Diabetes Mellitus Is Associated With Reduced High-Density Lipoprotein Sphingosine-1-Phosphate Content and Impaired High-Density Lipoprotein Cardiac Cell Protection

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Objective—The dyslipidemia of type 2 diabetes mellitus has multiple etiologies and impairs lipoprotein functionality, thereby increasing risk for cardiovascular disease. High-density lipoproteins (HDLs) have several beneficial effects, notably protecting the heart from myocardial ischemia. We hypothesized that glycation of HDL could compromise this cardioprotective effect.

Approach and Results—We used in vitro (cardiomyocytes) and ex vivo (whole heart) models subjected to oxidative stress together with HDL isolated from diabetic patients and nondiabetic HDL glycated in vitro (methylglyoxal). Diabetic and in vitro glycated HDL were less effective ($P<0.05$) than control HDL in protecting from oxidative stress. Protection was significantly, inversely correlated with the degree of in vitro glycation ($P<0.001$) and the levels of hemoglobin A1c in diabetic patients ($P<0.007$). The ability to activate protective, intracellular survival pathways involving Akt, Stat3, and Erk1/2 was significantly reduced ($P<0.05$) using glycated HDL. Glycation reduced the sphingosine-1-phosphate (S1P) content of HDL, whereas the S1P concentrations of diabetic HDL were inversely correlated with hemoglobin A1c ($P<0.005$). The S1P contents of in vitro glycated and diabetic HDL were significantly, positively correlated (both <0.01) with cardiomyocyte survival during oxidative stress. Adding S1P to diabetic HDL increased its S1P content and restored its cardioprotective function.

Conclusions—Our data demonstrate that glycation can reduce the S1P content of HDL, leading to increased cardiomyocyte cell death because of less effective activation of intracellular survival pathways. It has important implications for the functionality of HDL in diabetes mellitus because HDL-S1P has several beneficial effects on the vasculature. (***Arterioscler Thromb Vasc Biol*. 2016;36:817-824. DOI: 10.1161/ATVBAHA.115.307049.)**

Key Words: AGE ■ cardiomyocytes ■ glycation ■ HDL ■ ischemia reperfusion injury ■ sphingosine-1-phosphate ■ type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is one of the most important risk factors for developing cardiovascular disease.[1] The dyslipidemia found in T2DM has multiple etiologies and affects all lipoprotein subclasses. Unfavorable composition and quantities of lipoproteins are paralleled by deterioration of their function under the pressure of insulin resistance and processes of oxidation and glycation.[2,3] The complex process of protein glycation leads ultimately to accumulation of advanced glycation end products that have been correlated with the severity of coronary heart disease.[4]

Low plasma high-density lipoproteins (HDL) cholesterol is a strong risk factor for cardiovascular disease.[5] HDL cholesterol levels are decreased in T2DM with alterations of both the HDL proteome[6] and lipidome.[7] These modifications can be related to early markers of arterial disease.[8] HDL from T2DM patients also exhibit impaired anti-inflammatory and antioxidant properties.[9]

The protective effect of HDL has more recently been shown to extend into the field of cardiac hypoxic stress and ischemia. Oxidative stress, which is also induced by

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Nonstandard Abbreviations and Acronyms	
apoAI	apolipoprotein AI
apoM	apolipoprotein M
DOX	doxorubicin
HDL	high-density lipoproteins
IR	ischemia–reperfusion
MG	methylglyoxal
S1P	sphingosine-1-phosphate
T2DM,	type 2 diabetes mellitus

the anthracycline drug doxorubicin, leads to cardiotoxicity.[10,11] We and others have shown that HDL protects the cardiomyocyte against both doxorubicin-[12] and hypoxia[13]-induced oxidative stress in vitro. We have also shown that HDL protects the whole heart against ischemia–reperfusion (IR) injury by preserving mitochondrial integrity.[14] On the molecular level, the protective effects of HDL are mediated by inducing phosphorylation of intracellular prosurvival proteins.[15]

Sphingosine-1-phosphate (S1P) is a potent messenger molecule operating both intra- and intercellularly.[16] In plasma, it is mainly associated with HDL via apolipoprotein M (apoM).[17] S1P is enriched in small dense HDL3 with a positive correlation between HDL S1P concentration and apoptotic protection in endothelial cells.[18] Theilmeier et al[19] have also shown that HDL and its constituent S1P protect the heart during IR injury via an S1P receptor.

