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大鼠肝臟PXR基因表現量和維生素E代謝關係之探討(2/2)
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【中文摘要】

在 HepG2 細胞培養的實驗發現 CYP3A 會參與維生素 E 的代謝途徑。本實驗則以動物模式給予 CYP3A 誘導劑(inducer)或抑制劑 (KCZ)來探討α-生育醇代謝成α-CEHC 之過程是否確實與 CYP3A 有關。實驗一將動物分成正常維生素 E 組(50ppm)與維生素 E 補充組(500ppm)，飼養三週後每組再續分成 PCN 注射組 (P50, P500)與 DMSO 控制組(D50, D500)共四組，並於連續注射的三天收集尿液。以 Two-way ANOVA 分析結果，血漿與肝臟α-生育醇濃度皆顯著受到 PCN 因子所下降，然尿液α-CEHC 亦顯著受 PCN 因子所下降(p=0.0004)。而 PCN 注射則顯著增加了肝臟 TBARS 濃度。實驗二將大鼠以基礎飼料飼養三週後，分成兩組餵與含或不含 KCZ 飼料三天，最後一天收集尿液。結果 KCZ 組的肝臟α-生育醇顯著增加而尿液α-CEHC 含量則顯著下降約為控制組的 64%。實驗三將大鼠以基礎飼料飼養三週後，分成兩組予以單次注射 Dexamethasone (DEX 組)或 DMSO (控制組)並收集 24 小時尿液。結果血漿與肝臟α-生育醇皆顯著因 DEX 注射而顯著下降，但是尿液α-CEHC 濃度亦顯著下降。綜合上述實驗結果指出動物給予 CYP3A 的誘導劑或抑制劑皆會降低尿液α-CEHC 的排出量。

關鍵詞：α-生育醇、α-CEHC、大鼠、細胞色素 P450、PCN

【英文摘要】

CYP 3A has been reported to be involved in the metabolism of vitamin E in HepG2 cell. In this study the inducer (PCN) and inhibitor (KCZ) of CYP3A were used to investigate whether the metabolism of α-tocopherol to its metabolite, α-CEHC, is CYP3A-dependent in rats. In experiment 1, two groups of Wistar rats were respectively fed basal diet (50ppm α-tocopherol) and 10 fold α-tocopherol supplementation (500ppm vitamin E) diets for 3 wk. In the last week, each group above was divided into 2 groups by given a single i.p. of PCN at 75mg/kg/d (P50 & P500) or DMSO (D50 & D500), and the urine was collected for 3 days. The α-tocopherol levels in plasma and liver both significantly decreased by PCN factor ($p<0.0001$), the α-CEHC levels in urine also significantly decreased by PCN factor ($p=0.0004$) by Two-way ANOVA analysis. PCN injection significantly increased liver TBARS concentration. In experiment 2, Wistar rats were fed basal diet for 3 wk and in the last 3 days the rats were divided into two groups for control and KCZ (CYP3A inhibitor) diet, the urine was collected. The α-tocopherol levels in liver increased and the α-CEHC excretion in urine decreased in KCZ group compared with control group. The α-CEHC levels in KCZ group were about 64% in that of control group ($p<0.05$). In experiment 3, Wistar rats were fed basal diets for 3 wk. In the last day the rats were divided into two groups by given a single i.p. of dexamethasone (DEX) at 100mg/kg/d or DMSO (Control), and collected the 24h urine. The α-tocopherol in plasma and liver decreased and α-CEHC in urine also significantly decreased in DEX group. These results indicated that urinary α-CEHC excretion is decreased by both the inhibitor and inducer of CYP3A.

KEY WORDS: α-tocopherol, α-carboxyethyl hydroxychroman, rats, cytochrome P450, PCN

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【報告內容】

Modulation of Cytochrome P450-3A by pregnenolone (PCN) or ketoconazole (KCZ) both reduces the urine α-CEHC excretion in rats

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ABSTRACT CYP 3A has been reported to be involved in the metabolism of vitamin E in HepG2 cell. In this study the inducer (PCN) and inhibitor (KCZ) of CYP3A were used to investigate whether the metabolism of α-tocopherol to its metabolite, α-CEHC, is CYP3A-dependent in rats. In experiment 1, two groups of Wistar rats were respectively fed basal diet (50ppm α-tocopherol) and 10 fold α-tocopherol supplementation (500ppm vitamin E) diets for 3 wk. In the last week, each group above was divided into 2 groups by given a single i.p. of PCN at 75mg/kg/d (P50 & P500) or DMSO (D50 & D500), and the urine was collected for 3 days. The α-tocopherol levels in plasma and liver both significantly decreased by PCN factor ($p<0.0001$), the α-CEHC levels in urine also significantly decreased by PCN factor ($p=0.0004$) by Two-way ANOVA analysis. PCN injection significantly increased liver TBARS concentration. In experiment 2, Wistar rats were fed basal diet for 3 wk and in the last 3 days the rats were divided into two groups for control and KCZ (CYP3A inhibitor) diet, the urine was collected. The α-tocopherol levels in liver increased and the α-CEHC excretion in urine decreased in KCZ group compared with control group. The

α-CEHC levels in KCZ group were about 64% in that of control group ($p<0.05$). In experiment 3, Wistar rats were fed basal diets for 3 wk. In the last day the rats were divided into two groups by given a single i.p. of dexamethasone (DEX) at 100mg/kg/d or DMSO (Control), and collected the 24h urine. The α-tocopherol in plasma and liver decreased and α-CEHC in urine also significantly decreased in DEX group. These results indicated that urinary α-CEHC excretion is decreased by both the inhibitor and inducer of CYP3A.

KEY WORDS: α-tocopherol, α-carboxyethyl hydroxychroman, rats, cytochrome P450

INTRODUCTION

Vitamin E is a well known fat-soluble antioxidant in the body. Tissue preferentially retained the α-tocopherol over than other forms of tocopherol owing to the specific affinity to α-tocopherol transfer protein (α-TTP) in liver (Kayden and Traber 1993). We have described many dietary factors such as oxidized frying oil (Liu and Huang 1995), dietary low protein (Huang and Shaw 1994) could affect vitamin E status in tissues by regulating the expression of α-TTP (Shaw and Huang 1998), VLDL secretion rate (Shaw and Huang 2000), oxidative stress (Huang and Fwu 1993), absorption and the turnover rates (Liu and Huang 1996) of vitamin E. Besides, vitamin E is not accumulated in the liver to toxic levels (Traber 2004), suggesting that excretion and metabolism play an important factor in regulation of vitamin E status in tissues.

The main metabolites of vitamin E in urine has been reported to be carboxyethyl hydoxychromans (CEHCs), (Chiku et al. 1984, Schultz et al. 1995, Wechter et al. 1993, Lodge et al. 2001, Birringer et al. 2001, Sontage and Parker 2002) in which the phytyl tail is degradation without modification of the chromanol head group (Schonfeld et al. 1992, Wechter et al. 1996, Chiku et al. 1984). The tocopherols are metabolized first with the ω-hydroxylation followed by β-oxidation, and the cytochrom P450 (CYPs) were thought to exhibite the activity of ω-hydroxylase. The CYP3A was first reported to be involved in vitamin E metabolism in HepG2 cell by determination the changes of CEHC metabolites when CYP inhibitor (ketoconazole) or inducer (rifampicin) was added into the medium (Parker et al. 2000, Birringer et al. 2001). In these hepatocyte studies liver seems to be the main organ synthesize CEHCs. High dose of α-tocopherol feeding or injection induced CYP3A protein in animals (Kluth et al. 2005, Mustacich et al. 2006). Vitamin E plus Se-deficient rats also showed a down-regulation of CYP3A RNA in liver (Fischer et al. 2002). CYP3A in liver metabolize more than 50% of clinical drugs (Kliewer et al. 2002). This reveals that high-dose of α-tocopherol supplementation could be relative to drug metabolism, yet the role of CYP3A induction in α-tocopherol metabolism itself is still unclear. Many reports suggest that γ-tocopherol is more actively metabolized to CEHCs than is α-tocopherol (Traber et al. 1998, Lodge et al. 2001, Swanson et al. 1999). Therefore, we first studies to investigate whether the metabolism of α-tocopherol to its metabolite, α-CEHC, is CYP3A-dependent in rats.

