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Note

Changes in Distribution of α-Tocopherol and Cholesterol in Serum Lipoproteins and Tissues of Rats by Dietary PCB and Dietary Level of Protein

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Summary An experiment was conducted with growing rats to examine the influence of dietary addition of 0.1% polychlorinated biphenyls (PCB) and dietary level of protein on the distributions of α-tocopherol and cholesterol in serum lipoproteins and tissues. Addition of PCB increased serum α-tocopherol. This was mainly due to the increment in the fractions of chylomicrons/very-low-density lipoproteins (VLDL) and high-density lipoproteins (HDL). This chemical also increased serum cholesterol and HDL cholesterol. The contents of α-tocopherol and cholesterol in HDL fraction were higher in the high protein groups as compared to the low protein groups, regardless of PCB intake. PCB intake increased the concentrations of α-tocopherol in tissues including spleen, lung, kidney, testis, muscle, adipose tissue, and brain. High protein diet increased the levels of α-tocopherol in these tissues as compared to low protein diet. PCB intake also increased adrenal α-tocopherol, which was potentiated with low protein diet. The increment in liver α-tocopherol by PCB intake was observed only in the animals fed on low protein diet.

Key Words PCB, dietary protein, serum lipoproteins, α-tocopherol, cholesterol

Administration of PCB, Chloretone, caffeine, and some other xenobiotics to rats increases serum total and HDL cholesterol (*1*). Elevation of serum cholesterol by these xenobiotics is due to increased hepatic cholesterogenesis including 3-hydroxy-3-methylglutaryl-CoA reductase (*2, 3*). Increases in serum cholesterol of PCB-treated rats appear to be dependent on dietary level and quality of protein (*4, 5*). Increases in serum cholesterol are directly proportional to dietary protein level (*4*).

Recently we reported an increase in serum α-tocopherol of rats fed some xenobiotics such as PCB, Chloretone, and phenobarbital (*6*). Increases in serum α-tocopherol by these xenobiotics were positively correlated with changes in serum cholesterol (*6*). These metabolic changes of α-tocopherol seem to be some protective

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response against xenobiotics since PCB feeding increases liver lipid peroxidation and vitamin E requirement (*7*).

The present study was designed to investigate the influence of dietary addition of PCB and dietary level of protein on the distributions of α-tocopherol in serum lipoproteins and tissues of rats with special reference to cholesterol metabolism.

METHODS

After feeding commercial stock diet (MF Oriental Yeast Co., Ltd., Tokyo) for 3 days, male rats of Wistar strain, weighing 65 to 86 g, were used. Animals were housed at 24±1°C with a 12-h cycle of light (8:00 a.m.–8:00 p.m.) and dark. The diets and water were available *ad libitum*. The test diets were composed of 7 or 30% casein and other nutrients, described in Table 1. PCB (PCB-48, Tokyo Chemical Industries Ltd., Tokyo) was added to the diets at the level of 0.1%. Since influence of dietary PCB on tissue vitamin E was prominent in the rats fed the diets containing higher levels of the vitamin (*8*), the diets used here were supplemented with somewhat higher levels (5- to 10-fold) of tocopheryl acetate (30 mg/100 g) as compared with the diets generally used. After feeding the test diets, the diets were removed from the cages at 8:00 a.m. and the animals were lightly anesthetized with ether and killed between 1:00 p.m.–3:00 p.m. Blood was collected by heart puncture and serum was prepared by centrifugation. Tissues were immediately excised, weighed, and stored at −35°C until analysis.

The pooled sera were subjected to preparative ultracentrifugation and the lipoproteins, including chylomicrons plus very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL), were isolated by flotation (*9*). The concentrations of cholesterol in serum and serum lipoproteins were measured enzymatically using a commercially available kit (Cholesterol C-Test,

Table 1. Composition of the basal diets (%).

	High protein	Low protein
Casein[1]	30.0	7.0
α-Corn starch	55.0	78.0
Corn oil	5.0	5.0
Cellulose powder	4.0	4.0
Salt mixture[2]	4.0	4.0
Vitamin mixture[3]	2.0	2.0

[1] Vitamin-free casein (Oriental Yeast Co., Ltd., Tokyo). [2] Ebihara, K. *et al.* (1979): *J. Nutr.* **109**, 2106. [3] Provided the following (mg per 100 g diet): thiamine HCl, 2.4; riboflavin, 8; pyridoxine HCl, 1.6; D-biotin, 0.04; cyanocobalamin, 0.001; ascorbic acid, 60; menadione, 10.4; folic acid, 0.4; inositol, 12; Ca pantothenate, 10; *p*-aminobenzoic acid, 10; niacin, 12; choline chloride, 400; retinyl acetate, (1,000 IU per 100 g diet); cholecalciferol, (200 IU per 100 g diet); DL-α-tocopheryl acetate, 30.

