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Coronary Endothelial Dysfunction in the Insulin-Resistant State Is Linked to Abnormal Pteridine Metabolism and Vascular Oxidative Stress

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OBJECTIVES We investigated whether abnormal pteridine metabolism is related to coronary endothelial dysfunction in insulin-resistant subjects.

BACKGROUND Depletion of tetrahydrobiopterin (BH_4) and elevation of the 7,8-dihydrobiopterin (BH_2) (activating and inactivating cofactors of nitric oxide synthase [NOS], respectively) contribute to impairment of NO-dependent vasodilation through reduction of NOS activity as well as increased superoxide anion generation in insulin-resistant rats.

METHODS Thirty-six consecutive nondiabetic, normotensive and nonobese subjects with angiographically normal coronary vessels were studied. Traditional coronary risk factors, plasma pteridine levels, activities of erythrocyte dihydropteridine reductase (DHPR), the recycling enzyme that converts BH_2 to BH_4 and lipid peroxide (LPO) levels were measured and coronary endothelial function was assessed with graded infusions of acetylcholine (ACh).

RESULTS When we divided patients into tertiles based on insulin sensitivity, we observed stepwise decreases in the maximal ACh-induced vasodilation and plasma BH_4/7,8-BH_2 ratio, and increases in coronary LPO production as insulin sensitivity decreased. The ACh-induced vasodilation was positively correlated with insulin sensitivity, BH_4/7,8-BH_2 ratio and DHPR activity. Furthermore, BH_4/7,8-BH_2 was inversely correlated with DHPR activity and insulin sensitivity. In multiple stepwise regression analysis, BH_4/BH_2 was independently related to ACh-induced vasodilation and accounted for 39% of the variance. However, no significant correlation existed between other traditional risk factors and BH_4/7,8-BH_2.

CONCLUSIONS These results indicate that both abnormal pteridine metabolism and vascular oxidative stress are linked to coronary endothelial dysfunction in the insulin-resistant subjects. (J Am Coll Cardiol 2001;38:1821–8) © 2001 by the American College of Cardiology

Because defects of endothelium-dependent vascular reactivity are fundamental pathologic abnormalities in various insulin-resistant states such as diabetes, obesity, hypertension, and aging, endothelial damage has been implicated in preceding and likely contributing to the development of cardiovascular disease in these states (1–3). Recent clinical evidence has shown a possible link between diminished coronary vasodilator response to acetylcholine (ACh) and hyperinsulinemia in patients with vasospastic angina (4,5). In contrast, the response of coronary blood flow to N^G-monomethyl-L-arginine, a competitive inhibitor of nitric oxide synthase (NOS), was reduced in patients with vasospastic angina, and antioxidant treatment restored endothelial function in these patients (5,6). These results led to the proposal that impairment of insulin action on the endothelial nitric oxide (NO) system could contribute to the derangement of NO production/action in these states (1,2,7). However, the factors contributing to NO-mediated endothelial dysfunction in human insulin-resistant state are not fully defined.

There is increasing experimental evidence that (6R)-5,6,7,8-tetrahydrobiopterin (BH_4), the natural and essential cofactor of NOS, plays a crucial role not only in increasing the rate of NO generation by NOS but also in controlling the formation of superoxide anion (O_2^-) in endothelial cells (8). The biosynthesis of intracellular BH_4 is governed by two enzymes: GTP cyclohydrolase I (GTP-CH1), which regulates its rate of formation, and dihydropteridine reductase (DHPR), which regulates its rate of regeneration. Utilization of BH_4 in NO synthesis generates quinonoid dihydrobiopterin rather than dihydrobiopterin (BH_2) (8,9). Alternatively, quinonoid BH_2 may rearrange nonenzymatically to BH_2, which is no longer a substrate for DHPR (10). To maintain BH_4, increases in both enzyme activities are crucial. We have recently reported that under insulin-resistant conditions where the BH_4 levels are in a subnormal range, the excessive production of O_2^- by NO synthase may lead to hydroxyl radical production and oxidative tissue damage (11). This hypothesis was supported by the finding that long-term activation of endothelial NOS (eNOS) by oral administration of BH_4 prevents endothelial dysfunction

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Abbreviations and Acronyms

ACh	= acetylcholine
Ao	= descending aorta
BH_2	= 7,8-dihydrobiopterin
BH_4	= (6R)-5,6,7,8-tetrahydrobiopterin
BL	= borderline
CS	= coronary sinus
DHPR	= dihydropteridine reductase
eNOS	= endothelial nitric oxide synthase
GTP-CH1	= GTP cyclohydrolase I
IR	= insulin resistant
IS	= insulin sensitive
LPO	= lipid peroxide
NO	= nitric oxide
NOS	= nitric oxide synthase
NTG	= nitroglycerin
O_2^-	= superoxide anion
OGTT	= oral glucose tolerance test
TBARS	= thiobarbituric acid reactive substances
TRAP	= total radical-trapping antioxidant parameter
VSAP	= vasospastic angina

and vascular oxidative stress in the aortas of insulin-resistant rats (12).

