Plasma and fibroblasts of Tangier disease patients are disturbed in transferring phospholipids onto apolipoprotein A-I

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Abstract **Plasmas of patients with Tangier disease (TD) lack lipid-rich α-HDL which, in normal plasma, constitutes the majority of high density lipoprotein (HDL). Residual amounts of apolipoprotein (apo)A-I in TD plasma occur as lipid-poor or even lipid-free preβ-HDL. By contrast to normal plasma, TD plasma does not convert preβ-HDL into α-HDL. Moreover, fibroblasts of TD patients were found to be defective in secreting cholesterol or phospholipids in the presence of lipid-free apoA-I. We have therefore hypothesized that both defective conversion of preβ-HDL into α-HDL and defective lipid efflux from TD cells onto lipid-free apoA-I result from a disturbance in phospholipid transfer occurring in both cellular and extracellular compartments. To test this hypothesis we established an assay that measures the activity of plasma, cells, and cell culture media to transfer radiolabeled phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) from vesicles onto apoA-I, apoA-II, albumin, or reconstituted HDL. Plasmas, HDL, and lipoprotein-depleted plasma of normolipidemic probands as well as cell homogenates and culture media of normal fibroblasts were active at 37°C but not at 4°C in transferring radiolabeled PC, PI, and PE dose- and time-dependently onto either lipid-free apoA-I or reconstituted HDL. Transfer of glycerophospholipids onto apoA-II was much lower than onto apoA-I; transfer onto albumin was close to background. Compared to ten normolipidemic plasmas and four apoA-I-deficient plasmas, plasmas of six TD patients were significantly reduced by 40–50% in their glycerophospholipid transfer activities. Compared to eight normal fibroblast cell lines, homogenates and culture media of four TD fibroblast cell lines were reduced by 40–50% and 30–35%, respectively, in their activity to transfer PC, PI, or PE onto apoA-I. Our data suggest that in TD the same mechanism underlies both defective conversion of preβ-HDL into α-HDL and impaired efflux of cellular lipids, namely a defective phospholipid transfer.—**von Eckardstein, A., A. Chirazi, S. Schuler-Lüttmann, M. Walter, J. J. P. Kastelein, J. Geisel, J. T. Real, R. Miccoli, G. Noseda, G. Höbbel, and G. Assmann. **Plasma and fibroblasts of Tangier disease patients are disturbed in transferring phospholipids onto apolipoprotein A-I.** ***J. Lipid Res.*** **1998.** 39: **987–998.**

Supplementary key words prebeta-HDL • familial HDL deficiency • reverse cholesterol transport • cholesterol efflux • phospholipid transfer proteins

Tangier disease (TD) is a rare, high density lipoprotein (HDL) deficiency syndrome that is inherited as an autosomal-codominant trait and whose metabolic and genetic basis is unknown. Clinical symptoms in homozygotes include hyperplastic orange tonsils, hepatosplenomegaly, and relapsing peripheral neuropathy, probably due to cytoplasmic cholesteryl ester deposition in macrophages and Schwann cells, respectively (1, 2). The biochemical hallmark of TD plasma is the absence of α-migrating HDLs, i.e., α-LpA-I, which in normal plasma contain approximately 95% of apolipoprotein (apo) A-I and whose concentration is reflected by HDL-cholesterol. Residual amounts of apoA-I of

Abbreviations: apoA-I, apolipoprotein A-I; CETP, cholesteryl ester transfer protein; HDL, high density lipoprotein; LDL, low density lipoprotein; LpA-I, apoA-I-containing lipoprotein; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PLTP, phospholipid transfer protein; preβ-LpA-I, prebeta-LpA-I; rLpA-I, reconstituted LpA-I; TD, Tangier disease; VLDL, very low density lipoprotein.

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TD plasma are found in an HDL subclass, which in normolipidemic plasma accounts for less than 5% of total apoA-I and which because of its electrophoretic mobility is termed preβ_1-LpA-I (3). By contrast to lipid-rich α-HDL, which produce slow and unsaturable net cholesterol efflux only in the presence of lecithin:cholesterol acyltransferase (LCAT), this lipid-poor or even lipid-free particle promotes fast and saturable cholesterol efflux from certain but not all cell types, e.g., from fibroblasts and macrophages. This type of cholesterol efflux occurs independently of LCAT and in parallel with phospholipid efflux. It is sensitive to the treatment of cells with proteases and modulated by activation or inhibition of protein kinase C (4–16). Therefore, it was suggested that cholesterol efflux mediated by lipid-free apoA-I depends on specific protein–cell surface interactions.

Interestingly, lipid-free apoA-I was found completely inactive in releasing cholesterol and phospholipids from TD fibroblasts (17, 18). Compared to normal fibroblasts, the unspecific cholesterol efflux from TD fibroblasts promoted by native or trypsinized HDL was reduced by only 30–50% or even unaffected. Oram and Yokoyama (6) and Francis, Knopp, and Oram (17) hypothesized that the failure of lipid-free apoA-I to pick up phospholipids and cholesterol from TD cells caused not only cellular lipid accumulation and foam cell formation but also a block in the maturation of HDL. Actually, we have previously demonstrated that in contrast to plasmas of normolipidemic subjects or patients with other forms of familial HDL deficiency, TD plasma lacks the activity to convert lipid-poor preβ_1-LpA-I or lipid-free apoA-I into α-LpA-I (19). Therefore, and as preβ_1-LpA-I and α-LpA-I differ by the content and composition of phospholipids (4, 20–23), we hypothesize that plasmas and fibroblasts of TD patients are defective in transferring phospholipids onto apoA-I. To test this hypothesis we established an assay that measures the activities of plasmas, homogenized cells, and cell culture media to transfer glycerophospholipids from vesicles onto apoA-I and reconstituted HDL. By this test we were able to demonstrate significantly reduced phospholipid transfer activities in both plasmas and fibroblasts of TD patients.

MATERIALS AND METHODS

Subjects

Six patients with Tangier disease (TD), four patients with apoA-I deficiency, and ten normolipidemic volunteers participated in this study after informed consent and in accordance with the ethical standards laid down in the relevant version of the 1964 Declaration of Helsinki. Serum concentrations of lipids, lipoproteins, and apolipoproteins as well as plasma activities of lipid transfer enzymes are summarized in **Table 1**. Among the six TD patients, two German siblings (T1 and T2) and a man from Pakistan (T3) have been described previously (24, 25). T1 and T2 have clinically presented with hepatosplenomegaly, T3 with multiple peripheral motoric and sensory losses. Case reports of three other TD patients from Germany (T4), The Netherlands (T5), and Spain (T6) have not yet been described and are presented below. In all cases, isoelectric focusing of plasma and subsequent anti-apoA-I immunoblotting identified residual amounts of apoA-I and proapoA-I occurring with regular isoelectric points and at equal concentrations, which is a typical finding in TD (2).

The 60-year-old German TD patient (T4) suffered from myocardial infarction at the age of 49 years and underwent aortocoronary bypass surgery at the age of 50 years. In addition to HDL deficiency and hypertriglyceridemia (see Table 1), cardiovascular risk factors included overweight (body mass index = 29.0 kg/m^2), arterial hypertension, and hyperuricemia. Gingiva was hyperplastic. Spleen and liver were not enlarged. Neither the history nor the physical examination of the patient gave indications of peripheral neuropathy. No corneal opacifications were found upon inspection. Routine laboratory examinations over the last 10 years repeatedly led to the detection of thrombocytopenia (100.000 ± 10.000 platelets/μl). The patient has two sisters who are also deficient of HDL and two children who have half-normal HDL-cholesterol levels. DNA sequence analysis of the apoA-I gene revealed wild-type (J. Geisel, K. Oette, H. Funke, A. von Eckardstein, and G. Assmann, unpublished results).

The Dutch TD patient (T5) suffered from myocardial infarction of the inferior wall at the age of 38 years. Coronary angiography revealed an 80% proximal stenosis of the right coronary ar-

TABLE 1. Biographic and metabolic characteristics of the probands analyzed

	Normal	ApoA-I Deficiency	Tangier Disease Patients						
			mean	T1	T2	T3	T4	T5	T6
n, Sex	7m + 3f	2m + 1f	5m + 1f	m	f	m	m	m	m
Age (years)	27 ± 4	42 ± 9**	54 ± 11***	63	65	46	60	38	51
Cholesterol (mg/dl)	185 ± 29	173 ± 57##	70 ± 20**	57	106	63	72	74	48
Triglycerides (mg/dl)	105 ± 48	92 ± 48	193 ± 76*	237	288	138	256	127	110
HDL-chol. (mg/dl)	52 ± 9	<1***	<1***	<1	<1	<1	<1	<1	<1
ApoA-I (mg/dl)	142 ± 24	2 ± 2***	3 ± 1***	2	2	3	4	2	3
ApoA-II	45 ± 6	10 ± 2***	6 ± 4***	3	9	12	4	3	2
ApoB (mg/dl)	65 ± 13	92 ± 29*	78 ± 17	55	101	69	77	94	69
LCAT (nmol/h/ml)	17.4 ± 2.8	7.5 ± 4.4***	5.9 ± 1.8***	3.9	8.8	4.6	6.1	7.2	5.0
CETP (nmol/h/ml)	105 ± 18	90 ± 8	126 ± 36	135	149	177	121	98	75
PLTP (nmol/h/ml)	1579 ± 177	1240 ± 58	1399 ± 366	1879	1764	1500	1085	1125	n.d.

