JACC Vol. 25, No. 2
February 1995:356–61

Enhanced Insulin Response Relates to Acetylcholine-Induced Vasoconstriction in Vasospastic Angina

MICHIO SHIMABUKURO, MD, PHD, TATSUSHI SHINZATO, MD, SATOSHI HIGA, MD, TAKAO CHIBANA, MD, PHD, HISASHI YOSHIDA, MD, PHD, FUMIO NAGAMINE, MD, PHD, KEIJI MURAKAMI, MD, PHD,* NOBUYUKI TAKASU, MD, PHD

Okinawa and Kobe, Japan

Objectives. **This study investigated whether insulin response to an oral glucose load correlates to acetylcholine-induced coronary vasoconstriction in subjects with vasospastic angina.**

Background. **It has been suggested that coronary vasospasm is caused by augmented vascular responsiveness possibly exerted by atherosclerosis. Recently, insulin resistance syndrome has been proposed as a major promotor of atherosclerotic disease, potentially enhancing vascular smooth muscular tone.**

Methods. **Among subjects with angiographically smooth coronary arteries, we selected 14 subjects with vasospastic angina and 14 age- and gender-matched subjects with atypical chest pain. We compared coronary vasomotor response to acetylcholine infusion, glucose and insulin responses to an oral glucose load (75 g), serum lipid concentrations, obesity, heart rate, blood pressure and smoking habits in both groups.**

Results. **Fasting serum insulin concentrations and insulin response were higher in subjects with vasospastic angina than in those with atypical chest pain; however, glucose tolerance, obesity, heart rate, blood pressure and smoking habits did not differ between groups. In subjects with vasospastic angina, nearly all coronary segments, except distal segments of the left circumflex coronary artery, were constricted at peak acetylcholine infusion (20 to 100 μg), whereas all segments were dilated in subjects with atypical chest pain. Regression analysis for both groups demonstrated a correlation between coronary vasoconstriction and fasting serum insulin concentrations ($r = 0.52$, $p < 0.01$), insulin response ($r = 0.71$, $p < 0.001$), serum triglyceride concentrations ($r = 0.51$, $p < 0.05$) and atherogenic index ($r = 0.44$, $p < 0.05$).**

Conclusions. **Results show that acetylcholine-induced coronary vasoconstriction in subjects with vasospastic angina correlates with hyperinsulinemia and enhanced insulin response, suggesting insulin resistance syndrome as a feature of vasospastic angina.**

(J Am Coll Cardiol 1995;25:356–61)

It has been speculated (1–4) that coronary vasospasm plays an important role in the pathogenesis of ischemic cardiac events, ranging from variant angina to myocardial infarction. Injection of acetylcholine causes vasodilation in a normal coronary artery but vasoconstriction in an atherosclerotic or diseased coronary artery (5–7). This heterogeneous response to acetylcholine occurs even in angiographically smooth coronary arteries and has been thought to result from early atherosclerosis because of the presence of coronary risk factors, such as male gender, age, hypercholesterolemia and hypertension (8–11).

Insulin resistance syndrome has been proposed as a major promoter of atherosclerotic disease (12,13). Thus obesity, hypertension, glucose intolerance and dislipidemia have been suggested to promote atherosclerosis, with insulin resistance as the underlying intermediary. Furthermore, earlier studies have implied that hyperinsulinemia itself may enhance coronary vasomotor tone through sympathetic nerve activation (14,15), hypertrophic effects on smooth muscle cells (16,17) or modification of intracellular cation metabolism (18–20). Therefore, insulin resistance syndrome, at least in part, is speculated to relate to vasospastic angina through augmenting atherosclerosis or coronary vascular tone, or both.

To investigate whether insulin resistance syndrome correlates to coronary vasospasm, we evaluated coronary vasomotor response to acetylcholine and insulin response to a glucose load in angiographically normal subjects with vasospastic angina or atypical chest pain.

Methods

Study subjects. We studied 14 subjects with vasospastic angina and 14 control subjects with atypical chest pain. Subjects with vasospastic angina had a spontaneous episode of angina pectoris at rest accompanying ischemic electrocardiographic (ECG) changes and demonstrated >50% vasoconstriction by acetylcholine infusion in one or more segments of epicardial coronary arteries with ischemic ST segment changes. Age- and gender-matched control subjects had atypical chest pain without ischemic ECG changes or acetylcholine-induced vasospasm. Subjects from both groups were included if they

From the Second Department of Internal Medicine, School of Medicine, Faculty of Medicine, University of the Ryukyus, Okinawa and *Department of Internal Medicine, Suma Red Cross Hospital, Kobe, Japan.

Manuscript received April 13, 1994; revised manuscript received August 29, 1994, accepted August 31, 1994.