Little is known about how diabetes mellitus and the pathophysiological process of glycation affect HDL function in the setting of ischemia-induced oxidative stress. In the present work, we investigated the functionality of glycated HDL (glyHDL) during oxidative stress and ischemia. We hypothesized that glycation could compromise this function, which would contribute to an increased risk of vascular disease in diabetes mellitus. The study has identified a novel, physiopathological mechanism specific to diabetes mellitus. It also has wider implications for the functionality of HDL in diabetes mellitus.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Diabetic HDL Is Less Protective Than Control HDL

Previous studies have shown that HDL protects cells against oxidative stress induced by doxorubicin.[12] The effect of HDL on cell viability was quantified in primary cultures of cardiomyocytes after doxorubicin exposure, considering doxorubicin-only treated cells as 100% (Figure 1A). Cells exposed to doxorubicin+control HDL (cHDL) and doxorubicin+diabetic HDL (dmHDL) had mean viabilities of, respectively, 194.1±18.3% and 137.9±9.5% (P=0.011 for comparison). Thus, dmHDL was less effective in protecting against doxorubicin-induced cell death than cHDL. The characteristics of T2DM patients are given in the Table.

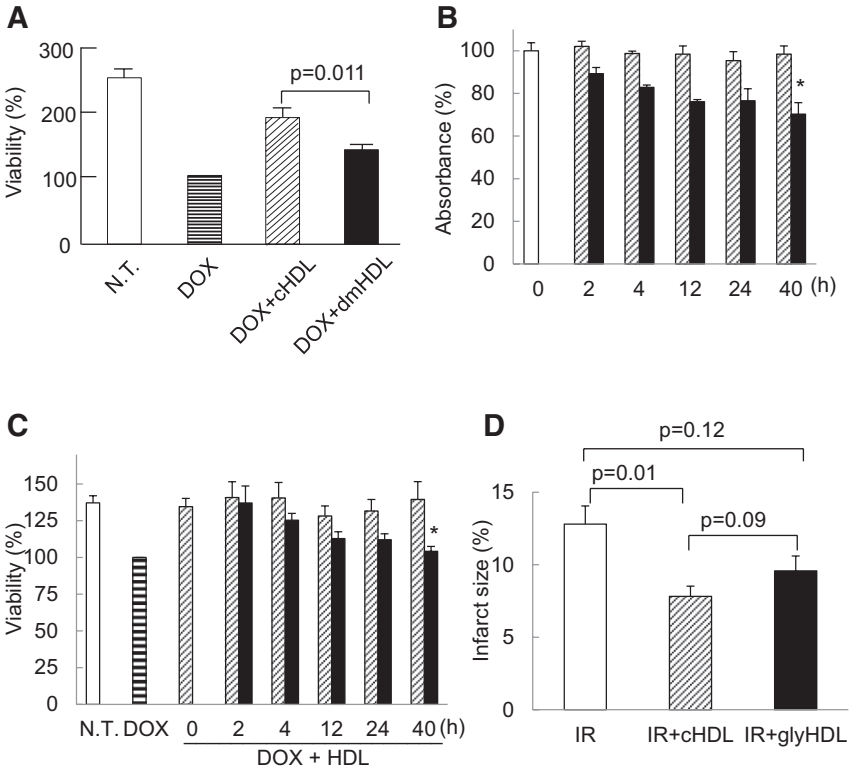

Figure 1. High-density lipoprotein (HDL) from type 2 diabetes mellitus (T2DM) patients and in vitro glycated HDL (glyHDL) are less protective than healthy HDL. **A**, Cell viability in primary cultures of cardiomyocytes after incubation (20 h) with doxorubicin (DOX; 0.5 μM) measured by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cardiomyocytes were preincubated with HDL (200 μg/mL) from diabetic patients (dmHDL) and from healthy controls (cHDL) for 30 min before addition of DOX. Results were normalized to DOX-treated cells. n=6. **B**, HDLs with increasing level of glycation were prepared in vitro by increasing methylglyoxal (MG) incubation times (2–40 h; black bars) in parallel with corresponding control HDLs (cHDL; hatched bars). The level of HDL glycation measured by the TNBS (2,4,6-trinitrobenzene sulfonic acid) assay with glycation being inversely correlated to absorbance. Mean absorbance for cHDL 0 h (open bar) was set to 100%. **C**, Cell viability in cardiomyocytes after a 20-h incubation with DOX and various HDL (200 μg/mL) preparations measured by the MTT assay. (n=3 for all groups). *P<0.001, analysis of variance (ANOVA) for trend across glyHDLs. **D**, Infarct sizes in mouse hearts after ex vivo ischemia–reperfusion (IR) injury. Isolated hearts were treated with 400 μg/mL cHDL (n=7) or glyHDL (n=8); control mice without treatment (IR, n=8). Quantification of infarct size is expressed as percentage of total heart area. P value was calculated by 1-way ANOVA, Bonferroni post hoc test. Student t test (unilateral, unpaired) was used to calculated difference between cHDL and glyHDL. N.T. indicates nontreated.

