Coagulation and inflammation in diabetes mellitus
Sommeijer, D.W.

Citation for published version (APA):
Anti-inflammatory effect of simvastatin on the vessel wall does not reduce arterial thrombus formation in diabetic mice

Dirkje W. Sommeijer\textsuperscript{1,2}, Hjalmar R. Hansen\textsuperscript{1,2}, Arnaud D. Hauer\textsuperscript{3}, Johan Kuiper\textsuperscript{3}, Pieter H. Reitsma\textsuperscript{1}, Hugo ten Cate\textsuperscript{1,4}

\textsuperscript{1}Laboratory for Experimental Internal Medicine, Academic Medical Center, University of Amsterdam, \textsuperscript{2}Department of Internal Medicine, Slotervaart Hospital, Amsterdam, \textsuperscript{3}Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, Leiden and \textsuperscript{4}Department of Internal Medicine and Cardiovascular Research Institute Maastricht, Academic Hospital and University of Maastricht, Maastricht, The Netherlands
Abstract

Endothelial dysfunction and a procoagulant state are potential contributors to the increased risk of atherothrombotic complications in diabetes mellitus. Clinical evidence shows that statin treatment reduces the incidence of atherothrombotic events in diabetic patients, but it remains uncertain which pleiotropic effects of statins are involved.

The aim of this study was to examine the antithrombotic and anti-inflammatory effect of simvastatin on in vivo arterial thrombus formation in experimental diabetic female C57Bl/6 mice (i.p. streptozotocin 200 mg/kg) treated with simvastatin (2 mg/kg/day s.c.) or control injections for two weeks. Diabetic mice had a mild thrombogenic phenotype. Simvastatin treatment did neither influence glucose or thrombin-antithrombin complex levels in blood, nor did it alter the time to occlusion of the carotid artery after ferric chloride. Statin treatment in diabetic mice induced anti-inflammatory effects on the vessel wall indicated by 66% (p=0.027) lower levels of mRNA encoding vascular adhesion molecule-1 (VCAM-1) while a trend for lower mRNA levels of E-selectin (-22%) and MCP-1 (-30%) was observed. Gene expression levels of interleukin-1β, tissue factor, intercellular adhesion molecule-1, monocyte chemoattractant peptide-1, E-selectin, P-selectin and endothelial nitric oxide synthase did not differ after statin treatment. Immunostaining for tissue factor and fibrin of vessel wall and thrombi was also unaltered by simvastatin treatment. We conclude that the pleiotropic effects of simvastatin are primarily based on anti-inflammatory, rather than on a direct antithrombotic effect in diabetic mice.
Introduction

Diabetes mellitus type 2 (DM2) is a leading cause of vascular morbidity and death. People with type 2 diabetes have a 2-4 times greater risk of coronary heart disease or stroke than the general population (1; 2). The majority of these large vessel complications result from acute thrombotic occlusion of an atherosclerotic artery (3). A contributor to this increased risk of thrombotic occlusions may be a so-called procoagulant state that is associated with DM2. Two major procoagulant features have been identified that may induce thrombogenicity in patients with DM2: first, a dysbalance of hemostatic and procoagulant factors in plasma and second, dysfunction and activation of vascular endothelial cells. Several studies have shown that patients with diabetes have increased levels of procoagulant markers such as prothrombin fragment 1+2 (4), D-dimer (4), thrombin anti-thrombin (TAT) complexes (5), fibrinogen (6), factor VII (7) and an increased expression of tissue factor (TF) on circulating monocytes (8). Although there is evidence that increased levels of these markers are associated with macro- and/or microvascular disease (9; 10), the contribution of changes in plasma coagulation proteins to the pathogenesis of vascular complications is controversial. Endothelial dysfunction and activation is frequently observed in patients with DM2 and is associated with hyperglycemia but also with other cardiovascular risk factors (11). Endothelial dysfunction is typically associated with decreased nitric oxide (NO) production and increased platelet and leukocyte adhesion, which may contribute to a prothrombotic phenotype in DM2 (12). In addition, activated endothelial cells can produce cytokines and express adhesion molecules (13) as well as TF (14) that alter the normal anticoagulant into a prothrombotic endothelial cell phenotype (15; 16). Furthermore, a relation between procoagulant proteins in plasma and increased markers of endothelial dysfunction, such as soluble thrombomodulin has been observed (4), suggesting that these two mechanisms are causally associated in diabetes mellitus.

