Original Article

Impact of Type 2 Diabetes on Nitric Oxide and Adrenergic Modulation of Myocardial Perfusion

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Type 2 diabetic patients are characterized by a reduced adenosine-induced hyperemic myocardial perfusion, which may contribute to their increased cardiovascular morbidity. We hypothesized that the reduced hyperemia can be explained by functional changes in endothelial or autonomic nervous regulation. In 12 type 2 diabetic patients without signs of ischemic heart disease and 14 age-matched control subjects, myocardial perfusion was measured at rest, during adenosine, and during adenosine and α-receptor blockade (phentolamine) using positron emission tomography on two separate days: *1*) with, and *2*) without nitric oxide (NO) inhibition with N^G-nitro-L-arginine methyl ester. Myocardial perfusion during adenosine was lower in type 2 diabetic patients compared with control subjects ($P = 0.05$). No significant effect of NO inhibition on myocardial perfusion during adenosine was found in any of the groups. In control subjects, α-receptor blockade increased hyperemic myocardial vascular resistance during NO inhibition, whereas no effect was observed in type 2 diabetic patients. At rest, a significant correlation was observed between rate-pressure product and myocardial perfusion in control subjects. NO inhibition and type 2 diabetes abolished this correlation. Endothelial and cardiac autonomic nerve function seems to play only a minimal role in the reduced hyperemic myocardial perfusion in type 2 diabetic patients. However, the linear correlation between resting perfusion and cardiac work appears to be abolished in type 2 diabetes and during NO synthase inhibition. *Diabetes* 56:468–475, 2007

Although the overall incidence of cardiovascular mortality has declined, the mortality attributable to diabetes increases. The etiology of the cardiac risk in diabetes is only partly understood and seems to consist of both structural and functional abnormalities in the cardiovascular system.

Studies of vascular reactivity in peripheral systemic arteries have documented that diabetic patients have a reduced vasodilatory response to direct stimulation of smooth muscle cells in the vessel wall and impairment of flow-mediated stimulation of the endothelium due to endothelial dysfunction (1–3). Whether these changes in the forearm vasculature reflect abnormalities in the myocardial perfusion is controversial (4).

Evaluation of the epicardial vascular reactivity with intracoronary ultrasound in diabetic patients has shown a reduction in maximal coronary flow reserve compared with nondiabetic control subjects (5). Furthermore, reduced vasodilation to acetylcholine stimulation of the endothelium and to a mixed sympathetic and endothelial stimulation with cold pressor testing in diabetic patients with angiographically normal coronary arteries has been demonstrated, indicating endothelial and possibly autonomic dysfunction (6,7). The requirement of coronary instrumentation has limited these studies to include only symptomatic or high-risk diabetic patients.

Studies of the myocardial microcirculation in diabetic patients compared with control subjects have consistently revealed a reduction in maximal myocardial perfusion induced by adenosine or dipyridamole (8–15). The mechanisms behind this phenomenon are unknown. Traditionally, adenosine- or dipyridamole-induced hyperemia has been considered a means of evaluating structural, endothelium-independent abnormalities of myocardial vessels, whereas the cold pressor test has been used as a tool for evaluating functional changes, because myocardial perfusion response to cold is mediated by a mixture of endothelial vasodilatory mechanisms and sympathetic stimulation. However, in a study by Buus et al. (16), it has recently been documented in young, healthy male volunteers that maximal myocardial perfusion during adenosine infusion is partly dependent on intact function of endothelial nitric oxide (NO) production. Furthermore, this study demonstrated that in young, healthy volunteers, a fine balance may exist between endothelial vasodilatory stimuli and sympathetic vasoconstrictory forces in regulation of hyperemic myocardial perfusion. It is unknown whether this regulatory system is influenced by diabetes and whether endothelial vasodilatory dysfunction or cardiac sympathetic neuropathy may constitute major components of the well-known reduced hyperemic response to adenosine in diabetic patients. A reduced response to stimulation of the endothelium (10–12,17,18) and alterations of cardiac sympathetic innervation and function (17–19) in diabetic patients has previously been observed.

Therefore, the aim of this study was to evaluate the impact of type 2 diabetes on the myocardial microvasculatory response to adenosine with and without NO inhibition. Second, we wanted to evaluate whether sympathetic

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L-NAME, N^G-nitro-L-arginine methyl ester; MVR, myocardial vascular resistance; PET, positron emission tomography; RPP, rate-pressure product.

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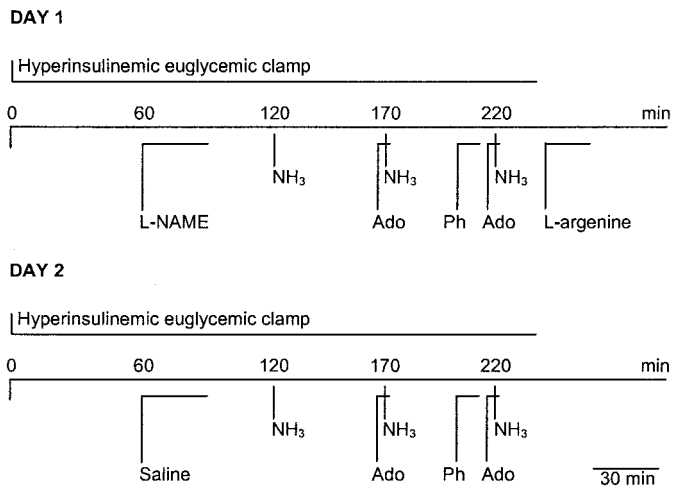

FIG. 1. Study days 1 and 2. Ado, adenosine; NH_3, [^{13}N]ammonia; Ph, phentolamine.

nerve activity has the same counterregulatory effect on endothelial vasodilation in diabetic patients as previously found in young, healthy control subjects. Finally, because the well-known coupling between cardiac work and myocardial perfusion has been shown to be disturbed in conditions with endothelial dysfunction, including smoking (20,21), we wanted to evaluate whether this coupling is influenced by type 2 diabetes and/or by pharmacological inhibition of the endothelial NO synthase.

