Direct Evidence for Insulin-Induced Capillary Recruitment in Skin of Healthy Subjects During Physiological Hyperinsulinemia

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It has been proposed that insulin-mediated changes in muscle perfusion modulate insulin-mediated glucose uptake. However, the putative effects of insulin on the microcirculation that permit such modulation have not been studied in humans. We examined the effects of systemic hyperinsulinemia on skin microvascular function in eight healthy nondiabetic subjects. In addition, the effects of locally administered insulin on skin blood flow were assessed in 10 healthy subjects. During a hyperinsulinemic clamp, we measured leg blood flow with venous occlusion plethysmography, skin capillary density with capillaroscopy, endothelium-(in)dependent vasodilatation of skin microcirculation with iontophoresis of acetylcholine and sodium nitroprusside combined with laser Doppler fluxmetry, and skin vasomotion by Fourier analysis of microcirculatory blood flow. To exclude nonspecific changes in the hemodynamic variables, a time-volume control study was performed. Insulin iontophoresis was used to study the local effects of insulin on skin blood flow. Compared to the control study, systemic hyperinsulinemia caused an increase in leg blood flow (−0.54 ± 0.93 vs. 1.97 ± 1.1 ml · min^{-1} · dl^{-1}; $P < 0.01$), an increase in the number of perfused capillaries in the resting state (−3.7 ± 3.0 vs. 3.4 ± 1.4 per mm^2; $P < 0.001$) and during postocclusive reactive hyperemia (−0.8 ± 2.2 vs. 5.1 ± 3.7 per mm^2; $P < 0.001$), an augmentation of the vasodilatation caused by acetylcholine (722 ± 206 vs. 989 ± 495%; $P < 0.05$) and sodium nitroprusside (618 ± 159 vs. 788 ± 276%; $P < 0.05$), and a change in vasomotion by increasing the relative contribution of the 0.01- to 0.02-Hz and 0.4- to 1.6-Hz spectral components ($P < 0.05$). Compared to the control substance, locally administered insulin caused a rapid increase (~13.5 min) in skin microcirculatory blood flow (34.4 ± 42.5 vs. 82.8 ± 85.7%; $P < 0.05$). In conclusion, systemic hyperinsulinemia in skin *1*) induces recruitment of capillaries, *2*) augments nitric oxide–mediated vasodilatation, and *3*) influences vasomotion. In addition, locally administered insulin *4*) induces a rapid increase in total skin blood flow, independent of systemic effects. *Diabetes* 51: 1515–1522, 2002

Insulin increases skeletal muscle blood flow in a time- and concentration-dependent fashion (1,2). Recently, it has been hypothesized that insulin's metabolic and vasodilatory actions are functionally coupled (2,3) with an important role for microcirculatory function (2,4–7). Specifically, it has been proposed that insulin, by reducing precapillary arteriolar tone and/or altering arteriolar vasomotion, redirects blood flow from nonnutritive vessels to nutritive capillary beds, with a resultant increase in the overall number of perfused capillaries, a process termed "functional capillary recruitment" (2,4). This increases the distribution volume of nutrients and enhances the access of insulin and glucose to a greater mass of muscle cells for metabolism (2,4,8).

Results from both experimental (5–7) and human (4,8) studies have suggested that insulin-mediated changes in muscle perfusion can modulate insulin-mediated glucose uptake. However, studies in which glucose uptake has been measured during hyperinsulinemia and manipulation of total limb blood flow have shown conflicting results (1,9). These differences have been ascribed to the fact that various vasoactive agents may change total flow, but may also have distinct effects on the microcirculation and distribution of blood flow in nutritive compared to nonnutritive vessels (1,6,7,9). Therefore, a better understanding of the putative effects of insulin on the microcirculation that permit modulation of glucose uptake is necessary.

At present, there is some evidence consistent with insulin-induced capillary recruitment (4,6–8). In the isolated-perfused rat hindlimb, insulin increased the metabolism of 1-methylxanthine, an indirect indicator of capillary recruitment (6,7). In humans, an increase in the distribution volume of glucose has been demonstrated in parallel with an increase in blood flow during supraphysiological hyperinsulinemia, suggesting capillary recruitment (8). However, these studies have provided only indirect evidence of insulin-mediated capillary recruitment.

