Absence of VLDL secretion does not affect α-tocopherol content in peripheral tissues

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Abstract α-Tocopherol is a lipid-soluble antioxidant that helps to prevent oxidative damage to cellular lipids. α-Tocopherol is absorbed by the intestine and is taken up and retained by the liver; it is widely presumed that α-tocopherol is then delivered to peripheral tissues by the secretion of VLDL. To determine whether VLDL secretion is truly important for the delivery of α-tocopherol to peripheral tissues, we examined α-tocopherol metabolism in mice that lack microsomal triglyceride transfer protein (*Mttp*) expression in the liver and therefore cannot secrete VLDL (*Mttp*$^{\Delta/\Delta}$ mice). *Mttp*$^{\Delta/\Delta}$ mice have low plasma lipid levels and increased stores of lipids in the liver. Similarly, α-tocopherol levels in the plasma were lower in *Mttp*$^{\Delta/\Delta}$ mice than in controls, whereas hepatic α-tocopherol stores were higher. However, α-tocopherol levels in the peripheral tissues of *Mttp*$^{\Delta/\Delta}$ mice were nearly identical to those of control mice, suggesting that VLDL secretion is not critical for the delivery of α-tocopherol to peripheral tissues. When fed a diet containing deuterated α-tocopherol, *Mttp*$^{\Delta/\Delta}$ and control mice had similar incorporation of deuterated α-tocopherol into plasma and various peripheral tissues. We conclude that the absence of VLDL secretion has little effect on the stores of α-tocopherol in peripheral tissues, at least in the mouse.—K. Minehira-Castelli, S. W. Leonard, Q. M. Walker, M. G. Traber, and S. G. Young. **Absence of VLDL secretion does not affect α-tocopherol content in peripheral tissues. *J. Lipid Res.* 2006.** 47: **1733–1738.**

Supplementary key words γ-tocopherol • microsomal triglyceride transfer protein • vitamin E

Vitamin E (tocopherols and tocotrienols) is a lipid-soluble antioxidant that helps to prevent oxidative damage to polyunsaturated fatty acids (1, 2). In the setting of vitamin E deficiency, neurodegeneration occurs and fertility is impaired (2, 3). Among the four main tocopherol isoforms (α, γ, δ, β), α-tocopherol is present in the highest concentrations, both in the plasma and in peripheral tissues (4–6). The α-tocopherol in the plasma is carried exclusively within the plasma lipoproteins (7, 8). Because vitamin E is an antioxidant and the oxidation of lipoproteins has been linked to atherogenesis, vitamin E has attracted interest as a potential therapy to prevent atherosclerosis (9, 10).

Dietary vitamin E is absorbed in the intestine and transported into the circulation by the intestinal triglyceride-rich lipoproteins (chylomicrons) (11, 12). During the hydrolysis of triglycerides by lipoprotein lipase, some tocopherols are probably transferred to other lipoproteins and/or taken up by peripheral tissues. However, most of the tocopherols are thought to be delivered to the liver by chylomicron remnants (11). Within the liver, a cytosolic protein, α-tocopherol transfer protein (α-TTP), selectively binds and retains α-tocopherol, whereas the other tocopherol isoforms are metabolized and/or secreted into the bile. α-TTP binding of α-tocopherol in the liver is physiologically important, as a genetic defect in α-TTP causes vitamin E deficiency in humans. Importantly, α-TTP has been thought to mediate the transfer of α-tocopherol for packaging into VLDL for delivery to peripheral tissues (13). The transport of tocopherol in rodents and humans is likely quite similar, even though rodents are known as "HDL animals" and tocopherol distribution in lipoproteins are slightly different between rodents and humans. Of note, in α-TTP-deficient mice, α-tocopherol levels in the plasma are only ∼5% of those in wild-type mice (14, 15). Thus, the α-TTP-deficient mouse model mimics the abnormality in humans with genetic defects in α-TTP, with both having very low plasma α-tocopherol concentrations, despite significant differences in the plasma lipoprotein profile.

Homozygous deficiency of α-TTP in humans (16) results in the inability to retain α-tocopherol in the liver,

Manuscript received 31 March 2006 and in revised form 4 May 2006.