Therefore, we examined the effect of insulin resistance on in vivo pteridine metabolism and endothelial dysfunction, which we evaluated by analyzing graded degrees of ACh-induced vasoconstriction in subjects with angiographically normal coronary arteries.

METHODS

Study subjects. Forty-four subjects were selected from consecutive patients who entered Osaka Police Hospital between 1998 and 2000 for coronary angiography because of atypical chest pain that occurred predominantly at rest, or for clinically suspected coronary vasospastic angina. Eight subjects initially evaluated were disqualified because of the presence of obstructive coronary artery disease (n = 3) or diabetes (n = 5). All blood specimens were taken after a 12-h fast, with no interruption of drug treatment. Patients who were taking lipid-lowering drugs, beta-adrenergic blocking drugs or diuretics, which might have adverse effects on carbohydrate and lipid metabolism, were excluded from the study. Patients with a history of hypertension, obesity (body-mass index >26 kg/m^2) or diabetes mellitus, and who were known to have impaired insulin sensitivity and endothelial dysfunction (13–15), were excluded from the study. Subjects with hypercholesterolemia (total cholesterol >6.2 mmol/l), myocardial infarction, unstable angina, valvular disease, hepatic, renal or endocrine dysfunction were also excluded. Finally, a total of 36 nondiabetic, normotensive and nonobese subjects (24 men, 12 women) with angiographically normal coronary vessels (<25% stenosis of the luminal diameter) fulfilled the criteria for participation in this study. All subjects gave their written informed consent, and the study protocol was approved by the Ethics Committee of the Osaka Police Hospital.

Baseline investigation. One day before cardiac catheterization, venous blood samples were drawn from each subject after an overnight fast for measurements of plasma glucose, insulin, total cholesterol, triglyceride and high-density lipoprotein cholesterol. A 75-g load of oral glucose (Trelan G 75, Shimizu, Shizuoka) was administered, and blood samples were drawn at 30, 60 and 120 min for determination of plasma glucose and insulin concentrations. Plasma glucose and insulin responses to glucose ingestion were evaluated by calculation of the areas under the curves of glucose and insulin throughout the 120 min of the test period. The definition of glucose tolerance was based on a 2-h oral glucose tolerance test (OGTT) according to the criteria of the American Diabetes Association (16). Hypertension was defined as systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg. As a cumulative estimate of tobacco consumption, cigarette-years (pieces/day × years) was used.

Coronary angiography. Coronary angiography was performed by the Judkins technique using a biplane cineangiography system in the morning when the patients were fasting (17). Thirty subjects (83.3%) had completely normal coronary arteries, and those of the others were nearly normal (<25% stenosis). Incremental doses of ACh (50 μg, 100 μg) were injected into the left main coronary artery through the catheter. Coronary spasm was defined as total or subtotal (a change in diameter ≥75%) vessel occlusion associated with chest pain or ischemic ST changes on the electrocardiogram or both. After the completion of the intracoronary injection of ACh, when the systemic hemodynamic parameters and the coronary arterial diameter on angiograms had returned to the baseline levels, 300 μg of nitroglycerin (NTG) was injected into the coronary artery, and coronary angiography was performed in multiple projections. The luminal diameter at the center of the left anterior descending artery was measured quantitatively with the use of a computer-assisted coronary angiographic analysis system (GE Medical Systems, Milwaukee, Wisconsin) by two observers blinded to the clinical history and risk-factor profile (18). Responses of coronary artery diameter to infusion of ACh and NTG were expressed as percentage changes from the baseline coronary diameter (i.e., 100 × [diameter after ACh or NTG − baseline diameter]/baseline diameter).

