Effects of Insulin on Regional Blood Flow and Glucose Uptake in Wistar and Sprague-Dawley Rats

Nathalie Gaudreault, Marta Santuré, Maryse Pitre, André Nadeau, André Marette, and Hélène Bachelard

The euglycemic-hyperinsulinemic clamp technique in conscious Sprague-Dawley and Wistar rats chronically instrumented with intravascular catheters and pulsed Doppler flow probes was used to examine insulin's actions on regional blood flow and glucose metabolism. The effect of insulin on in vivo and in vitro glucose utilization in individual muscles was estimated using [^{3}H]-2-deoxy-D-glucose. We found that in both strains, insulin (4, 32, and 64 mU · kg^{-1} · min^{-1}) causes similar cardiovascular changes characterized by slight increases in blood pressure (at high dose), vasodilation in renal and hindquarter vascular beds, and vasoconstriction (at high dose) in the superior mesenteric vascular bed. However, at the lowest dose of insulin tested, we found a smaller insulin sensitivity index and a lower insulin-stimulated in vivo glucose uptake in extensor digitorum longus (EDL) muscles of Wistar versus Sprague-Dawley rats. Higher insulin-stimulated glucose transport activity was found in isolated soleus muscle, while greater basal glucose transport was noted in isolated EDL muscle from Sprague-Dawley versus Wistar rats. These results provide further evidence for an insulin blood flow–regulatory effect and suggest that strain characteristics (differences in muscle perfusion, hindquarter composition, or fiber insulin sensitivity) constitute a major determinant in the variation in whole-body insulin sensitivity.
Copyright © 2001 by W.B. Saunders Company

BESIDES ITS KEY ROLE in the regulation of carbohydrate metabolism,[1] it has become clear that insulin has important cardiovascular effects. Several groups, using both invasive measurement techniques such as thermodilution[2-6] and dye dilution[7] and noninvasive techniques such as plethysmography[8-12] and positron emission tomography,[13,14] have reported that insulin increases net blood flow to skeletal muscle through a vasodilator effect and most probably via a muscle capillary recruitment mechanism. However, in contrast to the marked increase in skeletal muscle blood flow previously reported by Baron et al,[6,15] other studies have shown no increase[16-20] or a less marked increase[8,21] in blood flow in response to insulin. The failure to observe a significant increase in blood flow in some studies but not others could be explained, at least in part, by methodologic factors (such as the duration of the studies and the dose of insulin, the location, and the technique used for measurement of blood flow), as well as interindividual variation (especially in the proportion and composition of muscle).[11]

While most studies of insulin's hemodynamic actions have been made in humans or large animals, few studies have examined the hemodynamic effects of insulin in rat skeletal muscle in vivo. Such studies were mainly limited by the availability and reliability of the measurement methods for muscle flow. In one recent study in conscious, unrestrained and overnight-fasted rats, we examined the regional hemodynamic responses to insulin during a euglycemic-hyperinsulinemic clamp.[22] In this study, a direct and continuous recording of regional hemodynamics was made using pulsed Doppler flow probes chronically implanted around specific vessels. Thus, in Wistar Kyoto (WKY) rats, we found that the euglycemic infusion of insulin (4 and 16 mU · kg^{-1} · min^{-1}) caused vasodilation in the renal and hindquarter vascular beds but no changes in blood pressure, heart rate, or superior mesenteric vascular conductance. In contrast, in spontaneously hypertensive rats (SHRs), the same doses of insulin produced vasoconstriction in the superior mesenteric and hindquarter vascular beds and an increase in blood pressure. Moreover, at the lowest dose of insulin tested, we found a reduction in the insulin sensitivity index in SHRs compared with WKY rats.[22] In another study using the same method as ours to measure blood flow but a different strain of rat, the Sprague-Dawley rat, it was shown that euglycemic infusion of low-dose insulin (2 mU/min) in conscious unfasted rats produced an increase in blood pressure and hindquarter vascular resistance.[23] Other studies[24,25] using limited or indirect measures of blood flow have also failed to demonstrate any insulin-mediated increases in skeletal muscle blood flow in hindleg muscles during a euglycemic clamp in conscious rats. However, in contrast to the previous studies but similar to our results in WKY rats, a recent study using anesthetized Sprague-Dawley rats and measuring the metabolism of 1-methylxanthine to determine blood flow distribution within the hindleg muscles demonstrated that euglycemic infusion of insulin (10 mU · kg^{-1} · min^{-1}) significantly increased femoral blood flow and decreased hindleg vascular resistance.[26] We believe that the discrepancy between studies may be related, at least in part, to strain differences, as well as differences in study design (ie, fasted *v* unfasted animals, conscious *v* anesthetized rats, and direct *v* indirect measurement technique for muscle flow) and the inherent variability of blood flow measurements and the consequent difficulties in establishing a steady baseline measurement.

In continuity with our previous investigations and in light of the controversy regarding the blood flow effects of insulin in rats in vivo, we undertook a new series of experiments to investigate the acute heart rate, blood pressure, and regional hemodynamic responses to euglycemic infusion of insulin, as

From the Hypertension Research Unit, Diabetes Research Unit, and Department of Physiology and Lipid Research Unit, Laval University Hospital Research Center, Ste-Foy, Quebec, Canada.

Submitted February 21, 2000; accepted May 12, 2000.

Supported by grants from the Medical Research Council of Canada and the Heart and Stroke Foundation of Canada (H.B.) and by the Fonds de la Recherche en Santé du Québec (H.B.).

Address reprint requests to Hélène Bachelard, PhD, Hypertension Research Unit, Laval University Hospital Research Center, 2705 blvd. Laurier, Ste-Foy, Quebec, Canada, G1V 4G2.

Copyright © 2001 by W.B. Saunders Company

0026-0495/01/5001-0027$10.00/0

doi:10.1053/meta.2001.18569

well as insulin sensitivity, in two other strains of rats, Wistar and Sprague-Dawley. Moreover, in the present study, insulin-mediated glucose uptake in individual skeletal muscles was assessed in both strains in the presence or absence of blood flow influence. The rats were instrumented with intravascular catheters and Doppler flow probes to permit a continuous recording of blood pressure, heart rate, and regional blood flow. These in vivo conscious animal procedures involving both metabolic and vascular response determinations simultaneously in the same animal are original and constitute a unique and powerful tool to investigate the interactions among insulin sensitivity, blood flow, and the hemodynamic response to insulin.

