Transforming growth factor β is sequestered into an inactive pool by lipoproteins

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Abstract Elevated plasma concentrations of low density lipoprotein (LDL) and very-low density lipoprotein (VLDL) have been correlated with the development of atherosclerosis. These lipoproteins may promote atherogenesis by direct deposition of lipid in the vessel wall. In addition, previous data suggested that there was an inverse correlation between serum LDL-cholesterol concentration and the proportion of transforming growth factor β (TGF-β) in an active form (Grainger et al. 1995. *Nature Med.* **1:**74). Here we have investigated whether lipoproteins can affect the activity of TGF-β1 in plasma and show that TGF-β can associate with the lipoprotein fraction. In the plasma of healthy males, 16 ± 5% (mean ± standard deviation; n = 57) of the total plasma TGF-β1 was associated with the lipoprotein fraction, with the major proportion (64 ± 15%) in the HDL-3 subfraction. However, in ten diabetic subjects with moderately poor glucose control (Hb a1c > 8.0), the proportion of total plasma TGF-β in the lipoprotein fraction was 68 ± 21%. This large increase in TGF-β1 associated with the lipoprotein fraction was mainly due to association with VLDL, chylomicrons, and LDL. The lipoprotein fraction inhibits TGF-β1 binding to the type II TGF-β receptor extracellular domain in an ELISA and inhibits its TGF-β1 activity in the mink lung cell bioassay. We propose that sequestration of TGF-β into lipoproteins represents a novel mechanism by which TGF-β activity in circulation may be regulated. Lipoprotein sequestration of TGF-β may therefore contribute to the severe depression of TGF-β activity in advanced atherosclerosis.—**Grainger, D. J., C. D. Byrne, C. M. Witchell, and J. C. Metcalfe.** Transforming growth factor β is sequestered into an inactive pool by lipoproteins. *J. Lipid Res.* 1997. **38:** 2344–2352.

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Atherosclerosis is a disease of the major arteries, typified by changes in the structure and composition of the vessel wall. In the early stages of atherogenesis, the endothelium becomes focally damaged or dysfunctional, and at these sites smooth muscle cells migrate from the media of the vessel to form an expanded intima. Over a prolonged period, leukocytes, particularly monocytes, invade the expanded intima. In the later stages of the disease lipid from the circulation may be deposited into the developing lesion. Rupture of the lesions initiates thrombosis and can lead to myocardial infarction (reviewed in refs. 1, 2).

It is well established that in the early stage of atherogenesis the endothelial cells become activated (1). There is in vitro and in vivo evidence that inflammatory cytokines (such as TNF-α and IL-1β) activate endothelial cells (3, 4), and that TGF-β is a powerful suppressor of this activation (5, 6). We have proposed that TGF-β exerts a similar protective action on the endothelium to prevent the early stages of atherogenesis (2). TGF-β is produced as a latent precursor complex, which must be activated before it can exert its biological effects. The efficiency of conversion of latent TGF-β complexes into active TGF-β is therefore likely to be an important determinant of endothelial cell phenotype in vivo.

In the later stages of the disease, circulating lipid is accumulated into the intima to form the mature atherosclerotic plaque. Prospective epidemiological and clinical studies have shown that elevated levels of LDL and VLDL and reduced levels of HDL are associated with an increased risk of atherosclerosis (7, 8). This correlation is shown most clearly in familial hypercholesterolemia, in which the plasma concentration of LDL is markedly increased. Similarly, subjects with diabetes often exhibit an increased plasma concentration of triglyceride-rich lipoprotein (VLDL) and an associated increase in risk of cardiovascular disease (9–11).

Abbreviations: DMEM, Dulbecco's modification of Eagle's medium; Hb, hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; TGF-β, transforming growth factor β; VLDL, very low density lipoprotein.