Clinical trials have demonstrated that hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or statins significantly reduce the risk of cardiovascular complications in patients with and without diabetes (17-20). There is strong evidence that statins have additional actions beyond cholesterol lowering, the so-called pleiotropic effects of these agents. Such additional effects include improvement of endothelial function by increasing NO production (21-23) and by reducing the inflammatory response in endothelial cells (24) or in
atherosclerotic plaques (25-27). In addition, statins might display anti-thrombotic properties by reducing expression of TF in endothelial cells and leucocytes (28) and by inhibiting both the formation of thrombin and ex vivo clot formation (29-31). Although it is likely that these pleiotropic effects of statins contribute to their beneficial effects, there is only little evidence that the anti-inflammatory and anti-coagulant effects translate into a direct in vivo anti-thrombotic activity. Some studies have addressed this issue by using an ex-vivo flow perfusion system showing that ex vivo thrombus formation is reduced after statin treatment (32; 33). Gaddam et al showed that the time to thrombosis was increased in rats treated with atorvastatin 1.25 mg/kg for 10 days in a model of ferric chloride induced in vivo arterial thrombosis (34). The antithrombotic effect in the latter study appeared to be related to increased constitutive nitric oxide synthase (cNOS) expression. As far as we know, the effect of statins on in vivo experimental acute arterial thrombosis has not yet been studied in a model of diabetes. In the present study we first investigated the effect of hyperglycemia on arterial thrombus formation in mice and second we explored the potential antithrombotic action of a statin in the same diabetic mouse model.

**Mice, materials and methods**

**Animals and Treatment**

Female C57BL/6 mice were obtained from Charles River (Maastricht, The Netherlands). The study was approved by the Institutional Animal Care and Use Committee of the Academic Medical Center, University of Amsterdam, the Netherlands.

Diabetes was induced by a single intraperitoneal infusion of streptozotocin (STZ) (200 mg/kg bw) in 0.05 M citrate buffer (pH 4) (35) at the age of 8 weeks (n= 10 mice). If one STZ injection did not induce hyperglycemia (> 10 mmol/L) after 4 days, the injection was repeated. Control mice (n= 11 mice) were injected with citrate buffer alone. To investigate the effect of statin treatment on thrombus formation in diabetic mice, in a second experiment animals (n=8 mice) were treated daily with one subcutaneous injection of simvastatin (2 mg/kg) (Merck, Darmstadt, Germany) or control injection (simvastatin diluant) (n= 9 mice) for two weeks starting 8 weeks after the STZ injection. This dose was based on Endres et al. who showed that prophylactic treatment with 2 mg/kg simvastatin daily for
2 weeks reduces the size of ischemic strokes in mice after focal brain ischemia (23). Before injection the inactive, lacton form of simvastatin was activated according to the manufacturer's protocol. Simvastatin (50 mg) was dissolved in 1 ml ethanol. For activation 0.813 ml 1 M NaOH was added. Before injection pH was neutralized with 1 M HCL.

**Arterial thrombosis**

Ten weeks after STZ injection mice were anesthetized by intraperitoneal injection of 70 μl/g FFM mixture (Fentanyl (0.315 mg/ml) - Fluanisone (10 mg/ml) (Janssen Pharmaceutical, Beerse, Belgium), Midazolam (5 mg/ml) (Roche, Mijdrecht, The Netherlands)). Body temperature was monitored with a rectal probe and maintained at 37°C ± 1°C via a heating pad and a halogen-heating lamp. During anesthesia oxygen (1 L/min) was supplied via a tube placed at the nose of the mouse. Acute arterial thrombosis was induced by application of ferric chloride (25% FeCl₃) to the left carotid artery, as described (36) resulting in the formation of platelet and fibrin-rich thrombi (37). Before and after application of ferric chloride a Doppler flow probe was placed around the artery to measure blood flow. Time to occlusion (TTO) was defined as the time after initiation of arterial injury with ferric chloride required for blood flow to decline to < 0.2 mL/min (see Figure 1). Closing time was defined as the time from the start of the flow reduction i.e. thrombus formation to the final occlusion (see Figure 1).

