Role of Blood Flow in Regulating Insulin-stimulated Glucose Uptake in Humans

Studies Using Bradykinin, [^{15}O]Water, and [^{18}F]Fluoro-deoxy-glucose and Positron Emission Tomography

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Abstract

Defects in insulin stimulation of blood flow have been suggested to contribute to insulin resistance. To directly test whether glucose uptake can be altered by changing blood flow, we infused bradykinin (27 μg over 100 min), an endothelium-dependent vasodilator, into the femoral artery of 12 normal subjects (age 25±1 yr, body mass index 22±1 kg/m^2) after an overnight fast (n = 5) and during normoglycemic hyperinsulinemic (n = 7) conditions (serum insulin 465±11 pmol/liter, 0–100 min). Blood flow was measured simultaneously in both femoral regions using [^{15}O]-labeled water ([^{15}O]H_2O) and positron emission tomography (PET), before and during (50 min) the bradykinin infusion. Glucose uptake was measured immediately after the blood flow measurement simultaneously in both femoral regions using [^{18}F]-fluoro-deoxy-glucose ([^{15}F]FDG) and PET. During hyperinsulinemia, muscle blood flow was 58% higher in the bradykinin-infused (38±9 ml/kg muscle · min) than in the control leg (24±5, $P < 0.01$). Femoral muscle glucose uptake was identical in both legs (60.6±9.5 vs. 58.7±9.0 μmol/kg · min, bradykinin-infused vs. control leg, NS). Glucose extraction by skeletal muscle was 44% higher in the control (2.6±0.2 mmol/liter) than the bradykinin-infused leg (1.8±0.2 mmol/liter, $P < 0.01$). When bradykinin was infused in the basal state, flow was 98% higher in the bradykinin-infused (58±12 ml/kg muscle · min) than the control leg (28±6 ml/kg muscle · min, $P < 0.01$) but rates of muscle glucose uptake were identical in both legs (10.1±0.9 vs. 10.6±0.8 μmol/kg · min). We conclude that bradykinin increases skeletal muscle blood flow but not muscle glucose uptake in vivo. These data provide direct evidence against the hypothesis that blood flow is an independent regulator of insulin-stimulated glucose uptake in humans. (*J. Clin. Invest.* 1996. 97:1741–1747.) Key words: insulin sensitivity • bradykinin • tomography • imaging techniques

Introduction

Insulin stimulates limb glucose uptake by increasing both glucose extraction and blood flow (1). Although data regarding the ability of physiological (< 100 mU/liter) insulin concentrations to stimulate blood flow are controversial (1, 2), prolonged high-dose insulin infusions markedly increase blood flow both when measured across leg (3–6) and forearm (2) tissues. Defects in insulin stimulation of blood flow have been described in several insulin-resistant states including hypertension (4), obesity (3), and non–insulin-dependent diabetes mellitus (NIDDM)[1] (6). It has been suggested that these defects might contribute to decreased glucose uptake in these patients (1).

If blood flow was important for insulin action, one would have to assume that an increase in blood flow per se increases glucose uptake. Previous data regarding the effect of a change in blood flow on glucose uptake are controversial. Buchanan et al. (7) suggested that angiotensin II increases glucose utilization be increasing leg blood flow. However, in this study, angiotensin II decreased renal plasma flow by 22–38%. This decreased insulin clearance and resulted in significant and up to 33% higher insulin concentrations during angiotensin II infusions (7). The significant increase in insulin concentrations might have explained the observed increase in whole body glucose uptake. Also, as glucose uptake increases as a function of time (2), it is possible that the observed increase in glucose uptake by angiotensin II, which was infused during the latter part of the insulin infusion, would have occurred even in the absence of angiotensin II. Recently, Morris et al. (8) found that angiotensin II increases glucose uptake in patients with NIDDM independent of its hemodynamic effects, which suggests that angiotensin II may not merely act by increasing blood flow. In contrast to these data, Natali et al. (9) induced a 100% increase in blood flow under hyperinsulinemic conditions across the forearm using adenosine but failed to find any change in glucose uptake in mildly overweight patients with essential hypertension. However, adenosine did increase glucose uptake in these patients in the basal state, even when expressed as a ratio to concomitant oxygen uptake (9). This effect could have been due to the antilipolytic effect of adenosine (10) since in the basal state lowering of FFA by antilipolytic agents increases glucose uptake in human skeletal muscle (11). Alternatively, the failure of adenosine to increase glucose uptake under hyperinsulinemic conditions could have been due to the inability of adenosine to overcome insulin resistance.