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There is evidence to suggest that triglyceride-rich lipoproteins are directly involved in the second stage of atherogenesis, as a substrate for vessel wall lipid accumulation. For example, VLDL has been isolated from human atherosclerotic plaques by selected-affinity anti-apolipoprotein B immuno-absorption (12). However, there is also a suggestion from our earlier study (13) that there is an inverse correlation between serum LDL-cholesterol and the proportion of TGF-β in an active form. If this correlation arose because lipoprotein levels affect TGF-β activation, then lipoproteins could also contribute to the early stages of atherogenesis by this mechanism. It is well known that TGF-β in plasma can become associated with a variety of proteins (14–17), some of which affect the conversion of latent TGF-β to active TGF-β. For example, Nunes, Shapiro, and Rifkin (18) and Nunes et al. (19) have shown that association of latent TGF-β with members of the LTBP protein family promotes cell-mediated TGF-β activation. Similarly, Crookston et al. (20) and Gonias and colleagues (21) have extensively characterized the association of TGF-β with α2-macroglobulin in plasma and demonstrated that α2-macroglobulin-associated TGF-β is not available in an active form. As TGF-β is a relatively hydrophobic protein (22), we examined whether the inverse correlation between serum LDL-cholesterol concentration and TGF-β activation might be due to physical association between TGF-β and hydrophobic lipoprotein particles.

MATERIALS AND METHODS

Volunteer and patient groups

To obtain baseline data for the normal male population, plasma samples were obtained with local ethical committee permission from 57 healthy males (age range 42–76 years; mean 63.8 ± 3.2 (SEM) years), after fasting for 16 h. Specifically, these volunteers had normal glucose tolerance, confirmed by oral glucose tolerance testing using WHO criteria, and no history of cardiovascular disease.

In a second study, plasma samples from ten subjects with diabetes and moderately poor glucose control (Hb a1c > 8.0; age range 21–52 years; mean 36.1 ± 5.7 (SEM) years; six men and four women; four with type I and six with type II diabetes) were compared with plasma samples from ten volunteers with normal glucose tolerance (fasting plasma glucose < 5 mM; age range 21–54 years; mean 34.0 ± 5.8 (SEM) years; six men and four women). Neither of these groups had fasted prior to venesection, as the diabetic donors could not be fasted for ethical reasons.

Preparation of plasma and isolation of lipoproteins by density gradient ultracentrifugation

Blood was drawn from the ante-cubital vein between 8 AM and 12 noon. Platelet-poor plasma was prepared as described previously (23) and the absence of platelet degranulation (<0.02%) was confirmed by measurement of PF-4 in the plasma, using a specific ELISA (Asserchrom PF-4; Diagnostic Stago, Asnieres-sur-Seine, France). Plasma samples were stored at −80°C and after the samples were thawed all assays were performed within 24 h without further freeze-thawing.

The lipoprotein fraction (d < 1.215 g/ml) was prepared by density gradient ultracentrifugation, as previously described (24). Briefly, 1 ml of plasma was diluted to 4 ml in buffer A (0.15 M NaCl, 0.01% (wt/vol) sodium EDTA, and 0.02% (wt/vol) sodium azide at pH 7.2); the density was adjusted to 1.215 g/ml with KBr and the sample was centrifuged at 4°C for 48 h (235,000 *g*). The top 2 ml was collected as the lipoprotein fraction and the lower 2 ml as lipoprotein-deficient plasma. Efficient separation of these two fractions was confirmed by measuring cholesterol by enzymatic determination as previously described (25). More than 98% of the total cholesterol was in the lipoprotein fraction. Immunoglobulin (IgG) was measured by ELISA (I-6720; Sigma) in accordance with the manufacturer's instructions and more than 99% of the total IgG was in the lipoprotein-deficient plasma. The lipoprotein fraction was extensively dialyzed against serum-free Dulbecco's modification of Eagles' medium (DMEM). The triglyceride-rich lipoprotein (d < 1.006 g/ml), LDL (1.006 g/ml < d < 1.063 g/ml), and HDL (1.063 < d < 1.215 g/ml) subfractions were prepared from the lipoprotein fraction by further rounds of ultracentrifugation as previously described (26).

Lipoprotein-deficient human serum for the mink lung cell bioassay (see below) was prepared by density gradient ultracentrifugation (d > 1.215 g/ml) as for lipoprotein-deficient plasma, followed by extensive dialysis against DMEM.

