

Secretion of α -Tocopherol in VLDL Is Decreased by Dietary Protein Insufficiency in Young Growing Rats¹

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ABSTRACT The concentrations of α -tocopherol in plasma and most peripheral tissues were shown previously to be low in young growing rats fed a low protein diet. To examine the secretion rates of VLDL α -tocopherol and triglycerides, and lipoprotein lipase activity, weanling rats were fed a low protein (LP, 8 g/100 g lactalbumin) or a normal protein (NP, 20 g/100 g lactalbumin) diet for 6 wk. The absolute secretion rate of VLDL triglyceride ($\mu\text{mol/h}$) of the LP group was not significantly different from that of the NP group ($P > 0.05$), but was significantly higher ($P < 0.05$) when expressed relative to body weight [$\mu\text{mol}/(\text{h} \cdot \text{kg})$]. The secretion rates of VLDL α -tocopherol were significantly lower ($P < 0.05$) in the LP group than in the NP group. The activities of hepatic lipase, lipoprotein lipase and total heparin-releasable lipase in plasma of the LP group were only 50–60% those of the NP group ($P < 0.05$). The results demonstrated that the secretion rate of VLDL α -tocopherol and activities of lipases in postheparin plasma were significantly lower in rats fed a low protein diet. Thus, the redistribution of α -tocopherol from liver to peripheral tissues appears to have been impaired by dietary protein insufficiency. *J. Nutr.* 130: 3050–3054, 2000.

KEY WORDS: • vitamin E • low protein diet • VLDL secretion rate • lipoprotein lipase • rats

The transport of vitamin E by lipoproteins within the circulation has been well documented (Bjorneboe et al. 1990, Kayden and Traber 1993). Absorbed dietary vitamin E is incorporated into chylomicron particles (Bjorneboe et al. 1986, Traber et al. 1988) in enterocytes, then is released into lymphatics and subsequently into blood. Within the circulation, most of the absorbed dietary vitamin E remains in the chylomicron remnants (Bjorneboe et al. 1987a) and enters the liver when chylomicron remnants are taken up by that organ. In the liver, the newly absorbed vitamin E is repackaged into the VLDL particle, which is then secreted into the plasma (Bjorneboe et al. 1987b, Cohn et al. 1988, Traber et al. 1988). Accordingly, the redistribution of α -tocopherol from liver to peripheral tissues depends largely on VLDL secretion from the liver.

The α -tocopherol binding protein (α -TBP)⁴ is a cytosolic protein that binds specifically to RRR- α -tocopherol and is found in the liver of rats and humans (Behrens and Madère 1982, Catignani and Bieri 1977, Kaplowitz et al. 1989, Kuhlenskamp et al. 1993, Sato et al. 1991, Yoshida et al. 1992). The protein is also called α -tocopherol transfer protein (α -TTP) because it enhances the transfer of α -tocopherol between membranes (Sato et al. 1991). As mentioned above,

physiologic studies have indicated that liver is responsible for the preferential discrimination among the E vitamins (Burton and Traber 1990) and for the incorporation of RRR- α -tocopherol into nascent VLDL, which then distributes α -tocopherol to the peripheral tissues (Traber et al. 1990). This is consistent with the ligand specificity (Sato et al. 1991) and tissue distribution pattern of α -TTP (Yoshida et al. 1992). Taken together with the α -tocopherol transfer activity, α -TTP is proposed to be the mediator of preferential incorporation of RRR- α -tocopherol into VLDL (Kayden and Traber, 1993).

We showed previously that dietary protein insufficiency in rats significantly lowered α -tocopherol concentration in peripheral tissues but not in liver (Huang and Shaw 1994). The possibility that liver α -TTP might be impaired by dietary protein inadequacy was then examined, and the expression of α -TTP protein and mRNA were indeed depressed in the liver of rats fed a low protein diet (Shaw and Huang 1998). According to the proposed physiologic role of α -TTP, this could lead to a reduced redistribution of α -tocopherol from liver to peripheral tissues as a result of a decreased export of α -tocopherol through VLDL secretion from liver. To test the hypothesis the secretion rates of VLDL triglycerides and α -tocopherol were examined in this study in rats fed a low protein diet. The lipase activity in the postheparin plasma was also measured because it is involved in the clearance of the triglyceride-rich lipoprotein.