RESEARCH DESIGN AND METHODS

Fourteen patients with type 2 diabetes and 14 age- and sex-matched control subjects were included in the study and underwent myocardial perfusion assessment with positron emission tomography (PET). However, two of the diabetic patients showed signs of reversible ischemia on their PET adenosine stress and rest perfusion examinations (22) and were excluded from further participation in the study protocol and referred to coronary angiography. The remaining 12 diabetic patients (time from diagnosis of diabetes 5 ± 5 years) and 14 age- and sex-matched control subjects had normal PET stress and rest perfusion studies by visual analysis and completed the entire protocol, except for one male control subject who only accomplished the saline protocol, one male control subject who had no saline phentolamine-adenosine scan, and one male diabetic subject who had no N^G-nitro-L-arginine methyl ester (L-NAME) phentolamine-adenosine scan. None of the participants had a history of cardiovascular disease and all had a normal clinical examination, normal electrocardiographic findings, no clinical signs of neuropathy, and preserved left ventricular function defined as left ventricular ejection fraction >50% evaluated by echocardiography. The two groups had equal baroreflexor reactivity evaluated by power spectral analyses of heart rate variability data obtained during supine rest and 60° upright tilt (23). All patients with diabetes received antidiabetic treatment (diet, sulfonylurea, or insulin). Medical records and laboratory data were checked to confirm the diagnosis (according to the American Diabetes Association criteria [24]) and treatment. In the diabetic group, antidiabetic and cardiovascular medication consisted of metformin (three patients), insulin (one patient), sulfonylurea (two patients), aspirin (two patients), statins (five patients), ACE inhibitors (three patients), angiotensin II receptor antagonist (one patient), calcium channel blocker (one patient), and Clopidogrel (one patient). All medication was paused 24 h before the perfusion measurements except for insulin, which was paused for 8 h. None of the control subjects received any medication. Patients with albuminuria >30 mg albumin/24 h, a total cholesterol >7 mmol/l, and systolic blood pressure >160 mmHg and/or diastolic blood pressure >100 mmHg were excluded from the study. None of the participants were smokers (20,21), and all refrained from intake of methylxanthines including caffeine for at least 24 h before the perfusion measurements (25). The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee. All participants signed an approved consent form before entering the study.

Flow charts of the PET scans are illustrated in Fig. 1. After an overnight fast, myocardial perfusion was measured by PET. On two separate days in random order, myocardial perfusion was measured: *1*) at baseline, *2*) during intravenous infusion of adenosine (140 $\mu g \cdot kg^{-1} \cdot min^{-1}$ from 3 min before to 2 min after the injection of [^{13}N]ammonia), and *3*) during adenosine infusion after administration of the α-adrenergic receptor antagonist phentolamine, initially 2.5 mg/min for 2 min. If the observed drop in systolic blood pressure was <20 mmHg, 0.5 mg/min was infused until a 20 mmHg drop was observed. Infusion of phentolamine was titrated based on the observed effect on blood pressure on an individual basis due to safety reasons because the combination of phentolamine and adenosine in some subjects caused brief but extensive drop in blood pressure (40 mmHg), most pronounced in the diabetic patients. Therefore, the median phentolamine doses were 10.0 (9.0–10.0) mg in the control subjects vs. 6.8 (5.0–9.8) mg in the diabetic patients ($P = 0.02$). On day 1, inhibition of NO synthase with L-NAME (133 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 30 min, equal to a total of 4 mg/kg) was induced 60 min before commencing the perfusion measurements. After termination of the perfusion measurements in protocol 1, the participants received an infusion of L-arginine (200 mg/kg over 15 min) for reversal of the L-NAME effect (26). On day 2, L-NAME infusion was substituted with infusion of saline.

During both protocols, blood pressure and heart rate were determined by automated sphygmomanometry and continuous 12-lead surveillance electrocardiogram before and during the pharmacological interventions and during perfusion measurements. L-NAME (Clinalfa, Laufelfingen, Switzerland) has been shown to reduce stimulated NO production with ~70% (26). Adenosine and L-arginine were prepared at the Aarhus University Hospital Pharmacy (Aarhus, Demmark). Phentolamine was delivered by Novartis Healthcare (Copenhagen, Denmark).

Myocardial perfusion. Myocardial perfusion was quantified with a positron emission tomograph (model Exact HR 961; Siemens/CTI) with intravenous [^{13}N]ammonia as perfusion tracer. A detailed description of the perfusion scanning procedure has previously been published (27). For each perfusion scan, 740 MBq [^{13}N]ammonia diluted in 10 ml saline was injected over 15 s. At the time of injection, acquisition of a dynamic sequence of images (12 frames of 10 s) was started to obtain time-activity curves from the blood pool and from the myocardium. After this sequence, three short static frames were obtained (two frames of 30 s and one frame of 60 s). Finally, a 900-s-long static frame was acquired to obtain high-resolution images used for correct delineation of the contours of the left ventricle and assignment of regions of interest. Between the baseline scan and the first hyperemia scan with [^{13}N]ammonia, a 15-min transmission scan was obtained to correct for photon attenuation.

