Physiological Hyperinsulinemia in Dogs Augments Access of Macromolecules to Insulin-Sensitive Tissues

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Pharmacological doses of insulin increase limb blood flow and enhance tissue recruitment for small solutes such as glucose. We investigated whether elevating insulin within the physiological range (68 ± 6 vs. 425 ± 27 pmol/l) can influence tissue recruitment of [^{14}C]inulin, an inert diffusionary marker of molecular weight similar to that of insulin itself. During hyperinsulinemic-euglycemic clamps, transport parameters and distribution volumes of [^{14}C]inulin were determined in conscious dogs by applying a three-compartment model to the plasma clearance data of intravenously injected [^{14}C]inulin (0.8 μCi/kg). In a second set of experiments in anesthetized dogs with direct cannulation of the hindlimb skeletal muscle lymphatics, we measured a possible effect of physiological hyperinsulinemia on the response of the interstitial fluid of skeletal muscle to intravenously injected [^{14}C]inulin and compared this response with the model prediction from plasma data. Physiological hyperinsulinemia caused a 48 ± 10% (P < 0.005) and a 35 ± 15% (P < 0.05) increase of peripheral and splanchnic interstitial distribution volumes for [^{14}C]inulin. Hindlimb lymph measurements directly confirmed the ability of insulin to enhance the access of macromolecules to the peripheral interstitial fluid compartment. The present results show that physiological hyperinsulinemia will enhance the delivery of a substance of similar molecular size to insulin to previously less intensively perfused regions of insulin-sensitive tissues. Our data suggest that the delivery of insulin itself to insulin-sensitive tissues could be a mechanism of insulin action on cellular glucose uptake independent of and possibly synergistic with either enhanced blood flow distribution or GLUT4 transporter recruitment to enhance glucose utilization. Because of the differences between inulin and insulin itself, whether delivery of the bioactive hormone is increased remains speculative.
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In recent years, a growing body of work has demonstrated that insulin, besides its metabolic effects, can stimulate systemic blood flow to enhance the delivery of nutrients to peripheral tissues (1–6). Using the perfused rat hindlimb preparation, Clark et al. (7) identified two distinct groups of vasoconstrictors located in the muscle directing blood flow toward either nutritive or nonnutritive capillary networks. Clark et al. suggested that changes in microvascular blood flow distribution can occur within skeletal muscle absent changes in systemic blood flow. Supporting Clark's concept, Coggins et al. (8) found an increase of skeletal muscle microvascular (capillary) volume with physiological hyperinsulinemia despite unchanged total blood flow. Also, Vincent et al. (9) found that the observed increase of microvascular volume with physiological hyperinsulinemia precedes an increase in total blood flow by 60–90 min, suggesting differential regulation of blood flow distribution versus glucose utilization. Bonadonna et al. (10) reported an increase of the interstitial distribution volume of L-glucose at supraphysiological elevated insulin concentrations, favoring an effect of insulin on the recruitment of previously inaccessible interstitial volume and supporting the concept of insulin's microvascular actions in insulin-sensitive tissues.

The question arises whether the observed effect of insulin on the microvasculature is restricted to small solutes such as glucose or whether the distribution of larger molecules (the size of insulin itself) is affected. Before insulin can reach the target cells in skeletal muscle and adipose tissue to stimulate glucose disposal, it has to traverse the tight capillary endothelium between plasma and interstitial fluid that bathes cells (11,12), and it has been demonstrated that this transport mechanism is rate-limiting for the stimulation of glucose uptake (13,14). Therefore, it could be important if insulin would exploit its microvascular action not only to stimulate tissue recruitment for small metabolites but also to act as a stimulus for its own distribution to the cell. Both of these actions could enhance insulin's ability to stimulate glucose disposal in skeletal muscle.

In the present study, we examine whether elevating insulin in the physiological range has an effect to augment the vascular and extravascular distribution volume (tissue recruitment) of [^{14}C]inulin, an inert diffusionary marker of molecular weight very similar to that of insulin itself. We have investigated the effect of physiological insulin concentrations on the transport and distribution kinetics of

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[^{14}C]inulin in conscious and anesthetized dogs. In the conscious dog model, we investigated the effect of physiological hyperinsulinemia on the plasma clearance of intravenously injected [^{14}C]inulin and applied a three-compartment model to determine differences in transport parameters or distribution volumes. In a second set of experiments, we exploited the anesthetized dog model with direct cannulation of the hindlimb skeletal muscle lymphatics (15). We investigated the effect of physiological insulin concentrations on the response of intravenously injected [^{14}C]inulin both in plasma and in the interstitial fluid of skeletal muscle. Using this approach, we were able to measure directly an effect of physiological hyperinsulinemia on the response of the slow equilibrating peripheral distribution compartment and to compare this response with the model prediction from plasma data from the conscious dog model. These results support a potent effect of insulin per se to enhance the distribution volume of [^{14}C]inulin and presumably gain access to insulin-sensitive nutritive tissues.