Address for correspondence: Dr. Michio Shimabukuro, Second Department of Internal Medicine, School of Medicine, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-01, Japan.

0735-1097/95/$9.50
0735-1097(94)00381-Y

had angiographically normal coronary arteries to eliminate the dominant effect of atherosclerosis on the angiogram. All subjects showed no evidence of arrythmia, valvular heart disease or contractile dysfunction. Additionally, subjects with plasma glucose concentrations of <6.7 mmol/liter (120 mg/dl) at fasting and <11.1 mmol/liter (200 mg/dl) 120 min after a 75-g oral glucose load were included to omit possible deficits of insulin secretion in subjects with severe glucose intolerance. Other criteria for inclusion were age 40 to 69 years; no history of renal, hepatic and endocrine disorders; and no current medication that affects insulin sensitivity, such as thiazides and alpha- and beta-blockers. Written informed consent was obtained from all patients after the study protocol was explained.

Coronary vasomotor response to acetylcholine infusion. All patients stopped taking calcium channel blocking agents and long-acting nitrates at least 12 h before cardiac catheterization. After a standard protocol of right- and left-sided cardiac catheterization and coronary angiography, coronary vasomotor response to acetylcholine infusion was studied as follows. First, a pacing catheter set in the demand mode of 50 beats/min was placed in the right ventricular apex. Through 7F right and left Judkins catheters, graded doses of acetylcholine chloride (Dai-ichi Pharmaceutical Co., Tokyo, Japan) dissolved in 5 ml of saline solution were infused manually at 5 ml/30 s. Acetylcholine was infused at 20 and 50 μg in the right coronary artery and at 20, 50 and 100 μg in the left coronary artery. After acetylcholine-induced vasospasm occurred, the higher dosage of acetylcholine was discontinued. Throughout the study protocol, heart rate, aortic pressure and the ECG were monitored continuously.

When acetylcholine-induced vasospasm (>50% of vasoconstriction in one or more segments of epicardial coronary arteries) with ischemic ECG changes (>1-mV ST segment depression or elevation) occurred, or, if not, 3 min after each acetylcholine injection, coronary angiograms were recorded on 35-mm cinefilm (30 frames/s) with a cineangiographic system (Sim Trac, Siemens, Erlangen, Germany). A volume of 8 to 10 ml nonionic contrast medium (Omnipaque 350, Dai-ichi Pharmaceutical Co.) was injected into the coronary arteries at 4 to 5 ml/s. The optimal view for recording was carefully chosen based on the baseline angiogram, and that same position was used to document subsequent angiograms. After calibration with scaled grids in the fields of view, lumen diameters were measured with a caliper at end-diastole in the proximal, middle and distal segments of the left anterior descending and right coronary arteries and the proximal and distal segments of the left circumflex coronary artery. In each segment, the portion that exhibited the maximal change in diameter at peak acetylcholine dosage was chosen for subsequent measurement. Coronary vasomotor response to acetylcholine infusion was expressed as percent change in coronary artery diameter corrected for infused acetylcholine: % Change in coronary artery diameter = $(D_{after} - D_{before}) \times 100/D_{before}/-\log[\text{Acetylcholine (mol)}]$, where D_{after} and D_{before} = diameter before and after peak acetylcholine infusion (10). In this formula, positive and negative values reflect vasodilation and vasoconstriction, respectively. The sum of the percent diameter changes in eight coronary segments (sum of % diameter changes in coronary segments/$-\log[\text{Acetylcholine (mol)}]$) was used to assess total coronary vasomotor responses to acetylcholine.

Definition of risk factors. We measured the following risk factors 1 or 2 days before cardiac catheterization: plasma glucose and serum insulin responses to a glucose load (75 g), serum lipid concentrations, obesity, heart rate, blood pressure and current smoking habits. Heavy physical exercise, smoking and caffeine were restricted in all subjects for 2 days before the study. After an overnight fast, a 75-g oral glucose loading test was performed between 8 and 10 AM. Glucose and insulin areas were defined as follows: glucose or insulin area (mmol·h/liter or μU·h/ml) = $0.25 \times (C_0 + C_{120}) + 0.5 \times (C_{30} + C_{60} + C_{90})$, where $C_{0,30,60,90,120}$ = plasma glucose or serum insulin concentrations before and 30, 60, 90 and 120 min after a glucose load. Plasma glucose was determined with an autoanalyzer (model 736-60, Hitachi Medical Corporation, Tokyo, Japan) using a glucose oxidase method (Quickauto Neo Glu HK, Shinotest Co., Tokyo, Japan), and serum insulin was measured by radioimmunoassay using an anti-human insulin antibody (AB insulin beads, Eiken Co., Tokyo, Japan). After an overnight fast, serum concentrations of total cholesterol, triglycerides and high density lipoprotein (HDL) cholesterol fractionated using dextran magnesium sulfate were measured with enzymatic methods. Low density lipoprotein (LDL) cholesterol was calculated as follows: LDL cholesterol = Total cholesterol − HDL cholesterol − Triglyceride concentrations/5. Apolipoprotein A-I, A-II, B, C-II, C-III and E were measured with a single radial immunodiffusion method. Body mass index was defined as the weight/height^2 ratio (kg/m^2). Steady state blood pressure and heart rate were recorded in duplicate in the supine position after at least a 12-h cessation of medication. Current smoking habits were assessed by the index of cigarettes smoked/day × years.