Blood sampling. Blood samples were collected simultaneously from the coronary sinus (CS) and descending aorta (Ao) 1 min before and 1 min after ACh injection at the same speed and were placed on ice immediately after collection. A 10-ml blood sample was drawn into an ethylenediamine tetraacetate vacutainer and centrifuged at 3,000 rpm for 15 min at 4°C. After plasma was separated and the buffy coat was removed, erythrocytes were suspended in phosphate-buffered saline and centrifuged again. Thus, obtained packed erythrocytes and the separated

Table 1. Baseline Characteristics Categorized by Tertiles of Two-Hour Insulin Area in Patients With Angiographically Normal Coronary Arteries and No Other Risk Factors

	IS (n = 12)	BL (n = 12)	IR (n = 12)
Age (yr)	61.0 ± 1.8	55.1 ± 3.4	54.5 ± 3.8
Gender (male/female)	9/3	9/3	8/4
BMI (kg/m^2)	22.9 ± 0.7	23.2 ± 1.1	23.4 ± 0.8
Smoking (%)	33	75	83
Cigarette-years	122 ± 62	420 ± 124	479 ± 126*
Glucose tolerance (NGT/IGT)	8/4	4/8	6/6
Fasting glucose (mmol/l)	5.42 ± 0.14	5.20 ± 0.12	5.41 ± 0.12
2-h glucose area (mmol/l × h)	15.9 ± 0.7	17.6 ± 0.8	17.3 ± 1.2
Fasting insulin (pmol/l)	47.8 ± 7.5	46.1 ± 7.0	67.6 ± 8.7*‡
2-h insulin area (pmol/l × h)	452.3 ± 30.8	730.3 ± 17.7	1454.4 ± 140.8†§
Total cholesterol (mmol/l)	4.86 ± 0.24	4.98 ± 0.17	4.97 ± 0.26
HDL cholesterol (mmol/l)	1.57 ± 0.11	1.58 ± 0.18	1.41 ± 0.12
Triglycerides (mmol/l)	0.61 ± 0.10	0.93 ± 0.14	1.01 ± 0.17*
Systolic BP (mm Hg)	126.7 ± 3.2	129.0 ± 4.0	124.3 ± 3.2
Diastolic BP (mm Hg)	78.5 ± 1.6	72.8 ± 1.9	75.2 ± 2.7

*$p < 0.05$. †$p < 0.001$ vs. IS. ‡$p < 0.05$. §$p < 0.001$ vs. BL. Values are mean ± SEM or number of patients.
BL = borderline; BMI = body-mass index; BP = blood pressure; HDL = high density lipoprotein; IGT = impaired glucose tolerance; IR = insulin resistant; IS = insulin sensitive; NGT = normal glucose tolerance.

plasma were stored at −80°C until use. Coronary lipid peroxide (LPO) production during ACh infusion was calculated by the formula: CS − Ao difference = (thiobarbituric acid reactive substances [TBARS] level after ACh in the CS − that in the Ao) minus (TBARS level before ACh in the CS − that in the Ao). Plasma concentrations of biopterin derivative and total radical-trapping antioxidant parameter (TRAP) and erythrocyte DHPR activity were not significantly different between the CS and Ao regardless of ACh injection. Therefore, the levels of these variables were measured in the samples collected from the CS before ACh injection.

Plasma biopterin derivative levels and DHPR activities. Plasma biopterin levels were measured by high-performance liquid chromatography as previously described (11,12). The amount of BH_4 was estimated from the difference between the total (BH_4 + 7,8-BH_2 + oxidized biopterin) and alkaline-stable biopterin (7,8-BH_2 + oxidized biopterin). The DHPR, the recycling enzyme to convert BH_2 to BH_4, was assayed by the method of Arai et al. (19).

Measurement of LPO content and TRAP. Plasma TBARS content was measured by the fluorometric method described previously (20). Plasma antioxidant defenses were evaluated by measuring the TRAP (21).

Statistical analysis. All values are presented as means ± SE. All analyses were performed using a personal computer with the statistical software package SPSS, version 6.0. Group differences of categorical data were tested by chi-square analysis with the Yates' correction. The plasma glucose and insulin responses in the three groups during OGTT, blood pressure and lipid concentrations were compared using analysis of variance with a post hoc Scheffé comparison. The dose-dependent vascular responses were compared among the three groups using repeated-measures analysis of variance. The Pearson coefficient was used for normally distributed data, and the Spearman coefficient was used for abnormally distributed data to assess the relation between continuous factors. A p value < 0.05 was considered statistically significant.

RESULTS

Characteristics of the study groups. According to the tertiles of plasma insulin responses during OGTT, 12 subjects were classified as insulin resistant (IR) with 2-h insulin area ≥800 pmol/l × h, 12 as insulin sensitive (IS) with 2-h insulin area ≤600 pmol/l × h, and 12 as borderline (BL) with 2-h insulin area over 600 and <800 pmol/l × h. As shown in Table 1, subjects in the three groups were comparable with regard to age, gender, body-mass index, proportions of glucose tolerance and blood pressure. Both the percentage of active smokers and cumulative tobacco consumption were significantly higher in the IR than in the IS group. Analysis of the data from the OGTT showed that both fasting glucose level and 2-h glucose area were not significantly different among IS, IR and BL subjects, whereas the IR group had higher fasting insulin values than the other groups. There were no significant differences in plasma total cholesterol and HDL cholesterol among these three groups despite higher triglyceride concentration in IR subjects than in the other groups.