Table. Study Cohort

	Controls	Type 2 Diabetes Mellitus	*P* Value
n	6	6	
Sex (male/female)	3/3	3/3	
Age, y	50.5 (4.5)	55.7 (6.2)	0.49
BMI, kg/m^2	23.3 (1.3)	29.5 (2.6)	0.06
Triglycerides, mM	1.36 (0.45)	3.34 (0.98)	0.10
Total cholesterol, mM	5.59 (0.37)	5.23 (0.36)	0.69
LDLC,* mM	3.53 (0.22)	2.68 (0.44)	0.12
HDLC, mM	1.44 (0.17)	1.04 (0.10)	0.08
Diabetes mellitus, no of years	…	13 (1–33)	
Hemoglobin A1c, %/mmol/mol	…	8.8 (2.0) / 73 (9)	

Mean (SEM) or median (range). BMI indicates body mass index; HDLC, high-density lipoprotein cholesterol; and LDLC, low-density lipoprotein cholesterol.

*Calculated by Friedwald's formula.

HDL Glycated In Vitro Is Less Protective Than Control HDL

To examine specifically the effects of glycation, we used a model based on methylglyoxal (MG) modification of HDL. Using the TNBS (2,4,6-trinitrobenzene sulfonic acid) assay to monitor glycation, we observed that MG increased HDL glycation (P<0.001), whereas cHDLs showed no increase (P=0.73; Figure 1B). Increased glycation was associated with a significant decrease in HDL-mediated protection from doxorubicin (P<0.001; P=0.84 for cHDLs; Figure 1C). HDL glycation was inversely correlated with cell viability (r=–0.674, P=0.002, n=18). Immunoblots of apoM and apoAI after glycation of HDL are shown in Figure I in the online-only Data Supplement.

The protective effect of cHDL and glyHDL against IR injury was also evaluated in the ex vivo isolated heart perfusion model (Figure 1D). Control mice subjected to ischemia but without HDL treatment had an infarct size of 12.8±1.2%. Treatment with cHDL during reperfusion significantly decreased average infarct size by 39% compared with controls (P=0.01), whereas infarct size did not differ significantly between glyHDL and control mice.

Glycated HDL Does Not Induce Phosphorylation of Prosurvival Proteins as Effectively as Control HDL

The ability of HDL to activate prosurvival intracellular signaling pathways by inducing phosphorylation is important for cardiomyocytes during oxidative stress.[20] We examined phosphorylation of Akt, Stat3, and Erk1/2 in relation to HDL glycation by Western blotting (Figure 2). cHDL more efficiently induced phosphorylation of each respective pathway in comparison to glyHDL after both 5 and 30 minutes of stimulation (see also Figure II in the online-only Data Supplement for phosphorylation levels for Stat3 and Erk1/2 at 60 minutes).

Glycation Reduces HDL Binding to Cardiomyocytes

Given the role attributed to the scavenger receptor class B type I (SR-BI) receptor in certain HDL functions, we compared binding of cHDL and glyHDL to cardiomyocytes. Using ^{125}I-labeled HDL in the concentration range 1 to 40 μg/mL, saturable, specific binding of cHDL and glyHDL was observed (Figure 3A) with (at 40 μg/mL) 245±44 ng bound HDL/mg and 104±41 ng bound HDL/mg of cell lysate proteins, respectively, corresponding to a 58% decrease for glyHDL (P=0.023).

SR-BI Participates in the Binding of HDL to Cardiomyocytes But Does Not Influence Its Protective Effects

To study more specifically the role of SR-BI in cardiomyocytes under oxidative stress, we manipulated its expression by transfecting cardiomyocytes with siRNA-targeting SR-BI. SR-BI siRNA downregulated mRNA and protein levels to 14.0±7.0% and 32.7±9.7%, respectively, compared with Scr-siRNA (120.0±16.3% and 90.6±2.4%; the expression of lipofectamine-only transfected cells was arbitrarily set to 100%; Figure 3B and 3C). Specific binding of HDL was reduced 36% (Scr-siRNA cardiomyocytes, 510±56 ng/mg versus SR-BI-siRNA cells, 327±41 ng/mg; P=0.005; Figure 3D).