Thus, the question of whether glucose uptake can be altered by changing blood flow still needs to be tested in normal subjects under conditions where insulin clearance does not change and where the duration of the insulin infusion is not a confounding variable.

Due to the large variability in blood flow even in normal

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1. *Abbreviations used in this paper:* ACE, angiotensin-converting enzyme; NIDDM, non–insulin-dependent diabetes mellitus; PET, positron emission tomography.

subjects (2), one would ideally like to increase blood flow by a vasoactive substance locally in one limb and measure blood flow and glucose uptake in both legs simultaneously during such a maneuver. This approach has become feasible recently even in humans because of developments in positron emission imaging technology. Use of positron emission tomography (PET) combined with two radioactive tracers, [^{15}O]H_2O and [^{18}F]FDG, and appropriate modeling allows noninvasive quantitation of both blood flow and glucose uptake in vivo in humans (12–14).

Insulin increases blood flow via an endothelium-dependent mechanism since its effect can be blocked using N^G-monomethyl-L-arginine (LNMMA), which blocks nitric oxide synthesis in endothelial cells (15, 16). The effect of increasing blood flow with some other endothelium-dependent vasodilatator than insulin on glucose uptake is unknown since both angiotensin II and adenosine are endothelium-independent vasodilatators (17).

Bradykinin is a nonapeptide which produces dose-dependent increases in forearm blood flow (18) and venodilatation (19) in humans. This effect can be significantly inhibited by LNMMA (20). Bradykinin does not acutely enhance muscle glucose uptake in isolated myocytes (21) or rat soleus muscle (22), suggesting that bradykinin has no direct effect on muscle glucose extraction.

In this study we determined whether an increase in blood flow, after an overnight fast or under physiologic hyperinsulinemic conditions, induced by a local infusion of bradykinin into the femoral artery in healthy volunteers increases glucose uptake. These studies were performed by simultaneously visualizing and quantitating blood flow in both femoral regions using water labeled with the short-lived positron-emitting isotope [^{15}O]H_2O. Immediately after disappearance of this tracer, glucose uptake in skeletal muscle was determined in both femoral regions using PET-derived [^{18}F]FDG kinetics, as described previously (12). Our results demonstrated that an increase in blood flow does not increase glucose uptake per se.

Methods

Subjects

12 men volunteered for the studies (age 25±1 yr, body mass index 21.6±0.6 kg/m^2, mean±standard error of mean). The subjects were healthy as judged by history and physical examination and routine laboratory tests and were not taking any medications. The nature, purpose, and potential risks of the study were explained to all subjects before they gave their informed consent to participate. The study was approved by the Ethical Committee of the Turku University Hospital.

Study design

The subjects were studied after a 10–12-h overnight fast. In five subjects, bradykinin was infused into the femoral artery after an overnight fast (basal study) while the other seven subjects received a similar intraarterial infusion of bradykinin under normoglycemic hyperinsulinemic conditions (insulin study).

