Disposition Kinetics of α-Tocopherol in Apolipoprotein B Knockout Mice

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Received October 11, 2002; accepted November 14, 2002

Purpose. We examined the effects of apolipoprotein B (apoB) on the disposition kinetics of α-tocopherol by using apoB knockout mice.
Methods. The concentrations of α-tocopherol in plasma and tissues were measured by gas chromatography–mass spectrometry.
Results. In *apob* (–/–) mice, the endogenous levels of α-tocopherol in plasma and tissues (except liver) were significantly lower, and the liver concentration was significantly higher than those in wild-type mice. After single i.v. administration of α-tocopherol (25 mg/kg), the area under the plasma concentration–time curve (AUC) and the distribution volume at steady state were significantly decreased, whereas the total clearance of α-tocopherol was significantly increased in *apob* (–/–) vs. wild-type mice. α-Tocopherol was highly distributed to the liver, compared with other tissues. After an oral administration of α-tocopherol (100 mg/kg), the intestinal absorption of α-tocopherol was very low in apoB knockout mice, as the value of AUC_{0-32h} for *apob* (–/–) mice (17.7 ± 8.3 μg h/mL) was significantly less than that for *apob* (+/+) wild-type mice (96.5 ± 15.8 μg h/mL, mean ± SD of five experiments, $p < 0.01$). The biliary excretion of α-tocopherol was significantly greater in *apob* (+/–) mice than in *apob* (+/+) mice.
Conclusions. These results show that apoB plays a role in hepatic secretion and intestinal absorption of α-tocopherol.

KEY WORDS: apolipoprotein B; α-tocopherol; knockout mice; abetalipoproteinemia; disposition kinetics.

INTRODUCTION

Abetalipoproteinemia (ABL) is a human recessive genetic disease characterized by extremely low levels of plasma cholesterol and triglycerides (1,2). Patients are deficient in apoproprotein B (apoB)-containing lipoproteins such as chylomicrons, very low-density lipoproteins (VLDLs), and low-density lipoproteins (LDLs), and apoB is not present in blood. There have been reports that ABL is caused by a mutation of microsomal triglyceride transfer protein (MTP) (3–5). MTP is required for the formation of intracellular lipoprotein in the liver and intestine (6). After triglyceride is transferred to apoB by MTP in the liver, VLDLs are produced and secreted. In the absence of MTP, dietary fat absorbed from the intestine cannot be transferred to lipoprotein, so that the absorption of fat is impaired. Consequently, lipophilic vitamins absorbed concomitantly with fat are also decreased (7). Yang *et al.* reported that serum concentrations of apoB and vitamin E in a patient with ABL are extremely low compared with other apolipoproteins and lipophilic vitamins (8).

Vitamin E (α-tocopherol) acts as a chain-breaking antioxidant that prevents the propagation of free radical reactions (9). There are many reports on the disposition kinetics of α-tocopherol (10). α-Tocopherol is absorbed from the intestinal lumen by a passive diffusion process, then binds to chylomicrons and is transported in the lymphatic fluid (11). After the α-tocopherol-bearing chylomicrons are taken up into the liver as chylomicron remnants, α-tocopherol is bound with VLDLs in a process mediated by α-tocopherol transfer protein (α-TTP), and the α-tocopherol-bearing VLDLs are secreted into blood (12). Therefore, apoB is closely associated with intestinal absorption and tissue distribution of α-tocopherol, and ABL secondary to lack of apoB is now treated simply with large doses of α-tocopherol. However, little is known about the pharmacokinetics of α-tocopherol in ABL. It is important to understand the behavior of the drug in the body because this may lead to the development of new therapies or new drugs.

