α-Tocopherol Metabolism Is Abnormal in Scavenger Receptor Class B Type I (SR-BI)-Deficient Mice[1]

Pablo Mardones,* Pablo Strobel,† Soledad Miranda,† Federico Leighton,† Verónica Quiñones,* Ludwig Amigo,* Jaime Rozowski,** Monty Krieger‡ and Attilio Rigotti*[2]

*Departamentos de *Gastroenterología and **Nutrición, Diabetes y Metabolismo; †Facultad de Medicina and Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica, Santiago, Chile; and ‡Biology Department, Massachusetts Institute of Technology, Cambridge, MA 01239*

ABSTRACT Despite the physiologic importance of vitamin E, in particular its α-tocopherol (α-T) isoform, the molecular mechanisms involved in the cellular uptake of this antioxidant from plasma lipoproteins have not been well-defined. Recent studies have suggested that selective lipid uptake, rather than endocytosis, is important for α-T delivery to cells. Here we show that the scavenger receptor class B type I (SR-BI), which mediates cellular selective cholesteryl ester uptake from lipoproteins, facilitates efficient transfer of α-T from HDL to cultured cells. In SR-BI-deficient mutant mice, relative to wild-type control animals, there was a significant increase in plasma α-T levels (1.1- to 1.4-fold higher) that was mostly due to the elevated α-T content of their abnormally large plasma HDL-like particles. This increase in plasma α-T in SR-BI knockout mice was accompanied by a significant decrease (65–80%) in the α-T concentrations in bile and several tissues including ovary, testis, lung and brain. SR-BI deficiency did not alter the α-T concentrations of the liver, spleen, kidney or white fat. These data show that SR-BI plays an important role in transferring α-T from plasma lipoproteins to specific tissues. Also, in the case of the liver as was previously shown for SR-BI-dependent hepatic cholesterol transport, SR-BI-mediated uptake of α-T was primarily coupled to biliary excretion rather than to tissue accumulation. Defective tissue uptake of lipoprotein α-T in SR-BI-deficient mice may contribute to the reproductive and cardiovascular pathologies exhibited by these animals. J. Nutr. 132: 443–449, 2002.

KEYWORDS: • *lipoproteins* • *LDL receptor* • *vitamin E* • *mice* • *scavenger receptor class B type I*

Vitamin E is a family of essential micronutrients composed of lipid-soluble tocopherols and tocotrienols that have potent antioxidant activity (1–5). α-Tocopherol (α-T),[3] by far the most abundant and active vitamin E family member, is a chain-breaking antioxidant that prevents the propagation of free radical reactions in vitro (1). This has suggested that α-T protects against oxidative stress in vivo, and, thus, it may play a role in aging, atherosclerosis, neurodegeneration and cancer (2).

Dietary vitamin E is absorbed in the intestine and carried by lipoproteins to the liver. In the liver, the α-T fraction is incorporated into VLDL by α-T transfer protein (6,7) and then secreted into the bloodstream [reviewed in (1–5)]. The liver also excretes α-T and its oxidized metabolites and other vitamin E isoforms into the bile; however, the mechanisms that modulate biliary α-T output are not known (5). In the plasma, α-T can be transferred from apolipoprotein B-containing lipoproteins (VLDL and LDL) to HDL. In fact, LDL and HDL are the major carriers of α-T in the bloodstream. The control of the distribution and metabolism of α-T throughout the body is, therefore, closely linked to the complex mechanisms that mediate and regulate cholesterol, triglyceride and lipoprotein metabolism.

The detailed mechanisms of cellular α-T uptake from plasma lipoproteins are poorly understood. In vitro and in vivo studies have suggested that lipoprotein lipase (8,9), phospholipid transfer protein (10,11) and the LDL receptor (12,13) facilitate cellular uptake of lipoprotein α-T. However, plasma clearance and net uptake studies of α-T in LDL receptor-deficient Watanabe heritable hyperlipemic rabbits indicated that the LDL receptor pathway was not essential for tissue uptake of α-T (14,15), suggesting that tissue uptake of α-T is mediated primarily by LDL receptor-independent pathways, at least in rabbits.

Recent studies in cultured cells have shown that the uptake of α-T from both HDL and LDL exceeds the uptake of li-

[1] This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico, Chile (Grants 8990006 to A. R. and 2990085 to S. M.), Grant PUC-PBMEC 1999-2001, Chile to F. L., and the National Institutes of Health (Grants HL66105, HL64737 and HL52212 to M. K.).

[2] To whom correspondence should be addressed.
E-mail: arigotti@med.puc.cl

[3] Abbreviations used: α-T, α-tocopherol; G418, geneticin; IDL, intermediate density lipoproteins; KO, knockout; LPDS, lipoprotein-deficient serum; LDLR, LDL receptor; LDLR KO, LDL receptor-deficient; SR-BI, scavenger receptor class B type I; SR-BI KO, SR-BI-deficient.

Manuscript received 10 August 2001. Initial review completed 9 October 2001. Revision accepted 5 December 2001.

poprotein holoparticles (16,17), a process known as selective lipid uptake. Selective lipid uptake was initially described as a mechanism for delivery of lipoprotein cholesteryl esters to cells (18). The scavenger receptor class B type I (SR-BI) has been shown to bind HDL and LDL, mediate selective lipid uptake from these lipoproteins, and play an important role in HDL metabolism in vivo (19–23). Several findings suggest that SR-BI might be involved in the delivery of lipoprotein α-T to cells. First, SR-BI is predominantly expressed in those tissues (19) with high lipoprotein α-T uptake and α-T content (14,15,24). Second, SR-BI expression is regulated (suppressed) by cellular α-T (25,26). Third, selective α-T uptake by cultured brain endothelial cells is correlated with SR-BI expression (27).

Because the relevance of SR-BI for the selective uptake of α-T in vivo and for its tissue distribution had not been established, we evaluated α-T mass uptake in SR-BI-transfected cells and α-T concentrations in plasma, bile and tissues from SR-BI-deficient mice. During the preparation of this manuscript, similar results for the SR-BI-mediated uptake of α-T by cultured cells were reported (27). Those studies and ours indicate that SR-BI is a physiologically relevant lipoprotein receptor for controlling α-T transport and its accumulation in selected tissues in vivo.