The subjects were lying supine during the study. Three catheters were inserted, one in an antecubital vein for infusion of glucose and insulin and injection of [^{15}O]H_2O and [^{18}F]FDG, another in the opposite radial artery for blood sampling, and a third in the femoral artery for infusion of bradykinin (kindly provided by Hoechst AG, Frankfurt, Germany). The study for each subject consisted of a 30-min basal period (−30 to 0 min) and a 100-min (0–100 min) bradykinin infusion period (Fig. 1). Blood flow was measured using [^{15}O]H_2O during the 30-min basal period before start of the insulin and bradykinin infusions. The intraarterial infusion of bradykinin (0–6 min 30 ng/min, 6–12 min 100 ng/min, 12–100 min 300 ng/min, total dose 27 μg) was then started at 0 min and continued for 100 min in both studies (Fig. 1). The dose of bradykinin was selected based on preliminary studies which showed that this dose approximately doubles leg blood flow (data not shown). In the basal study saline was infused, while in the insulin study insulin was infused in a primed-continuous (1 mU/kg · min) fashion for 100 min. During hyperinsulinemia, normoglycemia was maintained using 20% glucose, and whole body glucose uptake was quantitated using the insulin clamp technique (23). In both studies, blood flow was measured using [^{15}O]H_2O and PET at 50 min (Fig. 1), and muscle glucose uptake was measured by giving an injection of [^{18}F]FDG at 60 min followed by scanning of the femoral region for 40 min (Fig. 1). Blood samples for the measurement of plasma glucose and serum insulin concentrations were taken as detailed below.

Measurement of muscle blood flow and glucose uptake with PET

Production of [^{15}O]H_2O. A cyclone 3 deuteron accelerator (Ion Beam Application Inc., Louvain-la-Neuve, Belgium) was used for [^{15}O]oxygen ($t_{1/2}$ 123 s) production. The [^{15}O]H_2O was synthesized in an oven in a palladium-catalyzed reaction from [^{15}O]oxygen and hydrogen gases at 200°C using a dialysis technique in a continuously working water module (24). A maximal production rate for [^{15}O]H_2O was 1.7 GBq/min. Sterility and pyrogen tests were performed to verify the purity of the product. The administered doses were monitored using a low voltage ionization chamber (25).

Production of [^{18}F]FDG. [^{18}F]FDG ($t_{1/2}$ of ^{18}F 109 min) was synthesized with an automatic apparatus as described by Hamacher et al. (26). The specific radioactivity at the end of the synthesis was 2 Ci/μmol and the radiochemical purity exceeded 98%.

Image acquisition. An eight-ring ECAT 931/08-tomograph (Siemens/CTI Corp., Knoxville, TN) was used. The scanner has an axial resolution of 6.7 mm and in-plane resolution of 6.5 mm. The images were obtained from the femoral regions. Before emission scanning, a transmission scan for the correction of photon attenuation was performed for 15 min with a removable ring source containing ^{68}Ge (total counts 15–30 × 10^6 in a plane).

For the flow studies, 30–35 mCi (1.3–1.3 GBq) [^{15}O]H_2O was injected intravenously, and a dynamic scan was performed for 6 min using 5-s frames. To obtain the input function, arterial blood was continuously withdrawn with a pump at a speed of 6 ml/min from the

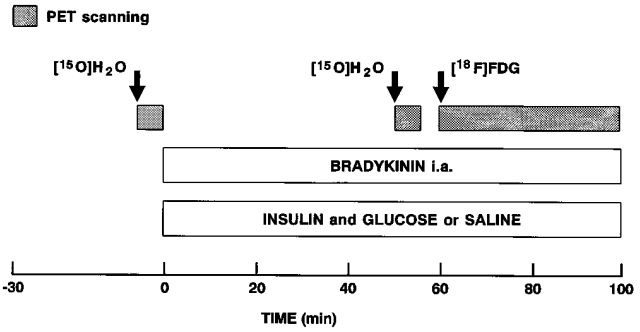

Figure 1. Design of the studies. Bradykinin was infused to the femoral artery under conditions of normoglycemic hyperinsulinemia (insulin study, n = 7) or during infusion of saline (basal study, n = 5). The arrows indicate the time of the [^{15}O]H_2O and [^{18}F]FDG injections. A dynamic scan of both femoral regions was performed to quantitate blood flow and glucose uptake in the bradykinin-infused and the control leg. Whole body glucose uptake was measured independent of the PET measurements using the euglycemic insulin clamp technique (insulin infusion rate 1 mU/kg · min, 0–100 min).