Male, m; female, f. Patient T2 was treated with fenofibrate.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; level of statistical significance by which data of HDL-deficient samples differ from data of normolipidemic control samples (calculated by Student's *t*-test).

##$P < 0.01$; level of statistical significance by which data differ from Tangier disease samples (calculated by Student's *t*-test).

tery and diffuse severe atherosclerosis of the left coronary artery. Subsequently, the patient underwent coronary bypass surgery and recovered uneventfully. Absence of HDL and severely reduced levels of apoA-I (Table 1) were the only coronary risk factors. Biopsy of both tonsil and rectal mucosa showed the presence of lipid-laden macrophages suggestive of TD. The patient's father and daughter had HDL-cholesterol levels below the 10th percentile of age-matched controls.

The 51-year-old Spanish TD patient (T6) came to medical attention in 1994 because of hepatosplenomegaly and thrombocytopenia (120.000 platelets/μl). Biopsies of bone marrow and spleen led to the identification of foamy histiocytes that were characterized as Niemann-Pick type. At the age of 20 years the patient underwent tonsillectomy because of yellowish and enlarged adenoid tissue. Physical examination revealed no signs of peripheral neuropathy. The patient's mother and four children had decreased HDL-cholesterol levels (<34 mg/dl).

Four patients from two families with apoA-I deficiency have been described previously. In one case a premature truncation at residue 28 led to apoA-I deficiency in a 32-year-old Italian woman (26). Two brothers and a sister from another Italian family are compound heterozygotes for an apoA-I null allele and an arginine for leucine substitution at residue 141, i.e., apoA-I$(L141R)_{Pisa}$ (27, 28).

Collection of blood samples

Blood samples were collected after overnight fasting and cooled immediately on ice. Sera and EDTA-plasmas were obtained by centrifugation at 4°C (800 *g*, 15 min), aliquoted, and stored at −70°C. When not sampled in Münster, plasmas and sera were shipped on dry ice to this laboratory.

Cell culture of fibroblasts

Fibroblasts were cultivated from skin biopsies that were obtained from hip skin of eight unrelated healthy normolipidemic volunteers and four patients with Tangier disease (T1, T2, T4, and T5) as decribed previously (29). The cells were grown and maintained in DMEM that contained 10% FCS and 1% antibiotics/antimycotics (Sigma Chemical Co., St. Louis, MO). Experiments described below were performed after three to ten passages.

Fibroblasts of the same donor were cultured in 75-cm^2 flasks. At the state of near confluence, cells were washed three times with PBS (pH 7.4) and then incubated for 24 h in 9 ml DMEM that contained 1% antibiotics/antimycotics and 30 g/l bovine serum albumin (BSA) as the basic medium and was supplemented with either no additive or 100 μg/ml LDL or 10% FCS. Thereafter the cells were washed three times with PBS and incubated again for 24 h in 9 ml lipoprotein-free DMEM. This medium was removed and centrifuged in a microcentrifuge for 10 min at 15,000 rpm to precipitate cell debris. The supernatants were frozen at −80°C. The cells were washed three times with PBS and then removed into 6 ml PBS with a rubber policeman. The cell suspension was also frozen at −80°C.

Isolation of lipoproteins, apoA-I, and apoA-II

Very low density lipoproteins (VLDL, $d < 1.006$ g/ml), intermediate and low density lipoproteins (IDL and LDL, $1.006 < d < 1.061$ g/ml), high density lipoproteins (HDL, $1.063 < d < 1.21$ g/ml), and lipoprotein-depleted plasma (LPDP, $d > 1.21$ g/ml) were isolated from plasma by sequential isopycnic ultracentrifugation (30).

ApoA-I and apoA-II were isolated from HDL by fast performance liquid chromatography as described previously (31) using a MonoQ HR5/5 column as the stable phase (Pharmacia, Bromma, Sweden). The mobile phase was run with a gradient consisting of buffer A (15 mm Tris-HCl, 10 mm NaCl, 5 m urea) and buffer B (15 mm Tris-HCl, 1 m NaCl, 5 m urea). The gradient was eluted within 30 min with a flow of 0.5 ml/min. Purity of apoA-I and apoA-II was ascertained by reversed-phase high performance liquid chromatography (HPLC) (32) and SDS-PAGE (33).

Determination of serum concentrations of lipids, lipoproteins, and (apolipo)proteins, and plasma activities of LCAT, cholesteryl ester transfer protein, and phospholipid transfer protein

Serum concentrations of triglycerides and cholesterol were quantified with an autoanalyzer (Hitachi/Boehringer, Mannheim, Germany). HDL-cholesterol was measured in the supernatant obtained by precipitation of apoB-containing lipoproteins with phosphotungstic acid/$MgCl_2$ (Boehringer Mannheim). Concentrations of apoA-I, A-II, and B were determined with a modified commercially available turbidimetric assay (Boehringer Mannheim). Plasma activity of lecithin:cholesterol acyltransferase (LCAT) was assayed as the esterification of [^{3}H]cholesterol (New England Nuclear, Boston, MA) that was incorporated into reconstituted, apoA-I-containing particles (31, 34). Cholesteryl ester transfer protein (CETP) activity was determined as the transfer of [^{3}H]cholesteryl oleate (New England Nuclear) from artificial apoA-I-containing proteoliposomes to LDL as reported previously (31, 35). Phospholipid transfer protein (PLTP) activity was monitored as the transfer of [^{14}C]phosphatidylcholine (New England Nuclear) from phospholipid vesicles to HDL_3 (36).

Measurement of glycerophospholipid transfers

In the course of this study we established assays to measure the activities of plasmas, cell culture homogenates, and cell culture media to transfer phosphatidylcholine (PC), phosphatidylinositol (PI), or phosphatidylethanolamine (PE) from donor vesicles onto immobilized acceptors. As the acceptors we used biotinylated apoA-I, biotinylated apoA-II, biotinylated human albumin (purchased from Sigma), or biotinylated rLpA-I that were conjugated to streptavidin-coated tubes (Boehringer Mannheim). Biotinylation of apoA-I, apoA-II, and albumin was done as recommended by the manufacturer of the biotinylation kit (Sigma). The solution containing biotinylated apoA-I was either directly incubated with streptavidin-coated tubes or used to prepare rLpA-I by the cholate-dialysis method (37). rLpA-I contained egg yolk PC (Sigma) and apoA-I at a weight per weight ratio of 7:1 (molar ratio approximately 250:1). Every streptavidin-coated tube was incubated for 24 h at 4°C with either 10 μg apoA-I, apoA-II, albumin, or rLpA-I that were solubilized in 300 μl assay buffer containing 10 mm Tris-HCl (pH 7.4), 150 mm NaCl, and 1 mm EDTA.

As the donor particles we used vesicles containing PC from egg yolk (Sigma) as well as unlabeled and radiolabeled glycerophospholipids. The relative composition of glycerophospholipids was adapted to that of VLDL in human plasma. Generally, alcoholic solutions with the labeled and unlabeled phospholipids were dried under nitrogen and then dissolved in 1 ml assay buffer with 10 mm Tris-HCl (pH 7.4), 150 mm NaCl, and 1 mm EDTA. This suspension was sonicated three times for 60 sec using Soniprep 150 (MSE, England). Specifically, for the measurement of PC transfer onto rLpA-I, we prepared vesicles with 7 mg egg yolk PC and 3.2 μg (0.5 μCi) l-α-dipalmitoyl-di[1-^{14}C]phosphatidylcholine (^{14}C-PC; DuPont New England Nuclear). For the measurement of PI transfer, we prepared vesicles with 7 mg egg yolk PC, 70 μg PI from bovine liver (Sigma), and 13 ng (0.25 μCi) l-3-phosphatidyl-[2-^{3}H-]inositol (^{3}H-PI; Amersham). For the measurement of PE transfer, we prepared vesicles with 7 mg egg yolk PC, 70 μg PE from bovine brain (Sigma), and 3.5 ng (0.25 μCi) 1,2-dioleoyl-l-3-phosphatidyl[2-^{14}C]ethanolamine (^{14}C-PE, Amersham). The transfer of phospholipids onto lipid-free apolipoproteins or albumin was measured with vesicles that contained 5-fold higher amounts of radiolabeled phospholipids.

Shortly before the start of the phospholipid transfer assay, plasmas or media were thawed and cells were homogenized by sonicating three times for 5 min with Soniprep 150. For the assays, 20 μl emulsion of donor vesicles and plasmas, cell homogenates, or cell culture media at volumes or masses described in the results section were placed in tubes containing immobilized rLpA-I or apoA-I and filled up with assay buffer to a final volume of 300 μl. The mixtures were incubated with an overhead shaker at 37°C and for 1 h when not otherwise indicated. The solutions were subsequently removed and the tubes were washed three times with assay buffer. Finally, the tubes were filled with scintillation liquid (Ultima Gold XR, Packard, Groningen, NL) for counting radioactivity. Measurements were performed in duplicate. In some indicated instances we modified these tests by incubation at 4°C.

