Insulin at physiological concentrations increases microvascular perfusion in human myocardium

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Liu Z. Insulin at physiological concentrations increases microvascular perfusion in human myocardium. Am J Physiol Endocrinol Metab 293:E1250–E1255, 2007. First published August 14, 2007; doi:10.1152/ajpendo.00451.2007.—Vascular endothelium regulates vascular tone and tissue perfusion in response to various physiological and pathological stimuli. Insulin and meal feeding increase microvascular perfusion and thus oxygen, nutrient, and hormone delivery to human skeletal muscle. Meal feeding also increases cardiac microvascular perfusion in healthy humans. To examine whether insulin at physiological concentrations increases microvascular perfusion in human myocardium, we studied 13 healthy, overnight-fasted, lean, young human volunteers by using myocardial contrast echocardiography (MCE) and insulin-clamp techniques. We measured cardiac microvascular blood volume (MBV), microvascular flow velocity (MFV), and microvascular blood flow (MBF) at baseline, 60 min, and 120 min after initiating insulin infusion at 1 mU·kg⁻¹·min⁻¹. MBF is the product of MBV and MFV and represents microvascular perfusion. Insulin increased myocardial MBV by 23% at 60 min (P < 0.01) and by 41% at 120 min (P = 0.001) without changing MFV. As a result, insulin-mediated myocardial MBF increased significantly at both 60 min (P < 0.01) and 120 min (P < 0.0005). Insulin also significantly increased brachial artery diameter, flow velocity, and total blood flow at 60 and 120 min (P < 0.05 for all). The changes in cardiac MBV correlated positively with quantitative insulin sensitivity check index (QUICKI) and negatively with body mass index but not with the steady-state glucose-infusion rates or the changes in brachial artery parameters. We conclude that insulin, at physiologically relevant concentrations, increases microvascular perfusion in human heart muscle by increasing cardiac MBV in healthy, insulin-sensitive adults. This insulin-mediated cardiac microvascular perfusion may play an important role in normal human myocardial oxygen and substrate physiology.

INSULIN ACTS ON THE VASCULAR endothelium to maintain normal vascular tone and integrity. In response to insulin, endothelial cells produce nitric oxide via the phosphatidylinositol 3-kinase/protein kinase B (Akt)/endothelial nitric oxide synthase (eNOS) pathway (11, 16, 17, 22, 39, 40), leading to vasodilation and increased tissue delivery of oxygen, nutrients, and hormones. It appears this effect is physiologically important because insulin at physiological concentrations stimulates the tyrosine phosphorylation of insulin receptor substrate-1, activates Akt and eNOS in cultured vascular endothelial cells (16, 17), and increases microvascular perfusion to the skeletal muscle in both humans and laboratory animals. This latter effect was first demonstrated by using 1-methylxanthine extraction to estimate endothelial surface exposure (23, 24) and was later confirmed with contrast-enhanced ultrasound imaging, which demonstrated an increase in microvascular blood volume (MBV) (4, 5, 30, 31). This action begins within 5–10 min (31) and is nitric oxide dependent because inhibition of eNOS with L-arginine methyl ester abolishes insulin-mediated microvascular perfusion (30, 31). These findings suggest that insulin normally plays an important role in maintaining tissue perfusion. Indeed, in the insulin-resistant states, insulin’s effect to increase muscle blood flow is impaired (2, 38).

Insulin also exerts a vasodilatory action on cardiac vasculature and regulates coronary blood flow in humans. In healthy humans, insulin increases coronary flow reserve by ~20–26% (12, 29). Although insulin-mediated increase in coronary blood flow was sustained in young patients with type 1 diabetes without microvascular complications or autonomic neuropathy (13, 28), it was blunted in patients with obesity (27) or type 2 diabetes (9). However, whether insulin actually regulates cardiac microvascular perfusion, especially MBV, remains unknown. This is important because the relative distribution in blood volume in the microcirculation only accounts for one-third of the coronary circulation (19, 34). Only microvasculature is related to oxygen, hormone, and nutrient delivery to the myocardium, and it is within the microvasculature that nutrient and oxygen exchange occur.

