ORIGINAL ARTICLE

Effect of statins on sirtuin 1 and endothelial nitric oxide synthase expression in young patients with a history of premature myocardial infarction

Erken miyokart enfarktüsü öyküsü olan genç hastalarda statinlerin sirtuin 1 ve endotel nitrik oksit sentaz ekspresyonu üzerine etkisi

Aylin Hatice Yamaç, M.D.,[1] Ülkan Kılıç, M.D.[2]

[1]Department of Cardiology, Bezmialem Foundation University Faculty of Medicine, İstanbul, Turkey
[2]Department of Medical Biology, Health Sciences University Faculty of Medicine, İstanbul, Turkey

ABSTRACT

Objective: The present study was an investigation of the effect of statins on the expression of circulating sirtuin 1 (SIRT1) and endothelial nitric oxide synthase (eNOS) proteins, and on the distribution of single nucleotide polymorphisms (SNPs) of the SIRT1 gene in patients with a history of premature myocardial infarction (PMI).

Methods: A total of 108 patients who had suffered from a premature ST-elevation myocardial infarction (STEMI) under the age of 45 years were enrolled in this study. While 79 patients had been taking statins since the index event, 29 patients had discontinued statin treatment after hospital discharge due to noncompliance or insufficient information about the importance of continuous statin therapy in post-MI patients. The control group consisted of 91 healthy patients without a previous cardiovascular event. The levels of SIRT1 and eNOS protein; oxidative stress markers, like total antioxidant status (TAS), total oxidant status (TOS), and the oxidative stress index (OSI); as well as the distribution of the SNPs rs7069102 and rs2273773 were measured and analyzed.

Results: A significant increase in the SIRT1 level ($p<0.001$) and a significant decrease in the eNOS level ($p=0.001$) was observed in all genotypes and alleles for both SNPs in patients who received statin therapy compared with the control group. Both SNPs were distributed in a similar frequency in the 2 MI groups, irrespective of statin treatment.

Conclusion: Statins induce SIRT1 protein, which might have a cardioprotective role after PMI. In addition, the eNOS protein level was low in all of the MI patients, suggesting that impairment of eNOS expression is disease-specific without a causal link to SIRT1.

ÖZET

Amaç: Bu çalışma ile, statinlerin, erken miyokart enfarktüsü (EME) geçiren hastalarda dolaşımdaki SIRT1 ve endotel nitrik oksit sentaz (eNOS) proteinlerinin ekspresyonu üzerindeki ve SIRT1 geninin tekli nükleotit polimorfizmlerinin (SNP) dağılımı üzerindeki etkisi araştırılmıştır.

Yöntemler: Bu çalışmaya, 45 yaşın altında ST yükselmeli miyokart enfarktüsü (STYEME) geçiren 108 hasta dahil edildi. İndeks olaydan sonra 79 hasta statin kullanırken, 29 hasta taburculuk sonrası uyumsuzluk ya da yetersiz bilgilendirme sonucu statin tedavisini bırakmıştır. Kontrol grubu, daha önce herhangi bir kardiyovasküler olayı bulunmayan 91 sağlıklı hasta tarafından oluşturuldu. Kan örnekleri en erken ME'den 3 ay sonra alındı. Oluşturulan 3 çalışma grubunda SIRT1 ve eNOS proteinlerinin miktarı, toplam antioksidan statüsü (TAS), toplam oksidan statüsü (TOS) ve oksidatif stres indeksi (OSI) gibi oksidatif stres belirteçlerinin yanı sıra SNP rs7069102 ve rs2273773'ün dağılımı incelendi.

Bulgular: Her iki SNP için statin tedavisi alan hastalarda, tüm genotiplerde ve allellerde, kontrol grubu ile karşılaştırıldığında, SIRT1 düzeylerinde anlamlı bir artış ($p<0.001$) ve eNOS düzeylerinde belirgin bir azalma gözlemledik ($p=0.001$). Her iki SNP de, statin tedavisinden bağımsız olarak, iki ME grubu arasında benzer bir frekansta dağılım gösterdi.

Sonuç: Statinler erken ME sonrası kalbi koruyucu bir rol oynayabilecek SIRT1 proteinini uyarmaktadır. Diğer taraftan, eNOS protein seviyeleri statin tedavisine bakılmaksızın ME geçirmiş olan hastalarda düşük bulunmuştur. Bu da eNOS ekspresyonundaki azalmanın SIRT1 ile nedensel bir bağı olmadan hastalığa özgü olduğunu düşündürmektedir.