MATERIALS AND METHODS

Materials. Reagents (and sources) were as follows: Ham's F-12 medium, fetal bovine serum, trypsin/EDTA, penicillin/streptomycin/amphotericin B, glutamine, geneticin (G418; Life Technologies, Rockville, MD); plastic ware for cell culture (Becton Dickinson, Franklin Lakes, NJ); α(+)-tocopherol standard (Sigma Chemicals, St. Louis, MO); heparin column (Amersham Pharmacia Biotech, Piscataway, NJ); Bradford reagent (Bio-Rad Laboratories, Hercules, CA); and Supelcosil LC-8 (15 cm × 4.6 cm, 3-μm) HPLC column (Sigma Chemicals). Human HDL was prepared by zonal centrifugation (28) from plasma obtained from normolipidemic blood donors. Apolipoprotein E-free HDL was isolated by heparin affinity chromatography (19). Lipoprotein-deficient serum (LPDS) was prepared by ultracentrifugation of fetal bovine serum (29). High purity solvents were purchased from Merck (Darmstadt, Germany). All other reagents were obtained from standard commercial sources.

Cells. ldlA-7 cells and ldlA-7 cells stably transfected with murine SR-BI cDNA (ldlA[mSR-BI]) were described previously (19). ldlA-7 cells were maintained in Ham's F-12 medium containing 10% fetal bovine serum, 2 mmol/L glutamine, 1×10^5 U/L penicillin, 100 mg/L streptomycin, and 0.25 mg/L amphotericin B (medium A). ldlA[mSR-BI] cells were cultured in medium A supplemented with 300 mg/L G418 (medium B). All incubations with cells were performed at 37°C in a humidified 5% CO_2/95% air atmosphere.

Animals. Mice with a targeted mutation in the *srbi* locus were maintained in a mixed genetic background (C57BL/6 × 129/Sv) by crossing heterozygous *srbi* mutant females with heterozygous or homozygous *srbi* mutant males. Homozygous *srbi* mutant (SR-BI KO) and control wild-type mice were screened by PCR (30). SR-BI KO mice (2- to 4-mo-old) as well as appropriate sex- and age-matched littermate control mice were used for most experiments. In some cases, SR-BI KO mice were compared with control mice derived from an independently maintained wild-type colony with mixed C57BL/6 × 129/Sv background. LDL receptor-deficient (LDLR KO) mice, which were initially purchased from The Jackson Laboratory (Bar Harbor, ME), were analyzed at 2–4 mo old. Because the LDLR KO mice were on a pure C57BL/6J background, experiments with these mice used C57BL/6J wild-type controls rather than the mixed background controls used with the SR-BI KO mice.

Mice were housed in a temperature-, humidity- and light-controlled room and consumed ad libitum standard nonpurified diet (<0.02% cholesterol, 75 IU/kg α-tocopheryl acetate, 4.5% fat; Prolab RMH3000; PMI Feeds, St. Louis, MO) and water. Mice were deprived of food overnight, and plasma, bile and tissue samples were harvested toward the middle/end of the dark phase of the light cycle. We have previously shown that there are no differences in bile flow between control and SR-BI-deficient mice (31). Protocols followed standard criteria for humane care of experimental animals and were approved by the review board for animal studies of the Pontificia Universidad Católica of Chile.

Mice were anesthetized with pentobarbital (4.5 mg/100 g body) by intraperitoneal injection. After laparotomy, a common bile duct fistula was installed and hepatic bile was collected for 30–60 min. Subsequently, blood was removed by puncture of the inferior vena cava, mice were killed and tissues were removed. Plasma was separated by low speed centrifugation for 10 min at 4°C and immediately subjected to fast performance liquid chromatography as previously described (30). Bile and remaining plasma were kept at −20°C while tissues were stored at −70°C until they were processed for biochemical analyses.

Cellular α-T uptake assay. On day 0, ldlA-7 and ldlA[mSR-BI] cells were plated in 100-mm dishes (2×10^6 cells/dish) in medium A or B, respectively. On day 1, media were changed to Ham's F-12 medium containing 3% LPDS, 2 mmol/L glutamine, 1×10^5 U/L penicillin, 100 mg/L streptomycin, 0.25 mg/L amphotericin B (medium C) supplemented with 300 mg/L G418 for ldlA[mSR-BI] cells. On day 3, cells were washed with 150 mmol/L NaCl in 30 mmol/L potassium phosphate, pH 7.4 (PBS) and incubated with Ham's F-12 medium containing 1% LPDS, 2 mmol/L glutamine, 1×10^5 U/L penicillin, 100 mg/L gentamycin, 0.25 mg/L amphotericin B (medium D) and 300 mg/L G418 for ldlA[mSR-BI] cells with or without apoE-free HDL supplementation (150 mg of HDL protein/L, 1–6 mg of α-T/g of HDL protein). After a 5 h incubation at 37°C, cells were washed, removed by scrapping in 1 mL cold 1.15% KCl, 50 mmol/L Tris-HCl, 0.001% butylated hydroxytoluene, pH 7.4 (buffer A) and sedimented by high speed centrifugation. Cell pellets were frozen for 1 h at −70°C, thawed in 250 μL buffer A, and lysed by 10 passes through a 1-mL syringe using a 25-gauge needle. These cell lysates were used for α-T and protein determinations as described below.

α-T analysis. Tissue samples were homogenized in cold buffer A (100 g tissue/L buffer A) with a teflon/glass Potter-Elvehjem homogenizer (Thomas Scientific, Swedesboro, NJ). Constant volumes of tissue homogenates (50–200 μL), cell lysates (100 μL), plasma (50 μL), and bile samples (20 μL) were mixed briefly with 500 μL methanol to precipitate proteins and extracted with 4 mL hexane, the organic phase was removed, and the solvent was evaporated at room temperature under a stream of nitrogen (32). The residue was resuspended in 100 μL methanol/ethanol (1:1 v/v) and filtered through a 0.22-μm pore membrane before analysis of 40–80 μL by HPLC.

The HPLC system consisted of a Hitachi L-6000 gradient pump (Hitachi Instruments, San José, CA), Supelcosil LC-8 column and precolumn, and a BAS-LC-4C amperometric electrochemical detector. The samples were eluted with 97.5% methanol-2.5% water-20 mmol/L lithium perchlorate at 1.5 mL/min. The electrochemical detector was set up in the oxidizing mode at +0.6 mV and full recorder scale at 10 nA. α-T calculations were performed by area comparisons with an α(+)-tocopherol standard curve of known concentrations.