![Figure 1. Typical example of flow pattern during one experiment with measurement of Time to Occlusion and Closing Time. 1: Time to Occlusion; 2: Closing Time](image)

**Plasma analysis**

Blood glucose levels were measured with a Glucometer® Elite (Bayer Diagnostics, Mijdrecht, The Netherlands) in a drop of blood from the tail vein. At the end of the experiment blood from the vena cava was sampled into a plastic syringe
containing 0.1 volume of 3.2% sodium citrate. Plasma levels of thrombin-antithrombin (TAT) complexes were measured by a specific murine sandwich TAT ELISA, developed in our laboratory (38): rabbits were immunized with a mixture of 500 μl Freund's Complete Adjuvant (Difco, Detroit, MI, USA) mouse thrombin (T) or rat antithrombin (AT) (Sigma, St.Louis, MO, USA) to obtain antithrombin and anti-AT antibodies. The combination of anti-thrombin antibodies as capture antibody and digoxigenin-conjugated anti-AT as detection antibody gave optimal discrimination between different TAT concentrations (39).

Tissue harvesting and histological analysis
At the end of the experiments, the left carotid arteries were formalin-fixed, embedded in paraffin and sectioned. For immunohistochemical analysis paraffin sections of 4 μm were deparaffinized and rehydrated. Sections were incubated with 1.5% H₂O₂ in PBS for 20 minutes and then blocked with TENG-T (10mM Tris, 5 mM EDTA, 0.15 M NaCl, 0.25% gelatin, 0.05% (vol/vol) Tween-20%, pH 8.0) for 30 minutes at room temperature. Thereafter sections were washed and incubated with primary antibodies against TF and fibrin O/N at 4° C. Rabbit anti-mouse TF antibody antibodies were developed in our laboratory by immunization of rabbits with a mixture of 500 ml Freund's Complete Adjuvant (Difco) and murine TF peptide P5 (38; 40). Final concentration of anti-TF antibody was 1.3 μg/ml. Rabbit anti-rat fibrin antibody was kindly provide by Dr. J. Emeis, TNO, The Netherlands and used in a 1:1000 dilution (41). After incubation with the primary antibodies tissue sections were washed and goat anti-rabbit (DAKO A/S, Glostrup, Denmark) was used as the secondary antibody in a 1:250 dilution for 1 hour at room temperature, and than washed with PBS. The sections were incubated with SABC complex (DAKO A/S) for one hour at room temperature, and washed. Enzyme activity was detected with AEC (Sigma) after incubation for 5 minutes. Specificity controls included normal rabbit immunoglobulin in place of specific primary antibodies. For evaluation of TF and fibrin immunostaining a semi quantitative score approach was chosen using a magnification of 20 times. For TF quantification the following criteria were used: 0: no staining in thrombus and vessel wall; 1: focal staining in thrombus; 2: diffuse staining in thrombus; 3: positive staining in thrombus and focal staining in vessel wall. For fibrin immunostaining the following criteria were used: 0: no staining in thrombus; 1: focal positive staining in thrombus. The degree of staining was evaluated blinded for treatment strategies.
Quantitative RT-PCR

Expression of genes involved in inflammation and coagulation, interleukin 1-Beta (IL-1β), TF, intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), monocyte chemoattractant peptide-1 (MCP-1), endothelial selectin (E-selectin), platelet selectin (P-selectin) and endothelial nitric oxide synthase (eNOS) was analyzed on mRNA level by quantitative real time PCR as earlier described (42). Total RNA was extracted from homogenates from the descending aorta using TriZol reagent (Life Technologies) according to the manufacturer’s instructions. Purified RNA was reverse transcribed (RevertAid M-Mul V Reverse Transcriptase) according to the protocols supplied by the manufacturer. Quantitative gene expression was performed on an ABI PRISM 7700 combination of hardware and software (Applied Biosystems, Foster City, CA) using SYBR Green technology. PCR primers were designed using Primer Express 1.7 software with the manufacturer’s default settings (Applied Biosystems) and validated for identical efficiencies (slope = -3.3 for a plot of Ct versus log ng cDNA). SYBR Green master mix (19 µl) was added to 5 µl cDNA (corresponding to 50 ng of total RNA input) and 300 nM of forward and reverse primers in water. The samples were heated for 2 min at 50°C and 10 min at 95°C. Subsequently 40 PCR cycles consisting of 15 sec at 95°C and 60 sec at 60°C were applied. At the end of the run, samples were heated to 95°C with a ramp time of 20 min to construct dissociation curves to check that single PCR products were obtained. The absence of genomic DNA contamination in the RNA preparations was confirmed by using total RNA samples that had not been subjected to reverse transcription. Acidic ribosomal phosphoprotein PO (36B4) was used as the standard housekeeping gene (42). Ratios of target gene and 36B4 expression levels (relative gene expression numbers) were calculated by subtracting the threshold cycle number (Ct) of the target gene from Ct of 36B4 and raising 2 to the power of this difference. Ct values are defined as the number of PCR cycles at which the fluorescent signal during the PCR reaches a fixed threshold. Target gene mRNA expressions are thus expressed relative to 36B4 expression.