Evaluation of TGF-β receptor binding and bioactivity

The effect of lipoprotein on TGF-β binding to the recombinant extracellular domain of the type II receptor (R2X; ref.27) was determined. Purified R2X-GST fusion protein was made as previously described (23, 27) and 1 μg of protein was coated onto each well of a Maxisorp ELISA plate (Gibco BRL) in 50 μl TBS overnight at room temperature, and a replicate set of wells was coated with GST alone under the same conditions. Wells were washed 3 times quickly (with ~300 μl for <10 sec) in TBS and blocked with TBS containing 3% bovine serum albumin (BSA, fatty-acid free; Sigma) for

30 min. A set of recombinant active TGF-β1 solutions (100 ng/ml to 1.5 ng/ml in 2-fold serial dilutions; R&D Systems) were prepared in TBS + 3% BSA. Further sets of TGF-β1 solutions at the same concentrations were prepared in TBS + 3% BSA also containing dialyzed lipoprotein at various concentrations. The lipoprotein concentrations were measured as total cholesterol using the enzymatic determination described previously (24). All sets of solutions containing the known concentrations of added TGF-β1 were incubated in the wells containing the R2X-GST fusion protein for 2 h, and also in the wells coated with GST alone. After three quick washes with TBS, the wells were incubated with TGF-β detection antibody (BDA 5; R&D Systems) at 1 μg/ml in TBS + 3% BSA (50 μl per well) for 1 h. After a further three washes in TBS, the wells were incubated with anti-rabbit IgG conjugated to horseradish peroxidase (A-6154; Sigma) at 1:5000 dilution in TBS + 3% BSA for 30 min. The wells were washed 3 times with TBS and visualized using 200 μl K-Blue Substrate (Elisa Technologies) per well for 20 min. After stopping the color reaction with 50 μl 3 M HCl, the absorbance was measured at 450 nm. Specific binding was calculated for each condition as the binding to R2X-GST fusion protein minus the binding to GST alone. All incubations were performed at room temperature with shaking (~300 rpm).

The effect of lipoprotein on TGF-β activity was determined using the mink lung bioassay. MvLu1 cells (passage 59–63; ATCC) were subcultured at 1:5 dilution into DMEM containing 10% FCS. After 24 h, the medium was removed and the cells were washed twice with serum-free DMEM. The cells were then incubated in DMEM containing 10% lipoprotein-deficient human serum and various concentrations of recombinant active TGF-β1 (10 ng/ml to 0.01 ng/ml in 3.3-fold dilutions; R&D Systems). A complete set of TGF-β1 concentrations were bioassayed with various concentrations of human total lipoprotein fraction added, measured as total cholesterol. After 23 h incubation, [^{3}H]thymidine (1 μCi/ml; Amersham International) was added to measure proliferation. Cells were harvested 1 h later and [^{3}H]thymidine incorporation into the TCA cold-precipitable fraction was determined as previously described (28).

Separation of lipoprotein classes by size

The lipoprotein classes in the lipoprotein fraction were also separated on the basis of size by gel filtration chromatography. Briefly, 200 μl of the lipoprotein fraction was loaded onto a 24 ml bed volume Sepharose 6B column equilibrated in buffer A, running at 0.4 ml/min. After a 3.4-min wait, 40 × 0.4 ml fractions were collected. Based on the elution of the apolipoproteins detected by Western blotting as previously described (29), VLDL and chylomicrons were present in fractions 1–10, LDL and IDL in fractions 11–20, and HDL in fractions after 20 (data not shown).

Detection of TGF-β in plasma and lipoproteins

TGF-β1 antigen was measured in lipoprotein-deficient plasma, lipoprotein fractions, and FPLC column fractions using the Quantikine TGF-β1 ELISA (R&D Systems) in accordance with the manufacturer's instructions, except that samples were activated with 5 M urea/1.3 M acetic acid and neutralized with 1.3 M NaOH/0.5 M HEPES. This assay detects plasma latent and active forms of TGF-β1.

Statistical analysis

As noted previously (13), many of the parameters associated with TGF-β measurement are not normally distributed in the whole population. Comparisons were therefore performed by unpaired non-parametric test (Mann-Whitney U test), taking $P < 0.05$ as significant. The central tendancy of these parameters is presented as median ±95% confidence interval unless stated otherwise. For other parameters that are normally distributed in the population as a whole, results are presented as mean ± standard deviation (unless stated otherwise) and comparisons are analyzed using the unpaired Student's *t*-test.