Wako Pure Chemical Industries Ltd., Osaka). Liver lipids were extracted by the method of Folch *et al.* (*10*) and used for the determination of liver cholesterol by the procedure of Pearson *et al.* (*11*). The concentrations of α-tocopherol in serum lipoproteins and tissues were measured by HPLC method described previously (*12*). The statistical significance of difference between values of the data was analyzed by Duncan's multiple-range test (*13*).

RESULTS

Table 2 represents the influence of dietary 0.1% PCB and dietary level of protein on the final body weight and the concentrations of lipids in tissues and serum. High protein diets stimulated the growth as compared to low protein diets, regardless of PCB addition. Dietary addition of 0.1% PCB caused no significant

Table 2. Effects of dietary 0.1% PCB and protein level on tissue and serum lipids in rats.

Groups	Normal		PCB	
	High protein	Low protein	High protein	Low protein
Final body weight		(g)		
	170 ± 3^{a}	88 ± 4^{b}	166 ± 3^{a}	84 ± 3^{b}
Tissue		(μg/g tissue)		
α-Tocopherol				
Liver	$83 \pm 4^{1,a}$	81 ± 11^{a}	103 ± 10^{ab}	135 ± 16^{b}
Adrenal	578 ± 30^{a}	502 ± 59^{a}	762 ± 23^{b}	920 ± 37^{c}
Spleen	84 ± 2^{a}	48 ± 3^{b}	125 ± 4^{c}	107 ± 4^{d}
Lung	85 ± 3^{a}	44 ± 3^{b}	115 ± 10^{c}	87 ± 6^{a}
Kidney	36 ± 1^{a}	22 ± 1^{b}	57 ± 3^{c}	49 ± 1^{d}
Testis	33 ± 3^{a}	24 ± 2^{b}	54 ± 3^{c}	44 ± 1^{d}
Muscle[2]	18.5 ± 0.5^{a}	11.5 ± 0.5^{b}	26.5 ± 1.4^{c}	20.9 ± 0.8^{a}
Adipose tissue[3]				
	11.9 ± 1.0^{a}	13.3 ± 1.4^{b}	28.0 ± 0.6^{c}	23.5 ± 2.8^{a}
Brain	11.3 ± 0.6^{a}	9.5 ± 0.8^{b}	16.0 ± 0.3^{c}	12.4 ± 0.4^{a}
Liver		(mg/g tissue)		
cholesterol				
	2.3 ± 0.1^{a}	2.1 ± 0.1^{a}	3.6 ± 0.2^{b}	4.1 ± 0.5^{b}
Serum		(mg/100 ml)		
α-Tocopherol				
	2.24[4]	1.15	4.65	3.82
Cholesterol		(mg/100 ml)		
	81 ± 4^{a}	70 ± 7^{a}	122 ± 20^{b}	98 ± 3^{ab}

[1] Mean ±SE. ($n=4$–7). Mean within a column not followed by the same letter are significantly different ($p<0.05$). [2] Soleus muscle. [3] Epididymal fat pads. [4] Pooled sample.