Statistics

Data are expressed as means ± SEM. Non-normal distributed and categorical data are expressed as medians with 25-75 quartiles. For comparison between normal distributed variables of interest the Student t test was used. The association
between non-normal distributed variables was assessed using the Mann-Whitney test. P values < 0.05 are considered statistically significant.

Results

Determination of levels of glucose and TAT complexes

Blood glucose levels (diabetic: 22.2 ± 2.0 vs. non-diabetic: 6.7 ± 2.5 mmol/l; p < 0.001) were significantly higher in the diabetic mice as compared to control mice. After the induction of carotid thrombosis the level of plasma TAT complexes was increased in diabetic mice (diabetic 2.2 (0.5; 21.2) vs. non-diabetic: 0.38 (0.2; 1.0) ng/ml; p=0.032). In the second experiment treatment with simvastatin had neither an effect on glucose levels (diabetic non-treated (n= 9): 20.6 ± 6.9 vs. diabetic simvastatin treated mice (n= 8): 16.0 ± 6.6 mmol/l; p=0.2) nor on the level of TAT complexes (diabetic non-treated: 0.67 (0.35; 1.85) vs. diabetic simvastatin treated mice: 0.69 (0.37; 1.25) ng/ml; p=0.6).

Acute arterial thrombosis

Ten weeks of hyperglycemia had no effect on time to occlusion (TTO) (Figure 2A). However, we observed a shortening of the time from the start of thrombus formation until occlusion (closing time) (Figure 2B) in the diabetic mice compared to control mice (non-diabetic mice 3.6 ± 0.5 vs. diabetic mice 2.0 ± 0.3 min; p=0.008). Treatment with simvastatin 2 mg/kg had neither a significant effect on TTO (non-treated: 8.9 ± 0.7 vs. treated: 7.5 ± 0.6 min) (p= 0.16) nor on closing time in diabetic mice (non-treated: 2.5 ± 0.2 vs. treated: 2.6 ± 0.3 min) (p > 0.81).

![Figure 2](image)

**Figure 2.** A) No effect of STZ induced diabetes on Time to Occlusion (TTO). B) More rapid thrombus formation in STZ induced diabetic mice, measured from the start of thrombus formation to final occlusion (Closing Time). Closing time was only measured when a thrombus was formed during the experiment (in 9 diabetic mice; 1 vessel did not occlude after ferric chloride and in 8 control mice; 3 vessels did not occlude).
Simvastatin and arterial thrombus

**Figure 3.** Carotid artery occluded by platelet rich (*) thrombus with red blood cells (**). Ferric chloride (***) is present in the vessel wall and in the vessel lumen.

Thrombus composition and immunohistochemistry

Serial sections of the left carotid artery segment that had been treated with ferric chloride showed total occlusion by a platelet rich thrombus (Figure 3). Occasionally some accumulation of erythrocytes amidst platelets was seen in adjacent sections. Ferric chloride was seen in the vessel wall and on the endothelium in the vessel lumen of the segment that had been treated. No difference could be observed in thrombus composition between mice with and without diabetes. Thrombus composition in arteries from mice with simvastatin treatment was also not different to those from non-simvastatin treated mice.

**Figure 4 A)** Example of focal TF staining (*) in thrombus. Positivity is observed at the borders of the thrombus.

**Figure 4 B)** Example of focal fibrin staining (*) in thrombus.

Occluded arteries from mice with and without diabetes showed similar immunostaining for fibrin and TF (Table 1, Figure 4A and B). No difference was observed in immunostaining for fibrin and TF in the occluded vessel from diabetic mice treated with simvastatin or control treatment (Table 1).
Table 1. Data are presented as medians with 25th–75th percentiles. No significant differences were observed in immunostaining of thrombi from non-diabetic mice, diabetic mice and diabetic mice with simvastatin treatment.