Published, JLR Papers in Press, May 18, 2006.
DOI 10.1194/jlr.M600125-JLR200

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Abbreviations: apoB, apolipoprotein B; *Mttp*, microsomal triglyceride transfer protein; pI-pC, polyinosinic-polycytidylic ribonucleic acid; α-TTP, α-tocopherol transfer protein.

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leading to low plasma and tissue levels of α-tocopherol and ultimately to severe disease phenotypes, such as spinocerebellar ataxia and retinal degeneration (17, 18). Mice lacking α-TTP also have low plasma and tissue levels of α-tocopherol and develop ataxia and cognitive abnormalities with aging (3, 18). The presumed mechanism for these disease phenotypes is that α-tocopherol is lost from the liver through the bile and therefore is not delivered to peripheral tissues by the hepatic lipoproteins.

An inability to secrete apolipoprotein B (apoB)-containing lipoproteins, as in abetalipoproteinemia or homozygous hypobetalipoproteinemia, also causes α-tocopherol deficiency. In these cases, the α-tocopherol deficiency could conceivably be caused both by the failure to absorb α-tocopherol from the intestine (because of the inability to make chylomicrons) and by the failure of the liver to distribute α-tocopherol to peripheral tissues (because of the inability to make VLDL). Studies from the laboratory of Kayden and Traber (19–21) suggested that the failure of the liver to produce VLDL was a key element in the development of α-tocopherol deficiency in the tissues of patients with abetalipoproteinemia.

To better define the physiologic importance of VLDL secretion in the delivery of α-tocopherol to peripheral tissues, we took advantage of the existence of mice that lack microsomal triglyceride transfer protein (*Mttp*) in the liver ($Mttp^{\Delta/\Delta}$ mice). These mice produce chylomicrons in the intestine but cannot assemble and secrete VLDL in the liver (22). We hypothesized that the delivery of α-tocopherol to peripheral tissues in $Mttp^{\Delta/\Delta}$ mice would be profoundly impaired, given the absence of VLDL secretion. In this study, we tested this hypothesis.

MATERIALS AND METHODS

Mice

Mice with a conditional *Mttp* allele ($Mttp^{flox}$) (22) were bred with Mx1-*Cre* transgenic mice (23, 24) to create $Mttp^{flox/flox}$Mx1-*Cre* mice (22, 25, 26). Five week old male $Mttp^{flox/flox}$Mx1-*Cre* mice were injected with polyinosinic-polycytidylic ribonucleic acid (pI-pC) (Sigma, St. Louis, MO), 500 μg every other day for 8 days. The pI-pC injections stimulate interferon production, inducing *Cre* expression in the liver and leading to the inactivation of *Mttp* (22, 24). The pI-pC-treated $Mttp^{flox/flox}$Mx1-*Cre* mice, which we have called $Mttp^{\Delta/\Delta}$ mice, lack *Mttp* expression in the liver and cannot secrete VLDL (22). These mice were compared with untreated littermate controls (i.e., $Mttp^{flox/flox}$Mx1-*Cre* mice). Mice were housed in a pathogen-free barrier facility with a 12 h light/12 h dark cycle and were fed rodent chow containing 4.5% fat (Ralston Purina, St. Louis, MO). The study protocol was approved by the Committee on Animal Research at the University of California, San Francisco.

Western blots and real-time RT-PCR

Plasma proteins were separated on SDS-polyacrylamide gels and transferred to a nitrocellulose membrane for Western blotting. ApoB-48 and apoB-100 were detected with a rabbit antiserum against mouse apoB (27) (1:5,000 dilution). After a 14 h incubation with the primary antibody at 4°C, the blots were washed and incubated for 1 h with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10,000) (Amersham Pharmacia Biotech, Piscataway, NJ). The membrane was developed with the ECL chemiluminescence system (Amersham Pharmacia Biotech) and exposed to X-ray film.

RNA was extracted from mouse liver with TRI Reagent (Sigma). The reverse transcription reaction was performed with the SuperScript II kit (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. Real-time RT-PCR with SYBR Green (Applied Biosystems, Foster City, CA) was carried out with an ABI Prism 7700 Sequence Detection System (Applied Biosystems). The *Mttp* oligonucleotide primers were 5′-ATGATCCTCTTGG-CAGTGCTT-3′ and 5′-TGAGAGGCCAGTTGTGTGAC-3′. Data were normalized to *18S* RNA expression.