In 1993, Homanics *et al.* (13) prepared mice with a modified *apob* allele. *apob* (+/+) mice have both apoB100 and B48, whereas *apob* (–/–) mice have apoB48 and truncated apoB (apoB70) but not apoB100; *apob* (+/–) mice have all the apoB types. The plasma concentrations of apoB, β-lipoproteins, total cholesterol, and total triglyceride in *apob* (–/–) and *apob* (+/–) mice are markedly decreased compared with those of *apob* (+/+) mice, and further, in *apob* (–/–) mice, the plasma concentration of α-tocopherol is also reduced. Therefore, these *apob*-modified mice are a good ABL model, and pharmacokinetic study of α-tocopherol in apoB knockout mice may be helpful for the development of new ABL pharmacotherapy.

In this study, we clarified the disposition kinetics of α-tocopherol in apoB knockout mice and confirmed the role of apoB in the pharmacokinetics of α-tocopherol.

MATERIALS AND METHODS

Materials

(±) α-Tocopherol, 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMC), and propylene glycol were purchased from Wako Pure Chemical Industries (Osaka, Japan). N,O-bis-TMS-trifluoroacetamide (MTBSTFA) was purchased from Tokyo Kasei Organic Chemicals Co. (Tokyo, Japan). HCO-60 was supplied from Nippon Chemicals Co. (Tokyo, Japan). Oligonucleotide primers were custom synthesized by Amersham Pharmacia Biotech (UK).

Animals and Propagation

Male and female *apob* (+/–) mice (C57BL/6J-Apo b^{tm1Unc}) as apoB knockout mice were purchased from The Jackson Laboratory (JAX MICE®, Maine). By mating male *apob*

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ABBREVIATIONS: ABL, abetalipoproteinemia; Apo-B, apoprotein B; AUC, the area under the blood concentration–time curve; CL_{tot}, the total body clearance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MRT, the mean residence; MTBSTFA, N,O-bis-TMS-trifluoroacetamide; MTP, microsomal triglyceride transfer protein; PMC, 2,2,5,7,8-pentamethyl-6-hydroxychroman; α-TTP, α-tocopherol transfer protein; VLDL, very low-density lipoprotein; $V_{d,ss}$, the distribution volume at steady state.

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(+/–) mice with female *apob* (+/–) mice, we obtained *apob* (–/–), *apob* (+/–), and *apob* (+/+) mice. Genotyping of littermates was performed according to the polymerase chain reaction (PCR) method using primers for *apob* (–/–) [5′-CAC CTC CTG TCC AAG CCG CCT ATC A-3′and 5′-CAG ATA TAC ATT GGC TTC ATT GGC A-3′ (400 bp)] and for *apob* (+/+) [5′-CAG ATA TAC ATT GGC TTC ATT GGC A-3′ and 5′-GCA GTA CAA ATT AGA GGG AAC ATC A-3′ (430 bp), according to the instructions of The Jackson Laboratory.

Animal Experiments

All animal experiments complied with the guidelines of the Institutional Animal Care and Use Committee of the University of Kanazawa.

Experiments were performed on 8-week-old *apob* (–/–), *apob* (+/–), and *apob* (+/+) mice. α-Tocopherol (25 or 100 mg/kg) in a solution of 50% ethanol, 10% HCO-60, and 10% propylene glycol was injected via the jugular vein in a volume of 40 μL or was orally administered in a volume of 200 μL. Blood samples were collected from the intraorbital venous plexus using a heparinized capillary tube under light ether anesthesia, at designated time intervals. The plasma was separated by centrifugation and stored at –30°C until assay. The tissues were quickly excised, rinsed well with ice-cold saline, blotted dry, and weighed. The samples were homogenized in ice-cold saline (10% w/v). For biliary recovery of α-tocopherol, the bile duct was cannulated with polyethylene tubing (type sp-8 O.D. 0.5 mm, Natsume, Tokyo, Japan) under light ether anesthesia. Cannulated mice were kept in a supine position on restraining plates. The samples were kept at –30°C until assay.