For the comparison of glycerophospholipid transfer activities in normal and patient plasmas the assays were performed at both 37°C and 4°C to correct for unspecific transfers. Fractional glycerophospholipid transfer rates were calculated by the following formula:

$$\%\ \text{glycerophospholipids/h} = (\text{cpm}_{\text{acceptor(37°C)}} - \text{cpm}_{\text{acceptor(4°C)}})/\text{cpm}_{\text{donor}}/1\ \text{h} \times 100\%$$

where $\text{cpm}_{\text{acceptor(37°C)}}$ and $\text{cpm}_{\text{acceptor(4°C)}}$ correspond to the radioactivity of ^{14}C-PC, ^{14}C-PE, or ^{3}H-PI present in the tube after incubation of donor vesicles at 37°C and 4°C, respectively, and $\text{cpm}_{\text{donor}}$ to the radioactivity present in the tube with donor vesicles before incubation with plasmas, cells, or media. The intraassay and interassay coefficients of variation of the assays were below 5% and 10%, respectively.

Other methods

Protein concentrations were determined by the method of Lowry et al. (38).

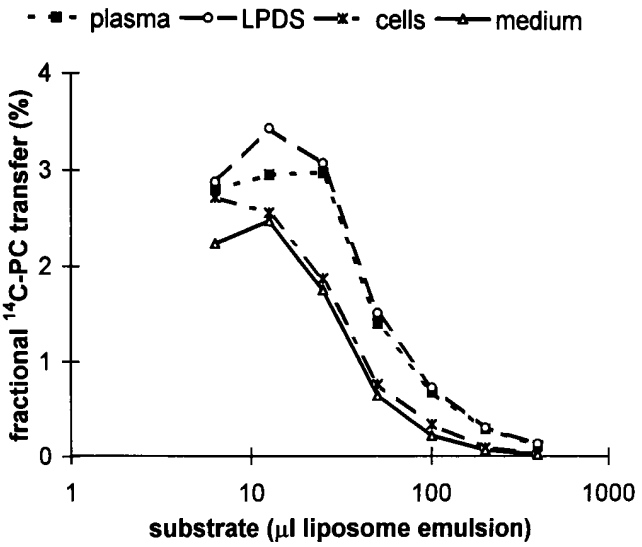

Fig. 1. Effect of increasing donor concentrations on transfer of [^{14}C]phosphatidylcholine onto lipid-free apoA-I by plasma, lipoprotein-depleted plasma, and homogenates and culture media of fibroblasts. Ten μg biotinylated, lipid-free apoA-I was immobilized in streptavidin-coated tubes. These were filled with increasing amounts of a PC vesicle-emulsion (7 mg/ml PC and 2.5 μCi/ml ^{14}C-PC). These tubes were incubated for 1 h at 37°C with 2 μl plasma, 50 mg LPDP, 20 μg homogenate, or 400 μl medium of fibroblasts that were cultivated in the presence of 10% FCS. The curves represent the fractional transfer of PC from the donor vesicle onto immobilized apoA-I.

Statistics

All data are presented either as medians or means ± standard deviations. The levels of significance for differences between patients and controls were calculated by ANOVA. All calculations were done with Excel (Microsoft) and an add-in program for Excel (Astute, 1993 DDU Software, The University of Leeds).

RESULTS

Assay characteristics

We established radiometric assays that monitor the activity of plasma, plasma fractions, cell homogenates, and cell culture media to transfer PC, PI, or PE from donor vesicles onto lipid-free apoA-I, apoA-II, albumin, or reconstituted apoA-I/PC complexes (rLpA-I). To separate donor particles from acceptor particles after incubation with the transfer activity, we immobilized lipid-free proteins or rLpA-I by the use of the streptavidin/biotin technology. The maximal binding of biotinylated acceptor particles was achieved by a 24-h incubation of 10 μg biotinylated apoA-I or rLpA-I with streptavidin-coated tubes (not shown). Subsequent washing removed 6–7 μg biotin apoA-I per tube. Another 0.5–1 μg biotinylated apoA-I (i.e., 15–25% of the biotin-apoA-I present in the assay) was removed during a 2-h assay incubation with vesicles and plasma, media, or cell homogenates.

Fractional transfer of PC from vesicles onto apoA-I by either plasma, LPDP, homogenates, or media of cultured fibroblasts peaked in the presence of 6–25 μl liposome emulsion that contained 40–225 μg PC (**Fig. 1**). Therefore, we used 20 μl of the liposome emulsion for all subsequently described experiments.

Transfer of PI, PC, and PE by normolipidemic and Tangier disease plasmas

Figure 2 demonstrates the time-dependent transfer of radiolabeled PC from vesicles onto immobilized apoA-I,

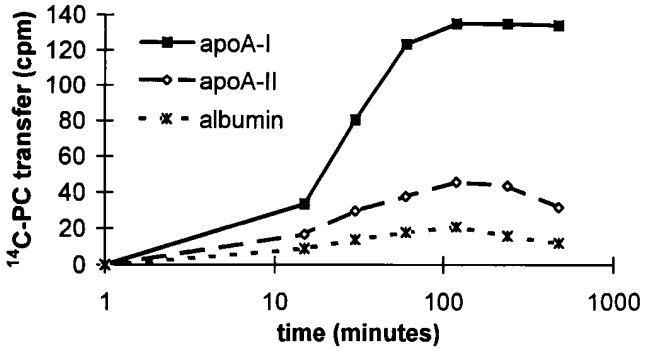

Fig. 2. Transfer of [^{14}C]phosphatidylcholine from vesicles onto lipid-free apoA-I, apoA-II and albumin by normal plasma. Ten μg biotinylated lipid-free apoA-I, 10 μg biotinylated lipid-free apoA-II, or 10 μg biotinylated albumin was immobilized in streptavidin-coated tubes. These were filled with 20 μl of a PC vesicle-emulsion (7 mg/ml PC and 2.5 μCi/ml ^{14}C-PC) and with 2 μl normolipidemic plasma. Incubations were performed for increasing time intervals. The curves represent the transfer of ^{14}C-PC from the donor vesicle onto the immobilized proteins.

apoA-II, or albumin by 2 μl plasma. The maximal transfer onto any acceptor was reached after 2 h incubation. However, at any time point, transfer rates onto apoA-I were 3- to 4-times higher than onto apoA-II and 8- to 10-fold higher than onto albumin.

Figures 3A and **3B** demonstrate the effects of increasing plasma volumes and incubation times on the transfer of radiolabeled PI from PI/PC vesicles onto either immobilized lipid-free apoA-I or immobilized rLpA-I. Maximal transfer of ^{3}H-PI was reached after 1 h incubation with 2 μl plasma. Incubation with more than 4 μl plasma as well as incubations longer than for 1 h resulted in lower transfer rates. Moreover, significantly more ^{3}H-PI was transferred onto lipidated rLpA-I as compared to lipid-free apoA-I (Fig. 3A). Incubations with plasma at 4°C led to a transfer of ^{3}H-PI onto rLpA-I that was much lower (Fig. 3B). Similar observations were made for the activities of plasma to transfer radiolabeled PC or PE onto rLpA-I or apoA-I (not shown). At any volume, plasmas of three TD patients (T1, T2, and T3) were significantly less active in transferring ^{3}H-PI onto either biotinylated rLpA-I or biotinylated apoA-I than normolipidemic plasmas (Fig. 3C). Similar to normal plasma, incubation with 2 μl TD plasma resulted in maximal PI transfer rates. Larger volumes decreased PI transfer (Fig. 3C). Maximal transfer by TD plasmas was reached by a 1-h long incubation at 37°C. This maximum, however, was 60% of that reached with normal plasma. Transfer of PI taking place in the presence of TD plasma at 4°C was as effective as in the presence of normal plasmas. Experiments on the transfer of PC or PE by TD plasma gave similar results (not shown).

Table 2 summarizes the data on transfers of PC, PI, and PE from donor vesicles onto rLpA-I by plasmas of ten normolipidemic probands, six patients with TD, and four patients with apoA-I deficiency. The results were corrected for the unspecific transfers taking place at 4°C (compare Fig. 3B and 3D). Plasmas of all six TD patients were less active in transferring glycerophospholipids onto rLpA-I than plasmas of normolipidemic controls. Transfers of ^{3}H-PI, ^{14}C-PE, and ^{3}H-PC were reduced by 39–62% (mean 50%), 30–64% (mean 48%), and 37–56% (mean 43%), respectively (all $P < 0.0001$, Student's *t*-test). All three glycerophospholipid transfer activities were also significantly decreased in plasmas of apoA-I-deficient patients which, however, had still significantly higher glycerophospholipid transfer activities than TD plasmas (Table 2).