MATERIALS AND METHODS

All surgical and experimental procedures were in accordance with institutional animal care guidelines. Male Sprague-Dawley rats (n = 36) and Wistar rats (n = 42) aged 12 to 14 weeks and weighing 250 to 300 g were purchased from Charles River Canada (St Constant, Quebec). The rats were anesthetized with a mixture of ketamine-xylazine (100 and 10 mg · kg^{-1}, respectively, intraperitoneally [IP]) and implanted with pulsed Doppler flow probes to monitor changes in renal, mesenteric, and hindquarter blood flows according to the method developed by Gardiner and Bennett[27] as previously described in detail.[22] At least 7 days later, the rats were reanesthetized with a mixture of ketamine and xylazine (100 and 10 mg · kg^{-1}, respectively, IP). The leads of the implanted probes were soldered to a microconnector (Microtech, Boothwyn, PA), and two separate catheters were implanted in the right jugular vein (for glucose and insulin infusions) and one catheter in the distal abdominal aorta via the left femoral artery (for measurement of blood pressure and heart rate). The catheters were tunneled subcutaneously to emerge at the same point as the probe wires. After a further 72-hour recovery, experiments were started in conscious unrestrained animals with free access to water but not food. Throughout the experiments, continuous recordings were made for phasic and mean blood pressure, instantaneous heart rate, and phasic and mean renal, mesenteric, and hindquarter Doppler shift signals using a modified[28] pulsed Doppler monitoring system (Crystal Biotech, Holliston, MA) and a Biopac Data Acquisition and Analysis system (model MP 100; Software Version 3.1, Santa Barbara, CA). At selected time points, the heart rate, mean blood pressure, and mean Doppler shifts were measured and related to the preclamp baseline value (absolute changes for the former 2 variables and percentages for Doppler shifts). In addition, the mean Doppler shift and corresponding mean blood pressure signals were used to calculate percentage changes in regional vascular conductance.

Euglycemic-Hyperinsulinemic Clamp Studies

The rats were deprived of food for 12 hours before the glucose clamp study. Before each experiment, blood glucose and plasma insulin were determined and the resting heart rate, blood pressure, and regional blood flow were recorded over 30 minutes in quiet, unrestrained, and unsedated rats. The euglycemic-hyperinsulinemic clamp was then performed over 2 hours while the heart rate, blood pressure, and regional blood flow were measured continuously. The rats were divided in 2 groups, with the first group represented by Wistar rats (n = 42) receiving insulin at a rate of 4 (n = 10), 32 (n = 10), and 64 (n = 11) mU · kg^{-1} · min^{-1} and the second group represented by Sprague-Dawley rats (n = 36) receiving insulin at a rate of 4 (n = 9), 32 (n = 9), and 64 (n = 8) mU · kg^{-1} · min^{-1}. In control experiments, subgroups of Wistar (n = 11) and Sprague-Dawley rats (n = 10) were infused with saline–0.2% bovine serum albumin (BSA) as an approximate match to the saline load delivered during the clamp studies. Thus, after basal measurements of blood glucose and plasma insulin, each rat received a continuous infusion of regular porcine insulin (Iletin II, 100 U/mL; Eli Lilly, Indianapolis, IN) at a constant rate (20 μL · min^{-1}). The insulin solution was diluted to the appropriate concentration in saline (0.9% NaCl) containing 0.2% BSA. Insulin was infused using a syringe infusion pump (model A-99; Razel, Stamford, CT) from a reservoir (5-mL syringe) through polyethylene tubing (0.28 mm ID; Clay Adams, Sparks, MD). Ten minutes after the insulin infusion started, a 30% glucose solution (with saline) was infused at a variable rate to maintain blood glucose at the baseline level according to frequent arterial blood glucose determinations performed at 10-minute intervals (Glucometer Elite; Miles Canada, Etobicoke, Ontario). The clamp studies were performed for 120 minutes to achieve steady-state glucose infusion rates, and the whole-body insulin sensitivity of each rat was assessed on the basis of data obtained over the last 60 minutes of each study. The amount of glucose required to maintain euglycemia during the last hour of the clamp, which corresponds to the steady-state concentration of insulin, was used as an index of insulin sensitivity. Blood samples (0.3 mL) were collected before starting the clamp and at timed (20-minute) intervals during the 120-minute euglycemic-hyperinsulinemic clamp for analysis of plasma insulin concentrations. Red blood cells from these samples were resuspended in saline after centrifugation and immediately returned to the rat.

In Vivo 2-Deoxy-D-Glucose Uptake

Measurement of steady-state insulin-stimulated glucose uptake in individual tissues was performed by measuring the incorporation of radiolabeled 2-deoxy-D-glucose (2-deoxy-D-[1,2-^{3}H(*N*)]glucose) based on the original method of Kraegen et al[29] as described previously.[30] Briefly, a bolus of [^{3}H]-2-deoxy-D-glucose 250 μCi/kg and [^{14}C]-sucrose 25 μCi/kg in 0.5 mL saline solution was injected during the last 25 minutes of some clamp studies performed at an insulin dose of 4 mU · kg^{-1} · min^{-1} (Wistar, n = 6; Sprague-Dawley, n = 5). Blood samples were taken at regular intervals (2.5, 5, 7.5, 10, 15, and 20 minutes) after the bolus injection for determination of plasma glucose and radiolabeled 2-deoxy-D-glucose and sucrose. Twenty-five minutes after the bolus, the animals were rapidly killed by decapitation, and the hindlimb muscles (soleus, extensor digitorum longus [EDL], gastrocnemius, and quadriceps) were rapidly excised, frozen in liquid nitrogen, and stored at −80°C. Subsequently, tissue samples (30 to 50 mg) were dissolved in 1 mL 0.5-mol/L ammonium hydroxide at 55°C for 16 to 18 hours. Thereafter, hydrogen peroxide (30% solution) was added for 60 minutes at 55°C to decrease quenching, followed by the addition of 10 mL scintillation fluid (BCS; Amersham, Mississauga, Ontario, Canada). The samples and the plasma (20 μL) were then analyzed in a liquid scintillation counter (Wallac 1409, Turku, Finland). The accumulation of [^{3}H]-2-deoxy-D-glucose in muscle, corrected for the extracellular space with [^{14}C]-sucrose, was used as an index of glucose uptake.[30]

Glucose Transport Activity in Isolated Rat Skeletal Muscle

Basal and insulin-stimulated glucose utilization were examined in isolated soleus and EDL skeletal muscle from overnight-fasted Wistar (n = 9) and Sprague-Dawley (n = 8) rats. Glucose transport in isolated muscles was measured with the glucose analog [^{3}H]-2-deoxy-D-glucose as described previously.[31] The rats were anesthetized with a mixture of ketamine-xylazine (100 and 10 mg · kg^{-1}, respectively, IP). Soleus and EDL muscles were dissected out and rapidly cut into 30- to 40-mg strips. The animals were then killed by intracardiac injection of ketamine-xylazine. Muscle strips were incubated in a shaking water bath at 30°C for 30 minutes in 25-mL flasks containing 3.0 mL oxygenated Krebs-Ringer bicarbonate (KRB) buffer supplemented with 8 mmol/L glucose, 32 mmol/L mannitol, and 0.1% BSA. The flasks were gassed continuously with 95% O_2-5% CO_2 throughout the experiment. After the initial incubation, the muscles were incubated for 30 minutes in

Table 1. Baseline Values for the Heart Rate, MAP, and Regional Doppler Shift and Vascular Conductance in Conscious Unrestrained Wistar and Sprague-Dawley Rats

Group	HR (bpm)	MAP (mm Hg)	Doppler Shift (kHz)			Vascular Conductance (kHz · mm Hg^{-1})10^3		
			Renal	Mesenteric	Hindquarter	Renal	Mesenteric	Hindquarter
Wistar (n = 42)	384 ± 6	97 ± 1	10.6 ± 0.9	16.3 ± 1.2	9.8 ± 1.1	109 ± 9	169 ± 12	101 ± 11
Sprague-Dawley (n = 36)	343 ± 5*	88 ± 2*	8.2 ± 0.7*	13.8 ± 0.8	7.0 ± 0.5*	95 ± 8	160 ± 10	81 ± 6

NOTE. Values are the mean ± SE. The groups represent those used to assess the hemodynamic effects of insulin intravenously infused during the euglycemic-hyperinsulinemic clamp studies.