Myocardial contrast echocardiography (MCE) has been widely used clinically to enhance ultrasound images and diagnostic accuracies by using various gas-containing microbubbles as contrast agents (7, 15, 19, 20, 34, 35). Because microbubbles are rheologically similar to red blood cells and efficiently reflect acoustic ultrasound waves within the capillaries without disrupting the local environment, they trace not only large blood vessels but also microvasculature (7, 19). Thus MCE permits a noninvasive, quantitative analysis of the spatial and temporal heterogeneity of blood flow and volumes within the microvasculature (7, 19).

In the current study, we performed MCE in combination with euglycemic hyperinsulinemic clamp to examine whether insulin at physiologically relevant concentrations increases cardiac microvascular perfusion in healthy humans. Our results provide strong evidence that insulin plays important role in regulating MBV and microvascular perfusion in normal human myocardium.

METHODS

The study protocol was approved by the human studies Institutional Review Board and the General Clinical Research Center (GCRC). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Advisory Committee at the University of Virginia. A total of 13 healthy subjects (6 female, 7 male) with no history of obesity, hypertension, diabetes, or hyperlipidemia were admitted to the GCRC the night before the study. All subjects gave written informed consent before the study. At the screening visit, blood samples were taken for plasma cholesterol, triglyceride, HDL cholesterol, insulin, and glucose measurements. Subjects were excluded from the study for family history of a first-degree relative with diagnosed diabetes or if they were smokers or taking any medications or supplements known to affect either endothelial function or glucose metabolism. On admission, height, weight, and hip and waist circumferences were measured and body composition was determined by using an air-displacement chamber (BOD-POD; Life Management, Concorde, CA).

**Hyperinsulinemic-euglycemic clamp.** After overnight fast, catheters were placed in peripheral arm veins for blood sampling and infusions of insulin, glucose, and microbubbles according to the protocol described in Fig. 1. After baseline blood samples were obtained, brachial artery measurements were taken and MCE was performed. This was followed by 2 h of intravenous infusion of regular insulin at 1 mU·min⁻¹·kg⁻¹. Blood samples were taken for plasma glucose measurements every 5 min throughout the 120-min insulin infusion, and 20% dextrose was infused at variable rates to maintain euglycemia. Heating the hand to arterialize sampled blood was avoided because this alters blood flow both in the heated and contralateral arm (1). Recognizing that when arterial glucose is maintained constant during insulin clamp, venous glucose concentrations decline progressively with time and that the magnitude of this arteriovenous difference is proportional to the glucose-infusion rate (GIR) required to maintain euglycemia, we clamped plasma glucose at 10 mg/dl below the basal level to avoid arterial hyperglycemia. At 60 min and at end of the clamp, brachial arterial flow measurements and MCE were again performed.

**Brachial artery diameter, blood-flow velocity, and blood-flow measurements.** Brachial artery diameter and flow velocity were measured while the subject was in the supine position by using a SONOS 7500 ultrasound system with an L11-3 linear array transducer (Philips Medical Systems, Andover, MA) with a transmit frequency of 7.5 MHz. Artery diameter was measured as the distance between each inside edge of the arterial intima by using two-dimensional imaging of the brachial artery in the longitudinal axis at peak systole and online video calipers. A pulsed-wave Doppler sample volume was placed in the center of the vessel, and the time-averaged mean brachial artery blood velocity was determined. Brachial artery blood flow (Q) was calculated from the averages of three diameter (d) and velocity (v) measurements by using the equation Q = vπ·(d/2)².

