Changes in Skin Blood Flow in Type 2 Diabetes Induced by Prostacyclin: Association With Ankle Brachial Index and Plasma Thrombomodulin Levels

Yoshimasa Aso, Kazumi Tayama, Keishi Takanashi, Toshihiko Inukai, and Yoshihiro Takemura

In a previous study, we reported that beraprost sodium (BPS), a stable prostaglandin I_2 (PGI_2) analog, increases skin blood flow in the feet of both control subjects and patients with type 2 diabetes, and that the flow increase induced by BPS is lower in diabetic patients than in controls. The present study was undertaken to clarify factors associated with smaller increases in skin blood flow in the feet of patients with type 2 diabetes after the administration of BPS, and to investigate the relationship between microalbuminuria and the changes in skin blood flow induced by the PGI_2 analog. We studied 61 patients with type 2 diabetes: 10 received placebo (control) and 51 (31 with normoalbuminuria and 20 with microalbuminuria) received BPS. Using laser Doppler flowmetry, we measured the skin blood flow at the pulp of the right big toe before and 90 minutes after administration of 40 μg BPS, and calculated the change in blood flow, ie, delta flux (peak flux at 90 minutes − basal flux at 0 minutes). Plasma concentrations of soluble thrombomodulin (TM) were determined using an enzyme immunoassay (EIA) sandwich method. BPS significantly increased skin blood flow in the treatment group compared with the placebo group ($P < .01$). The delta flux was positively correlated with the value of the ankle brachial index (ABI) ($r = .41$, $P < .0038$) and was negatively correlated with plasma TM levels ($r = -.53$, $P < .0001$). By multiple regression analysis both the ABI value and the plasma TM level retained a significant influence on delta flux. Furthermore, both the delta flux and the ABI value in patients with microalbuminuria were lower than in patients with normoalbuminuria ($P < .05$). The results suggest that BPS increases the skin blood flow of the toe of patients with type 2 diabetes and that the increased flow is independently influenced by the value of the ABI and the plasma TM levels; in addition, microalbuminuria is associated with the impairment of vasodilation in the feet in response to BPS.

PROSTACYCLIN (prostaglandin I_2 [PGI_2]) is believed to act directly on PGI_2 receptors in arterial smooth muscles of all vessels, leading to the dilation of arteries and arterioles.[1,2] In our previous study using laser Doppler flowmetry, we showed that beraprost sodium (BPS), a stable PGI_2 analog, significantly increases skin blood flow in the feet of both control subjects and patients with type 2 diabetes, and that the flow increase induced by BPS is lower in diabetic patients than in controls.[3] It is, however, unclear why the increase in skin blood flow of the toe induced by BPS is impaired in patients with type 2 diabetes. Therefore, we sought to clarify the factors that influence the increase in skin blood flow of the toe caused by the PGI_2 analog in type 2 diabetic patients.

Elevated concentrations of plasma thrombomodulin (TM), which is a soluble form of endothelial TM,[4] are found in a variety of clinical diseases that are associated with systemic vascular disorders, such as diabetes mellitus[5-7] and microscopic polyarteritis.[8] Unlike von Willebrand factor, which is secreted by platelets as well as by endothelial cells,[9] TM antigen is expressed only by endothelial cells of various tissues.[10] Soluble TM in plasma is produced by proteolytic cleavage of the membrane TM. Therefore, plasma TM concentration may be a more specific and sensitive marker for vascular endothelial injury. Furthermore, it is possible that plasma TM could be a marker for premature atherosclerosis, since vascular endothelial injury and/or dysfunction play a crucial role in the initial stage of atherosclerosis formation.[11] To date, there have been no published reports on the relationship between skin blood flow and plasma TM concentration.

The present study was therefore undertaken to clarify factors associated with the changes in skin blood flow in patients with type 2 diabetes after the administration of BPS, and to investigate the relationship between skin blood flow, determined by laser Doppler flowmetry, and the plasma levels of TM. Furthermore, since diabetic patients with microalbuminuria are at risk for vascular injury, such as coronary artery disease and cerebrovascular disease,[12-14] we also investigated the relationship between microalbuminuria and the change in skin blood flow after BPS administration. We demonstrate that the plasma TM levels as well as ankle brachial index (ABI) are closely related to the change in the skin blood flow of the toe in patients with type 2 diabetes after the administration of BPS.