We postulated that if the CYP3A is a key enzyme in the catabolism of α-tocopherol, then the CYP3A induction enhance the production of α-CEHC excretion in urine of rats treated with PCN (CYP3A inducer). We here show the α-CEHC excretion are both reduced by CYP3A induction or

inhibition.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats (3 weeks old) were pursed from BioLASCO Co. (Taipei, Taiwan). They were housed individually in stainless-steel wire cages in a room maintained at 23± 2℃ with a controlled 12-h light/dark cycle and allowed free access to water and food. Body weights were recorded weekly. Animal care and handling conformed to accepted guidelines (NRC 1985). All rats were fed a purified basal diet (Table 1) before the individual treatment.

Experiment 1. The rats (body weight about93±7 g) were randomly assigned to two groups, rats were respectively fed basal diet (D50 group) and a high level of vitamin E (500mg/kg all-*rac*- α -tocopheryl acetate in basal diet, D500 group) diets for 3 wk (252 ±22 g body weight). In the last 3 days, each group was divided into 2 groups by given a single i.p. of PCN (5-pregnen-3b-01-20-one-16a-carbonitrite, Sigma) at 75mg/kg/d (P50 & P500) or DMSO (Dimethyl sulphoxide, Sigma) (D50 & D500) per day for 3d (Johnson et al. 2002).

Experiment 2. The rats (115±8g) were fed basal diets for 3 wk (300±19 g body weight). In the last 3 days the rats were divided into two groups for control (basal diet, n=8) and KCZ diet (1% ketoconazole in basal diet, Ikeda et al. 2002, n=8), and the urine was collected in the last 24h.

Experiment 3. Wistar rats (88±1g) were fed basal diets for 3 wk (264±19 g body weight). In the last day the rats were divided into two groups by given a single i.p. of dexamethasone (Sigma) at 100mg/kg/d (Choudhuri et al. 1995)(DEX, n=6) or DMSO (Control, n=5), and collected the 24h urine.

Tissue sampling and preparation. Urine was ice-cold incubated during the collected period, and the urine collected was added with ascorbic acid (100mg/mL urine) and stored at -20℃ under nitrogen until used for α-CEHC assay. At the termination of feeding, food was withheld overnight and the rats were killed by carbon dioxide asphyxiation in the morning. Blood was collected from the abdominal vena cava into a heparinized tube, and centrifuged at 1,000×g for 10 min. Then the separated plasma was stored at -80℃. A small piece of fresh liver was homogenized in ice-cold 0.01 mol/L phosphate buffer (pH 7.4, containing 11.5 g/L KCl) using a Potter-Elvehjem-type homogenizer with a Teflon pestle.

Measurement of α-tocopherol and TBARS concentrations. The concentration of α-tocopherol in plasma and liver was analyzed by HPLC as previously described (Huang and Shaw 1994). TBARS concentrations in liver homogenate were determined according to the method of Oteiza et al. (1995).

Measurement of α-CEHC levels.

α-CEHC concentration was determined using HPLC with an electrochemical detector, and the standard of α-CEHC was generously donated by Dr. Huang (Taipei, Taiwan). Urine (1mL) was added to a 50mL glass tube, then 1mL 6M HCl and 9mL diethyl ether was added into the tube, vortex vigorously, after centrifugation, aspirated ether layer to a new tube. Solvent layer was removed in a freeze-dryer and the residue was dissolved in 200μL mobile phase (methanol/H_2O, v/v, 43/57; 50mM sodium acetate, pH 4.5 was contained in water phase) for analysis. The HPLC

was a Jasco model with a ESA Coulochem II electrochemical detector and the Keystone BetaBasic ™ 18 column. The flow rate was 1mL/min and the retention time of the α-CEHC standard peak was 23.58±1.83 min.

Statistical analysis. Data are expressed as means±SD. The significance of difference among the four groups was analyzed by one-way ANOVA and Duncan's multiple range test using the General Linear Model of the SAS Package (SAS Institute, Cary, NC). Student's test was used for the comparison between the two groups. Two-way ANOVA was conducted to confirm the effects of dietary vitamin E and PCN injection and their interaction. Differences were considered significant at $P<0.05$.

RESULTS

Experiment 1. PCN injection for 3 days increased liver weight and relative liver weight significantly (Table 2). The α-tocopherol in plasma and liver of the PCN groups (P50, P500) was significantly reduced with PCN administration by two-way ANOVA results. The liver TBARS concentration was the highest in the rats of P50 group among the four groups.

There were no significantly differences in the excretion of urine volume for three days among the four groups of rats. The excretion of α-CEHC was measured as acid-releasable α-CEHC. The urinary α-CEHC concentration was significantly decreased in the groups of PCN treatment (Table 3). The concentrations of urine α-CEHC (presented as either per mL urine, per day or per mol creatinine) of the P500 or P50 group was only about 41% or 34% of that of the D500 or D50 group, respectively. The 10 fold addition of α-tocopherol in rat diets increased the α-CEHC excretion in urine. The concentrations of urine α-CEHC of the D500 and P500 groups were about 12~14 fold higher than that of the D50 and P50 groups. The vitamin E and PCN factor are both effect the α-CEHC excretion significantly by Two-way ANOVA results.

Experiment 2 and experiment 3. The liver weight, relative liver weight and plasma α-tocopherol concentration in experiment 2 were not significantly different between two groups in exp 3. The α-tocopherol in plasma and liver of DEX group were significantly lower than that of control group. The liver and relative liver weight in rats of DEX group were significantly higher than that of control group (Table 4). So, we calculated the α-tocopherol concentration in the whole liver, as the data showed, the whole liver α-tocopherol levels was significantly higher in the rats of KCZ group than that of control group in experiment 2. However, there was no significant difference in whole liver vitamin E and TBARS concentration between the two groups in experiment 3.

The results of urine α-CEHC excretion were showed in Table 5. Though the concentration of α-CEHC excretion per mL urine showed no significant difference between the two groups in exp.2 and exp. 3. We found that the α-CEHC concentration presented as per day or per mol creatinine were significantly reduced in rats with KCZ administration or DEX injection. The α-CEHC concentration of rats in KCZ group was only about 65 to 68% of those of control group in exp. 2. Similarly, The α-CEHC concentration of rats in DEX group was only about 64 to 67% of those of control group in exp. 3. The volume of urine excretion was not significantly different

between the two groups both in exp2 and exp3.