Protein analysis. Protein contents of cells were determined with the Bradford reagent. Protein concentrations in lipoprotein preparations were measured by the Lowry method modified by Markwell et al. (33). Bovine serum albumin was used as a reference standard.

Statistics. Results are presented as mean ± SEM. The significance of the differences between the means of the experimental groups were tested using the Student's *t* test for unpaired data. A difference was considered significant at $P < 0.05$.

RESULTS

Uptake of HDL α-T in ldlA and ldlA[mSR-BI] cells. To determine whether SR-BI could mediate the cellular uptake of α-T from HDL, we measured cellular α-T content in transfected cells overexpressing murine SR-BI (ldlA[mSR-BI]) and their untransfected controls (ldlA-7) after incubation for 5 h with and without unlabeled apoE-free HDL (**Fig. 1**). The basal levels of cellular α-T did not differ between cell types when

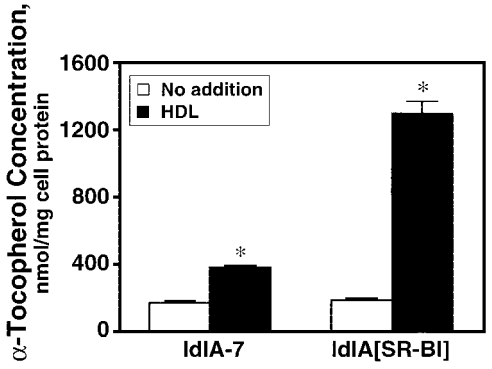

FIGURE 1 HDL α-tocopherol uptake by ldlA[mSR-BI] and control untransfected ldlA cells. On day 3 of cell growth, ldlA-7 and ldlA[mSR-BI] cells were incubated for 5 h at 37°C without and with apoE-free HDL (150 mg of HDL protein/L). After incubation, cells were removed and lysed for cell-associated α-tocopherol measurements by HPLC. Data shown are means ± SEM, $n = 5$. This experiment was repeated four times with similar results. *Different from cells not incubated with HDL, $P < 0.05$.

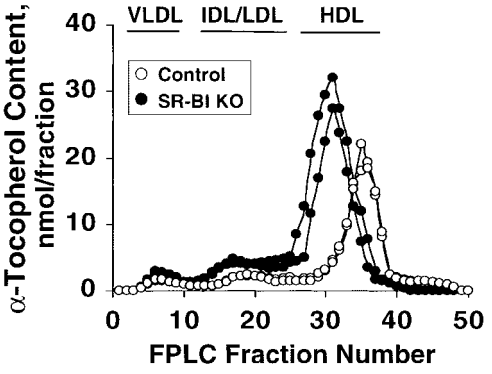

FIGURE 3 Plasma lipoprotein α-tocopherol distributions in control and SR-BI KO mice. Male C57BL/6 × 129/Sv control and SR-BI-deficient littermate mice were deprived of food overnight, blood was removed and plasma was obtained. Plasma lipoproteins of two individual mice from each genotype were separated by Superose-6 size chromatography. FPLC fractions were extracted with methanol and α-tocopherol concentration was measured and expressed as nmol/fraction. Approximate elution positions of VLDL, IDL+LDL and HDL are shown.

incubated in the absence of HDL. In the presence of HDL, total α-T content increased 1.3-fold in untransfected cells and 6.1-fold in ldlA[mSR-BI] cells, indicating that SR-BI can facilitate substantial net cellular uptake of α-T from native HDL.

Plasma levels and lipoprotein distribution of α-T in SR-BI-deficient mice. To determine whether the capacity of SR-BI to mediate cellular uptake of vitamin E from lipoproteins influences α-T metabolism in vivo, we compared the plasma α-T concentration in control and SR-BI knockout (KO) mice, both of which are on a mixed C57BL/6 × 129/Sv genetic background. Plasma total α-T concentrations in both male and female SR-BI-deficient mice were significantly higher than in their controls littemates (1.1- to 1.4-fold greater; **Fig. 2**). Large differences in plasma α-T also were observed when SR-BI KO mice were compared with nonlittermate controls (16.2 ± 1.6 vs. 4.2 ± 1.5 μmol/L, $P = 0.0014$).

To determine the distribution of α-T among plasma lipoproteins, we size fractionated plasma samples from control and SR-BI KO mice by FPLC and measured the amounts of α-T in each fraction (**Fig. 3**). In control mice, most plasma α-T was associated with HDL, with only small amounts detected in the intermediate density lipoproteins (IDL)+LDL and VLDL fractions. In SR-BI KO mice, most plasma α-T was found in an abnormally large and heterogeneous peak of HDL particles, a distribution virtually identical to that previously reported for plasma cholesterol from SR-BI KO mice (30).

α-T concentrations in nonhepatic tissues of SR-BI-deficient mice. The SR-BI-mediated uptake of HDL α-T in cultured cells and the increase in plasma lipoprotein α-T in SR-BI KO mice raised the possibility that SR-BI might play an important role in delivering lipoprotein α-T to tissues in vivo. **Figure 4** shows the relative concentrations of α-T in several nonhepatic tissues in control and SR-BI KO mice. The levels of α-T in the kidney, spleen, and adipose tissue from SR-BI

FIGURE 2 Plasma total α-tocopherol concentrations in male and female control and SR-BI KO mice. C57BL/6 × 129/Sv control and SR-BI-deficient littermate mice were deprived of food overnight and blood was removed for plasma separation. Total plasma was extracted with methanol and α-tocopherol was measured in extracts by HPLC as described in the Materials and Methods section. Values are means ± SEM, $n = 3$–5. *Different from control mice, $P < 0.05$.