Myocardial perfusion was quantified using a semiautomatic delineation technique and two-compartment model described in detail previously (28–30). In brief, the left ventricle was resliced according to the transaxial plane. Subsequently, the entire myocardium was delineated automatically from base to apex. The wall thickness was set according to the left ventricular wall thickness measurements obtained from echocardiography in each subject. After delineating the entire left ventricle, a polar map of the myocardium was created, and three regions of interest representing areas supplied by each of the three major coronary arteries were defined. Subsequently, myocardial perfusion was quantified by fitting the tissue and the blood pool time-activity curves to a validated two-compartment model for [^{13}N]ammonia. Correction for spill-over and partial volume is incorporated in this mathematical model. Because the participants had normal and homogenous tracer uptake in all scans, an average value for the entire myocardium is reported.

Hyperinsulinemic-euglycemic clamp. We used the glucose clamp method to obtain stable and comparable metabolic conditions during the experimental protocol because myocardial perfusion may be influenced by hyperglycemia and hyperinsulinemia (31,32). Insulin (Actrapid 100 IE/ml; Novo Nordisk, Gentofte, Denmark) infusion rate was 2 $mU \cdot kg^{-1} \cdot min^{-1}$ in the 1st hour followed by 1 $mU \cdot kg^{-1} \cdot min^{-1}$ for the rest of the examination. Plasma glucose concentration was clamped at 5 mmol/l by a variable infusion rate of 20% glucose (33). Every 5 min, plasma glucose concentrations were analyzed in duplicate on a Beckman glucose analyzer (Beckman Instruments, Palo Alto, CA) immediately after blood sampling using blood drawn from an arterialized venous canula. Whole-body glucose uptake given as M values (in mg glucose · kg total body $wt^{-1} \cdot min^{-1}$) was assessed using the glucose infusion rates from the final 30-min period of the study when the rate of glucose infusion approximates whole-body glucose disposal (time 209–239 min).

Calculations and statistical analysis. Data are presented as means ± SD or median (interquartile range) and squared correlation coefficients. Myocardial vascular resistance (MVR) is calculated as mean arterial blood pressure (diastolic pressure + 1/3 × [systolic blood pressure − diastolic blood pressure]) divided by myocardial perfusion and given in $mmHg \cdot ml^{-1} \cdot min^{-1} \cdot g^{-1}$. The rate-pressure product (RPP) is defined as the product of systolic blood pressure and heart rate and given in mmHg/min. For comparison between groups, Student's t test or the nonparametric Mann-Whitney rank sum test was used, as appropriate. The one-way ANOVA for repeated

TABLE 1
Baseline characteristics of control subjects and diabetic patients

	Control subjects	Diabetic patients
n	14	12
Age (years)	53 ± 6	57 ± 6
Men (*n*)	12	8
BMI (kg/m^2)	25.6 ± 2.0	29.0 ± 5.0
Heart rate (beats/min)	63 ± 11	89 ± 12
Systolic blood pressure (mmHg)	136 ± 12	141 ± 20
Diastolic blood pressure (mmHg)	86 ± 7	89 ± 12
Total cholesterol (mmol/l)	5.5 ± 0.8	4.3 ± 1.4
HDL cholesterol (mmol/l)	1.8 ± 0.4	1.3 ± 0.4
LDL cholesterol (mmol/l)	3.2 ± 0.8	2.6 ± 0.9
Triglycerides (mmol/l)	1.1 ± 0.3	1.7 ± 1.4
A1C (%)	5.5 ± 0.4	7.0 ± 1.5
Fasting plasma insulin (pmol/l)	42.5 ± 29.7	62.8 ± 30.3
Fasting plasma glucose (mmol/l)	5.3 ± 0.4	8.0 ± 2.7
M value (mg glucose · g^{-1} · min^{-1})	10.2 ± 4.6	6.0 ± 3.8

Data are means ± SD.

measures or the paired *t* test was used for comparisons of the effect of saline and L-NAME on outcome measurements. For nonparametric data, the repeated-measures ANOVA on ranks or the Wilcoxon's signed rank sum test was used. Comparisons of more than two mean values were modified according to Bonferroni if demonstrated to be significant. Correlations between RPP and myocardial perfusion were sought using Spearman's rank order correlation. *P* values <0.05 were considered statistically significant. All statistical tests were performed using Sigma Stat 3.1 from Systat Software.

RESULTS

Baseline and metabolic characteristics. Baseline characteristics of the two study groups are shown in Table 1. The diabetic patients had lower circulating total cholesterol and *p*-HDL values than the control subjects. Furthermore, the diabetic group had lower whole-body glucose uptake (*M* value, $P = 0.02$) and higher fasting plasma glucose ($P < 0.001$), fasting plasma insulin ($P = 0.06$), and A1C ($P < 0.01$) compared with the control group.

Hemodynamic results. Hemodynamic results are shown in Table 2. At baseline, no difference was found in heart rate between the two groups, although a trend was found toward a higher heart rate in the diabetic patients ($P = 0.17$). NO synthase inhibition significantly reduced heart rate and increased mean arterial pressure in both diabetic patients and control subjects in all three scan sequences. Adenosine alone and in combination with α-blockade with phentolamine increased heart rate and RPP in the two groups. However, in the diabetic patients, there was a trend toward a decreased response in reflex heart rate and in RPP from rest to the combined α-blockade and adenosine infusion compared with control subjects (increase in heart rate 21 ± 15 vs. 32 ± 13 beats/min, $P = 0.06$; increase in RPP 853 ± 3,047 vs. 2,809 ± 2,401 mmHg/min, $P = 0.09$). After NO inhibition, no differences in heart rate or RPP responses were found between the groups.

