Dose-Dependent Vasodilating Effects of Insulin on Adenosine-Stimulated Myocardial Blood Flow

Jan Sundell,[1,2] Pirjo Nuutila,[1,2] Hanna Laine,[1,2] Matti Luotolahti,[3] Kari Kalliokoski,[1] Olli Raitakari,[1,3] and Juhani Knuuti[1]

In the peripheral vasculature, insulin induces time- and dose-dependent vasodilation. We have recently demonstrated that insulin potentiates adenosine-stimulated myocardial blood flow. However, it is unknown whether insulin's effects on the coronary vasculature are dose dependent. In this study, we quantitated myocardial blood flow and adenosine-stimulated coronary flow (140 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 5 min) in 10 healthy men (age, 32 ± 6 years; BMI, 24.1 ± 1.8 kg/m^2) using positron emission tomography and ^{15}O-labeled water. Hyperemic myocardial blood flow was measured in the basal state, during euglycemic physiological hyperinsulinemia (serum insulin ~65 mU/l) and during supraphysiological hyperinsulinemia (serum insulin ~460 mU/l). Basal myocardial blood flow was 0.84 ± 0.17 $ml \cdot g^{-1} \cdot min^{-1}$. Physiological hyperinsulinemia increased the adenosine-stimulated flow by 20% (from 3.92 ± 1.17 to 4.72 ± 0.96 $ml \cdot g^{-1} \cdot min^{-1}$; $P < 0.05$). Supraphysiological hyperinsulinemia further enhanced the adenosine-stimulated flow by 19% (to 5.61 ± 1.03 $ml \cdot g^{-1} \cdot min^{-1}$; $P < 0.05$). These effects were not explained by changes in systemic hemodynamics, since coronary resistance decreased during each insulin infusion ($P < 0.05$). In addition, hyperemic myocardial blood flow responses during insulin stimulation were positively correlated with whole-body glucose uptake. The results demonstrate that insulin is able to enhance hyperemic myocardial blood flow in a dose-dependent manner in healthy subjects. These effects might contribute to the known beneficial dose-dependent effects of insulin on myocardial ischemia. *Diabetes* 51:1125–1130, 2002

Insulin is known to be vasoactive in the peripheral vasculature, but its effects on myocardial perfusion are poorly known. Glucose-insulin-potassium (GIK) therapy has been found to be beneficial in the treatment of acute myocardial ischemia (1,2). Several metabolic mechanisms, such as changes in glucose (3,4) and free fatty acid (5,6) metabolism, may explain the beneficial effects of insulin. In addition to these actions on myocardial substrate metabolism, we have recently shown that insulin directly affects myocardial perfusion (7). In addition, Marano et al. (8) demonstrated using SPECT (single-photon emission computed tomography) that GIK therapy improved regional myocardial perfusion and function mainly in segments adjacent to the recently infarcted area.

Insulin induces a dose-dependent vasodilation in the peripheral arteries (9–11) that is blunted in insulin-resistant states (10,12,13). Insulin causes endothelium-dependent vasodilation by the L-arginine–nitric oxide pathway (14,15), and another important mediator of insulin-induced vasodilation is the sympathetic nervous system (14). Unlike studies of insulin's action on skeletal muscle perfusion, studies addressing insulin's action on myocardial perfusion are sparse. Because differences in the regulation of vasodilation between coronary and peripherial arteries have been observed (7), previous studies targeting insulin's effects on the skeletal muscle vasculature cannot be directly applied to the coronary vasculature.

The present study was designed to examine whether insulin-induced increases in hyperemic coronary blood flow are dose dependent in healthy humans. Myocardial blood flow, hyperemic adenosine-stimulated flow, and coronary vascular resistance were determined after an overnight fast and during euglycemic physiological and supraphysiological hyperinsulinemia using positron emission tomography (PET) and ^{15}O-labeled water ($[^{15}O]H_2O$).

RESEARCH DESIGN AND METHODS

Subjects. Ten nonsmoking asymptomatic men volunteered for the study. The characteristics of the subjects are shown in Table 1. The subjects were healthy as judged by history and physical examination and were not taking any medication. All subjects were normotensive and had normal glucose tolerance, blood counts, and electrolytes. All of the electrocardiograms, stress echocardiograms, and echocardiographically determined left ventricular masses, dimensions, and functions were normal in studied subjects.