Assessment of skin microcirculation with capillary microscopy enables direct visualization of capillary density and capillary recruitment. By using this technique, we recently demonstrated that capillary recruitment during postocclusive reactive hyperemia is associated with insu-

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CV, coefficient of variation; DBP, diastolic blood pressure; M, whole-body glucose uptake; MAP, mean arterial pressure; M/I, whole-body glucose uptake per unit of plasma insulin concentration; PRH, postocclusive reactive hyperemia; PU, arbitrary perfusion units.

TABLE 1
Characteristics of the healthy volunteers

	Study 1	Study 2
n (male/female)	8 (6/2)	10 (10/0)
Age (years)	23 ± 3.6	25 ± 3.3
Waist-to-hip ratio	0.90 ± 0.10	0.89 ± 0.04
BMI (kg/m^2)	22.5 ± 2.5	23.1 ± 3.1
Systolic blood pressure (mmHg)	123 ± 9	114 ± 10
Diastolic blood pressure (mmHg)	71 ± 9	78 ± 9
M/I value (mg · kg^{-1} · min^{-1} per pmol/l) × 100	2.2 ± 0.6	—
Fasting plasma glucose (mmol/l)	4.9 ± 0.3	4.8 ± 0.5
Fasting serum total cholesterol (mmol/l)	4.5 ± 0.7	4.8 ± 0.9
Fasting HDL cholesterol (mmol/l)	1.5 ± 0.3	1.4 ± 0.3
Fasting serum triglycerides (mmol/l)	1.0 ± 0.4	1.0 ± 0.3

Data are n or means ± SD. M/I value, glucose infusion rate during a hyperinsulinemic clamp, expressed per unit of plasma insulin concentration.

lin's metabolic and vascular actions in both normal and hypertensive subjects (10,11). Human skin also allows the continuous assessment of changes in the frequency of vasomotion (12).

The current study examined the effects of systemic physiological hyperinsulinemia on skin capillary density and capillary recruitment during postocclusive reactive hyperemia as assessed by capillary microscopy. The effects of insulin on vasomotion and endothelium-(in)dependent vasodilatation of skin microcirculation were measured with laser Doppler fluxmetry. In addition, iontophoresis of insulin was used to investigate the local effects on total skin blood flow independent of its systemic actions.

RESEARCH DESIGN AND METHODS

Subjects. Characteristics of the study subjects in each study are given in Table 1. All subjects were determined to be healthy based on their medical history; nondiabetic, according to American Diabetes Association criteria (13), and normotensive, as determined by triplicate office blood pressure measurements. They did not use medication and all were nonsmokers. None had a first-degree relative with type 2 diabetes. The study protocol was approved by the local Ethics Committee and conformed with the principles outlined in the Declaration of Helsinki.

Study 1.

Study design. All subjects underwent the experimental protocol as shown in Fig. 1. The measurements were conducted in a quiet, temperature-controlled room (T = 23.4 ± 0.4°C) at 8:00 A.M., after a 12-h fast, with the subjects in the supine position and after subjects had emptied their bladder. The subjects had abstained from caffeine- and alcohol-containing drinks overnight. All microcirculatory measurements were performed with the investigated, nondominant hand at heart level. Baseline measurements were obtained after allowing 30 min of rest and acclimatization after the insertion of two polytetrafluoroethylene catheters (Venflon; Viggo, Gothenborg, Sweden): one in the right antecubital vein and one in a vein of the opposite forearm. To exclude nonspecific changes in leg blood flow and microcirculatory function, a time- and volume-control study was performed 1 week after the clamp procedure in an identical fashion with the infusion of the same amounts of fluid (0.65% saline) and with blood sampling at the same time intervals, but without insulin or glucose infusion. The control experiments and hyperinsulinemic clamp experiments were not randomized, because the amount of fluid to be infused during the control study depended on the volume of glucose infused during the hyperinsulinemic clamp study.

Whole-body glucose uptake. Sensitivity to insulin-mediated glucose uptake was assessed by a hyperinsulinemic clamp technique, as previously described (10). Whole-body glucose uptake (M) was expressed per unit of plasma insulin concentration (M/I), thereby correcting for differences in steady-state plasma insulin levels (14). For convenience, the M/I ratio was multiplied by 100.