MATERIALS AND METHODS

We studied 61 patients with type 2 diabetes mellitus diagnosed according to the criteria of the World Health Organization. All patients who fulfilled the following inclusion criteria were considered for the study: (1) no episodes of ketoacidosis, (2) diagnosis of diabetes after more than 30 years of age, and (3) if any, insulin therapy started after at least 5 years of known disease. Ten of these patients were randomly selected as a placebo group, to confirm that skin blood flow does not change significantly during an acclimatization period. Although the number of subjects in the placebo group was relatively small, we considered it enough to analyze the natural changes in skin blood flow during the acclimatization period. Therefore, we administered BPS, a PGI_2 analog, to 51 diabetic patients (BPS group). Excluded from this study were patients who were receiving medications that could affect skin blood flow, such as Ca^{2+}-channel blockers, nitrates, anticoagulants, and antiplatelet agents. To exclude patients with a falsely high ABI due to medial artery calcification,[15,16] only those whose ABI was less than 1.3 were included in the study. Also excluded were patients with overt proteinuria, ie, macroalbuminuria, because the plasma TM

From the Department of Medicine, Koshigaya Hospital, Dokkyo University School of Medicine, Koshigaya, Japan.

Submitted July 13, 2000; accepted November 10, 2000.

Address reprint requests to Yoshimasa Aso, MD, Department of Medicine, Koshigaya Hospital, Dokkyo University School of Medicine, 2-1-50 Minami-Koshigaya, Koshigaya, Saitama 343-8555, Japan.

0026-0495/01/5005-0008$35.00/0
doi:10.1053/meta.2001.22517

concentration is strongly influenced by an impairment of the renal clearance of TM.[17] In the BPS group, 13 diabetic patients had clinical evidences of diabetic neuropathy, including symptoms such as pain, numbness, and paresthesia, and signs of absent ankle reflexes and reduced nerve conduction velocities. That is, they had decreased median motor nerve conduction velocity (<50 m/s) and/or decreased peroneal motor nerve conduction velocity (<40 m/s). In the placebo group, 3 diabetic patients had diabetic neuropathy. All patients gave informed consent and the local ethical committee approved the study.

The ABI was defined as an ankle (dorsalis pedis or posterior tibial artery)/brachial systolic pressure ratio. According to the regular criteria, ie, an ABI less than 0.90, only 3 patients fell in peripheral vascular disease category. Urinary albumin excretion (UAE) was measured with an immunoturbidimetric assay technique (Hoechst Japan, Tokyo, Japan). According to the rate of UAE in a 24-hour urine collection, normoalbuminuria was defined as UAE less than 30 mg/24 h, microalbuminuria as UAE from 30 to 299 mg/24 h, and macroalbuminuria as a UAE more than 300 mg/24 h.

Measurement of Plasma TM Levels

Venous blood was obtained between 7 AM and 8 AM after an overnight fast and collected in a tube containing 3.8% sodium citrate (1 mL/9 mL blood). The samples were centrifuged at 2,500 rpm for 15 minutes and the supernatant was stored at −70°C until use. The concentration of soluble TM in the plasma was measured by an enzyme immunoassay (EIA) sandwich method with mouse monoclonal antibodies against human placental TM (TM MGCC kit; Mitsubishi Gas Chemical, Tokyo, Japan).[17,18] This EIA sandwich method captures almost all species of circulating TM fragments in plasma of molecular size 105, 85, 80, 56, 33, and 28 kd. The intra-assay and interassay coefficients of variation of this method were 4.3% and 4.8%, respectively.[17,18]

Skin Blood Flow

The diabetic subjects had to refrain from food or liquid intake during the 2 hours preceding the test. All measurements were performed with the subject in the supine position in a room maintained at a temperature of 23 to 25°C in the morning. After 30 minutes of acclimatization, skin blood flow, ie, flux, was measured on the pulp of the big toe of the right foot using laser Doppler flowmetry (Moor type MBF-3; Moor Instruments, Axminister, UK).[19] Since diabetic foot ulceration is most frequently found on the tips of the toe, the measurements of skin blood flow were taken at the pulp of big toe. The flux was expressed in arbitrary units (AU). The coefficient of variation of this technique was 15% to 25%. Skin temperature was also measured simultaneously at an adjacent pulp site of the big toe of the right foot. Patients whose skin temperature was between 32 and 34°C were included in the study.