In both groups, mean arterial pressure was reduced during adenosine in combination with α-blockade compared with rest. This reduction was more pronounced in the diabetic patients than in the control subjects, although not significant (reduction in mean arterial pressure 23 ± 17 vs. 15 ± 12 mmHg, respectively, $P = 0.14$). During NO inhibition the reduction in mean arterial pressure was similar (diabetic patients 13 ± 10 mmHg; control subjects 12 ± 11 mmHg, $P = 0.5$).

Myocardial perfusion and MVR. Myocardial perfusion values are shown in Table 2, Fig. 2, and Fig. 3, and MVR values are shown in Table 2 and Fig. 4. No differences were found in resting myocardial perfusion between diabetic patients and control subjects either with or without NO synthase inhibition with L-NAME. During adenosine infusion, myocardial perfusion was lower in the diabetic patients than in the control group. NO inhibition abolished this difference between the two groups, since perfusion in the control subjects showed a slight, insignificant decrease, whereas perfusion in the diabetic patients showed a slight and insignificant increase to 3.02 ml · g^{-1} · min^{-1} (mainly driven by a single outlier seen in Fig. 3*B*). During α-blockade, there was no difference in myocardial perfusion between the two groups during adenosine infusion, either with or without NO inhibition. In the control subjects, α-blockade had no significant effect on myocardial perfusion. In the diabetic patients, however, α-blockade without NO inhibition induced a reduction in hyperemic

TABLE 2
Hemodynamic parameters, myocardial perfusion, and MVR

	Scan	HR (beats/min)	RPP (mmHg/min)	MAP (mmHg)	MP (mL · g^{-1} · min^{-1})	MVR (mmHg · ml^{-1} · min^{-1} · g^{-1})
Control subjects						
Saline	Rest	61 ± 13	7,807 ± 2,478	94 ± 8	0.81 ± 0.23	123 ± 24
	Ado	89 ± 17*	11,463 ± 2,335*	92 ± 10	3.25 ± 0.80*†	30 ± 6*
	Ado +Ph	94 ± 19*	10,717 ± 3,090*	79 ± 13*‡	2.90 ± 0.78*	29 ± 8*
L-NAME	Rest	47 ± 5§‖	7,086 ± 1,406‖	114 ± 14§	0.65 ± 0.15§	181 ± 38§
	Ado	68 ± 18*§	10,057 ± 2,904*	110 ± 12§	2.98 ± 0.75*	38 ± 7*§
	Ado +Ph	77 ± 16*§	10,875 ± 2,409*	102 ± 8*‡§	3.31 ± 0.81*	33 ± 8*‡
Diabetic patients						
Saline	Rest	68 ± 10	8,996 ± 2,254	96 ± 14	0.93 ± 0.15	106 ± 23
	Ado	87 ± 13*	10,478 ± 2,576*	87 ± 12	2.66 ± 0.58*	34 ± 10*
	Ado +Ph	89 ± 20*	9,849 ± 3,788*	73 ± 14*‡	2.19 ± 0.61*‡	36 ± 13*
L-NAME	Rest	55 ± 8§	8,855 ± 2,464	120 ± 18§	0.68 ± 0.13§	181 ± 40§
	Ado	73 ± 12*§	11,694 ± 3,448*	118 ± 23§	3.02 ± 0.68*	41 ± 12*
	Ado +Ph	79 ± 13*¶	11,698 ± 2,749*	107 ± 17*‡§	2.98 ± 0.62*§	38 ± 10*

Data are means ± SD. Ado, adenosine; Ado + Ph, adenosine with phentolamine; HR, heart rate; MAP, mean arterial pressure; MP, myocardial perfusion. *$P < 0.05$ vs. rest; †$P = 0.05$, diabetic patients vs. control subjects; ‡$P < 0.01$, adenosine vs. adenosine + phentolamine; §$P < 0.01$, L-NAME vs. saline in same scan sequence (i.e. L-NAME rest vs. saline rest); ‖$P < 0.05$, diabetic patients vs. control subjects; ¶$P = 0.03$, L-NAME vs. saline in same scan sequence.