Vitamin E metabolic studies

After the last injection of pI-pC, 2 month old mice were fed a chow diet for 6 weeks. After a 4 h fast, 1.0 ml of blood was drawn from the retro-orbital plexus into tubes containing EDTA, and plasma was separated by centrifugation. Plasma lipoprotein fractions were separated by fast-performance liquid chromatography (28). Triglyceride and cholesterol levels were measured with commercial kits (Roche Diagnostics, Indianapolis, IN). Liver, kidney, lung, heart, adrenal gland, skeletal muscle, epididymal fat, brain, and gallbladder were also removed, frozen in liquid nitrogen, and stored at −80°C until tocopherol levels were measured.

In a separate study, mice were fed a diet containing deuterated α- and γ-tocopheryl acetate. This diet was made commercially (Harlan Teklad, Madison, WI) with tocopherol-stripped corn oil; deuterated tocopherols were the only source of vitamin E (15). α-5,7-$(CD_3)_2$-Tocopheryl acetate (d_6-α-tocopheryl acetate) and unlabeled α-tocopheryl acetate (d_0-α-tocopheryl acetate) were gifts from Dr. James Clark (Cognis Nutrition and Health). γ-3,4-(D)-Tocopheryl acetate (d_2-γ-tocopheryl acetate) was prepared from γ-tocopherol labeled with two deuterium atoms, as described (29). The d_6-α- and d_2-γ-tocopheryl acetates were mixed in a 1:1 molar ratio and added to the tocopherol-stripped corn oil, and the diet was prepared. The internal standard, α-5,7,8-$(CD_3)_3$-tocopheryl acetate (d_9-α-tocopheryl acetate), was synthesized by Isotec (Miamisburg, OH). The measured dietary concentrations of d_6-α- and d_2-γ-tocopheryl acetates were 23.8 ± 1.2 and 19.8 ± 0.2 mg/kg, respectively. Two weeks after the last injection of pI-pC, the mice (then 2 months old) were switched to the deuterated tocopherol diet. Mice were euthanized 1, 14, or 28 days later, and blood and tissues were collected for analysis of labeled (deuterated) and unlabeled α- and γ-tocopherol levels.

Measurement of vitamin E concentrations

Plasma and tissue α- and γ-tocopherol concentrations in chow-fed mice were analyzed by HPLC with electrochemical detection, as described (30). To measure deuterated and unlabeled α- and γ-tocopherols, plasma and tissues were extracted (30), a fixed amount of internal standard (d_9-*ambo*-tocopherol) was added to the extracts, and extracts were analyzed by liquid chromatography-mass spectrometry with negative atmospheric pressure chemical ionization, as described (15, 31).

Statistical analysis

Values are expressed as means ± SEM. Differences in tocopherol levels in control and $Mttp^{\Delta/\Delta}$ mice on a chow diet were analyzed with a *t*-test. For the deuterated tocopherol diet studies, a Mann-Whitney *U* test was used (StatView 5.0; SAS Institute, Cary, NC).

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RESULTS

Inactivation of *Mttp* abolishes hepatic VLDL production

After inducing *Cre* expression with pI-pC, hepatic *Mttp* transcripts were reduced by >90% (**Fig. 1A**), apoB-100 virtually disappeared from the plasma, and apoB-48 levels were somewhat reduced (Fig. 1B), consistent with earlier studies (22). The plasma cholesterol and triglyceride levels were also reduced (**Table 1**), and triglyceride-rich VLDL particles virtually disappeared from the plasma, as judged by fast-performance liquid chromatography fractionation studies (Fig. 1C). Finally, there was a uniform accumulation of neutral lipids in the parenchymal cells of the liver (Fig. 1D). These findings suggested that we had largely eliminated VLDL production by the hepatocytes of $Mttp^{\Delta/\Delta}$ mice.