<table>
<thead>
<tr>
<th>Staining of thrombus in carotid artery</th>
<th>Non-diabetic mice (N=11)</th>
<th>Diabetic mice (N=19)</th>
<th>Diabetic mice with simvastatin treatment (N=8)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue factor</td>
<td>1.0 (1.0-2.5)</td>
<td>2.0 (1.0-3.0)</td>
<td>2.0 (1.0-3.0)</td>
<td>0.72</td>
</tr>
<tr>
<td>Fibrin</td>
<td>1.0 (0.0-1.0)</td>
<td>1.0 (0.25-1.0)</td>
<td>1.0 (0.25-1.0)</td>
<td>0.76</td>
</tr>
</tbody>
</table>

RT-PCR
Gene expression of VCAM-1, ICAM-1, TF, MCP-1, E-selectin, P-selectin, eNOS and IL-1β in the aorta was compared between all control mice (n=11), diabetic mice (n=19) and diabetic mice treated with simvastatin (n=8). In contrast to our expectations the relative gene expression levels of mRNA encoding VCAM-1, ICAM-1, TF, MCP-1, E-selectin, P-selectin and eNOS and did not differ between diabetic and non-diabetic aortas (Figure 5A-G). The level of IL-1β mRNA was even significantly lower in aortas from diabetic mice (0.42 \times 10^{-3}, 0.22 \times 10^{-3}, 0.93 \times 10^{-3}) as compared to aortas from control mice (1.0 \times 10^{-3}, 0.6 \times 10^{-3}, 1.8 \times 10^{-3}) (P=0.04) (Figure 5H, black and white bars).

After treatment with simvastatin a significant reduction was observed in the level of VCAM-1 mRNA (-66%) (P=0.027) (Figure 5A) in diabetic mice as compared to non-treated diabetic mice (n=18). Furthermore, we observed a trend towards reduced levels of mRNA encoding E-selectin (-22%) (P=0.076) and MCP-1 (-30%) (P=0.091) (Figure 5E and D). Gene expression levels of ICAM-1, TF, MCP-1, P-selectin, eNOS and IL-1β did not differ after statin treatment.

Discussion
A prothrombotic state, characterized by an increased level and activity of procoagulant plasma proteins (10) as well as an enhanced expression of procoagulant, adhesion and other inflammatory proteins on the endothelial cell surface (43) has been suggested to contribute to the increased risk of vascular thrombotic complications in patients with DM2. Accumulating evidence suggests that treatment with statins reduces the incidence of cardiovascular events not only by its cholesterol-lowering effects but also by its antithrombotic and anti-inflammatory properties (44-46). By using a model of ferric chloride induced endothelial injury, we studied the effect of simvastatin treatment on in vivo arterial thrombus formation and vascular wall inflammation in mice with STZ induced diabetes mellitus.
Figure 5. Relative mRNA levels in aorta's from control mice (control; n = 11), diabetic mice without simvastatin treatment (DM; n=19) and diabetic mice treated with simvastatin (DM+statin; n=8). * significantly lower VCAM-1 mRNA in diabetic mice with simvastatin treatment as compared to diabetic mice without simvastatin treatment (p = 0.027).

* significantly lower MCP-1 mRNA and IL-1β mRNA in diabetic mice after simvastatin as compared to non diabetic control mice without simvastatin treatment (p = 0.036 and p=0.0037).

# significantly lower IL-1β mRNA in non-treated diabetic mice (p=0.039) as compared to non-diabetic control mice.
First, we demonstrated that mice with STZ induced diabetes have a mild thrombogenic phenotype indicated by an increased rate of induced arterial thrombosis and an increased level of TAT complexes after thrombus formation as compared to control mice. The mechanism of this thrombogenic phenotype is not explained by altered vessel wall properties, because we did not detect any changes in the levels of mRNA encoding TF or pro-inflammatory cytokines in the aortas from mice with diabetes. The IL-1β mRNA level was even lower in the vessel wall of diabetic mice. The mRNA data are supported by immunohistochemical staining, which did not reveal any increase in TF staining in the vessel walls of diabetic as compared to the vessels of control mice. These observations are in apparent contrast with data from mainly in vitro studies showing that hyperglycemia enhances the expression of TNFα, IL-1β and TF in endothelial and other vascular cells (43).