**MCE.** MCE was performed by using a SONOS 7500 ultrasound system and an S3 phased-array transducer (Philips Medical Systems) while the subject was in the left decubitus position. The contrast agent (Definity; Bristol-Myers Squibb, Princeton, NJ) used with MCE is commercially produced microbubbles that contain a lipid shell and a perfluorocarbon gas. Definity microbubbles were delivered intrave-

![Fig. 2. Representative 4-chamber view of MCE with region of interest delineated with dashed line.](http://ajpendo.physiology.org/ by 10.221.33.1 on October 29, 2017)

**β-value of A/LVO.** The β-value of A/LVO was calculated by using QLAB software (Philips Medical Systems). All MCE images, including images obtained through 4-, 2-, and 3-chamber views, were randomly coded and blindly analyzed by using QLAB software. The code was broken after all images were analyzed. Because all subjects were healthy and young, homogenous perfusion in all myocardial segments was assumed, and results from all three views were averaged. This was decided before image analysis. Two subjects had no analyzable MCE images at 120 min, one received insulin infusion for only 1 h because of an infusion-line occlusion, and one had unanalyzable images. The video intensity, i.e., the intensity of signal generated from the rupture of microbubbles, in the region of interest was determined (Fig. 2) at each pulsing interval. After background subtraction, the pulsing interval (time) vs. video-intensity curve was generated and was fitted to an exponential function, y = A(1 − e⁻³βt), where y is the video intensity in decibels at a pulsing interval t, A is the plateau video intensity representing MBV, and β is the rate constant reflecting the rate of rise of video intensity (i.e., microvascular flow velocity, MFV). MCE is unique in that it provides information on the three most important indices of microvascular flow: MBV, which reflects the volume of the capillary bed; MFV, which corresponds to the rate of blood flow through this microvascular bed; and microvascular blood flow (MBF) which is the product of MBV and MFV (i.e., MBF = MBV × MFV).

To ascertain that the infusion rate of microbubbles used in the study indeed led to optimal LVO and video-intensity plateau in the microvasculature, the A values were normalized to video intensity obtained from the left ventricle, and the resultant A/LVO values correlated extremely well with the A values (r = 0.96). As a result, A values were used for all analyses except when calculating percent change when A values were converted to acoustic intensity (AI) by using the formula AI = [anti-log (dB/10)] × AI₀ref, where AI₀ref is the reference
Table 1. Biometric parameters for 13 study subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>22.3 ± 1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>66.3 ± 3.9</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.76 ± 0.3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.2 ± 0.8</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>19.8 ± 2.8</td>
</tr>
<tr>
<td>Fat weight, kg</td>
<td>12.7 ± 1.8</td>
</tr>
<tr>
<td>Lean weight, kg</td>
<td>53.5 ± 4.1</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>75.4 ± 2.2</td>
</tr>
<tr>
<td>Hip, cm</td>
<td>92.9 ± 1.9</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.8 ± 0.02</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>120 ± 2.7</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>74.3 ± 2</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>176.2 ± 8.1</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>111.2 ± 17</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>109.2 ± 7.6</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>48.6 ± 2.1</td>
</tr>
<tr>
<td>Plasma lactate, mM</td>
<td>0.82 ± 0.07</td>
</tr>
<tr>
<td>Plasma glucose, mM</td>
<td>5.27 ± 0.09</td>
</tr>
<tr>
<td>Plasma insulin, pM</td>
<td>28.5 ± 6.3</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.4 ± 0.01</td>
</tr>
<tr>
<td>Steady-state GIR, mg·kg⁻¹·min⁻¹</td>
<td>6.8 ± 0.6</td>
</tr>
</tbody>
</table>

BMI, body mass index; QUICKI, quantitative insulin sensitivity check index; GIR, glucose infusion rate.

AI that remained constant before and after insulin infusion because overall gain, time-gain compensation, and lateral-gain compensation were not adjusted and dB is the video intensity in decibels.