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Correspondence: Dr. Aylin Hatice Yamaç. Bezmialem Vakıf Üniversitesi Tıp Fakültesi, Kardiyoloji Anabilim Dalı, İstanbul, Turkey.
Tel: +90 212 - 691 52 62 e-mail: haticeyamac80@yahoo.de

Statins, which are widely used as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, decrease the low-density lipoprotein (LDL) level by inhibiting cholesterol synthesis. They are very well known to reduce adverse cardiovascular events and mortality in patients with stable coronary artery disease (CAD) and in patients with an acute coronary syndrome (ACS), the latter group receiving a high-dose statin very early.[1–3] As the prevalence of risk factors for CAD, such as impaired glucose tolerance and obesity, is increasing in young people,[4] it has been suggested that the incidence of premature myocardial infarction (PMI) will also rise in the coming years. Thus, primary and strict secondary prevention in young adults with myocardial infarction (MI) are of key importance.

Post-MI patients with high normal or only mildly elevated lipid levels also benefit from statin therapy.[5] In addition, a retrospective study has suggested that there may be substantial benefit from statin therapy in previously untreated patients with a baseline LDL ≤80 mg/dL (2.1 mmol/L).[6] This observation is consistent with the recommendations that all patients with ACS should be treated with statin therapy, and is also consistent with the hypothesis that the pleiotropic effects of statins contribute to the therapeutic benefit. In addition to lowering LDL, statins exhibit a number of pleiotropic effects, including the improvement of vascular endothelial function,[7–9] the attenuation of vascular inflammation,[7,10] plaque stabilization,[11] correction of prothrombotic tendencies[2,11] and the attenuation of myocardial remodeling.[12]

One such pleiotropic effect was demonstrated in our previous work, indicating a link between statins and the SIRT1 protein in patients with stable CAD and PMI.[13]

SIRT1, known as a longevity gene, protects cells against oxidative stress and promotes DNA stability by binding and deacetylating several substrates.[14] In the cardiovascular system, SIRT1 has been recognized as a key regulator of vascular endothelial homeostasis, angiogenesis, endothelial senescence, and dysfunction.[15,16] It prevents atherosclerosis by improving endothelium relaxation through up-regulating endothelial nitric oxide synthase (eNOS) expression and the production of nitric oxide (NO).[17]

In cardiomyocytes, due to its antioxidant activity, nuclear SIRT1 increases the resistance of the myoblast to oxidative stress by enhancing the expression of manganese-dependent superoxide dismutase[18] or activation of the FoxO1-dependent pathway,[19] the latter yielding reduced cardiac infarct volume and improved functional recovery after ischemia/reperfusion in mice.[20]

Abbreviations:

ACS	*Acute coronary syndrome*
BMI	*Body mass index*
CAD	*Coronary artery disease*
eNOS	*Endothelial nitric oxide synthase*
HDL	*High-density lipoprotein*
LDL	*Low-density lipoprotein*
LVEF	*Left ventricle ejection fraction*
MI	*Myocardial infarction*
NFκB	*Nuclear factor kappa B*
NO	*Nitric oxide*
OSI	*Oxidative stress index*
PCR	*Polymerase chain reaction*
PMI	*Premature myocardial infarction*
SIRT1	*Sirtuin 1*
SNP	*Single nucleotide polymorphism*
STEMI	*ST-elevation myocardial infarction*
TAS	*Total antioxidant status*
TG	*Triglycerides*
TOS	*Total oxidant status*
VLDL	*Very low-density lipoprotein*

In our previous study, SIRT1 protein was significantly increased in patients with stable CAD,[21] whereas in patients receiving statin therapy, SIRT1 was significantly decreased to levels similar to those seen in healthy participants, while eNOS expression was increased.[13] There was no association between the frequencies of SIRT1 single nucleotide polymorphisms (SNPs) and the cardioprotective effect of statins.[13] These results indicated that statin treatment could mediate its cardioprotective effect through the inhibition of SIRT1 expression in patients with chronic CAD.

However, no studies examining the relationship between statin treatment and the expression levels of SIRT1 protein, and SIRT1 SNPs in post-MI patients at young age have been reported. The present study was an investigation of the effect of statins on the expression of circulating SIRT1 and eNOS proteins, and on the distribution of the SNP genotype frequencies rs7069102 and rs2273773 in patients with a history of PMI.

METHODS

Study groups

The retrospective study groups consisted of 108 patients who had suffered from a premature ST-elevation MI (STEMI) before the age of 45 years (87.0% men; mean age: 40.74±3.82 years) and 91 control subjects (57.1% men; mean age: 32.66±6.31 years). The power analysis of this study was performed with 80% power and a 95% confidence interval. The difference

in SIRT1 protein between the 2 groups (STEMI vs control) was at least 0.7 ng/mL for any subgenotype, with a SD of 1.7. Corresponding to the power analysis, a minimum of 94 patients in the STEMI group was necessary to calculate a significant difference.