MATERIALS AND METHODS

Animals and diets. Male Long-Evans weanling rats purchased from the Laboratory Animal Center, College of Medicine, National Taiwan University (Taipei, Taiwan) were housed individually in

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⁴ Abbreviations used: apo, apolipoprotein; α -TTP (α -TBP), α -tocopherol transfer protein (α -tocopherol binding protein); HL, hepatic lipase; LP, low protein; LPL, lipoprotein lipase; NP, normal protein control.

TABLE 1

Composition of test diets

Component	Diet	
	Normal protein	Low protein
	g/kg diet	
Lactalbumin ¹	200	80
Cornstarch ¹	570	690
Soybean oil ¹	150	150
Cellulose ¹	30	30
AIN-76 vitamin mixture ²	10	10
AIN-76 mineral mixture ²	35	35
Choline chloride ¹	3	3

¹ Ingredient sources: lactalbumin and choline chloride, Sigma Chemical (St. Louis, MO); α -cellulose, ARBOCEL, type BE 600/300, J. Bettenmaier & Söhne, Germany; soybean oil, Taiwan Sugar Company (Taipei, Taiwan); cornstarch, Roquette, France.

² American Institute of Nutrition (1977).

stainless steel wire cages in a room maintained at $25 \pm 2^\circ\text{C}$ with a controlled 12-h light: dark cycle. The rats were divided into two groups as follows: the NP group was fed a control diet containing a normal level of protein (20 g/100 g lactalbumin); the LP group was fed a low protein diet (8 g/100 g lactalbumin). Diets and tap water were freely available. Body weights and food intake were recorded weekly. The compositions of the test diets are shown in Table 1. Animal care and handling conformed to NIH guidelines (NRC 1985).

Blood sampling and chemical analysis. After 6 wk of feeding, rats from each group were deprived of food overnight. The next morning, rats were anesthetized by intraperitoneal injection of pentobarbital (Nembutal Sodium Solution, Abbot Laboratories, North Chicago, IL; 62.5 mg/kg body), and a catheter was implanted into the jugular vein. After blood was collected as the 0-h sample, a dose of heparin (250 U/kg body) was infused (Krauss et al. 1973). The postheparin blood was drawn after 5 min. Tyloxapol (200 g/L in saline; Sigma Chemical, St. Louis, MO), which is the same polyethylene glycol as Triton WR-1339, was infused via the catheter to block VLDL degradation, at a dose of 400 mg/kg of body (Shiomi et al. 1987). Blood samples were collected 2 and 4 h after Tyloxapol infusion. All blood samples were collected in heparin-containing tubes and the plasma was isolated by low speed centrifugation at $1000 \times g$ for 15 min. The VLDL fraction was isolated by ultracentrifugation (Oda et al. 1991). Aliquots of plasma were added to the thin-walled polyallomer tubes (13×64 mm), and a NaBr solution ($d = 1.006$ kg/L) was layered over the surface. After capping, the samples were centrifuged at $121,000 \times g$ for 16 h (50.4 Ti rotor, XL-90 ultracentrifuge, Beckman, Fullerton, CA). The VLDL fraction was recovered from the top layer of each tube.

Plasma and VLDL triglycerides concentrations were determined by enzymatic analysis using a reagent kit (Randox Laboratory, Crumlin, UK). The concentration of α -tocopherol in plasma and VLDL was analyzed by HPLC as previously described (Huang and Shaw 1994).

Calculation of the VLDL secretion rate. The secretion rate of VLDL triglycerides and α -tocopherol were calculated according to Oda et al. (1991) using the following equation: secretion rate of VLDL triglyceride ($\mu\text{mol/h}$) or α -tocopherol (nmol/h) = $(C_4 - C_0)V/4$, where C_0 and C_4 are the concentrations of triglyceride (or α -tocopherol) before (0 h) and 4 h after Tyloxapol administration, and V is the plasma volume derived on the basis of 4 mL plasma/100 g body weight. Because the body weight of rats fed the low protein diet was significantly lower than that of the control rats, the secretion rate obtained was further divided by the body weight of each rat and expressed as $\mu\text{mol}/(\text{h} \cdot \text{kg body})$ for VLDL triglyceride secretion or $\text{nmol}/(\text{h} \cdot \text{kg body})$ for VLDL- α -tocopherol secretion.