Statistical analysis. Results are expressed as mean value ± SEM. Two-tailed unpaired Student *t* and Fisher exact probability tests were used to compare group mean values. Glucose and insulin responses to a glucose load were compared between groups using two-way analysis of variance and within a group by one-way analysis of variance for repeated measures followed by a Bonferroni multiple comparison test. Simple and stepwise multiple regression analyses were performed to test correlations between total coronary vasomotor response and risk factors in both groups. A p value < 0.05 was considered significant.

Results

Clinical characteristics. The two study groups did not differ in body mass index, heart rate, blood pressure and cigarette smoking habits (Table 1). Obesity (body mass index >25 kg/m^2), hypertension (systolic blood pressure >160 mm Hg or diastolic blood pressure >95 mm Hg, or both) and cigarette smoking (cigarettes smoked/day × year >400) analyzed with the

JACC Vol. 25, No. 2
February 1995:356–61

Table 1. Clinical Characteristics in Subjects With Vasospastic Angina or Atypical Chest Pain

	Subjects With Atypical Chest Pain (n = 14)	Subjects With Vasospastic Angina (n = 14)
Age (yr)	54 ± 2	52 ± 3
Gender (M/F)	7/7	8/6
Body mass index (kg/m^2)	24.1 ± 0.6	23.4 ± 0.7
Heart rate (beats/min)	73 ± 4	68 ± 4
Blood pressure (mm Hg)	139/79 ± 8/4	133/77 ± 8/3
Smoking (cigarettes/day × year)	144 ± 69	281 ± 125

Data presented are mean value ± SEM or number of patients. F = female; M = male.

Fisher exact probability test also showed no significant difference between groups (data not shown). Serum concentrations of total, HDL and LDL cholesterol, triglycerides and apolipoproteins and atherogenic index did not differ between the groups (Table 2).

Glucose and insulin responses. Plasma glucose and serum insulin responses to an oral glucose load (75 g) are shown in Figure 1. Fasting plasma glucose concentration and glucose response (expressed by peak glucose concentration and glucose area) were similar between both groups (Table 3). However, fasting serum insulin concentration and insulin response (expressed by peak insulin concentration, insulin area, insulin area/glucose area and insulinogenic index) were significantly higher in subjects with vasospastic angina than in those with atypical chest pain.

Coronary vasomotor response to acetylcholine. All 14 subjects with vasospastic angina had acetylcholine-induced vasoconstriction >50% with ischemic ST segment changes (the left anterior descending coronary artery in 8 [2 with vasoconstriction >90%]; the right coronary artery in 5 [1 with vasoconstriction >90%]; and the left anterior descending and right

Table 2. Serum Concentrations of Cholesterol, Triglycerides and Apolipoproteins in Subjects With Vasospastic Angina or Atypical Chest Pain

	Subjects With Atypical Chest Pain (n = 14)	Subjects With Vasospastic Angina (n = 14)
Total cholesterol (mmol/liter)	5.69 ± 0.28	5.74 ± 0.27
HDL (mmol/liter)	1.40 ± 0.08	1.33 ± 0.13
LDL (mmol/liter)	3.59 ± 0.23	3.57 ± 0.23
Triglycerides (mmol/liter)	1.55 ± 0.14	1.96 ± 0.29
Apo-AI (mg/100 ml)	136 ± 8	137 ± 9
Apo-AII (mg/100 ml)	32 ± 2	33 ± 3.0
Apo-B (mg/100 ml)	106 ± 4	97 ± 11
Apo-CII (mg/100 ml)	3.50 ± 0.40	4.36 ± 0.58
Apo-CIII (mg/100 ml)	7.3 ± 0.8	11.0 ± 1.7
Apo-E (mg/100 ml)	5.40 ± 0.90	6.70 ± 0.70
Atherogenic index	3.10 ± 0.19	3.80 ± 0.50

Data presented are mean value ± SEM. Apo = apolipoprotein; Atherogenic index = (Total cholesterol − High density lipoprotein [HDL] cholesterol)/HDL cholesterol; LDL = low density lipoprotein cholesterol.