radial artery, and the concentration of radioactivity in blood was measured using a two-channel detector system (Scanditronix, Uppsala, Sweden), which was cross-calibrated with a well counter (Bicron 3MW3/3; Bicron Inc., Newbury, OH). To obtain the actual input function, $C_a(t)$, for the tissue element, internal dispersion from the arteries and external dispersion from the tubing in the blood sampling system were corrected for by using an exponential dispersion function. In this function, we used an internal dispersion time constant of 5 s (27). The external dispersion time constant was 3 s based on the phantom studies. The delay between the input curve and the tissue curve was solved by fitting (27).

For the [^{18}F]FDG study, 4–9 mCi of [^{18}FDG ($t_{1/2}$ 109 min) was injected intravenously over 2 min and dynamic scanning for 40 min was started (12 × 15 s, 4 × 30 s, 3 × 60 s, 1 × 120 s, 6 × 300 s). Blood samples for measurement of plasma radioactivity were withdrawn once during each time frame and the radioactivity was measured with the well counter.

Calculation of blood flow. The method to measure blood flow with [^{15}O]H_2O is based on the principle of inert gas exchange between blood and tissues (28). Earlier studies have shown that for a small volume element the gas exchange can be modeled mathematically with a single compartment model for a small volume element (27). The autoradiographic method has been widely used to solve numerically the flow (f, ml/min· 100 grams) from the following equation (29):

$$\int_0^T C_t(t)\,dt = \int_0^T f C_a(t) * \exp\left(-\frac{f}{pt}\right) dt.$$

The right side of the equation can be solved using the measured arterial time-activity curve ($C_a(t)$, nCi/gram) and an assumed fixed value for the distribution constant of water (p) in muscle tissue (30). The left side is the integrated tissue time activity concentration ($C_t(t)$, nCi/gram) obtained from dynamic images. The symbol * denotes the convolution integral. A table look-up procedure from the left side to the right side finally gives a unique rate constant f within the integration time scale T (29). Blood flow was calculated pixel by pixel into parametric flow images, which were produced by the autoradiographic technique described above. A 200-s integration time was applied to achieve optimal statistics.

Image processing. All data were corrected for dead time, decay, and measured photon attenuation as described previously (12) and reconstructed into a 128 × 128 matrix using a Hanning filter with a cut-off frequency of 0.5. The estimated resolution of the final images was 8 mm.

Regions of interest were drawn in the posterior, anterolateral, and anteromedial muscular compartments of the femoral region in both legs. Large vessels were avoided when outlining the muscle areas. The localization of the muscle compartments was verified by comparison of the flow images with the transmission image, which provides a topographical distribution of tissue density. The regions of interest outlined in the flow images were copied to the FDG images to obtain quantitative data from identical regions.

Comparison of blood measurements using PET and [^{15}O]H_2O and plethysmography. To compare PET-derived flow rates with those obtained with strain-gauge plethysmography, we measured calf blood flow using mercury in rubber strain gauge venous occlusion plethysmography (model EC-4; Hokanson Plethysmography, Issaquah, WA) (31), as described previously in detail (2), and femoral blood flow using PET and [^{15}O]H_2O, as described above, in 11 normal subjects under basal conditions and during a 140-min high-dose insulin infusion (5 mU/kg · min, serum insulin 470±24 mU/liter). Flow was measured with PET and [^{15}O]H_2O basally and after 75 min of hyperinsulinemia. Blood flow was measured with plethysmography immediately before the [^{15}O]H_2O injection. In addition, blood flow was measured in the basal state after an overnight fast in five subjects using both techniques. The correlation coefficient between all blood flow measurements performed with PET [^{15}O]H_2O and plethysmography was 0.68 (n = 27) and the coefficient of variation between the two methods was 18±3%. When determined by plethysmography (n = 11), insulin increased calf blood flow from 25±2 to 36±3 ml/kg leg tissue·min ($P < 0.001$). In the femoral region, leg blood flow, as measured with [^{15}O]H_2O and PET, increased in these subjects from 28±6 to 49±11 ml/kg leg · min ($P < 0.001$).