Leg blood flow. At t = −60 and 120 min, leg blood flow was measured by mercury-in-silastic strain gauge venous occlusion plethysmography (EC6; Hokanson, Bellevue, WA) with the subjects in the supine position (11,15). An occlusive cuff was placed proximally around the right leg, and the strain gauge was placed around the calf at the largest circumference. A pediatric cuff inflated to suprasystolic pressure was placed at the ankle to exclude foot circulation. Hokanson-dedicated software was used (NIVP3) to automatically balance the plethysmograph, inflate the cuff to 50 mmHg using a E20 Rapid Cuff Inflator and an AG101 Air Source, capture the inflow waveform, and calculate blood flow. Nine separate recordings of leg blood flow were made once every 15 s. Flow measurements were expressed in terms of milliliter flow per deciliter, and represent the average of seven to nine separate recordings. In our hands, this technique has a coefficient of variation (CV) of 10–14% (present study, 15%). We noninvasively determined systolic blood pressure, diastolic blood pressure, mean arterial pressure, and heart rate (Colin Press-Mate BP-8800; Colin, Komaki City, Japan) with every leg blood flow

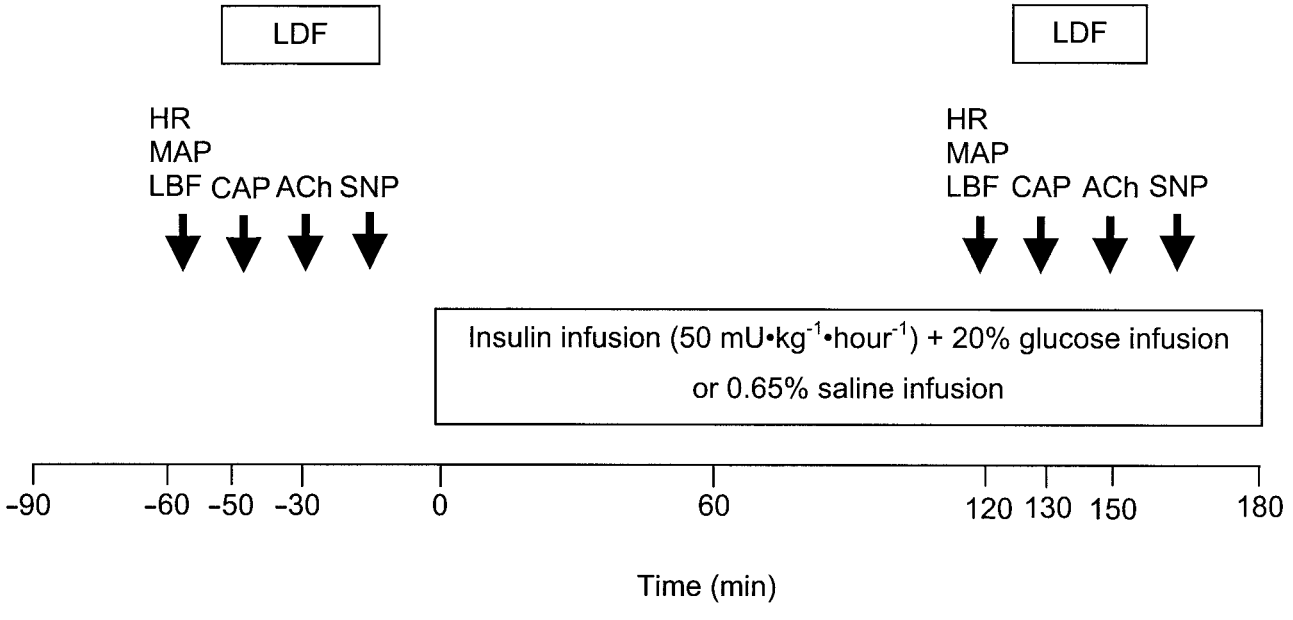

FIG. 1. Design of the study. Measurements started after 30 min of acclimatization. ACh, iontophoresis of acetylcholine; CAP, intravital capillaroscopy; HR, heart rate; LBF, leg blood flow assessed with venous occlusion plethysmography; LDF, laser Doppler flow measurement used to assess vasomotion; SNP, iontophoresis of sodium nitroprusside. A time- and volume-control study was performed 1 week after the clamp procedure in an identical fashion, but without insulin and glucose infusion.

determination. The average of four consecutive blood pressure and heart rate readings during each period was used for further analysis.