The impact of SR-BI silencing on cell viability was examined after HDL+doxorubicin exposure (Figure 3E). SR-BI–downregulated cardiomyocytes had similar viability to Scr-siRNA–treated cells, 119.4±7.0% and 121.6±8.3%, respectively (P=0.86), in the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay with cHDL. Cardiomyocyte viability was also evaluated by DNA fragmentation after 8 hours of HDL+doxorubicin exposure. SR-BI-siRNA– and Scr-siRNA–transfected cells displayed similar fragmentation levels of 81.6±4.4% and 83.0±3.0%, respectively (P=0.81). A final evaluation of the role of SR-BI exploited SR-BI knockout mice using the ex vivo heart exposed to IR injury (Figure 3F). Without HDL treatment during reperfusion, wild-type and SR-BI knockout mouse did not differ in infarct size (P=0.23). HDL treatment reduced the infarct size by 37% (P=0.002) and 46% (P=0.044) in wild-type and SR-BI knockout mice respectively.

In addition, we investigated the involvement of SR-BI in activating HDL prosurvival pathways by comparing the phosphorylation of proteins in Scr-siRNA– and SR-BI-siRNA–transfected cardiomyocytes (Figure III in the online-only Data Supplement). There were no significant differences in stimulation responses for any of the phosphorylated proteins.

In Vitro Glycation of HDL Decreases Its S1P Content Which Inversely Correlates to Its Protective Capacity

Given the reported importance of S1P, we next studied the consequences of glycation on HDL S1P content. We could find no evidence for the presence of derivatives of the S1P molecule by liquid chromatography mass spectrometry (data not shown). However, S1P content in the glyHDLs decreased with increasing incubation time (P<0.001; Figure IV in the online-only Data Supplement). S1P content in the cHDL preparations did not differ between groups (P=0.49).

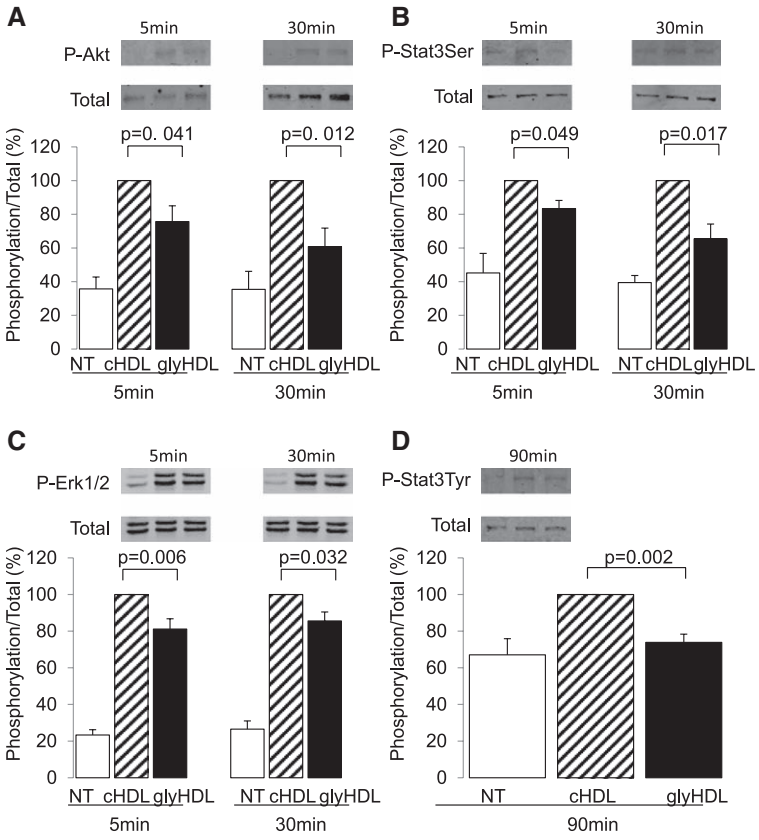

Figure 2. Glycated high-density lipoprotein (glyHDL) is less efficient than control HDL in inducing phosphorylation of signal pathway proteins. Primary cultures of cardiomyocytes were stimulated with control HDL (cHDL) or glyHDL during 5 and 30 min for (**A**) AktSer^{473}, (**B**) Stat3Ser^{727}, (**C**) Erk1/2, Thr^{202}/Tyr^{204}, or 90 min for (**D**) Stat3Tyr^{705} before cells were lysed, and 30 μg of protein was analyzed by SDS-PAGE. A representative gel is shown with all bands from same gel (lane removed). Specific phospho-protein expression was quantified and normalized to respective protein content. cHDL-stimulated cells were set to 100%. n=5 to 7. NT indicates nontreated.