DISCUSSION

The present data shows the concentration of α-tocopherol in plasma and liver of rats were reduced significantly with treatment of PCN and dexamethasome. These drugs, cytochrome P450 3A inducer, also increased liver weight and relative liver weight as previously reports (Lake et al. 1998, Bjondahl 1976) due to the SER proliferation in hepatocyte. Thus the concentration of α-tocopherol in liver (per gram liver) would be decreased. Nevertheless, PCN still lower the total α-tocopherol levels at about 50% of control rats when calculated as the α-tocopherol amount in the whole liver of rats. PCN treatment increased the liver TBARS at the same time, this suggests that PCN injection in rats would increase lipid peroxidation and this could be one of the reasons why vitamin E status is reduced markedly with PCN treatment. The reaction steps of CYPs involve one-electron transfers which can give rise to by-products such as superoxide, hydrogen peroxide (Porter and Coon 1991, Halkier 1996, White and Coon 1980). The excess production of those reactive oxygen species is able to damage the lipids, proteins and DNA. Dexamethasone are found to elevate MDA levels in liver (Talas et al. 2002) and soleus muscles (Pereira et al. 1999) and decrease the levels of reduced glutathione in liver homogenate of rats (De et al. 2004). Though lipid peroxidation of PCN haven't been investigated directly, CYP3A microsome is reported to owe the highest rate of superoxide production than CYP1A1 microsome (Puntarulo and Cederbaum 1998). Johnson et al. (2002) also reported that PCN injection could increase the rate of biliary SH excretion in rats. All these data suggests that the oxdative stress was elevated by PCN and Dex due to the induction of CYP. The α-tocopherol, a well-known lipid-soluble antioxidant, would be consumed largely for protecting the cell from the attack of reactive oxygen species. This could explain the lower concentration of α-tocopherol in plasma and liver of rats injected by PCN or DEX in this study.

The specific CYPs involved in vitamin E metabolism are still unclear. Parker et al (2000) reported that the metabolism of α-, γ-, and δ-tocopherols was inhibited by the ketoconazole in cultured primary hepatocyte. Ikeda et al. (2002) showed that γ-tocopherol is metabolized by CYP3A in rats fed ketoconazole, an inhibitor of CYP 3A proteins. All these reports suggest that γ-tocopherols are more likely metabolized by CYP3A. Our study first aims at the role of CYP3A in α-tocopherol metabolism with ketoconazole in vivo. We found dietary ketoconazole decreased the urinary excretion of α-CEHC and elevated the concentration of α-tocopherol in liver. It seems CYP3A may be a potent enzyme involved in α-tocopherol metabolism. Birringer et al. (2001) found that rifampicin, a CYP3A inducer, stimulated α-CEHC release from HepG2 cell supplemented with α-tocopherol. So, we hypothesis that CYP3A proteins induced in rats with PCN or DEX administration should increase the metabolism rate of α-tocopherol, and the increased amount of α-CEHC excretion in urine could be observed. In contrast, the results in the present study showed the excretion of urine α-CEHC in rats markedly reduced with PCN. We re-confirmed this results by DEX, another PXR mediated inducer of CYP3A, in experiment 2, and got the same results. This observation reveals that the metabolite of α-tocopherol won't be

elevated by CYP3A induction, in contrast, the production of α-CEHC seems to be inhibited.

CYP3A genes are all induced by DEX and PCN in the rats (Waxman 1999) mediated by PXR nuclear receptors. The PXR receptor can function as regulators in regulating phase I and phase II enzymes, and drug transporters (Xie et al. 2004). The proteins include CYPs (phase I), UDP-glucuronsyl-transferase (UGTs) (Sugatani et al. 2001, Huang et al. 2003), sulfotransferase (SULTs) (Sonoda et al. 2002, Saini et al. 2004), the transporters multidrug resistance proteins (Synold et al. 2001, Geick et al. 2001, Dussault et al. 2001). The glucuronide-conjugated and sulfate-conjugated forms of α-CEHC have been reported in human urine (Lodge et al. 2001). The amount of conjugated form of α-CEHC may be varied in the phase II enzyme modulation. The extraction procedure of α-CEHC in rat urine has been well-established in our lab (unpublished). The HCl was added in the urine to hydrolyze various conjugated forms of α-CEHC and then the ascorbic acid was also added to inhibit the production of α-tocopherolactone from α-CEHC oxidation. Thus, the acid-releasable α-CEHC we detected could be presented as the total amount of α-CEHC in urine. The uncomplete extraction would not be the factor to decrease the urine α-CEHC in rats with PCN or Dex treatment.

Bjorneboe et al.(1987) suggested that about 14% of the injected α-^{3}H-tocopherol was recovered in bile. It shows bile is one of the excretion way of tocopherol, although the percentages of secreted tocopherol in the bile to α-tocopherol concentration in the liver were only about 0.3-0.7% (Yamashita et al. 2000). The multidrug resistance (MDR) protein P-glycoprotein in liver participates in the biliary excretion of various drugs and xenobiotics (Gill et al. 1992, Abraham et al. 1993). Mdr2 knockout mice was reported to have lower biliary α-tocopherol levels than wild-type mice (Mustacich et al. 1998). Mustacich et al. (2006) found that hepatic MDR1 protein increases coincided with the decrease in α-tocopherol concentrations in liver and serum. These data indicates the MDR transporter, a member of ABC transporter family, should be responsible for the α-tocopherol excretion from bile. Furthermore, PCN injection increased the bile flow mediated by MDR2 induction in rats (Johnson et al. 2002). DEX can activate mouse mdr1, mdr2 and human MDR1 in hepatoma cells (Zhao et al. 1993). Whether the MDR induction by PCN or DEX could increase the bile excretion of vitamin E and its metabolite is the important role in vitamin E metabolism needs to be further investigated.

Many CYPs have been studied in vitamin E metabolism. CYP3A was first reported to be involved in γ-CEHC excretion by KCZ treatment in primary hepatocyte culture (Parker et al. 2000), and CYP2C showed no effect on vitamin E metabolism. Sesamin could inhibited γ-tocopherol metabolism in HepG2 cell (Parker et al.2000), and which also inhibited the ω-hydroxylation activity of CYP4F2 by decreased the γ-CEHC in rat liver microsome (Sontage & Parker 2002). This revealed that the reduction of γ-CEHC secretion by sesamin is not specific to CYP3A inhibition in cell culture. Recombinant CYP4F exihibited substrate preference for γ-tocopherol over α-tocopherol in liver microsome (Sontag and Parker 2002). Birringer et al. (2001) showed the *all-rac*-α-tocopherol is much more degraded to α-CEHC than

RRR-α-tocopherol in HepG2 cell with rifampicin (CYP3A inducer) treatment. The tocotrienols are the most potent activator of PXR in HepG2 cell (Landes et al. 2003). All these data from in vitro studies showed that CYPs mainly participated in the γ-tocopherol metabolism other than α-form metabolism. Despite those results reveal that the metabolism of α-tocopherol is not so directly relative to CYPs, some papers showed α-tocopherol do induce the CYP protein.

It has been reported that α-tocopherol injection increased activities of CYP1A1, 2C and 2B in rat liver microsome (Sidorova et al. 2003). Kluth et al. (2005) also reported high dose (200mg) of α-tocopherol in diet for 3 month would induce cyp3a11 in mice when compared to the mice fed α-tocopherol deficienct diet. Mustacich et al. (2006) found that α-tocopherol supplemention induced liver CYP3A, 2B in rats but did not increase α-tocopherol metabolites. They also measured the intermediate metabolite in plasma and urine, but those metabolites still did not increase. Our data also show the similar results, that is CYP3A induced with PCN and DEX didn't increase the levels of α-CEHC in urine, the metabolite of α-tocopherol.

Though α-tocopherol could induce many forms of CYP protein as described above, except the CYP4F, in mice and rats (Traber et al. 2005, Mustacich et al. 2006). All these CYPs induced by α-tocopherol metabolism, preferentially metabolize the other forms vitamers such as γ-tocopherol (Traber et al. 2005). Obviously, the homeostasis of α-tocopherol in vivo is closely relative to its metabolism. This suggests that α-tocopherol plays a role as CYP inducer but dose not act as the substrate of the CYP which induced by itself.