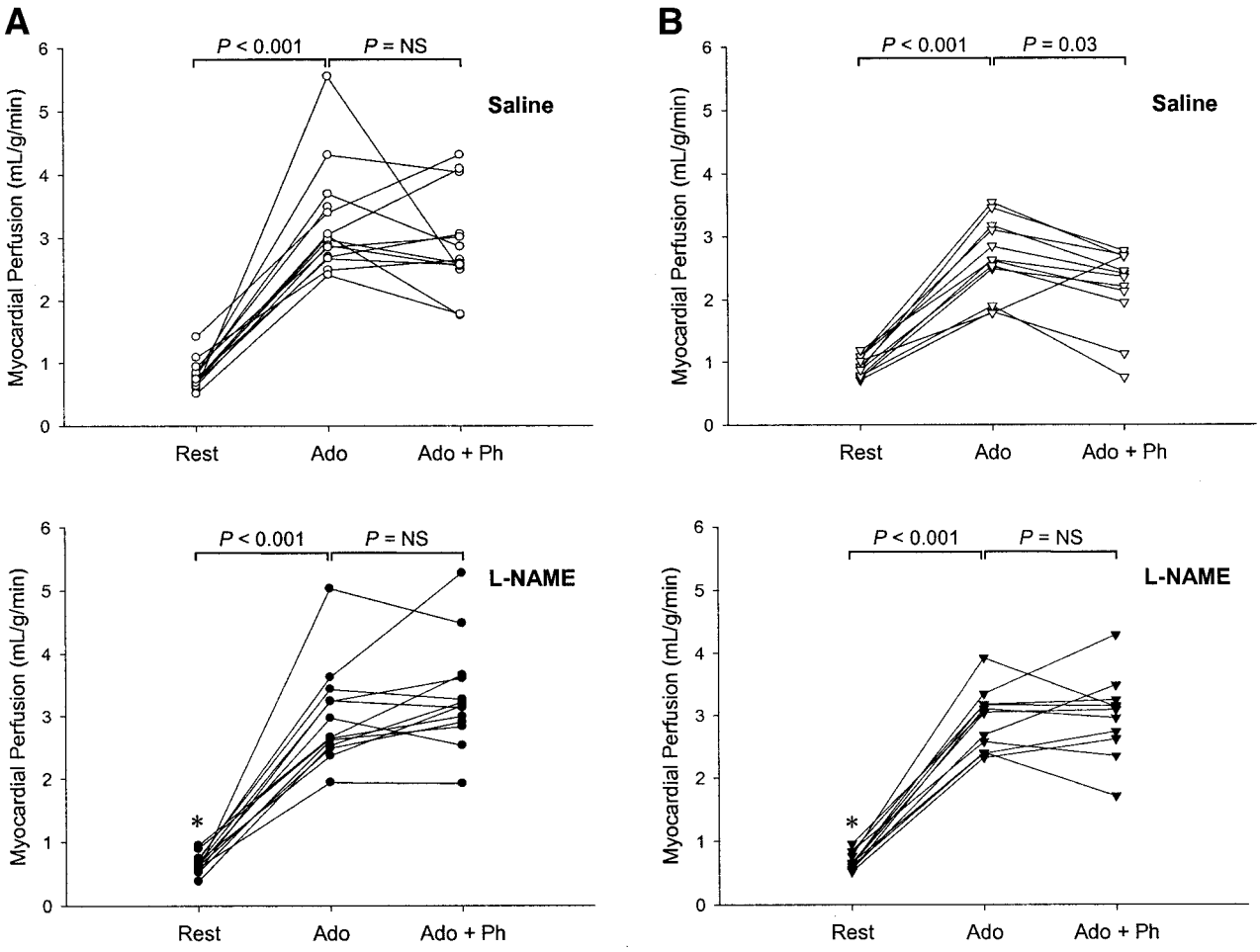

FIG. 2. Myocardial perfusion during PET studies. *A*: Control subjects. *B*: Diabetic patients. *Top*: Perfusion during saline. *Bottom*: Perfusion during NO synthase inhibition with L-NAME. Ado, adenosine; Ado + Ph, adenosine with phentolamine. **P* < 0.01, L-NAME vs. saline in same scan sequence (L-NAME rest vs. saline rest).

perfusion, whereas in the presence of NO inhibition, no perfusion changes were observed.

As shown in Fig. 4, NO inhibition mediated an increase in MVR at rest in both diabetic patients and control subjects (of 76 ± 44 and 58 ± 31 mmHg · ml^{-1} · min^{-1} · g^{-1}, respectively, $P = 0.3$ between groups). During adenosine infusion, a substantial reduction in MVR compared with rest was observed in both groups with the largest reduction during NO inhibition (reduction in control subjects 141 ± 38 vs. 71 ± 19 mmHg · ml^{-1} · min^{-1} · g^{-1}; diabetic patients 142 ± 40 vs. 93 ± 27 mmHg · ml^{-1} · min^{-1} · g^{-1}, $P <$ 0.001 in both groups). In the control subjects, NO inhibition caused a slight but significant increase in MVR during adenosine. No difference was found in MVR during adenosine between the two groups either with or without NO inhibition. Without NO inhibition, α-blockade did not affect MVR during adenosine infusion in any of the groups. During NO inhibition, α-blockade induced a slight but significant reduction in MVR in the control group. In the diabetic group, however, no such effect of NO inhibition during α-blockade was observed.

In Fig. 5, correlations between RPP and myocardial perfusion at rest are shown for the control subjects (Fig. 5*A*) and the diabetic patients (Fig. 5*B*) with and without the presence of NO synthase inhibition with L-NAME. Without NO inhibition, a significant correlation was found between myocardial perfusion and RPP in the control subjects. However, when NO synthase was inhibited, no such correlation could be demonstrated. In the diabetic patients, no correlation was found between RPP and myocardial perfusion either with or without NO inhibition. From Fig. 5*A*, the correlation found in the control group may look as if driven primarily by the outlying subject with high rate pressure and perfusion values. However, when performing the correlation test without this subject, the correlation is still intact ($r^2 = 0.35$, $P = 0.03$).