Patients enrolled in this study were selected from people who underwent primary PCI at the departments of cardiology of Bezmialem Vakif University Hospital and Mehmet Akif Ersoy Heart Hospital between January 2012 and May 2015. Twenty-nine patients had discontinued statin treatment after hospital discharge due to noncompliance or insufficient information about the importance of continuous statin therapy in post-MI patients. Seventy-nine patients had been taking statins, such as simvastatin or atorvastatin, since the index event. Patients with malignancies, major trauma or surgery in the previous 6 months, acute or chronic infectious disease, or any kind of immune-mediated disease were excluded. The randomly selected healthy controls were also recruited from people who came to Bezmialem Vakif University Hospital for a routine examination and they had no prior history of cardiovascular diseases.

This study was approved by the ethics committee of the Bezmialem Vakif University Faculty of Medicine. All of the participants, after providing written informed consent, completed a structured questionnaire in order to collect demographic data. The study was conducted in accordance with the ethical principles described in the Declaration of Helsinki.

Biochemical and demographic analysis

Blood samples were obtained in uncoated tubes after 12 hours of fasting (Vacuette, Greiner Greiner Bio-One GmbH, Kremsmünster, Austria). The samples were centrifuged for 5 minutes at 4500 rpm at +4°C, followed by the separation of serum and plasma, and then were stored at -20°C. The following biochemical parameters were determined in both the control and experimental groups at Bezmialem Vakif University Hospital using standard laboratory methods: fasting glucose, total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, and very low-density lipoprotein (VLDL). Body mass index (BMI) was calculated by dividing weight by height square (kg/m^2) and categorized according to the World Health Organization recommendations.

Determination of SIRT1 and eNOS protein levels

The level of SIRT1 and eNOS proteins in the samples was analyzed using enzyme-linked immunosorbent assay kits from USCN Life Science, Inc. (Catalog no: E94912Hu for SIRT1 and SEA868Hu for eNOS, Wuhan, China). Standards and samples were incubated with antibody-coated 96-well plates. Enzyme-linked antibodies for the proteins were then added. The intensity of the color was measured in a microplate reader (Chromate 4300; Awareness Technology, Inc., Palm City, FL, USA) at a wavelength of 450 nm.

Measurement of total antioxidant and oxidant status

The total antioxidant status (TAS) and the total oxidant status (TOS) of serum were determined using an automated measurement method (Rel Assay Diagnostics, Gaziantep, Turkey) (28,29) and the Chromate analyzer.[20] The data were expressed as mmoL Trolox equivalent/L. The oxidative stress index (OSI) was calculated using the formula OSI=((TOS)/(TASx1000)) x100.[20]

DNA isolation

Blood samples of all of the participants were drawn into tubes containing ethylenediaminetetraacetic acid and the genomic DNA was isolated from peripheral blood leukocytes with a DNA isolation kit (EasyDNA™ gDNA Purification Kit, Invitrogen, Carlsbad, CA, USA). All purified DNA samples were stored at +4°C until the polymerase chain reaction (PCR) technique was performed, as described previously.[21]

Determination of SIRT1 gene polymorphisms

SIRT1 rs7895833 A>G in the promoter region, rs7069102 C>G in the intron 4, and rs2273773 C>T in the exon 5 gene polymorphisms were analyzed using PCR with confronting two-pair primers assay, as described previously, with minor modifications (20, 32) Three different segments of the SIRT1 gene encompassing rs7895833 A>G, rs7069102 C>G, and rs2273773 C>T polymorphic sites were amplified by PCR using the primers as described.[21]

Statistical evaluation

Statistical analyses of differences in the distribution of the genotypes or alleles in the SIRT1 gene between patients and the control group were tested using a chi-square test for categorical variables. If the expected

values were less than 5 in 2x2 tables, the Fisher's exact test was used. Normality tests, such as the Kolmogorov-Smirnov test, were used to illustrate the distribution of the variables. Parametric assumption tests were used as appropriate.

Clinical characteristics and protein levels were compared using Student's t-test and one-way analysis of variance. The relative risk was calculated as an odds ratio with a 95% confidence interval. Pearson's correlation test was also applied to determine the relationship between the SIRT1 expression level and other parameters. The accuracy and significance of the correlation coefficient was tested with a t-test. The Hardy-Weinberg equilibrium was assessed with a chi-square test. The analyses were performed using a standard software package (PASW Statistics for Windows, Version 18.0; SPSS Inc., Chicago, IL, USA). The test results were accepted as significant if the p value was <0.05.