The α-CEHC levels in urine of rats fed ten-fold vitamin E supplemented diets (D500 & P500) were about 12-15 folds higher than that of rats fed normal vitamin E diets (D50 & P50). When rats were injected with PCN (P50 & P500) for 3 days, the α-CEHC excretion in urine was reduced to at about 34-40% of that in rats of control groups (D50 & D500). This suggests that the dietary vitamin E level is much more the critical factor which affect the amount of α-CEHC excretion than PCN administration. The PCN also can reduce the α-CEHC excretion at a constant percentage. Whether the pathway of α-tocopherol metabolism is inhibited or the substrats of metabolism is limited by PCN administration is still unknown.

From these data together, the production of α-CEHC is actually reduced by CYP3A inducer (PCN, DEX) and inhibitor (KCZ). Though, KCZ is not so specific to CYP3A but also inhibited 2B6, 2C9, 3A4 protein (Duret et al. 2006). And PCN, the CYP3A inducer, also slightly induced the mRNA expression of 2B2, 2C6, 2C11 in rat liver (Chen et al. 1995). The pathway of metabolism of α-tocopherol could not be CYP3A-dependent. Nevertheless, the vitamin E status is markedly reduced by CYP3A inducer (PCN & DEX), due to the increased lipid peroxidation and which could increase the consumption of α-tocopherol in liver.

In conclusion, both CYP3A inducers and inhibitor would decrease the α-CEHC excretion in urine. The ten folds of α-tocopherol supplemented in diet would increase the α-CEHC excretion and protect the livers of rats from the damage of lipid peroxidation during the metabolism of CYP3A-induced drug.

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【附表及附圖】

Table 1 Composition of the basal diet

Component	Diet[1]
	g/kg diet
Cornstarch	620
Casein	200
Corn oil (Vitamin E stripped)	100
Cellulose	30
AIN-76 vitamin mixture	35
AIN-76 mineral mixture	10
DL-Methionine	3
Choline chloride	2

[1] All the ingredient sources were the products of MP Biomedicals (CA, USA) except DL-Methionine and choline chloride purchased from Sigma Co. (St. Louis, MO).

Table 2 The liver weight, relative liver weight and α-tocopherol concentration in plasma and liver, whole liver α-tocopherol and liver TBARS concentrations of rats in experiment 1

	Group				Two-way results		
	D50	P50	D500	P500	Vit. E factor	PCN factor	Vit.E ×PCN
Liver*(g)*	7.54±0.56 ^{b}	10.15±1.59 ^{a}	7.91±1.32 ^{b}	10.72±1.67 ^{a}	0.3246	<.0001*	0.8238
RelativeLiver weight (%)	3.46±0.36 ^{b}	4.87±0.82 ^{a}	3.76±0.67 ^{b}	4.97±0.76 ^{a}	0.4012	<.0001*	0.6662
Plasma α-tocopherol *(nmol/L)*	16.5±3.5 ^{b}	10.4±1.1 ^{c}	26.5±3.5 ^{a}	18.4±4.2 ^{b}	<0.0001*	<0.0001*	0.4024
Liver α-tocopherol *(umol/g)*	30.6±6.3 ^{bc}	12.8±4.5 ^{c}	113.7±29.4 ^{a}	49.2±24.0 ^{b}	<0.0001*	<0.0001*	0.0023*
TBARS*(nmol/g Liver)*	118.4 ± 51.6 ^{b}	308.3 ± 69.7 ^{a}	79.1 ± 9.0 ^{b}	122.4 ± 46.8 ^{b}	<.0001*	0.0013*	0.0480 *

1.Each value represents Mean±SD.
2.D50, P50: rats fed diet containing 50 ppm α-tocopherol acetate with DMSO (D50) or PCN injection (P50). D500, P500: rats fed diet containing 500 ppm α-tocopherol acetate with DMSO (D500) or PCN injection (P500). n =8 for D50 and P50 groups, n =9 for D500 and P500 groups. (For TBARS, n=6)
3.Analyzed by Two way ANOVA among the four groups. * denotes p<0.05.
4.Values not sharing common superscript letters a, b are significantly different from one another among the four groups by oneway ANOVA and Duncan's Multiple Range Test (p<0.05).# denotes significantly different from D50 group by student's T test..

Table 3 The urine volume and acid-releasable α -CEHC concentration in urine of rats in experiment 1

	Group				Two-way results		
	D50	P50	D500	P500	Vit. E factor	PCN factor	Vit.E ×PCN
Urine Volume *(ml/day)*	22.1±4.6	23.5±5.2	25.4±6.2	25.2±9.3	0.2197	0.7062	0.8089
Acid-releasable α -CEHC							
(nmol/mL urine)	3.91±1.09 [c]	1.28±0.55 [c]	46.23±25.0 [a]	19.18±7.34 [b]	<0.0001*	0.0021*	0.0130*
(nmol/d)	82.29±19.51 [c]	28.72±14.36 [c]	1089.61±503.60 [a]	443.48±133.26 [b]	<0.0001*	0.0004*	0.0032*
(nmol/mol creatinine)	0.82±0.25 [c]	0.27±0.12 [c]	10.20±5.90 [a]	4.29±1.99 [b]	<0.0001*	0.0045*	0.0213*

1.Each value represents Mean±SD.
2.D50, P50: rats fed diet containing 50 ppm α -tocopherol acetate with DMSO (D50) or PCN injection (P50). D500, P500: rats fed diet containing 500 ppm α-tocopherol acetate with DMSO (D500) or PCN injection (P500). n =8 for D50 and P50 groups, n =9 for D500 and P500 groups.
3.Analyzed by Two way ANOVA among the four groups. * denotes p<0.05.
4.Values not sharing common superscript letters a, b are significantly different from one another among the four groups by one way ANOVA and Duncan's Multiple Range Test (p<0.05)

Table 4 Liver weight, relative liver weight, a-tocopherol levels in plasma, liver and whole liver and liver TBARS concentration of rats in experiment 2 and experiment 3

	Experiment 2		Experiment 3	
	Control	KCZ	Control	DEX
Liver (g)	10.4±0.8	10.6±1.1	10.1±1.4	14.8±1.9*
Liver/body wt.(%)	3.37±0.26	3.48±0.23	4.0±0.4	6.3±0.4*
n	8	8	5	6
Plasma α -tocopherol *(umol/L)*	19.2±4.4	20.1±3.6	15.8±3.0	12.3±1.1*
Liver α -tocopherol *(nmol/g)*	50.0±11.4	65.5±16.6*	46.8±4.3	26.5±4.5*
Whole liver α -tocopherol *(nmol/g)*	516.3±101.6	695.5±202*	466.1±90.9	393.0±91.3
TBARS *(nmol/g Liver)*	105.4 ±21.8	104.4±17.2	45.98±7.70	56.10±31.9

1. Each value represents Mean±SD. * denotes $p<0.05$

Table 5 The urine volume and acid-releasable α -CEHC concentration in urine of rats in experiment 2 and experiment 3

	Experiment 2		Experiment 3	
	Control	KCZ	Control	DEX
Urine volume *(ml/day)*	31.5±9.1	31.4±13.9	37.0±15.4	44.6±17.9
Acid-releasable α -CEHC				
(nmol/mL urine)	3.21±0.59	2.36±1.16	4.03±1.98	2.38±1.58
(nmol/d)	99.6±28.6	64.9±23.6*	124.9±19.13	83.9±24.0*
(nmol/mol creatinine)	1.109±0.302	0.759±0.208*	1.38±0.51	0.88±0.22*

1.Each value represents Mean±SD. * denotes $p<0.05$

【計畫成果自評部份】

許多細胞培養的結果皆指出 PXR、細胞色素 P450 3A 與維生素 E 之代謝有關係。但是透過 PXR 誘導劑之動物實驗的驗證結果發現，維生素 E 代謝產物確實受到 PCN 藥物所影響，然而結果並未如預期會因為 PCN 之注射而增加，爲了證明此點，本研究又多養了一批大鼠並給予同樣屬於 PXR 誘導劑的其他藥物(Dexamethasone)。本實驗結果雖然與預期不相符和，但誠為本領域的新發現，這可能是推翻細胞實驗認為 CYP3A 參與維生素 E 代謝的觀點，因此已經將實驗結果整理以便發表。

【附錄】

Huey-Mei Shaw and Yi-Jen Li (2007) Modulation of Cytochrome P450-3A by pregnenolone (PCN) or ketoconazole (KCZ) both reduces the urine alpha-CEHC excretion in rats. 10th Asian Congress of Nutrition Abstract Book, p104, B35 poster.