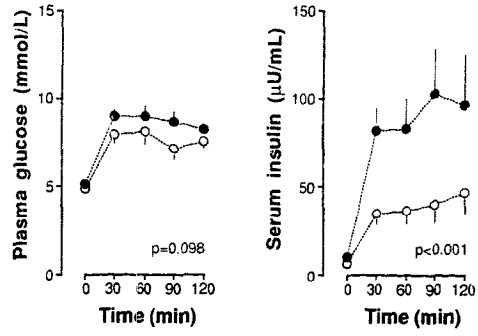


Figure 1. Plasma glucose (**left**) and serum insulin (**right**) responses to an oral glucose load (75 g) in subjects with vasospastic angina (**solid circles,** n = 14) or atypical chest pain (**open circles,** n = 14). Data represent mean value ± SEM. Two-way analysis of variance between-group p values are shown.

coronary arteries in 1). Subjects with atypical chest pain showed vasodilation of the right (acetylcholine up to 50 μg) and left (acetylcholine up to 100 μg) coronary arteries. Group mean coronary diameter changes during acetylcholine infusion are shown in Figure 2. Subjects with vasospastic angina showed vasoconstriction of nearly all segments except for distal segments of the left circumflex coronary artery, whereas those with atypical chest pain had vasodilation of all segments at the maximal dose of acetylcholine. Total coronary vasomotor response to acetylcholine ranged from −40.2 to −6.7 (−18.4 ± 9.5) in subjects with vasospastic angina and from 32.2 to 2.4 (11.2 ± 8.4) in those with atypical chest pain.

Multiple regression analysis. Simple regression analysis for both groups also indicated that age, body mass index, heart rate (data not shown), systolic and diastolic blood pressures and fasting plasma glucose concentrations did not correlate with total coronary vasomotor response to acetylcholine (Fig. 3). On the contrary, the analysis demonstrated that total coronary vasomotor response to acetylcholine significantly correlated with serum fasting ($r = -0.52$, $p < 0.01$) and peak insulin concentrations ($r = -0.71$, $p < 0.001$), insulin area ($r = -0.71$, $p < 0.001$), insulin area/glucose area ($r = -0.71$, $p <$

Table 3. Plasma Glucose and Serum Insulin Responses to an Oral Glucose Load (75 g) in Subjects With Vasospastic Angina or Atypical Chest Pain

	Subjects With Atypical Chest Pain (n = 14)	Subjects With Vasospastic Angina (n = 14)
Fasting plasma glucose (mmol/liter)	4.87 ± 0.10	5.12 ± 0.09
Fasting plasma insulin (μU/ml)	6.5 ± 0.8	10.2 ± 1.4*
Peak plasma glucose (mmol/liter)	9.10 ± 0.62	9.90 ± 0.44
Peak plasma insulin (μU/ml)	46 ± 7	123 ± 25†
Glucose area (mmol·h/liter)	14.8 ± 0.9	16.7 ± 0.7
Insulin area (μU·h/mliter)	61 ± 10	161 ± 31†
Insulin area/glucose area	3.99 ± 0.53	9.24 ± 1.46†
Insulinogenic index	0.54 ± 0.13	1.05 ± 0.15*

*$p < 0.05$, †$p < 0.01$ versus subjects with atypical chest pain. Data presented are mean value ± SEM.

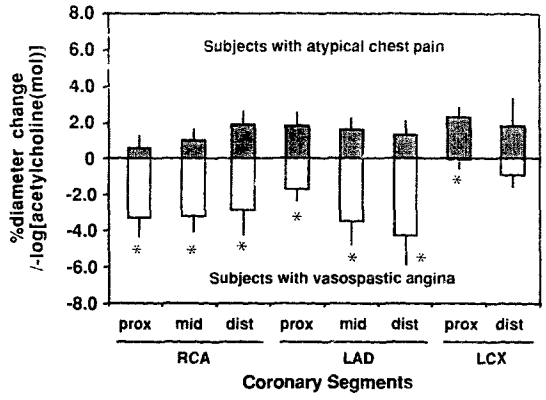
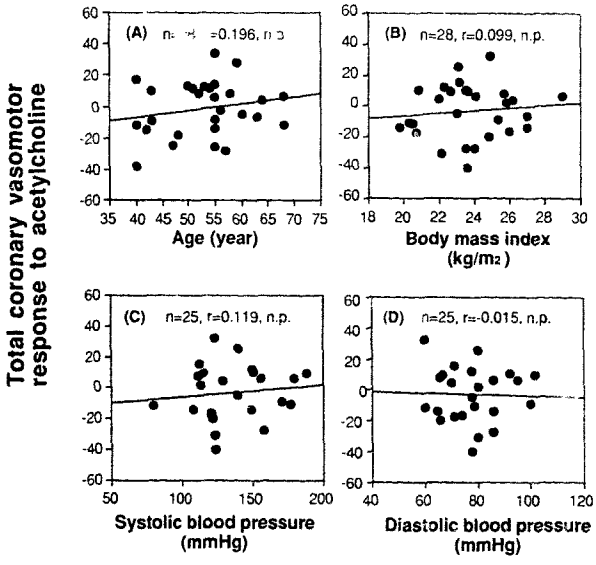