Assay for α-Tocopherol

Concentrations of α-tocopherol in plasma, bile, and tissues were determined by gas chromatography–mass spectrometry (GC-MS, Model GC-17 system Class 5000, Shimadzu, Kyoto, Japan). The assay for α-tocopherol was carried out according to Nakanishi *et al.* (14).

Aliquots of 100 μL of plasma, bile, or tissue homogenates were each mixed with 100 μL of 1% ascorbic acid in water, 100 μL of 0.1 μg/mL PMC in ethanol, as an internal standard, and 1 mL of *n*-hexane. The mixture was shaken for 20 s and centrifuged for 5 min at 3000 × *g*. The supernatant organic phase was transferred to another glass tube and preconcentrated under a stream of nitrogen gas at 37°C in a heating block. Then, 150 μL of acetone and 50 μL of MTBSTFA were added to the residue, and the mixture was shaken vigorously. The sample was transferred to an automated-sampler microvial and incubated for 12 h at room temperature. An aliquot (1 μL) of sample was injected into the GC-MS system.

Analyses were carried out in the selected-ion monitoring mode, monitoring ions at *m/z* 502 and *m/z* 292 for α-tocopherol and PMC, respectively. Chromatographic separation of α-tocopherol was achieved with a 5% phenylmethylpolysiloxane-crosslinked capillary column (DB-5; 30 m × 0.315 mm I.D.; J&W Scientific Inc., USA) in a gas chromatograph equipped with a splitless injector. The oven temperature was set at 60°C for 1 min and then programmed up to 280°C at 20°C/min. The final temperature was maintained for 12 min.

Data Analysis

The pharmacokinetic parameters were estimated according to model-independent moment analysis as described by Yamaoka *et al.* (15). The data were analyzed using Student's *t* test to compare the unpaired mean values of two sets of data. The number of determinations is noted in each table and figure. A value of $p < 0.05$ was taken to indicate a significant difference between sets of data.

RESULTS

Endogenous Plasma and Tissue Concentration of α-Tocopherol

Figure 1 shows the endogenous plasma and tissue concentrations of α-tocopherol in the three genotypes. The concentrations of α-tocopherol in the brain, lung, heart, spleen, fat, and plasma in *apob* (–/–) mice were significantly lower than those in *apob* (+/+) mice. The concentrations in *apob* (+/–) mice were intermediate between those of *apob* (–/–) mice and *apob* (+/+) mice. However, only the concentration in the liver was significantly higher in *apob* (–/–) mice than in *apob* (+/+) mice.

Plasma Concentration-Time Course of α-Tocopherol after an I.V. Administration

After an i.v. administration of α-tocopherol (25 mg/kg), plasma concentration of α-tocopherol was measured. Figure 2 represents the increased values after the endogenous concentration has been subtracted from the observed concentration at each time. The plasma concentration time courses in all three genotypes showed rapid decreases until 2 h after administration, and thereafter, the plasma concentrations in *apob* (–/–) mice and *apob* (+/–) mice were significantly lower than that in *apob* (+/+) mice. The plasma concentrations in all

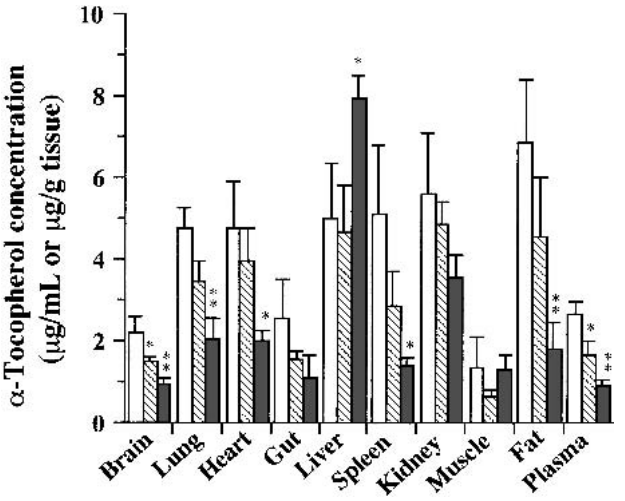

Fig. 1. Tissue and plasma concentrations of endogenous α-tocopherol in *apob* (+/+), *apob* (+/–), and *apob* (–/–) mice. Each *column* with *bar* represents the mean ± SE of four mice. *,**Significantly different from *apob* (+/+) mice at $p < 0.05$ and 0.01, respectively. Key: *open bars*, *apob* (+/+) mice; ▨, *apob* (+/–) mice; ■, *apob* (–/–) mice.