RESULTS

Biochemical and demographic analysis

The demographic and clinical characteristics of the study participants are summarized in Tables 1 and 2. The statin-positive and statin-negative groups were predominantly male, whereas gender was balanced in the control group (87% vs 86% vs 57.1%, STEMI vs control; p=0.003). The control group also tended to be younger compared with the overall STEMI population (40.74±3.82 years vs 32.7±6.3 years; p<0.001). There was statistically no significant difference in the prevalence of smoking history (88.6% vs 82.7%; p=0.410), diabetes mellitus (17.7% vs 20.7%; p=0.900), or hypertension (39.2% vs 31%; p=0.566) between the statin-positive and statin-negative groups. Patients under statin therapy had a higher frequency of a positive family history for CAD (64.6% vs 41.4%; p=0.048) and of dyslipidemia, compared with untreated MI patients (78.5% vs 58.6%; p=0.042). There was no significant difference in the level of fasting blood glucose, triglycerides (TG), total cholesterol, HDL-cholesterol, VLDL, TG/HDL, or uric acid between the statin-positive and statin-negative groups (p>0.05 for all parameters). The LDL level was markedly lower in patients taking statins compared with the untreated patients (p=0.035), who had levels comparable to those of the control group. The baseline values of patients treated with statins were not determined.

There was no significant difference in left ventricle ejection fraction (LVEF) between the statin groups (53.0±8.7% vs 50.3±9.3%; p=0.178).

Expression levels of SIRT1 and eNOS proteins, and levels of TAS, TOS, and OSI

The SIRT1 level increased (p<0.001), whereas the eNOS level decreased significantly (p<0.001) in patients under statin therapy compared with the controls (Table 3). In statin-negative patients, the SIRT1 level was higher and the eNOS level was lower in comparison with the control group, but did not reach a significant value. There was no remarkable difference in SIRT1 and eNOS protein expression between the statin groups; however, the SIRT1 level tended to be higher in patients treated with statins (Table 3). Pearson's test demonstrated a significant, negative correlation between SIRT1 expression and TG/HDL values in the statin-negative patients (p=0.05), while the correlation was not significant in the statin-positive group (p=0.572) (Table 4). Further, there was a positive correlation between the SIRT1 and TOS levels in the statin-negative patients (p=0.032). A positive correlation between the eNOS level and TAS was found in both groups, irrespective of statin treatment (statin-negative: p=0.018, statin-positive: p=0.013). In patients treated with statins there was a negative correlation between the eNOS and LDL levels (p=0.021) and a positive correlation between the eNOS level and TG/HDL (p=0.029). In the non-CAD controls, a negative correlation between the eNOS level and BMI was detected (p=0.039). Not all correlations are shown in Table 4.

Frequency of SIRT1 (rs7069102 and rs2273773) gene variants and their relationships to SIRT1, eNOS, and TAS levels

The frequency of genotypes and alleles of the SIRT1 gene in all of the groups is shown in Figure 1.

For rs2273773, the homozygous wildtype genotype AA was more frequently found in PMI patients than in controls, whereas the heterogeneous AG genotype was more often seen in the non-CAD control patients (Fig. 1a). There was no statistically significant difference in the genotype and allele distribution between the statin-positive and statin-negative patients.

For rs7069102, in the control population, the frequency of the homozygous mutant genotype GG was

Table 1a. Demographic and clinical characteristics of the study participants

	Statin + (n=79)	Statin – (n=29)	Control (n=91)	*p*
Age (years)	40.7±3.8	40.7±3.9	32.7±6.3	n.s
Male gender (%)	69 (87.3)	25 (86.3)	52 (57.1)	0.991
Body mass index (kg/m^2)	28.9±4.2	27.9±3.6	24.7±3.9	0.450
Family history (%)	51 (64.6)	12 (41.4)*	28 (30.8)	0.048
History of smoking (%)	70 (88.6)	24 (82.7)	58 (63.8)	0.410
Diabetes mellitus (%)	14 (17.7)	6 (20.7)	3 (3.3)	0.900
Dislipidemia (%)	62 (78.5)	17 (58.6)*	11 (12.1)	0.042
Arterial hypertension (%)	31 (39.2)	9 (31)	2 (2.2)	0.566
Recurrent myocardial infarction	10 (12.7)	2 (6.9)	–	0.433
Percutaneous coronary intervention at FU	14 (17.7)	3 (10.3)	–	0.403
Heart failure development at FU	1 (1.3)	2 (6.9)	–	0.117
Hospitalization at FU	17 (21.5)	5 (17.2)	–	0.629
Localization of MI (anterior/inferior/other) (%)	40/14/25	16/7/6	–	0.339
	(50.6/17.7/31.6)	(55.2/24.1/20.7)		
Left ventricular ejection fraction (%)	53.0±8.7	50.3±9.3	61.3±3.6	0.178

Statistical evaluation performed using one-way analysis of variance with post-hoc Tukey's test. Results are shown as mean±SD. Significant at *p<0.05 statin- group compared with statin+ group. The p values represent the results of analysis between the statin + and statin - groups.
MI: Myocardial infarction; FU: Follow-up.