DISCUSSION

Our findings of a reduced adenosine-induced maximal hyperemic myocardial perfusion in diabetic patients without signs of ischemic heart disease compared with age-matched healthy control subjects confirm findings from previous studies (8–15). A main finding in the present study is that adenosine-induced hyperemia seems not to be NO dependent in type 2 diabetic patients and only to a minor extent in a matched middle-aged control group. NO inhibition increased MVR during adenosine infusion in the control subjects only and thereby abolished the difference observed in adenosine-induced hyperemia between the two groups. However, in absolute terms, the effects of NO inhibition on MVR in the two groups were small and thus may not reflect biological differences between the two groups. Furthermore, no significant effect of NO inhibition

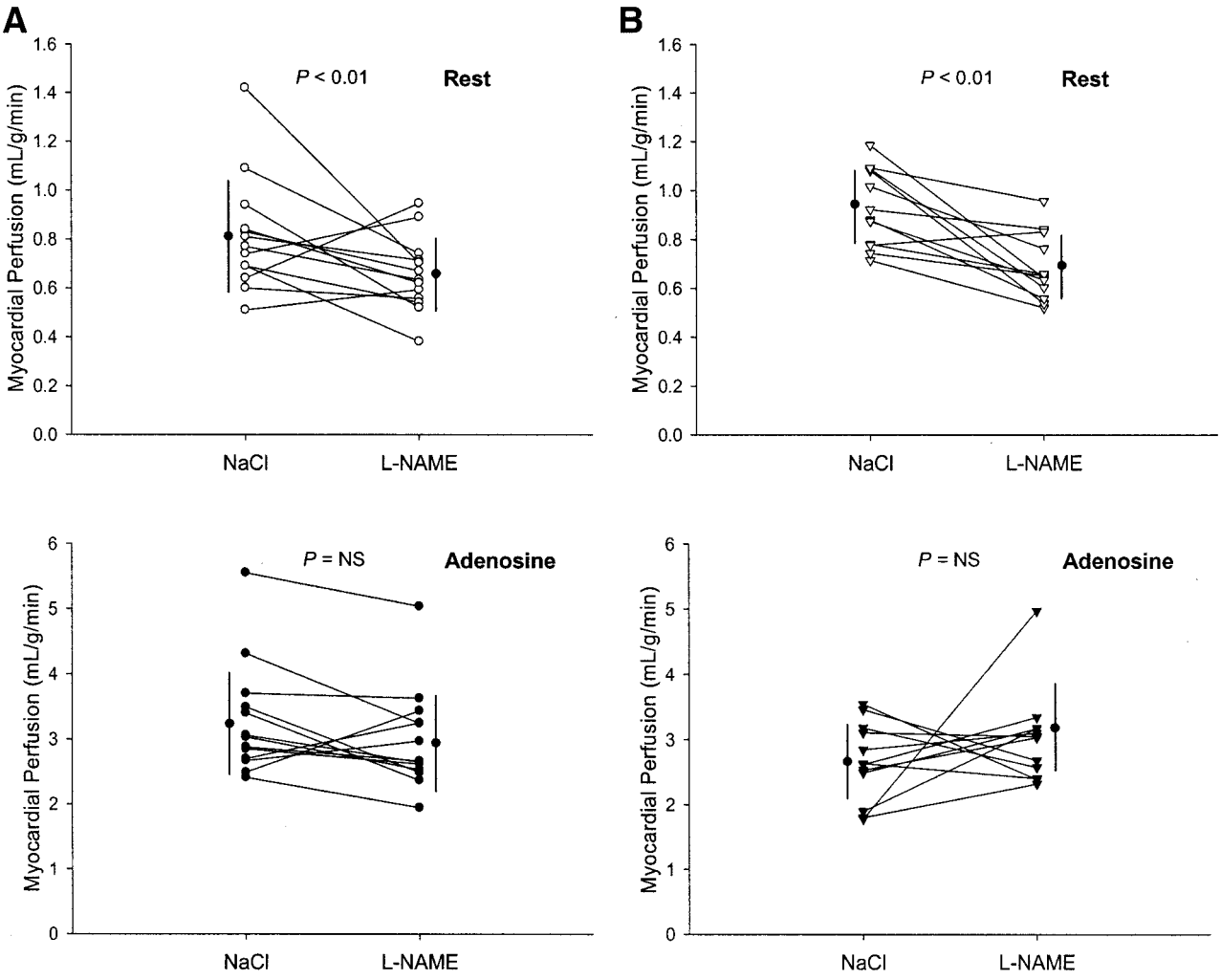

FIG. 3. Myocardial perfusion during saline and during NO synthase inhibition with L-NAME. *A*: Control subjects. *B*: Diabetic patients. *Top*: Perfusion during rest. *Bottom*: Perfusion during adenosine. Black circles with vertical bars indicate mean ± SD.

on myocardial perfusion was observed in any of the groups. This seems to be in contrast to the results by Buus et al. (16), who found that NO inhibition significantly reduced myocardial perfusion during adenosine by ~20% in young, healthy volunteers (age 23–27 years), whereas the reduction in the present study was only ~10% in the middle-aged healthy control subjects. Our results, however, are in accordance with previous results showing a declining myocardial perfusion reserve (27) and a decreasing endothelium-dependent dilation of the coronary arteries with increasing age (34). Therefore, although several studies have shown signs of endothelial dysfunction in diabetic patients compared with nondiabetic subjects (10–12,17,18), this does not seem to affect adenosine-induced myocardial hyperemia in diabetic patients.

In young, healthy volunteers it has previously been demonstrated that in the presence of NO inhibition, α-adrenergic blockade decreases MVR during adenosine infusion (16). These findings indicate that in young subjects, a balance exists between α-adrenergic vasoconstriction and endothelium-dependent vasodilation during adenosine-induced hyperemia. In diabetic patients, previous studies have revealed possible interactions between sympathetic autonomic dysfunction and disturbances in myocardial perfusion. A PET study of patients with type 1 diabetes showed a decreased sympathetic innervation assessed with uptake of [^{11}C]meta-hydroxyephedrine in the heart together with an abnormal myocardial perfusion response to adenosine and to sympathicus-mediated endothelium stimulation with cold pressor test (19). The same was observed in a study of both type 1 and type 2 diabetic patients (18). In the present study, α-adrenergic blockade had no effect on myocardial perfusion during adenosine in the control subjects. In the diabetic patients, however, an 18% reduction in hyperemic myocardial perfusion was found, which was abolished by L-NAME. Most likely, the reduction was due to changes in hemodynamic parameters despite the lower phentolamine doses used in the diabetic patients compared with the control subjects. In agreement, after correction for hemodynamic changes, no effects of α-adrenergic blockade on MVR were observed in the diabetic patients either with or without NO inhibition.