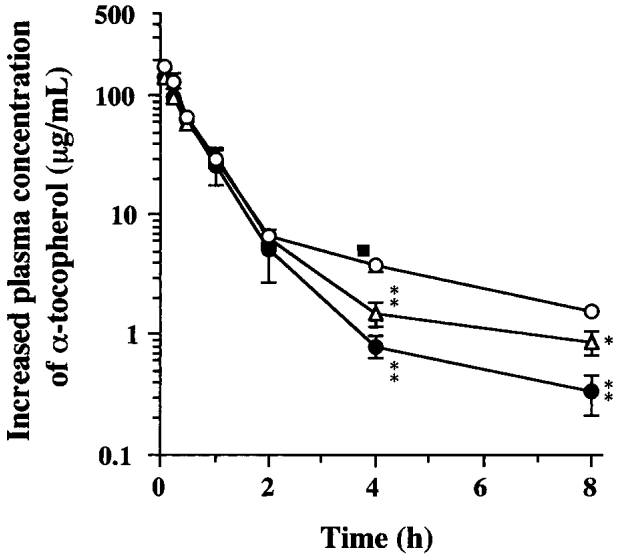

Fig. 2. Increased plasma concentration–time courses of α-tocopherol after i.v. administration of α-tocopherol (25 mg/kg) in *apob* (+/+), *apob* (+/–), and *apob* (–/–) mice. Each *point* with *bar* represents the mean ± SE of four mice. *,**Significantly different from *apob* (+/+) mice at $p < 0.05$ and 0.01, respectively. Key: ○, *apob* (+/+) mice; △, *apob* (+/–) mice; ●, *apob* (–/–) mice.

these mice decreased to the endogenous level within 24 h after administration.

The pharmacokinetic parameters of α-tocopherol in mice of the three genotypes are listed in Table I. The areas under the plasma concentration–time curve (AUC) from zero to 8 h for *apob* (+/–) mice and *apob* (–/–) mice were significantly less than that for *apob* (+/+) mice. The values of plasma total clearance (CL_{tot}) of α-tocopherol for *apob* (+/–) mice and *apob* (–/–) mice were significantly higher than that for *apob* (+/+) mice, whereas the value of distribution volume at steady state ($V_{d,ss}$) for *apob* (–/–) mice was significantly less than those in the others.

Tissue Distribution of α-Tocopherol after an I.V. Administration

Figure 3 shows the tissue concentrations of α-tocopherol at 2 h after an i.v. administration of α-tocopherol (25 mg/kg) in the three genotypes of mice. In tissues except for the liver, the α-tocopherol concentrations tended to be lower in the mutant mice than in *apob* (+/+) mice, but the differences were not statistically significant. In the liver, as shown in Fig. 4, the α-tocopherol concentration reached a peak at 2 h after administration in all the mice that was very much higher than those in other tissues and about 30 times higher than that in the plasma. Thereafter, whereas the concentration in the liver of *apob* (+/+) mice decreased to the endogenous level by 24 h after administration, the rates of decrease in *apob* (–/–) mice and *apob* (+/–) mice were very slow, i.e., α-tocopherol was accumulated in the liver of mutant mice.