Capillary microscopy. The capillaroscopy studies were conducted at $t = -50$ and 130 min. Skin temperature was monitored. Nailfold capillaries in the dorsal skin of the third finger were visualized by an epi-illuminated microscope (10,11,16). Capillaries were visualized ~1.5 mm proximal to the terminal row of capillaries in the middle of the nailfold of the third finger. This distance was the width of one visual field of the microscope. At this spot, capillaries appear mostly as dots with only a small part of the arteriolar and venular limb being visible. Subsequently, a characteristic capillary (i.e., a capillary that was constantly perfused and had an eye-catching morphological feature) was kept on the same spot exactly in the center of the visual field (marked by a dot on the monitor) to ensure that capillary density was measured in the exact same visual field during the entire experiment and the control studies. Two separate visual fields of 1 mm^2 were recorded before and after 4 min of arterial occlusion, and the images were stored on videotape. The number of capillaries was counted off-line by two experienced investigators (E.H.S. and G.D.V.) from a freeze-framed reproduction of the videotape and from the running videotape when it was uncertain whether a capillary was present or not. The investigators counting the capillaries were unaware of whether the videotapes were from the hyperinsulinemic clamp or control study. Capillary density was defined as the number of erythrocyte-perfused capillaries per square millimeter of nailfold skin. During direct intravital microscopy without dyes, some capillaries seemed to continuously fill with erythrocytes, whereas others were intermittently perfused. Capillary density in the resting state was counted during a 3-min period, counting all, continuously and intermittently, perfused capillaries. Postocclusive reactive hyperemia (PRH) after 4 min of arterial occlusion with a digital cuff (Hokanson) was used to assess functional recruitment of capillaries (10,11,16). In this case, the number of capillaries in the resting state was counted during a 15-s period, counting only continuously perfused capillaries, as previously described (16). Directly after release of the cuff, the number of perfused capillaries was counted. (The increase in capillary number occurs within a few seconds.) The absolute capillary recruitment during PRH was assessed by subtracting the capillary density in the resting state from the peak capillary density during PRH. The procedure was then repeated using a visual field adjacent to the first visual field. Data concerning capillary densities are the mean of two measurements. Intrasubject CVs of the capillary density in the resting state (15-s vs. 3-min count) and the absolute capillary recruitment during PRH were 2.3 ± 1.8 vs. 5.7 ± 4.3 and 6.2 ± 4.3%, respectively (present study; measured on two separate days in eight subjects; data not shown).

Endothelium-(in)dependent vasodilatation of skin microcirculation. This was evaluated at $t = -30$ and 150 min by iontophoresis of acetylcholine and sodium nitroprusside in combination with laser Doppler fluxmetry, as previously described in more detail (10,11). A protocol of multiple fixed doses (current intensity × delivery time) was used, resulting in an incremental dose-response curve (10,11). During the iontophoresis procedure, a thermostatic laser Doppler probe was heated to 30°C to prevent a rapid decrease in skin temperature. Acetylcholine (1%; Miochol; Bournonville Pharma, the Hague, the Netherlands) was delivered using an anodal current; seven doses (0.1 mA for 20 s) were delivered, with a 60-s interval between each dose. Sodium nitroprusside (0.1%; Nipride, Roche, Mijdrecht, the Netherlands) was delivered using a cathodal current; nine doses (0.2 mA for 20 s) were delivered, with a 90-s interval between each dose. Acetylcholine- and nitroprusside-dependent laser Doppler flux were measured on dorsal skin of the middle phalanx of the third and fourth fingers, respectively. Dorsal skin was chosen because we were interested in the nutritive function of skin microcirculation as opposed to its thermoregulatory function. Dorsal skin is considered devoid of arteriovenous anastomoses, which serve the thermoregulatory function (17). During hyperinsulinemia and the control study, the same fingers were used as during the first baseline measurement. The increase from baseline to the final 2 and 3 min of the plateau phase were used for further analyses of the blood flow responses to acetylcholine and sodium nitroprusside, respectively. To exclude possible nonspecific microcirculatory reactivity, responses to the vehicles for both acetylcholine (mannitol 3%) and sodium nitroprusside (water for injection) were tested before and during hyperinsulinemia using identical protocols to those used for both drugs.

Skin microcirculatory vasomotion. Blood flow was recorded at $t = -50$ and 130 min using the Periflux 4000 laser Doppler system (Perimed, Stockholm, Sweden) in combination with a Periflux tissue heater set to 30°C (PF 4005 PeriTemp) to prevent a rapid decrease of skin temperature. The light, emitted from a near-infra-red laser diode with a power of 1.0 mW at a wavelength of 780 nm, was delivered by an optical-fiber probe (PF 408). The probe, combined with a thermostatic probeholder (PF450), was positioned on the dorsal side of the wrist of the nondominant arm and was kept in place during the entire study period. A bandpass filter with cutoff frequencies at 20 Hz and 20 kHz, and a time constant of 0.2 s was selected. Perisoft dedicated software (PSW) was used for data acquisition on a personal computer. The signal was sampled for 15 min at 32 Hz.