Calculation of regional glucose uptake. We used the three compartment model of [^{18}F]FDG kinetics (32) and graphical analysis according to Patlak and Blasberg (33) as previously described (12). Plasma and tissue time–activity curves were analyzed graphically to quantitate the fractional rate of tracer phosphorylation K_i (12, 34, 35). K_i is equal to $(k_1 \times k_3)/(k_2 + k_3)$, where k_1 is the transfer coefficient from vascular space into the tissue, k_2 is the initial clearance and efflux coefficient, and k_3 is the phosphorylation rate constant. The rate of the glucose uptake is obtained by multiplying K_i by the plasma glucose concentration $[Glc]_p$ divided by a lumped constant term (LC): $rGU = [Glc]_p/LC) \times K_i$. The lumped constant accounts for differences in the transport and phosphorylation of [^{18}F]FDG and glucose. A lumped constant value of 1.0 for skeletal muscle was used as described previously (12, 36).

Calculation of muscle glucose extraction. Glucose uptake can be calculated using the Fick equation by multiplying the glucose arteriovenous difference (glucose extraction) by blood flow (37). Since we measured glucose uptake and flow directly in the same region of femoral muscle, the amount of glucose extracted (millimoles per liter) by muscle tissue was calculated using the same principle by dividing muscle glucose uptake by muscle blood flow.

Whole body glucose uptake

For determination of the rate of whole body glucose uptake, the euglycemic insulin clamp technique was used as described previously (23, 38). During hyperinsulinemia, normoglycemia was maintained using an infusion of 20% glucose based on determination of plasma glucose (39) in arterial blood at 5–10-min intervals. The rate of glucose uptake during hyperinsulinemia was calculated during the time period when the measurements of blood flow and muscle glucose uptake were performed (50–100 min). Serum insulin concentrations were measured in the basal state and at 30-min intervals during the insulin infusion (40).

Statistical procedures

Statistical comparisons between measurements performed in the basal state and during hyperinsulinemia were performed using the nonparametric Wilcoxon's signed rank test. Correlations between selected study variables were calculated using Spearman's rank correlation coefficient. Data are expressed as mean±standard error of mean.

Results

Glucose and insulin concentrations. Normoglycemia was maintained both in the basal (4.8±0.1 mmol/liter) and insulin (5.3±0.1 mmol/liter) studies. Serum insulin concentrations averaged 28±2 pmol/liter in the basal study and 45±7 and 465±11 pmol/liter before and during hyperinsulinemia in the insulin study.

Muscle blood flow. In the basal state before the bradykinin or insulin infusions, femoral muscle blood flow was similar in both legs (23±3 vs. 21±3 ml/kg muscle · min, catheterized vs. control leg). During hyperinsulinemia, muscle blood flow was 58% higher in the bradykinin-infused (38±9 ml/kg muscle · min) than the control leg (24±5, $P < 0.01$). Compared with the basal state, bradykinin increased muscle blood flow by 61%, or by 21±4 ml/kg muscle · min ($P < 0.05$). The increase in femoral blood flow was confined to the muscle compartment (Fig. 2). In the basal study, blood flow was 98%

