (2007) Clinical, biochemical and histological correlations in a group of non-drinker subjects with non-alcoholic fatty liver disease. Acta Gastroenterol. Belg. 70,277–284.

27. Dianzani, M.U., Muzio, G., Biocca, M.E. and Canuto, R.A. (1991) Lipid peroxidation in fatty liver induced by caffeine in rats. Int. J. Tissue React. 13,79–85.

28. Laurent, A., Nicco, C., Tran Van Nhieu, J., Borderie, D., Chereau, C., Conti, F., Jaffray, P., Soubrane, O., Calmus, Y., Weill, B. and Batteux, F. (2004) Pivotal role of superoxide anion and beneficial effect of antioxidant molecules in murine steatohepatitis. Hepatology 39,1277–1285.

29. Liao, C.H., Shaw, H. M. and Chao, P.M. (2008) Impairment of glucose metabolism in mice induced by dietary oxidized frying oil is different from that induced by conjugated linoleic acid. Nutrition 24, 744–752.

30. Huang, C.J. and Shaw, H.M. (1994) Tissue vitamin E status is compromised by dietary protein insufficiency in young growing rats. J. Nutr. 124,571–579.

31. Ohkawa, H., Ohisi, N. and Yagi, K. (1997) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.

32. Oteiza, P.I., Olin, K.L., Fraga, C.G. and Keen, C.L. (1995) Zinc deficiency causes oxidative damage to proteins, lipids and DNA in rat testes. J. Nutr. 125,823–829.

33. DeLany, J.P., Blohm, F., Trueit, A.A., Scimeca, J.A. and West, D.B. (1999) Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. Am. J. Physiol. 276,R1172-R1179.

34. Tsuboyama-Kasaoka, N., Takahashi, M., Tanemura, K., Kim, H.J., Tange, T., Okuyama, H., Kasai, M., Ikemoto, S. and Ezaki, O. (2000) Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. Diabetes 49,1534–1542.

35. Clément, L., Poirier, H., Niot, I., Bocher, V., Guerre-Millo, M., Krief, S., Staels, B. and Besnard, P. (2002) Dietary trans-10, cis-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. J. Lipid Res. 43, 1400–1409.

36. Bruno, R.S., Dugan, C.E., Smyth, J.A., DiNatale, D.A. and Koo, S.I. (2008) Green tea extract protects leptin-deficient, spontaneously obese mice from hepatic steatosis and injury. J. Nutr. 138,323–331.

37. Frank, J., Lundh, T., Parker, R.S., Swanson, J.E., Vessby, B. and Kamal-Eldin, A. (2003) Dietary (+)-catechin and BHT markedly increase α-tocopherol concentrations in rats by a tocopherol-ω-hydroxylase-independent mechanism. J. Nutr. 133,3195–3199.

38. Choi, E.J., Chee, K.M. and Lee, B.H. (2003) Anti- and prooxidant effects of chronic quercetin administration in rats. Eur. J. Pharmacol. 482,281–285.

39. Frank, J., Budek, A., Lundh, T., Parker, R.S., Swanson, J.E., Lourenco, C.F., Gago, B., Laranjinha, J., Vessby, B. and Kamal-Eldin, A. (2006) Dietary flavonoids with a catechol structure increase α-tocopherol in rats and protect the vitamin from oxidation in vitro. J. Lipid Res. 47,2718–2725.

40. Su, N.D., Liu, X.W., Kim, M.R., Jeong, T. S. and Sok, D.E. (2003) Protective action of CLA against oxidative inactivation of Paraoxonase 1, an antioxidant enzyme. Lipids 38, 615–622.

41. Wilson, T.A., Nicolosi, R.J., Chrysam, M. and Kritchevsky, D. (2000) Conjugated linoleic acid reduces early aortic atherosclerosis to a greater degree than linoleic acid in hypercholesterolemic hamsters. Nutr. Res. 20, 1795–1805.

42. Choi, J.S., Koh, I.U., Jung, M.H. and Song, J. (2007) Effects of three different conjugated linoleic acid preparations on insulin signaling, fat oxidation and mitochondrial function in rats fed a high-fat diet. Br. J. Nutr. 98, 264–275.

43. Smedman, A., Vessby, B. and Basu, S. (2004) Isomer-specific effects of conjugated linoleic acid on lipid peroxidation in humans: regulation by alpha-tocopherol and cyclo-oxygenase-2 inhibitor. Clin. Sci.