RESEARCH DESIGN AND METHODS

Experiments were conducted on 11 male mongrel dogs (28.5 ± 2.0 kg; range, 18.7–39.3). The animals were housed under controlled kennel conditions (12-h light/dark cycle) in the University of Southern California Keck Medical School Vivarium. Animals were accepted into the study only when judged to be in good health as determined by visual observation, body temperature, and hematocrit. All experimental protocols were approved by the University Institutional Animal Care and Use Committee.

Diet. Dogs had free access to water and were given daily a can (415 g) of Hill's Prescription Diet (9% protein, 8% fat, 10% carbohydrates, and 73% moisture [Hill's Pet Nutrition, Topeka, KS]) and ~825 g dry chow (26.4% protein, 14.7% fat, 39.6% carbohydrates, 2.9% fiber, and 16.5% moisture [Wayne Dog Food; Alfred Mills, Chicago, IL]). Thus, the total diet consisted of ~3,900 calories: 27% from proteins, 35% from fat, 39% from carbohydrates. Food was withdrawn 18 h before experiments.

Chronic surgery. One week before experimentation, with the dogs under general anesthesia, chronic catheters were implanted as previously described (16). Indwelling silastic catheters were placed in the right carotid artery for sampling and also into the left jugular vein and into the left femoral vein for infusions as described in the experimental protocol section. On the morning of the experiment, an acute catheter was inserted into the saphenous vein for the variable infusion of glucose and for the injection of [^{14}C]inulin.

Protocol 1 (conscious study, randomized crossover approach with plasma access). A total of $n = 9$ dogs entered this protocol. With each animal, two euglycemic glucose clamps were performed on separate days with one of the two possible insulin infusion rates: 1.2 pmol · min^{-1} · kg^{-1} (replacement clamp) or 6.0 pmol · min^{-1} · kg^{-1} (hyperinsulinemic clamp). Thus, 18 conscious dog studies are reported herein. Insulin doses were chosen in randomized order, and experiments were separated by 1 week. Hyperinsulinemic clamp experiments started at 0700 (equals −270 min) with a primed infusion of [3-^{3}H]glucose (priming dose, 25 μCi; infusion, 0.25 μCi/min; DuPont-NEN, Boston, MA) into the femoral vein catheter to determine glucose turnover. After three basal samples from −150 to −120 min, a continuous infusion of somatostatin (1.0 μg · min^{-1} · kg^{-1}; Bachem, Torrance, CA) was begun to suppress endogenous insulin secretion, and porcine insulin was infused into the femoral vein and maintained from −120 to 180 min for a total period of 5 h. During this insulin infusion, glucose was measured in 10-min intervals during the first 60 min and in 15-min intervals thereafter, and plasma glucose was clamped to basal levels by variable infusion of 50% glucose labeled with [3-^{3}H]-D-glucose (2.7 μCi/g glucose). Also, at 0 min, an intravenous bolus of [^{14}C]inulin (0.8 μCi/kg body wt) was given into a saphenous vein, and rapid samples for the measurement of the kinetic profile of [^{14}C]inulin were collected at 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 17, 20, 25, 30, 35, 40, 45, 50, and 60 min and in 15-min intervals until the end of the experiment at 180 min.

Protocol 2 (anesthetized study, sequential approach with interstitial fluid access). A euglycemic-hyperinsulinemic clamp was performed under general anesthesia in a total of seven animals (five from protocol 1). First, surgery was performed at 0700. Dogs were preanesthetized with acepromazine maleate (Prom-Ace; Aueco, Fort Dodge, IA; 0.22 mg/kg) and atropine sulfate (Western Medical, Arcadia, CA; 0.11 ml/kg). Anesthesia was induced with sodium pentobarbital (Nembutal, Abbott Laboratories, Chicago, IL; 0.44 ml/kg) and was maintained with halothane and nitrous oxide. Indwelling catheters were implanted in the left carotid artery (sampling) and left or right jugular vein (saline drip). Left and right cephalic vein intracatheters were inserted for various infusions as detailed below. A perivascular ultrasonic flowprobe (2 mm diameter; Transonic, Ithaca, NY) was placed around the right femoral artery distal to the femoral catheter for measurement of blood flow. Left hindlimb muscle lymphatic fluid was sampled via a small polyethylene catheter (PE10–PE90, predominantly PE50) inserted into a deep lymph vessel as described previously (15). Blood pressure, heart rate, and respiratory CO_2 were monitored continuously. Dogs received a saline drip throughout both the surgery and the experiment to improve stability (~1 l was administered during the first 60 min of surgery and a slow drip thereafter). After experiments, animals were killed by an overdose of sodium pentobarbital (Eutha-6, Western Medical; 65 mg/kg).