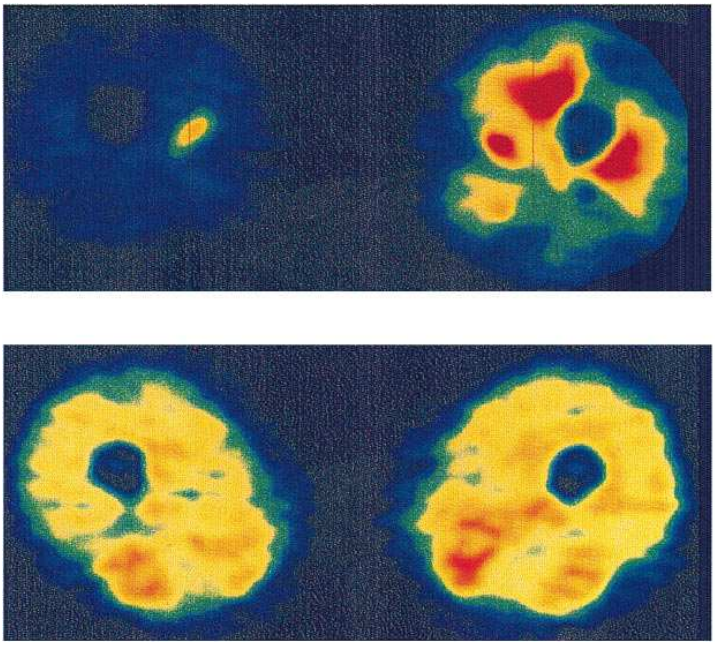

Figure 2. Cross-sectional images of blood flow and glucose uptake in the femoral region of a normal subject as seen during PET scanning. The top shows a parametric flow image obtained using $[^{15}O]H_2O$ and PET, and the bottom shows glucose uptake measured with $[^{18}F]$FDG and PET under euglycemic hyperinsulinemic conditions. The bradykinin-infused leg is shown on the right and the control leg is shown on the left.

higher in the bradykinin-infused (58±12 ml/kg muscle · min) than the control leg (28±6 ml/kg muscle · min, $P < 0.01$, Fig. 3).

Muscle glucose uptake. Muscle glucose uptake was identical in the insulin study in both femoral regions (60.6±9.5 vs. 58.7±9.0 μmol/kg muscle · min, bradykinin-infused vs. control leg, NS, Figs. 2 and 3). The ratio between glucose uptake in the bradykinin-infused and the control leg averaged 0.98±0.04 (NS vs. 1). Rates of muscle glucose uptake between the bradykinin-infused and control leg were highly correlated ($r = 0.97$, $P < 0.001$). The rates of glucose uptake in the control leg ($r = 0.78$, $P < 0.05$) and the bradykinin-infused leg ($r = 0.80$, $P < 0.05$) were significantly correlated with whole body glucose uptake (32.2±2.9 μmol/kg body weight · min). In the basal study, glucose uptake was also identical in both legs (10.1±0.9 vs. 10.6±0.8 μmol/kg · min, bradykinin-infused vs. control leg, NS).

Glucose extraction. We calculated glucose extraction (millimoles per liter) using the Fick principle (uptake = flow · glucose extraction). Glucose extraction was significantly lower during hyperinsulinemia in the bradykinin-infused (1.8±0.2 mmol/liter) than the control leg (2.6±0.2 mmol/liter, $P < 0.01$, Fig. 3). In the basal study, glucose extraction was also lower in the bradykinin-infused (0.27±0.1 mmol/liter) than the control leg (0.49±0.1 mmol/liter, $P < 0.01$).

Discussion

The increasing number of studies demonstrating defects in insulin-induced vasodilatation as potential causes of insulin resistance (3, 4, 6) and beneficial effects of vasodilatory drugs such as α_1-blockers (41) and angiotensin-converting enzyme (ACE) inhibitors (42–44) on insulin action promoted us to directly test whether glucose uptake can be increased simply by increasing blood flow. For this purpose we infused bradykinin, an endothelium-dependent vasodilatator (17, 20), into the femoral artery of healthy volunteers and measured the effect of bradykinin on blood flow and glucose uptake. Bradykinin increased blood flow significantly both in the basal state and during hyperinsulinemia but had no effect on glucose uptake under either condition because of a concomitant decrease in glucose extraction.