Study design. All PET studies were performed after an overnight fast. The subjects were instructed to avoid all caffeine-containing drinks and foods for 12 h before the PET studies. Myocardial perfusion was measured four times (Fig. 1): once at rest and three times during intravenous infusion of adenosine (140 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 5 min). The perfusion measurements during adenosine infusion were performed during saline infusion, physiological hyperinsulinemia (1 $mU \cdot kg^{-1} \cdot min^{-1}$ insulin infusion), and supraphysiological hyperinsulinemia (5 $mU \cdot kg^{-1} \cdot min^{-1}$ insulin infusion). The duration of each insulin infusion was 60 min. Each subject's electrocardiogram and heart rate were monitored continuously during the studies. Blood pressure was measured at rest and during each adenosine infusion. Blood pressure was monitored with an automatic oscillometric blood pressure monitor (OMRON HEM-705C; Omron Healthcare, Hamburg, Germany) during all PET studies. Each subject gave written informed consent. The study was conducted according to the guidelines of the Declaration of Helsinki, and the study protocol was accepted by the Ethics Committee of the Turku University Central Hospital.

From the [1]Turku PET Centre, Turku University, Turku, Finland; the [2]Department of Medicine, Turku University, Turku, Finland; and the [3]Department of Clinical Physiology, Turku University, Turku, Finland.

Address correspondence and reprint requests to Dr. Pirjo Nuutila, Turku PET Centre, Turku University Central Hospital, P.O. Box 52, FIN-20521 Turku, Finland. E-mail: pirnuu@utu.fi.

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GIK, glucose-potassium-insulin; PET, positron emission tomography; ROI, region of interest.

TABLE 1
Characteristics of the study subjects

Age (years)	32 ± 6
BMI (kg/m^2)	24.1 ± 1.8
$V_{O_{2max}}$ (ml · kg^{-1} · min^{-1})	35.9 ± 3.1
Blood pressure (mmHg)	125/77 ± 11/10
Blood HbA_{1c} (%)	5.2 ± 0.4
Total cholesterol (mmol/l)	5.0 ± 0.7
LDL cholesterol (mmol/l)	3.0 ± 0.6
HDL cholesterol (mmol/l)	1.5 ± 0.3
Triglycerides (mmol/l)	1.1 ± 0.5

Data are means ± SD.

Production of [^{15}O]H_2O. Using a low-energy deuteron accelerator Cyclone 3 (Ion Beam Application, Louvain-la-Neuve, Belgium), ^{15}O-labeled water was produced using dialysis techniques in a continuously working water module (16). Sterility and pyrogenicity tests for water and chromatographic analysis for gases were performed to verify the purity of the products.

Image acquisition, processing, and corrections. The subjects were positioned supine in a 15-slice ECAT 931/08–12 tomograph (Siemens/CTI, Knoxville, TN). After the transmission scan, myocardial perfusion was measured with an intravenous injection of [^{15}O]H_2O (~1.5 GBq) at rest and 60 s after each intravenous administration of adenosine (140 μg · kg^{-1} · min^{-1} for 5 min). Each dynamic scan lasted for 6 min (6 × 5 s, 6 × 15 s, and 8 × 30 s). All data were corrected for deadtime, decay, and photon attenuation and reconstructed into a 128 × 128 matrix. The final in-plane resolution in the reconstructed and Hann-filtered (0.3 cycles/s) images was 9.5 mm (full width, half maximum).

Calculation of regional blood flow and coronary vascular resistance. Regions of interest (ROIs) were drawn on the lateral, anterior, and septal wall of the left ventricle in four representative transaxial slices in each study, as previously described (17). The ROIs drawn on the baseline images were copied to the images obtained after each adenosine administration. Values of regional myocardial blood flow (expressed in ml · g tissue^{-1} · min^{-1}) were calculated according to the previously published method using the single-compartment model (18,19). The arterial input function was obtained from the left ventricular time-activity curve using a previously validated method (20), in which corrections were made for the limited recovery of the left ventricular ROI and spillover from the myocardial signals. The average blood flow of the lateral and anterior part of the myocardium was calculated and used in further analysis. Coronary vascular resistance values were calculated both at baseline and after each adenosine infusion without or with simultaneous insulin infusions by dividing the mean arterial blood pressure by the respective flow value.