Table 1b. Demographic and clinical characteristics of the study participants

	Statin + (n=79)	Statin – (n=29)	Control (n=91)	*p*
Fasting blood glucose	138.08±97.48	132.61±56.65	100.31±35.72	0.931
Total cholesterol	178.89±52.14	192.93±52.14	183.27±40.13	0.376
Low-density lipoprotein	104.05±47.05*	124.76±38.30	118.85±36.04	0.035
High-density lipoprotein	37.63±9.75	36.69±9.68	49.19±13.92	0.938
Triglycerids	193.49±117.69	201.17±106.88	142.37±127.98	0.955
Very low-density lipoprotein	38.69±23.53	38.68±22.36	30.26±28.74	1.000
Triglycerides/high-density lipoprotein	6.18±5.71	6.04±3.81	3.27±3.34	0.990

Statistical evaluation performed using one-way analysis of variance with post-hoc Tukey's test. Results are shown as mean±SD. Significant at *p<0.05 compared with statin - group. The p values represent the results of analysis between the statin + and statin – groups.

slightly greater than in both PMI groups, without reaching a significant value. There was no statistically significant difference in the genotype distribution between the statin groups (Fig. 1b). The heterogeneous CG genotype was more frequent in PMI patients than in controls (p<0.001). In patients carrying both allele types, the frequency of the CC wild-type genotype was low in all study participants (statin treated patients: n=9, non-treated patients: n=4, controls: n=10), limiting the analysis of the variation in SIRT1 level with respect to statin therapy in patients carrying the wild-type genotype.

The association of SIRT1 and eNOS levels and the distribution of genotypes and alleles for both SNPs are provided in Table 5.

In patients who received statin treatment, the increase in SIRT1 expression for rs7895833 was significant in all of the screened genotypes and alleles (mutant or wildtype) compared with the control group (p<0.05). In patients who carried both allele types and did not receive a statin, the SIRT1 level was similar to the level found in the healthy control patients.

The expression of the eNOS protein in patients who

Table 2. Demographic and clinical characteristics of the study participants

	Statin + (n=79)	Statin – (n=29)	Control (n=91)	*p*
Beta-blocker	69 (87.3)	25 (86.2)	0	1.000
ACE-inhibitor or ARB	60 (75.9)	22 (75.9)	1 (1.1)	0.992
Spironolactone	3 (3.8)	2 (6.9)	0	0.609
Other diuretics	1 (1.3)	1 (3.4)	0	0.467
Acetylsalicylic acid	78 (98.7)	18 (96.6)	1 (1.1)	0.467
Other antithrombotic agents	38 (48.1)	19 (65.5)	0	0.122
Oral antidiabetes drug	9 (11.4)	0	3 (3.3)	0.724
Insulin	2 (2.5)	0	0	0.287

Statistical evaluation performed using a chi-square or Fisher's exact test. Results are shown as mean±SD. The p values represent the results of analysis between the statin + and statin - groups. ACE: Angiotensin-converting enzyme; ARB: Angiotensin II receptor blocker.

Table 3. Comparison of SIRT1, eNOS protein, TAS, TOS, and OSI levels in the study population

	Statin + (n=79)	Statin – (n=29)	Control (n=91)	*p*
SIRT1 protein (ng/mL)	1.33±1.15*	0.99±0.73	0.70±0.46	<0.001
eNOS protein (pg/mL)	258.46±265.09*	320.72±426.76	509.95±571.03	<0.001
TAS (mmol Trolox Equiv./L)	1.87±0.32	1.91±0.38	1.83±0.32	0.817
TOS (mmol H2O2 Equiv./L)	14.08±7.19	13.60±7.99	12.92±5.69	0.944
OSI (Arbitrary units)	0.80±0.56	0.72±0.37	0.71±0.32	0.692

Statistical evaluation performed using analysis of variance. The results are shown as mean±SD. *P<0.05 vs control group. There was no statistically relevant difference between the statin + and statin - groups. eNOS: Endothelial nitric oxide synthase; OSI: Oxidative stress index; SIRT1: Sirtuin 1; TAS: Total antioxidant status; TOS: Total oxidant status.

carried all of the screened allele types and who were treated with a statin was significantly lower than in the control group (p<0.05). Statin treatment did not significantly influence the expression level of eNOS protein in patients carrying the AA genotype (p=0.08).

Similar results were found for variant rs7069102 regarding the effect of statin treatment on SIRT1 and eNOS protein expression. In patients who did receive statin treatment, the increase in SIRT1 expression was significant in all of the screened genotypes and alleles (mutant or wildtype) compared with the control group (p<0.05). ENOS protein expression was significantly lower in patients under statin therapy compared with control subjects, except in patients with the GG genotype. For the GG genotype, the mean level of eNOS protein tended to be lower in PMI patients, regardless of statin therapy; however, in comparison with the control group, the difference was not statistically significant. It should be noted that the eNOS protein level varied a lot within the control group, which might mask a potential statistical significance between the groups.