Baseline hemodynamic parameters and responses of epicardial coronary diameter to ACh and NTG. Baseline values of heart rate, mean blood pressure and left ventricular ejection fraction were not different among the three groups. Seven patients showed coronary spasm and 16 subjects had completely normal coronary arteries with neither coronary spasm (a change in diameter <50%) nor ischemic ST change in the ACh-provocation test. As shown in Figure 1, the constrictor responses of the epicardial coronary arteries to ACh were dose-dependently increased in all groups. The maximal ACh-induced coronary vasoconstriction induced

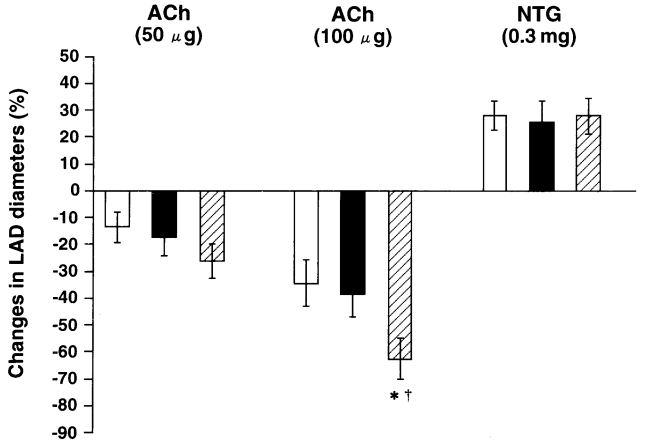

Figure 1. Percent changes in left anterior descending artery (LAD) diameters from baseline values in response to intracoronary infusion of graded dose (50 μg, 100 μg) of acetylcholine (ACh) and to 0.3 mg of nitroglycerin (NTG) categorized by tertiles of insulin sensitivity in patients with normal coronary angiogram (n = 36). IS = insulin sensitive **(open bars)**; BL = borderline **(solid bars)**; IR = insulin resistant **(hatched bars)**. *p < 0.05 vs. IS. †p < 0.05 vs. BL.

by injection of ACh at a dose of 100 μg in IR subjects was 1.8-fold and 1.6-fold higher (p < 0.05) than that in IS and BL subjects, respectively. Conversely, no significant difference existed in the magnitude of the coronary dilator response to NTG among the three groups.

Plasma concentrations of biopterin derivatives and DHPR activity. To determine whether changes occurred in the levels of pteridine metabolites as determinants of ACh-induced coronary vasoconstriction, we measured the plasma levels of biopterin derivatives in the three groups. No significant difference was found among the three groups in the absolute values of concentrations of neopterin, total biopterin, or BH_4 (Table 2). However, the plasma level of biopterin plus 7,8-BH_2, an oxidized form of BH_4, in IR subjects was 1.43-fold and 1.24-fold higher than that in IS or BL subjects, respectively. The ratio of BH_4 to 7,8-BH_2 plus biopterin (BH_4/7,8-BH_2 ratio) as well as the ratio of BH_4 to total biopterin was significantly lower in IR subjects than in IS or BL subjects.

Consistent with these results, IR subjects exhibited a significant decrease in the activity of DHPR when compared with the other groups. In separate analyses, plasma levels of biopterin derivatives and erythrocyte DHPR activity in patients with vasospastic angina (VSAP) were compared with those in control subjects (chest pain syndrome). The BH_4/7,8-BH_2 ratio (control, 4.62 ± 0.33; VSAP, 3.45 ± 0.46, p < 0.05) and DHPR activity (control, 3.57 ± 0.13; VASP, 2.81 ± 0.14 nmol nicotinamide adenine dinucleotide/min/mg hemoglobin, p < 0.01) were also significantly reduced in patients with VSAP compared to control subjects.

Lipid peroxidation and antioxidant system components. The increase in the plasma levels of TBARS above the basal value after ACh injection was slightly but not significantly higher in IR subjects than in the other groups (IS, 2.30 ± 0.59; BL, 2.24 ± 0.46; IR, 3.13 ± 0.38 nmol/ml). In contrast, there was a trend toward decreased plasma levels of TRAP as insulin sensitivity decreased, and the TRAP level in IR subjects was significantly lower than that in IS subjects (IS, 303 ± 39; BL, 247 ± 27; IR, 197 ± 32 μmol/l, p < 0.05).