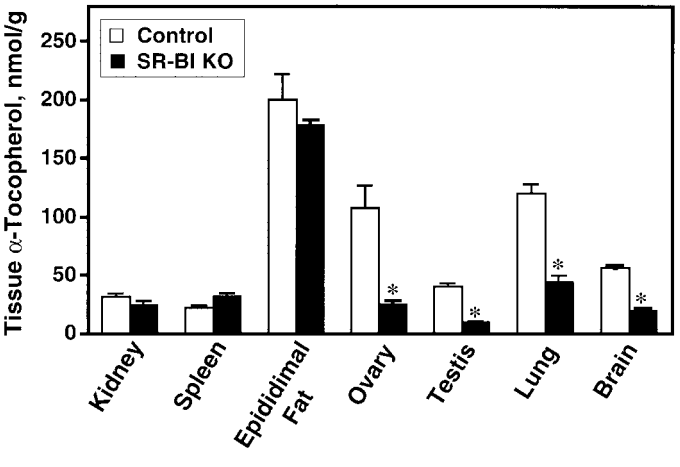

FIGURE 4 Tissue α-tocopherol concentrations in control and SR-BI KO mice. C57BL/6 × 129/Sv control and SR-BI-deficient mice were deprived of food overnight before harvesting tissues for α-tocopherol determinations. Tissues were homogenized and extracted and α-tocopherol was measured by HPLC. While renal, ovarian, testicular, pulmonary and brain α-tocopherol concentrations were measured in littermate mice, splenic and epididimal fat α-tocopherol was determined in nonlittermate animals. Values are means ± SEM, $n = 3$–6. *Different from control mice, $P < 0.05$.

KO mice were not significantly different than those from controls. In contrast, α-T concentrations in ovarian and testicular steroidogenic tissues were substantially lower (by ~77%) in SR-BI KO mice than in controls. In addition, the α-T levels were significantly lower than in controls in the lungs (64%) and brains (67%) of the SR-BI KO mice. Thus, SR-BI plays an important role in the transport of α-T to select tissues.

α-T concentrations in liver and bile of SR-BI-deficient mice. To assess the influence of SR-BI on α-T metabolism in the liver, we measured both hepatic and biliary α-T levels in control and SR-BI KO mice (**Fig. 5**). There was no significant difference between the levels of α-T in the livers of control and SR-BI KO littermates, whereas biliary α-T concentrations were 74–81% lower in SR-BI-deficient mice than in nonlittermate controls. Similar differences in biliary α-T concentration were obtained when control and SR-BI KO littermates were compared (results not shown). These data indicate that SR-BI plays an important role in controlling secretion of α-T into the bile.

Plasma, biliary and tissue α-T in LDLR-deficient mice. To evaluate the relative importance of SR-BI and the LDL receptor in α-T metabolism, we also measured plasma, biliary and tissue α-T levels in LDLR KO mice and their C57BL/6 controls (see the Materials and Methods section; **Fig. 6**). Plasma α-T levels were 2.3-fold higher in LDL receptor-deficient mice than in controls. In contrast to the significant effects of SR-BI deficiency on α-T levels in the bile and in ovaries, testes, lung and brain (Figs. 4 and 5), LDL receptor deficiency did not influence the steady-state concentrations of α-T in the bile and in these tissues (Fig. 6). These findings indicate that LDL receptor expression does not play an essential role in controlling α-T delivery to these tissues in vivo.

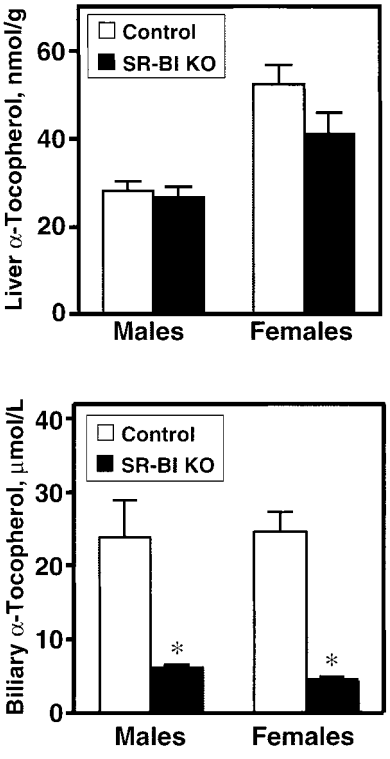

FIGURE 5 Hepatic and biliary α-tocopherol concentrations in male and female control and SR-BI KO mice. C57BL/6 × 129/Sv control and SR-BI-deficient mice were deprived of food overnight before liver and bile sampling for α-tocopherol measurements in methanolic extracts. While hepatic α-tocopherol concentration was measured in littermate mice, biliary α-tocopherol was determined in nonlittermate animals. Values are means ± SEM, n = 3–6. *Different from control mice, $P < 0.05$.

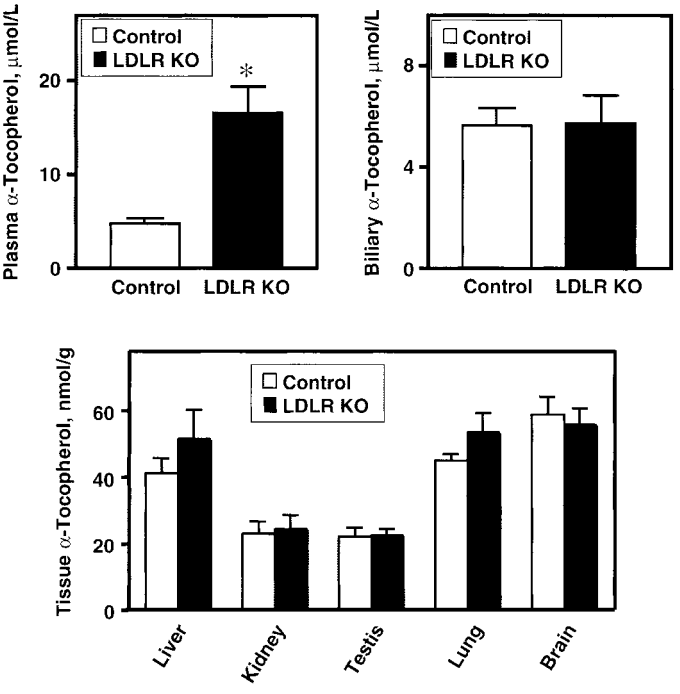

FIGURE 6 Total plasma, biliary and tissue α-tocopherol concentrations in control and LDLR KO mice. C57BL/6 control and LDLR KO (in a C57BL/6 background) mice were deprived of food overnight and plasma and tissues were removed. Samples were extracted for α-tocopherol determinations as described in the Material and Methods section. Values are means ± SEM, n = 3–6. *Different from control mice, $P < 0.05$.