Abbreviation: HR, heart rate.

*$P < .05$, Sprague-Dawley *v* Wistar, Student's *t* test for unpaired data.

oxygenated KRB buffer in the presence or absence of insulin (Humulin R) at 4 different concentrations (0.002, 0.02, 0.2, and 2 mU/mL). The muscles were next washed for 10 minutes at 29°C in 3 mL KRB buffer containing 40 mmol/L mannitol and 0.1% BSA. They were then incubated for 20 minutes at 29°C in 3 mL KRB buffer containing 8 mmol/L [^{3}H]-2-deoxy-D-glucose (2.25 μCi/mL), 32 mmol/L [^{14}C]-mannitol (0.3 μCi/mL), 2 mmol/L sodium pyruvate, and 0.1% BSA. Insulin was present throughout the wash and uptake incubations (if it was present in the previous incubation medium). After the incubation, the muscles were rapidly blotted at 4°C, clamp-frozen, and stored at −80°C until processed. Muscles were processed by boiling for 10 minutes in 1 mL water. Extracts were transferred to an ice bath, vortexed, and then centrifuged at 1,000 × *g*. Triplicate 200-μL aliquots of the muscle extract supernatant and of the incubation medium were counted for radioactivity using a Wallac 1409 counter. [^{3}H]-2-deoxy-D-glucose uptake rates were corrected for extracellular trapping using [^{14}C]-mannitol.[32]

Analytic Methods

Blood samples for plasma glucose and insulin determinations in the basal state and during insulin infusion were obtained, placed in untreated polypropylene tubes, and centrifuged with an Eppendorf microcentrifuge (Minimax; International Equipment, Needham Heights, IL). The plasma was stored at −20°C until assay. The glucose concentration of the supernatant was measured by the glucose oxidase method[33] using a glucose analyzer (Bayer RA-XT, Tarrytown, NY), and the plasma insulin level was measured by radioimmunoassay using porcine insulin standards and polyethylene glycol for separation.[34]

Data Analysis

Results are the mean ± SE, n is the number of observations. Data describing the biologic characteristics of the rats were evaluated using Student's *t* test for unpaired data, whereas results obtained over time, such as those for the cardiovascular response to insulin, were analyzed for statistical significance by ANOVA for repeated measures. Post hoc comparisons were made using Fisher's test. A *P* value less than .05 was used to indicate a significant difference. Linear regression analysis was used to correlate the various parameters shown in Table 3.

RESULTS

Resting values for cardiovascular variables in Wistar and Sprague-Dawley rats are listed in Table 1. We found that basal mean blood pressure was lower in Sprague-Dawley rats than in Wistar rats. This was accompanied by a lower basal heart rate and lower basal renal and hindquarter blood flow in Sprague-Dawley versus Wistar rats. However, there was no significant difference in the basal renal, superior mesenteric, or hindquarter vascular conductance between the strains.

Hemodynamic Responses to Insulin Infusion During the Euglycemic-Hyperinsulinemic Clamp Period

Important cardiovascular responses to insulin were observed in conscious unrestrained Wistar and Sprague-Dawley rats in which arterial blood glucose was maintained at baseline levels throughout the experiments by administering glucose during insulin infusion. Figures 1 and 2 show the maximum cardiovascular changes elicited by increasing doses of insulin in Wistar and Sprague-Dawley rats. Thus, we found that the lowest dose of insulin tested (4 mU · kg^{-1} · min^{-1}) in Wistar rats caused a slight but significant increase in renal blood flow (significant at 15 to 45 minutes and 90 to 120 minutes) but had no significant effect on the mean arterial blood pressure, heart rate, or superior mesenteric or hindquarter blood flow compared with the control infusion of vehicle (saline-BSA 0.2%; Fig 1A). This response was associated with a slight but significant increase in renal vascular conductance (significant at 15, 45, 90, and 120 minutes), but no significant changes were found for the superior mesenteric or hindquarter vascular conductance (Fig 2A). In Sprague-Dawley rats, the same infusion of insulin had no effect on the heart rate, mean arterial blood pressure, or superior mesenteric or hindquarter blood flow, while a late but significant increase in renal flow (significant at 90 to 120 minutes) was observed as compared with the control infusion of saline-BSA 0.2% (Fig 1B). However, no significant changes were found for renal, superior mesenteric, or hindquarter vascular conductance (Fig 2B). These findings were not different from those observed in Wistar rats, although in the latter, there was a significant increase in renal vascular conductance.

Infusion of insulin at a dose of 32 mU · kg^{-1} · min^{-1} produced more pronounced cardiovascular effects in both strains. In Wistar rats, there were increases in renal (significant at 15 to 120 minutes) and hindquarter (significant at 15 and 45 to 120 minutes) blood flow, while no changes in the mean arterial blood pressure, heart rate, or superior mesenteric blood flow were observed (Fig 1A). These effects were associated with increases in renal (significant at 15 to 120 minutes) and hindquarter (significant at 15, 45 to 75, and 105 to 120 minutes) vascular conductance (Fig 2A). Superior mesenteric vascular conductance did not change significantly. In Sprague-Dawley rats, insulin infusion at a dose of 32 mU · kg^{-1} · min^{-1} produced a significant increase in mean arterial blood pressure (significant at 60 to 120 minutes) and long-lasting increases in renal (significant at 15 to 120 minutes) and hindquarter (sig-