Plasma and tissue α- and γ-tocopherol concentrations

$Mttp^{\Delta/\Delta}$ mice had significantly lower plasma levels of α-tocopherol than control mice, likely reflecting the lower levels of VLDL and other apoB-100-containing lipoproteins ($P < 0.001$) (**Table 1**). The ratio of plasma α-tocopherol to total plasma cholesterol was also lower in $Mttp^{\Delta/\Delta}$ mice, demonstrating that the reduction in α-tocopherol was greater than that of plasma cholesterol (Table 1). Plasma α-tocopherol was largely confined to the HDL fraction in both control and $Mttp^{\Delta/\Delta}$ mice (**Fig. 2**). Plasma γ-tocopherol levels were low in both groups.

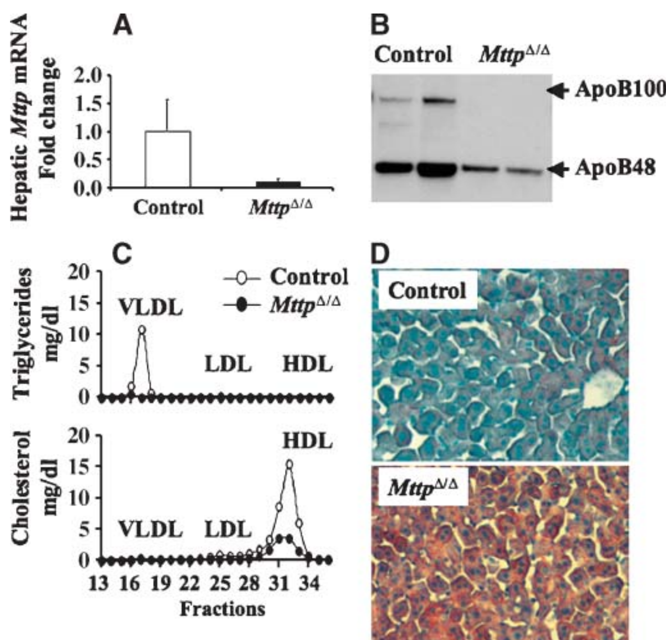

Fig. 1. Microsomal triglyceride transfer protein (*Mttp*) expression, plasma apolipoprotein B (apoB) levels, cholesterol and triglyceride concentrations in plasma lipoprotein fractions, and lipid staining of hepatocytes. A: *Mttp* expression in the liver measured by real-time RT-PCR. Values shown are means ± SEM. B: Western blot of mouse plasma with a rabbit antiserum against mouse apoB. C: Triglyceride and cholesterol concentrations in plasma lipoprotein fractions obtained by fast-performance liquid chromatography. D: Oil Red O staining revealing increased numbers of lipid droplets (red) in hepatocytes of $Mttp^{\Delta/\Delta}$ mice. Original magnification, 10×.

TABLE 1. Plasma lipid and α- and γ-tocopherol concentrations

Lipids and Tocopherols	Control (n = 8)	$Mttp^{\Delta/\Delta}$ (n = 9)
Triglycerides (mg/dl)	64.1 ± 3.5	16.5 ± 4.8[a]
Cholesterol (mg/dl)	123.7 ± 4.8	67.6 ± 4.0[a]
α-Tocopherol (μmol/l)	3.76 ± 1.38	0.96 ± 0.43[a]
γ-Tocopherol (μmol/l)	0.04 ± 0.01	0.03 ± 0.01
α-Tocopherol-cholesterol ratio	1.16 ± 0.13	0.56 ± 0.09[a]
γ-Tocopherol-cholesterol ratio	0.01 ± 0.00	0.02 ± 0.01

$Mttp^{\Delta/\Delta}$, microsomal triglyceride transfer protein-deficient mice. Values shown are means ± SEM. Ratio was calculated by dividing plasma tocopherol concentration (μmol/l) by the total plasma cholesterol concentration (mmol/l).

[a] $P < 0.05$ versus control (*t*-test).

Hepatic α-tocopherol stores were significantly higher in $Mttp^{\Delta/\Delta}$ mice than in control mice ($P = 0.002$) (**Fig. 3**), in parallel with the increased stores of neutral lipids. Hepatic γ-tocopherol levels tended to be higher in $Mttp^{\Delta/\Delta}$ mice (0.39 ± 0.15 vs. 0.05 ± 0.02 nmol/g; $P = 0.059$).