TABLE 2

Body weight gain, food intake, feed efficiency and plasma albumin of rats fed normal (NP) or low (LP) protein diets for 6 wk¹

	NP	LP
Initial body weight, g	69.2 $\pm$ 5.5	69.2 $\pm$ 6.4
Final body weight, g	334.7 $\pm$ 39.9	215.0 $\pm$ 33.3*
Body weight gain, g/d	6.32 $\pm$ 0.89	3.47 $\pm$ 0.76*
Food intake, g/d	15.29 $\pm$ 1.73	12.75 $\pm$ 2.60*
Feed efficiency, g gain/g feed	0.41 $\pm$ 0.03	0.27 $\pm$ 0.01*
Plasma albumin, g/L	31.12 $\pm$ 3.07	26.41 $\pm$ 2.61*

¹ Values are means $\pm$ SD, $n = 7$. * Significantly different from the NP group, $P < 0.05$.

Lipase assays. Lipase activity was determined as described by Nilsson-Ehle and Schotz (1976) with some modifications. The substrate was freshly prepared as follows: calculated amounts of labeled triolein (glycerol tri[9,10(n)-³H] oleate, 0.66 TBq/mmol; Amersham Pharmacia Biotech, Uppsala, Sweden) and unlabeled triolein in chloroform (Sigma Chemical) were mixed and the solvent evaporated under a stream of nitrogen, then sonicated after the addition of a gum arabic solution. The reaction mixture for total lipase activity contained the following: triolein, 5 mmol/L; gum arabic, 5 g/L; albumin, 100 g/L; heated serum (normal rat serum preheated for 60 min at $56 \pm 1^\circ\text{C}$), 10 g/100 g; Tris buffer, 0.2 mol/L, pH 8.6; CaCl_2 , 0.01 mol/L; NaCl, 0.1 mol/L and 0.01 mL of plasma sample in a final volume of 0.5 mL. The mixture was incubated at 25°C for 30 min and the reaction was terminated by the addition of 3.25 mL methanol:chloroform:heptane (1.41:1.25:1, v/v/v) and 0.75 mL of 0.1 mol/L potassium carbonate-borate buffer (pH 10.5). After vigorous mixing for 15 s, the mixture was centrifuged at $800 \times g$ for 10 min, and the upper phase separated and counted in a Beckman LS 5000 CE counter for radioactivity. This procedure detects heparin-releasable lipase activity of both hepatic and nonhepatic origin.

Hepatic lipase (HL) activity was determined as described above except that plasma samples were preincubated with 1 mol/L NaCl for 30 min, the reaction mixture maintained a final NaCl concentration of 1 mol/L and the heated serum [apolipoprotein (apo) C source] was omitted. It is well established that lipoprotein lipase (LPL), but not HL, is strongly inhibited under these conditions (Krauss et al. 1973). Thus, the activity of LPL could be calculated by subtracting HL activity from total lipase activity. Enzyme activity (1 mU) is defined as the amount of enzyme that catalyzes the release of 1 nmol of oleic acid per min at 25°C .

Statistical analysis. Data were expressed as means $\pm$ SD. The significance of difference ($P < 0.05$) between the two groups was analyzed by Student's t test.

RESULTS

In agreement with our previous data, food intake, body weight gain, feed efficiency and plasma albumin concentration were significantly lower in the LP group than in the NP group ($P < 0.05$, Table 2). The final body weight of rats fed the LP diet was $\sim 60\%$ that of the NP group. These data indicated the protein-insufficient status of the LP group.

There was no significant difference in plasma triglyceride concentration between the two groups before Tyloxapol infusion (Table 3). The α -tocopherol concentration and the ratio of α -tocopherol to triglyceride in plasma were significantly lower in rats of the LP group than those of the NP group, also consistent with our previous report (Huang and Shaw 1994).