The experimental protocol consisted of three temporal phases: a basal replacement phase (−300 to 0 min), an insulin activation phase (0–300 min), and an insulin deactivation phase (300–420 min). At −300 min, a primed (25 μCi) tracer infusion of HPLC-purified, [3-^{3}H]-D-glucose (0.25 μCi/min; DuPont-NEN) was started and maintained throughout the study to assess glucose turnover. At the same time (−300 min), a continuous infusion of somatostatin (1.0 μg · min^{-1} · kg^{-1}; Bachem) was started to suppress endogenous insulin release, and a basal replacement infusion of porcine insulin (1.2 pmol · min^{-1} · kg^{-1}; Novo Nordisk, Bagsvaerd, Denmark) was initiated. Both the somatostatin and the replacement insulin infusion were maintained throughout the study. From −300 min until the end of the experiment (420 min), arterial glucose was clamped at basal levels by exogenous infusion of 50% glucose labeled with [3-^{3}H]-D-glucose (2.7 μCi/g glucose) (17). We have previously shown that femoral blood flow, glucose infusion rate, and glucose turnover are constant for a 12-h glucose clamp, indicating that the anesthetized dog preparation is stable for the time of these experiments. After steady-state replacement insulin levels were established, at time −180 min, an intravenous bolus of [^{14}C]inulin (4 μCi/kg body wt; American Radiolabeled Chemicals, St. Louis, MO) was administered to quantify transport and distribution kinetics of [^{14}C]inulin in arterial plasma and hindlimb lymph for a period of 3 h. At time 0 min, a primed intravenous infusion of porcine insulin (6 pmol · min^{-1} · kg^{-1}; Novo Nordisk) was started and maintained for a period of 5 h. At time 120 min, after steady-state insulin concentrations were established, a second intravenous bolus of [^{14}C]inulin (4 μCi/kg body wt) was given. At time 300 min, or 5 h after the start of the primed insulin infusion, the insulin infusion was terminated and the clearance of insulin from plasma and hindlimb lymph was measured for 2 h or until 420 min, when the experimental protocol ended. Arterial sampling (~4 ml blood) was coupled with hindlimb lymph sampling (continuously from ~2 min before to ~2 min after arterial sample time, 400–900 μl of lymphatic fluid). For the first 60 min after insulin activation and deactivation and after the [^{14}C]inulin boluses, arterial blood samples were drawn according to the schedule as described for protocol 1; hindlimb lymph samples were taken in 5-min intervals during these periods. For the remainder of the experimental protocol, both arterial plasma and hindlimb lymph samples were taken in 15-min intervals.

Assays. Arterial blood samples for assay of glucose, insulin, [3-^{3}H]glucose, and [^{14}C]inulin as well as hindlimb lymph samples for assay of porcine insulin and [^{14}C]inulin were collected in microtubes that were precoated with lithium-heparin (Becton Dickinson, Franklin Lakes, NJ). Arterial sample tubes were additionally precoated with EDTA (Sigma Chemicals). Blood samples were centrifuged immediately, and the supernatant was transferred and after measurement of plasma glucose was stored at −20°C until further assay. Hindlimb lymph samples were stored at −20°C immediately after sampling. On-line plasma glucose was assayed using glucose oxidase on an automated analyzer (model 2700; Yellow Springs Instrument Co., Yellow Springs, OH). Porcine insulin was measured in plasma with an enzyme-linked immunospecific assay. The assay for the analysis of [3-^{3}H]glucose and [^{14}C]inulin has been described earlier (13). Glucose turnover data are not presented in this study. For the determination of [^{14}C]inulin in plasma and hindlimb lymph samples, 0.15 ml of sample was mixed with 1 ml of tissue solubilizer (NCS; Beckman, Fullerton, CA). After 30 min of incubation at room temperature, samples were counted in 10 ml of Ready Organic scintillation fluid (Beckman) on a dual-channel liquid scintillation counter (Beckman).

Pharmacokinetic data analysis: [^{14}C]inulin distribution model. Distribution and elimination of inulin was best described using a three-compartment model (Fig. 1), as originally proposed by Henthorn et al. (18) and recently applied by Steil et al. (19). Henthorn et al. characterized the distribution of inulin in extracellular space by a three-compartment model with a central compartment representing the plasma volume (V_1) and a slow (V_2) and a rapid (V_3) equilibrating compartment representing the interstitial fluid space. The

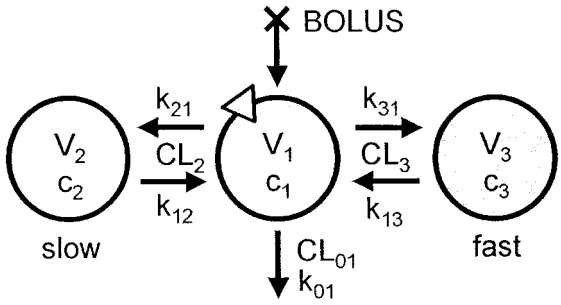

FIG. 1. Three-compartment model for the distribution and clearance of [^{14}C]inulin as proposed by Henthorn et al. (18).