Insulin infusions. Insulin and glucose were infused in a catheter inserted in the right antecubital vein. The left hand was kept in a heated pillow, and arterialized venous blood was withdrawn from a heated left antecubital vein. During each insulin infusion, insulin (Actrapid Human; Novo Nordisk, Copenhagen, Denmark) was administered in a primed continuous manner at a rate of either 1 or 5 mU · kg^{-1} · min^{-1} for 60 min. Normoglycemia was maintained using a variable rate of 20% glucose. The rate of the glucose infusion was adjusted according to plasma glucose determinations performed every 5–10 min from arterialized venous blood. Samples for serum insulin and free fatty acids were taken every 30 min. Whole-body glucose uptake was calculated from the glucose infusion rate after correcting for changes in the glucose pool size (21).

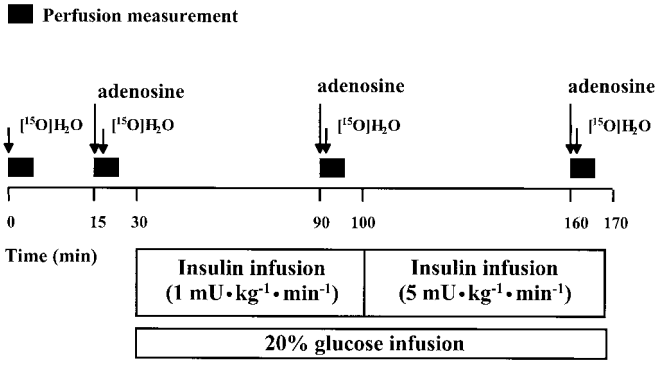

FIG. 1. Design of the study. Myocardial perfusion was measured with [^{15}O]H_2O four times: once during basal conditions and three times during adenosine stimulation without and with simultaneous insulin infusion. Insulin was infused at two rates: 1 and 5 mU · kg^{-1} · min^{-1}.

TABLE 2
Metabolic and hormonal characteristics of the study subjects

	Basal	Insulin infusion 1 mU · kg^{-1} · min^{-1}	Insulin infusion 5 mU · kg^{-1} · min^{-1}
Plasma glucose (mmol/l)	5.3 ± 0.5	5.1 ± 0.4	5.5 ± 0.3
Serum insulin (mU/l)	7 ± 2	65 ± 11*	457 ± 72*†
Serum FFA (mmol/l)	0.59 ± 0.36	0.10 ± 0.04‡	0.04 ± 0.01*†
Whole-body glucose uptake (μmol · kg^{-1} · min^{-1})		33.6 ± 12.9	74.1 ± 27.0†

Data are means ± SD. *$P < 0.001$, ‡$P < 0.05$ vs. basal; †$P < 0.001$ vs. 1 mU · kg^{-1} · min^{-1}.

Echocardiographic examination. To rule out silent ischemia, the subjects underwent a rest and a bicycle exercise echocardiographic examination. All echocardiographic recordings and analyses were performed by the same experienced investigator (M.L.) using a commercially available ultrasound scanner (Acuson 128XP/10; Acuson, Mountain View, CA). Standard echocardiographic views of the left ventricle were obtained, and resting cardiac dimensions were measured. Thereafter, an upright bicycle-ergometer exercise test was performed by increasing the work load by 20 W at 1-min intervals. The test was continued until extreme fatigue or 90% of the predicted maximum heart rate. The echocardiograms were recorded before and immediately after the exercise. All subjects had a normal exercise capacity, were asymptomatic, had no diagnostic ST-changes in electrocardiograms, and exhibited no wall motion abnormalities either at rest or immediately after exercise.

Analytical methods. During insulin clamp, plasma glucose was determined every 5 min by the glucose oxidase method (22). Serum insulin was measured every 30 min by radioimmunoassay kit (Pharmacia, Uppsala, Sweden). Plasma epinephrine and norepinephrine levels were measured at rest and after each adenosine infusion as previously described (23). Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were measured using standard enzymatic methods (Boehringer Mannheim, Mannheim, Germany) with a fully automated analyzer (Hitachi 704; Hitachi, Tokyo, Japan). LDL cholesterol concentration was calculated using the Friedewald formula (24).