After the administration of Tyloxapol, triglyceride and α -tocopherol concentration in plasma (data not shown) and in VLDL (Fig. 1) increased linearly up to 4 h. The VLDL

TABLE 3

The plasma triglyceride and α -tocopherol concentrations and the molar ratio of α -tocopherol to triglycerides in rats fed normal (NP) or low protein (LP) diets for 6 wk¹

	NP	LP
Triglyceride, mmol/L	0.497 $\pm$ 0.101	0.555 $\pm$ 0.107
α -Tocopherol, μ mol/L	4.88 $\pm$ 2.23	2.69 $\pm$ 0.54*
α -Tocopherol/triglyceride, μ mol/mmol	10.55 $\pm$ 6.41	4.95 $\pm$ 1.16*

¹ Values are means $\pm$ SD, $n = 7$. * Significantly different from the NP group, $P < 0.05$.

triglyceride concentrations at 2 and 4 h after Tyloxapol administration were significantly higher ($P < 0.05$) in the LP group than in the NP group. Nevertheless, the α -tocopherol concentration at 4 h after Tyloxapol administration was significantly lower ($P < 0.05$) in the LP group than in the NP group. Consequently, the difference between the two groups was magnified when these data were expressed as the ratio of α -tocopherol/triglycerides in VLDL after Tyloxapol treatment (Fig. 1). The secretion rates of VLDL triglyceride and α -tocopherol were calculated on the basis of these data. The absolute secretion rate of VLDL-triglyceride (μ mol/h) did not differ between the two groups (Table 4). However, the LP group had a significantly higher ($P < 0.05$) relative VLDL-triglyceride secretion rate [μ mol/(h $\cdot$ kg body)]. On the other hand, the absolute and relative secretion rates of VLDL α -tocopherol were significantly lower in rats fed the LP diet than in those fed the NP diet (Table 4).

Total lipase activity in postheparin plasma of the LP group was $\sim 54\%$ that of the NP group ($P < 0.05$, Table 5). Both HL and LPL activities also were significantly lower in the LP group than in the NP group ($P < 0.01$).

DISCUSSION

The reduction in food intake of the LP group might have resulted in a reduced vitamin E intake. Nevertheless, this may not be a major contributing factor to the compromised vitamin E status of the LP group because the food intake/body weight ratio was actually higher in the LP group than in the NP group. In other words, the vitamin E intake per unit body tissue of the LP group was not reduced.

The Triton technique was used in this study to measure VLDL secretion in vivo. Triton WR-1339 blocks the degradation of lipoproteins, either by coating the plasma lipoproteins (Schotz et al. 1957) or by direct inhibition of LPL activity (Borensztajn et al. 1976). The validity of this technique has been questioned (Palmer et al. 1978) and the calculated rates of secretion may be too low by 5–10% (Otway and Robinson 1967). However, the results are consistent with those from other independent methods and describe metabolic responses adequately (Bird et al. 1984, Huang and Williams 1980). We conducted these experiments with anesthetized rats because our preliminary experiments showed that the VLDL-triglyceride secretion rate of anesthetized rats was not different from that of awake animals (data not shown).

The VLDL triglyceride concentrations at 2 and 4 h after Tyloxapol injection were significantly higher in the LP group than in the NP group (Fig. 1). When the absolute secretion rate (μ mol/h) was calculated by multiplying the rate of the concentration change by plasma volume, the groups did not