Table I. Pharmacokinetic Parameters of α-Tocopherol after I.V. Administration of α-Tocopherol (25 mg/kg) in Mice[a]

	apob (+/+)	*apob* (+/–)	*apob* (–/–)
$AUC_{0-8\ h}$ (μg h/mL)	130 ± 11	103 ± 10**	93.3 ± 13.2**
MRT (h)	1.66 ± 0.13	1.17 ± 0.15**	0.791 ± 0.19**
$V_{d,ss}$ (mL/kg)	318 ± 39	285 ± 46	212 ± 62*
CL_{tot} (mL/h/kg)	192 ± 16	243 ± 21**	268 ± 33**

[a] Each value represents the mean ± SD ($n = 4$).
*,** Significantly different from the *apob* (+/+) mice at $p < 0.05$ and 0.01, respectively.

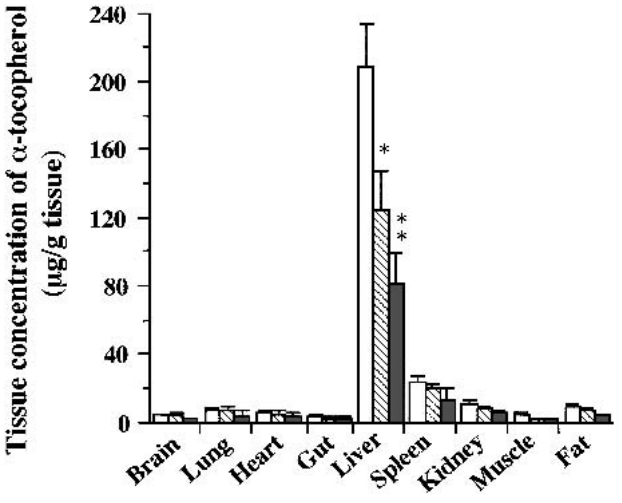

Fig. 3. Tissue concentrations of α-tocopherol at 2 h after an i.v. administration of α-tocopherol (25 mg/kg) in *apob* (+/+), *apob* (+/–), and *apob* (–/–) mice. Each *column* with *bar* represents the mean ± SE of four mice. *,**Significantly different from *apob* (+/+) mice at $p < 0.05$ and 0.01, respectively. Key: □, *apob* (+/+) mice; ▨, *apob* (+/–) mice; ■, *apob* (–/–) mice.

Plasma Concentration Time Course of α-Tocopherol after Oral Administration

α-Tocopherol (100 mg/kg) was orally administered to *apob* (+/+) mice and *apob* (–/–) mice, and the plasma con-

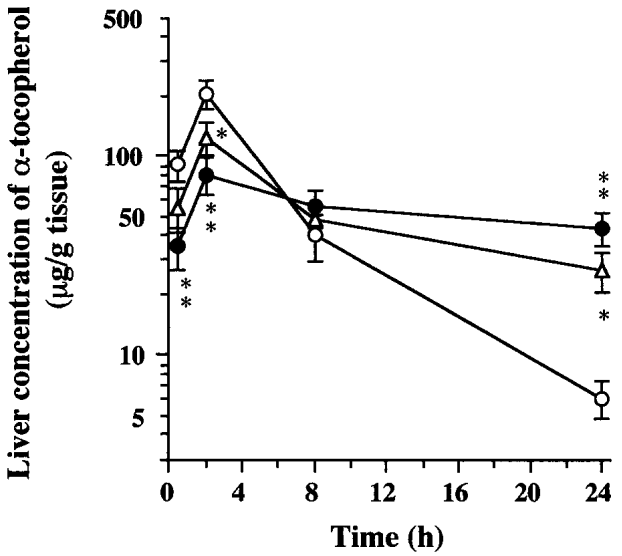

Fig. 4. Liver concentration time courses of α-tocopherol after i.v. administration of α-tocopherol (25 mg/kg) in *apob* (+/+), *apob* (+/–), and *apob* (–/–) mice. Each *point* with *bar* represents the mean ± SE of four mice. *,**Significantly different from *apob* (+/+) mice at $p < 0.05$ and 0.01, respectively. Key: ○, *apob* (+/+) mice; △, *apob* (+/–) mice; ●, *apob* (–/–) mice