faster equilibrating compartment is assumed to be supplied by capillaries in the splanchnic bed that are porous and lack a continuous investment of the basement membrane, whereas skeletal muscle is assumed to account for a major component of the slowly equilibrating interstitial fluid compartment. Parameter k_{01} represents the irreversible clearance of inulin from the plasma compartment by the kidney (18,20), and transport between plasma and slow (k_{21}, k_{12}) and rapid (k_{31}, k_{13}) equilibrating compartments was assumed to occur by passive diffusion ($k_{12} \times V_2 = k_{21} \times V_1 = CL_2$; $k_{13} \times V_3 = k_{31} \times V_1 = CL_3$) (18). [^{14}C]inulin was assumed not to be transported into an intracellular pool. Under these assumptions, the equations that describe the three-compartment model are

$$\frac{dc_1}{dt} = -(k_{01} + k_{21} + k_{31})c_1 + k_{21}c_2 + k_{31}c_3 \quad (1)$$

$$\frac{dc_2}{dt} = k_{21}\frac{V_1}{V_2}(c_1 - c_2) \quad (2)$$

$$\frac{dc_3}{dt} = k_{31}\frac{V_1}{V_3}(c_1 - c_3) \quad (3)$$

where V_i is volume and c_i is concentration of compartment I, and with the initial conditions:

$$c_1(0) = \frac{\text{BOLUS}}{V_1}, \quad (1)$$

$c_2(0) = 0$, and $c_3(0) = 0$; the six parameters V_1, V_2, V_3, k_{01}, k_{21}, and k_{31} are a priori identifiable. Parameters k_{12} and k_{13} can be derived from the diffusion assumptions. Distribution volumes were expressed as ml/kg body wt, and clearance and tissue-specific transport parameters were expressed as ml/min ($CL_{01} = k_{01} \times V_1$, $CL_2 = k_{21} \times V_1$, $CL_3 = k_{31} \times V_1$). Model parameters of protocol 1 (conscious study) and protocol 2 (anesthetized study) were analyzed using plasma data only. The hindlimb lymph data of the anesthetized study were used to confirm the model-predicted differences of the response of the slow equilibrating interstitial fluid compartment.

Numerical methods and data analysis. Data are reported as mean ± SE. Paired Student's t tests were used to calculate statistical significance within and between subsets of data. $P < 0.05$ was considered significant. Statistical data analysis was performed using Minitab (State College, PA) and Excel (Microsoft, Redmond, WA) software. Parameter identification for both models was obtained using a modified Gauss-Newton algorithm with inverse-variance weights. Accuracy of individual parameter estimates was evaluated as a fractional standard deviation. Model parameters were identified using the Windows version of the SAAM program (National Institutes of Health, Bethesda, MD) implemented on a personal computer.

RESULTS

Protocol 1 (conscious study). Plasma glucose concentrations were not different between low and high insulin infusion experiments (low insulin, 96.5 ± 1.3 mg/dl; high insulin, 99.3 ± 1.2 mg/dl; NS) and were constant during the 3 h after administration of the [^{14}C]inulin bolus (low insulin, cv = 5.8 ± 1.5%; high insulin, cv = 8.6 ± 0.6%). By definition, plasma insulin concentrations were physiologically elevated in the high insulin infusion experiments (low insulin, 68 ± 6 pmol/l; high insulin, 425 ± 27 pmol/l; $P < 0.001$) and were constant during the [^{14}C]inulin clearance period (low insulin, cv = 11.6 ± 1.5%; high insulin, cv = 8.5 ± 1.1; NS). The mean washout curves for [^{14}C]inulin at low and high insulin concentrations are shown in Fig. 2*A*. From the raw data, the washout curve seemed altered by elevated insulin. As previously determined (19), the Henthorn three-compartment model was able to account for the [^{14}C]inulin kinetics with no statistical residuals (data not shown). Modeling the data identified a significant enhancement of insulin distribution volumes V_2 and V_3 (Table 1). With an ~sixfold increase of the plasma insulin concentration, V_2 and V_3 increased 47.6 ± 10.0% and 35.2 ± 14.8%, respectively. In contrast to the peripheral volumes, the intravascular distribution volume V_1, the irreversible clearance of [^{14}C]inulin from the plasma CL_{01}, and both tissue-specific transport parameters CL_2 and CL_3 were not changed by hyperinsulinemia (Table 1, Fig. 2*C*). The reduced predicted peak [^{14}C]inulin levels in the remote distribution compartments under hyperinsulinemic conditions are consistent with an insulin effect to enhance tissue distribution of large molecules (Fig. 2*B*).

Protocol 2 (anesthetized study). Protocol 1 suggested that volume of the interstitial compartment is enhanced by hyperinsulinemia and that the concentration of the large molecule, inulin, would be reduced in peripheral interstitial compartments as a result of dilution in a larger pool. Because modeling is an indirect approach, we performed an additional series of studies that provided direct access to an interstitial compartment. In our second study, we applied the hindlimb lymph cannulation technique in the anesthetized animal to measure directly the effect of physiological insulin concentrations on the response of the slow equilibrating interstitial fluid compartment to intravenous injection of [^{14}C]inulin. Because of methodological limitations and the high variability between animals, a sequential rather than a randomized crossover design was used for protocol 2.