Our data show a 50% reduction in hyperemic response in MVR to α-adrenergic blockade in healthy, middle-aged control subjects compared with that previously found in the young volunteers (16). This may reflect age-related attenuation of NO production and cardiovascular autonomic function (35). The lack of response on MVR in the diabetic patients in the present study might also be due to even more pronounced cardiovascular autonomic alterations compared with the middle-aged control subjects. However, we were unable to demonstrate a difference in

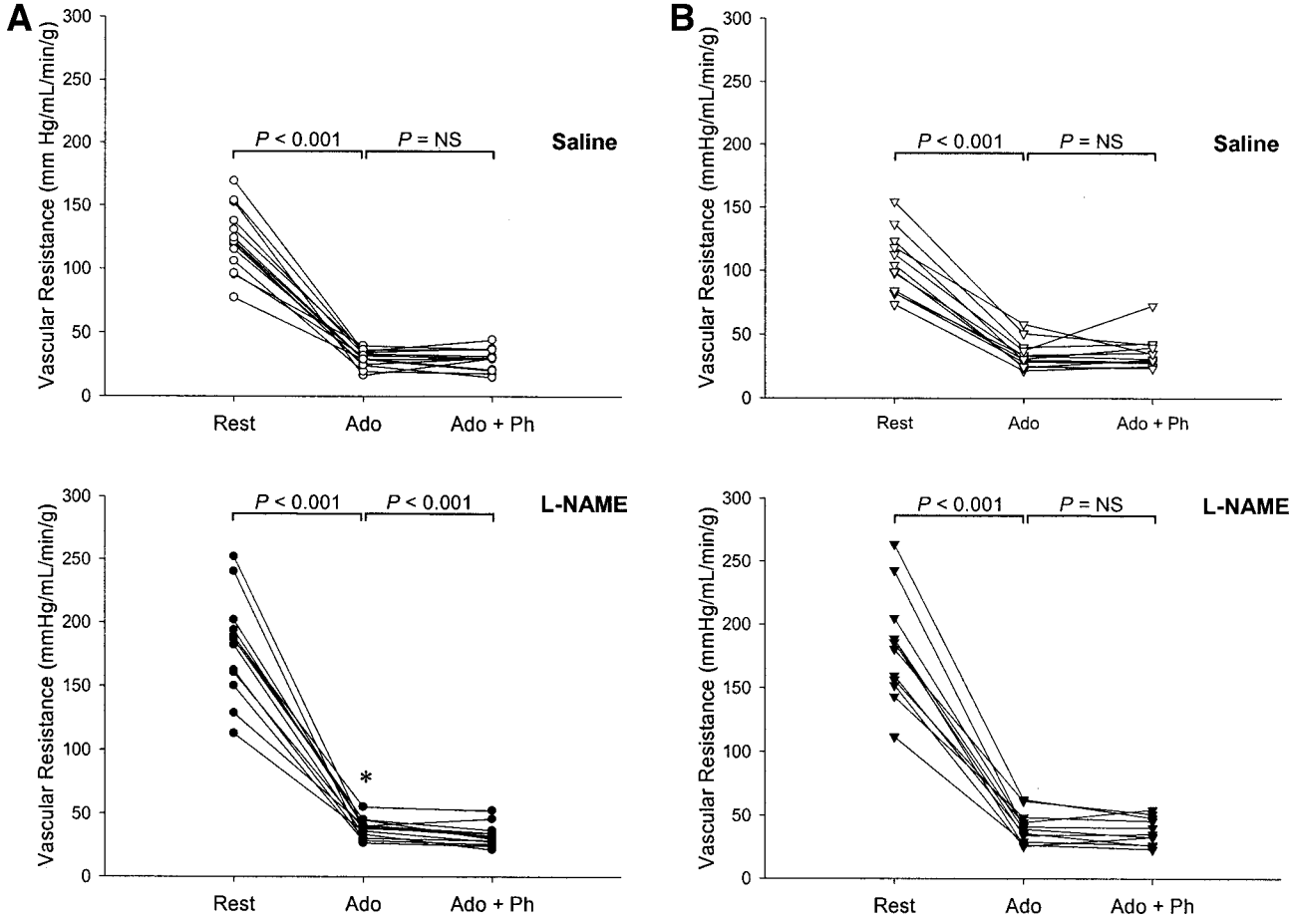

FIG. 4. MVR during PET studies. *A*: Control subjects. *B*: Diabetic patients. *Top*: Perfusion during saline. *Bottom*: Perfusion during NO synthase inhibition with L-NAME. Ado, adenosine; Ado + Ph, adenosine with phentolamine. *$P < 0.001$, L-NAME vs. saline in same scan sequence (L-NAME adenosine vs. saline adenosine).

heart rate variability reflecting baroreceptor function between control subjects and diabetic patients.