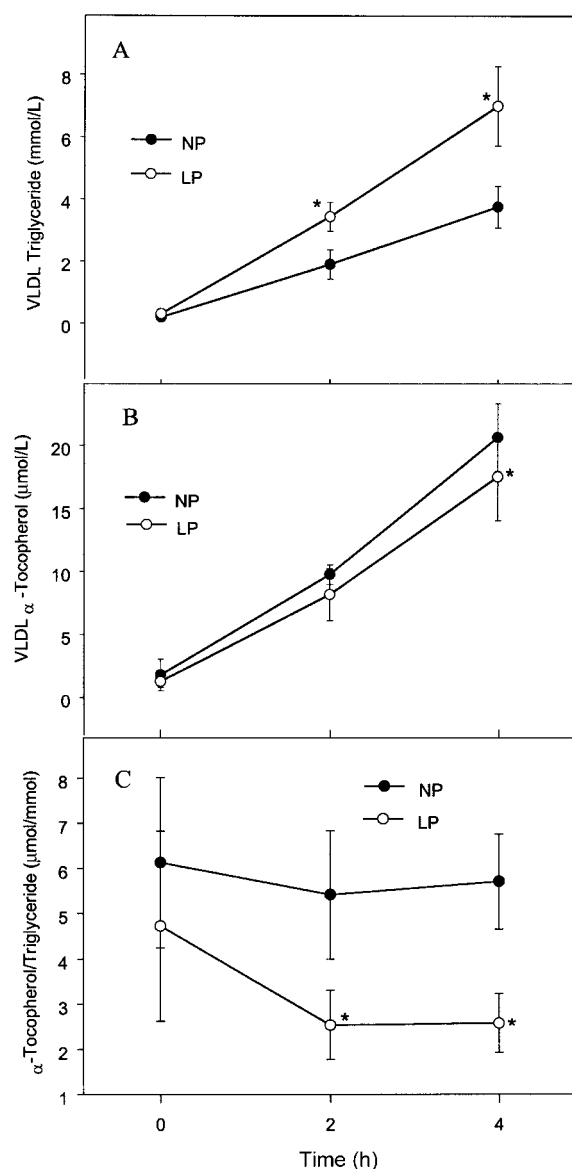


FIGURE 1 VLDL α -tocopherol and triglyceride concentrations and ratio in rats fed control (NP) or low protein (LP) diets after Tyloxapol administration. The NP rats were fed a normal protein (20 g/100 g lactalbumin) diet; the LP rats were fed a low protein (8 g/100 g lactalbumin) diet. After 6 wk of feeding, Tyloxapol was injected into the jugular vein at a dose of 400 mg/kg body. Values represent means $\pm$ SD, $n = 7$. *The value differed significantly ($P < 0.05$) from the NP group.

differ. This likely was because the plasma volume was estimated as 4% of the body weight and the body weight of the LP group was significantly lower than that of the NP group (Table 4). Because rats fed an 8% lactalbumin diet had significantly lower liver weight (Huang and Fwu 1993, Huang and Shaw 1994), the secretion rate per g liver or per 100 g body weight, in fact, is higher in the LP group than in the NP group.

The fatty liver observed in kwashiorkor has been attributed to decreased export of lipid from liver. Severe protein deficiency (≤ 6 g/100 g casein) in rats resulted in significantly lower apo B and C, the major apolipoproteins in VLDL. Hence, decreased synthesis of apolipoprotein in the liver is considered to be a major cause of fatty liver in severe protein malnutrition such as kwashiorkor (Gouache et al. 1991, Meghelli-Bouchenak et al. 1987). On the basis of these data, it is conceivable that α -tocopherol secretion from liver could

TABLE 4

Absolute and relative VLDL secretion rates of triglycerides and α -tocopherol in rats fed normal (NP) or low (LP) protein diets for 6 wk¹

	NP	LP
VLDL-triglyceride		
$\mu\text{mol/h}$	12.9 $\pm$ 2.1	15.6 $\pm$ 3.7
$\mu\text{mol}/(\text{h} \cdot \text{kg body weight})$	35.5 $\pm$ 6.7	66.9 $\pm$ 12.6*
VLDL α -tocopherol		
nmol/h	69.1 $\pm$ 15.6	37.7 $\pm$ 10.1*
$\text{nmol}/(\text{h} \cdot \text{kg body weight})$	187.4 $\pm$ 34.4	162.4 $\pm$ 38.0*

¹ Values are means $\pm$ SD, $n = 7$. * Significantly different from the NP group, $P < 0.05$.

be reduced as a result of lower VLDL secretion in protein deficiency. Should this be the case, the α -tocopherol/triglyceride ratio in plasma should be unaffected. Results of the present study and our previous report (Huang and Shaw 1994), however, do not support this speculation. Not only was the plasma triglyceride level not significantly lower in the LP group than in controls, but there was also a 50% reduction in the plasma α -tocopherol/triglyceride ratio in the LP group.