In the 51 diabetic patients, BPS,[20,21] a PGI_2 analog, was administered orally at a dose of 40 μg, which is a safe dose and has a pharmacologic effect. It was reported that the administration of BPS at a dose of more than 40 μg gives rise to adverse effects, including skin flush, palpitation, headache, and hypotension.[22] This analog has a long biologic half-life and is orally effective in relaxing smooth muscle of large and small sized arteries and arterioles, as well as in inhibiting platelet aggregation responses to adenosine diphosphate and/or collagen.[21] It is therefore possible to evaluate the vasodilatory capacity of any size artery and arteriole by the administration of BPS. Skin blood flow and skin temperature measurements were performed at 0 minutes (basal), 30, 60, 90, and 120 minutes after BPS administration. The patients remained lying down throughout the study. We found the peak flux at 90 minutes. The change in skin blood flow was calculated for each patient as follows; delta flux (AU) = flux at 90 minutes (AU) − basal flux (AU).

In a preliminary study, we examined 20 age-matched healthy control subjects. The control subjects had a similar response to BPS at a dose of 40 μg, with the skin blood flow increasing from 103.1 ± 27.9 AU at 0 minutes to 123.1 ± 19.3 AU at 30 minutes, 124.8 ± 17.3 AU at 60 minutes, 125.0 ± 29.3 AU at 90 minutes, and 109.4 ± 30.7 AU at 120 minutes after BPS administration (data not shown).[3]

Statistical Analysis

Data are presented as the mean ± SD. Differences between groups were analyzed by the paired or unpaired Student's *t* test. Correlations were determined by linear regression analysis after performing a log transformation on values of ABI and plasma TM. Multiple regression analysis was performed to assess the relationship between the change in skin blood flow induced by BPS and various clinical parameters. A *P* value less than .05 was accepted as statistically significant.

RESULTS

The clinical characteristics of all subjects, including basal skin blood flow and plasma TM levels, are summarized in Table 1.

There was no difference in the skin blood flow between 0 and 90 minutes in the placebo group (73.6 ± 13.6 AU *v* 71.3 ± 17.4 AU) (Fig 1). However, in the BPS group, the skin blood flow of the toe increased significantly from 70.8 ± 24.3 AU at 0 minutes to 95.6 ± 25.7 AU at 90 minutes after BPS administration ($P < .0001$) (Fig 1). The flux at 90 minutes was significantly higher than that of the placebo group ($P = .01$) (Fig 1). The skin temperature in the BPS group also rose from 33.0 ± 1.1°C at 0 minutes to 33.8 ± 1.0°C at 90 minutes ($P < .0001$) (data not shown).

We compared the changes in skin blood flow between the diabetic patients without and with neuropathy to determine whether diabetic neuropathy influences the increased flow induced by BPS. Although there was no significant difference in the basal flux or the flux at 90 minutes between diabetic

Table 1. Clinical Characteristics of Diabetic Subjects With Normoalbuminuria and Microalbuminuria