RESULTS

Association of TGF-β with lipoprotein particles

Platelet-poor plasma was prepared from peripheral venous blood drawn from 57 apparently healthy male donors, fasted for 16 h. The total fraction containing lipoproteins (d < 1.215 g/ml) was separated from the plasma protein fraction by density gradient ultracentrifugation and TGF-β1 antigen was measured separately for the lipoprotein fraction and the lipoprotein-deficient plasma fraction. The Quantikine ELISA was used to detect TGF-β1 antigen after treatment of the fractions with 5 M urea/1.3 M acetic acid. The median concentration of TGF-β1 antigen in platelet-poor plasma from healthy individuals was 4.9 ± 1.0 ng/ml (n = 57; range 3.0–8.8 ng/ml). In 56/57 individuals TGF-β was detected in the lipoprotein fraction. The proportion of TGF-β associated with the lipoprotein fraction varied from 6% to 30% with a mean of 16% (standard deviation 5%). We conclude that TGF-β1 can co-fractionate with lipoprotein.

Biological activity of lipid-associated TGF-β

We next investigated whether the lipoprotein fraction was able to inhibit the binding of TGF-β to its type II signalling receptor. For these experiments, the total lipoprotein fraction was prepared from an individual with a very low plasma concentration of TGF-β (<1 ng/ml TGF-β in plasma; individual I in ref. 23). The specific binding of TGF-β to the extracellular domain of its type II signalling receptor (R2X) was estimated from the binding to R2X-GST fusion protein minus the binding to GST alone, under conditions where equilibrium had been reached. The equilibrium dissociation constant (K_D) for the binding of recombinant TGF-β1 to the recombinant extracellular domain of the type II TGF-β receptor (R2X) was 17 ± 3 ng/ml. Binding of recombinant TGF-β1 to R2X was measured in the presence of increasing concentrations of the total lipoprotein fraction purified from the plasma of individual I. The presence of lipoprotein caused a dose-dependent increase in the apparent K_D for TGF-β binding to R2X (**Fig. 1a**) to a maximal value of 42 ± 6 ng/ml when lipoprotein equivalent to 3 mM total cholesterol was added. Note that a change of 2-fold in the apparent K_D would require sequestration of at least 50% of the TGF-β present. The concentration of lipoprotein that caused a half-maximal increase in the apparent K_D was equivalent to approximately 1 mM cholesterol. We conclude that the presence of the lipoprotein fraction reduces the apparent K_D for TGF-β1 binding to its receptor.

We therefore examined whether the lipoprotein fraction could inhibit the activity of TGF-β in the mink lung bioassay. (23, 28, 30) Proliferation of mink lung cells, assayed by [^{3}H]thymidine incorporation, was half-maximally inhibited by recombinant active TGF-β1 with an ID_{50} of 0.12 ± 0.04 ng/ml (n = 6). Addition of lipoprotein purified from the plasma of individual I caused a dose-dependent increase in the ID_{50} of TGF-β. The ID_{50} was maximal at 0.52 ± 0.08 ng/ml when 3 mM total cholesterol was added (Fig. 1b) and the concentration of lipoprotein that half-maximally increased the ID_{50} was equivalent to approximately 0.8 mM cholesterol. The inhibitory activity of the lipoprotein fraction therefore occurred over a similar concentration range in both the receptor binding assay and the bioassay.

TGF-β in different lipoprotein classes

To determine whether TGF-β1 co-fractionated with any specific lipoprotein classes, the lipoprotein in plasma from 56 of the 57 healthy individuals were separated by further rounds of density gradient ultracentrifugation (26). The average distribution of TGF-β1 among the lipoprotein classes was 64 ± 2% in HDL, 21 ± 2% in LDL, and 14 ± 2% in the VLDL/chylomicron