Modulation of Cytochrome P450-3A by Pregnenolone (PCN) or Ketoconazole (KCZ) Both Reduces the Urine α-CEHC Excretion in Rats

Huey-Mei Shaw[1], Yi-Jen Li[2]

time management

行政院國家科學委員會補助專題研究計畫 ☑成果報告

大鼠肝臟 PXR 基因表現量和維生素 E 代謝關係之探討(2/2)

計畫類別：☑ 個別型計畫　　□ 整合型計畫
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共同主持人：
計畫參與人員：　黃玟綺

成果報告類型(依經費核定清單規定繳交)：□精簡報告　☑完整報告

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
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□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：嘉南藥理科技大學

中　華　民　國　九十六 年 十　月 十 日

【中文摘要】

本實驗目的為探討炸油餵食之動物體內維生素 E 營養狀況惡化的原因是否與維生素 E 尿液代謝產物增加有關。採用 24 隻 Wistar 雄性大鼠，依飼料不同分為新鮮油組 (CO)、炸油組 (FO)、炸油＋維生素 E 組(FOE)三組，飼養期六週並於最後一天收集尿液。在六週的炸油餵飼下，動物的飼料效率、飼料攝取量與體重增加量皆顯著降低；兩組炸油組的終體重約為 CO 組的 88%左右。FO 與 FOE 兩炸油組的血漿α-生育醇濃度皆顯著下降，約分別為 CO 組的 47%和 65%，但 FO 與 FOE 兩組間並無顯著差異。而肝臟α-生育醇含量以每克肝臟表示或是以總肝臟量表示皆以 FO 組顯著最低，約為 CO 組的 25-30%；FOE 組次之，約為 CO 組的 40-50%左右，然而肝臟 TBARS 以 FOE 組和 FO 組相當且兩組皆顯著高於 CO 組。三組間的肝臟α-TTP 蛋白質表現量相當並無顯著差異。尿液α-CEHC 不管是以每天排出量(mmol/d)、每公升尿液濃度(umol/L)、每克體重排出量或是以尿液肌酸酐作校正之濃度(nmol/mmol)，結果皆以 FO 組顯著最低，而 CO 組與 FOE 組相當。從上述結果發現，雖然炸油會顯著降低動物維生素 E 的營養狀況，但是並非透過降低α-生育醇轉移蛋白之表現量或是增加維生素 E 的尿液代謝產物排出量等途徑所造成。
關鍵詞：大鼠、炸油、尿液α-CEHC、α-生育醇轉移蛋白、細胞色素 P450 3A

【英文摘要】

The purpose of this study is to investigate the effect of frying oxidized oil on the vitamin E metabolite, α-CEHC excretion levels. Three groups of male weanling Wistar rats are fed with control diet containing 15%(w/w) fresh soy oil (CO), 15% frying oxidized oil (FO) or 15% frying oxidized oil+vitamin E (FOE) for 6 weeks and the urine of rats was collected at the last day. The final body weights of the two frying oxidized oil groups were significantly lower than CO group. The CO group showed significantly higher α-tocopherol concentration in plasma, and between the FO and FOE groups showed no significantly difference. The liver α-tocopherol concentration of FO group was significantly lower than that of FOE group, and the FOE group showed significantly lower than CO group in liver α-tocopherol concentration. The TBARS concentration in liver of FOE and FO groups showed significantly higher than that of CO group. There were no significant difference in α-TTP and CYP3A protein levels among the three groups. The urine α-CEHC excretion levels were significantly lower in the FO group. The results indicated that the reduced vitamin E status can not be attribute to the increasing of α-tocopherol metabolite, α-CEHC excretion in urine of rats fed frying oxidized oil.

Key words: Rat, Frying oxidized oil, urine α-CEHC, α-TTP, CYP3A

【目錄】

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【成果內容】

一、前言

在動物餵食炸油模式中，已知會降低維生素 E 營養，原因之一是組織的氧化壓力增加造成對維生素 E 的氧化消耗亦增加以及加速組織維生素 E 之 turnover，目前許多研究指出維生素 E 的代謝反應可能與細胞色素 P450 有關;而炸油餵食的研究亦指出肝臟 cytochrom P450 的量會受誘導增加。因此本實驗希望在炸油餵食模式中，探討動物體內維生素 E 營養狀況惡化的原因是否與代謝產物增加有關。

二、研究目的：了解炸油餵食之動物，鼠體組織中之維生素 E 含量降低，是否因代謝增加所致，因此測定尿液中 alpha-CEHC 此維生素 E 代謝產物作為指標。

三、文獻探討

經高溫油炸之食品因兼具香味、色澤與特殊嚼感而廣受歡迎。由於社會變遷使外食人口遽增，使得如炸雞排、鹹酥雞等各種油炸物充斥日常三餐。但是油炸過程難免會影響食品中的營養成分，高溫對於多元不飽和脂肪酸亦會造成氧化裂解且生成具細胞毒性的物質，而且會被人體吸收並進入循環(Grootveld et al. 1998)。油脂如果反覆油炸，將使氧化劣變情形更為嚴重，其反應複雜包括水解、氧化、異構化、熱分解及聚合等作用，進而改變了油脂的物性與化學性質。在實驗室中以極端條件製備之炸油，經由餵食發現對動物體有不良的影響，包括生長受阻、飼料效率降低、油脂吸收不良、下痢、脫毛、肝腎腫大等效應。

有報告指出炸油餵食會影響肝臟及血漿中脂質含量，如降低 VLDL 和 LDL 三酸甘油脂，但是 HDL 未受影響，而肝臟總脂質雖不變但三酸甘油脂則下降 (劉和黃 1995)。由於油炸時會破壞維生素 E 此抗氧化劑，除了增加動物體內脂質過氧化情形外，亦降低維生素 E 營養狀況 (Liu & Huang 1995, Quiles et al.2002, Sheehy et al. 1994)。可能炸油中的氧化物會促進體內脂質過氧化反應發生，並且加速了體內維生素 E 之轉換率與消耗量(Liu & Huang 1996)。至於動物體在炸油餵食下會增加對維生素 E 轉換率的原因為

何？目前並未有文獻做進一步的探討。動物組織中的維生素E含量是同時受到與α-TTP親和力的強弱以及被酵素代謝的活性所控制，也就是說，飲食中若含有可以影響代謝維生素E之酵素如 CYP3A 活性的成分，必然也會影響到組織或血液中的維生素E含量。例如飲食中的芝麻子，不但富含γ-生育醇同時亦具有抑制 CYP3A 之能力，因此會減少γ-CEHC 的排泄並增加動物組織的γ-生育醇濃度(Ikeda et al 2002)。