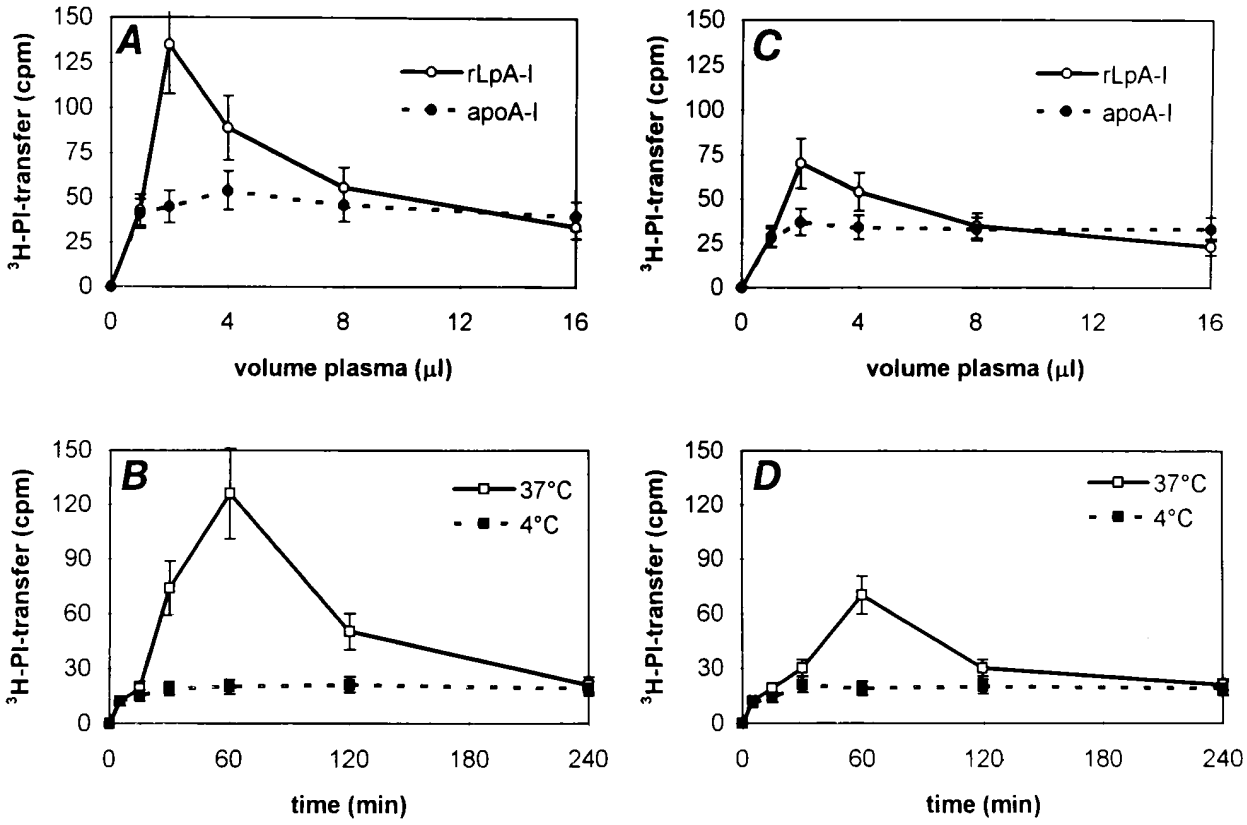

Fig. 3. Transfer of [^{3}H]phosphatidylinositol from vesicles onto lipid-free apoA-I and reconstituted LpA-I by normal or TD plasmas. Ten μg biotinylated, lipid-free apoA-I (broken line and closed symbols) or 10 μg biotinylated rLpA-I (closed line and open symbols) was immobilized in streptavidin-coated tubes. These were filled with 20 μl of a PI/PC vesicle-emulsion (7 mg/ml PC, 70 μg/ml PI, and 0.25 μCi/ml ^{3}H-PI). Increasing volumes of three normal (A) or three TD plasmas (patients T1, T2, and T3, C) were incubated in these tubes for 1 h at 37°C. Alternatively, tubes with immobilized rLpA-I were incubated for increasing time intervals with 2 μl plasma of three normal probands (B) or three TD patients (D) and at 37°C (closed line and open symbols) or 4°C (broken line and closed symbols). In all figures, symbols and bars represent mean values and standard deviations, respectively, of experiments on three normolipidemic plasmas and three TD plasmas.

TABLE 2. Fractional glycerophospholipid transfer rates in plasmas of normal probands and Tangier disease patients

Subjects	PC Transfer	PI Transfer	PE Transfer
		%/h	
Normolipidemic controls			
Mean (n = 10)	6.54 ± 1.16$^{\#\#\#\#}$	5.26 ± 1.00$^{\#\#\#\#}$	5.99 ± 0.74$^{\#\#\#\#}$
Range	5.55–8.24	4.18–6.36	4.80–6.78
ApoA-I deficiency			
Mean (n = 4)	5.14 ± 0.21$^{*,\#\#\#}$	3.43 ± 0.16$^{**,\#}$	5.38 ± 0.48$^{\#\#\#}$
Range	5.06–5.51	3.26–3.66	5.06–6.14
Tangier disease			
Mean (n = 6)	3.70 ± 0.48****	2.61 ± 0.50****	3.12 ± 0.67****
Range	2.87–4.14	1.98–3.22	2.16–4.18

Two μl plasma was incubated for 1 h in tubes with immobilized rLpA-I (acceptor) and with phospholipid vesicles containing either ^{14}C-PC, ^{3}H-PI, or ^{14}C-PE (donor). After incubation, plasmas and vesicles were removed and the radioactivity in the immobilized acceptor particles was counted (for details, see Methods). Fractional transfer rates are presented as %/h, which was calculated by the following formula: % glycerophospolipids/h = $(cpm_{acceptor\ (37°C)} - cpm_{acceptor\ (4°C)}/cpm_{donor}/1\ h \times 100\%$.

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$; differences of mean values for glycerophospholipid transfer activities differ significantly from plasmas of normal controls (Student's *t*-test).

$^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$, $^{\#\#\#\#}P < 0.0001$; differences of mean values for glycerophospholipid transfer activities differ significantly from plasmas of TD patients (Student's *t*-test).

When the PC transfer assay was carried out with lipid-free apoA-I or apoA-II, the transfer activities of TD plasma were about 50% lower than that of normal plasmas. The transfer onto albumin was close to background with any plasma.

To rule out the presence of an inhibitor in TD plasma that interferes with the transfer of glycerophospholipids, we mixed normolipidemic plasma with TD plasma at different volume ratios. At any ratio we recovered 100% transfer activity (not shown). By univariate regression analysis we tested the dependence of glycerophospholipid transfer activities of normal plasmas on one another and on other variables of lipid metabolism (**Table 3**). Highly significant positive correlations were found among all three glycerophospholipid transfer activities. The positive correlation between PC transfer activity and apoA-I concentration, as well as negative correlations between all glycerophospholipid transfer activities with the plasma PLTP activity, were also significant (Table 3).

TABLE 3. Univariate coefficients of correlation between glycerophospholipid transfer activities and other variables of plasma lipoprotein metabolism in normolipidemic plasmas (n = 10)

	PC Transfer	PI Transfer	PE Transfer
Cholesterol	−0.410	−0.310	−0.084
Triglycerides	−0.574	−0.537	−0.286
HDL-cholesterol	0.573	0.520	0.519
LDL-cholesterol	−0.443	−0.328	−0.173
ApoA-I	0.611*	0.562	0.562
ApoB	−0.238	−0.218	−0.007
LCAT	0.235	0.110	0.110
CETP	0.273	0.331	0.224
PLTP	−0.874***	−0.841**	−0.707**
PC transfer	1.000	0.939***	0.875***
PI transfer	0.939***	1.000	0.947***
PE transfer	0.875***	0.947***	1.000

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (Pearson's *t*-test).

Transfer of PI, PC, and PE by plasma lipoproteins and lipoprotein-depleted plasmas

Table 4 as well as **Fig. 4** summarize data on the distribution of glycerophospholipid transfer activities among plasma lipoprotein subfractions. In normal plasma, transfer activities for PC, PI, and PE onto rLpA-I were encountered in every lipoprotein fraction. Similar to total plasma, increases in lipoprotein mass did not lead to equivalent increases in glycerophospholipid transfer when they exceeded threshold levels. The highest specific transfer activities were found in the presence of 1 μg protein of either VLDL, LDL, or HDL (Table 4) and 50 μg protein of LPDP (Fig. 4).

Similiar to total plasma, transfer of radiolabeled phospholipids mediated by lipoproteins from vesicles onto rLpA-I decreased during incubations that lasted longer than 1 h (not shown). Incubation with 50 μg LPDP resulted in maximal transfer of PC (Fig. 4B) (and PI or PE, not shown) after 1 h long incubation. By contrast to incubations with plasma and lipoprotein fractions, this maximum persisted up to 16 h. Based on glycerophospholipid transfer activities measured in the presence of 1 μg VLDL, 1 μg IDL/LDL, and 1 μg HDL or 50 μg LPDP (all by protein) we estimate that in normal plasma about 54% of glycerophospholipid transfer activities reside in HDL, 25% in LPDP, 14% in IDL/LDL, and 7% in VLDL. With the exception of the IDL/LDL fraction, all plasma fractions of TD patient T1 contained lower glycerophospholipid transfer activities than the respective fractions of normal plasma (Table 4 and Fig. 4). We estimated the following distribution of glycerophospholipid transfer activities: 24% in VLDL, 40% in IDL/LDL, 1% in the fraction which was isolated at the density of HDL, and 35% in LPDP.

We also measured the LPDP-mediated transfer of phospholipids onto lipid-free apoA-I, apoA-II, and albumin. Similar to the data shown for plasma in Fig. 2, transfer of radiolabeled PC onto apoA-I was 3-fold higher than

TABLE 4. Distribution of glycerophospholipid transfer activities among plasma lipoproteins

	VLDL			IDL/LDL			HDL		
	1 μg	10 μg	100 μg	1 μg	10 μg	100 μg	1 μg	10 μg	100 μg
		cpm			*cpm*			*cpm*	
^{14}C-PC transfer									
Normal	98	125	112	43	138	129	49	115	148
TD	79	94	78	68	148	135	(13	44	55)
^{3}HPI-transfer									
Normal	95	115	108	36	129	115	42	98	118
TD	68	85	78	57	136	127	(14	45	53)
^{14}C-PE transfer									
Normal	94	119	105	38	133	121	42	98	118
TD	71	88	80	62	142	132	(12	41	52)

Indicated amounts of lipoproteins (protein mass as determined by the method of Lowry et al. (38)) were incubated with phospholipid vesicles and with biotinylated rLpA-I that was immobilized in streptavidin-coated tubes. After 1 h incubation at 37°C lipoproteins and vesicles were removed and the radioactivity in the immobilized tubes was counted. Note that in normal plasma the highest specific transfer rates (cpm/μg protein) were measured in the presence of only 1 μg protein. Higher amounts decreased specific activities. In VLDL and IDL/LDL even total activity decreased after incubation with 100 μg protein.

the transfer onto apoA-II and 6- to 7-fold higher than onto albumin.