S1P concentration in HDL was significantly and inversely correlated to its glycation level (r=–0.810; P<0.001) and significantly and positively correlated to its protective capacity (r=0.715; P<0.001; Figure 4A and 4B).

Similar results were achieved using albumin (BSA), instead of HDL, as a vehicle for S1P. S1P was resuspended with BSA into BSA:S1P complexes and then glycated with MG, which led to a significant glycation compared with control complexes (Figure VA in the online-only Data Supplement). Glycation also led to a decrease in S1P content by 22.7±6.3% compared with control (P=0.03), and the protection capacity decreased from 141.1±5.7% to 121.9±4.6% (P=0.036; Figure VB in the online-only Data Supplement).

HDL S1P Content in T2DM Patients Show Correlations to Both Their Hemoglobin A1c and Their Protective Capacities

HDL was individually prepared from a second cohort of T2DM patients (n=26) with a range of hemoglobin A1c (HbA1c) levels (Figure 5). Using HbA1c as a measure of in vivo glycation, we observed that patient HDL S1P content correlated inversely with their HbA1c levels (Figure 5A; r=–0.551; P=0.005). The S1P content of dmHDL was also correlated to cell viability (Figure 5B; r=0.557, P=0.007).

Adding S1P to Glycated HDL Improves Its Protective Capacity

In vitro glycated HDL and HDL from T2DM patients were resuspended with S1P and the HDL S1P content, and their protective capacities were evaluated. S1P content increased similarly in glyHDL (86.7±1.7 to 150.5±7.1 pmol/mg; P<0.001) and dmHDL (123.2±12.1 to 189.9±12.3 pmol/mg; P=0.006; Figure 6A and 6C). Concomitantly with the S1P increase, the protective capacity increased for both glyHDL (122.6±3.7% to 135.6±4.3%; P=0.034) and dmHDL (135.2±13.3% to 158.8±15.6%; P=0.028; Figure 6B and 6D).

Discussion

The main findings of this study are that the cardioprotective function of HDL manifested during acute oxidative stress and IR injury is impaired by glycation in vitro and inversely correlates in vivo with measures of glycation. On a molecular level, we demonstrate that glycation of HDL impairs its capacity to stimulate previously identified, prosurvival signaling pathways. Finally, we demonstrate that impaired function is directly related to loss of S1P from HDL, which correlates with measures of glycation in vitro and in vivo.

HDL isolated from diabetic patients was defective in protecting cardiomyocytes from stress. As diabetes mellitus can modify HDL in several ways, we used in vitro modification with MG to study specifically the consequences of glycation. It is a widely accepted methodology provoking protein glycation comparable to that observed in diabetic patients.[21,22] Indeed, we observed similar levels of HDL glycation of our in vitro preparations and the diabetic patient group. Moreover, recent studies have reported that in vivo glycated products derived from MG best differentiate diabetic and nondiabetic HDL.[23,24] In our studies, the consequence of such in vitro glycation was to reduce by 20% to 30% the protective capacity of HDL in both in vitro and