centration time courses are shown in Fig. 5. The plasma concentrations in wild-type mice gradually increased until 24 h after oral administration, whereas the plasma concentrations in *apob* (–/–) mice increased only a little. The value of AUC_{0-32h} for *apob* (+/+) mice (96.5 ± 15.8 μg h/mL, mean ± SD of five experiments, $p < 0.01$) was significantly larger than that for *apob* (–/–) mice (17.7 ± 8.3 μg h/mL).

Biliary Excretion of α-Tocopherol

Table II shows the cumulative amount of α-tocopherol in bile after i.v. administration of α-tocopherol (25 mg/kg) to *apob* (+/+) mice and *apob* (+/–) mice. Before administration, the biliary excretion of α-tocopherol was significantly less in *apob* (+/–) mice than in *apob* (+/+) mice, whereas after administration the excretion was significantly greater in *apob* (+/–) mice than in *apob* (+/+) mice.

DISCUSSION

Kayden *et al.* (16) reported that an ABL patient, who poorly absorbed α-tocopherol, had extremely low levels of plasma and adipose α-tocopherol. Traber *et al.* (17) also reported that the plasma concentration of α-tocopherol after the oral administration of vitamin E in patients with ABL was below 10% of that in normal subjects. In this study, we found that the endogenous levels of α-tocopherol in plasma and tissues, except for liver, of *apob* (–/–) mice were significantly lower than those in *apob* (+/+) mice (Fig. 1), and the intestinal absorption of α-tocopherol was very low in *apob* (–/–) mice (Fig. 5). It has been reported that α-tocopherol binds to chylomicrons on the intestinal epithelial cells and then is absorbed via the lymphatic system (18). Microsomal triglyceride transfer protein (MTP), which plays a role in chylomicron formation by transferring lipids to apoB, is present in the intestine (6). These findings indicate that apoB is essential for the utilization of α-tocopherol and that apoB knockout mice are useful as an ABL model to study the disposition kinetics of α-tocopherol.

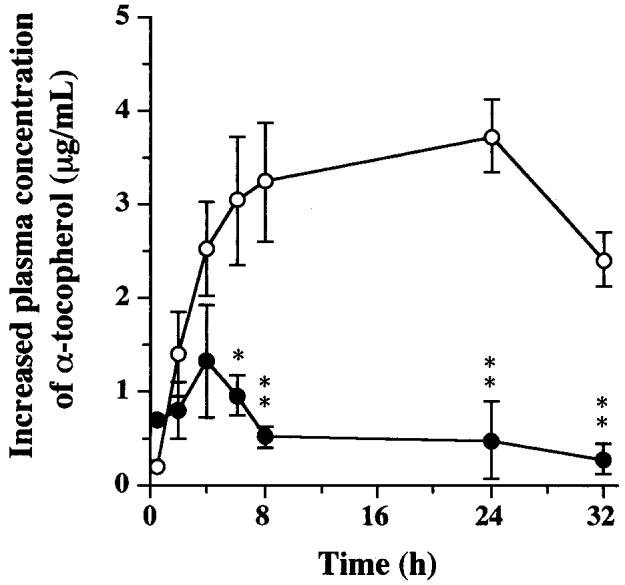

Fig. 5. Increased plasma concentration time courses of α-tocopherol after oral administration of α-tocopherol (100 mg/kg) in *apob* (+/+) and *apob* (–/–) mice. Each *point* with *bar* represents the mean ± SE of four mice. *,**Significantly different from *apob* (+/+) mice at $p < 0.05$ and 0.01, respectively. Key: ○, *apob* (+/+) mice; ●, *apob* (–/–) mice.