Effect of LDL on the activity of normal fibroblasts to transfer radiolabeled PI, PC, and PE

We also monitored the activity of homogenized fibroblasts to transfer PC, PI, or PE onto rLpA-I. In initial experiments we analyzed the phospholipid transfer activity of homogenized fibroblasts that were cultivated in either the presence or absence of lipoproteins. **Figure 5** shows the data on PE transfer as an example. Both cell homogenates (hatched lines in Fig. 5A) and cell culture media (hatched lines in Fig. 5B) exerted only moderate transfer activities that were close to the unspecific transfer activity measured at 4°C. After fibroblasts had been pre-incubated with medium containing either 10% FCS or 100 μg/ml LDL, both cell homogenates (solid lines in Fig. 5A) and media (solid lines in Fig. 5B) exerted time-dependent transfer activities. Maximal transfers of either ^{14}C-PC, ^{3}H-PI, or ^{14}C-PE from phospholipid vesicles onto immobilized rLpA-I were reached by 60-min incubations (Fig. 5). At this time point the transfer activities in either cell homogenates or media from LDL- or FCS-treated fibroblasts were 4- to 5-fold higher than in those of untreated fibroblasts.

Like plasma and LPDP, homogenates (**Fig. 6A**) and culture media (Fig. 6B) of FCS-treated fibroblasts mediated time-dependent transfer of PC (and PE or PI) onto lipid-free apoA-I and, to a lesser degree, onto apoA-II. Again the transfer of phospholipids onto albumin was very low.

Phospholipid transfer activities of homogenates and media from TD fibroblasts

Figure 7 compares dose- and time-dependent PI transfers by homogenates from fibroblasts of normal donors

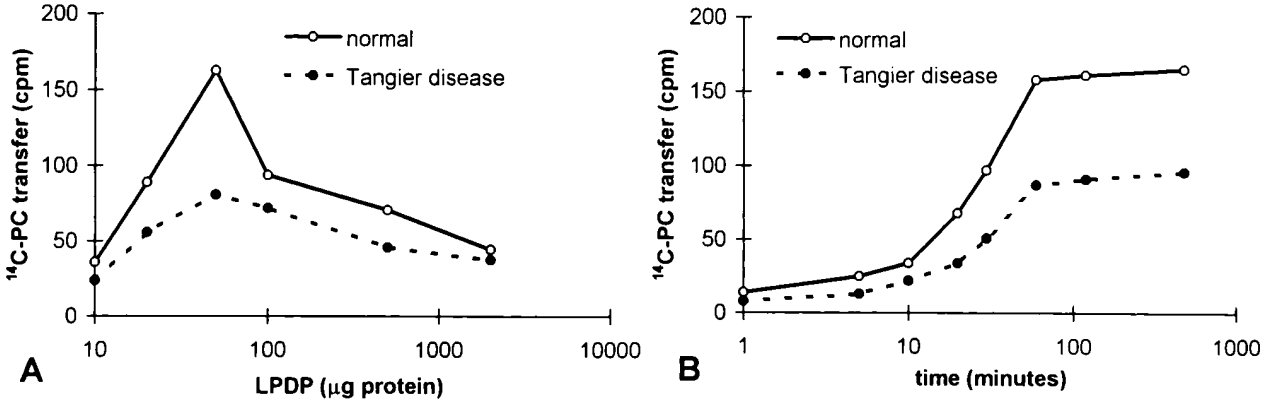

Fig. 4. Dosage- (A) and time-dependent transfers (B) of [^{14}C]phosphatidylcholine from vesicles onto reconstituted LpA-I by lipoprotein-depleted plasmas of a normal proband and a TD patient. Ten μg biotinylated rLpA-I was immobilized in streptavidin-coated tubes which were then filled with 20 μl of vesicle emulsion containing either ^{14}C-PC. In (A) increasing amounts of normal (closed line and open symbols) or TD LPDP (broken line and closed symbols, patient T1) were incubated for 1 h at 37°C. In (B) the tubes with immobilized rLpA-I were incubated for increasing time intervals with 50 μg LPDP protein of a normal proband (closed line and open symbols) or TD patient T1 (broken line and closed symbols). Similar results were obtained by experiments where vesicles contained ^{3}H-PI or ^{14}C-PE (not shown).

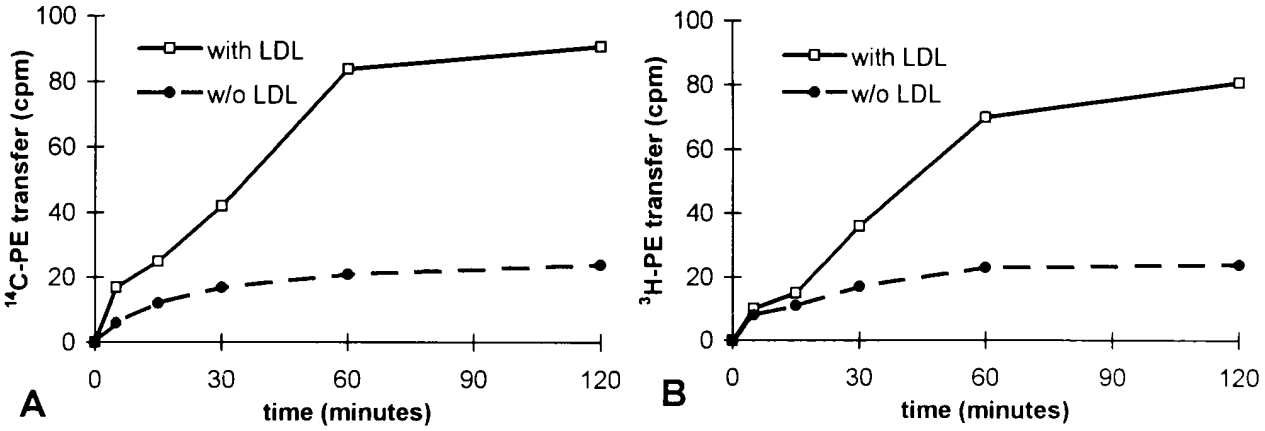

Fig. 5. Effect of LDL treatment on time-dependent transfers of [^{14}H]phosphatidylethanolamine from vesicles onto reconstituted LpA-I by homogenates (A) and culture media of fibroblasts (B). Confluently grown fibroblasts of a normal proband were pre-incubated for 24 h either in lipoprotein-free medium (hatched line and closed symbols) or in the presence of 100 μg/ml LDL (closed line and open symbols). After extensive washing the cells were incubated for another 24 h in lipoprotein-free medium. Media were removed; cells were homogenized and solubilized. Aliquots with 20 μg cell protein (A) or 400 μl cell culture media (B) were incubated for increasing time intervals in streptavidin-coated tubes that contained 10 μg biotinylated rLpA-I and 20 μl of vesicle emulsion with ^{14}C-PE. Similar results were obtained by experiments where vesicles contained ^{3}H-PI or ^{14}C-PC or where cells were pre-incubated for 24 h with 10% FCS instead of LDL (not shown).

and TD patients that were pre-incubated with LDL. Transfers of PI (Fig. 7A), PE, and PC (not shown) by cell homogenates increased dose-dependently until a dosage of 20 μg cell protein. Higher dosages decreased the amount of transferred ^{14}C-PC, ^{3}H-PI, or ^{14}C-PE. At any amount of cell protein, TD cells were less active than control cells in transferring ^{14}C-PC, ^{3}H-PI, or ^{14}C-PE from donor vesicles onto biotinylated rLpA-I (Fig. 7A). The time-dependent transfer of PI (Fig. 7B), PE, and PC (not shown) reached its maximum after 1 h. Longer incubations did not cause a decrease in radiolabel transfer. TD cells reached only about 50% of the maximum in transfer that was reached by normal fibroblasts, even after prolonged incubation (Fig. 7B). **Table 5** summarizes the data on transfers of radiolabeled PC, PI, and PE from donor vesicles onto rLpA-I by homogenates of LDL-loaded fibroblasts from eight healthy and normolipidemic donors and from four TD patients. Glycerophospholipid transfer activities in all four TD fibroblast cell lines were reduced by at least three standard deviations compared to the mean values of phospholipid transfer activities in normal cells. Compared to normal cells, the mean transfer activities of TD cells were reduced by 35%, 40%, and 45% for ^{3}H-PI, ^{14}C-PE, and ^{14}C-PC, respectively (Table 5). As fibroblasts of TD patients and normal donors that were cultivated in lipoprotein-free medium did not differ in their residual phospholipid transfer activities (not shown), we subtracted these basal transfer activities from those of cholesterol-enriched cells. After this adjustment, the differences in mean transfer activities of normal and TD fibroblasts became even greater: Mean ^{3}H-PI transfer in TD cells was reduced by 42% and mean transfers of ^{14}C-PE and ^{3}H-PC by 47%. When the assays were carried out with lipid-free apoA-I or apoA-II, the transfer activity of TD cells was 45% lower than that of normal cells. The very low transfer onto albumin did not differ among normal and TD cells.