Plasma glucose was clamped throughout protocol 2 and was comparable during low and high insulin concentrations (low insulin, 99.5 ± 1.7 mg/dl; high insulin, 103.1 ±

TABLE 1
Effects of insulin on tissue recruitment

	Glucose (mg/dl)	Insulin (pmol/l)	V_1 (ml/kg)	V_2 (ml/kg)	V_3 (ml/kg)	CL_{01} (ml/min)	CL_2 (ml/min)	CL_3 (ml/min)
Low insulin	96.5 ± 1.3	67.7 ± 6.3	57.0 ± 4.2	101.5 ± 7.2	37.4 ± 2.1	84.1 ± 6.3	74.4 ± 10.3	268.0 ± 68.4
High insulin	99.3 ± 1.2	424.6 ± 26.6	60.7 ± 3.2	147.9 ± 12.5	49.0 ± 3.7	96.8 ± 7.2	70.5 ± 4.6	257.3 ± 35.7
P value	NS	<0.001	NS	<0.005	<0.05	NS	NS	NS

Data are mean ± SE. Results from protocol 1 (randomized crossover approach with plasma access, conscious study) during low- and high-insulin concentrations ($n = 9$).

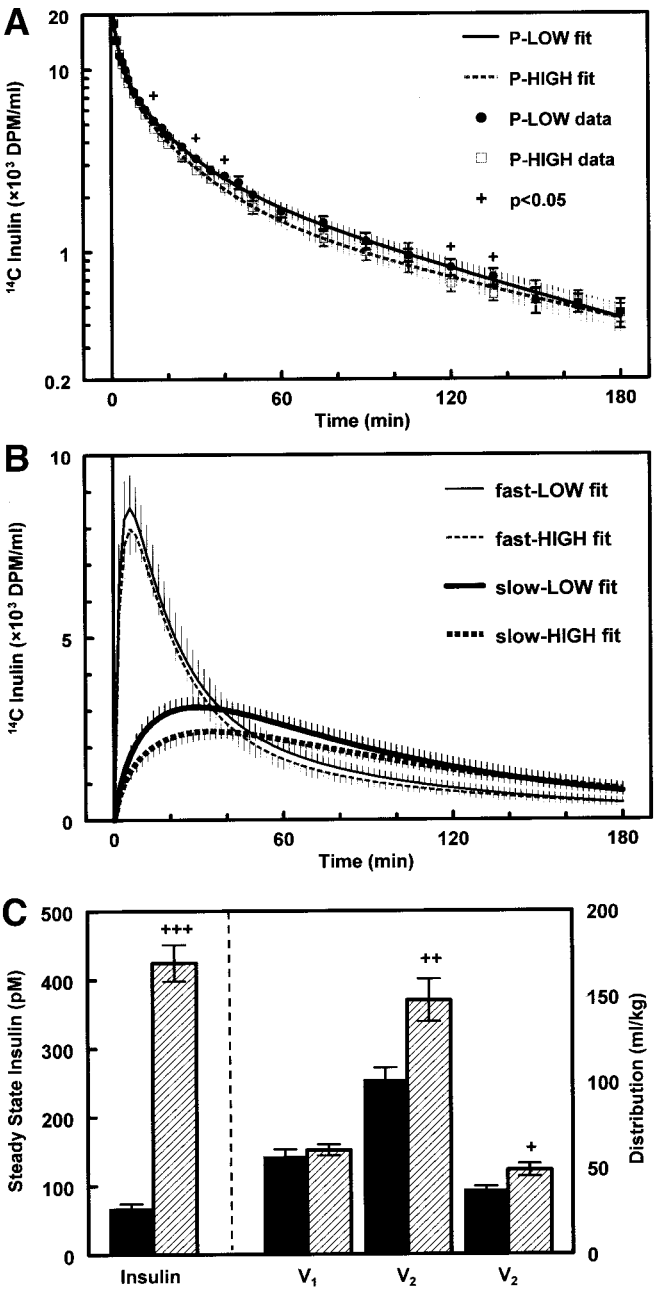

FIG. 2. Results from protocol 1. *A*: Plasma clearance of [^{14}C]inulin for low (●) and high (□) plasma insulin concentrations. Solid black line and dashed gray line represent plasma clearance for low and high plasma insulin concentrations, respectively, as suggested by compartmental modeling. *B*: Response of the fast (thin lines) and slow (bold lines) peripheral distribution compartments for low (black solid lines) and high (gray dashed lines) plasma insulin concentrations, as predicted by compartmental modeling of plasma [^{14}C]inulin data. *C*: Steady-state plasma insulin concentrations and model-predicted distribution volumes during low (■) and high (▨) insulin concentrations. All data are presented as mean ± SE. Vertical lines in *A* and *B* indicate 95% CI.