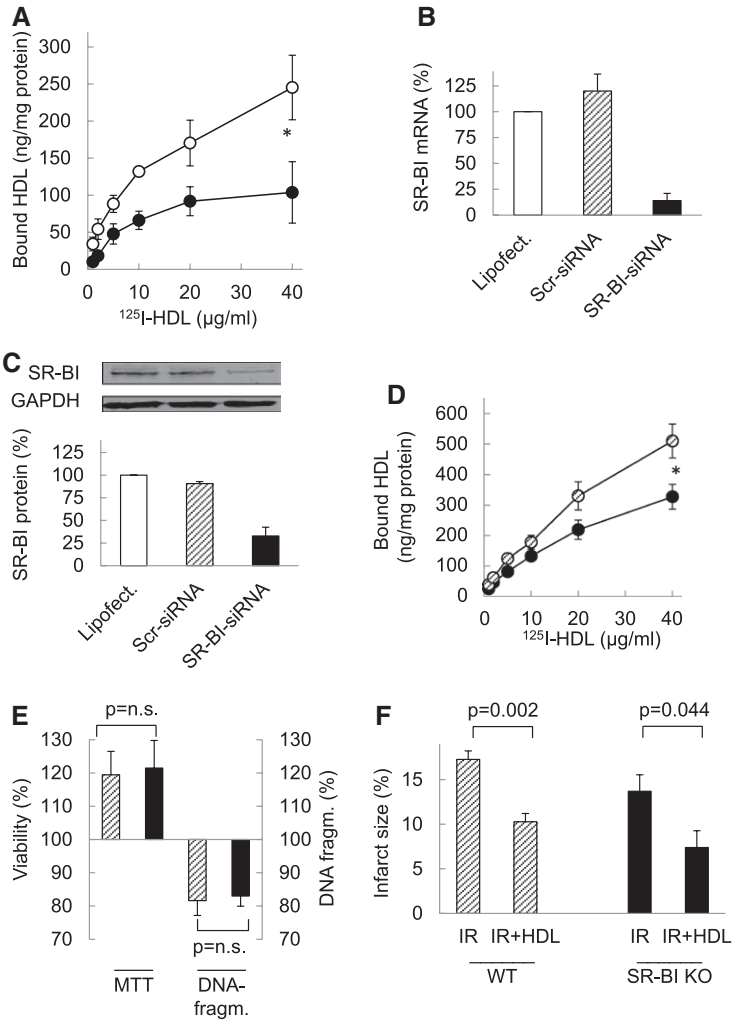

Figure 3. The role of scavenger receptor class B type I (SR-BI) in cardiomyocyte high-density lipoprotein (HDL) binding and HDL protective effect. **A**, Cardiomyocytes bind in vitro glycated HDL (methylglyoxal [MG], 24 h) less efficiently than control HDL. White circles=specific ^{125}I-cHDL binding; black circles=specific ^{125}I-glyHDL binding (n=4). **P*=0.023 (repeated measurement ANOVA interaction concentration×group). Mean expression levels of SR-BI in cardiomyocytes of mRNA (**B**) and protein (**C**) after transient transfection with scramble (Scr) siRNA, SR-BI-siRNA, or lipofectamine only (Lipofect; n=3). **D**, Specific ^{125}I-HDL binding to cardiomyocytes transfected with Scr-siRNA or SR-BI-siRNA cells (n=4). **P*=0.005 (repeated measurement ANOVA for interaction concentration×group). **E**, Cell survival after oxidative stress in cardiomyocytes knocked down for SR-BI. Cells were exposed to doxorubicin (DOX) and HDL for 20 h (n=4) or 8 h (n=3), and cell viability and DNA fragmentation were quantified, respectively. **F**, Infarct sizes in wild-type (WT) and SR-BI knockout (KO) mice hearts after ischemia–reperfusion (IR) injury ex vivo. n=7 for WT IR+HDL, n=5 for other groups. Hatched bars/circles=Scr-siRNA treated cells; black bars/circles=SR-BI-siRNA treated cells; Student *t* test. cHDL indicates control high-density lipoprotein; glyHDL, glycated high-density lipoprotein; and n.s., not significant.

ex vivo models of acute oxidative stress. It was linked to compromised activation of Akt, Stat3, and Erk1/2, protein kinases that have key roles in the SAFE (survivor activating factor enhancement) and RISK (reperfusion injury salvage kinase) myocardial prosurvival pathways and that we previously identified as important in HDL-mediated protection of cardiomyocytes.[20]

One potential explanation for the reduced protective capacity of HDL could be variations in apoM concentrations. The role of apoM in the association and function of S1P in HDL has been demonstrated in several models, even beyond the vascular field.[25,26] However, we could observe no variations in apoM concentrations (Figure VI in the online-only Data Supplement), in accordance with previous reports that apoM is not decreased in non-MODY3 diabetic patients.[27–29]

An alternative explanation may be defective interaction with cell receptors. Glycation did reduce binding of HDL to cardiomyocytes. SR-BI, the major HDL receptor, is expressed in the heart.[30] Complementary studies showed that siRNA-mediated reduction of SR-BI expression in cardiomyocytes did not affect the efficacy of HDL. This was confirmed in SR-BI knockout mice where absence of the receptor did not affect the protective function of HDL. Thus, we cannot demonstrate a major role for SR-BI in our model, although its central importance to the impact of HDL elsewhere in the vasculature, notably endothelium function, is clear.[31] We cannot exclude, however, a possible, secondary role of anchoring HDL to the membrane to facilitate interaction of S1P with its receptor.[31] This data suggest that the impact of S1P is independent of