DISCUSSION

In the present study, we investigated the effect of statins on the expression of SIRT1 and eNOS proteins, and on the distribution of the genotype frequencies rs7069102 and rs7895833 SNPs in young adults with a history of PMI. Our previous study demonstrated that SIRT1 protein expression was markedly elevated in patients with CAD.[13] SIRT1 protein expression was thought to be disease-related.

In the present study, patients with PMI who were taking statins had a markedly higher level of SIRT1 compared with the controls. There was no significant difference between the statin-negative and the control group patients, although the SIRT1 level tended to be higher in the statin-negative PMI group compared with the control group. These results suggest that SIRT1 expression is disease-related and is induced by statins.

This observation contrasts with the findings of our previous study, in which a normal level of SIRT1

Table 4. Results of Pearson's correlation between expression level of SIRT1 and other parameters

	Statin +		Statin –		Control	
	r	p	r	p	r	p
Endothelial nitric oxide synthase protein (pg/mL)	-0.148	0.200	-0.025	0.259	0.147	0.166
Total antioxidant status (mmol Trolox Equiv./L)	-0.177	0.143	0.022	0.917	-0.101	0.339
Total oxidant status (mmol H2O2 Equiv./L)	0.075	0.540	0.399	0.043*	0.007	0.949
Oxidative stress index	0.007	0.951	0.284	0.160	0.038	0.720
Age	0.224	0.005*	0.215	0.282	-0.120	0.258
Body mass index	0.049	0.674	0.099	0.624	-0.185	0.079
Fasting blood glucose	-0.036	0.757	-0.174	0.386	-0.144	0.186
High-density lipoprotein	-0.058	0.629	0.321	0.102	0.047	0.670
Low-density lipoprotein	0.012	0.923	-0.377	0.053	-0.068	0.535
Triglycerides	0.024	0.842	-0.339	0.084	-0.094	0.388
Triglycerides/high-density lipoprotein	-0.068	0.572	-0.380	0.050*		
SIRT1 protein (pg/mL)	-0.148	0.200	-0.025	0.259	0.147	0.166
Total antioxidant status (mmol Trolox Equiv./L)	0.296	0.013*	0.456	0.019*	-0.005	0.960
Total oxidant status (mmol H2O2 Equiv./L)	-0.100	0.410	-0.184	0.367	0.025	0.817
Oxidative stress index	-0.130	0.282	-0.299	0.137	0.024	0.821
Age	0.185	0.107	-0.108	0.591	-0.082	0.442
Body mass index	0.136	0.239	0.274	0.166	-0.217	0.039*
Fasting blood glucose	-0.148	0.202	-0.040	0.845	-0.040	0.712
High-density lipoprotein	-0.078	0.518	-0.157	0.435	0.036	0.744
Low-density lipoprotein	-0.271	0.021*	0.033	0.869	-0.090	0.412
Triglycerides	-0.005	0.969	0.290	0.142	-0.076	0.484

n, number of individuals. *p<0.05.

was restored with statin treatment.[13] Statins, known as cardio-protective agents, were supposed to reverse the pathomechanism of atherosclerosis by decreasing SIRT1.[13]

This discrepancy might be the result of some key differences in the patient characteristics in the 2 studies. In the present study, the patients were younger and had a history of an acute MI, mostly characterized

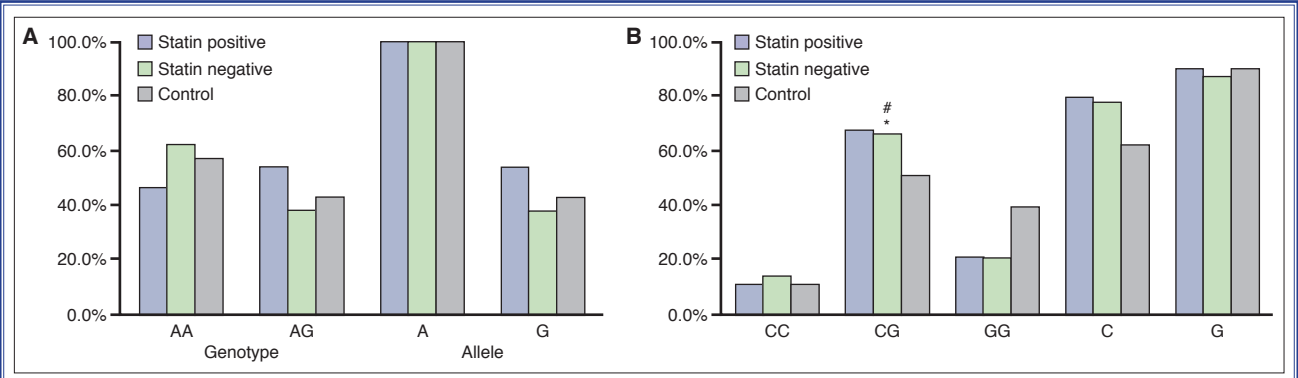

Figure 1. (A) Frequency of genotypes and alleles of the Sirtunin 1 gene in the study groups. There was no statistically significant difference in genotype and allele distribution between the statin-positive and statin-negative patients. **(B)** Frequency of genotypes and alleles of the Sirtunin 1 gene in the study groups. Statistical evaluation performed using analysis of variance. *P<0.001: Distribution of CG genotype compared with GG genotype. #P<0.001: Distribution of CG genotype compared with CC genotype.