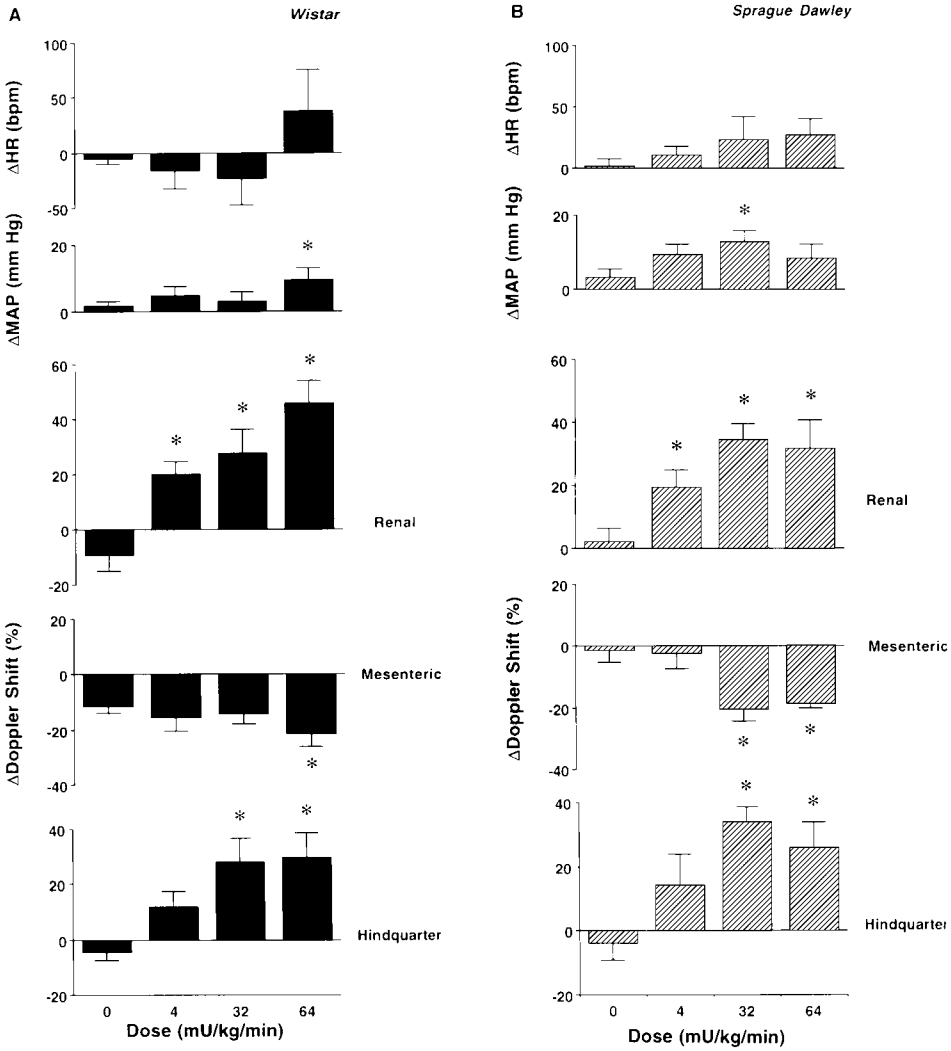

Fig 1. Bar graphs showing (A) maximum cardiovascular changes elicited by control intravenous (IV) infusion of saline-0.2% BSA (n = 11) or euglycemic infusion of insulin at a rate of 4 (n = 10), 32 (n = 10), or 64 (n = 11) mU · kg^{-1} · min^{-1} in conscious Wistar rats, and (B) maximum cardiovascular changes elicited by control IV infusion of saline-0.2% BSA (n = 10) or euglycemic infusion of insulin at a rate of 4 (n = 9), 32 (n = 9), or 64 (n = 8) mU · kg^{-1} · min^{-1} in conscious Sprague-Dawley rats. Effects of saline-0.2% BSA or insulin were assessed relative to baseline values. Values are the mean ± SE shown by vertical lines. HR, heart rate. *P < .05, insulin-infused group *v* vehicle-infused group, ANOVA followed by Fisher's test.

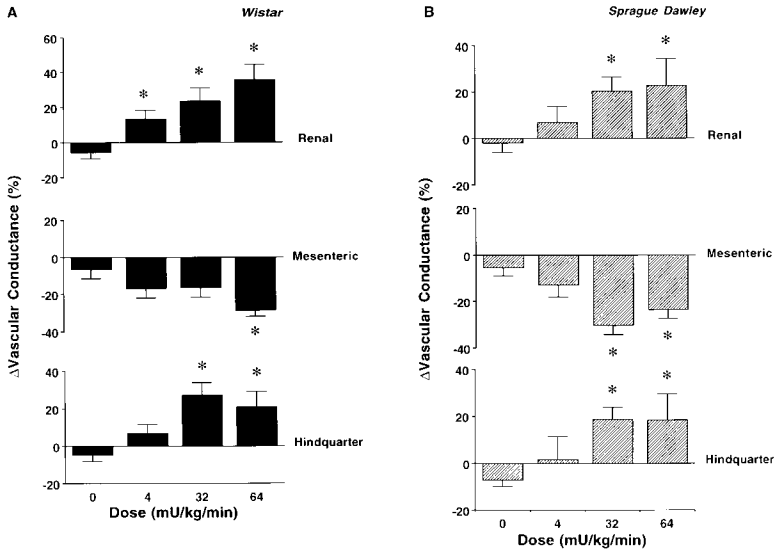

Fig 2. Bar graphs showing (A) maximum changes in renal, superior mesenteric, and hindquarter vascular conductance elicited by control intravenous (IV) infusion of saline-0.2% BSA (n = 11) or euglycemic infusion of insulin at a rate of 4 (n = 10), 32 (n = 10), or 64 (n = 11) mU · kg^{-1} · min^{-1} in conscious Wistar rats, and (B) maximum changes in regional vascular conductance elicited by control IV infusion of saline-0.2% BSA (n = 10) or euglycemic infusion of insulin at a rate of 4 (n = 9), 32 (n = 9), or 64 (n = 8) mU · kg^{-1} · min^{-1} in conscious Sprague-Dawley rats. Data are derived from the data in Fig 3. Effects of saline-0.2% BSA or insulin were assessed relative to baseline values. Values are the mean ± SE shown by vertical lines. *P < .05, insulin-infused group *v* vehicle-infused group, ANOVA followed by Fisher's test.

Table 2. Blood Glucose, Plasma Insulin, and Glucose Infusion Rate During the Euglycemic-Hyperinsulinemic Clamp in Conscious Wistar and Sprague-Dawley Rats

Insulin Dose ($mU \cdot kg^{-1} \cdot min^{-1}$)	Blood Glucose (mmol/L)			Plasma Insulin (pmol/L)		$GIR_{60\text{-}120}$ ($mg \cdot kg^{-1} \cdot min^{-1}$)
	Basal	60-120 min	CV (%)	Basal	60-120 min	
Wistar						
4 (n = 10)	4.1 ± 0.1	4.1 ± 0.1	7.5 ± 1.0	55 ± 10	477 ± 37	25.3 ± 2.0
32 (n = 10)	4.2 ± 0.2	4.1 ± 0.2	9.9 ± 1.3	67 ± 6	7,904 ± 1,189	46.0 ± 2.5
64 (n = 11)	3.7 ± 0.1	3.7 ± 0.1	7.4 ± 1.4	47 ± 7	22,850 ± 1,199	47.7 ± 6.6
Sprague-Dawley						
4 (n = 9)	4.2 ± 0.1	4.0 ± 0.1	9.1 ± 0.8	54 ± 9	482 ± 48	32.2 ± 1.9*
32 (n = 9)	4.1 ± 0.2	3.8 ± 0.1	7.6 ± 1.5	50 ± 6	7,770 ± 991	45.4 ± 1.8
64 (n = 8)	4.0 ± 0.1	4.2 ± 0.1	9.8 ± 1.4	51 ± 7	24,462 ± 4,514	47.8 ± 2.4

NOTE. Values are the mean ± SE.

Abbreviations: CV, coefficient of variation of blood glucose from 60-120 min; $GIR_{60\text{-}120}$, glucose infusion rate required to maintain euglycemia during steady-state (60-120 min) plasma insulin.

*$P < .01$, Sprague-Dawley *v* Wistar, Student's *t* test for unpaired data.

nificant at 15 to 120 minutes) blood flow, but no significant change in heart rate (Fig 1B). The blood pressure response differed significantly in Sprague-Dawley rats versus Wistar rats, in which insulin infusion had no effect on mean arterial blood pressure. Moreover, there was a decrease in superior mesenteric flow (significant at 15 to 120 minutes), which was not different from that found in Wistar rats, although in the latter, this effect did not reach the level of significance (Fig 1B). These cardiovascular responses to insulin were associated with long-lasting increases in renal (significant at 30 to 120 minutes) and hindquarter (significant at 15 to 30 and 75 to 120 minutes) vascular conductance and a substantial decrease in superior mesenteric (significant at 15 to 120 minutes) vascular conductance (Fig 2B). The superior mesenteric vasoconstriction was greater in Sprague-Dawley rats versus Wistar rats.