Compared to media of normal cells, culture media of LDL-loaded TD fibroblasts were also significantly reduced in their activity to transfer glycerophospholipids onto

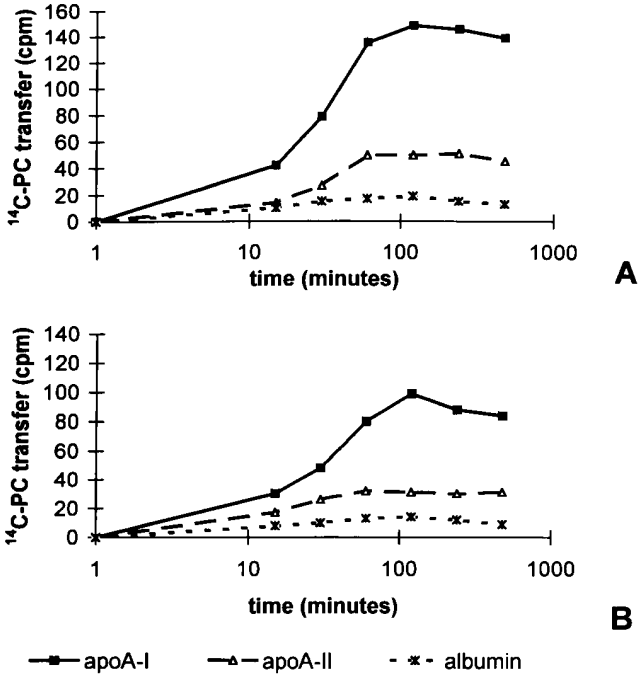

Fig. 6. Transfer of [^{14}C]phosphatidylcholine from vesicles onto lipid-free apoA-I, apoA-II, and albumin by homogenates (A) and culture media of fibroblasts (B). Ten μg biotinylated lipid-free apoA-I, 10 μg biotinylated lipid-free apoA-II or 10 μg biotinylated albumin were immobilized in streptavidin-coated tubes. These were filled with 20 μl of a PC vesicle emulsion (7 mg/ml PC and 2.5 μCi/ml ^{14}C-PC) and with either 20 μg homogenized cell protein (A) or 400 μl medium (B) of fibroblasts that were cultivated in 10% FCS. Incubations were performed for increasing time intervals. The curves represent the transfer of ^{14}C-PC from the donor vesicle onto the immobilized proteins.

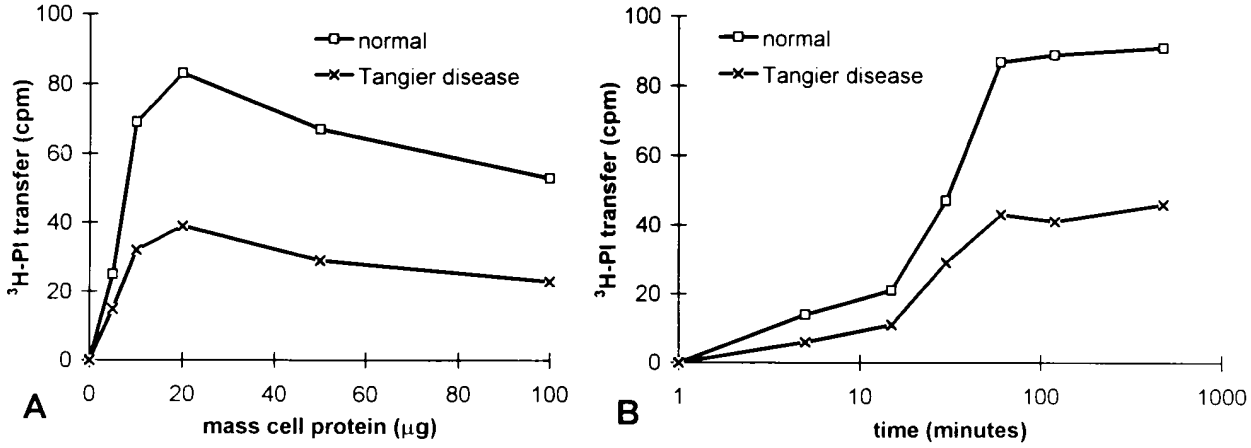

Fig. 7. Dose- (A) and time-dependent transfers (B) of [^{3}H]phosphatidylinositol from vesicles onto reconstituted LpA-I by fibroblasts of normal donors and TD patients. Confluently growing fibroblasts of a normal proband (open symbols) and from a TD patient (crosses) were incubated for 24 h in the presence of 100 μg/ml LDL. After extensive washing the cells were incubated for another 24 h in lipoprotein-free medium. The cells were then removed, homogenized, and solubilized. In (A) increasing dosages of cell protein were incubated for 1 h in streptavidin-coated tubes that contained 10 μg biotinylated rLpA-I and 20 μl of vesicle emulsion with ^{3}H-PI. In (B) aliquots with 20 μg cell protein were incubated for increasing time intervals.

rLpA-I (**Table 6**), namely by 32% (^{3}H-PI), 33% (^{14}C-PE), and 30% (^{3}H-PC). After correction for the basal transfer activities encountered in media of unloaded cells, which again did not differ between TD and normal fibroblasts, the differences increased to 49% (^{3}H-PI), 51% (^{14}C-PE), and 43% (^{3}H-PC). The transfer of PC onto lipid-free apoA-I or apoA-II was about 50% lower in the presence of media from TD fibroblasts as compared to media of normal cells. The transfer onto albumin was close to background in the presence of either medium.

DISCUSSION

In this study we have demonstrated that normolipidemic plasma as well as cell homogenates and culture media of fibroblasts from normal volunteers transfer radiolabeled PI, PE, or PC in a dose- and time-dependent manner from donor vesicles onto rLpA-I, lipid-free apoA-I and, although to a much lesser degree, lipid-free apoA-II. Plasmas of TD patients as well as cell homogenates and culture media of TD fibroblasts had significantly lower glycerophospholipid transfer activities than the respective specimens from normolipidemic probands. The similar kinetics for phospholipid transfer in plasmas and cells (Figs. 1 and 4), in LPDP and cell culture media (Figs. 3 and 4) suggest that glycerophospholipid transfers are exerted by the same mechanism. This assumption is further supported by the fact that phospholipid transfer activities were decreased by a similiar extent in all four specimens when they were obtained from TD patients.

TABLE 5. Fractional glycerophospholipid transfer rates by homogenates of fibroblasts of normal probands and Tangier disease patients

	PC Transfer	PI Transfer	PE Transfer
		%/h	
Normal cells (n = 8)			
Mean ± SD	2.73 ± 0.25	2.70 ± 0.33	2.93 ± 0.24
Range	2.38–3.07	2.17–3.29	2.66–3.33
Tangier disease (n = 4)			
Mean ± SD	1.38 ± 0.18	1.57 ± 0.20	1.63 ± 0.13
Range	1.15–1.60	1.39–1.80	1.52–1.81

Homogenized fibroblasts with 20 μg cell protein were incubated for 2 h in tubes with immobilized rLpA-I (acceptor) and with phospholipid vesicles containing either ^{14}C-PC, ^{3}H-PI, or ^{14}C-PE (donor). After incubation, cells and vesicles were removed and the radioactivity in the immobilized acceptor particles was counted. For details see Methods. Presented are fractional transfer rates that were calculated by the following formula: % glycerophospholipids/h = ($cpm_{acceptor(37°C)}$ − $cpm_{acceptor(4°C)}$)/cpm_{donor}/1 h × 100%. Differences of mean values for glycerophospholipid transfer activities differ significantly between TD cells and normal cells ($P < 0.001$; Student's *t*-test).

TABLE 6. Fractional glycerophospholipid transfer rates by cell culture media of fibroblasts of normal probands and Tangier disease patients

	PC Transfer	PI Transfer	PE Transfer
		%/h	
Normal cells (n = 8)			
Mean ± SD	2.23 ± 0.19	2.28 ± 0.19	2.43 ± 0.25
Range	1.88–2.50	1.94–2.50	2.04–2.80
Tangier disease (n = 4)			
Mean ± SD	1.56 ± 0.27	1.47 ± 0.26	1.57 ± 0.29
Range	1.23–1.84	1.30–1.85	1.38–2.00

Four hundred-μl aliquots of media of cultivated and LDL-loaded fibroblasts were incubated for 2 h in tubes with immobilized rLpA-I (acceptor) and with phospholipid vesicles containing either ^{14}C-PC, ^{3}H-PI, or ^{14}C-PE (donor). After incubation, cells and vesicles were removed and the radioactivity in the immobilized acceptor particles was counted. For details, see Methods. Presented are fractional transfer rates that were calculated by the following formula: % glycerophospholipids/h = ($cpm_{acceptor(37°C)}$ − $cpm_{acceptor(4°C)}$)/cpm_{donor}/1 h × 100%. Differences of mean values for glycerophospholipid transfer activities differ significantly between media of TD cells and media of normal cells ($P < 0.001$; Student's *t*-test).