Statistical methods. The results are expressed as means ± SD. The responses to adenosine infusion with and without hyperinsulinemia and the interaction of these variables were tested using repeated-measures ANOVA (25). For correlation analysis, Spearman's correlation coefficients were calculated. Paired t tests were used when appropriate. P values <0.05 were interpreted as statistically significant. All statistical tests were performed with SAS statistical analysis system (SAS Institute, Gary, NC).

RESULTS

Metabolic and hormonal characteristics. Metabolic and hormonal characteristics are given in Table 2 and plasma catecholamines in Table 3. During insulin infusions, serum insulin concentrations increased and serum free fatty acid concentrations decreased significantly and dose dependently, whereas plasma glucose concentrations remained unaltered (Table 2). Whole-body glucose uptake increased by 120% during the 5 mU · kg^{-1} · min^{-1} insulin infusion compared with the 1 mU · kg^{-1} · min^{-1} insulin infusion ($P < 0.001$) (Table 2). Adenosine infusion increased plasma epinephrine and norepinephrine concentrations ($P < 0.05$). Simultaneous hyperinsulinemia potentiated the increase of these values ($P < 0.05$). Furthermore, during supraphysiological hyperinsulinemia, the catecholamine responses were higher than during physiological hyperinsulinemia ($P < 0.05$) (Table 3).

Hemodynamic measurements during PET. Blood pressures, heart rates, and rate-pressure products (systolic blood pressure × heart rate) are presented in Table 4.

TABLE 3
Plasma catecholamine concentrations during PET studies

	Basal	Adenosine stimulation		
		Saline	1 mU · kg^{-1} · min^{-1}	5 mU · kg^{-1} · min^{-1}
Plasma epinephrine (nmol/l)	0.10 ± 0.10	0.17 ± 0.16*	0.23 ± 0.16*	0.34 ± 0.22†‡§
Plasma norepinephrine (nmol/l)	1.6 ± 0.6	2.0 ± 0.4*	2.2 ± 0.4*‡	2.6 ± 0.5†‖§

Data are means ± SD. *$P < 0.05$, †$P < 0.001$ vs. basal; ‡$P < 0.05$, ‖$P < 0.001$ vs. saline; §$P < 0.05$ vs. 1 mU · kg^{-1} · min^{-1}.

Adenosine infusion increased heart rate both without and with simultaneous insulin infusions ($P < 0.001$ vs. basal). During supraphysiological hyperinsulinemia, heart rate was higher than during physiological hyperinsulinemia ($P < 0.05$). Diastolic and systolic blood pressure increased during insulin infusions ($P < 0.05$). Consequently, the rate-pressure product significantly increased after adenosine infusion and further increased during each insulin infusion in a dose-dependent manner ($P < 0.05$) (Table 4).

Myocardial blood flow and coronary vascular resistance. Myocardial blood flow results are presented in Fig. 2*A*. Basal myocardial blood flow was 0.84 ± 0.17 ml · g^{-1} · min^{-1}. Physiological hyperinsulinemia increased the adenosine-stimulated flow by 20% (from 3.92 ± 1.17 to 4.72 ± 0.96 ml · g^{-1} · min^{-1}; $P < 0.05$). Supraphysiological hyperinsulinemia further increased the adenosine-stimulated flow by 19% (to 5.61 ± 1.03 ml · g^{-1} · min^{-1}; $P < 0.05$) (Fig. 2*A*).

Coronary vascular resistance results during adenosine stimulation are presented in Fig. 2*B*. Basal coronary vascular resistance was 113.6 ± 24.2 mmHg · min · g · ml^{-1}. Adenosine infusion decreased coronary vascular resistance by 77% (to 26.0 ± 10.2 mmHg · min · g · ml^{-1}; $P < 0.001$). Consequently, hyperinsulinemia decreased the adenosine-stimulated coronary vascular resistance by 16% (to 21.8 ± 6.5 mmHg · min · g · ml^{-1}; $P < 0.05$), and supraphysiological hyperinsulinemia further decreased it by 12% (to 19.2 ± 4.9 mmHg · min · g · ml^{-1}; $P < 0.05$) (Fig. 2*B*).