	Placebo	BPS	
	Diabetics	Normoalbuminuric	Microalbuminuric
No. (male/female)	10 (5/5)	31 (16/15)	20 (12/8)
Age (yr)	48.3 ± 11.7	53.4 ± 1.0.	48.2 ± 13.3
BMI	23.6 ± 2.8	22.6 ± 2.6	22.0 ± 4.1
Duration (yr)	5.8 ± 5.1	7.1 ± 5.4	8.6 ± 5.5
FPG (mmol/L)	9.6 ± 2.8	10.1 ± 2.9	10.3 ± 3.0
HbA_{1c} (%)	9.3 ± 1.6	9.1 ± 1.7	9.9 ± 2.0
SBP (mm Hg)	124.2 ± 18.0	121.4 ± 18.6	122.7 ± 21.1
DBP (mm Hg)	77.2 ± 10.1	75.0 ± 8.7	76.6 ± 11.0
Serum Cr (mg/dL)	0.60 ± 0.15	0.60 ± 1.19	0.65 ± 0.18
UAE (mg/24 h)	34.4 ± 32.3	13.4 ± 6.9	104.9 ± 88.3*†
Plasma TM (U/mL)	18.9 ± 5.6	20.0 ± 5.4	21.7 ± 6.4
Basal flux (AU)	73.6 ± 13.6	70.9 ± 20.9	68.5 ± 28.8
Tx (D/OHA/Ins)	2/4/4	6/13/12	1/8/11

NOTE. Data are means ± SD.

* $P < .01$ *v* placebo.

† $P < .001$ *v* normoalbuminuric.

Abbreviations: BPS, beraprost sodium; BMI, body mass index; FPG, fasting plasma glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; Cr, creatinine, UAE, urinary albumin excretion; TG, triglyceride; TM, thrombomodulin; Tx, treatment; D, diet alone; OHA, oral hypoglycemic agents; Ins, insulin.

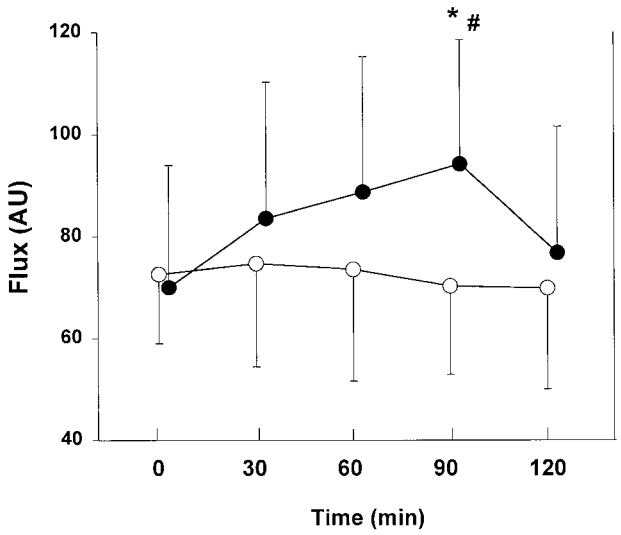

Fig 1. Changes in skin blood flow of the toe measured by laser Doppler flowmetry before and after the administration of 40 μg BPS (●) or placebo (○) in patients with type 2 diabetes. *$P < .0001$ *v* basal flux (0 min), #$P = .01$ *v* peak flux at 90 minutes of placebo group. Data are means ± SD.

patients without and with neuropathy, the delta flux was significantly lower in patients with neuropathy (Table 2).

The basal flux (flux at 0 minutes) was not correlated with the ABI value or plasma TM levels (data not shown). In contrast to the basal flux, the delta flux was positively correlated with the ABI value ($r = .41$, $P < .0038$) (Fig 2A). In addition, the delta flux was inversely correlated with plasma TM levels ($r = -.53$, $P < .001$) (Fig 2B). However, we found no correlation between the ABI value and plasma TM levels ($r = .01$, not significant) (data not shown). Multiple regression analysis demonstrated that the ABI value and plasma TM levels were the independent variables that retained a significant influence on delta flux ($P < .0001$ and $P = .0023$, respectively) (Table 3).

We then compared a variety of parameters, including ABI, plasma TM levels, and delta flux, between patients with normoalbuminuria and microalbuminuria, since microalbuminuria is believed to be associated with widespread vascular damage such as coronary artery diseases.[12-14] As shown in Table 1, plasma total cholesterol levels were higher in patients with microalbuminuria than in those with normoalbuminuria. Plasma TM levels were similar between patients with normoalbuminuria and microalbuminuria. There was no difference in the basal flux between patients with normoalbuminuria and

Table 2. Comparison of Changes in Skin Blood Flow (delta flux) Induced by BPS in Diabetic Patients Without and With Neuropathy

	Without Neuropathy	With Neuropathy
No.	38	13
Basal flux (AU)	68.7 ± 21.9	74.6 ± 28.9
Flux at 90 min (AU)	96.5 ± 23.7	91.1 ± 28.6
Delta flux (AU)	27.8 ± 16.9	16.5 ± 7.9*

NOTE. Date are means ± SD.
*$P < .01$ *v* without neuropathy.