We used $[^{15}O]H_2O$ and a single compartment model combined with an autoradiographic method to quantitate blood flow. The short half-life of $[^{15}O]$ (123 s) enables sequential measurements of blood flow. In addition, after decay of this isotope, $[^{18}F]$FDG can be used to determine glucose uptake. This autoradiographic method for measurement of blood flow has been validated against the steady state flow method as well as against strain gauge plethysmography (14, 45). Regarding $[^{18}F]$FDG, we found as in previous studies (13, 32, 36, 46), a close correlation between muscle and whole body glucose uptake. The glucose uptake measurements with $[^{18}F]$FDG have been validated previously against the forearm balance technique (12).

Bradykinin and other kinins are thought to predominantly

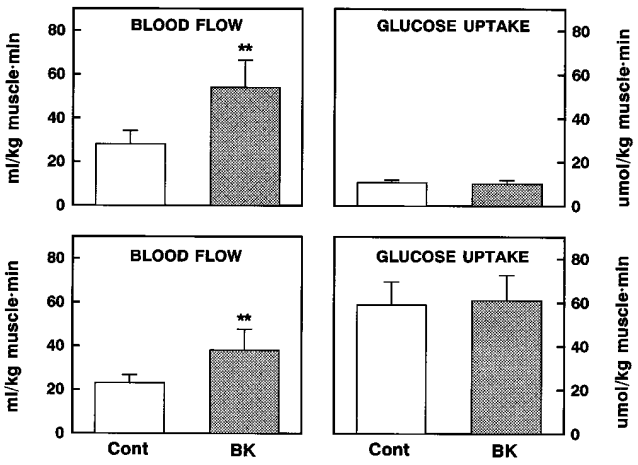

Figure 3. Mean rates of muscle blood flow and glucose uptake in the basal (*top*) and insulin (*bottom*) studies in the bradykinin-infused (*BK*) and the control (*Cont*) leg. $^{**}P < 0.01$ for BK vs. Cont.

act in a paracrine fashion, making estimation of physiological concentrations difficult. Because of the rapid degradation of kinins in the systemic circulation (47), it was not possible to determine the bradykinin concentration achieved in the present study. Cockcroft et al. (18) infused increasing doses of bradykinin intraarterially into normal volunteers. A 2.6-μg dose of bradykinin increased blood flow approximately fivefold within 24 min in the forearm, which contains ~ 0.6 kg of muscle in a 70-kg person (48). In the present study, a dose of ~ 13 μg increased muscle blood flow ~ 1.6-fold within 50 min in the leg which contains ~ 7.7 kg muscle (30). Thus, both the dose of bradykinin (4.3 vs. 1.7 μg/kg muscle for forearm vs. leg) and the increase in flow were lower per kilogram of muscle in this study than in that of Cockcroft et al. (18). Bradykinin has been suggested to mediate, either via its vasodilatory effect or independent of this effect, the possible beneficial effects of ACE inhibitors on peripheral glucose uptake (49). The present data suggest that, at least acutely, bradykinin induced increases in blood flow have no effect on glucose uptake. In an early study by Jauch et al. (50), a single dose (25 mg) of captopril increased the forearm arterio-venous difference for glucose by 270% (2.2 vs. 0.8 mmol/liter) with no change in blood flow in patients with NIDDM compared with placebo during an insulin clamp similar to that in the present study. While planning this study, we initially wished to use an ACE inhibitor and determine its acute effect on glucose uptake. However, in these preliminary studies in eight normal volunteers, we were unable to demonstrate any enhancement in glucose uptake or blood flow during a 6-h period after 25 mg of captopril (51). The inability of ACE inhibitors to change blood flow acutely suggests that the increase in local bradykinin concentrations after inhibition of kininase II (ACE) is not high enough to measurably change blood flow. The present data demonstrate that even if the bradykinin concentration increased sufficiently to stimulate blood flow, this alone would not acutely enhance glucose uptake.