Correlation between whole-body glucose uptake and hyperemic myocardial blood flow. A significant correlation was found between whole-body glucose uptake (measured during physiological hyperinsulinemia) and hyperemic myocardial blood flow during hyperinsulinemia (physiological, $r = 0.61$, $P = 0.06$; supraphysiological, $r = 0.87$, $P < 0.003$) (Fig. 3). The correlation between whole-body glucose uptake and adenosine-stimulated myocardial perfusion did not reach statistical significance ($r = 0.47$, $P = 0.17$).

DISCUSSION

The present data demonstrate that insulin is able to enhance adenosine-stimulated myocardial perfusion and that insulin induces a dose-dependent increase in hyperemic myocardial blood flow in healthy men. We found that physiological hyperinsulinemia (serum insulin ~65 mU/l) significantly increased adenosine-stimulated myocardial blood flow and that supraphysiological hyperinsulinemia (serum insulin ~460 mU/l) further enhanced hyperemic myocardial blood flow. In addition, hyperemic myocardial blood flow responses during insulin stimulation correlated positively with whole-body glucose uptake.

The results of available studies concerning the effects of insulin on coronary vasculature are controversial. In animal studies, myocardial blood flow has been found to be either unchanged (26,27) or increased (28,29) by insulin. Studies of insulin action on myocardial perfusion in humans are sparse. In an early study, an intravenous bolus of 2 units did not change coronary sinus flow or coronary resistance (30). Moreover, physiological hyperinsulinemia for 100 min had no effect on basal myocardial perfusion (31). However, according to a meta-analysis of 75 articles focusing on the peripheral vasculature, significant vasodilation has been observed after either a longer period (>2 h) or a higher dose of insulin than in the previous study (11). Thus, it is unlikely that basal myocardial blood flow would have been significantly enhanced after 1 h of physiological hyperinsulinemia in the present study.

In the present study, the effects of insulin were measured during adenosine-induced hyperemia. The adenosine-induced coronary flow response reflects a combined effect of endothelial-mediated vasodilatory function (32) and vascular smooth muscle relaxation (33) and has been used as an integrating measure of coronary reactivity (34,35). Recently, Buus et al. (36) found that a significant amount of the adenosine response is endothelium dependent. In contrast to resting conditions where flow and myocardial work (oxygen consumption) are tightly coupled, during adenosine stimulation the metabolic control of myocardial blood flow is lost. However, endothelial and neurogenic controls are still functional. In addition, flow is directly dependent on blood pressure and modulated by mechanical forces within the myocardial wall (37). In the peripheral vasculature, insulin enhances the effect of

TABLE 4
Hemodynamic data during PET studies

	Basal	Adenosine infusion		
		Saline	1 mU · kg^{-1} · min^{-1}	5 mU · kg^{-1} · min^{-1}
Heart rate (beats/min)	57 ± 7	100 ± 12*	106 ± 9*	112 ± 8*†‡
Systolic blood pressure (mmHg)	125 ± 11	127 ± 12	135 ± 16§	145 ± 14*‖‡
Diastolic blood pressure (mmHg)	77 ± 10	75 ± 12	80 ± 13†	83 ± 10†
Rate-pressure product (mmHg/min)	7,084 ± 857	12,721 ± 2,192*	14,259 ± 2,009*†	16,170 ± 1,836*‖¶

Data are means ± SD. *$P < 0.001$, §$P < 0.05$ vs. basal; †$P < 0.05$, ‖$P < 0.001$ vs. saline; ‡$P < 0.05$, ¶$P < 0.001$ vs. 1 mU · kg^{-1} · min^{-1}.