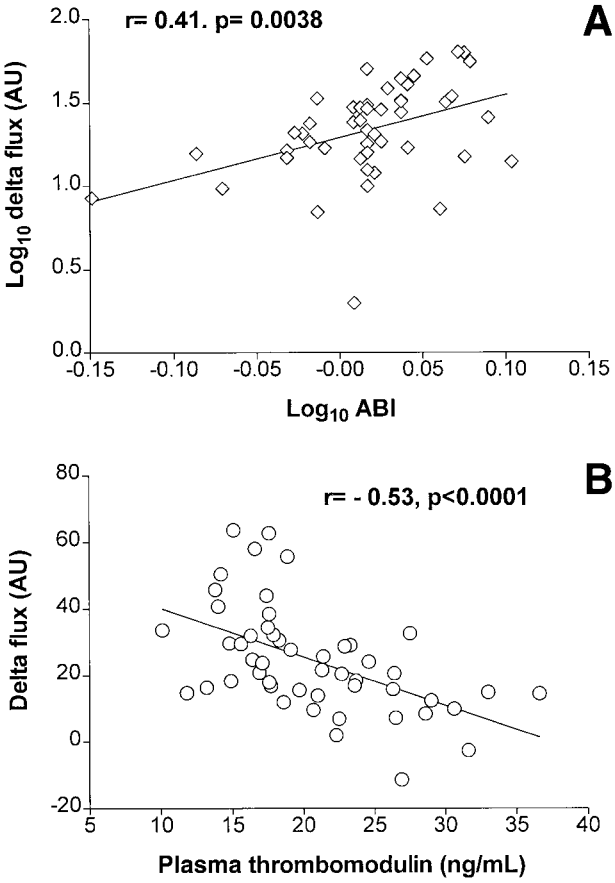

Fig 2. Correlation between the change in the skin blood flow before and after BPS administration (delta flux) and ABI (A) or plasma TM levels (B) in diabetic patients. (A) $r = .41$, $P < .0038$; (B) $r = -.53$, $P < .001$.

microalbuminuria (Table 1). The ABI value was significantly lower in microalbuminuric patients than in normoalbuminuric patients ($P = .0388$) (Fig 3A). The delta flux, the increase in the blood flow induced by BPS, was significantly lower in microalbuminuric patients than in normoalbuminuric patients ($P = .024$) (Fig 3B). There were no obvious bleeding episodes throughout the study.

DISCUSSION

The present study demonstrates that BPS significantly increases the blood flow of the skin of the feet in patients with type 2 diabetes, since the skin blood flow in the BPS group was significantly higher than that of the placebo group. Furthermore, we investigated the relationship between the increase in skin blood flow by BPS and various clinical parameters to determine which factors are associated with the blood flow change. We found a significant correlation between the delta flux, the change in blood flow induced by BPS, and the ABI values. This suggests that the change in the skin blood flow after BPS administration is influenced by atherosclerosis of the middle-sized arteries, since the ABI values are calculated using the ratio of the systolic pressure of the dorsalis pedis artery or

Table 3. Multiple Regression Analysis of the Relationship Between the Change in Skin Blood Flow Induced by BPS (delta flux) and Clinical Variables

Variables	Delta Flux	
	Partial Coefficient	*P* Value
Age (yr)	−0.723	.6573
BMI	−.0468	.7741
Diabetes duration (yr)	−.2562	.1105
MBP (mm Hg)	.1309	.4209
ABI	.5708	.0001
HbA_{1c} (%)	−.2596	.1057
T. Chol (mg/dL)	.2603	.1048
TG (mg/dL)	−.0004	.9979
HDL-Chol (mg/dL)	−.0727	.6559
UAE (mg/24 h)	−.0944	.5622
TM (U/mL)	−.4700	.0023

Abbreviations: BMI, body mass index; MBP, mean blood pressure; ABI, ankle brachial index; T. Chol, total cholesterol; TG, triglyceride; HD-Chol, high-density lipoprotein cholesterol; UAE, urinary albumin excretion.

posterior tibial artery to that of the brachial artery. BPS is known to dilate all sizes of arteries and arterioles similarly via the PGI_2 receptors in their smooth muscle.[1,2] We therefore speculate that BPS increases the skin blood flow in the toe by relaxing vascular smooth muscles of the middle-sized arteries, such as the dorsalis pedis and posterior tibial arteries.