黃氏等人(Huang et al. 1988)發現長期餵食炸油之老鼠，肝臟 Total cytochrome P450 量增加，phase II 酵素亦有提升。由於 cyptochrome P450 為藥物代謝系統的 phase I 部分，如藥物、農藥、學物質或毒素，可經由氧化、還原、水解、氫氧基化、去浣基化等反應來增加極性以利排出體外。吳氏(1996)發現炸油會顯著誘發 CYP2B 之蛋白質量。雖然目前推測 CYP3 是參與維生素E代謝的主要酵素，但是不排除還有其他種類的 CYP 酵素之參與。有報告指出炸油可經由誘發 CYP450 3A 的活性而加速維生素 A 之代謝(湯 1994)，由於維生素E之代謝亦有 CYP 酵素的參與，因此炸油餵食除了氧化消耗需求增加外，本人推測，炸油是否亦誘發了某些與維生素E代謝相關的酵素而促進對維生素E的分解代謝？

四、研究方法：

(一) 炸油與脫除維生素E沙拉油之製備

將新鮮大豆沙拉油置於鐵鍋中直接加熱，將油溫維持在 205±5℃以供油炸麵片。麵片製作原料為 1.5 公斤高筋麵粉、200 公克砂糖、5 公克發粉與 600cc 水混勻，和成麵糰後以桿麵機桿成厚度 0.15 公分的麵皮，切割成 10 公分×4.5 公分的麵片，切開中間反折成巧果狀，投入熱油鍋中，每次一片待炸成金黃後取出，再丟入另一片重複進行。每天炸 6 小時，連續炸 4 天，待冷卻後將得到的 24 小時炸油收集後充入氮氣，放置冷凍庫保存，作為日後的實驗用油。

脫除維生素E沙拉油之製備方法如下。取大豆沙拉油 500mL 至燒杯中，加入等體積的 n-hexane，3-5%活性碳，以電磁攪拌器進行 3-5 小時的攪拌。接著以單層濾紙將大量的活性碳濾掉，再改用雙層濾紙過濾掉剩餘的活性碳，將所得之澄清濾液在 37℃下以減壓濃縮去除溶劑，約三個小時直至沒有 n-hexane 的味道，再將由分裝充氮並保存於-20℃，作為日後新鮮油組的油脂來源。

(二) 動物分組與飼養

採用購自國家動物中心的 Wistar 雄性大鼠，依飼料不同分為新鮮油組 (CO)、炸油組 (FO)、炸油＋維生素E組(FOE)三組。飼料組成如表一所示，維生素E的補充劑量為正常飼料的兩倍，由於參考前人文獻(Liu & Huang 1996)發現炸油組的維生素E吸收率會降低約 50%，因此在本實驗中為了排除吸收問題以及進入體內維生素E含量不同而設計此組，使進入體內的維生素E與新鮮油組相當以作比較。飼養期為 6 週。第六週的最後一天收集尿液，記錄體積並保存於-20℃。

(三) 樣品處理與分析項目

1. 血液與組織前處理

動物犧牲前一夜先行禁食約 12 小時。犧牲當天秤過老鼠體重後，將之以 CO_2 窒息再自腹腔靜脈採血，並快速取下臟器秤重。血液以低速離心將血漿分離，分裝後貯存於 -20℃，以供日後各項測定。肝臟經秤重後，於冰上剪下定量的組織並記錄重量後，以均質緩衝液 (potassium-phosphate buffer 0.01 M, pH 7.4) 進行均質，並定量體積使成為 25% (W/V) 組織均質液。取適量組織均質液於 4°C 下 12,000×g 20 分鐘，取出定量上清液再於 4℃下進行 105,000 ×g 離心 60 分鐘，所得細胞質上清液貯存於 -80°C 以供α-生育醇轉移蛋白之分析，沉澱物則為肝微粒體貯存於-80℃以供細胞色素 P450 蛋白質分析。

2. 血漿脂質與白蛋白測定測定：採用市售試劑組之呈色法分析總脂質、三酸甘油脂、膽固醇與白蛋白含量。

3. 尿液中α-CEHC 代謝產物之測定

採用酸加熱處理法：1 ml 尿液樣品（含 ascorbic acid, 20mg/ml），加入 1ml 12N HCl，混勻，充氮氣，於 60℃水浴反應 1 小時，冰浴冷卻，再加入 9 ml 乙醚劇烈萃取 1 min，離心，取上層 6 ml 有機層，抽乾溶劑，以 0.2 ml mobile phase 回溶，適當稀釋，由 HPLC-ECD 偵測維生素 E 代謝產物 (α-CEHC)的含量，並以α-CEHC 外部標準曲線來計算濃度。以市售肌酸酐分析試劑測定肌酸酐作為校正。

4. 維生素 E 濃度與 TBARS 測定

取得血漿或肝臟均質液加入酒精後再以 n-hexane 萃取，以 HPLC 分析維生素 E 含量。另以 Thiobarbiturate method 測定 TBARS 作為脂質過氧化指標。

5. 肝臟細胞色素 P450 3A1 蛋白質量之測定

肝微粒體經過適當稀釋後測定蛋白質含量。取 10μg 蛋白質以 7.5% SDS-PAGE 進行電泳，轉印至 PVDF 後，以 3A1 之專一性抗體進行免疫反應，最後以 ECL 試劑 (Amersham)進行冷光顯影反應，底片再以電腦分析個別色帶的顯影強度。

6. 肝臟細胞質α-生育醇轉移蛋白含量測定

α-生育醇轉移蛋白以 Western blot 進行測定，方法參照前人文獻 (Shaw & Huang 2000)，簡述如下：取含 10μg 蛋白質之細胞質液進行 4%~15% SDS Polyacrylamide gel 蛋白質電泳，條件為 150 伏特下進行電泳 1.5 小時。經轉印至 PVDF 上，先後與一級抗體(anti-α-TTP) 、二級抗體培養，再以 ECL 試劑 (Amersham)進行冷光顯影反應。

(四) 統計與分析

實驗結果皆以平均值±標準偏差表示，並以 SAS 軟體進行 one way ANOVA 來檢定組間差異顯著性，$p<0.05$ 表示有顯著差異。

五、結果與討論

1. 飲食設計說明

本實驗的飼料雖然以 AIN-76 飼料配方作為基礎，但是為了與前人研究相比較，所以採用 15%的油脂百分比；由於炸油的製備過程會大量破壞油中的維生素 E，所以為了與控制組之間的維生素 E 含量必須調整到一致，避免成為比較維生素 E 營養狀況時的不平等因素，因此在控制組所使用的油為「脫除維生素 E 沙拉油」。另外炸油餵食鼠能出現吸收不良現象，其維生素 E 吸收率平均約為控制組的 50%，因此增加一組 FOE 使其吸收進入體內的維生素 E 與控制組相當，因此該組與控制組有所差別之處應是炸油因素所致。

2. 動物生長

在六週的炸油餵飼下，動物的飼料效率、飼料攝取量與體重增加量皆顯著降低；兩組炸油組的終體重約為 CO 組的 88%左右，且並未因為維生素 E 的添加(FOE)而有所改善。在器官與重量方面，兩炸油組以肝臟重顯著增為 CO 組的 1.23 倍，但由於炸油組體重下降因此以器官相對體重百分比來表示時，其肝臟百分比約為控制組的 1.42 倍，顯示本實驗所製備的炸油會造成肝臟種大現象。脾臟與腹腔脂肪(RE)重皆以炸油組顯著下降，但是換算成相對體重百分比時，三組間則無顯著差異；然而睪丸與腦重在組間雖無顯著差異，換算成相對體重百分比時卻以炸油兩組顯著高於 CO 組。表五為炸油餵食對血漿白蛋白、三酸甘油酯與膽固醇結果指出 FO 組的白蛋白含量顯著高於其餘兩組；然三組間的三酸甘油酯與膽固醇濃度皆無顯著差異。