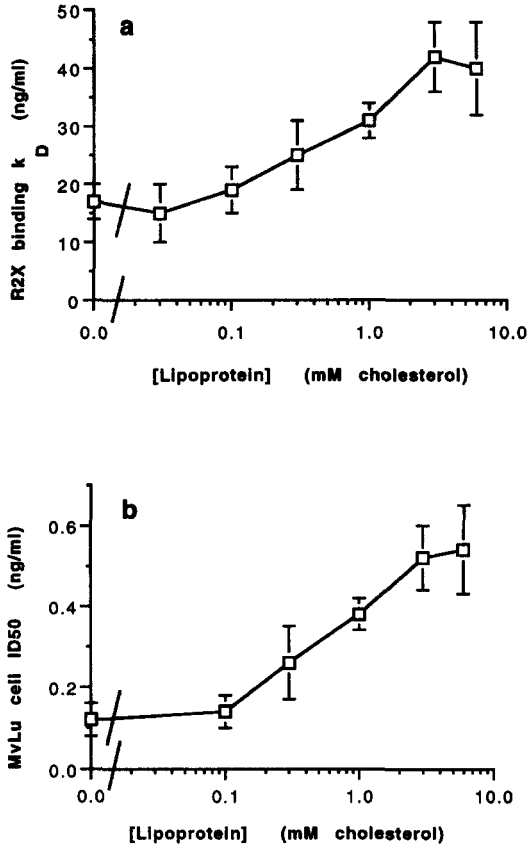

Fig. 1. Lipoprotein reduces the binding of TGF-β to its receptors and reduces its biological activity. a: The effect of lipoprotein on the binding of recombinant active TGF-β to the recombinant extracellular domain of the type II TGF-β receptor (R2X). Lipoprotein concentration is reported as total cholesterol and TGF-β binding to the R2X protein is reported as the apparent K_D (the apparent equilibrium dissociation constant for TGF-β binding to R2X). b: The effect of lipoprotein on the inhibition of mink lung cell proliferation mediated by recombinant active TGF-β. Inhibition of the mink lung cell proliferation by TGF-β is reported as the apparent ID_{50} (the concentration of TGF-β resulting in half-maximal inhibition). Note that a change of 2-fold in the apparent K_a or ID_{50} would require sequestration of at least 50% of the TGF-β present.

fraction (mean ± standard error; n = 56). For most of the individuals (46/56) the majority of the TGF-β1 in the lipoprotein fraction was present in the HDL fraction. Further density gradient ultracentrifugation showed that for all 56 individuals, >90% of the TGF-β1 in the HDL fraction, co-fractionated with the HDL-3 particles (98 ± 2%; n = 56). The remaining (i.e., non-HDL) TGF-β1 in the lipoprotein fraction was distributed to widely varying extents between the VLDL/chylomicron fraction and the LDL fraction.

Gel filtration was used to separate the lipoproteins by size, as an independent method to test whether TGF-β1 would co-fractionate with the same lipoprotein classes as determined by the density gradient centrifugation method. Separation of the lipoprotein classes by