1.9 mg/dl; cv <5%; NS). Plasma insulin concentrations were stable during the [^{14}C]inulin washout periods (low insulin, cv = 12.6 ± 1.7%; high insulin, cv = 10.9 ± 1.7%) and were significantly different between low and high insulin infusion (low insulin, 106 ± 10 pmol/l; high insulin, 761 ± 83 pmol/l; $P < 0.05$; Fig. 3*A*). With high insulin,

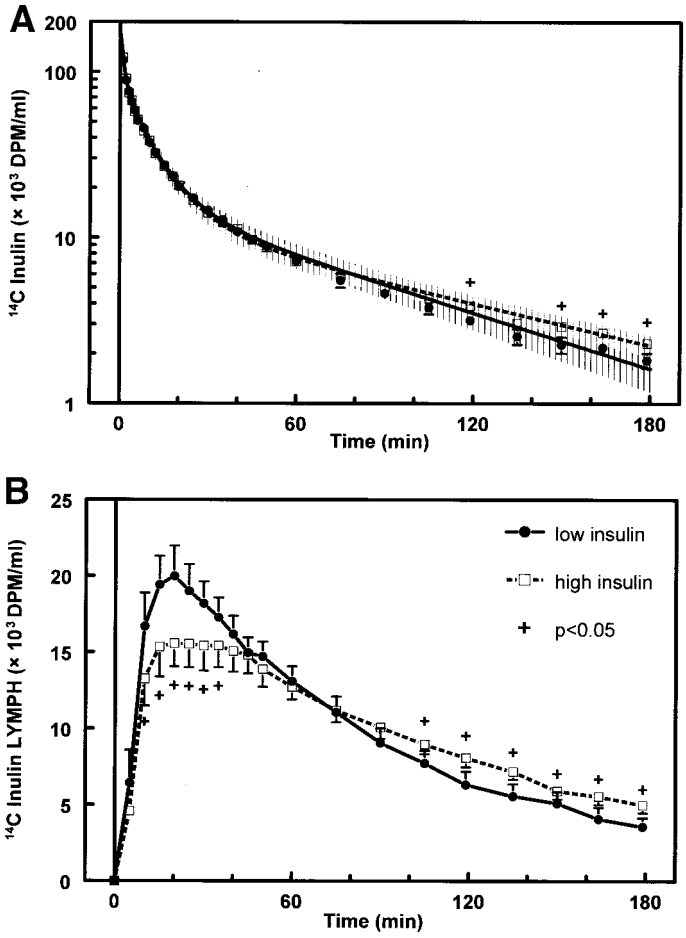

FIG. 3. Results from protocol 2. *A*: Plasma clearance of [^{14}C]inulin for low (●) and high (□) plasma insulin concentrations. Solid black line and dashed white line represent plasma clearance for low and high plasma insulin concentrations respectively, as suggested by compartmental modeling. *B*: Response of the interstitial fluid compartment of skeletal muscle, as represented by the hindlimb lymph compartment, for low (●) and high (□) plasma insulin concentrations. Data are presented as mean ± SE.

[^{14}C]inulin in interstitial fluid of skeletal muscle peaked at a lower level in response to the intravenous [^{14}C]inulin bolus (peak response: low insulin, 20.9 ± 1.8 × 10^3 dpm/ml; high insulin, 15.8 ± 1.5 × 10^3 dpm/ml; $P < 0.01$; Fig. 3*B*). To test whether applying the three-compartment model will also describe a comparable model-predicted response to our hindlimb lymph data, we applied the model to the individual plasma washout data only. Figure 4*A* compares the model-predicted response of the slow equilibrating interstitial fluid compartment at low and high plasma insulin concentrations together with the measured hindlimb lymph dynamics. Both independently accumulated data sets were strongly correlated (low insulin, r^2 = 0.90 ± 0.02; high insulin, r^2 = 0.88 ± 0.03), suggesting that hindlimb lymph measurements represent the slow equilibrating interstitial fluid compartment and that physiological insulin concentrations indeed have a significant effect on the peripheral distribution volume of [^{14}C]inulin. The model parameters are summarized in Table 2 and Fig. 4*B*. Similar to protocol 1, with physiological elevated plasma insulin concentrations, all transport and clearance parameters and the intravascular distribution compartment remained unchanged, whereas a significant increase of the