Table 5. The association between Sirtuin 1 and endothelial nitric oxide synthase levels and the distributions of genotypes and alleles for both single nucleotide polymorphismss

	Statin +	Statin –	Control	*p*
rs7895833 A>G Genotype				
SIRT1 protein (ng/mL) Genotype				
AA	1.35±1.24*	1.11±0.68	0.71±0.49	0.002
AG	1.33±1.10*	0.81±0.80	0.69±0.43	0.003
GG	n.a			
Allele				
A	1.34±1.16*	0.98±0.73	0.70±0.46	<0.001
G	1.33±1.10*	0.81±0.80	0.69±0.43	0.003
eNOS protein Genotype				
AA	252.60±166.01	322.89±507.60	503.17±682.03	0.08
AG	267.34±334.00*	317.54±294.99	518.99±384.52	0.003
GG	n.a			
Allele				
A	260.36±266.32*	320.72±426.76	509.95±571.04	0.001
G	267.34±334.00*	317.54±294.99	518.99±384.52	0.003
rs7069102 C>G Genotype				
SIRT1 protein (ng/mL) Genotype				
CC	1.57±0.68*	1.20±0.76	0.66±0.40	0.008
CG	1.29±1.32*	0.97±0.81	0.70±0.47	0.011
GG	1.33±0.75*	0.88±0.43	0.72±0.48	0.002
Allele				
C	1.33±1.25*	1.01±0.79	0.69±0.46	0.001
G	1.30±1.20*	0.95±0.73	0.71±0.47	<0.001
eNOS protein Genotype				
CC	196.97±126.12*	470.37±451.59	479.73±416.91	0.008
CG	265.14±297.21*	334.14±477.91	428.06±304.50	0.011
GG	285.24±210.92	152.67±40.46	626.22±819.25	n.s
Allele				
C	250.87±279.62*	358.90±465.75	437.29±323.58	0.009
G	270.17±276.80*	294.69±427.374	513.68±589.20	0.005

Statistical evaluation performed using one-way analysis of variance with post-hoc Tukey's test. Results are shown as mean±SD. *Significant at p<0.05 compared with the control group. The level of significance in the binary comparison was accepted as 0.016 with a Bonferroni correction.

eNOS: Endothelial nitric oxide synthase; SIRT1: Sirtuin 1.

by single-vessel disease without diffuse atherosclerosis. Most of the patients recovered with an acceptable LVEF. In contrast, the patients in the previous study were older, had comorbidities, and suffered from chronic CAD, characterized by diffuse atherosclerosis with ongoing ischemia. It has been reported that the cross-talk between reactive oxygen species, as found in atherosclerosis, and the SIRT1 protein could be responsible for the pathology underlying cardiovascular disease.[22] Alcendor et al.[22] demonstrated that pathological levels of SIRT1 expression resulted in oxidative stress and apoptosis, and increased cardiac hypertrophy. However, it has to be underlined that in the present study, SIRT1 was induced moderately, and not to pathological levels.

Consistent with this explanation, we found a positive correlation between SIRT1 and TOS in the statin-negative patients. Yet there was no difference de-

tected between the absolute level of TOS, TAS, and OSI in patients receiving statins compared with the untreated patients with PMI and the control subjects. As the PMI patients were not marked by diffuse atherosclerosis and ongoing coronary ischemia, it is not surprising that the levels of oxidative stress markers did not differ between the 3 groups.

In addition to key differences in patient characteristics between the 2 studies, there are also differences in lipid management strategy. The PMI patients were mostly treated with high dose atorvastatin, up to 80 mg after the acute event for more than 3 months with a dose reduction in the follow up period. The therapy seemed to be very effective, as the LDL level was significantly lower ($p<0.05$) in the statin-positive group compared with the untreated PMI patients. Moreover, even the healthy controls had a higher LDL level than patients taking statins. Oxidized LDL, known as an atherogenic factor, impairs SIRT1; thus, statins might decrease the oxidized LDL level, which accordingly yields inhibition of SIRT1 impairment.[23] SIRT1 activation therefore results in improved glucose tolerance and lipid homeostasis and reduced inflammatory tone, which all are also atheroprotective.[24,25]