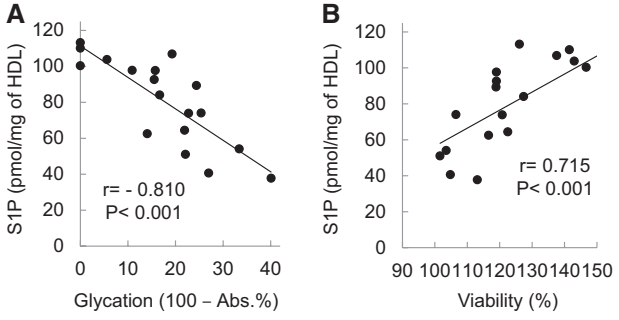

Figure 4. Correlations between high-density lipoprotein (HDL) sphingosine-1-phosphate (S1P) content and glycation level (**A**) and protective capacity (**B**) of in vitro glycated HDL (n=18). Content of S1P was analyzed from in vitro glycated HDL and correlated to its level of glycation measured by TNBS (2,4,6-trinitrobenzene sulfonic acid; **A**) and its protective capacity measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (**B**), as described in Figure 1 (n=18).

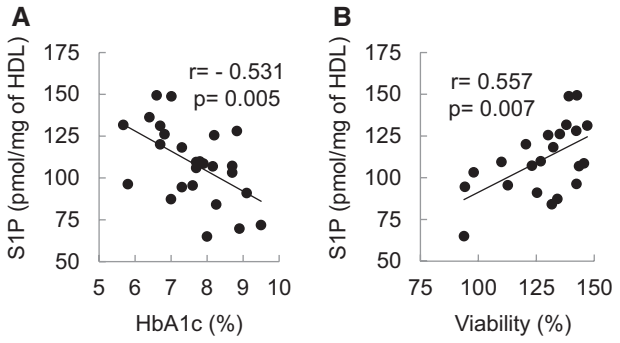

Figure 5. Sphingosine-1-phosphate (S1P) content in high-density lipoprotein (HDL) from type 2 diabetes mellitus (T2DM) patients shows correlations to the (**A**) hemoglobin A1c (HbA1c) levels and (**B**) HDL protective capacity (viability). HDL was isolated from a group of diabetes mellitus patients (n=26) with a range of HbA1c between 5.7% and 9.5%. Viability was measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, as described in Figure 1.

HDL function, where the lipoprotein seems to serve primarily a transport vector role. It is supported by our observations with BSA:S1P complexes, which were also able to protect cardiomyocytes from oxidative stress despite the absence of HDL (Figure V in the online-only Data Supplement). Nevertheless, in vivo, it seems that HDL-associated S1P is the principal, bioactive form of the lipid in plasma.[32]

We also considered whether there could be direct glycation of S1P. The molecule has one amine group potentially susceptible to modification by MG, thus creating S1P-MG derivatives. In collaboration with the Sciences Mass Spectrometry platform (core facility of the University of Geneva), we developed a mass spectrometry procedure to try to detect glycated derivatives of S1P. Several attempts were made to identify derivates in S1P–albumin complexes subjected to glycation. No derivatives suggestive of direct glycation of S1P were detected. Neither were derivatives observed in HDL preparations. On the other hand, our in vitro data clearly demonstrate an inverse relationship between the level of HDL glycation, its S1P content, and its cardioprotective capacity. The clinical relevance of these findings is underlined by the inverse correlation we observed between S1P concentrations of HDL from diabetic patients and their HbA1c levels. This may seem surprising as HbA1c integrates glycemic control over a longer period than the plasma residence time of HDL (estimated to 4 days). However, several studies have shown a strong correlation between HbA1c and HDL-apoAI glycation products measured by the mass spectrometry procedure.[23,33] Moreover, a recent study reported an inverse correlation between HbA1c levels and the concentration of S1P in HDL.[34] Overall, these observations, together with data underlining the importance of S1P for HDL-mediated protection of cardiomyocytes, clearly identify loss of S1P from HDL as one explanation for impaired HDL function. Glycation itself would seem to be an important factor because (1) in vitro, it was sufficient to compromise HDL function and (2) it had the same effects in our simplified BSA:S1P model in the absence of HDL (loss of S1P which is associated with the degree of glycation and impaired protection).