Instead, the marked effect on the blood pressure of combined adenosine-infusion and α-adrenergic blockade in the diabetic patients may be due to an increase in arterial α-adrenergic tone. Hogikyan et al. (36) found that despite comparable concentrations of plasma norepinephrine, arterial α-adrenergic tone was higher and the vasoconstrictory response to intra-arterial norepinephrine was greater in type 2 diabetic patients compared with matched control subjects.

Despite the fact that no differences were found between diabetic and healthy subjects regarding RPP and myocardial perfusion at rest, a discrepancy was observed between the two groups. Although cardiac work (RPP) correlated with myocardial perfusion in our healthy, middle-aged control subjects, this correlation seemed to be abolished in the presence of NO inhibition and in the

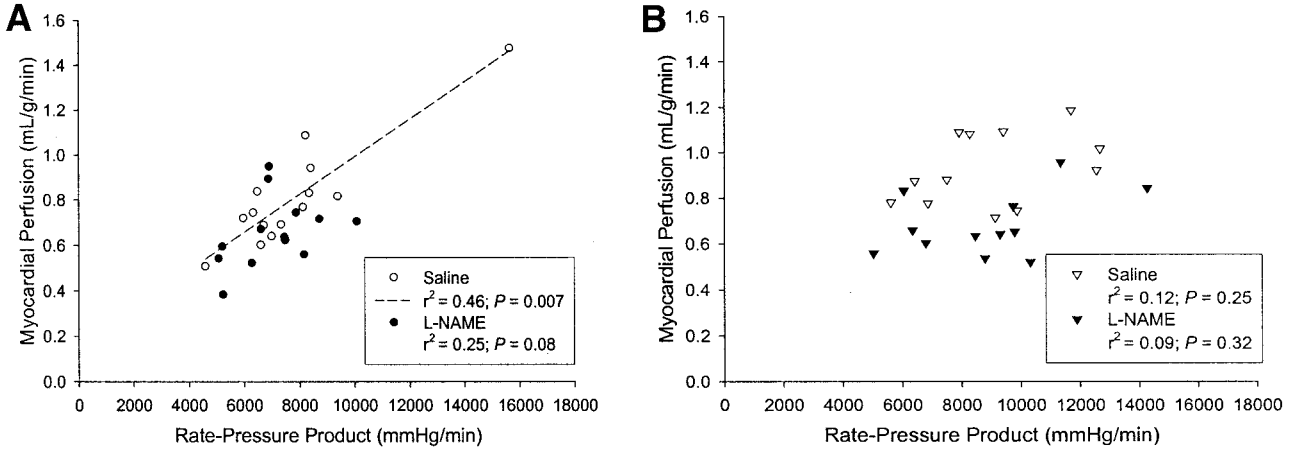

FIG. 5. RPP at rest plotted against myocardial perfusion at rest. *A*: Control subjects. *B*: Diabetic patients. White circles, control subjects, saline; black circles, control subjects, L-NAME; white triangles, diabetic patients, saline; black triangles, diabetic patients, L-NAME.

diabetic patients. Canine studies have indicated that NO is of importance in maintaining the balance between exercise-induced oxygen demand and supply (37,38). Studies in young and in middle-aged smokers demonstrating reduced endothelium-dependent myocardial perfusion have also revealed lack of correlation between RPP and myocardial perfusion (20,21). In a recent study by Prior et al. (12), it was demonstrated that despite comparable increases in RPP in diabetic and nondiabetic subjects, myocardial perfusion in response to cold-induced stimulation of the endothelium failed to increase or even declined in the diabetic group in contrast to the significant increase found in nondiabetic subjects. The present results are in accordance with and further expand on these previous observations of a possible dependency of intact endothelial function on preserving a significant correlation between cardiac work and myocardial perfusion.

Study limitations. The differences between the diabetic patients and the control subjects observed in this study could be due to several other differences between the two groups than diabetes per se because type 2 diabetes is known to be associated with increasing age, obesity, hypertension, dyslipidemia, nephropathy, early development of coronary atherosclerosis, and left ventricular dysfunction. However, in this study, great care was taken to match patients with diabetes to the control group, and no differences were found between the two groups regarding age, sex, BMI, blood pressure, or heart rate at the time of inclusion. Diabetic patients with albuminuria were excluded from participation, and although the participants were not evaluated by coronary angiography, all had normal stress perfusion PET scans and normal left ventricular function.

The results may be blunted by the fact that L-NAME exerts only a 70% inhibition of NO synthase, although it is the most potent NO synthase inhibitor known (26). If a full blockade of NO synthase had been possible, we cannot exclude that a more pronounced difference between the two study groups might have appeared.

It cannot be ruled out that the different phentolamine doses used in the two groups have influenced the results of the study. However, phentolamine was found to exert a dramatic effect on the diabetic patients regarding systemic hemodynamic and myocardial perfusion values, and due to ethical considerations, administration of higher phentolamine doses was impossible.

The results of this study might be influenced by the fact that the diabetic patients received antidiabetic and cardioprotective medication. This effect was sought to be minimized by discontinuation of all medication at least 24 h before the PET scans.

In conclusion, the present study demonstrates that adenosine-induced hyperemia seems not to be the precedent in type 2 diabetic patients without signs of ischemic heart disease and only to a minor extent in a matched, middle-aged control group. Furthermore, α-adrenergic blockade appears to exert only discrete effects on myocardial vasoreactivity in middle-aged healthy control subjects, whereas no direct cardiac effects are found in diabetic patients. Finally, the correlation between cardiac work and myocardial perfusion found in healthy control subjects seems to be altered by systemic NO synthase inhibition and in type 2 diabetic patients.

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