Figure 2. Coronary artery diameter responses to acetylcholine infusion in subjects with vasospastic angina (**open bars,** n = 14) or atypical chest pain (**dotted bars,** n = 14). Data represent mean value ± SEM. *Significantly different from the corresponding values in subjects with atypical chest pain ($p < 0.01$). dist = distal; LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery; mid = middle; prox = proximal; RCA = right coronary artery.

0.001), insulinogenic index ($r = -0.46$, $p < 0.05$) and glucose area ($r = -0.52$, $p < 0.01$) (Fig. 4). Plasma triglyceride concentrations and atherogenic index were also related to total coronary vasomotor response (Fig. 5). Stepwise multiple regression analysis showed that only insulin area correlated with total coronary vasomotor response (standard coefficient -0.732, $p < 0.001$). Values not included in the model were age (partial coefficient -0.030), body mass index (-0.248), systolic

Figure 3. Correlation between total coronary vasomotor response to acetylcholine and age (**A**), body mass index (**B**) and systolic (**C**) and diastolic (**D**) blood pressure in subjects with angiographically normal coronary arteries. Total coronary vasomotor response to acetylcholine is expressed as sum of percent diameter changes in eight coronary segments/−log [acetylcholine (mol)], where positive and negative values reflect vasodilation and vasoconstriction, respectively. n.p. = not predictable.



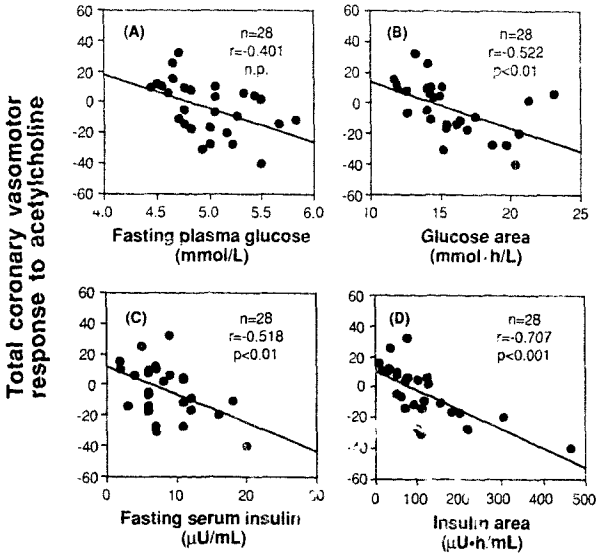


Figure 4. Correlation between total coronary vasomotor response to acetylcholine and fasting plasma glucose concentrations (**A**), glucose area (**B**), serum insulin concentrations (**C**) and insulin areas (**D**) in subjects with angiographically normal coronary arteries. Total coronary vasomotor response to acetylcholine and positive and negative values as in Figure 3. n.p. = not predictable.

blood pressure (0.074), smoking habits (-0.235), glucose area (-0.036) and total cholesterol (-0.050) and triglyceride (-0.096) concentrations. For the final regression model, total coronary vasomotor response to acetylcholine was $-0.129 \times$ (Insulin area) + 12.48 (Adjusted $R^2 = 0.514$).

Discussion

In this study, angiographically normal subjects with vasospastic angina demonstrated hyperinsulinemia and enhanced insulin response to an oral glucose load. Regression analysis in subjects with vasospastic angina or atypical chest pain showed that acetylcholine-induced vasoconstriction correlated with fasting serum insulin concentrations, insulin response and serum triglyceride concentrations but not with obesity, heart rate, blood pressure or smoking habits.