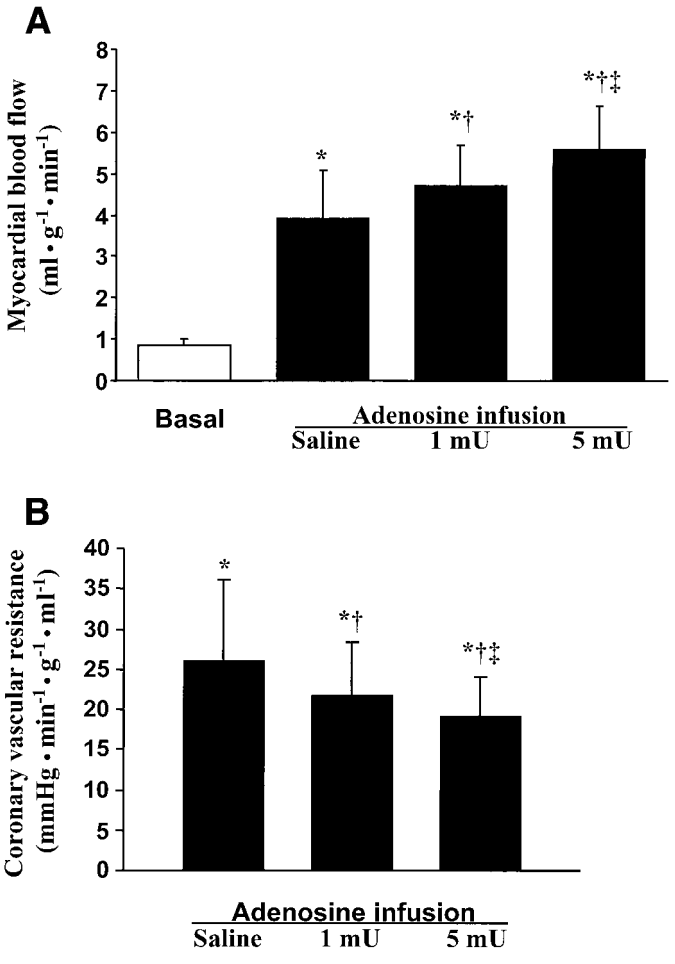

FIG. 2. Myocardial blood flow (*A*) and coronary vascular resistance (*B*) during basal conditions and during adenosine stimulation without and with simultaneous insulin infusion. Insulin was infused at two rates: 1 and 5 mU · kg^{-1} · min^{-1}. For comparison, basal coronary vascular resistance was 113.6 ± 24.2 mmHg · min · g · ml^{-1}. *P < 0.001 vs. basal; †P < 0.05 vs. saline; ‡P < 0.05 vs. 1 mU · kg^{-1} · min^{-1}.

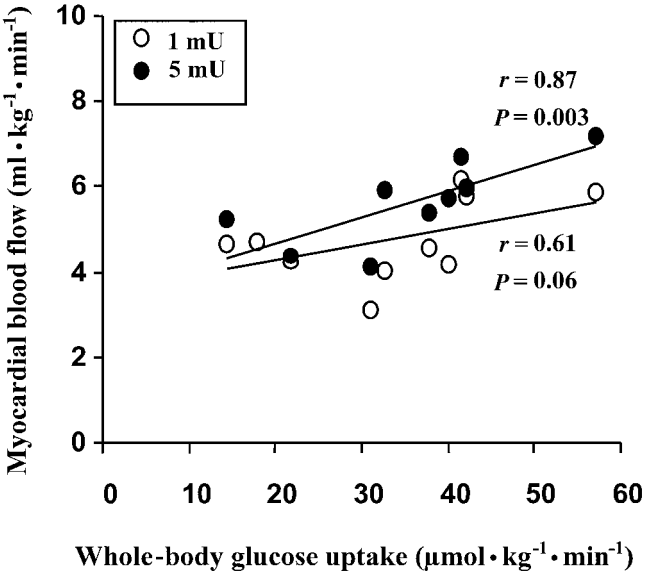

FIG. 3. Whole-body glucose uptake measured during physiological insulin infusion was associated with hyperemic myocardial blood flow during both physiological (○) (r = 0.61, P = 0.06) and supraphysiological (●) (r = 0.87, P < 0.003) hyperinsulinemia.

endothelium-dependent vasodilation before any changes occur in basal blood flow (38,39). In our recent study (7), we found that physiological hyperinsulinemia for 1 h increases adenosine-induced myocardial blood flow in healthy humans. Thus, it appears that hyperemic flow responses are enhanced already after 1 h of physiological hyperinsulinemia. In addition, the synergistic effect of adenosine and insulin on myocardial perfusion demonstrates that adenosine alone is not able to induce "maximal" hyperemia.