Although the change in the skin blood flow after BPS administration in diabetic patients is significantly influenced by the ABI value, the present study demonstrated that there is another strong correlation between plasma TM levels and the change in skin blood flow after BPS administration. However, we found no relationship between plasma TM levels and ABI values in patients with type 2 diabetes. Multiple regression analysis confirmed that not only the ABI value but also plasma TM levels are significant independent factors for regulating the BPS-induced blood flow change. TM is an endothelial cell surface receptor for thrombin, and plays an important role in the regulation of coagulation via protein C activation.[23,24] The plasma levels of soluble form of the endothelial cell surface TM have been proposed to be a useful and specific marker for endothelial cell injury.[17] On the basis of the response to injury hypothesis proposed by Ross,[11,25] endothelial dysfunction and/or damage is a pivotal factor in the early stage of atherosclerosis. It has been suggested that plasma TM levels may be a marker for atherogenesis.[26] The present study showed that the increase in skin blood flow of the toe induced by BPS is independently influenced by plasma TM levels. The relationship between plasma TM levels and the change in skin blood flow by BPS suggests that the plasma TM level in patients with type 2 diabetes is associated with peripheral vascular disease, distinct from the ABI as a marker for peripheral vascular disease. The membrane TM is expressed predominantly on the endothelium of small vessel.[23,24] BPS relaxes the arterial smooth muscle in vessels of all sizes, particularly small arteries and arterioles.[1,2] Taken together, plasma TM levels may reflect the vasodilatory capacity of small arteries, eg, metatarsal or digital arteries, and arterioles in response to BPS, while the effect on the middle-sized arteries is more likely to influence the ABI value. Compared with nondiabetic individuals, diabetic patients are thought to have smaller vessel disease in arteriosclerosis formation.[27,28] It is possible that elevated plasma levels of soluble TM reflect the development of small vessel disease in patients with type 2 diabetes mellitus.

Since laser Doppler flowmetry measures not only nutritive capillary flow but also arteriovenous shunting flow, skin blood flow may be influenced by sympathetic nerve function.[29,30] The blood flow in the feet of diabetic patients with neuropathy is considered to be increased, as a result of the dilation of the arteriovenous shunt due to sympathetic neuropathy.[31-33] It is therefore possible that diabetic neuropathy influences changes in skin blood flow induced by BPS. The present study showed that the changes in blood flow induced by BPS, the delta flux, was significantly lower in the patients with neuropathy than those without neuropathy, suggesting that diabetic patients with neuropathy are in a vasodilated state, resulting in a less increased flow in response to BPS.

In both type 1 and 2 diabetes, microalbuminuria is not only a predictor of diabetic nephropathy but also a strong marker for risk of widespread vascular damage such as coronary artery