Biochemical analysis. Plasma cholesterol, HDL cholesterol, and triglycerides were measured by the University of Virginia clinical chemistry laboratories. Plasma glucose and lactate were measured using a YSI glucose/lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured using an ELISA assay with an interassay coefficient of variation of <4%. The quantitative insulin-sensitivity check index (QUICKI) was calculated as \( 1/\log(I_o) + \log(G_o) \), where \( I_o \) is fasting plasma insulin and \( G_o \) is fasting plasma glucose (10). Plasma free fatty acids were quantitated by using an in vitro enzymatic colorimetric assay with a Wako HR Series NEFA-HR kit (Wako Diagnostics, Richmond, VA).

Statistical analysis. Data are presented as means ± SE. Statistical analyses were performed by using SigmaStat 3.1 software (Systat Software). Comparisons among measurements made at baseline, 60 min, and 120 min were made with repeated-measures ANOVA. Paired t-test was used to compare substrate concentrations between baseline and clamp. Pearson product moment correlation was used to assess the strength of the association between pairs of variables. A P value of <0.05 was considered statistically significant.

RESULTS

Subject characteristics. The anthropomorphic characteristics and serum chemistries of all subjects at baseline or during the insulin clamp are shown in Tables 1 and 2. Although all subjects were young and had normal body mass index (BMI, ranging from 18 to 25.2 kg/m²), their insulin sensitivity, as assessed by using QUICKI, ranged from 0.32 to 0.44. This corresponded to a wide range of steady-state GIR during the last 30 min of insulin clamp, ranging from 2.7 to 9.54 mg·kg⁻¹·min⁻¹ (r = 0.71, P = 0.01). Insulin infusion raised plasma insulin concentrations from 28.5 ± 6.3 pM to 203 ± 28.5 pM, a high physiological concentration comparable with that seen postprandially and sufficient to markedly decrease plasma free fatty acid concentrations.

Brachial artery parameters. Brachial artery diameter, flow velocity, and blood flow are shown in Fig. 3. Insulin infusion significantly increased brachial artery diameter at 60 min (from 3.38 ± 0.10 to 3.53 ± 0.11 mm; P < 0.01) and 120 min (to 3.59 ± 0.13 mm; P = 0.03). This was accompanied by a 30% increase in flow velocity at 60 min (P = 0.01) and a 53% increase at 120 min (P < 0.02). As a result, insulin infusion increased brachial arterial blood flow by 43 ± 10% (P < 0.002) at 60 min and 75 ± 23% (P < 0.01) at 120 min.

Cardiac microvascular parameters. As shown in Fig. 4A, insulin infusion significantly increased MBV at 60 min (from 3.08 ± 0.19 to 3.84 ± 0.18 dB; P < 0.01) and at 120 min (to 4.59 ± 0.27 dB; P = 0.001). These correspond to a 23 ± 6%

Table 2. Plasma substrate concentrations during insulin clamp

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal</th>
<th>120 min</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steady-state glucose, mM</td>
<td>5.3 ± 0.1</td>
<td>4.7 ± 0.2</td>
<td>&lt;0.007</td>
</tr>
<tr>
<td>Insulin, pM</td>
<td>28.5 ± 6.3</td>
<td>203 ± 28.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Free fatty acids, meq/l</td>
<td>0.53 ± 0.07</td>
<td>0.08 ± 0.01</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>0.82 ± 0.08</td>
<td>1.05 ± 0.06</td>
<td>=0.01</td>
</tr>
</tbody>
</table>

Fig. 3. Brachial artery diameter (A), velocity (B), and flow (C) at baseline, at 60 min after initiating insulin infusion, and at end of 120-min insulin infusion. Compared with baseline, *P = 0.008, **P = 0.03, #P = 0.01, ##P < 0.02, @P < 0.002, and @@P < 0.007.

AJP-Endocrinol Metab • VOL 293 • NOVEMBER 2007 • www.ajpendo.org
increase in AI at 60 min and a 41 \pm 9\% increase in AI at 120 min. In contrast, MFV did not change at either 60 or 120 min (Fig. 4B). As a result, cardiac MBF (Fig. 4C) increased significantly at both 60 min (\(P < 0.01\)) and 120 min (\(P < 0.0005\)). Because MFV did not change during insulin infusion, insulin-induced changes in MBF were entirely attributable to changes in MBV.