44. Parker, R.S., Sontag, T.J. and Swanson, J.E. (2000) Cytochrome P4503 A-dependent metabolism of tocopherol and inhibition by sesamin. Biochem. Biophys. Res. Commun. 277, 531–534.

45. Sontage, T.J. and Parker, R.S. (2002) Cytochrome P450 omega-hydroxylase pathway of tocopherol catabolism. Novel mechanism of regulation of vitamin E status. J. Biol. Chem. 277, 25290–25296.

46. Kamal-Eldin, A. and Appelqvist, L.A. (1994) Variations in the composition of sterols, tocopherols, and ligans in seed oils from four Sesamum species. J. Am. Oil Chem. Soc. 71, 149–156.

47. Ikeda, S., Tohyama, T. and Yamashita, K. (2002) Dietary sesame seed and its lignands inhibit 2, 7, 8-trimethyl-2(2'-carboxyethyl)-6-hydroxychroman excretion into urine of rats fed γ-tocopherol. J. Nutr. 132, 961–966.

48. Belury, M.A., Moya-Camarena, S.Y., Liu, K.L. and Vanden Heuvel, J.P. (1997) Dietary conjugated linoleic acid induces peroxisome-specific enzyme accumulation and ornithine decarboxylase activity in mouse liver. J. Nutr. Biochem. 8,579–584.

49. Peters, J.M., Park, Y., Gonzalez, F.J. and Pariza, M.W. (2001) Influence of conjugated linoleic acid on body composition and target gene expression in peroxisome proliferator-activated receptor alpha-null mice. Biochim. Biophys. Acta 1533,233–242.

50. Finck, B.N. and Kelly, D.P. (2002) Peroxisome proliferator-activated receptor alpha (PPAR-alpha) signaling in the gene regulatory control of energy metabolism in the normal and diseased heart. J. Mol. Cell Cardiol. 34,1249–12587.

51. Qian, J., Morley, S., Wilson, K., Nava, P., Atkinson, J. and Manor, D. (2005) Intracellular trafficking of vitamin E in hepatocytes, the role of tocopherol transfer protein. J. Lipid Res. 46,2072–2082.

52. Wu, C.G., Hoek, F.J., Groenink, M., Reitsma, P.H., van Deventer, S.J. and Chamuleau, R.A. (1997) Correlation of repressed transcription of alpha-tocopherol transfer protein with serum alpha-tocopherol during hepatocarcinogenesis. Int. J. Cancer 71, 686–690.

53. Takenaka, A., Kita, A., Ikeya, M., Arai, H. and Igarashi, K. (2007) Galactosamine-induced acute liver injury in rats reduces hepatic α-tocopherol transfer protein production. J. Nutr. Sci. Vitaminol. 53,366–371.

54. Fechner, H., Schlame, M., Guthmann, F., Stevens, P.A. and Rustow, B. (1998) α- and δ- tocopherol induce expression of hepatic α-tocopherol-transfer-protein mRNA. Biochem. J. 331,577–581.

55. Cantwell, H., Devery, R., O'Shea, M. and Stanton, C. (1999) The effect of conjugated linoleic acid on the antioxidant enzyme defense system in rat hepatocytes. Lipids 34,833–839.

56. Eder, K., Flader, D., Hirche, F. and Brandsch, C. (2002) Excess dietary vitamin E lower the activities of antioxidative enzymes in erythrocytes of rats fed salmon oil. J. Nutr. 132,3400–3404.

57. Gohil, K., Schock, B.C., Chakraborty, A.A., Terasawa, Y., Raber, J., Farese, R.V., Packer, L., Cross, C.E. and Traber, M.G. (2003) Gene expression profile of oxidant stress and neurodegeneration in transgenic mice deficient in α-tocopherol transfer protein. Free Radical Biol. Med. 35,1343–1354.

Huey-Mei Shaw

Department of Health and Nutrition
Chia-Nan University of Pharmacy and Science
60 Erh-Jen Road
Sec. 1
Jen-Te Hsiang, Tainan
Taiwan
Tel: +886 6 2664911, ext 3403
Fax: +886 6 2667327
E-Mail:
mei@mail.chna.edu.tw
meimei.shaw@gmail.com