DISCUSSION

This study describes the important role of SR-BI both in supplying α-T to cells and, consequently, in murine whole-body α-T homeostasis. We found that SR-BI mediates the uptake of lipoprotein α-T by cultured cells and that SR-BI deficiency in vivo increases levels of plasma α-T, decreases levels of α-T in some tissues (testes, ovary, lung and brain), but not others (liver, kidney, spleen and fat), and reduces biliary α-T. These observations strongly suggest that there are SR-BI-dependent and SR-BI-independent pathways for tissue α-T uptake and that the relative importance of these mechanisms for maintaining tissue α-T varies among tissues. SR-BI and the LDLR apparently play different roles in α-T transport and metabolism, because there were no differences in tissue α-T concentrations in control and LDL receptor-deficient mice (14,15 and this study). The effects of SR-BI deficiency on the amounts (~100% elevation) and lipoprotein distribution of α-T in the plasma were almost identical to those on plasma cholesterol (30). Because the changes in plasma cholesterol in SR-BI KO mice are attributed to loss of SR-BI-mediated selective uptake [reviewed in (20–23)], the changes in plasma α-T are also likely to be a consequence of the loss of SR-BI-mediated selective uptake of this lipid.

This study complements recent work that reported that overexpression of SR-BI in cultured cells by transfection or recombinant adenovirus transduction resulted in selective up-

take of [^{3}H]α-T from lipoproteins (27). Compared with LDL, HDL-associated α-T is delivered more efficiently to HepG2 and brain endothelial cells (16,17) and to SR-BI-transfected cells (27). Consistent with these latter findings, murine SR-BI deficiency had a more substantial effect on the levels of plasma HDL α-T, than on those of LDL α-T (Fig. 3). Future detailed analysis of the kinetics of α-T turnover should provide further insights into the relationship between cholesterol and vitamin E transport in SR-BI KO and wild-type mice.

Biliary secretion of α-T represents an important pathway for α-T disposal from the body (4,34). Although the final step of hepatic transport of α-T from the circulation into the bile seems to depend on the canalicular mdr2 P-glycoprotein (35), many of the mechanisms preceding this step, including the potential role of lipoprotein receptors, have not been well-defined. Although the role of SR-BI in controlling biliary α-T levels might be indirect, because of the abnormal lipoprotein structure in the SR-BI KO mice (30), it seems likely that SR-BI directly mediates the hepatic uptake of lipoprotein α-T for subsequent biliary secretion. It seems unlikely that the reduced biliary α-T was due to decreased α-T absorption by the intestines without a change in capacity of the liver to take up α-T from the circulation, because plasma α-T levels were greater in the SR-BI KO mice than in their controls. Interestingly, the extent of reduction of biliary α-T in SR-BI KO mice relative to controls (74–81%; Fig. 5) is greater than that previously found for biliary cholesterol in these mice (reduction by 55%) (31). These findings indicate that excretion of α-T into the bile has a greater dependence on SR-BI than does that of cholesterol and/or that alternative compensatory mechanisms for α-T and cholesterol homeostasis are differentially expressed in SR-BI-deficient mice.

Despite the substantial effects of SR-BI deficiency on plasma and biliary α-T concentrations, hepatic α-T concentration was not affected by SR-BI deficiency. These results raise the possibility that there are SR-BI-independent homeostatic mechanisms, including those involving uptake from lipoproteins, that maintain the steady-state levels of α-T in the liver. These mechanisms presumably contribute to hepatic α-T supply and compensate for the lack of SR-BI expression in SR-BI KO mice. In addition, renal, splenic and adipose α-T concentrations were not affected by SR-BI deficiency, which is fully consistent with the relatively low level of SR-BI protein expressed in these tissues in control mice (19,36).

Because α-T can serve as an effective antioxidant [reviewed in (1–5)], it is possible that metabolic activities of gonadal tissues (e.g., steroidogenesis in the ovary and testes), lungs and the brain generate requirements for increased receptor-mediated delivery of HDL α-T that are not shared by the kidney, spleen and epididimal fat. The decreased concentration of α-T in gonadal tissues of SR-BI KO mice is consistent with the normally high expression levels of SR-BI in steroidogenic cells in control animals [reviewed in (20–23)]. Because type II pneumocytes in the lungs (26,37), endothelial cells of the blood brain barrier [(27) and Miranda, S., Mardones, P., Leighton, F. & Rigotti, A., unpublished data] and microglia and astrocytes in the brain (38–40) have all been shown to express SR-BI in wild-type animals, SR-BI deficiency in these cell types probably contributes to the lower levels of α-T seen in these tissues in SR-BI KO mice. Two striking pathologies in SR-BI KO mice may be influenced by abnormal α-T transport: accelerated atherogenesis in the context of an atherosclerosis prone genetic background, apoE deficiency (41), and female infertility (41) involving production of abnormal oocytes and preimplantational embryos due to an abnormal lipoprotein profile (41,42). LDL lipid peroxidation has been proposed to play a role in atherogenesis and α-T can have either anti- and/or pro-oxidative activities during radical-triggered LDL lipid peroxidation [reviewed in (1–5,43–45)]. If not counterbalanced by other redox-active molecules, increased plasma α-T concentration in SR-BI KO mice may facilitate α-T-mediated lipoprotein peroxidation (43–46) in the circulation or within the artery wall, and, thus, possibly accelerate atherogenesis. Alternatively, SR-BI, which can be expressed in atherosclerotic lesions (47–49), may be critical for delivering lipoprotein α-T to the artery wall to serve as a cellular antioxidant (50,51) and/or modulator of intracellular signal transduction (52,53).

Vitamin E is an essential micronutrient for zygote implantation and female fertility in rodents, as can be seen in α-T transfer protein-deficient mice (51,54,55). Indeed, dietary α-T (51,55) and antioxidant supplementation (55) can prevent placental failure and infertility in α-T transfer protein-deficient female mice. In contrast, dietary α-T supplementation (1000 IU α-tocopheryl acetate/kg of diet) does not restore fertility to SR-BI KO females (Mardones, P., Rozowski, J., Krieger, M. & Rigotti, A., unpublished data). Dietary α-T supplementation may have been ineffective because tissue α-T deficiency is not responsible for the infertility of the SR-BI KO mice, or because adequate amounts of α-T could not be delivered to key reproductive tissues in the absence of SR-BI.