3. 血漿α-生育醇與肝臟α-生育醇、TBARS 及α-生育醇轉移蛋白

如表六所示，FO 與 FOE 兩炸油組的血漿α-生育醇濃度皆顯著下降，約分別為 CO 組的 47%和 65%，但 FO 與 FOE 兩組間並無顯著差異。而肝臟α-生育醇含量以每克肝臟表示或是以總肝臟量表示以 FO 組顯著最低，約為 CO 組的 25-30%；FOE 組次之，約為 CO 組的 40-50%左右，結果指出 FOE 組確實有比 FO 組攝入較多的維生素 E，然而在肝臟 TBARS 的結果發現，FOE 組和 FO 組相當且兩組皆顯著高於 CO 組。上述結果說明，FOE 組雖然肝臟α-生育醇濃度有顯著高於 FO 組，但是在炸油的生理影響下該組的α-生育醇貯存量亦只有 CO 組的一半，尚不足以發揮較 FO 組還強的脂質抗氧化能力。根據前人研究指出，炸油餵飼鼠在補充 10 倍維生素 E(500ppm)後六週 (劉與黃, 1995)或十二週(湯與黃, 1998)皆可以使肝臟維生素 E 含量提升至比新鮮油組高，且使肝臟 TBARS 含量亦下降與新鮮油組相當(劉與黃, 1995)，然而肝臟總 P450 活性仍顯著高於新鮮油組(湯與黃, 1998)。

肝臟α-生育醇轉移蛋白 (α-TTP)含量的表現如圖一所示，α-TTP 為分子量 34kD 的蛋白質，本實驗以β-actin 作為校正蛋白質。結果指出，三組間的α-TTP 蛋白質表現量相當並無顯著差異，表示炸油對於肝臟雖然造成較高的氧化壓力並降低維生素 E 的含量，但是並未對α-TTP 產生誘導或抑制作用。由於α-TTP 對於肝臟維生素 E 含量的保留有關 (Kayden and Traber 1993)，且本人先前研究發現維生素 E 缺乏會抑制其蛋白質表現量 (Shaw and Huang 1998)。本實驗結果說明炸油降低肝臟維生素 E，增加維生素 E 汰換的原因與α-TTP 此蛋白質並無顯著關係。

4. 尿液α-生育醇代謝產物(α-CEHC)排出量

本實驗在飼養期最後一天收集尿液，所測定的肌酸酐結果如表七所示。三組的尿液排出量並無顯著差異，在肌酸酐排出方面，三組間亦無顯著差異。在維生素 E 代謝產物方面，我們測定了尿液α-CEHC 排出量，結果如圖八所示。α-CEHC 不管是以每天排出量(mmol/d)、每公升尿液濃度(umol/L)、每克體重排出量或是以尿液肌酸酐作校正之濃度(nmol/mmol)，結果皆以 FO 組顯著最低，而 CO 組與 FOE 組相當。可見尿中α-CEHC 排出量與維生素 E 攝入量及肝臟維生素 E 含量有相關。

假設炸油會促進維生素 E 的代謝情形，理應會增加該產物在尿液中的排出量，但是在本實驗結果並未有此發現，似乎炸油對維生素 E 的消耗途徑不包括增加代謝排出此途徑。由於炸油會誘導不同型的 cytochrome P450 ，故本實驗測定了 CYP3A 以探討其與維生素 E 代謝的關係，在圖二的結果發現三組間的 CYP3A1 蛋白質表現量並無顯著差異，表示炸油餵食六週後未能明顯誘導 CYP3A 此酵素蛋白質；如果 CYP3A 確實參與維生素 E 的代謝，則上述在代謝產物排出量與 CYP3A 表現的結果是十分一致的。

炸油餵食對於維生素 E 的營養狀況有很顯著的影響，此點從肝臟維生素 E 含量有非常明確的證據證明。炸油的影響原因包括抗氧化能力下降可大量消耗維生素 E 之外，還會促進其在組織中的汰換速率(Liu and Huang 1996)，本實驗發現並非透過誘導肝臟 CYP 相關酵素以促進代謝產物來達成，因此應該是透過其他方式而增加維生素 E 的消耗狀況，究竟是何種方式呢？如肝臟釋出 VE 進入血液的途徑？週邊組織的攝入途徑？VE 釋入膽汁的途徑？或是吸收過程的某種影響？仍有待進一步探討。

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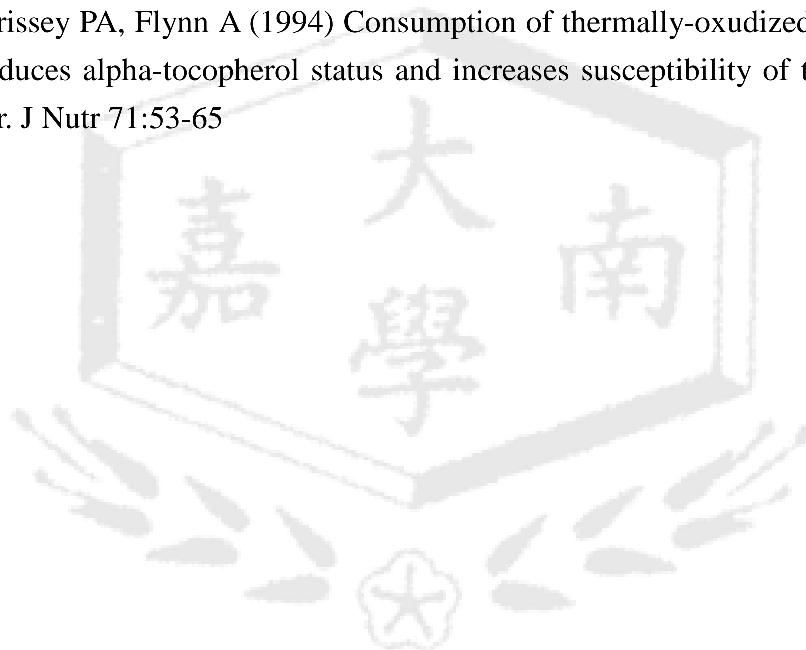


【附表與附圖】

表一 飼料組成

Group/ Ingredients	CO	FO	FOE
	g/kg Diet		
Fresh Oil	150		
Frying Oil		150	150
Corn starch	572	572	572
Casein	200	200	200
Cellulose	30	30	30
AIN-76Vitamin mixture	10	10	10
AIN-76Mineral mixture	35	35	35
Choline	3	3	3
D,L-Methionine	3	3	3
All-rac-α-Tocopheryl Acetate			

表二 餵食新鮮油(脫除維生素 E 黃豆油，CO)、炸油(FO)或補充維生素 E 之炸油(補充 50ppm 維生素 E 之炸油，FOE)，飼料六週後對老鼠體重增加、總攝食量及飼料利用效率之影響

	Body final weight (g)	Body weight gain (g)	Food intake (g/d)	Feed efficiency (%)
CO	400.91±26.22	7.809±0.818^{a}	21.03±1.44^{a}	37.10±2.33^{a}
FO	352.15±14.89^{b}	6.473±1.742^{b}	18.80±4.75^{b}	34.60±8.31^{b}
FOE	353.70±25.99^{b}	6.511±1.707^{b}	19.28±4.73^{ab}	33.81±8.24^{b}

表三 餵食新鮮油(脫除維生素 E 黃豆油，CO)、炸油(FO)或補充維生素 E 之炸油(補充 50ppm 維生素 E 之炸油，FOE)，飼料六週後對老鼠組織重量之影響