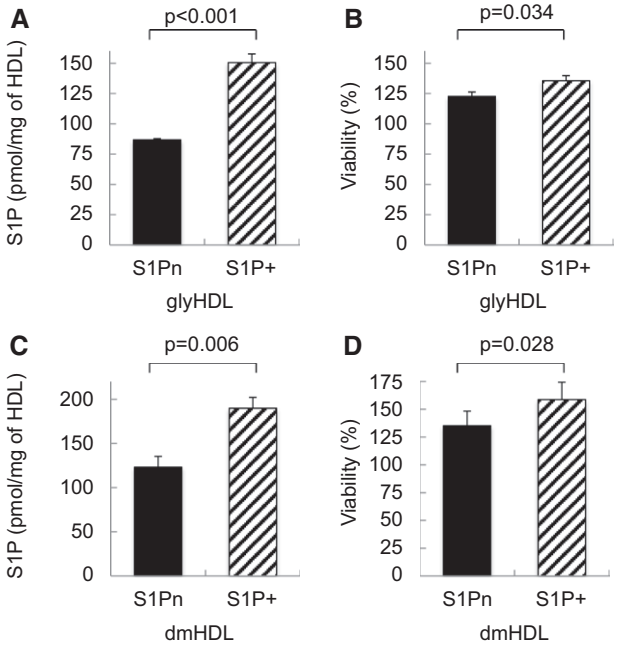

Figure 6. Adding sphingosine-1-phosphate (S1P) to glycated HDL (glyHDL) improves its protective capacity. In vitro glycated HDL (**A** and **B**, n=6) and pooled HDL from T2DM patients (dmHDL) with similar hemoglobin A1c (HbA1c; **C** and **D**, n=5) were resuspended with 60 pmol/mg of S1P (S1P+) or not (S1Pn), and their S1P contents (**A** and **C**) and protective capacities (**B** and **D**) were measured.

The pathophysiological relevance of loss of S1P is underlined by the observation that HDL function can be recovered by restoring SIP to HDL. It highlights the need for a better understanding of the metabolic factors that influence the S1P content of HDL. The potential clinical consequences are illustrated by recent studies demonstrating increased risk of restenosis in patients with reduced HDL-S1P.[35]

The question arises as to how glycation may provoke loss of S1P from HDL. The major determinant of S1P binding to HDL is apoM. HDL-apoM could be susceptible to glycation-induced changes in HDL, which can destabilize HDL structure, modifying particle size and protein composition.[36] Glycation also reduces the lipid-binding capacity of HDL,[21] perhaps arising from an increase in hydrophilicity. The latter may influence binding of apoM to HDL, which is achieved via its hydrophobic signal sequence. However, we could not demonstrate a correlation between HDL–apoM concentrations and HbA1c levels in diabetic patients (Figure VI in the online-only Data Supplement), and as mentioned previously, apoM concentrations did not differ between diabetic patients and controls. Alternatively, apoM could be vulnerable to direct glycation. A model[37] of its association with HDL shows the apolipoprotein protruding from the lipoprotein surface with the S1P-binding pocket facing outwards. In vitro glycation of apoAI[21] and HDL[38] strongly modifies tryptophan residues inducing conformational changes. Tryptophan residues of apoM have been implicated in binding S1P.[37] We were able to confirm glycation of apoM in our dmHDL samples. When

challenged with an antibody to advanced glycation end products, advanced glycation end products were detected in apoM from diabetic patients, whereas no advanced glycation end products were evident in nondiabetic HDL (Figure VII in the online-only Data Supplement).

The failure in recent years to show clinical benefit from raising HDL cholesterol[39,40] has prompted intense re-evaluation of the role of the lipoprotein in vascular disease. Although previous studies had established a protective role for HDL-S1P in ischemia, the present study is the first to show the potential pathological impact of a diabetes mellitus–associated modification of HDL on this function. Moreover, we identify a mechanism, loss of S1P, that could have wider implications for the role of HDL in vascular disease. The S1P component of HDL has been associated with several beneficial effects, including endothelial function, endothelial barrier integrity, and anti-inflammatory reactions. More recent studies have extended the potential impact of HDL-S1P to other cells, including pancreatic beta cells, where it was shown to improve insulin secretion, notably under conditions of endoreticulum stress.[41]

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Disclosures

None.

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Significance

The present study is the first to show the potential pathological impact of a diabetes mellitus–associated modification of high-density lipoprotein (HDL) on the protective role for HDL sphingosine-1-phosphate (S1P) in ischemia. Moreover, we identify a mechanism, loss of S1P, that could have wider implications for the role of HDL in vascular disease. The S1P component of HDL has been associated with several beneficial effects, including endothelial function, endothelial barrier integrity, and anti-inflammatory reactions. Recent data demonstrate a direct correlation between low S1P-HDL and cardiac disease. Our data underline the diagnostic potential of measuring the S1P content of HDL as a marker of HDL functionality. They also have therapeutic implications with respect to the addition of S1P to reconstituted HDL currently used in clinical trials.