Despite the low plasma α-tocopherol concentrations in $Mttp^{\Delta/\Delta}$ mice, α-tocopherol stores in the extrahepatic tissues were similar in $Mttp^{\Delta/\Delta}$ mice and controls (Fig. 3). Extrahepatic γ-tocopherol content was also similar in both groups of mice.

Incorporation of deuterated tocopherol into peripheral tissues

To further understand α- and γ-tocopherol transport, we fed $Mttp^{\Delta/\Delta}$ mice and control mice a diet containing deuterated α- and γ-tocopherols for 1, 14, or 28 days. Plasma deuterated α-tocopherol concentrations in $Mttp^{\Delta/\Delta}$ mice were approximately half those in control mice at all time points, but the ratio of deuterated tocopherol to total plasma tocopherol was similar in both groups (**Fig. 4A**). The majority of deuterated α-tocopherol was in HDL (Fig. 4B).

The percentage enrichment of deuterated α-tocopherol was slightly reduced in liver, lung, kidney, heart, muscle, epididymal fat, and adrenal gland of $Mttp^{\Delta/\Delta}$ mice at day 1 (**Table 2**), but was similar in both groups at days 14 and 28 (**Fig. 5**). The percentage deuterated α-tocopherol enrichment in brain was similar for both groups at all time points.

TABLE 2. Plasma and tissue α-tocopherol concentrations on day 1

Plasma and Tissues	Control	$Mttp^{\Delta/\Delta}$
	μmol/l	
Plasma	7.6 ± 0.4 (53%)	3.6 ± 0.4[a] (46%)
	nmol/g	
Liver	18.6 ± 1.7 (39%)	28.2 ± 2.6[a] (26%)[a]
Lung	23.3 ± 1.9 (27%)	22.1 ± 1.2 (22%)
Kidney	18.1 ± 3.6 (31%)	17.8 ± 0.8 (19%)[a]
Muscle	8.4 ± 0.8 (10%)	9.5 ± 0.4 (6%)[a]
Brain	7.2 ± 0.5 (3%)	7.7 ± 1.2 (3%)
Heart	29.3 ± 1.1 (18%)	28.7 ± 1.6 (10%)[a]
Adrenal gland	36.8 ± 6.3 (22%)	33.2 ± 11.5 (8%)[a]
Epididymal fat	22.7 ± 1.4 (4%)	25.7 ± 3.6 (1%)[a]

Values shown are means ± SEM (n = 4 control and 3–4 $Mttp^{\Delta/\Delta}$ mice). Values in parentheses represent percentage enrichment of deuterated α-tocopherol.

[a] $P < 0.05$ versus control (Mann-Whitney *U* test).

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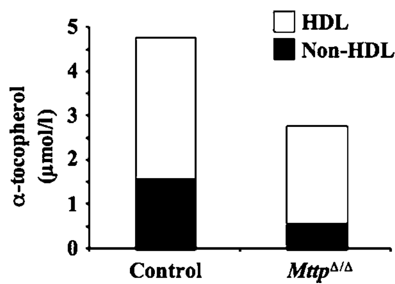

Fig. 2. α-Tocopherol concentration in lipoprotein fractions. Fresh plasma samples from five mice per group were pooled. HDL ($d <$ 1.21 g/ml) and non-HDL fractions were prepared by ultracentrifugation, and α-tocopherol was measured in each fraction. The percentage of α-tocopherol carried by HDL was 80% in *Mttp*$^{\Delta/\Delta}$ mice and 67% in controls. γ-Tocopherol levels were below the limit of detection.

Plasma deuterated γ-tocopherol was not detectable at any time point. Like α-tocopherol, deuterated γ-tocopherol accumulated in the liver of *Mttp*$^{\Delta/\Delta}$ mice at all time points [1 day, 0.3 ± 0.0 vs. 1.0 ± 0.1 μmol/g ($P = 0.02$); 14 days, 0.2 ± 0.0 vs. 0.4 ± 0.1 μmol/g ($P = 0.09$); 28 days, 0.1 ± 0.0 vs. 0.4 ± 0.1 μmol/g ($P = 0.01$, control vs. *Mttp*$^{\Delta/\Delta}$)]. γ-Tocopherol was detected in the lung, adrenal gland, gallbladder, and epididymal fat at 1 day but only in epididymal fat at 14 and 28 days (data not shown).