In this study, insulin alone didn't increase blood flow significantly. We measured blood flow after 50 min of physiological hyperinsulinemia (serum insulin 465 pmol/liter). It is clear from previous studies that the ability of insulin to stimulate blood flow is critically dependent on both the concentration of insulin used and the duration of the insulin infusion (1, 2). In studies where the insulin infusion rate has been similar to that in this study (1 mU/kg·min or 40 mU/m^2 · min), Vollenweider et al. (52) observed an ~ 15% increase in blood flow at 45 min, Anderson et al. (53) a 20% increase at 60 min, Scherrer et al. (16) a 26% increase at 60 min, Baron et al. (54) a 57% increase after 180 min, and a 105% increase after 200 min (55). In contrast to these data, several other investigators found no significant increase in blood flow after 120 min using this insulin dose (2, 48, 56–61). Thus, our present finding of no change in blood flow is consistent with previous data showing small or no increases in blood flow during the first hour of a 1 mU/kg · min insulin infusion. The reason for the discrepant results is unclear. One reason is the large variability in blood flow even within lean healthy subjects. For example, in the study of Utriainen et al. (2), individual increases in blood flow during a 1 mU/kg · min insulin infusion varied from −12 to 60% at 120 min. This variation was closely correlated with forearm muscularity (2). However, longer exposure to insulin (1–3, 54) markedly stimulates blood flow. In keeping with this, a significant increase in leg blood flow was observed in studies comparing flow values measured with plethysmography with those measured with PET and $[^{15}O]H_2O$ (see Methods). This increase amounted to 44% when measured with plethysmography in the calf and to 75% when measured with PET and $[^{15}O]H_2O$ in the femoral region. The reason for the lower increase in blood flow by plethysmography remains uncertain but could involve factors such as greater proportion of nonmuscle tissue in the calf as compared with the femoral region (2) or higher blood flow in femoral than calf muscles.

The lack of an effect of increased blood flow on glucose uptake in this study does not exclude the possibility that defects in blood flow contribute to diminished glucose uptake in patients with defects in insulin-stimulated blood flow. Such defects have been described in several insulin-resistant conditions including hypertension (4), obesity (3), and NIDDM (6). The significance of these hemodynamic defects for insulin-stimulated glucose uptake awaits studies where flow is normalized by vasoactive agents and the impact of this intervention on glucose uptake is assessed. In keeping with this possibility, postoperative insulin resistance was ameliorated by bradykinin in the study of Jauch et al. (62). Under such conditions, glucose uptake might be dependent on tissue perfusion, although vascular autoregulation appears to maintain oxygen uptake constant over a wide range of blood flow in mammalian skeletal muscle (63).

In this study bradykinin had no effect acutely on either basal or insulin-stimulated glucose uptake. These data are consistent with the lack of effect of bradykinin on glucose transport in isolated cardiac myocytes (21) or on glucose transport, glycolysis or glycogen synthesis in rat soleus muscle (22). This does not exclude the possibility that chronic exposure of tissues to increased bradykinin concentrations enhances insulin action. Indeed, in spontaneously hypertensive rats infusion of bradykinin for 5 d was reported to improve glucose uptake (49). Kinins appear responsible for improvement in insulin sensitivity during treatment with ACE inhibitors in rats (49). The mechanisms underlying such kinin effects remain speculative but could involve structural alterations in muscle tissue such as changes in myosin isoforms and consequently fiber types (64–66), antiproliferative effects in response to endothelial injuries (67, 68) and prevention of vascular rarefaction (69).

In conclusion, the present data demonstrate that insulin-stimulated glucose uptake cannot be improved by simply increasing blood flow in normal subjects. Furthermore, we found that bradykinin has no acute insulin-like effect on glucose uptake in human skeletal muscle, in keeping with the in vitro data in rat muscle (21, 22). These data imply that improvements in insulin sensitivity cannot be induced simply by increasing glucose delivery to muscle tissue and suggest that the mechanism underlying the improvement in insulin sensitivity by ACE inhibitors is unlikely to be a consequence of an acute insulin-like action of bradykinin.

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