Analyses of risk factors for endothelial dysfunction. Univariate analysis showed that the constrictor response of the coronary diameter to ACh (100 μg) had a significant positive correlation with the 2-h insulin area, TBARS level (Fig. 2) and cigarette-years (r = 0.36, p < 0.05). Conversely, an inverse correlation between the response to ACh and either BH_4/7,8-BH_2 ratio or DHPR activity was observed. To exclude the possibility that angiographic results in the spasm patients might affect the results of the present study, these univariate analyses were carried out in subjects without vasospasm. As a result, a similar tendency was observed in these subjects. A 2-h insulin area as a marker of insulin resistance was positively correlated with 7,8-BH_2 plus biopterin and TBARS level, and this was inversely correlated with BH_4/7,8-BH_2 ratio, BH_4/total biopterin and DHPR activity. Interestingly, lower BH_4/7,8-BH_2 ratio was correlated with higher cumulative tobacco consumption. Similarly, DHPR activity was associated with an increase in BH_4/7,8-BH_2 ratio, and a decrease in TBARS level.

Multivariate analyses using stepwise regression models were carried out to analyze the relationships between the percent

Table 2. Concentrations of Plasma Pteridine Derivatives and Erythrocyte Dihydropteridine Reductase Activity in the Coronary Sinus

	IS (n = 12)	BL (n = 12)	IR (n = 12)
Neopterin (nmol/l)	35.5 ± 3.2	36.7 ± 1.7	37.5 ± 1.8
Total biopterin (nmol/l)	42.4 ± 2.9	44.7 ± 2.7	44.3 ± 3.1
BH_4 (nmol/l)	34.7 ± 2.3	35.8 ± 2.2	33.4 ± 3.3
7,8-BH_2 + biopterin (nmol/l)	7.67 ± 0.76	8.87 ± 0.67	11.0 ± 0.59†‡
Neopterin/total biopterin	0.84 ± 0.05	0.92 ± 0.09	0.84 ± 0.06
BH_4/total biopterin	0.83 ± 0.01	0.80 ± 0.01	0.766 ± 0.02†‡
BH_4/7,8-BH_2 + biopterin	4.85 ± 0.31	4.24 ± 0.38	3.21 ± 0.31†‡
DHPR activity (nmol NADH/min/mg Hb)	3.35 ± 0.58	3.27 ± 0.71	2.75 ± 0.46*‡

*p < 0.05. †p < 0.001 vs. IS. ‡p < 0.001 vs. BL.
BH_2 = dihydrobiopterin; BH_4 = tetrahydrobiopterin; BL = borderline; DHPR = dihydropteridine reductase; Hb = hemoglobin; IR = insulin resistant; IS = insulin sensitive; NADH = nicotinamide adenine.