Table II. Cumulative Amount of α-Tocopherol in Bile after I.V. Administration of α-Tocopherol (25 mg/kg) in Mice[a]

	apob (+/+)	*apob* (+/–)
For 30 min before administration	19.1 ± 4.5	12.6 ± 1.4**
For 30 min after administration	27.5 ± 4.5	51.1 ± 11.5**
For 60 min after administration	58.1 ± 9.1	87.7 ± 19.7*

[a] Each value represents the mean ± SD (ng) of four mice.
*,** Significantly different from *apob* (+/+) mice at $p < 0.05$ and 0.01, respectively.

We found that the plasma concentrations of α-tocopherol in *apob* (–/–) and *apob* (+/–) mice decreased more rapidly than that in *apob* (+/+) mice after an i.v. administration, so that CL_{tot} was significantly higher and $V_{d,ss}$ was lower in apoB knockout mice than in wild-type mice (Fig. 2 and Table I). Moreover, α-tocopherol was highly distributed in the liver 2 h after administration but was little distributed to other tissues (Fig. 3). It is known that highly lipophilic α-tocopherol is present in blood in bound forms with various lipoproteins, such as HDL, LDL, and VLDL, and one of the constituents of LDL and VLDL is apoB (19). Homanics *et al.* (13) reported that lipids, HDL-cholesterol, and β-lipoprotein are decreased in plasma of *apob* (–/–) and *apob* (+/–) mice, which were used in this study. It has been clarified that HDL-associated α-tocopherol is taken up into cells via scavenger receptor SR-BI acting as an HDL receptor (20), whereas LDL-associated α-tocopherol is distributed into tissues via LDL receptor (21). Therefore, the high clearance of α-tocopherol and the decreased tissue distribution in apoB knockout mice may result from the low lipoprotein concentration in plasma of these mice.

On the other hand, the plasma concentration of α-tocopherol did not differ among *apob* (+/+), *apob* (+/–), and *apob* (–/–) mice in the distribution phase, 2 h after the i.v. administration, while the liver concentration of knockout mice was lower than that of wild-type mice. These results may suggest that α-tocopherol transport is partly dependent on apoB and is also performed by other mechanisms such as transport of the unbound form, etc.

The concentration of α-tocopherol in the liver of *apob* (+/+) mice decreased rapidly, whereas the elimination from the liver in *apob* (–/–) and *apob* (+/–) mice was very slow (Fig. 4). Bjorneboe *et al.* (22,23) clarified in rat experiments that most of the α-tocopherol associated with nascent VLDL is secreted from the liver, and the α-tocopherol concentration of the liver is increased when this secretion pathway is inhibited. Traber *et al.* (24) reported that the serum concentration of α-tocopherol is regulated by secretion from the liver. Therefore, it seems that the secretion of α-tocopherol from the liver into blood is inhibited because VLDL cannot be synthesized in the liver because of the apoB deficiency, so that α-tocopherol accumulates in the liver and is lowered in serum of apoB knockout mice.

Cohn (18) reported that unchanged α-tocopherol is excreted into the bile in humans. In this study, the biliary excretion of α-tocopherol after i.v. administration in *apob* (+/–)

mice was significantly increased compared with that in *apob* (+/+) mice, although the amount was less than about 0.02% of the dose because of short-term collection of bile for only 60 min (Table II). α-Tocopherol is also metabolized via β-oxidation (25) and by CYP3A (26). Schuelke *et al.* (27) reported that the urinary metabolites of α-tocopherol in patients with α-TTP deficiency are increased compared with those in normal subjects. Therefore, we suggest that the increased CL_{tot} of α-tocopherol in apoB knockout mice is caused by accelerated biliary excretion and hepatic metabolism because α-tocopherol is accumulated in the liver.

In conclusion, we demonstrated using apoB knockout mice that the disposition kinetics of α-tocopherol is strongly dependent on the role of apoB in α-tocopherol secretion from the liver into blood as well as on the intestinal absorption.

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