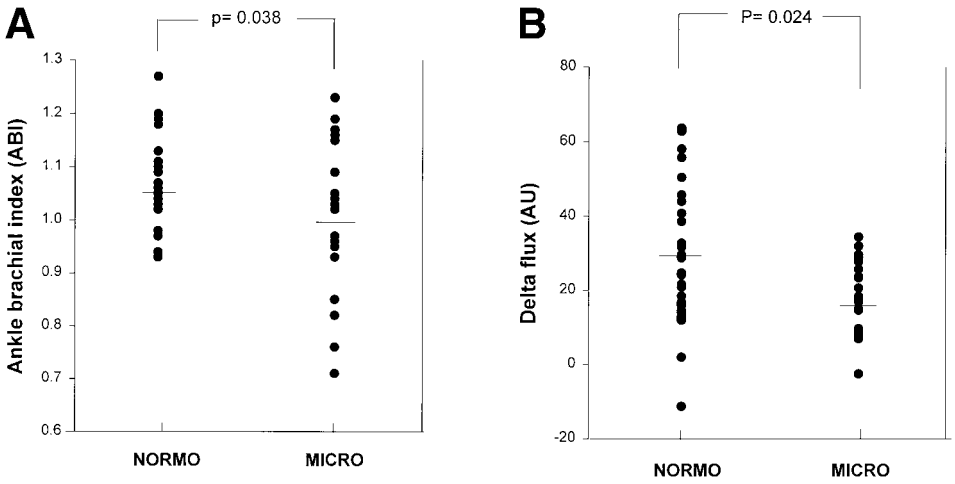

Fig 3. Comparison of the ABI (A) and the change in skin blood flow before and after BPS administration (delta flux) (B) between diabetic patients with normoalbuminuria and microalbuminuria. Statistical significance of the differences is indicated in the figure.

disease.[12-14,34] We therefore investigated the relationship between microalbuminuria and peripheral vascular disease in type 2 diabetes. We found that both the increase in the skin blood flow of the toe induced by BPS and the ABI values were significantly lower in microalbuminuric patients than in normoalbuminuric patients. This suggests that in type 2 diabetes, microalbuminuria is related to the development of peripheral vascular disease. The present study is the first to demonstrate a relationship between microalbuminuria and an impairment of vasodilation in the feet of patients with type 2 diabetes.

Finally, the present study shows that the delta flux, but not the basal flux, is associated with ABI values and plasma TM levels. The vasodilatory capacity of middle- and small-sized arteries and arterioles, ie, the development of potential atherosclerosis, can be determined by analysis of the change in skin blood flow of the feet in patients with type 2 diabetes after BPS administration, using laser Doppler flowmetry. Analysis of the change in the cutaneous blood flow after BPS administration may help to detect latent peripheral vascular disease.