_	CO	FO	FOE
		weight(g)	
Liver	13.13±2.14[b]	16.23±0.74[a]	16.53±1.79[a]
Kidney	3.14±0.46	2.93±0.19	3.09±0.22
Lung	1.86±0.36	2.05±0.55	1.73±0.25
Spleen	0.87±0.89[a]	0.74±0.09[b]	0.77±0.67[b]
Heart	1.34±0.14	1.25±0.13	1.23±0.08
Testis	3.30±0.26	3.47±0.30	3.35±0.11
E P	6.97±1.61	5.66±1.17	5.76±1.18
RE	7.53±2.03[a]	5.07±1.86[b]	4.99±2.36[b]
Brain	1.84±0.10	1.78±0.06	1.86±0.12

表四 餵食新鮮油(脫除維生素 E 黃豆油，CO)、炸油(FO)或補充維生素 E 之炸油(補充 50ppm 維生素 E 之炸油，FOE)，飼料六週後對老鼠組織相對重量之影響

	CO	**FO**	**FOE**
		Relative tissue weight(%)	
Liver	3.42±0.36[b]	4.88±0.23[a]	4.89±0.53[a]
Kidney	0.82±0.10	0.88±0.05	0.91±0.05
Lung	0.49±0.10	0.62±0.17	0.51±0.07
Spleen	0.23±0.03	0.22±0.02	0.23±0.02
Heart	0.34±0.02	0.37±0.05	0.36±0.02
Testis	0.87±0.07[b]	1.04±0.10[a]	0.99±0.07[a]
EP	1.81±0.31	1.69±0.31	1.69±0.30
RE	1.96±0.42	1.51±0.53	1.45±0.63
Brain	0.48±0.02[b]	0.53±0.04[a]	0.55±0.03[a]

表五 餵食新鮮油(脫除維生素 E 黃豆油，CO)、炸油(FO)或補充維生素 E 之炸油(補充 50ppm 維生素 E 之炸油，FOE)，飼料六週後對老鼠血漿中白蛋白、三酸甘油酯及膽固醇含量之影響

	Albumin (g/dL)	Triglyceride (mmol/L)	Cholesterol (mmol/L)
CO	3.835±0.228[b]	0.708±0.136	2.015±0.361
FO	4.197±0.297[a]	0.694±0.136	2.337±0.322
FOE	4.050±0.274[ab]	0.618±0.182	2.064±0.567

表六 餵食新鮮油(脫除維生素 E 黃豆油，CO)、炸油(FO)或補充維生素 E 之炸油(補充 50ppm 維生素 E 之炸油，FOE)，飼料六週後對老鼠血漿、肝臟中α-tocopherol 與 TBARS 濃度之影響

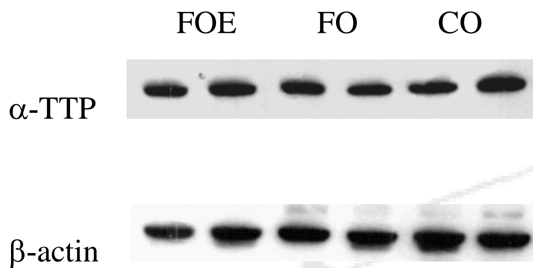
	Plasma α-tocopherol	Liver α-tocopherol		Liver TBARS
	μmol/L	nmol/g	nmol/liver	nmol/g
CO	16.31±6.07[a]	38.67±3.37[a]	508.7±101.8[a]	98.69±11.28[b]
FO	7.82±2.99[b]	9.75±1.34[c]	159.0±27.9[c]	135.13±12.52[a]
FOE	10.52±3.82[b]	15.25±3.08[b]	251.1±50.2[b]	124.55±12.65[a]

表七 餵食新鮮油(脫除維生素 E 黃豆油，CO)、炸油(FO)或補充維生素 E 之炸油(補充 50ppm 維生素 E 之炸油，FOE)，飼料六週後對老鼠收集一天尿液體積及肌酸酐含量之影響

	Urine volume	Creatinine excretion		
	mL/d	mmol/L	mmol/d	umol/g BW
CO	38.7±18.4	205±77	6.90±1.98	17.3±5.2
FO	30.3±11.1	230±70	6.46±1.50	18.3±4.0
FOE	35.5±19.4	258±115	7.60±1.77	21.5±4.8

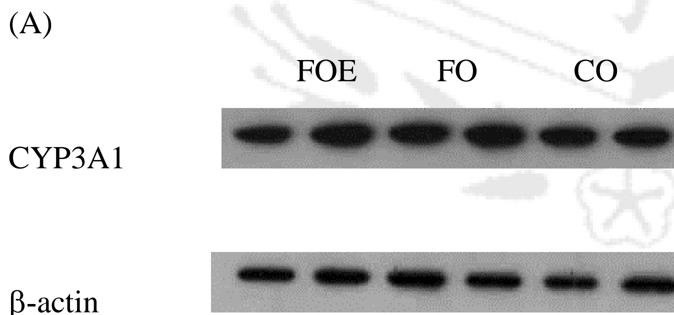
表八 餵食新鮮油(脫除維生素 E 黃豆油，CO)、炸油(FO)或補充維生素 E 之炸油(補充 50ppm 維生素 E 之炸油，FOE)，飼料六週後對老鼠一天尿液中α–CEHC 含量之影響

	Acid-releasable α-CEHC excretion			
	μmol/d	μmol/L	nmol/mmol	nmol/g BW
CO	0.112±0.032 [a]	3.53±1.97 [a]	17.78±7.81 [a]	0.283±0.087 [a]
FO	0.065±0.019 [b]	2.30±0.81 [b]	9.94±1.21 [b]	0.183±0.050 [b]
FOE	0.123±0.018 [a]	4.78±3.84 [a]	17.06±5.29 [a]	0.350±0.065 [a]

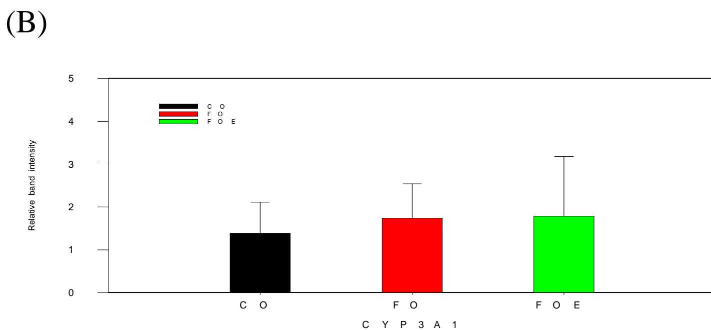


圖一 餵食新鮮油(脫除維生素 E 黃豆油，CO)、炸油(FO)或補充維生素 E 之炸油(補充 50ppm 維生素 E 之炸油，FOE)，飼料六週後對老鼠肝細胞質液α-TTP 蛋白含量的影響。

(A)



(B)



圖二 餵食新鮮油(脫除維生素 E 黃豆油，CO)、炸油(FO)或補充維生素 E 之炸油(補充 50ppm 維生素 E 之炸油，FOE)，飼料六週後對老鼠肝微粒體細胞色素 P450 酵素 3A1 蛋白含量的影響。(A) 底片經冷光顯影後的圖片。(B)定量分析成數據後之結果，每組數值為 8 隻老鼠的平均值。

【成果自評】

本研究之分組設計與原先計畫有所不同，其特點亦在「結果與討論」中加以說明。本實驗結果與原本預期雖然有所不同，但是仍提供了證據說明炸油對維生素 E 代謝的影響可能不是單純透過α-CEHC 此代謝途徑而造成顯著影響，因此極具學術價值且可提供日後繼續探討的方向，我們將再進行一些確認實驗之後即整理發表。

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