SR-BI is expressed on both the maternal (e.g., decidual and trophoblast cells) and embryonic (yolk sac visceral endoderm and placental chorionic labyrinth) sides of the maternal-fetal interface during pregnancy (56,57). Thus, it may play a role during postimplantational embryonic development by controlling the transfer of lipoprotein α-T to the growing embryo. This might contribute to the lower than expected yield of SR-BI homozygous null mice from matings of male and female heterozygous null animals (30). Previous studies have shown that placental α-T concentration increases progressively during gestation (58), a process susceptible to oxygen-free radical damage that requires efficient antioxidant defense mechanisms for normal intrauterine fetal development (59–61).

α-T deficiency has been associated with oxidative stress (62) and dysfunction of the central nervous system (e.g., human spinocerebelar ataxia and myopathies) (63–68) and the lungs (69,70). Additional studies will be required to determine whether SR-BI KO mice are predisposed to similar disorders because of the reduced levels of α-T in their brains and lungs.

In summary, this study demonstrates that the SR-BI-mediated lipid uptake pathway regulates α-T metabolism in vivo. The identification of this novel role of SR-BI should contribute to our understanding of the physiological and pathophysiological relevance of this lipoprotein receptor. SR-BI KO mice represent a new model for selective tissue vitamin E deficiency and, thus, may be useful for addressing the potential relevance of α-T, other antioxidants and oxidative stress not only in atherosclerosis and female infertility, but also in normal and disease conditions of the biliary tree, lung and central nervous system.

ACKNOWLEDGMENT

We thank Bernardo Trigatti for critical review of the manuscript.

LITERATURE CITED

1. Burton, G. W. & Traber, M. G. (1990) Vitamin E: antioxidant activity, biokinetics, and bioavailability. Annu. Rev. Nutr. 10: 357–382.

2. Brigelius-Flohe, R. & Traber, M. G. (1999) Vitamin E: function and metabolism. FASEB J. 13: 1145–1155.

3. Bjorneboe, A., Bjorneboe, G. E. & Drevon, C. A. (1990) Absorption, transport and distribution of vitamin E. J. Nutr. 120: 233–242.

4. Kayden, H. J. & Traber, M. G. (1993) Absorption, lipoprotein transport, and regulation of plasma concentrations of vitamin E in humans. J. Lipid Res. 34: 343–358.

5. Traber, M. G. & Arai, H. (1999) Molecular mechanisms of vitamin E transport. Annu. Rev. Nutr. 19: 343–355.

6. Sato, Y., Hagiwara, K., Arai, H. & Inoue, K. (1991) Purification and characterization of the alpha-tocopherol transfer protein from rat liver. FEBS Lett. 288: 41–45.

7. Arai, H., Miyata, A., Tokita, S., Yamamoto, K., Tanabe, T. & Inoue, K. (1993) Primary structure of alpha-tocopherol transfer protein from rat liver: homology with cellular retinaldehyde-binding protein. J. Biol. Chem. 268: 17705–17710.

8. Traber, M. G., Olivecrona, T. &. Kayden, H. J. (1985) Bovine milk lipoprotein lipase transfers tocopherol to human fibroblasts during triglyceride hydrolysis in vitro. J. Clin. Invest. 75: 1729–1734.

9. Sattler, W., Levak-Frank, S., Radner, H., Kostner, G. M. & Zechner, R. (1996) Muscle-specific overexpression of lipoprotein lipase in transgenic mice results in increased α-tocopherol levels in skeletal muscle. Biochem. J. 318: 15–19.

10. Kostner, G. M., Oettl, K., Jauhiainen, M., Ehnholm, C., Esterbauer, H. & Dieplinger, H. (1995) Human plasma phospholipid transfer protein accelerates exchange/transfer of α-tocopherol between lipoproteins and cells. Biochem. J. 305: 659–667.

11. Desrumaux, C., Deckert, V., Athias, A., Masson, D., Lizard, G., Palleau, V., Gambert, P. & Lagrost, L. (1999) Plasma phospholipid transfer protein prevents vascular endothelium dysfunction by delivering α-tocopherol to endothelial cells. FASEB J. 13: 883–892.

12. Traber, M. G. & Kayden, H. J. (1984) Vitamin E is delivered to cells via the high affinity receptor for low-density lipoprotein. Am. J. Clin. Nutr. 40: 747–751.

13. Thellman, C. A. & Shireman, R. B. (1985) In vitro uptake of [3H]α-tocopherol from low density lipoprotein by cultured human fibroblasts. J. Nutr. 115: 1673–1679.

14. Cohn, W., Goss-Sampson, M. A., Grun, H. & Muller, D. P. (1992) Plasma clearance and net uptake of α-tocopherol and low-density lipoprotein by tissues in WHHL and control rabbits. Biochem. J. 287: 1247–1254.

15. Cohn, W. & Kuhn, H. (1989) The role of the low density lipoprotein receptor for α-tocopherol delivery to tissues. Ann. N. Y. Acad. Sci. 570: 61–71.

16. Goti, D., Reicher, H., Malle, E., Kostner, G. M., Panzenboeck, U. & Sattler, W. (1998) High-density lipoprotein (HDL3)-associated α-tocopherol is taken up by HepG2 cells via the selective uptake pathway and resecreted with endogenously synthesized apo-lipoprotein B-rich lipoprotein particles. Biochem. J. 332: 57–65.

17. Goti, D., Hammer, A., Galla, H. J., Malle, E. & Sattler, W. (2000) Uptake of lipoprotein-associated α-tocopherol by primary porcine brain capillary endothelial cells. J. Neurochem. 74: 1374–1383.

18. Glass, C., Pittman, R. C., Weinstein, D. B. & Steinberg, D. (1983) Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal, and gonads. Proc. Natl. Acad. Sci. U.S.A. 80: 5435–5439.

19. Acton, S., Rigotti, A., Landschulz, K. T., Xu, S., Hobbs, H. H. & Krieger, M. (1996) Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. Science 271: 518–520.

20. Krieger, M. (1999) Charting the fate of the good cholesterol: identification and characterization of the high-density lipoprotein receptor SR-BI. Annu. Rev. Biochem. 68: 523–558.

21. Williams, D. L., Connelly, M. A., Temel, R. E., Swarnakar, S., Phillips, M. C., de la Llera-Moya, M. & Rothblat, G. H. (1999) Scavenger receptor BI and cholesterol trafficking. Curr. Opin. Lipidol. 10: 329–339.