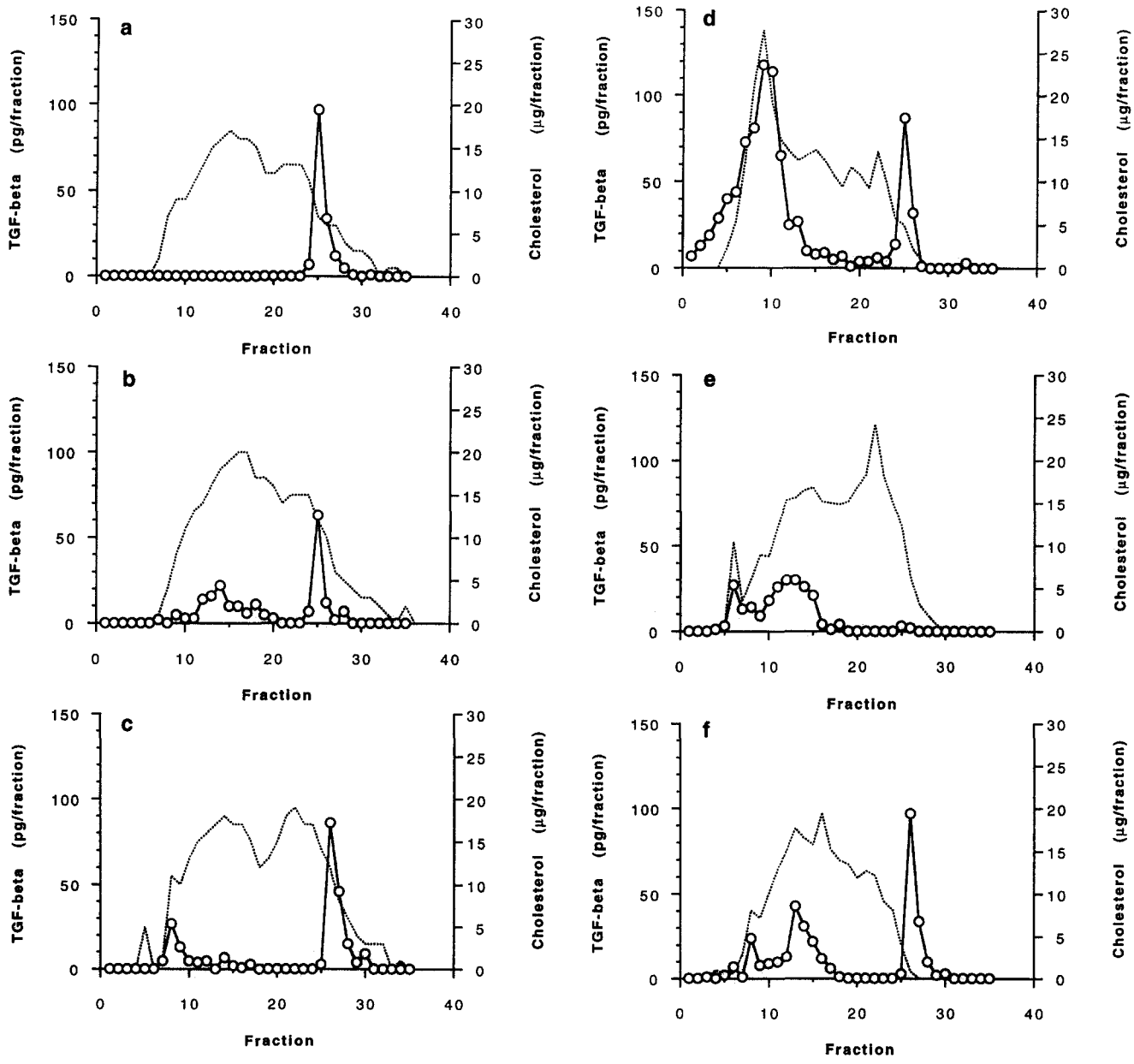

Fig. 2. Association of TGF-β with different lipoprotein classes. Profile of lipoprotein particle elution measured as total cholesterol (.....) and TGF-β elution (open circles) after separation of lipoprotein particles, on the basis of size, from (a–c) individuals with normal glucose tolerance and (d–f) individuals with diabetes.

size was performed for a random selection of 10/57 individuals and typical lipoprotein gel filtration profiles from three of these individuals are shown in **Fig. 2.** For the individual with the profile shown in Fig. 2a, for example, 27% of the total plasma TGF-β1 was in the lipoprotein fraction, with the remaining 83% in the lipoprotein-deficient plasma. Of the TGF-β1 in the lipoprotein fraction, >95% eluted with a subfraction of HDL which had the smallest size of all of the lipoprotein particles analyzed (Fig. 2a), consistent with the expected elution properties of the HDL-3 particles. This pattern, in which the majority of the TGF-β1 in the lipoprotein fraction co-eluted with an HDL subfraction was typical of all the healthy donors tested (Fig. 2b,c), although some individuals had small, variable amounts of TGF-β associated with LDL (Fig. 2b) and with the large, triglyceride-rich particles (Fig. 2c).

The co-fractionation of TGF-β1 with the HDL-3 par-

ticles by two independent methods suggests that TGF-β1 may be physically associated with the HDL-3 lipoprotein.

TGF-β in the lipoprotein fraction from diabetic patients

Subjects with diabetes mellitus often have low plasma concentrations of HDL and elevated concentrations of triglyceride-rich lipoprotein (VLDL and chylomicrons). We therefore examined whether the altered lipoprotein profiles of these individuals was correlated with an altered distribution of TGF-β1 in the lipoprotein fraction.