REFERENCES

1. Weksler BB: Prostacyclin. Prog Hemost Thromb 6:113-138, 1982
2. Kimura T, Yoshida Y, Toda N: Mechanism of relaxation by prostaglandins in isolated canine arteries. Am J Obstet Gynecol 167: 1409-1416, 1992
3. Aso Y, Inukai T, Takemura Y: Evaluation of microangiopathy of the skin in patients with non–insulin-dependent diabetes mellitus by laser Doppler flowmetry: Microvasodilatory responses to beraprost sodium. Diabetes Res Clin Pract 36:19-26, 1997
4. Ishii H, Majerus PW: Thrombomodulin is present in human plasma and urine. J Clin Invest 76:2178-2181, 1985
5. Iwashima Y, Sato T, Watanabe K, et al: Elevation of plasma thrombomodulin level in diabetic patients with early diabetic nephropathy. Diabetes 39:983-988, 1990
6. Shimano H, Takahashi K, Kawakami M, et al: Elevated plasma and urinary thrombomodulin levels in patients with non–insulin-dependent diabetes mellitus. Clin Chim Acta 225:89-96, 1994
7. Inukai T, Fujiwara Y, Tayama K, et al: Clinical significance of measurements of urinary and plasma thrombomodulins in patients with non–insulin-dependent diabetes mellitus. Diabetes Res Clin Pract 33: 99-104, 1996
8. Aso Y, Sato A, Tanaka K, et al: Thrombomodulin and thrombin–antithrombin-III complex: Tool for possible markers of disease activity in microscopic polyarteritis and polyarteritis nodosa. Dokkyo J Med Sci 23:201-206, 1996
9. Mannucci PM: Von Willebrand factor. A marker of endothelial damage? Arterioscler Thromb Vasc Biol 18:1359-1362, 1998
10. Maruyama I, Bell CE, Majerus PW: Thrombomodulin is found on endothelium of arteries, veins, capillaries, and lymphatics, and on syncytiotrophoblast of human placenta. J Cell Biol 101:363-371, 1985
11. Ross R: The pathogenesis of atherosclerosis: A perspective for 1990s. Nature 362:801-809, 1993
12. Yudkin JS, Forrest RD, Jackson CA: Microalbuminuria as predictor of vascular disease in non-diabetic subjects. Lancet 2:530-533, 1988
13. Stehouwer CDA, Nauta JJ, Zeldenrust GC, et al: Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non–insulin-dependent diabetes mellitus. Lancet 340:319-323, 1992
14. Zander E, Heinke P, Gottschling D, et al: Increased prevalence of elevated urinary albumin excretion rate in type 2 diabetic patients suffering from ischemic foot lesions. Exp Clin Endocrinol Diabetes 105:S51-S53, 1997 (suppl)
15. Edmonds ME: The diabetic foot: Pathophysiology and treatment. Clin Endocrinol Metab 15:889-916, 1986
16. Edmonds ME, Morrison N, Law JW, et al: Medial arterial calcification and diabetic neuropathy. Lancet 284:928-930, 1982
17. Aso Y, Inukai T, Takemura Y: Mechanisms of elevation of serum and urinary concentrations of soluble thrombomodulin in diabetic patients: Possible application as a marker for vascular endothelial injury. Metabolism 47:362-365, 1998
18. Ishii H, Nakano M, Tsubouchi J, et al: Establishment of enzyme immunoassay of human thrombomodulin in plasma and urine using monoclonal antibodies. Thromb Haemost 63:157-162, 1990
19. Aso Y, Inukai T, Takemura Y: Evaluation of skin vasomotor reflexes in response to deep inspiration in diabetic patients by laser Doppler flowmetry: A new approach to the diagnosis of diabetic peripheral autonomic neuropathy. Diabetes Care 20:1324-1328, 1997
20. Ohno K, Nagase H, Matsumoto K, et al: Stereoselective synthesis of 5,6,7-trinor-4,8-inter-m-phenylene-PGI_2 derivatives and their inhibitory activities to human platelet aggregation. Adv Prostaglandin Leukotriene Res 5:279-281, 1985
21. Akiba T, Miyazaki M, Toda N: Vasodilatory action of TRK-100, a new prostaglandin I_2 analogue. Br J Pharmacol 89:703-711, 1986
22. Kato R, Uji Y, Matsumoto K: Phase I study of beraprost sodium (TRK-100), a prostacyclin derivative-repeated administration for 10 days. Jpn Clin Pharmacol Ther 20:529-539, 1989
23. Esmon CT, Owen WG: Identification of an endothelial cofactor for thrombin-catalyzed activation of protein C. Proc Natl Acad Sci USA 78:2249-2252, 1981
24. Dittman WA, Majerus PW: Structure and function of thrombomodulin: A natural anticoagulant. Blood 75:329-336, 1990
25. Ross R: Atherosclerosis—An inflammatory disease. N Engl J Med 340:115-126, 1999
26. Salomaa V, Matei C, Aleksic N, et al: Soluble thrombomodulin as a predictor of coronary heart disease and symptomless carotid artery atherosclerosis in the Atherosclerosis Risk in Communities (ARIC) study: A case-cohort study. Lancet 353:1729-1734, 1999
27. Moor JM, Frew IDO: Peripheral vascular lesions in diabetes mellitus. Br Med J 2:19-23, 1965
28. Faris I: Small and large vessel disease in the development of foot lesions in diabetics. Diabetologia 11:249-253, 1975
29. Rowell LB: Reflex control of the cutaneous vasculature. J Invest Dermatol 69:154-166, 1977
30. Flynn MD, Edmonds ME, Tooke JE, et al: Direct measurement of capillary blood flow in the diabetic neuropathic foot. Diabetologia 31:652-656, 1988
31. Boulton AJM, Scarpello JHB, Ward JD: Venous oxygenation in diabetic neuropathic foot: Evidence of arteriovenous shunting. Diabetologia 22:6-8, 1982
32. Ward JD: The diabetic leg. Diabetologia 22:141-147, 1982
33. Archer AG, Roberts VC, Watkins PJ: Blood flow patterns in painful diabetic neuropathy. Diabetologia 27:563-567, 1984
34. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, et al: Albuminuria reflects widespread vascular damage. The Steno hypothesis. Diabetologia 32:219-226, 1989