Coronary vasospasm and atherosclerosis. Coronary vasospasm has been hypothesized to depend on the extent of atherosclerosis because predictors for vasospastic angina or acetylcholine-induced vasoconstriction are similar to coronary risk factors for atherosclerosis (6–11). Impaired release of endothelium-derived relaxing factor (EDRF) has been reported in atherosclerotic coronary arteries, suggesting atherosclerosis-elicited endothelial dysfunction as a cause of coronary vasospasm (21,22). However, several investigators (23,24) have reported preserved endothelium-dependent vasodilation in vasospastic sites in patients with vasospastic angina, implicating coronary vasospasm mainly on the basis of a supersensitivity of vascular smooth muscle. An augmented contractility of vascular smooth muscle has been shown to be

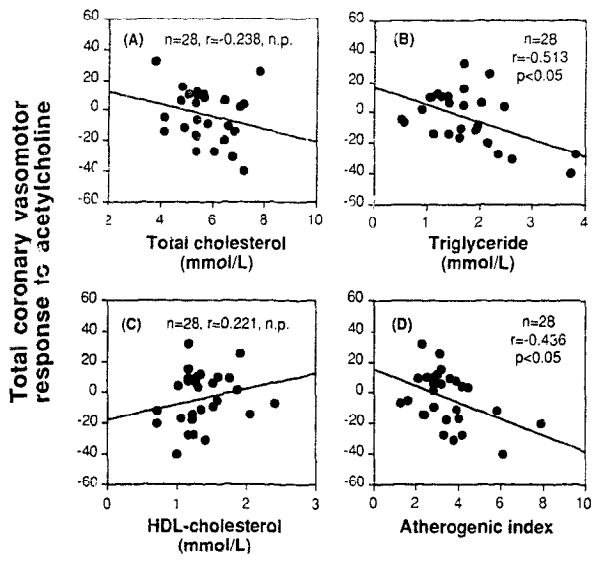


Figure 5. Correlation between total coronary vasomotor response to acetylcholine and serum total cholesterol (**A**), triglyceride (**B**) and high density lipoprotein (HDL) cholesterol (**C**) concentrations and atherogenic index [(Total cholesterol − HDL cholesterol)/HDL cholesterol] (**D**) in subjects with angiographically normal coronary arteries. Total coronary vasomotor response to acetylcholine and positive and negative values as in Figure 3. n.p. = not predictable.

evoked by atherosclerosis (25). The atherosclerosis-elicited hyperreactivity of vascular smooth muscle may play some role in the pathogenesis of coronary vasospasm (26).

Insulin resistance syndrome and atherosclerosis. Even were overt hyperglycemia not established, hyperinsulinemia and increased responses in plasma glucose and insulin obviously suggest the involvement of insulin resistance in subjects with vasospastic angina (27). Insulin resistance syndrome was observed to be a significant contributor to atherogenesis when accompanied by other risk factors, namely glucose intolerance, dislipidemia, obesity and hypertension (12,13). Up to now serum total cholesterol (9–11) and apolipoprotein concentrations (28,29) have been considered indicators for acetylcholine-induced vasoconstriction or vasospastic angina. However, the present study demonstrated that serum triglyceride concentrations and atherogenic index, regardless of total cholesterol or apolipoprotein concentrations, correlated with acetylcholine-induced vasoconstriction (30). Numerous studies (27,31–34) have shown that insulin resistance syndrome affects lipoprotein metabolism through several mechanisms. Thus, insulin stimulates hepatic very low density lipoprotein cholesterol (VLDL) secretion, whereas peripheral insulin resistance causes a reduced breakdown of triglyceride-rich lipoprotein and a decrease in HDL cholesterol synthesis (33). Also, an increase in atherogenic small, dense LDL (27,32) and stimulated uptake of LDL receptor-mediated cholesterol (34) have been demonstrated. In fact, regression analysis in the present study subjects revealed that fasting serum insulin concentrations and insulin area correlated with plasma triglyceride ($r = 0.51$, $p < 0.01$; $r = 0.65$, $p < 0.001$, respectively) and HDL cholesterol ($r = -0.49$, $p < 0.01$; $r = -0.41$, $p < 0.05$) concentrations and atherogenic index ($r = 0.67$, $p < 0.001$; $r = 67$, $p < 0.001$). In vitro studies have demonstrated that insulin exerts a direct hypertrophic effect on both vascular endothelium and smooth muscle cells (16,17). Insulin resistance syndrome may promote atherogenesis through dislipidemia or primary hypertrophic effects, or both, involving a vasospastic response to acetylcholine through an augmented contractility of vascular smooth muscle, although abnormal lipid metabolism itself could be considered a cause of acetylcholine-induced vasoconstriction (9–11).