We measured the amount of TGF-β1 in both the lipoprotein fraction and the lipoprotein-deficient plasma from ten subjects with diabetes and moderately poor glucose control (hemoglobin a1c > 8.0). These individuals represented a broad cross section of diabetic individuals (four with type I diabetes and six with type II diabetes, males and females over a wide age range). Furthermore, as it was impractical to collect fasting blood samples from subjects with diabetes, all samples were collected without prior fasting. All the individuals had dyslipidemia as a common characteristic (total plasma triglyceride levels 2.86 (1.90–5.21) mmol/l (median and 95% confidence interval; n = 10)). The proportion of TGF-β1 in the lipoprotein fraction was 68 ± 21% (n = 10).

The high proportion of TGF-β1 in the lipoprotein fraction in subjects with diabetes compared with the fasted, healthy donors might result either from their unfasted status or from diabetes. We therefore selected a control group of healthy, but unfasted, donors of age and sex distribution similar to the group with diabetes and measured the proportion of TGF-β1 in their lipoprotein and lipoprotein-deficient plasma fractions. The proportion of TGF-β1 in the lipoprotein fraction (16 ± 6%; n = 10) was much lower than for the diabetic donors ($P < 0.001$; Student's unpaired *t*-test). Furthermore, the proportion of TGF-β in the lipoprotein fraction for unfasted, healthy subjects was very similar to that of the larger, fasted group described above (16 ± 6% versus 16 ± 5%; $P = 0.91$; Student's unpaired *t*-test). It is therefore likely that the high proportions of TGF-β1 in the lipoprotein fraction of the subjects with diabetes was due to diabetes rather than their unfasted status.

In light of the very high proportion of TGF-β1 in the lipoprotein fraction of the subjects with diabetes, we determined the distribution of the TGF-β1 between the lipoprotein classes for three of the ten subjects with diabetes, selected at random. The lipoprotein gel filtration profiles are shown for one individual with diabetes and severe hypertriglyceridemia (Fig. 2d; triglyceride 10.2 mmol/l) and two with relatively normal plasma triglyceride (Fig. 2e, f; 2.6 and 1.9 mmol/l, respectively). The individual with hypertriglyceridemia had more than 50% of total plasma cholesterol in the largest triglyceride-rich lipoprotein particles (Fig. 2d). This individual had 78% of plasma TGF-β1 associated with the lipoprotein fraction, but only 20% of this TGF-β1 was in the HDL fraction and the remaining 80% co-eluted with the large triglyceride-rich particles (Fig. 2d). Of the remaining two subjects with diabetes, one had 92% of plasma TGF-β associated with the lipoprotein fraction, but very little of this TGF-β1 (<5%) co-eluted with the HDL. Approximately 60% of the TGF-β1 co-eluted with the largest triglyceride-rich lipoprotein particles and the remainder with the LDL particles (Fig. 2e). The other diabetic had 52% of plasma TGF-β1 in the lipoprotein fraction and a lipoprotein gel filtration profile similar to some of the healthy individuals, with only a moderately increased amount of TGF-β1 associated with the VLDL/chylomicron fraction (Fig. 2f). The data (summarized in **Table 1**) suggest that much more TGF-β1 is associated with triglyceride-rich lipoprotein in plasma from the subjects with diabetes than from the healthy individuals.

DISCUSSION

The data show that lipoprotein can inhibit the binding of TGF-β1 to the type II TGF-β receptor and also inhibits TGF-β activity in the mink lung cell bioassay. Furthermore, in healthy subjects, a significant proportion of plasma TGF-β1 is associated with the lipoprotein fraction. The majority of TGF-β associated with lipoprotein co-fractionated with the HDL-3 subfraction using two independent methods of separation. These observations suggest that endogenous TGF-β1 in plasma can become physically associated with HDL-3, although the mechanism(s) of interaction remain to be established.