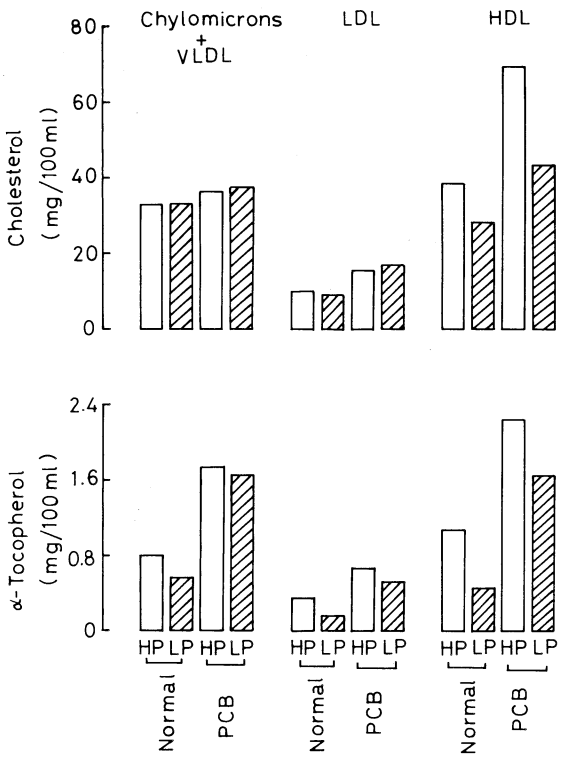

Fig. 1. Effects of dietary 0.1% PCB and dietary level of protein on the distributions of cholesterol and α-tocopherol in serum lipoproteins. HP, high protein diet; LP, low protein diet.

influence on the growth. Liver concentrations of α-tocopherol were not significantly affected with dietary level of protein. A significant increase in liver α-tocopherol by PCB was observed only in the animals fed the low protein diet. PCB intake also increased adrenal α-tocopherol and this effect was prominent in the animals fed on the low protein diet. The concentrations of α-tocopherol in other tissues examined here were significantly increased by PCB and increasing dietary protein.

Liver and serum cholesterol were also increased in the PCB-treated group. There was a trend of increment in serum cholesterol and α-tocopherol with increasing dietary protein.

Distributions of cholesterol and α-tocopherol in lipoprotein fractions were illustrated in Fig. 1. Dietary addition of PCB markedly raised HDL-cholesterol. HDL-cholesterol was also increased with increasing dietary protein. The levels of cholesterol in the fractions of chylomicrons/VLDL and LDL were slightly increased by PCB. PCB intake increased α-tocopherol in all of lipoprotein fractions examined here. High protein diets clearly increased HDL α-tocopherol, regardless of PCB intake. The levels of α-tocopherol in other fractions were also slightly raised by

high protein diet.

DISCUSSION

Previous study by our group showed an increment in serum and HDL cholesterol by PCB and other xenobiotics with heparin-Mn precipitation method (*1*). The present study using ultracentrifugation method confirmed our previous study. The present study further demonstrated increased α-tocopherol in serum or serum lipoprotein fractions and in several tissues by PCB. We recently showed an increase in intestinal absorption of this vitamin in the rats that received xenobiotics (Katayama and Kato, to be published). Therefore, an increase in serum lipoprotein and tissue α-tocopherol might relate to the increased intestinal absorption of it.

α-Tocopherol is absorbed in the small intestine and transported in lymph associated with chylomicrons (*14*). Studies have revealed that α-tocopherol is transported in serum located in chylomicrons/VLDL and HDL in rats (*14*). It has also been demonstrated that α-tocopherol may be transferred directly between lipoproteins *in vitro* (*15*).

It has been suggested that α-tocopherol associated with HDL or secretory products from the liver were taken up by peripheral tissues (*16*). This was supported by the present study demonstrating similar changes in the levels of α-tocopherol in HDL fraction and in most extrahepatic tissues (Table 2, Fig. 1).

It has been reported that dietary protein is required for the intestinal absorption of α-tocopherol and increasing dietary protein increases the concentrations of α-tocopherol in kidney, lung, and muscle of rats (*17, 18*). These studies were confirmed by the present study showing similar changes in tissue α-tocopherol by dietary protein level in the rats without receiving PCB (Table 2). Interestingly, in PCB-treated rats, there was a trend of increment in liver α-tocopherol by low protein diet, and PCB intake caused a significant increase in liver α-tocopherol only when the animals were fed on low protein diet (Table 2). Similarly, accumulation of liver cholesterol by PCB was also prominent in the low protein diet. These effects of low protein diet might be due to the depressed secretion of cholesterol and α-tocopherol with lipoproteins from the liver. It is also of interest that an increase in adrenal α-tocopherol by PCB was prominent in the low protein diet (Table 2). Apparently, further study is necessary to clarify the underlying mechanisms of these effects of protein level on liver and adrenal α-tocopherol.

We previously reported a positive correlation between serum cholesterol and α-tocopherol in the rats fed several xenobiotics (*1*). The present study further showed similar trends in the changes of cholesterol and α-tocopherol in serum and HDL fraction by dietary manipulation (Fig. 1). An increase in serum lipoprotein cholesterol by xenobiotics might relate to the increase in serum vitamin E. Further study is in progress to examine this possibility.

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