The highest dose of insulin tested (64 $mU \cdot kg^{-1} \cdot min^{-1}$) in Wistar rats produced cardiovascular effects characterized by long-lasting increases in mean arterial blood pressure (significant at 15 and 60 to 120 minutes) and renal (significant at 15 to 120 minutes) and hindquarter (significant at 30 to 120 minutes) blood flow and a slight decrease in superior mesenteric flow (significant at 15, 75, and 90 minutes), but no significant change was found for the heart rate compared with the control infusion of saline-BSA 0.2% (Fig 1A). Furthermore, long-lasting increases were noted in renal (significant at 15 to 120 minutes) and hindquarter (significant at 45 to 105 minutes) vascular conductance (Fig 2A), while a significant decrease in superior mesenteric vascular conductance (significant at 15, 45, and 75 to 120 minutes) was found. In Sprague-Dawley rats, insulin infusion at the same dose had no effect on the heart rate or mean arterial blood pressure, but caused significant increases in renal (significant at 30 to 120 minutes) and hindquarter (significant at 15 to 120 minutes) blood flows and a decrease in superior mesenteric flow (significant at 15, 60 to 75, and 105 minutes) compared with the control infusion of saline-BSA 0.2% (Fig 1B). These changes were accompanied by significant increases in renal (significant at 30 to 120 minutes) and hindquarter (significant at 15 to 120 minutes) vascular conductance and a decrease in superior mesenteric vascular conductance (Fig 2B). These findings are not different from those observed in Wistar rats.

In summary, the overall patterns of regional hemodynamic responses to hyperinsulinemia were similar in both strains (eg, slight blood pressure increase, vasodilation in renal and hindquarter vascular beds, and vasoconstriction in superior mesenteric vascular beds).

Responses During Euglycemic-Hyperinsulinemic Clamp

Table 2 shows that there was no significant difference for basal blood glucose or plasma insulin between the 6 insulin subgroups studied. Moreover, during the euglycemic-hyperinsulinemic clamp, we found that for each dose of insulin tested, fasting plasma insulin in both Wistar and Sprague-Dawley rats increased acutely and achieved similar plateaus, whereas blood glucose was maintained close to the basal level in every subgroup. The coefficient of variation for blood glucose during hyperinsulinemia (60 to 120 minutes) varied from 7% to 10%. The mean glucose infusion rates required to maintain euglycemia during the last hour of the clamp ($GIR_{60\text{-}120}$), the conditions of which closely approximate a steady-state insulin concentration and represent whole-body glucose utilization, were similar in both strains at the high doses of insulin tested (32 and 64 $mU \cdot kg^{-1} \cdot min^{-1}$), while a significantly lower glucose infusion rate was found for Wistar rats versus Sprague-Dawley rats at the lowest dose of insulin (4 $mU \cdot kg^{-1} \cdot min^{-1}$) (Table 2).

Correlation Analysis

Table 3 shows the correlation analysis. Because whole-body insulin-mediated glucose uptake occurs principally in skeletal muscle and because insulin's hemodynamic action has been proposed as an important determinant of insulin action on glucose metabolism, it was of value to examine the relationship between the metabolic parameters ($GIR_{60\text{-}120}$) and the hemodynamic responses to insulin measured over all insulin infusions studied. Thus, in both strains of rats, we found that the change in mean arterial pressure (ΔMAP) was not correlated with the $GIR_{60\text{-}120}$. In contrast, the percent increment in hindquarter blood flow and vascular conductance in response to insulin was positively correlated with the $GIR_{60\text{-}120}$ in both Wistar and Sprague-Dawley rats.

Table 3. Correlation Analysis

	GIR_{60-120}			
	Wistar		Sprague-Dawley	
Parameter	*r*	*P*	*r*	*P*
ΔMAP	.081	.6783	.003	.9895
%ΔHDS	.502	.0049	.693	<.0001
%ΔHC	.461	.0111	.658	.0003

NOTE. Correlation analysis includes all data points over all insulin infusion rates studied in Wistar (n = 29) and Sprague-Dawley (n = 24) rats.

Abbreviations: GIR_{60-120}, glucose infusion rate required to maintain euglycemia during steady-state (60-120 min) plasma insulin; ΔMAP, maximum change from baseline in mean arterial pressure; %ΔHDS, percent change from baseline in hindquarter blood flow; %ΔHC, percent change from baseline in hindquarter vascular conductance.

Effect of Insulin on In Vivo 2-Deoxy-D-Glucose Uptake

Figure 3 shows that in both strains of rats, the euglycemic infusion of insulin at a rate of 4 $mU \cdot kg^{-1} \cdot min^{-1}$ elicited significant increases in glucose uptake in several individual muscles compared with the control infusion of vehicle (saline-BSA 0.2%). Thus, in both strains, we found significant increases in glucose uptake in soleus, quadriceps, gastrocnemius, and EDL muscles. The increase in EDL muscle was significantly greater in Sprague-Dawley rats than in Wistar rats. The correlation analysis shows that in both strains, the percent change in hindquarter blood flow in response to insulin was not related to the rate of in vivo glucose uptake in soleus (Wistar, $r = .245$, P = nonsignificant [NS]; Sprague-Dawley, $r = -.359$, P = NS), quadriceps (Wistar, $r = .085$, P = NS; Sprague-Dawley, $r = .287$, P = NS), gastrocnemius (Wistar, $r = .163$, P = NS; Sprague-Dawley, $r = .267$, P = NS), and EDL (Wistar, $r = .151$, P = NS; Sprague-Dawley, $r = .126$, P = NS).

Effect of Insulin on In Vitro 2-Deoxy-D-Glucose Uptake

The basal glucose transport activity measured in soleus muscle isolated from Wistar rats (1.41 ± 0.09 μmol/g/h) was not different from that found in Sprague-Dawley rats (1.35 ± 0.10 μmol/g/h). However, we found a significantly lower basal glucose transport activity in EDL muscles isolated from Wistar rats (1.51 ± 0.09 μmol/g/h) versus Sprague-Dawley rats (2.20 ± 0.15 μmol/g/h). Figure 4 shows the effect of increasing doses of insulin on glucose transport activity in isolated skeletal muscles (soleus and EDL) from Wistar and Sprague-Dawley rats, as measured with the glucose analog [^{3}H]-2-deoxy-D-glucose. Thus, at insulin doses of 0.02 to 2 mU/mL, we found significantly lower insulin-stimulated glucose transport activity in soleus muscles isolated from Wistar rats versus Sprague-Dawley rats. Similarly, in EDL muscles isolated from Wistar rats, we found significantly lower insulin-stimulated glucose transport activity versus the level in Sprague-Dawley rats. However, if we correct for the lower basal glucose transport activity observed in Wistar rat EDL muscles, the results indicate no differences in insulin-stimulated glucose transport activity between the strains.