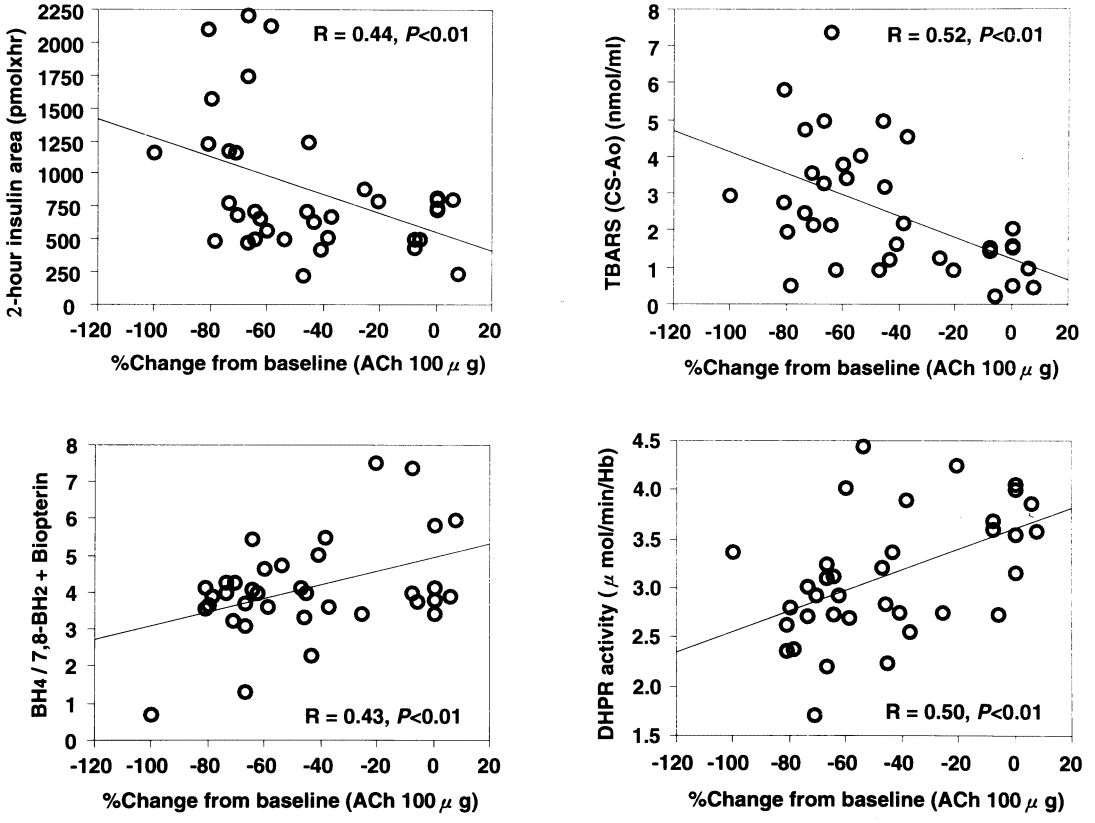

Figure 2. Correlation between percent changes in left anterior descending artery (LAD) diameters from baseline values in response to intracoronary infusion of 100 μg acetylcholine (ACh) and 2-h insulin area, plasma lipid hydroperoxide (TBARS) level of coronary sinus-arterial (CS-Ao) difference, BH_4/7,8-BH_2 + biopterin, and dihydropteridine reductase (DHPR) activity in study subjects (n = 36). BH_4 = tetrahydrobiopterin; BH_2 = dihydrobiopterin.

changes of ACh-induced coronary diameters and a set of variables selected on the basis of the above-cited correlation analyses (TBARS level, BH_4/7,8-BH_2 ratio, and DHPR activity) and other recognized risk factors. As shown in Table 3, BH_4/7,8-BH_2 ratio and DHPR activity were independently associated with the constrictor response to ACh and explained 12% to 25% of the variation in the response to ACh. Cigarette-years, which showed positive correlation with response to ACh in univariate analyses, did not show any substantial correlation in multivariate analyses.

Table 3. Multiple Stepwise Regression Analysis of Variables Significantly Associated With Percent Changes of Acetylcholine-Induced Left Anterior Descending Artery Diameters

	β	SE	F	Partial R^2	p Value
Model 1					
TBARS (CS-Ao)	−0.460	2.507	11.05	0.273	< 0.001
BH_4/7,8-BH_2 + biopterin	0.345	3.204	6.19	0.115	< 0.001
			Multiple R^2 = 0.388		
Model 2					
TBARS (CS-Ao)	−0.408	2.553	8.38	0.251	< 0.001
DHPR activity	0.379	677.7	7.20	0.116	< 0.001
			Multiple R^2 = 0.367		
Model 3					
DHPR activity	0.412	712.8	7.70	0.251	< 0.001
2-h insulin area	−0.305	0.067	4.23	0.086	< 0.001
			Multiple R^2 = 0.337		

Model 1 includes age, body mass index, mean blood pressure, cigarette-years, total cholesterol, 2-h glucose area, 2-h insulin area, thiobarbituric acid reactive substances (TBARS) coronary sinus-descending aorta (CS-Ao) and (6R)-5,6,7,8-tetrahydrobiopterin (BH_4)/7,8-BH_2 + biopterin as independent variables. Model 2 includes age, body mass index, mean blood pressure, cigarette-years, total cholesterol, 2-h glucose area, 2-h insulin area, TBARS coronary sinus-descending aorta (CS-Ao) and dihydropteridine reductase (DHPR) activity as independent variables. Model 3 includes age, body mass index, mean blood pressure, cigarette-years, total cholesterol, 2-h glucose area, 2-h insulin area and DHPR activity as independent variables.

β = standardized regression coefficient; R = multiple correlation coefficient; SE = standard error.

JACC Vol. 38, No. 7, 2001
December 2001:1821–8

DISCUSSION

This study showed that responses of angiographically normal coronary arteries to ACh were impaired in subjects with insulin resistance. Furthermore, not only decrements of the plasma BH_4/7,8-BH_2 ratio and DHPR but also enhancement of oxidative stress and defects of antioxidant defenses were observed in these subjects. Conversely, ACh-induced vasodilation was positively correlated with insulin sensitivity, BH_4/7,8-BH_2 ratio and DHPR activity. Thus, in addition to overproduction of LPO in the coronary artery, decreased BH_4/7,8-BH_2 ratio also appeared to be an independent predictor of impaired epicardial vasodilator responses to ACh, suggesting that these factors exerted additional adverse effects on the endothelial function of the coronary artery. Moreover, the unaltered sensitivity to NTG among the three different groups (Fig. 1) suggested that the reactivity of smooth muscle cell per se was not significantly altered and the abnormal pteridine levels were specific for endothelium-dependent pathways in the insulin-resistant state. All these findings are compatible with the hypothesis that inactivation of NO through abnormal pteridine metabolism might be partly responsible for the impairment of coronary vasodilation in patients with insulin resistance.