Insulin and coronary vasomotor tone. Another possible mechanism for a correlation between hyperinsulinemia and acetylcholine-induced vasoconstriction is the finding that hyperinsulinemia itself may augment coronary vasomotor tone. Yanagisawa-Miwa et al. (35) demonstrated that a thromboxane A_2 analogue accentuated contraction of the coronary artery preincubated with a physiologic concentration of insulin (30 to 300 μU/ml). Because attenuation had been observed only at lower concentrations of Mg^{2+} (0.5 to 1.5 mmol/liter), these investigators (35) hypothesized that Mg^{2+}-modulated insulin action on glucose uptake and Ca^{2+} metabolism was the mechanism. An earlier study (36) reported that thromboxane B_2, an inactive metabolite of thromboxane A_2, was increased in the peripheral and coronary sinus blood of patients with vasospastic angina and suggested thromboxane A_2 as a possible mediator of coronary vasospasm. Furthermore, insulin has been demonstrated to modify intracellular cation metabolism, presumably through the mechanism of inhibited Ca^{2+}/Mg^{2+} ATPase and stimulated Ca^{2+}/Na^{+} exchanger activities (18–20). A potential alteration in intracellular calcium metabolism may modulate coronary vasomotor tone (27,37). Recently, hyperinsulinemia has been implicated in the pathogenesis of microvascular angina (syndrome X) (38–40). The term syndrome X refers to patients with angiographically normal findings who have anginal chest pain and positive exercise test results on the ECG. The presence of the insulin-resistant state in coronary vasospasm and syndrome X may indicate similar underlying mechanisms despite their different clinical manifestations.

Conclusions. The present study demonstrated that hyperinsulinemia and an enhanced insulin response to an oral glucose load correlated with acetylcholine-induced vasoconstriction in subjects with vasospastic angina. Although the role of the insulin-resistant state in the pathogenesis of coronary vasospasm cannot be ascertained on the basis of this study, we may speculate that insulin resistance syndrome is a feature of vasospastic angina. Further studies are necessary to elucidate the role of insulin resistance syndrome in the development or modification of coronary vasospasm.