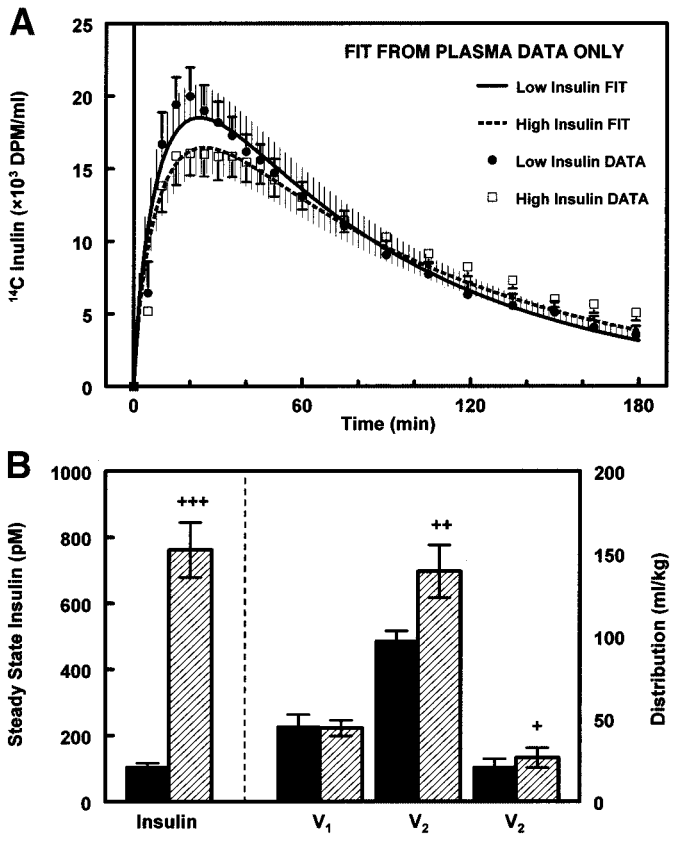

FIG. 4. Results from protocol 2. *A*: Response of the interstitial fluid compartment of skeletal muscle, as represented by the hindlimb lymph compartment, for low (●) and high (□) plasma insulin concentrations. Black solid line and gray dashed line represent the response of the slow equilibrating distribution compartment to the intravenous bolus of [^{14}C]inulin for low and high plasma insulin concentrations, respectively, as predicted from plasma inulin data only. Vertical lines of the model fit represent 95% CI. *B*: Steady-state plasma insulin concentrations and model-predicted distribution volumes during low (■) and high (▨) insulin infusion. Data are presented as mean ± SE.

slow equilibrating distribution compartment of [^{14}C]inulin was suggested. With elevated plasma insulin concentrations (Table 2), the fast equilibrating distribution compartment showed only a tendency to be increased. This finding might be explained by the different study approaches (protocol 1 was performed in conscious dogs [$n = 9$], whereas in protocol 2, dogs were investigated during anesthetized conditions [$n = 6$]). Mean arterial pressure was unchanged between low and high insulin concentrations (low insulin, 78.4 ± 6.3 mmHg; high insulin, 79.4 ± 4.6 mmHg; NS), whereas the systemic femoral artery blood flow indicated a substantial increase of 30.1 ± 7.7% (low insulin, 152.9 ± 30.6 ml/min; high insulin, 189.5 ± 26.3 ml/min; $P < 0.05$) with insulin stimulation.

DISCUSSION

In the present study, we examined the whole-body transport and distribution kinetics of an extracellular marker of similar size to insulin. Our results indicate that at physiological concentrations, insulin stimulates specifically the extravascular distribution of [^{14}C]inulin. In contrast, we obtained no evidence for insulin effects on vascular distribution volume or fractional clearance from the plasma compartment. It has been understood that insulin can enhance the delivery rate of small metabolites. The present results show that insulin can also enhance the delivery of substances of similar size to insulin to previously less intensively perfused regions of insulin-sensitive tissues. Provided that these tissues are insulin-sensitive, the delivery of insulin itself would be an added mechanism beyond transporter recruitment to enhance glucose utilization and other insulin effects.

The results of the present study are based on two approaches. One was a study in conscious dogs that allowed us to compare directly the effect of insulin on the plasma clearance of [^{14}C]inulin using a randomized crossover design. The second approach was done in anesthetized dogs and allowed us access to hindlimb lymph. Despite the differences in protocol, the outcomes of the two studies regarding the ability of insulin to stimulate tissue recruitment of [^{14}C]inulin were remarkably similar: an increase in extravascular distribution volume of [^{14}C]inulin. We observed an ~31% increase in the fast equilibrating compartment in conscious dogs, whereas the increase of ~25% in the anesthetized dogs did not quite reach statistical significance despite the higher insulin concentration. Despite this minor difference in the size of the effect of insulin on the extravascular distribution compartments between conscious and anesthetized animals, overall the qualitative effect was remarkably similar: increased access to extravascular distribution compartments for large molecules the size of insulin itself.

In the present study, [^{14}C]inulin was used as an extracellular marker to examine the effects of different plasma insulin concentrations on the distribution compartment of large-size molecules. Inulin not only possesses a molecular weight similar to insulin but also has a molecular size that is comparable to the size of the insulin monomer (Stokes Einstein radius: 14 vs. 21 Å, respectively [lsqb[21,22). Thus, it is attractive to speculate that the observed alteration in the distribution volume of [^{14}C]inulin will reflect changes in the distribution volume of similar macromolecules of molecular weight in the range of 5,000 Da. However, molecules that are transported out of the endothelium by mechanisms other than simple diffusion, possibly as a result of binding to specific receptors or binding to albu-

TABLE 2
Effects of insulin on tissue recruitment

	Glucose (mg/dl)	Insulin (pmol/l)	V_1 (ml/kg)	V_2 (ml/kg)	V_3 (ml/kg)	CL_{01} (ml/min)	CL_2 (ml/min)	CL_3 (ml/min)
Low insulin	99.5 ± 1.7	106.4 ± 9.7	45.7 ± 7.0	97.7 ± 5.5	21.2 ± 4.6	91.4 ± 9.4	78.0 ± 8.0	210.6 ± 66.1
High insulin	103.1 ± 1.9	761.4 ± 83.2	44.4 ± 4.8	139.3 ± 16.0	26.5 ± 6.0	87.3 ± 9.5	80.4 ± 10.4	218.6 ± 61.0
P value	NS	<0.001	NS	<0.05	NS	NS	NS	NS

Data are mean ± SE. Results from protocol 2 (sequential approach with interstitial fluid access, anesthetized study) during low and high insulin concentrations ($n = 6$).

min, may not be affected similar to inulin, but the present results give credence to the concept that the hemodynamic effects of insulin may play an import role in its action on various metabolic processes.