Insulin resistance and vascular oxidative stress. Several lines of evidence now link excess vascular oxidative stress to the impairment of NO action in subjects with insulin resistance (2,5). We have previously reported that insulin resistance causes oxidative stress to cardiovascular tissues and the release of oxygen free radicals from endothelial cells (11). Moreover, not only was there an increase in the LPO level, but also marked activation of redox-sensitive transcription factors in cardiovascular tissues of insulin-resistant rats (12). In separate analyses, plasma levels of TBARS were also significantly increased in patients with VSAP compared with control subjects (control, 1.70 ± 0.29; VSAP, 3.34 ± 0.53 nmol/ml, $p < 0.05$). These findings suggest that increased oxidative stress associated with insulin resistance contributes to endothelial dysfunction in patients with VSAP.

Pteridine metabolism and eNOS activity. Previous data from our laboratory and other groups suggest abnormal pteridine metabolism as a possible mechanism linking insulin resistance to vascular disease (11,12). In vivo data suggest that increased plasma BH_4 levels can augment endothelial NO production (22). Endothelial cells constitutively release substantial amounts of BH_4 and neopterin (23). Neopterin is an oxidized product of 7,8-dihydroneopterin-triphosphate, the intermediate generated by GTP-CH1. The constancy of the neopterin to biopterin ratio and total biopterin levels (Table 2) suggest that the activity of GTP-CH1 is not decreased in the insulin-resistant state. These results suggest that reduction in the BH_4/7,8-BH_2 ratio rather than depletion of BH_4 is associated with impaired eNOS activity and thereby contributes to the defective ACh-induced vasodilation observed in human insulin-resistant states. The plasma 7,8-BH_2 levels in IR subjects showed highly significant increases, suggesting that the insulin-resistant state leads to increases in the synthesis of BH_2.

As illustrated in Figure 3, because BH_4 is rapidly oxidized to 7,8-BH_2, a lack of sufficient DHPR activity would lead to accumulation of 7,8-BH_2, which has been shown to inhibit the stimulatory effects of BH_4 on NO synthase (24). In addition, high concentrations of 7,8-BH_2 inhibit GTP-CH1 and hence de novo synthesis of BH_4 (9). Therefore, although it remains uncertain to what extent endothelial cells are responsible for and/or are affected by these declines, the present results support the hypothesis that insulin resistance induces vascular dysfunction through alterations in the BH_4/7,8-BH_2 ratio (14).

Potential mechanisms underlying the abnormal pteridine metabolism in subjects with insulin resistance. It is important to note that in the present study the BH_4/7,8-BH_2 ratio and DHPR activity were significantly reduced in patients with VSAP compared with subjects with chest pain syndrome. The reason for this reduced BH_4/7,8-BH_2 ratio is not clear. One possible cause is the increased production of reactive oxygen species in the insulin-resistant state, resulting in enhanced oxidation of BH_4 to BH_2 (25). In agreement with other reports (5,6), coronary LPO production emerged in our study as a stronger predictor of endothelial dysfunction.

In the present study, DHPR activity was correlated with the BH_4/7,8-BH_2 ratio. Interestingly, it has been reported that physiological concentrations of glutathione increase the synthesis and biological activity of NOS by activating DHPR (26). Consistent with these results, administration of either GSH or vitamin C improves the impairment of endothelium-dependent vasodilation in patients with coronary spastic angina (5,6). Although the multivariate analysis was negative for cigarette smoking, higher cumulative tobacco consumption was correlated with lower BH_4/7,8-BH_2 ratio and the maximal ACh-induced vasoconstriction. Therefore, a high frequency of smokers among IR subjects would be an explanation for decreased DHPR activity and BH_4/7,8-BH_2 ratio. In this context, it is conceivable that the abnormal intracellular redox state in the insulin-resistant state, which is unfavorable for reduction of the oxidized biopterin, impairs the endothelial recycling of BH_4, and an optimal ratio of BH_4/7,8-BH_2 is critical for eNOS activation.