Several phenomena indicate that glycerophospholipid transfer occurred by a directed rather than a diffusional process. First, the fractional transfer onto rLpA-I was higher than the transfer onto lipid-free apoA-I and even more so than onto apoA-II or albumin. Thus, the assay monitors a specific transfer onto apolipoproteins rather than nonspecific transfer to the tube. Second, significant phospholipid transfer was observed at 37°C but not at 4°C where most enzymes are inactive (Figs. 3B and 3D). Third, in all specimens, phospholipid transfer occurred dependent on time and dosage. Fourth, phospholipid transfer activity in plasma was not dispersed throughout all lipoprotein subfractions but was found predominantly in HDL and LPDP. Fifth and finally, in cell homogenates and culture media significant transfer activities were only assessed after fibroblasts were pre-incubated with FCS or LDL, indicating that enrichment of cells with cholesterol induces the transfer activity (Fig. 5).

Several intracellular and extracellular phospholipid transfer proteins have been identified (39–42). As most of them translocate phospholipids from lipid-rich donor membranes or vesicles to lipid-poor acceptor membranes or particles, it is likely that several of them nonselectively contribute to the phospholipid transfer from vesicles onto rLpA-I or even lipid-free apoA-I, which was measured by our assay. Possibly they account for the about 50% residual phospholipid transfer activity encountered in plasmas and fibroblasts of TD patients. However, the reduced phospholipid transfer activity of TD plasmas as well as of cell homogenates and culture media of TD fibroblasts strongly suggests that a considerable proportion of the phospholipid transfer activity in our assay is exerted by a specific factor that is impaired in TD. In the plasma compartment, this specific proportion of glycerophospholipid transfer activity appears to be mediated independently of CETP and PLTP, as both activities were normal in TD plasma (Table 1). Moreover, in normal plasma, glycerophospholipid transfer activities did not correlate with CETP activity and even negatively with PLTP activity (Table 3). Finally, as apoA-I-deficient plasma had significantly higher phospholipid transfer activity than TD plasma, it is unlikely that the low phospholipid transfer activity in TD plasma is secondary to HDL deficiency.

Phospholipid transfer onto apoA-I was saturated at relatively low amounts of plasma or cell protein. Incubation with more than 2 μl plasma or 20 μg lysed cells even caused a decay of radioactivity from the immobilized acceptor particle. Several reasons may account for this phenomenon. First, plasmas and cells may contain a factor that inhibits the transfer of glycerophospholipids onto apoA-I. By mixing TD plasmas and normal plasmas, we have at least ruled out that such an inhibitor is responsible for defective phospholipid transfer in TD. Second, plasma lipoproteins or cell membranes may have served as secondary acceptors of radiolabeled phospholipids and have removed phospholipids from rLpA-I. Actually, small amounts of isolated lipoproteins (e.g., 10 μg VLDL) led to a more effective transfer of radiolabeled glycerophospholipids from donor vesicles than larger amounts (e.g., 100 μg VLDL) (Table 4). Third, endogenous phospholipids of either plasma or cells may compete with exogenous radiolabeled phospholipids for transfer. In agreement with this explanation, radioactivity did not disappear from the acceptor particles during prolonged incubation with either LPDP (Fig. 2B), cell homogenates (Figs. 3A and 4B), or cell culture media (Fig. 3B) when the amount of possible competitors did not increase. The disappearance of labeled phospholipids from the immobilized acceptor in the presence of plasma (Figs. 1B and 1D) may also be caused by the presence of excess apoA-I and several lipid transfer enzymes in plasma. In this case, immobilized rLpA-I will probably acquire not only glycerophospholipids but also additional apoA-I molecules that have a high affinity to phospholipids. Thus the de novo formed particles may be further processed by lipid transfer enzymes and thereby sequester radiolabeled phospholipids. It was previously shown that interconversions of mature HDL by phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP) are associated with the release of apoA-I or preβ_1-LpA-I (43–48).

Is impaired transfer of glycerophospholipids onto apoA-I by cells and plasmas of TD patients consistent with previously described disturbances in Tangier disease? Unlike normal cells, TD fibroblasts do not release cholesterol and phospholipids in the presence of lipid-free apolipoproteins (6, 20, 21). Oram and colleagues have suggested that this defect interferes with the maturation of HDL (6, 17). We have previously reported that TD plasma is defective in converting lipid-free apoA-I or lipid-poor preβ_1-LpA-I into lipid-rich α-LpA-I (19). This conversion involves not only esterification of cholesterol by LCAT but also enrichment with phospholipids (4, 20–23, 49, 50). In view of these cellular and plasmatic disturbances in TD we conclude from the data of this study that phospholipids of either cell membranes or plasma lipoprotein surfaces are transferred onto apoA-I by a transfer protein whose function is lost in TD. The mediation of phospholipid transfer by an identical protein in both intracellular and extracellular defect is not an unprecedented phenomenon. For example, the macrophage surface antigen CD14 exists as a soluble plasma isoform that assists lipopolysaccharide binding protein in the transfer of endotoxins and phospholipids (40, 51, 52). We hypothesize that defective phospholipid transfer interferes with maturation of HDL as well as efflux of cellular cholesterol and thereby causes deficiency of HDL cholesterol and foam cell formation, respectively. Abnormal plasma membrane phospholipid content observed in TD erythrocytes (53) as well as abnormalities in agonist-induced phospholipid breakdown by phospholipases (29, 54) observed in TD fibroblasts may occur secondary to an inadequate supply of phospholipid.