Almost all the available evidence suggests that a high HDL to LDL ratio is associated with a reduced risk of ischaemic heart disease (7, 8). HDL can be divided in subfractions either on the basis of density (HDL-1, HDL-2, and HDL-3 in order of increasing density) or protein composition (particles containing only apoA-I or both apo-I and apoA-II) (31). Although the denser subfractions are somewhat enriched in apoA-I/apoA-II-containing particles compared with the less dense subfractions, subfractions defined by density and subfractions defined by protein composition do not correspond (32). Associations between the various HDL subfractions and coronary heart disease has been extensively analyzed, and although some reports pro-

TABLE 1. Proportion plasma TGF-β1 sequestered into lipoprotein in plasma from healthy and diabetic individuals

Group Studied	n	% TGF-β1 in Lipoprotein	% Sequestered TGF-β1 in		
			VLDL	LDL	HDL
Non-diabetic (fasted)	57	16 ± 5	14 ± 11[a]	21 ± 13[a]	64 ± 15[a]
Non-diabetic (unfasted)	10	16 ± 6	n.d.	n.d.	n.d.
Diabetic (unfasted)	10	68 ± 21[c]	57 ± 26[b]	23 ± 19[b]	24 ± 22[b]

The proportion of plasma TGF-β1 (mean ± standard deviation) sequestered into the total lipoprotein fraction is shown for plasma samples drawn from healthy, non-diabetic individuals and from diabetic individuals. The proportion of the lipoprotein-associated TGF-β1 that co-fractionated with various lipoprotein subfractions (mean ± standard deviation) is also shown.

[a]Values are for 56 of the 57 individuals studied, where the remaining individual had no detectable TGF-β1 associated with the total lipoprotein fraction.

[b]Values are for 3 of the 10 individuals selected at random.

[c]$P < 0.001$; Student's unpaired *t*-test versus non-diabetic, unfasted individuals; n.d. not determined.

vide conflicting evidence for association of one or other subfraction with disease (33–37), the majority of the evidence suggests that no subfraction of HDL (based on either density or protein composition) is more strongly associated with coronary heart disease than total HDL-cholesterol (33–41). It is therefore unclear what role, if any, the HDL-3 particles, with which plasma TGF-β1 may be associated, play in the pathogenesis of atherosclerosis.

Minor proportions of plasma TGF-β1 from the healthy subjects were associated in varying amounts with VLDL, chylomicron, and LDL fractions. Elevated levels of plasma triglyceride and the most triglyceride-rich lipoprotein particles (particularly VLDL, chylomicrons, and their remnants) have been implicated in the increased risk of cardiovascular disease among individuals with diabetes (9–11). We therefore investigated whether the association between TGF-β1 and lipoprotein was altered in a broad cross-section of individuals with diabetes and moderately poor glucose control, with a common characteristics of dyslipidemia. The data show that association of TGF-β1 with VLDL and LDL occurs to a much greater extent in subjects with diabetes compared to subjects with normal glucose tolerance. The high levels of association of plasma TGF-β1 with the VLDL and LDL fractions would account for most or all of the high proportion of plasma TGF-β associated with the lipoprotein fraction from the subjects with diabetes. This observation suggests that in addition to depositing lipid into the atherosclerotic plaque (12), the atherogenic lipoproteins VLDL and LDL may also contribute to atherogenesis by depressing TGF-β activity. It is unlikely, however, that the increased amount of TGF-β associated with the VLDL and LDL fractions in plasma from diabetic subjects is a direct consequence of elevated concentration of VLDL and LDL particles. We have recently shown that when apparently healthy individuals undertake a standardized fat tolerance test, the concentration of plasma cholesterol in the VLDL fraction goes up by more than 2-fold but there is no accompanying increase in the fraction of plasma TGF-β associated with VLDL (D. J. Grainger and C. D. Byrne, unpublished observations). This suggests that the VLDL concentration may not be the limiting factor in determining the amount of TGF-β associated with the VLDL fraction. There is limited evidence that VLDL particles isolated from the plasma of diabetic individuals are qualitatively, as well as quantitatively, abnormal (42) and this altered constitution of the VLDL particle may be the cause of the increased amount of TGF-β associated with VLDL particles in plasma from diabetic individuals.

Taken together, the data suggest that lipoproteins in individuals with diabetes may reduce TGF-β activity in vivo. Such a reduction would represent a novel mechanism by which diabetics with poor glucose control would have an increased risk of cardiovascular disease. Increased levels of triglyceride-rich lipoproteins, or alteration of the composition of the lipoprotein particles, in non-diabetic individuals, for example due to diet (43), may also increase the risk of cardiovascular disease by a similar mechanism. Further studies are required to determine whether increased plasma concentrations of triglyceride are associated with increased sequestration of TGF-β1, irrespective of the degree of glucose tolerance.

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