22. Trigatti, B., Rigotti, A. & Krieger, M. (2000) The role of the high density lipoprotein receptor SR-BI in cholesterol metabolism. Curr. Opin. Lipidol. 11: 123–131.

23. Trigatti, B. L., Rigotti, A. & Braun, A. (2000) Cellular and physiological roles of SR-BI, a lipoprotein receptor which mediates selective lipid uptake. Biochim. Biophys. Acta 1529: 276–286.

24. Bjorneboe, A., Bjorneboe, G. E., Bodd, E., Hagen, B. F., Kveseth, N. & Drevon, C. A. (1986) Transport and distribution of α-tocopherol in lymph, serum and liver cells in rats. Biochim. Biophys. Acta 889: 310–315.

25. Witt, W., Kolleck, I., Fechner, H., Sinha, P. & Rustow, B. (2000) Regulation by vitamin E of the scavenger receptor BI in rat liver and HepG2 cells. J. Lipid Res. 41: 2009–2016.

26. Kolleck, I., Schlame, M., Fechner, H., Looman, A. C., Wissel, H. & Rustow, B. (1999) HDL is the major source of vitamin E for type II pneumocytes. Free Radic. Biol. Med. 27: 882–890.

27. Goti, D., Hrzenjak, A., Levak-Frank, S., Frank, S., Van der Westhuyzen, D. R., Malle, E. & Sattler, W. (2001) Scavenger receptor class B, type I is expressed in porcine brain endothelial capillary endothelial cells and contributes to selective uptake of HDL-associated vitamin E. J. Neurochem. 76: 498–508.

28. Goldstein, J. L., Basu, S. K. & Brown, M. S. (1983) Receptor-mediated endocytosis of low-density lipoprotein in cultured cells. Methods Enzymol. 98: 241–260.

29. Havel, R. J., Eder, H. S. & Bragdon, J. H. (1955) The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J. Clin. Invest. 3: 1345–1353.

30. Rigotti, A., Trigatti, B. L., Penman, M., Rayburn, H., Herz, J. & Krieger, M. (1997) A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. Proc. Natl. Acad. Sci. U.S.A. 94: 12610–12615.

31. Mardones, P., Quiñones, V., Amigo, L., Moreno, M., Miquel, J. F., Schwarz, M., Miettinen, H. E., Trigatti, B., Krieger, M., Van Patten, S., Cohen, D. E. & Rigotti, A. (2001) Hepatic cholesterol and bile acid metabolism and intestinal cholesterol absorption in scavenger receptor class B type I-deficient mice. J. Lipid Res. 42: 170–180.

32. Motchnik, P. A., Frei, B. & Ames, B. N. (1994) Measurement of antioxidants in human blood plasma. Methods Enzymol. 234: 267–279.

33. Markwell, M. K., Hass, S., Bieber, L. L. & Tolbert, N. E. (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. 87: 206–210.

34. Bjorneboe, A., Bjorneboe, G. E. & Drevon, C. A. (1987) Serum half-life, distribution, hepatic uptake and biliary excretion of α-tocopherol in rats. Biochim. Biophys. Acta 921: 175–181.

35. Mustacich, D. J., Shields, J., Horton, R. A., Brown, M. K. & Reed, D. J. (1998) Biliary secretion of α-tocopherol and the role of the mdr2 P-glycoprotein in rats and mice. Arch. Biochem. Biophys. 350: 183–192.

36. Landschulz, K. T., Pathak, R. K., Rigotti, A., Krieger, M. & Hobbs, H. H. (1996) Regulation of the scavenger receptor class B type I, a high density lipoprotein receptor, in liver steroidogenic tissues of the rat. J. Clin. Invest. 98: 984–995.

37. Kolleck, I., Witt, W., Wissel, H., Sinha, P. & Rustow, B. (2000) HDL and vitamin E in plasma and the expression of SR-BI on lung cells during rat perinatal development. Lung 178: 191–200.

38. Nakagawa-Toyama, Y., Hirano, K., Okamoto, Y., Nishida, M., Miyagawa, J., Fujimura, H., Yamashita, S. & Matsuzawa, Y. (2000) Expression on high density lipoprotein receptor scavenger receptor class B type I in human central nervous system. Atherosclerosis 151: 295.

39. Husemann, J. & Silverstein, S. C. (2001) Expression of scavenger receptor class B type I (SR-BI) by astrocytes and vascular smooth muscle cells in normal adult mouse and human brain and in Alzheimer's disease brain. Am. J. Pathol. 158: 825–832.

40. Husemann, J., Loike, J. D., Kodama, T. & Silverstein, S. C. (2001) Scavenger receptor class B type I (SR-BI) mediates adhesion of neonatal murine microglia to fibrillar β-amyloid. J. Neuroimmunol. 114: 142–150.

41. Trigatti, B., Rayburn, H., Viñals, M., Braun, A., Miettinen, H., Penman, M., Hertz, M., Schrenzel, M., Amigo, L., Rigotti, A. & Krieger, M. (1999) Influence of the HDL receptor SR-BI on reproductive and cardiovascular pathophysiology. Proc. Natl. Acad. Sci. U.S.A. 96: 9322–9327.

42. Miettinen, H. E., Rayburn, H. & Krieger, M. (2001) Abnormal lipoprotein metabolism in HDL receptor (SR-BI)-deficient mice and reversible female infertility. J. Clin. Invest. 108: 1717–1722.

43. Stocker, R. (1999) The ambivalence of vitamin E in atherogenesis. Trends Biochem. Sci. 24: 219–223.

44. Upston, J. M., Terentis, A. C. & Stocker, R. (1999) Tocopherol-mediated peroxidation of lipoproteins: implications for vitamin E as a potential antiatherogenic supplement. FASEB J. 13: 977–994.

45. Neuzil, J., Thomas, S. R. & Stocker, R. (1997) Requirement for, promotion, or inhibition by α-tocopherol of radical-induced initiation of plasma lipoprotein lipid peroxidation. Free Radic. Biol. Med. 22: 57–71.