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REFERENCES

1. Fredrickson, D. S., P. H. Altrocchi, L. V. Avioli, D. S. Goodman, and H. C. Goodman. 1961. Tangier disease—combined clinical staff conference at the National Institutes of Health. *Ann. Intern. Med.* **55:** 1016–1021.
2. Assmann, G., A. von Eckardstein, and H. B. Brewer, Jr. 1995. Familial HDL deficiency. Tangier disease. *In* The Metabolic and Molecular Basis of Inherited Disease, 7th ed. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 2053–2072.
3. von Eckardstein, A., Y. Huang, S. Wu, H. Funke, G. Noseda, and G. Assmann. 1995. Reverse cholesterol transport in plasmas of patients with different forms of familial high density lipoprotein deficiency. *Arterioscler. Thromb. Vasc. Biol.* **15:** 691–703.
4. Fielding, C., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
5. von Eckardstein, A. 1996. Cholesterol efflux from macrophages and other cells. *Curr. Opin. Lipidol.* **7:** 308–319.
6. Oram, J. F., and S. Yokoyama. 1997. Apolipoprotein-mediated removal of cellular cholesterol and phospholipids. *J. Lipid Res.* **37:** 2473–2491.
7. Castro, G. R., and C. J. Fielding. 1988. Early incorporation of cell-derived cholesterol into pre-β-migrating high density lipoprotein. *Biochemistry.* **27:** 25–29.
8. Huang, Y., A. von Eckardstein, and G. Assmann. 1993. Cell-derived cholesterol cycles between different HDLs and LDL for its effective esterification in plasma. *Arterioscler. Thromb.* **13:** 445–458.
9. Kawano, M., T. Miida, C. J. Fielding, and P. E. Fielding. 1993. Quantitation of preβ-HDL-dependent and nonspecific components of the total efflux of cellular cholesterol and phospholipid. *Biochemistry.* **32:** 5025–5028.
10. Hara, H., and S. Yokoyama. 1991. Interaction of free apolipoproteins with macrophages: formation of high density lipoprotein-like lipoproteins and reduction of cellular cholesterol. *J. Biol. Chem.* **266:** 3080–3086.
11. Czarnecka, H., and S. Yokoyama. 1995. Lecithin:cholesterol acyltransferase reaction on cellular lipid released by free apolipoprotein-mediated efflux. *Biochemistry.* **34:** 4385–4392.
12. Czarnecka, H., and S. Yokoyama. 1996. Regulation of cellular cholesterol efflux by lecithin:cholesterol acyltransferase reaction through nonspecific lipid exchange. *J. Biol. Chem.* **266:** 2023–2028.
13. Yancey, P. G., J. K. Bielicki, W. J. Johnson, S. Lund-Katz, M. N. Palgunachari, G. M. Anantharamaiah, J. P. Segrest, M. C. Phillips, and G. H. Rothblat. 1995. Efflux of cellular cholesterol and phospholipid to lipid-free apolipoproteins and class A amphipathic peptides. *Biochemistry.* **34:** 7955–7965.
14. Li, Q., and S. Yokoyama. 1995. Independent regulation of cholesterol incorporation into free apolipoprotein-mediated cellular lipid efflux in rat vascular smooth muscle cells. *J. Biol. Chem.* **269:** 26216–26223.
15. Mendez, A. J., J. F. Oram, and E. L. Bierman. 1991. Protein kinase C as a mediator of high density lipoprotein dependent efflux of intracellular cholesterol. *J. Biol. Chem.* **266:** 10104–10111.
16. Walter, M., H. Reinecke, J-R. Nofer, U. Seedorf, and G. Assmann. 1995. HDL_3 stimulates multiple signaling pathways in human skin fibroblasts. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1975–1986.
17. Francis, G. A., R. H. Knopp, and J. F. Oram. 1995. Defective removal of cellular cholesterol and phospholipids by apolipoprotein A-I in Tangier disease. *J. Clin. Invest.* **96:** 78–87.
18. Remaley, A. T., K. Schumacher, J. A. Stonik, B. D. Farsi, H. Nazih, and H. B. Brewer, Jr. 1997. Decreased reverse cholesterol transport from Tangier disease fibroblasts. Acceptor specificity and effect of brefeldin on lipid efflux. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1813–1821.
19. Huang, Y., A. von Eckardstein, S. Wu, and G. Assmann. 1995. Generation of $preβ_1$-high density lipoprotein (HDL) and conversion into α-HDL: evidence for disturbed $preβ_1$-HDL conversion in Tangier disease. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1746–1754.
20. Barrans, A., B. Jaspard, R. Barbaras, H. Chap, B. Perret, and X. Collet. 1996. Pre-β HDL: structure and metabolism. *Biochim. Biophys. Acta.* **1300:** 73–83.
21. Davidson, W. S., D. L. Sparks, S. Lund-Katz, and M. C. Philips. 1994. The molecular basis for the difference of charge between preβ- and α-migrating high density lipoproteins. *J. Biol. Chem.* **269:** 8959–8965.
22. Sparks, D. L., W. S. Davidson, S. Lund-Katz, and M. C. Phillips. 1993. Effect of cholesterol on the charge and structure of apolipoprotein A-I in high density lipoprotein particles. *J. Biol. Chem.* **268:** 23250–23257.
23. Jaspard, B., X. Collet, R. Barbaras, J. Manent, C. Vieu, G. Pontonnier, H. Chap, and B. Perret. 1996. Biochemical characterization of pre $β_1$- high-density lipoprotein from human ovarian follicular fluid: evidence for the presence of a lipid core. *Biochemistry.* **35:** 1352–1357.
24. Assmann, G., E. Smootz, K. Adler, A. Capurso, and K. Oette. 1977. The lipoprotein abnormality in Tangier disease. Quantitation of A apoproteins. *J. Clin. Invest.* **59:** 565–575.
25. Gibbels, E., H. E. Schaefer, U. Runne, J. M. Schröder, W. F. Haupt, and G. Assmann. 1985. Severe polyneuropathy in Tangier disease mimicking syringomyelia or leprosy. Clinical, biochemical, electrophysiological, and morphological evaluation, including electron microscopy of nerve, muscle, and skin biopsies. *J. Neurol.* **232:** 283–290.
26. Römling, R., A. von Eckardstein, H. Funke, C. Motti, G. Noseda, G. Fragiacomo, and G. Assmann. 1994. A nonsense mutation in the apolipoprotein A-I gene is associated with high density lipoprotein deficiency but not with coronary artery disease. *Arterioscler. Thromb.* **14:** 1915–1923.
27. Navalesi, R., R. Miccoli, L. Odoguardi, H. Funke, A. von Eckardstein, H. Wiebusch, and G. Assmann. 1995. Genetically determined absence of HDL-cholesterol and coronary atherosclerosis (letter). *Lancet.* **346:** 708–709.
28. Miccoli, R., A. Bertolotto, R. Navalesi, L. Odoguardi, A. Boni, J. Wessling, H. Funke, H. Wiebusch, A. von Eckardstein, and G. Assmann. 1996. Hemizygosity for a structural apolipoprotein A-I-variant, $apoA\text{-}I(L141R)_{Pisa}$, causes high density lipoprotein deficiency, corneal opacifications, and coronary heart disease. *Circulation.* **94:** 1622–1628.
29. Walter, M., H. Reinecke, U. Gerdes, J. R. Nofer, G. Höbbel, U. Seedorf, and G. Assmann. 1996. Defective regulation of phosphatidylcholine-specific phospholipases C and D in a kindred with Tangier disease. Evidence for the involvement of phosphatidylcholine breakdown in HDL-mediated cholesterol efflux mechanisms. *J. Clin. Invest.* **98:** 2315–2323.
30. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
31. von Eckardstein, A., H. Funke, A. Chirazi, C. Chen-Haudenschild, H. Schulte, R. Schönfeld, E. Köhler, S. Schwarz, A. Steinmetz, and G. Assmann. 1994. Sex-specific effects of the glutamine / histidine polymorphism in apolipoprotein A-IV on high density lipoprotein metabolism. *Arterioscler. Thromb.* **14:** 1114–1120.
32. von Eckardstein, A., M. Walter, A. Benninghoven, and G. Assmann. 1991. Site specific methionine sulfoxide formation is the structural basis of chromatographic heterogeneity in apolipoproteins A-I, C-II, and C-III. *J. Lipid Res.* **32:** 1465–1476.
33. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond).* **227:** 680–685.
34. Pritchard, P. H., R. McLeod, J. Frohlich, M. C. Park, B. J. Kudchodkar, and A. G. Lacko. 1988. Lecithin:cholesterol acyltransferase in familial HDL-deficiency. *Biochim. Biophys. Acta.* **958:** 227–234.
35. Kato, H., T. Nakanishi, and H. Arai. 1989. Purification, microheterogeneity, and stability of human lipid transfer protein. *J. Biol. Chem.* **264:** 4082–4087.
36. Jauhiainen, M., J. Metso, R. Pahlman, S. Blomqvist, A. van Tol, and C. Ehnholm. 1993. Human plasma phospholipid transfer protein causes high density lipoprotein conversion *J. Biol. Chem.* **268:** 4032–4036.
37. Matz, C. E., and A. Jonas. 1982. Micellar complexes of human apolipoprotein A-I with phosphatidylcholines and cholesterol prepared from cholate–lipid dispersions. *J. Biol. Chem.* **257:** 4535–4540.
38. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.

39. Tall, A. 1995. Plasma lipid transfer enzymes. *Annu. Rev. Biochem.* **64:** 235–257.
40. Albers, J. J., A. Y. Tu, G. Wolfbauer, M. C. Cheung, and S. M. Marcovina. 1996. Molecular biology of phospholipid transfer protein. *Curr. Opin. Lipidol.* **7:** 88–93.
41. Wirtz, K. W. A. 1991. Phospholipid transfer enzymes. *Annu. Rev. Biochem.* **60:** 73–92.
42. Alb, J. G., M. A. Kearns, and V. A. Bankaitis. 1996. Phospholipid metabolism and membrane dynamics. *Curr. Opin. Cell Biol.* **8:** 534–541.
43. Hennessy, L. K., S. T. Kunitake, and J. P. Kane. 1993. Apolipoprotein A-I-containing lipoproteins, with or without apolipoprotein A-II, as progenitors of preβ-high density lipoprotein particles. *Biochemistry.* **32:** 5759–5765.
44. Liang, H-Q., K-A. Rye, and P. J. Barter. 1994. Dissociation of lipid-free apolipoprotein A-I from high density lipoproteins. *J. Lipid Res.* **35:** 1187–1199.
45. Liang, H-Q., K-A. Rye, and P. J. Barter. 1995. Cycling of apolipoprotein A-I between lipid-associated and lipid-free pools. *Biochim. Biophys. Acta.* **1257:** 31–37.
46. Francone, O. L., L. Royer, and M. Haghpassand. 1996. Increased preβ-HDL levels, cholesterol efflux, and LCAT mediated esterification in mice expressing the human cholesteryl ester transfer protein (CETP) and human apolipoprotein A-I (apoA-I) transgenes. *J. Lipid Res.* **37:** 1268–1277.
47. von Eckardstein, A., M. Jauhiainen, Y. Huang, J. Metso, C. Langer, P. Pussinen, S. Wu, C. Ehnholm, and G. Assmann. 1996. Phospholipid transfer protein-mediated conversion of high density lipoproteins (HDL) generates generation of preβ$_1$-HDL. *Biochim. Biophys. Acta.* **1301:** 255–261.
48. Jiang, X-J., O. L. Francone, C. Bruce, R. Milne, J. Mar, A. Walsh, J. L. Breslow, and A. R. Tall. 1996. Increased preβ-high density lipoprotein, apolipoprotein A-I, and phospholipid in mice expressing the human phospholipid transfer protein and human apolipoprotein A-I transgenes. *J. Clin. Invest.* **98:** 2373–2380.
49. Clay, M. A., and P. J. Barter. 1996. Formation of new HDL particles from lipid-free apolipoprotein A-I. *J. Lipid Res.* **37:** 1722–1732.
50. Liang, H-Q., K. A. Rye, and P. J. Barter. 1996. Remodelling of reconstituted high density lipoproteins by lecithin:cholesterol acyltransferase. *J. Lipid Res.* **37:** 1962–1970.
51. Wurfel, M. M., and S. D. Wright. 1997. Lipopolysaccharide-binding protein and soluble CD14 transfer lipopolysaccharide to phospholipid bilayers: preferential interaction with particular classes of lipid. *J. Immunol.* **158:** 3925–3934.
52. Yu, B., E. Hailman, and S. D. Wright. 1997. Lipopolysaccharide binding protein and soluble CD14 catalyze exchange of phospholipids. *J. Clin. Invest.* **99:** 315–324.
53. Reinhart, W. H., U. Gössi, P. Bütikofer, P. Ott, H. Sigrist, H. J. Schatzmann, H. U. Lutz, and W. Straub. 1989. Haemolytic anaemia, in analphalipoproteinaemia (Tangier disease): morphological, biochemical, and biophysical properties of the red blood cell. *Br. J. Haematol.* **72:** 272–276.
54. Drobnik, W., C. Möllers, T. Resink, and G. Schmitz. 1995. Activation of phosphatidylinositol-specific phospholipase C in response to HDL$_3$ and LDL is markedly reduced in cultured fibroblasts from Tangier patients. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1369–1377.