DISCUSSION

α-Tocopherol is bound and retained by a specific protein within hepatocytes, α-TTP, and it is widely assumed that this lipid-soluble vitamin is then packaged into the neutral lipid core of VLDL particles for delivery to peripheral tissues (7). In this study, we tested the hypothesis that the delivery of α-tocopherol to peripheral tissues would be abnormal in mice lacking the capacity to assemble and secrete VLDL. We suspected that the α-tocopherol stores in peripheral tissues would be depleted in the absence of VLDL, but this suspicion was not upheld. α-Tocopherol stores were normal in extrahepatic tissues of mice lacking VLDL, and the delivery of a deuterated form of α-tocopherol to peripheral tissues was, at most, minimally delayed in these mice.

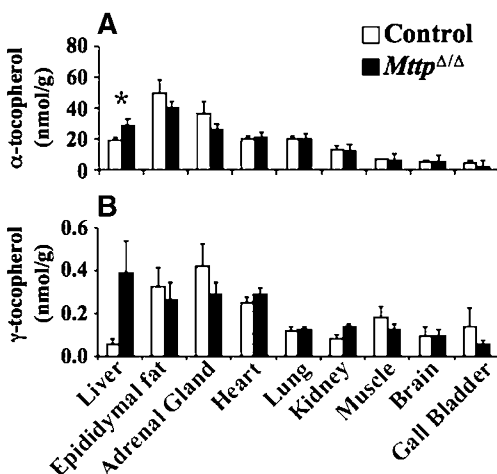

Fig. 3. Tissue content of α- and γ-tocopherols in mice on a chow diet. A: α-Tocopherol content. B: γ-Tocopherol content. Values shown are means ± SEM. * $P < 0.05$ versus control.

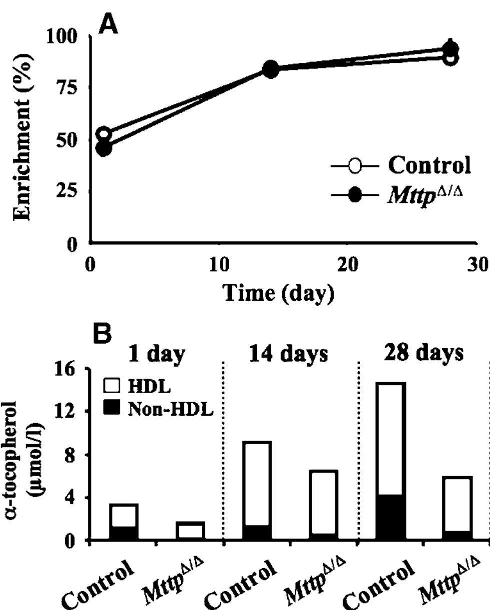

Fig. 4. Deuterated α-tocopherol levels in mice fed a diet containing deuterated tocopherols for 1, 14, or 28 days. A: Plasma levels of deuterated α-tocopherol. B: Deuterated α-tocopherol in lipoprotein fractions.

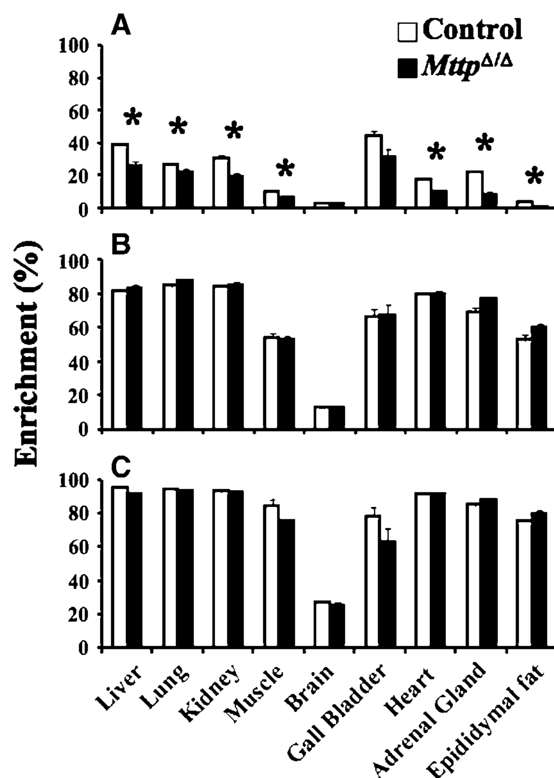

Fig. 5. Tissue α-tocopherol content in mice fed a diet containing deuterated α-tocopherol for 1 day (A), 14 days (B), or 28 days (C). Values shown are means ± SEM. * $P < 0.05$ versus control.