It is unclear why the relative VLDL triglyceride secretion rate [$\mu\text{mol}/(\text{h} \cdot \text{kg})$] in the LP group was significantly higher than that of the control group. In agreement of this result of the present study, Portman et al. (1981) also observed an increased secretion rate of VLDL triglyceride [$\text{mg}/(\text{kg} \cdot 24 \text{ h})$] in rhesus monkeys fed a 4% casein diet for a long time compared with that of monkeys fed a normal protein diet. It is not clear whether the increased rate of VLDL triglyceride secretion in protein-inadequate animals observed in these two studies is related to the high dietary fat level ($\geq 15 \text{ g}/100 \text{ g}$) used, or to the relatively greater amount of carbohydrate in the LP diet, which may lead to an increase in hepatic lipogenesis.

The present study clearly demonstrated that the absolute and relative secretion rates of VLDL- α -tocopherol were significantly lower in the LP group than in the NP control group. The significantly lower ratio of α -tocopherol to triglyceride in plasma of rats fed the LP diet may be secondary to the higher VLDL triglyceride secretion rate. However, this may not be the case because the VLDL α -tocopherol concentration 4 h after Tyloxapol treatment was significantly lower in the LP group than in the NP group (Fig. 1). In addition, the plasma α -tocopherol concentration of rats of the LP group was only

55% that of the control rats (Table 3). The result indicates that the redistribution of α -tocopherol from liver to peripheral tissues was impaired in rats fed the LP diet.

Kayden and Traber (1993) proposed that α -TTP is the major mediator responsible for preferential incorporation of RRR- α -tocopherol into nascent VLDL, which are then secreted from the liver. In support of this view, Arita et al. (1997) demonstrated that the secretion of α -tocopherol was more efficient in cells expressing α -TTP than in matched cells lacking α -TTP. We have found that the α -TTP protein level and mRNA expression were significantly lower in rats fed a low protein diet (Shaw and Huang 1998). The result of the present study that secretion rate of VLDL α -tocopherol was lower in rats fed a low protein diet further supports the proposed physiologic role of α -TTP.

LPL is important in the metabolism of triglyceride-rich lipoprotein, including VLDL and chylomicrons. Consistent with previous reports (Lamri et al. 1995, Portman et al. 1981), the activities of total lipase, HL and LPL in the postheparin plasma were significantly lower in rats fed a low protein diet. It is not clear why such a paradoxical situation existed in that the plasma triglyceride concentration was not significantly higher in protein-deficient rats despite a significantly greater VLDL-triglyceride secretion rate accompanied by a significantly lowered plasma heparin-releasable lipase activities. Portman et al. (1981) also observed similar puzzling results.

Traber et al. (1985) demonstrated that LPL enhanced the uptake of α -tocopherol by cells in culture. Although the tissue origin of the LPL in the postheparin plasma cannot be specified, the lower activity of LPL in rats fed a low protein diet may account in part for the lower α -tocopherol concentration in peripheral tissues (Huang and Shaw 1994).

Vitamin E deficiency occurs in human patients with fat malabsorption and in individuals with various genetic disorders (Sokol 1993, Traber et al. 1993). Impaired digestion, absorption, transportation, tissue uptake and storage of vitamin E, rather than inadequate dietary intake, seem to underlie most circumstances of human vitamin E deficiency. Our observations concerning the importance of protein nutrition in the utilization of dietary vitamin E may be relevant for humans. Karla et al. (1998) showed that children with protein-energy malnutrition were vitamin E deficient, as revealed by a low serum α -tocopherol, which correlated with neurological signs.

In conclusion, we have demonstrated that the secretion rate of VLDL α -tocopherol was decreased significantly in growing rats fed a low protein diet. The results support the role of liver α -tocopherol binding protein in mediating the incorporation of α -tocopherol into nascent VLDL for secretion.

TABLE 5

Total lipase, hepatic lipase and lipoprotein lipase activities in post-heparin plasma of rats fed normal (NP) or low (LP) protein diets for 6 wk¹

	NP	LP
	<i>u/L plasma²</i>	
Total lipase	199.4 $\pm$ 40.3 ¹	107.5 $\pm$ 37.0*
Hepatic lipase	66.0 $\pm$ 9.0	37.7 $\pm$ 12.3*
Lipoprotein lipase	133.4 $\pm$ 36.8	70.0 $\pm$ 27.4*

¹ Values are means $\pm$ SD, $n = 7$. * Significantly different from the NP group, $P < 0.05$.

² 1 u = 1 μmol fatty acid released/min at 25°C.

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