DISCUSSION

The present study clearly demonstrates that euglycemic infusion of insulin has definite acute hemodynamic effects in conscious Wistar and Sprague-Dawley rats. In both strains, we found that insulin infusion at a rate of 4, 32, or 64 $mU \cdot kg^{-1} \cdot min^{-1}$ elicited similar cardiovascular changes characterized by vasodilation in renal and hindquarter vascular beds. A small but significant increase in blood pressure, probably secondary to the slight superior mesenteric vasoconstrictor effect of insulin, was also noted in either group at one of the two highest doses of insulin. The hindquarter hemodynamic responses reported here are consistent with previous findings indicating that insulin, over a range of physiologic and supraphysiologic concentrations, stimulates blood flow and exerts vasodilator effects in muscle.[2,8,15,21,35-37] Although we need to be careful in extrapolating our findings for the highest doses of insulin to the usual physiologic situation, the current data indicating a high correlation between whole-body glucose disposal and the increment in hindquarter blood flow in response to insulin support the contention that insulin's hemodynamic effect in muscle is an important physiologic determinant of its action on glucose metabolism.[2,15]

With regard to the renal hemodynamic responses to insulin

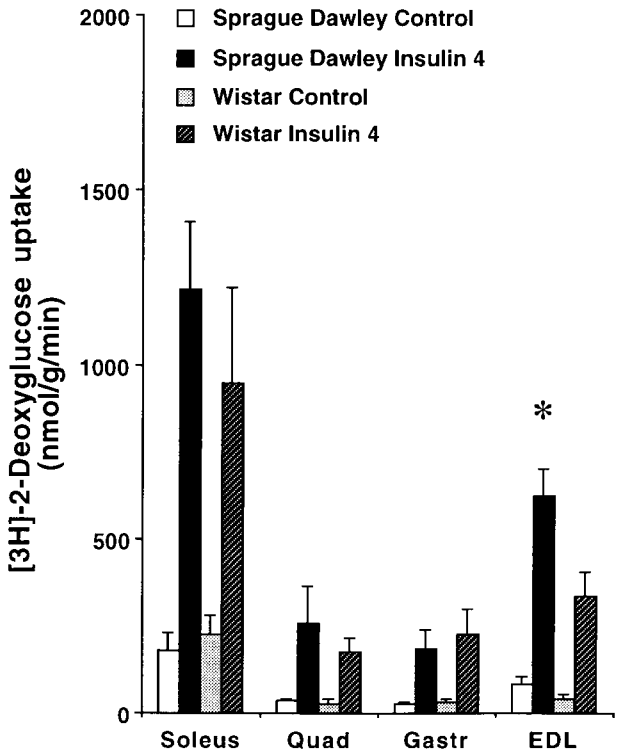

Fig 3. Bar graphs showing in vivo [^{3}H]-2-deoxy-D-glucose uptake in various skeletal muscles during a control intravenous (IV) infusion of saline-0.2% BSA (Wistar, n = 5; Sprague-Dawley, n = 6) or a euglycemic infusion of insulin at a rate of 4 $mU \cdot kg^{-1} \cdot min^{-1}$ in conscious rats (Wistar, n = 6; Sprague-Dawley, n = 5). Bars represent the mean ± SE shown by vertical lines. Comparisons were made for insulin-evoked responses in Wistar *v* Sprague-Dawley rats. *$P < .05$, Wistar *v* Sprague-Dawley, ANOVA followed by Fisher's test. Quad, quadriceps; Gastr, gastrocnemius.

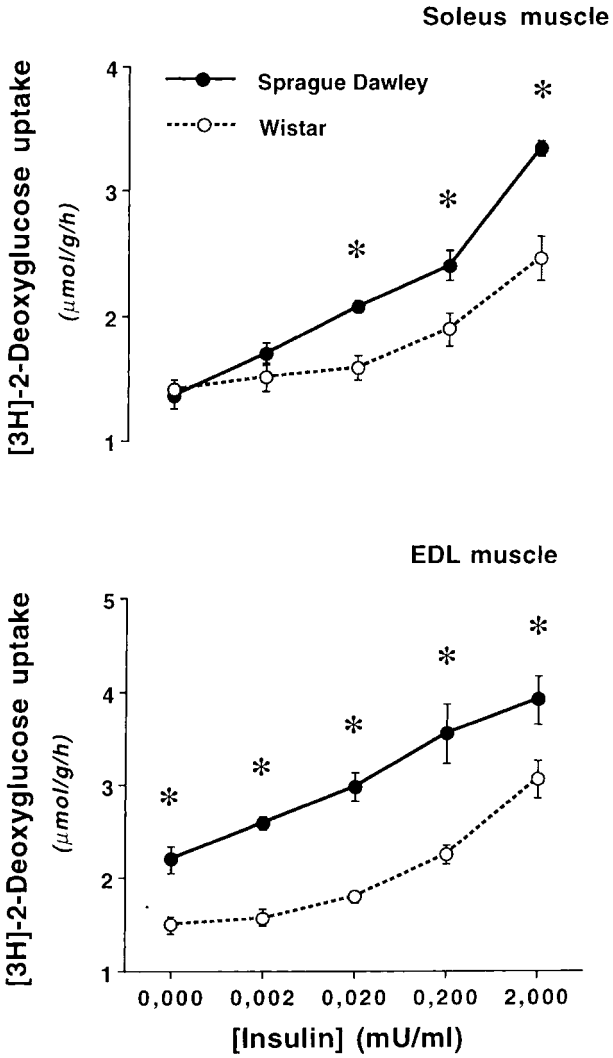

Fig 4. Insulin dose-response curve for stimulation of glucose uptake in soleus and EDL muscles. Muscles were from Wistar rats (n = 9) and Sprague-Dawley rats (n = 8). Values are the mean ± SE shown by vertical lines. Comparisons were made for insulin-evoked responses in Sprague-Dawley *v* Wistar rats. *P < .05, Sprague-Dawley *v* Wistar, ANOVA followed by Fisher's test.

in Wistar and Sprague-Dawley rats, it is interesting to note that similar findings were reported in WKY rats[22] and in dogs following euglycemic infusion of insulin.[38] Moreover, a previous study in isolated perfused rat kidney showed that insulin produced vasodilation and increased the glomerular filtration rate.[39] Although the significance of the insulin-mediated renal hemodynamic effect is unclear, it is tempting to propose that this effect represents an important mechanism of insulin's overall action to dispose of glucose and possibly also other substrates by the kidney.[40-42] Previous studies in overnight-fasted dogs[40] and normal postabsorptive humans[43] have shown that under euglycemic conditions, physiologic hyperinsulinemia can simultaneously suppress renal glucose production and stimulate glucose utilization, which results in net uptake of glucose by the kidney.