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Thus, VLDL and LDL do not appear to be particularly important for α-tocopherol delivery to peripheral tissues.

The animal model that we used for these studies, *Mttp*$^{\Delta/\Delta}$ mice, does not express *Mttp* in the liver and therefore cannot assemble and secrete VLDL (22). Transmission electron micrographs of the hepatocytes of these mice has revealed a virtually complete absence of triglyceride-rich VLDL particles in the secretory organelles (22). *Mttp*$^{\Delta/\Delta}$ mice have a striking reduction in VLDL triglycerides and a nearly complete absence of apoB-100 in the plasma. Because they cannot make VLDL, *Mttp*$^{\Delta/\Delta}$ mice have mild to moderate hepatic steatosis, even on a low-fat chow diet.

We expected that *Mttp*$^{\Delta/\Delta}$ mice would have low plasma levels of α-tocopherol, because α-tocopherol partitions almost exclusively into lipids and because *Mttp*$^{\Delta/\Delta}$ mice have abnormally low lipid levels in the plasma. Indeed, this was the case. We also suspected that hepatic stores of α-tocopherol in *Mttp*$^{\Delta/\Delta}$ mice would be increased, for the simple reason that their hepatocytes have increased stores of neutral lipids. This was also the case.

The α-tocopherol levels in peripheral tissues were similar in *Mttp*$^{\Delta/\Delta}$ mice and control mice, indicating that VLDL and apoB-100-containing lipoproteins are not crucial in α-tocopherol transport. This view was further supported by the finding of virtually normal levels of deuterated α-tocopherol delivery to peripheral tissues. One potential explanation for these findings is that chylomicrons and their remnants suffice for α-tocopherol delivery. We studied α-tocopherol transport by postprandial chylomicrons in *Mttp*$^{\Delta/\Delta}$ mice after deuterated α-tocopherol injection by gavage. After 2 h, the majority of the α-tocopherol was in chylomicrons; after 6 h, the majority was present in the HDL fraction (data not shown).

Another potential explanation for these findings is that HDL suffices for α-tocopherol delivery in the mouse. In this study, we found that the HDL contained ~65% of the total α-tocopherol in controls and nearly 80% in *Mttp*$^{\Delta/\Delta}$ mice (based on pooled samples from six mice in each group). The fact that mice lacking a HDL receptor (scavenger receptor class B type I) had increased α-tocopherol concentrations in the plasma but decreased α-tocopherol stores in several tissues (ovary, testis, lung, and brain) (32) also suggests that HDL plays a significant role in α-tocopherol transport. Also, in phospholipid transfer protein-deficient mice, HDL levels were decreased, and liver, brain, and aorta α-tocopherol levels were low (33, 34).