References

1. Hills LD, Braunwald E. Coronary artery spasm. N Engl J Med 1978;299: 695–702.

2. Conti CR, Curry RC. Coronary artery spasm and myocardial ischemia. Mod Concepts Cardiovasc Dis 1980;49:1–6.
3. Maseri A, Chierchia S. Coronary artery spasm: demonstration, definition, diagnosis and consequences. Prog Cardiovasc Dis 1982;25:169–92.
4. Maseri A, Davies G, Hackett D, Kaski SC. Coronary artery spasm and vasoconstriction: the case for a distinction. Circulation 1990;81:1983–91.
5. Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986;315:1046–51.
6. Yasue H, Horio Y, Nakamura N, et al. Induction of coronary artery by acetylcholine in patients with variant angina: possible role of the parasympathetic nervous system in the pathogenesis of coronary spasm. Circulation 1986;74:955–63.
7. Arbustini E, Grasso M, Diegoli M, et al. Morphological changes induced by acetylcholine infusion in normal and atherosclerotic coronary arteries. Am J Cardiol 1993;71:1382–90.
8. Scholl JM, Benacerraf A, Ducimetiere P, et al. Comparison of risk factors in vasospastic angina without fixed coronary narrowing to significant fixed coronary narrowing and no vasospastic angina. Am J Cardiol 1986;57:199–202.
9. Yasue H, Matsuyama K, Matsuyama K, Okumura K, Morikami Y, Ogawa H. Responses of angiographically normal human coronary arteries to intracoronary injection of acetylcholine by age and segment: possible role of early coronary atherosclerosis. Circulation 1990;81:482–90.
10. Vita JA, Treasure CB, Nabel EG, et al. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation 1990;81:491–7.
11. Zeiher AM, Drexler H, Wollschlanger H, Just H. Modulation of coronary tone in humans: progressive endothelial dysfunction with different early stages of coronary atherosclerosis. Circulation 1991;83:391–401.
12. Raven GM. Role of insulin resistance in human disease. Diabetes 1988;37: 1595–1607.
13. Kaplan NM. The deadly quartet: upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. Arch Intern Med 1989;149:1514–20.
14. Rowe JW, Young JB, Minalker KL, Stevens AL, Pallota J, Landsberg L. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. Diabetes 1981;30:219–25.
15. Lansberg L, Young JB. Insulin-mediated glucose metabolism in the relationship between dietary intake and sympathetic nervous system activity. Int J Obes 1985;9:63–8.
16. Stout RW, Bierman EL, Ross R. Effect of insulin on the proliferation of cultured primate arterial smooth muscle cells. Circ Res 1975;36:319–27.
17. King GL, Goodman AD, Buzney S, Moses A, Kahn C. Receptors and growth-promoting effects of insulin and insulin-like growth factors on cells from bovine retinal capillaries and aorta. J Clin Invest 1985;75:1028–36.
18. Moore RD. Effects of insulin upon ion transport. Biochim Biophys Acta 1983;737:1–49.
19. Pershandsingh HA, McDonald JM. Direct action of insulin inhibits a high affinity Ca^{2+}-ATPase in isolated adipocyte plasma membranes. Nature 1979;281:495–7.
20. Pershandsingh HA, McDonald JM. (Ca^{2+} + Mg^{2+})-ATPase in adipocyte plasmalemma: inhibition by insulin and concanavalin A in the intact cell. Biochem Int 1981;2:243–8.
21. Chester AH, O'Neil GS, Moncada S, Tadjkarimi S, Yacoub MH. Low basal and stimulated release of nitric oxide in atherosclerotic epicardial coronary arteries. Lancet 1990;336:897–900.
22. Bossaller C, Habib GB, Yamamoto H, Williams C, Wells S, Henry PD. Impaired muscarinic endothelium-dependent relaxation and cyclic guanosine 5-monophosphate formation in atherosclerotic human coronary artery and rabbit aorta. J Clin Invest 1987;79:170–4.
23. Yamamoto H, Yoshimura H, Noma M, et al. Preservation of endothelium-dependent vasodilation in the spastic segment of the human epicardial coronary artery by substance P. Am Heart J 1992;123:298–303.
24. Egashira K, Inou T, Yamada A, Hirooka Y, Takeshita A. Preserved endothelium-vasodilation at the vasospastic site in patients with variant angina. J Clin Invest 1992;89:1047–52.
25. Heistad DD, Armstrong ML, Marcus ML, Piegors DJ, Mark AL. Augmented response to vasoconstrictor stimuli in hypercholesterolemic and atherosclerotic monkeys. Circ Res 1984;54:711–8.
26. Satoh S, Tomoike H, Mitsuoka W, Egashira K, Tagawa H, Kuga T, Nakamura M. Smooth muscle from spastic coronary artery segments show hypercontractility to histamine. Am J Physiol 1990;259:H9–13.
27. Ferrari P, Weidmann P. Insulin, insulin sensitivity and hypertension. J Hypertens 1990;8:491–500.
28. Yoshida H, Murakami K, Mimura G. Study on lipid and glucose metabolism in patients with vasospastic angina. Jpn J Med 1989;28:348–54.
29. Tasaki H, Nakashima Y, Nandate H, Yashiro A, Kawashima T, Kuroiwa A. Comparison of serum lipid values in variant angina pectoris and fixed coronary artery disease with normal subjects. Am J Cardiol 1989;63:1441–5.
30. Yamamoto S, Murakami K, Nagamine F, Mimura G. Study on lipoprotein and apoprotein metabolism by the ultracentrifugation method in patients with vasospastic angina (in Japanese). J Jpn Atheroscler Soc 1992;20:45–51.
31. Austin MA. Epidemiologic associations between hypertriglyceridemia and coronary heart disease. Semin Thromb Hemost 1988;14:137–42.
32. Tobey TA, Greenfield M, Kraemer F, Raeven DM. Relationship between insulin resistance, insulin secretion, very low density lipoprotein kinetics and plasma triglyceride levels in normotriglyceridemic man. Metabolism 1981; 30:165–71.
33. Golay A, Zech L, Shi MZ, Chiou YA, Reaven GM, Chen YD. High density lipoprotein (HDL) metabolism in non-insulin-dependent diabetes mellitus: measurement of HDL turnover using tritiated HDL. J Clin Endocrinol Metab 1987;65:512–8.
34. Oppenheimer MJ, Sundquist K, Bierman EL. Downregulation of high density lipoprotein receptor activity of cultured fibroblasts by insulin and IGF-1. Diabetes 1989;38:117–22.
35. Yanagisawa-Miwa A, Ito H and Sugimoto T. Effects of insulin on vasoconstriction induced by thromboxane A_2 in porcine coronary artery. Circulation 1990;81:1654–9.
36. Toda M, Kuzuya T, Inoue M, et al. Elevation of thromboxane B_2 levels in patients with classic and variant angina pectoris. Circulation 1981;64:1107–14.
37. Draznin B, Sussman KE, Eckel RH, Kao M, Yost T, Sherman NA. Possible role of cytosolic free calcium concentrations in mediating insulin resistance of obesity and hyperinsulinemia. J Clin Invest 1988;82:1848–52.
38. Dean JD, Jones CJH, Hutchinson SJ, Peters JR, Henderson AH. Hyperinsulinaemia and microvascular angina ("syndrome X"). Lancet 1991;337: 456–7.
39. Swan JW, Walton C, Godsland IF, Crook D, Oliver MF, Stevenson JC. Insulin resistance syndrome as a feature of cardiological syndrome X in non-obese men. Br Heart J 1994;71:41–4.
40. Chauhan A, Foote J, Petch MC, Schofield PM. Hyperinsulinemia, coronary artery disease and syndrome X. J Am Coll Cardiol 1994;23:364–8.