**Correlation analyses.** Because insulin infusion increased both cardiac microvascular perfusion and the brachial artery flow, the relationship between the changes in cardiac microvascular parameters and the changes in brachial artery parameters after 120 min insulin infusion were explored. Changes in brachial artery diameter, velocity, and flow were not correlated with changes in cardiac MBV, MFV, and MBF, respectively. I further examined whether insulin-induced changes in cardiac MBV were associated with the steady-state glucose infusion rates, BMI, QUICKI, percent body fat, or waist-hip ratios. As shown in Fig. 5, changes in MBV were correlated negatively with BMI (\(r = -0.606; P < 0.05\)) and positively with QUICKI (\(r = 0.684; P = 0.02\)).

**DISCUSSION**

By using a combination of MCE and hyperinsulinemic-euglycemic clamp techniques, the current study demonstrates that insulin at physiologically relevant concentrations significantly increases cardiac microvascular perfusion in humans and that this insulin-mediated increase in total MBF is entirely due to an increase in MBV, because MFV did not change during insulin infusion. In addition, the insulin-mediated increase in MBV correlates negatively with BMI and positively with insulin sensitivity measured in the fasting state (QUICKI).

Although insulin has been repeatedly shown to cause coronary vasodilation and to increase coronary blood flow (8, 14), it remained unclear before the current study whether insulin regulates blood volume and flow in the coronary microcirculation. In human hearts, coronary blood volume is distributed almost equally among the arterial, microcirculatory, and venous compartments, and most of the arterial and venous blood volumes are located on the epicardial surface of the heart (34).
MCE has proved to be a unique, effective, and sensitive method for noninvasively quantifying MBV and microvascular perfusion within the human myocardium without radioisotope or X-ray exposure. It differs from nuclear scans, positron emission tomography scans, or stress echo in that it uses gas-containing microbubbles as a direct vascular tracer and the contribution of large and small vessels can be separated. By using this technique, the current study demonstrates that insulin at physiological concentrations does potentely increase myocardial MBV and microvascular perfusion in healthy humans.

The current finding is consistent with two recent reports demonstrating a significant increase in myocardial microvascular perfusion in healthy humans 2 h after a standardized meal (25, 26). Meal feeding increased MBV by 30% and MBF by 77%. Because plasma glucose did not change but insulin levels increased by more than eightfold, most likely insulin contributed significantly to the observed changes in cardiac microvascular parameters. This study differs from those previous studies in that a standardized meal induced a 37% increase in MFV and no change was observed in MFV in this study despite comparable changes in the plasma insulin concentrations. This is not surprising because meal response is more complex than simple insulin infusion. Ingestion of a mixed meal not only stimulates insulin secretion, it also alters the secretion of incretins, glucagon, catecholamines, and other hormones; increases plasma concentrations of amino acids (including arginine); and affects protein and lipid metabolism.

MCE can also be used to noninvasively quantify coronary blood-flow reserve in humans. In an earlier study, Cheriﬁ and colleagues (3) performed contrast echocardiography with sonicated meglumine diatrizoate sodium as a contrast agent during coronary angiography before and after intracoronary injection of papaverine and found that papaverine increased peak contrast intensity by $\sim$53% in 13 patients with normal coronary angiograms. In the current study, insulin at physiologically relevant concentrations increased the acoustic intensity generated by the microbubbles to a comparable extent. This suggests that insulin may play an important physiological role in maintaining myocardial perfusion and thus adequate delivery of oxygen, nutrients, and hormones, especially in the postprandial state when insulin concentrations increase significantly.