The findings in the diabetic mice in these studies suggest that in the STZ induced diabetes model, hyperglycemia does not induce a state of vascular inflammation/activation even after 10 weeks of hyperglycemia. A limitation of the present study is that we did not investigate the effects of hyperglycemia against a background of atherosclerosis, which obviously is a clinically more relevant condition. However, even clinical studies of the influence of hyperglycemia on large arterial vessel disease are still inconclusive (47-50) and the debate on the importance of hyperglycemia as an independent risk factor in diabetes has not been settled yet (51; 52).

It is likely that other factors play a role in the observed enhanced closure rate and thrombin generation in diabetic mice. Such factors may relate to the influence of hyperglycemia on phospholipids surfaces, which in the case of platelets or microparticles may enhance the rate of thrombin generation thus leading to an increased tendency of thrombus formation (53-55). The influence of these circulating factors on thrombus formation is the subject of ongoing investigations in this mouse model.

Accumulating data suggest that statins have direct vascular protective effects by increasing blood flow via increased bioavailability of nitric oxide, attenuating the level of expression of pro-inflammatory genes and proteins also diminishing leukocyte adherence (25-27). A possible mechanism involves the inhibition of production of isoprenoids (44; 46), which will affect cell proliferation and activation in the vessel wall that may influence vasomotor function, inflammatory
responses and plaque stability. These mechanisms may contribute to the reduction of atherothrombotic complications by statin treatment (45). Gaddam and co-workers showed, using the ferric chloride induced arterial thrombosis model in rats, that the time to thrombus formation was increased in animals treated with atorvastatin (1.25 mg/kg) for 10 days as compared to control rats (34). The antithrombotic effect of atorvastatin in this model appeared to be related to increased expression of constitutive nitric oxide synthase (cNOS). Under basal conditions, nitric oxide (NO) is produced mainly by cNOS (neuronal NOS (nNOS) and endothelial NOS (eNOS)). The authors suggested that the increased expression of cNOS reduces the formation of platelet rich thrombi via inhibition of platelet aggregation and increased vasodilatation. In our experiment the same ferric chloride model of in vivo acute arterial thrombosis was used, however eNOS mRNA expression was not influenced by simvastatin (2 mg/kg) for 2 weeks. The absence of an effect in our study on NOS may be explained by differences in statins, doses or other factors. However, also various other studies on NO related effects of statins in diabetes mellitus have produced controversial findings. Treatment with simvastatin for 6 (56) and 24 weeks (57) or treatment with atorvastatin for 4 weeks (58) did not result in NO dependent endothelial function changes. In contrast, other studies did report NO dependent endothelial function changes in diabetes even after a few days (59-61).

Our study revealed another important anti-inflammatory effect of simvastatin in the aorta vessel wall of diabetic mice indicated by a reduced level of VCAM-1 mRNA. In addition, trends towards reduced levels of mRNA for E-selectin and MCP-1 appear to support the anti-inflammatory action of simvastatin in this model, independent from the presence of hyperglycemia. Assuming that the effect on mRNA translates into protein, the net result may be attenuated leukocyte activation and adhesion, which may play a very important role in the risk of atherosclerosis and its long term complications. However, these anti-inflammatory effects do apparently not influence the thrombogenic properties of the arterial vessel wall. This may also be related to unaltered levels of TF mRNA and protein after simvastatin treatment.

In conclusion, we demonstrate that simvastatin did not reduce acute arterial thrombus formation or thrombin formation in diabetic mice with a mild prothrombotic phenotype, while we did observe reduced aortic mRNA levels of
VCAM-1 and a trend towards lower levels of E-selectin and MCP-1. These data suggest that beneficial clinical effects of statins in preventing atherothrombotic complications in the presence of hyperglycemia may be based on the anti-inflammatory action on the vessel wall rather than on a direct antithrombotic effect.

Acknowledgments

We like to thank Joost Daalhuisen en Ingvild Kopp from the Academic Medical Center for their invaluable assistance. Hugo ten Cate is a Clinical Established Investigator of the Netherlands Heart Foundation (1998T13). Johan Kuiper is an Established Investigator of the Netherlands Heart foundation (2000T040). Hjalmar Hansen and Arnaud Hauer were supported by grants from the Netherlands Heart Foundation (2000B062 and 2000.198, respectively).

References