It has been demonstrated that insulin-induced vasodilation is time and dose dependent in the peripheral vasculature (9–11). In the present study, the increase of hyperemic myocardial blood flow might also be partly due to a time-dependent response to hyperinsulinemia. In the peripheral vasculature, the flow response to insulin appears to be determined by the total exposure of insulin (the dose of insulin and the duration of the insulin infusion) (40). In the present study, the delay between the two measurements was only 1 h, whereas the serum insulin concentration was sevenfold higher during the high-dose insulin infusion. Taking these results together, the significantly higher level of hyperemic myocardial blood flow during supraphysiological hyperinsulinemia was likely caused by the dose-dependent rather than the time-dependent response to insulin infusion.

The mechanism of insulin's ability to increase adenosine-stimulated myocardial perfusion cannot be answered directly by the present study. Insulin induces vasodilation via an endothelial-dependent mechanism by activating L-arginine transport and nitric oxide synthase (15). In vascular smooth muscle cells, insulin increases cyclic adenosine monophosphate and cyclic guanosine monophosphate concentrations (41,42). Recently, it was demonstrated that insulin also increases large vessel compliance as measured by pulse wave analysis (43). Interestingly, this effect of insulin occurred within 30 min, whereas peripheral vasodilation in resistance vessels appears to require a longer period of time (43).

The other mechanism by which insulin induces vasodilation is via the sympathetic nervous system. Insulin increases plasma norepinephrine concentrations (44,45) and muscle sympathetic nerve activity (13,44,45). Additionally, using power spectral analysis of R-R intervals, insulin has also been found to stimulate cardiac sympathetic activity (46). This insulin-induced increase in sympathetic tone has been suggested to be mediated via the central nervous system (44,45,47). For example, Natali et al. (47) demonstrated that local forearm insulin administration did not stimulate flow, whereas systemic insulin infusion was able to increase muscle blood flow. However, we have recently demonstrated that centrally mediated sympathetic activation does not appear to play a major role in regulating insulin action on hyperemic myocardial perfusion in healthy subjects (7).

Insulin has both inotropic and chronotropic effects on myocardium. Insulin has been reported to increase stroke volume and cardiac output in a dose-dependent manner (48–51). In addition, high-dose insulin infusion has been found to increase heart rate (48). The findings of insulin's effects on blood pressure are controversial. Mean arterial

pressure has been found to be decreased (44,48), unchanged (13,30,31,46,52–54), or increased (51). In the present study, hyperinsulinemia and simultaneous adenosine infusion significantly increased blood pressure values, heart rates, and plasma catecholamine concentrations. However, the hyperemic myocardial blood flow responses to insulin cannot be explained by changes in systemic hemodynamics, since coronary vascular resistance, which takes into account changes in blood pressure, was also significantly changed by insulin.

Intravenous insulin therapy has been shown to be beneficial in the treatment of patients with acute myocardial infarction (2,55). At least in theory, the hyperemic effects of insulin on the coronary vasculature might partly contribute to this benefit. In concert with our findings, the beneficial effects of insulin have been found to be dose dependent in those large clinical trials (2,55). Hyperinsulinemia increased catecholamines, blood pressure values, and heart rates, indicating an increase of sympathetic activity. However, in clinical situations where GIK therapy is given, β-blockers are routinely used, and these agents are likely to prevent the undesirable effects of insulin therapy on sympathetic activity.

In summary, the results of the present study demonstrate that insulin is able to enhance adenosine-stimulated myocardial perfusion and that this response is dose dependent in healthy subjects. These effects might contribute to the known beneficial dose-dependent effects of insulin on myocardial ischemia. In addition, hyperemic myocardial blood flow responses during insulin stimulation were positively correlated with whole-body glucose uptake, suggesting that sensitivity of coronary reactivity to insulin parallels the insulin sensitivity at the whole-body level. Further human studies are needed to investigate the effects of insulin on coronary function in patients with insulin resistance, diabetes, and coronary artery disease.

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