We are confident, based on our current findings and previous studies (22, 28), that the hepatic secretion of triglyceride-rich lipoproteins and apoB-100 was largely abolished in *Mttp*$^{\Delta/\Delta}$ mice. But it is important to note several caveats. First, very rare hepatocytes in *Mttp*$^{\Delta/\Delta}$ mice retain VLDL secretion, as judged by electron microscopy (22), almost certainly because those cells escaped *Cre*-mediated inactivation of *Mttp*. We seriously doubt that the trace amount of VLDL secreted by those rare *Mttp*$^{\text{flox/flox}}$ cells could account for the normal α-tocopherol delivery, simply because we believe that VLDL secretion was negligible in the *Mttp*$^{\Delta/\Delta}$ mice. One reason that we believe that VLDL secretion was largely abolished is that LDL cholesterol was absent in LDL receptor-deficient *Mttp*$^{\Delta/\Delta}$ mice (whereas LDL receptor-deficient *Mttp*$^{\text{flox/flox}}$ mice had a large amount of LDL) (28). A second caveat relates to the synthesis of apoB-48 in the livers of *Mttp*$^{\Delta/\Delta}$ mice. Previously, we showed that primary hepatocytes from *Mttp*$^{\Delta/\Delta}$ mice secrete small amounts of lipid-poor apoB-48 particles with a buoyant density in the HDL range (but they secrete no apoB-48-VLDL particles). Although the apoB-48-HDL particles might conceivably account for some α-tocopherol transport in the *Mttp*$^{\Delta/\Delta}$ mice, we doubt that these lipid-poor HDL particles contributed substantially to α-tocopherol transport. Of note, we generated *Mttp*$^{\Delta/\Delta}$ mice that lacked the LDL receptor and were homozygous for an apoB-100 allele (28). These mice were genetically incapable of making apoB-48, yet they had normal levels of α-tocopherol in peripheral tissues (data not shown). The normal levels of α-tocopherol in peripheral tissues in those mice could not have been derived from the apoB-48-HDL particles, because they made none.

We considered the possibility that *Mttp*$^{\Delta/\Delta}$ mice maintain normal tissue α-tocopherol by decreasing the rate of peripheral tissue α-tocopherol export. However, by measuring deuterated tocopherol replacement in several tissues for 28 days, we observed that >90% of tocopherol was replaced by the deuterated tocopherol in lung, kidney, spleen, and heart. Thus, it seems unlikely that the release of α-tocopherol from peripheral tissues is the mechanism for the normal tocopherol content in peripheral tissues.

Our findings imply that VLDL does not play a crucial role in transporting α-tocopherol to peripheral tissues. However, *Mttp*$^{\Delta/\Delta}$ mice incorporated less deuterated α-tocopherol in several peripheral tissues after 1 day of deuterated α-tocopherol feeding. The difference was minor but significant. After 14 days, however, the incorporation was similar in both groups. We speculate that very soon after tocopherol absorption, VLDL secreted by the liver might enhance α-tocopherol transport to peripheral tissues, thereby explaining the lower deuterated α-tocopherol levels in *Mttp*$^{\Delta/\Delta}$ mice at the 1 day time point. Alternatively, the newly absorbed deuterated α-tocopherol in *Mttp*$^{\Delta/\Delta}$ mice might simply take longer to equilibrate with the α-TTP, because the livers of those mice contain larger stores of tocopherol. However, the incorporation of deuterated α-tocopherol into peripheral tissues of *Mttp*$^{\Delta/\Delta}$ mice was normal after 14 and 28 days. Thus, over the long term, our data suggest that HDL and/or chylomicrons suffice for the delivery of α-tocopherol to peripheral tissues.

This study, like a study examining Golgi-VLDL enrichment with α-tocopherol (13), implies that VLDL synthesis is not required for the delivery of α-tocopherol from the liver to peripheral tissues. α-TTP might facilitate the movement of α-tocopherol to the hepatocyte plasma membrane, where any lipoprotein could acquire α-tocopherol. Horiguchi et al. (35) suggested that hepatic α-TTP acquires α-tocopherol from late endosomes and then an α-TTP-α-tocopherol complex moves to the plasma membrane, whereupon α-tocopherol is transferred to the membrane. In the *Mttp*$^{\Delta/\Delta}$ mice, HDL could be the main acceptor of

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α-tocopherol from the plasma membrane. Of note, there is some evidence that ABCA1, a transporter involved in the regulation of cellular cholesterol, could play a role in the enrichment of HDL with α-tocopherol (36).

In summary, blocking VLDL secretion decreased plasma α-tocopherol levels and led to an accumulation of α-tocopherol in the liver. Despite low plasma α-tocopherol levels, peripheral α-tocopherol content was normal in $Mttp^{\Delta/\Delta}$ mice. Therefore, VLDL does not appear to be critical for tocopherol delivery to peripheral tissues.

This work was supported by a fellowship award from the American Heart Association, Western States Affiliate, and by the Swiss National Science Foundation. The authors thank Ms. Kelsey Yorks for technical assistance.

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