With regard to the metabolic measurements, differences were noted for the insulin sensitivity index at the lowest dose of insulin tested. The glucose infusion rate required to maintain euglycemia was lower (~31%) in Wistar versus Sprague-Dawley rats, although this difference was no longer observed at higher doses of insulin. The insulin sensitivity index in Wistar rats (25.3 ± 2.0 mg · kg^{-1} · min^{-1}) was similar to that previously reported by us in WKY rats (24.9 ± 0.7 mg · kg^{-1} · min^{-1}) for the 4-mU · kg^{-1} · min^{-1} dose of insulin.[22] No immediate explanation for the interstrain difference in whole-body insulin sensitivity is available. However, as the amount of glucose metabolized during a euglycemic clamp is the sum of infused plus endogenously produced (by the liver) glucose, we cannot exclude the possibility that differential suppression of hepatic glucose production during hyperinsulinemia might explain some of the species differences we found. In the present study, we did not measure hepatic glucose production, as we expected that hepatic gluconeogenesis was suppressed by the high circulating insulin concentrations achieved during the clamp.[44,45] This has been shown in another laboratory during hyperinsulinemia comparable to our study.[44] On the other hand, insulin-mediated glucose uptake occurs principally in skeletal muscle,[16,46] where it is determined mainly by the ability of insulin to increase the cellular permeability to glucose (by recruiting and activating glucose transport proteins to the plasma membrane) and muscle perfusion (which determines insulin and glucose delivery). Therefore, any change or defect in these two components could potentially influence the degree of insulin sensitivity. Sprague-Dawley rats are characterized by lower basal blood pressure, heart rate, and renal and hindquarter blood flow compared with Wistar rats (Table 1). Thus, the higher insulin sensitivity index in Sprague-Dawley rats may well result from the lower blood pressure, as an inverse relation has been reported between basal blood pressure and insulin sensitivity,[15] and from a lower blood flow velocity in skeletal muscle. This would contribute to increase insulin and glucose transit time through the capillary network, which would in turn produce an increase in the efficiency of glucose exchange. This is of course reliant on a similarity in the relative distribution of blood flow in muscles between the strains.

Alternatively, the difference in insulin sensitivity between the strains may result from a difference in insulin regulation of glucose extraction at the level of skeletal muscle, which accounts for most of the peripheral glucose use. It has been shown that whole-body insulin sensitivity is positively correlated with the percentage of body weight that is muscle.[47] Moreover, previous studies examining the effect of insulin on glucose metabolism in metabolically distinct muscles indicated a marked heterogeneity in insulin sensitivity and responsiveness among muscles of different fiber composition.[36,48] Indeed, an insulin-induced increase in total peripheral glucose disposal occurs predominantly in muscles containing a high proportion of oxidative fibers. Thus, it is likely that strain characteristics such as hindquarter composition and differences in fiber insulin sensitivity constitute a major factor in the variation of whole-body insulin sensitivity. This is consistent at least with the higher insulin-stimulated glucose transport and maximal responsiveness to insulin that we found in isolated soleus muscles

and the higher basal glucose transport we noted in isolated EDL muscles from Sprague-Dawley versus Wistar rats.

In addition, in a few experiments, we have combined the [^{3}H]-2-deoxy-D-glucose technique with the euglycemic clamp which permits the maintenance of insulin at a steady-state level during investigation of its effect on glucose uptake in muscles of different fiber composition. We found that insulin infused at a rate of 4 mU $\cdot$ kg^{-1} $\cdot$ min^{-1} produced a significant increase in glucose uptake in all muscles tested in both strains. Although we observed a greater rate of glucose uptake in EDL muscle from Sprague-Dawley versus Wistar rats, there was no significant difference in the maximal response to insulin between the strains in the other muscles tested. However, as demonstrated in a previous study using lower doses of insulin than we used,[36] it is likely that in the present study, the maximal rate of insulin-stimulated glucose uptake was achieved in all muscles tested. This may have contributed to blunt any interstrain variability in insulin sensitivity among muscles of different fiber composition.

Another point that needs to be discussed is the fact that at the lowest dose of insulin tested, we found no changes in total blood flow or vascular conductance in the hindquarter, while a substantial increase in whole-body glucose utilization was noted in both strains. Although porcine insulin is quite potent in the rat, we cannot exclude the possibility that different effects resulting from a potency difference between porcine and native insulin would have been obtained with rat insulin, and further study will be necessary to clarify this point. On the other hand, these results might indicate that at low dose, insulin stimulates glucose uptake mainly by increasing glucose extraction instead of increasing blood flow. The present study is limited to measurements of total blood flow into muscle and does not address whether changes in blood flow distribution occur within the muscle. Capillary recruitment without changes in total flow may occur and may explain the present results. Previous in vitro studies have clearly demonstrated that skeletal muscle metabolism can be significantly altered by changes in blood flow distribution into the muscle in the absence of altered total blood flow.[49] This is consistent with a biologic action of insulin to recruit muscle capillaries.[26]

In summary, euglycemic infusion of insulin in Sprague-Dawley and Wistar rats causes similar cardiovascular changes characterized by renal and hindquarter vasodilation. A slight increase in blood pressure, probably secondary to a mesenteric vasoconstrictor effect, was also observed at the high doses of insulin. These results provide further support for an insulin blood flow–regulatory effect. At the lowest dose of insulin tested, the insulin sensitivity index was smaller in Wistar versus Sprague-Dawley rats but similar to that previously found by us in WKY rats, suggesting that Sprague-Dawley rats may be the most sensitive to the glucose-lowering action of insulin, at least at low doses, since differences were no longer observed at higher doses. Although differences in basal blood pressure and blood flow velocity in insulin-sensitive tissues between Wistar and Sprague-Dawley rats may account for the variation in insulin sensitivity, this could also result from a difference in insulin regulation of glucose extraction at the level of skeletal muscle, as demonstrated in isolated EDL and soleus muscles. Thus, differences in muscle perfusion, hindquarter composition, and fiber insulin sensitivity may explain some of the species differences we report. Further study is needed to ascertain this last point.

ACKNOWLEDGMENT

The authors wish to thank Rachelle Duchesne for expert assistance in performing the plasma glucose and insulin determinations.

REFERENCES

1. Dinneen S, Gerich J, Rizza R: Carbohydrate metabolism in non–insulin-dependent diabetes mellitus. N Engl J Med 327:707-713, 1992

2. Laakso M, Edelman SV, Brechtel G, et al: Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. J Clin Invest 85:1844-1852, 1990

3. Laakso M, Edelman SV, Olefsky JM, et al: Kinetics of in vivo muscle insulin mediated glucose uptake in human obesity. Diabetes 39:965-974, 1990

4. Laakso M, Edelman S, Brechtel G, et al: Impaired insulin mediated skeletal muscle blood flow in patients with NIDDM. Diabetes 41:1076-1083, 1992

5. Laakso M, Edelman SV, Brechtel G, et al: Effects of epinephrine on insulin-mediated glucose uptake in whole body and leg muscle in humans: Role of blood flow. Am J Physiol 263:E199-E204, 1992

6. Baron AD, Steinberg H, Brechtel G, et al: Skeletal muscle blood flow independently modulates mediated glucose uptake. Am J Physiol 266:E248-E253, 1994

7. Capaldo B, Napoli R, Di Maino L, et al: Epinephrine directly antagonizes insulin-mediated activation of glucose uptake and inhibition of free fatty acid release in forearm tissues. Metabolism 41:1146-1149, 1992

8. Vollenweider P, Tappy L, Randin D, et al: Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. J Clin Invest 92:147-154, 1993

9. Scherrer U, Vollenweider P, Randin D, et al: Suppression of insulin induced sympathetic activation and vasodilation by dexamethasone in humans. Circulation 88:388-394, 1993

10. Randin D, Vollenweider P, Tappy L, et al: Effects of adrenergic and cholinergic blockade on insulin induced stimulation of calf blood flow in humans. Am J Physiol 266:R809-R816, 1994