The finding that insulin increases only MBV but not MFV suggests that insulin increases the microvascular-exchange surface area, which is important in ensuring adequate exchange of these substances delivered to the myocardium between the plasma and the interstitial compartments. Thus insulin-mediated increase in coronary blood flow appears to be different from that mediated by the vasodilator adenosine (21, 37). Wei et al. (36) measured coronary blood-flow reserve by using quantitative coronary angiography and MCE in 11 patients with angiographically normal epicardial coronary arteries and found that adenosine infusion increased MBF entirely via increasing MFV because they observed no significant changes in MBV or plateau video intensity. This difference is not surprising because insulin exerts a vasodilatory effect via a nitric oxide-dependent mechanism, whereas adenosine causes coronary vasodilation in a predominantly flow-mediated fashion (21).

QUICKI is a widely accepted clinical index of insulin sensitivity in humans (10). In the current study, insulin-induced changes in MBV positively correlated with QUICKI and negatively correlated with BMI. This suggests that insulin-mediated cardiac microvascular perfusion is subjective to regulation by many factors affecting insulin sensitivity. We have previously reported that even mild-to-moderate insulin resistance as seen in simple obesity blunts insulin-mediated microvascular perfusion in the skeletal muscle in humans (4). In addition, with the use of laser Doppler and nail-bed capillaroscopy, de Jongh et al. (6) demonstrated that capillary recruitment and acetylcholine-mediated vasodilation in human skin were also positively correlated with insulin sensitivity. Thus it is very likely that insulin resistance could also lead to decreased blood flow and thus oxygen, nutrient, and hormone delivery to the myocardial microcirculation, especially postprandially, and this may contribute to the increased cardiac morbidity and mortality seen in various insulin-resistant states. The lack of correlation between $\Delta$MBV and GIR is not surprising because GIR mostly reflects skeletal muscle glucose uptake and $\Delta$MBV reflects insulin sensitivity in the microvascular bed in the heart.

Although insulin significantly increased brachial artery diameter, blood-flow velocity, and arterial blood flow, which are similar to a recent report by Clerk et al. (4), changes in cardiac MBV did not correlate with changes in brachial artery diameter. Similarly, changes in cardiac MBF did not correlate with brachial artery blood flow. A likely explanation is that different arterial segments respond differently to insulin, with microvascular perfusion being regulated by precapillary arterioles, brachial artery being a conduit artery, and brachial artery flow being dependent on dilation of intermediate-diameter resistance vessels. It has been reported in rats that insulin-mediated skeletal muscle precapillary arteriole relaxation precedes increases of total arterial blood flow as well as precapillary arterioles, being more sensitive to insulin than resistance artery (32, 41). By using a radioaective microsphere method, Liang and colleagues (18) demonstrated that in dogs, insulin-mediated increase in myocardial blood flow is mediated via adrenergic mechanisms, whereas in skeletal muscle it is mediated via mechanisms unrelated to sympathetic stimulation. Thus it remains a possibility that insulin may have exerted this effect via sympathetic activation. Although data on the hemodynamic status of the participants were not collected in the current study, in a separate study ($n = 5$) of subjects with similar age and BMI ranges, I found that 2 h of insulin clamp did not alter systolic blood pressure ($107 \pm 4$ vs. $109 \pm 4$ mmHg, basal vs. insulin), diastolic blood pressure ($63 \pm 3$ vs. $62 \pm 2$ mmHg), and heart rates ($58 \pm 5$ vs. $61 \pm 6$ beats/min). Whether insulin-mediated microvascular responses in the myocardium correlate to those observed in the skeletal muscle remains to be investigated.

In conclusion, the current study provides strong evidence that insulin at physiologically relevant concentrations increases microvascular perfusion in human heart muscle via increasing MBV in healthy, insulin-sensitive adults and that the changes correlate with body insulin sensitivity. This insulin-mediated cardiac microvascular perfusion likely plays a significant role in nutrient and oxygen delivery to the normal human heart.

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AJP-Endocrinol Metab • VOL 293 • NOVEMBER 2007 • www.ajpendo.org
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