We were fortunate in that the kinetics of [^{14}C]inulin distribution have been defined carefully by previous investigators. That is, we were able to use the three-compartment distribution model provided by Henthorn et al. (18). Henthorn's model includes a central compartment that corresponds to the intravascular space and a rapid and a slowly equilibrating peripheral distribution compartment. Sedek et al. (20) reported an ~50% reduction of the rapid equilibrating compartment in the dog but no significant reduction of the slow equilibrating compartment. Sedek's results suggest that the rapidly equilibrating extravascular fluid space is supplied by porous splanchnic capillaries, whereas the slowly equilibrating space includes skeletal muscle, which is supplied by less porous capillaries with a continuous basement membrane (23) that show a more restricted permeability and therefore only slow equilibration for inulin.

Our studies support the conclusion (20) that the slowly equilibrating compartment is skeletal muscle. We report (Fig. 4*A*) a strong and highly significant correlation between the [^{14}C]inulin response of the slowly equilibrating compartment from the three-compartment model using plasma data only and the [^{14}C]inulin response directly measured in the hindlimb lymph compartment. As hindlimb lymph fluid to a large extent emanates from skeletal muscle (15,24), these results strongly support the hypothesis that the interstitial fluid compartment of skeletal muscle is best described by the slow equilibrating compartment of [^{14}C]inulin. Thus, any insulin-dependent increase in this compartment may be interpreted as increased access by interstitial fluid of muscle of macromolecules. It remains to be proved that this access can also apply to bioactive molecules such as polypeptide hormones.

Insulin may increase muscle access by increases in total blood flow (vasoactive effects) versus tissue recruitment (i.e., distribution of blood flow). Extensive studies by Baron (5) indicated that insulin is a vasoactive peptide, but other reports suggested no vasoactive activity of insulin (25) or effects only at supraphysiological insulin concentrations or after long exposure (26). In the present study, using a ultrasonic flowprobe (27), we saw a significant increase (+30%) of the femoral artery blood flow with insulin stimulation. However, in our hands, this increase is ephemeral. Steil et al. (28) reported no increase of the femoral artery blood flow, and Hamilton-Wessler et al. (29) reported only a modest increase with insulin stimulation. Taken together, our results indicate that changes in blood flow per se may not explain insulin's ability to access receptors under hyperinsulinemic conditions.

That insulin's effects are more due to blood distribution was supported by measurements of macrovascular capillary volume made by Barrett and his collaborators using contrast enhanced ultrasound. Coggins et al. (8) reported that insulin can stimulate microvascular volume without increasing systemic blood flow, supporting the concept of the existence of nutritive and nonnutritive flow routes within skeletal muscle tissue as proposed by Clark et al. (6). This effect might also explain our observation of an increased extravascular distribution compartment of [^{14}C]inulin with systemic hyperinsulinemia despite an unchanged vascular distribution compartment.

Regarding the mechanism underlying the observed increase of the peripheral distribution volume of [^{14}C]inulin, blood flow in the microcirculatory system is regulated by vasomotive mechanisms, i.e., rhythmic opening and closing of precapillary sphincters. Studies of rhythmic diameter changes along the arteriolar network have indicated a complex superposition of waveforms between 0.3 and 15 cycles per minute (30). Insulin effect on vasomotion in insulin-sensitive tissues has been reported recently (31,32). Using the present approach, the clearance kinetics of [^{14}C]inulin are measured over a period of 180 min, giving an integrated picture of this rhythmic regulation of the microcirculation. We speculate that the intensity of vasomotion (frequency and amplitude) might contribute to the regulation of tissue perfusion.

In summary, the results reported in the present study suggest that physiological hyperinsulinemia increases limb blood flow and recruits additional extravascular space to the splanchnic and peripheral distribution of [^{14}C]inulin in the dog. However, it remains unknown whether insulin can increase access of insulin-sensitive tissues to the bioactive hormone. We have previously concluded that insulin leaves the capillary via passive diffusion, rather than being transported via receptor binding (28,29), Also, insulin is a charged molecule; therefore, its transport properties may be very different from inulin. Thus, it remains speculative whether insulin can enhance its own access to insulin receptors on skeletal muscle as a result of changes in distribution volume. Nonetheless, we believe that the present data add to the potential for insulin activation of glucose uptake and other metabolic processes via hemodynamic as opposed to purely biochemical processes. A possible role of this putative mechanism on the metabolic actions of insulin or whether this mechanism is altered during insulin-resistant states remains to be investigated.

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