Other studies have demonstrated that atorvastatin up-regulates SIRT1 expression via inhibition of miR-34a, possibly contributing to the beneficial effects of atorvastatin on endothelial function in CAD.[26] Furthermore, a high concentration of simvastatin promoted the expression of SIRT1 and increased the proliferation of endothelial progenitor cells via SIRT1.[27]

Pharmacological SIRT1 inhibition has been reported to increase thrombosis by inhibiting tissue factor activation via nuclear factor kappa B (NFκB).[28] Similarly, cyclooxygenase-2-derived prostacyclin and peroxisome proliferator-activated receptor delta activation were found to decrease arterial thrombus formation by suppressing tissue factor in a sirtuin-1-dependent manner.[29] Thus, activation of SIRT1 protects against arterial thrombosis. In the context of atherosclerosis, pharmacological SIRT1 activation lowered plasma LDL levels by inhibiting proprotein convertase subtilisin/kexin 9 secretion, thereby increasing hepatic LDL-receptor availability and consecutive LDL cholesterol clearing.[30]

The TG/HDL ratio is a prognostic marker of all-cause mortality after ACS and is a risk factor for cardiovascular events.[31] The TG/HDL ratio is also predictive of the severity of CAD.[31] It could help predict in-hospital new-onset heart failure incidents of CAD patients.[32] In the present study, we detected a negative correlation between the SIRT1 level and the TG/HDL ratio in statin-negative patients, indicating that SIRT1 induction yielded degradation of TG/HDL. In statin-treated patients this effect is lost (Table 4), possibly due to its specific effects on lipid metabolism.

Taken together, the induction of SIRT1 by statins is thought to provide a protective role in the development of atherosclerosis, arterial thrombosis, and endothelial dysfunction.

The first evidence of a connection between SIRT1 and endothelial cells was that SIRT1 activated eNOS.[33] Later, studies in genetically engineered mouse models demonstrated that SIRT1 exerts atheroprotective effects by activating eNOS or by diminishing NFκB activity in endothelial cells and macrophages.[34–36] Moreover, pharmacological SIRT1 activation protected endothelial cells from senescence induced by disturbed flow.[37] Another report assigned SIRT1 in vascular smooth muscle cells a protective role against DNA damage, medial degeneration, and atherosclerosis.[38]

In the present study, we observed a significant decrease in the level of eNOS protein in patients who received statins compared with healthy controls. The eNOS level was also lower in the statin-negative group compared with the control group, but without statistical significance. There was no significant difference in eNOS expression between the statin-positive and statin-negative groups.

Studies have shown that vascular eNOS protein expression is usually diminished in atherosclerosis.[39] In a vascular disease, NO is degraded rapidly by a reaction with .O2_. .NO and .O2_ react rapidly to form peroxinitrite, which in turn leads to eNOS uncoupling and enzyme dysfunction due to oxidative stress.[40]

In our study, the level of eNOS protein was considerably lower in PMI patients compared with the control group (Table 3), whereas no correlation was found between SIRT1 and eNOS (Table 4), emphasizing that the modulation of eNOS expression is independent of SIRT1 in PMI patients.

In our previous publication, a positive correlation was demonstrated between eNOS and SIRT1 expres-

sion in patients receiving statins. Furthermore there was a positive correlation between eNOS expression and TAS level in patients treated with statins. However, a significant negative correlation was found between eNOS expression and TAS level in patients who did not receive statin therapy.

In the present study, there was a positive correlation between eNOS expression and TAS in both groups, irrespective of statin treatment. This implies that eNOS has an antioxidative effect that is not statin dependent.

In addition, we observed a negative correlation between LDL and eNOS levels in patients taking statins, indicating that less LDL is protective for the endothelium. The positive correlation between eNOS expression and the TG/HDL ratio suggested compensatory upregulation of eNOS under statin therapy with a greater TG/HDL ratio.

To summarize, statins induce SIRT1 protein, which has a cardioprotective role after MI. The eNOS protein level is low in MI patients, regardless of statin treatment, suggesting that impairment of eNOS expression is disease-specific and without a causal link to SIRT1. LDL reduction with statins is associated with increased eNOS expression, which particularly shows a LDL-dependent, but pleiotropic, cardioprotective effect of statins.

To understand the epigenetic effects of statins on the CAD phenotype with a genetic background, 2 SNPs in the SIRT1 gene were evaluated.

With respect to the relationship between genotype and phenotype, we observed a significant increase in the SIRT1 level and a significant decrease in the eNOS level in all genotypes and alleles for both SNPs in patients who received statin therapy compared with the control group.

In both patient groups, the SIRT1 and eNOS levels differed (Tables 3, 5), whereas the genotype frequencies were similar. These results suggest that the effect of statins on SIRT1 expression occurs post-transcriptionally or post-translationally, rather than through these SNPs. Individual variations due to epigenetic factors might explain the observed phenotypes.[41] Further studies are needed to better characterize the pleiotropic effects of statins on SIRT1 expression in CAD patients.

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