Clinical usefulness and limitations of biopterin derivatives as markers for vascular function. The interpretation of changes in plasma and tissue biopterin derivative levels as reflections of tissue contents of those derivatives remains speculative in the present study. However, it has been shown that when DHPR activity is decreased, more 7,8-BH_2 appears in the plasma, and the plasma 7,8-BH_2 is low when de novo synthesis of BH_4 is low (27). Furthermore, we have also reported that plasma and tissue biopterin levels are closely associated with each other (12). These results suggest that measurement of pteridines would provide a

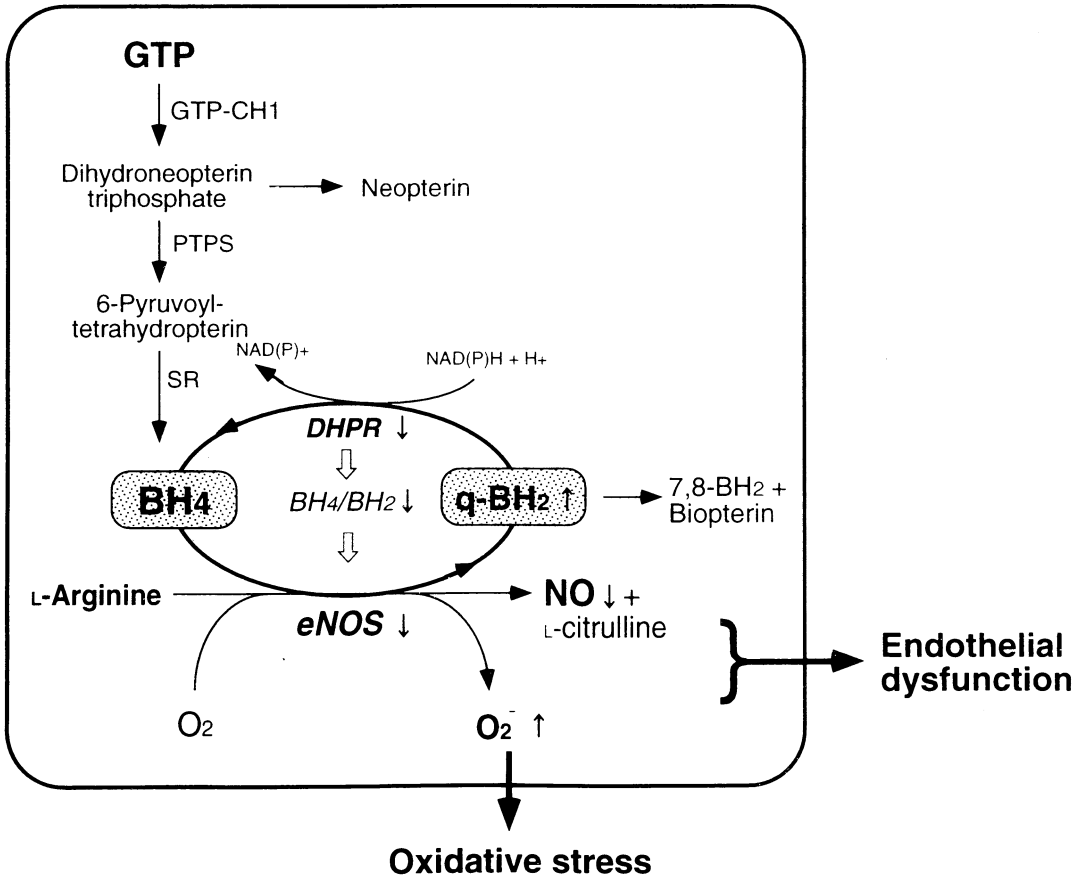

Figure 3. Hypothetical scheme illustrating the possible mechanism of impaired endothelial function in the insulin-resistant state. Increased quinonoid dihydrobiopterin (qBH_2) synthesis in the insulin-resistant state is closely associated with a decrement in the activity of dihydropteridine reductase (DHPR), the recycling enzyme that converts BH_2 to tetrahydrobiopterin (BH_4). As a result, both BH_4/BH_2 ratio and BH_4 to total biopterin (BH_2 + BH_4) were significantly decreased in the insulin-resistant subjects. These results indicate that decreased nitric oxide (NO)-dependent vasodilation in the insulin-resistant state may be related to abnormal pteridine metabolism and vascular oxidative stress. GTP = guanosine triphosphate; eNOS = endothelial nitric oxide synthase; O_2^- = superoxide anion; PTPS = 6-pyruvoyltetrahydropterine synthase; SR = sepiapterin reductase.

sensitive and informative measure of changes in the function of vascular cells. Moreover, the hypothesis that patients with abnormal pteridine metabolism are at increased risk of developing cardiovascular disease, including high blood pressure, VSAP, and atherosclerosis, must be further investigated.

Conclusions. Finally, the novel observation of this study is that deranged endothelial responses to ACh in the insulin-resistant state are, at least in part, due to impairments of the NO system caused by abnormal pteridine metabolism and vascular oxidative stress.

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