11. Utriainen T, Malmström R, Mäkimattila S, et al: Methodological aspects, dose-response characteristics and causes of interindividual variation in insulin stimulation of limb blood flow in normal subjects. Diabetologia 38:555-564, 1995

12. Scherrer U, Randin D, Vollenweider P, et al: Nitric oxide release accounts for insulin's vascular effects in humans. J Clin Invest 94: 2511-2515, 1994

13. Nuutila P, Raitakari M, Laine H, et al: 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. J Clin Invest 97:1741-1747, 1996

14. Raitakari M, Nuutila P, Ruotsalainen U, et al: Evidence for a dissociation of insulin stimulation of blood flow and glucose uptake in human skeletal muscle: Studies using [^{15}O]H_2O, [^{18}F]fluoro-2-deoxy-D-glucose, and positron emission tomography. Diabetes 45:1471-1477, 1996

15. Baron AD, Brechtel-Hook G, Johnson A, et al: Skeletal muscle blood flow. A possible link between insulin resistance and blood pressure. Hypertension 21:129-135, 1993

16. DeFronzo RA, Jacot E, Jequier E, et al: The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry

and hepatic and femoral venous catheterization. Diabetes 30:1000-1007, 1981

17. Kelley DE, Reilly JP, Veneman T, et al: Effects of insulin on skeletal muscle glucose storage, oxidation, and glycolysis in humans. Am J Physiol 258:E923-E929, 1990

18. Capaldo B, Napoli R, Di Bonito P, et al: Dual mechanism of insulin action on human skeletal muscle: Identification of an indirect component not mediated by FFA. Am J Physiol 260:E389-E394, 1991

19. Buchanan TA, Thawani H, Kades W, et al: Angiotensin II increases glucose utilization during acute hyperinsulinemia via a hemodynamic mechanism. J Clin Invest 92:720-726, 1993

20. Natali A, Buzzigoli G, Taddei S, et al: Effects of insulin on hemodynamics and metabolism in human forearm. Diabetes 39:490-500, 1990

21. Anderson EA, Hoffman RP, Balon TW, et al: Hyperinsulinemia produces both sympathetic neural activation and vasodilatation in normal humans. J Clin Invest 87:2246-2252, 1991

22. Pitre M, Nadeau A, Bachelard H: Insulin sensitivity and hemodynamic responses to insulin in Wistar-Kyoto and spontaneously hypertensive rats. Am J Physiol 271:E658-E668, 1996

23. Qaadir E, Porter JP: Effect of insulin on regional vascular resistances in conscious rats. Am J Physiol 270:R450-R455, 1996

24. James DE, Burleigh KM, Storlein LH, et al: Heterogeneity of insulin action in muscle: Influence of blood flow. Am J Physiol 251: E422-E430, 1986

25. Youn JH, Kim JK, Steil GM: Assessment of extracellular glucose distribution and glucose transport activity in conscious rats. Am J Physiol 268:E712-E721, 1995

26. Rattigan S, Clark MG, Barrett EJ: Hemodynamic actions of insulin in rat skeletal muscle. Evidence for capillary recruitment. Diabetes 46:1381-1388, 1997

27. Gardiner SM, Bennett T: Regional haemodynamic responses to adrenoceptor antagonism in conscious rats. Am J Physiol 255:H813-H824, 1988

28. Gardiner SM, Compton AM, Bennett T, et al: Can pulsed Doppler technique measure changes in aortic blood flow in conscious rats? Am J Physiol 259:H448-H456, 1990

29. Kraegen EW, James DE, Jenkins AB, et al: Dose-response curves for in vivo insulin sensitivity in individual tissues in rats. Am J Physiol 248:E353-E362, 1985

30. Roy D, Perreault M, Marette A: Insulin stimulation of glucose uptake in skeletal muscles and adipose tissues in vivo is NO dependent. Am J Physiol 274:E692-E699, 1997

31. Kapur S, Bédard S, Marcotte B, et al: Expression of nitric oxide synthase in skeletal muscle: A novel role for nitric oxide as a modulator of insulin action. Diabetes 46:1691-1700, 1997

32. Hansen PA, Gulve EA, Holloszy JO: Suitability of 2-deoxyglucose for in vitro measurement of glucose transport activity in skeletal muscle. J Appl Physiol 76:979-985, 1994

33. Richterich R, Dauwalder H: Zur bertimmung der plasmaglucokonzentration mit der hexokinase-glucose-6-phosphat-dehydrogenase methode. Schweiz Med Wochenschr 101:615-618, 1971

34. Desbuquois B, Aurbach GD: Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J Clin Endocrinol Metab 37:732-738, 1971

35. Richter EA, Mikines KJ, Galbo H, et al: Effect of exercise on insulin action in human skeletal muscle. J Appl Physiol 66:876-885, 1989

36. James DE, Jenkins AB, Kraegen EW: Heterogeneity of insulin action in individual muscles in vivo: Euglycemic clamp studies in rats. Am J Physiol 248:E567-E574, 1985

37. Liang CS, Doherty JU, Faillace R, et al: Insulin infusion in conscious dogs: Effects on systemic and coronary hemodynamics, regional blood flows, and plasma catecholamines. J Clin Invest 69: 1321-1336, 1982

38. Hall JE, Brands MW, Kivlighn SD, et al: Chronic hyperinsulinemia and blood pressure: Interaction with catecholamines? Hypertension 15:519-527, 1990

39. Cohen AJ, McCarthy DM, Staff JS: Direct hemodynamic effect of insulin in the isolated perfused kidney. Am J Physiol 257:F580-F585, 1989

40. Cersosimo E, Judd R, Miles J: Insulin regulation of renal glucose metabolism in conscious dogs. J Clin Invest 93:2584-2589, 1994

41. Dzurik R, Krajci-Lazary B, Niederland T: Glucose metabolism in rat kidney: Influence of insulin and adrenaline. J Physiol (Lond) 168:782-786, 1963

42. Kleinzeller A, McDooy E: Glucose transport and metabolism in rat renal proximal tubules: Multicomponent effects of insulin. Biochim Biophys Acta 856:545-555, 1986

43. Cersosimo E, Garlick P, Ferretti J: Insulin regulation of renal glucose metabolism in humans. Am J Physiol 276:E78-E84, 1999

44. Buchanan TA, Sipos GF, Madrilejo N, et al: Hypertension without peripheral insulin resistance in spontaneously hypertensive rats. Am J Physiol 262:E14-E19, 1992

45. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 6:E214-E223, 1979

46. Baron AD, Brechtel G, Wallace P, et al: Rates and tissue sites of non–insulin and insulin mediated glucose uptake in humans. Am J Physiol 255:E769-E774, 1988

47. Yki-Järvinen H, Koivisto VA: Effect of body composition on insulin sensitivity. Diabetes 32:965-969, 1983

48. Hom FG, Goodner CJ: Insulin dose-response characteristics among individual muscle and adipose tissues measured in the rat in vivo with 2-[^{3}H]deoxyglucose. Diabetes 33:153-159, 1984

49. Clark MG, Colquhoun EQ, Rattigan S, et al: Vascular and endocrine control of muscle metabolism. Am J Physiol 268:E797-E812, 1995