46. Neuzil, J., Christison, J. K., Iheanacho, E., Fragonas, J. C., Zammit, V., Hunt, N. H. & Stocker, R. (1998) Radical-induced lipoprotein and plasma lipid oxidation in normal and apolipoprotein E gene knockout (apoE$^{-/-}$) mice: apoE$^{-/-}$ mouse as a model for testing the role of tocopherol-mediated peroxidation in atherogenesis. J. Lipid Res. 39: 354–368.

47. Ji, Y., Jian, B., Wang, N., Sun, Y., Moya, M. L., Phillips, M. C., Rothblat, G. H., Swaney, J. B. & Tall, A. R. (1997) Scavenger receptor BI promotes high density lipoprotein-mediated cellular cholesterol efflux. J. Biol. Chem. 272: 20982–20985.

48. Hirano, K., Yamashita, S., Nakagawa, Y., Ohya, T., Matsuura, F., Tsukamoto, K., Okamoto, Y., Matsuyama, A., Matsumoto, K., Miyagawa, J. & Matsuzawa, Y. (1999) Expression of human scavenger receptor class B type I in cultured human monocyte-derived macrophages and atherosclerotic lesions. Circ. Res. 85: 108–116.

49. Chinetti, G., Gbaguidi, F. G., Griglio, S., Mallat, Z., Antonucci, M., Poulain, P., Chapman, J., Fruchart, J. C., Tedgui, A., Najib-Fruchart, J. & Staels, B. (2000) CLA-1/SR-BI is expressed in atherosclerotic lesion macrophages and regulated by activators of peroxisome proliferator-activated receptors. Circulation 101: 2411–2417.

50. Pratico, D., Tangirala, R. K., Rader, D. J., Rokach, J. & Fitzgerald, G. A. (1998) Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in ApoE-deficient mice. Nat. Med. 4: 1189–1192.

51. Terasawa, Y., Ladha, Z., Leonard, S. W., Morrow, J. D., Newland, D., Sanan, D., Packer, L., Traber, M. G. & Farese, R. V. (2000) Increased atherosclerosis in hyperlipidemic mice deficient in α-tocopherol transfer protein and vitamin E. Proc. Natl. Acad. Sci. U.S.A. 97: 13830–13834.

52. Traber, M. G. & Packer, L. (1995) Vitamin E: beyond antioxidant function. Am. J. Clin. Nutr. 62: 1501S–1509S.

53. Azzi, A. & Stocker, R. (2000) Vitamin E: non-antioxidant roles. Prog. Lipid Res. 39: 231–255.

54. Evans, H. M. & Bishop, B. S. (1922) On the existence of a hitherto unrecognized dietary factor essential for reproduction. Science 56: 650–651.

55. Jishage, K., Arita, M., Igarashi, K., Iwata, T., Watanabe, M., Ogawa, M., Ueda, O., Kamada, N., Inoue, K., Arai, H. & Suzuki, H. (2001) α-Tocopherol transfer protein is important for the normal development of placental labyrinthine trophoblasts in mice. J. Biol. Chem. 276: 1669–1672.

56. Wyne, K. L. & Woollett, L. A. (1998) Transport of maternal LDL and HDL to the fetal membranes and placenta of the Golden Syrian hamster is mediated by receptor-dependent and receptor-independent processes. J. Lipid Res. 39: 518–530.

57. Hatzopoulos, A. K., Rigotti, A., Rosenberg, R. D. & Krieger, M. (1998) Temporal and spatial pattern of expression of the HDL receptor SR-BI during murine embryogenesis. J. Lipid Res. 39: 495–508.

58. Qanungo, S., Sen, A. & Mukherjea, M. (1999) Antioxidant status and lipid peroxidation in human feto-placental unit. Clin. Chim. Acta 285: 1–12.

59. Hagay, Z. J., Weiss, Y., Zusman, I., Peled-Kamar, M., Reece, E. A., Eriksson, U. J. & Groner, Y. (1995) Prevention of diabetes-associated embryopathy by overexpression of the free radical scavenger copper zinc superoxide dismutase in transgenic mouse embryos. Am. J. Obstet. Gynecol. 173: 1036–1041.

60. Hassoun, E. A., Walter, A. C., Alsharif, N. Z. & Stohs, S. J. (1997) Modulation of TCDD-induced fetotoxicity and oxidative stress in embryonic and placental tissues of C57BL/6J mice by vitamin E succinate and ellagic acid. Toxicology 124: 27–37.

61. Nicol, C. J., Zielenski, J., Tsui, L. C. & Wells, P. G. (2000) An embryoprotective role for glucose-6-phosphate dehydrogenase in developmental oxidative stress and chemical teratogenesis. FASEB J. 14: 111–127.

62. Murray, C. A. & Lynch, M. A. (1998) Dietary supplementation with vitamin E reverses the age-related deficit in long term potentiation in dentate gyrus. J. Biol. Chem. 273: 12161–12168.

63. Traber, M. G. & Sies, H. (1996) Vitamin E in humans: demand and delivery. Annu. Rev. Nutr. 16: 321–347.

64. Terasawa, Y., Liang, D. B., Cases, S., Raber, J., Traber, M. G., Packer, L. & Farese, R. V. (1999) α-Tocopherol transfer protein knockout mice: a gene-targeted model of vitamin E deficiency. Circulation 100: S47.

65. Xie, Z. & Sastry, B. R. (1993) Induction of hippocampal long-term potentiation by α-tocopherol. Brain Res. 604: 173–17.

66. Xie, Z. & Sastry, B. R. (1995) Impairment of long-term potentiation in rats fed with vitamin E-deficient diet. Brain Res. 681: 193–196.

67. Christen, Y. (2000) Oxidative stress and Alzheimer disease. Am. J. Clin. Nutr. 71: 621S–629S.

68. Miranda, S., Opazo, C., Larrondo, L. F., Munoz, F. J., Ruiz, F., Leighton, F. & Inestrosa, N. C. (2000) The role of oxidative stress in the toxicity induced by amyloid β-peptide in Alzheimer's disease. Prog. Neurobiol. 62: 633–648.

69. Martin, W. J., Gadek, J. E., Hunninghake, G. W. & Crystal, R. G. (1981) Oxidant injury of lung parenchymal cells. J. Clin. Invest. 68: 1277–1288.

70. Suntres, Z. E., Hepworth, S. R. & Shek, P. N. (1992) Protective effect of liposome-associated α-tocopherol against paraquat-induced acute lung toxicity. Biochem. Pharmacol. 44: 1811–1818.