Study 2.

Insulin iontophoresis. All subjects underwent iontophoresis of regular insulin (0.18 ml Actrapid 100 IU/ml; Novo Nordisk, Bagsvaerd, Denmark) and a control substance (0.18 ml diluting medium for soluble insulin injection; Novo Nordisk) in a double-blind randomized order. The diluting medium (control substance) had the same composition as Actrapid but did not contain insulin molecules. A period of ~10 min elapsed between the two measurements. The first measurement was performed on dorsal skin of the middle phalanx of the third finger, whereas the second measurement was performed on the middle phalanx of the fourth finger. The substances were delivered using a cathodal current (18,19). To minimize nonspecific current-induced vasoreactivity, nine doses (0.2 mA for 20 s) were delivered, with a 90-s interval between each dose, resulting in an incremental dose-response curve. Blood flow was recorded continuously using the Periflux 4000 laser Doppler system (Perimed) with the thermostatic probe (PF 481-2; recently modified by Perimed to improve the backscatter/flux ratio) set to 30°C. Although systemic glucose-lowering effects of iontophoresed insulin could not be demonstrated in humans (20), plasma glucose levels were measured before and after the iontophoresis procedures. The measurements were conducted under the same conditions as study 1, but with the subjects in the sitting position.

Analytical methods. In study 1, all serum samples were directly centrifuged at −4°C and stored at −80°C. Insulin levels were measured with an immunoradiometric assay (Medgenix Diagnostics, Fleurus, Belgium). Glucose levels during the hyperinsulinemic clamp were determined by the glucose oxidase method with a glucose analyzer YSI2300 (YSI, Yellow Springs, OH). In study 2, glucose levels were measured with the HemoCue Glucose Analyzer (HemoCue AB, Angelholm, Sweden).

Data analysis. Data are expressed as means ± SD, unless otherwise stated. Comparison of microvascular measurements before and during the insulin and control study was performed with a paired t test. In addition, ANOVA for repeated measurements was used to compare the dose-response curve. Fourier transformation was used to determine the frequency components within the laser Doppler signal (12). The frequency band was studied at 0.01–1.6 Hz, which was divided into a set of intervals. For each interval, a short-time Fourier transform with a different window length was used. On the basis of a recently published study (12), we chose five frequency intervals: 0.01–0.02 Hz, which is thought to contain local endothelial activity; 0.02–0.06 Hz, which is thought to contain neurogenic activity; 0.06–0.15 Hz, which is associated with the myogenic response of the smooth muscle cells in the vessel wall; 0.15–0.4 Hz, which is the frequency interval of respiratory function; and 0.4–1.6 Hz, which contains the heart beat frequency. The Mann-Whitney (Wilcoxon) paired statistical test was used to compare differences in energy densities obtained with Fourier analysis. A two-tailed P value of <0.05 was considered significant. All analyses were performed on a personal computer using the statistical software package SPSS version 9.0.

RESULTS

Study 1

Metabolic and hemodynamic variables. Table 2 shows the metabolic and hemodynamic variables before and during the hyperinsulinemic clamp and time-volume control studies. Both mean arterial pressure (MAP) and diastolic blood pressure (DBP) decreased significantly during hyperinsulinemia ($P < 0.05$); however, this decrease was not significantly different ($P = 0.75$) from the nonsignificant decrease in MAP and DBP observed during the control study. Heart rate increased during the insulin-infusion study ($P = 0.07$), but not during the control study ($P = 0.55$). The increase in leg blood flow during hyperinsulinemia was significantly different from the change in leg blood flow observed during the control study (1.97 ± 1.1 vs. −0.54 ± 0.93 ml · min^{-1} · dl^{-1}; $P < 0.01$).

Capillary microscopy. Capillaroscopy data are shown in Fig. 2. At $t = -50$, baseline (15-s count) and peak capillary density during PRH were not significantly different between the insulin and control study (37.7 ± 5.4 vs. 37.9 ± 5.4 and 53.0 ± 6.3 vs. 53.1 ± 6.5 per mm^2, respectively). During the insulin study, baseline capillary density (both

TABLE 2
Metabolic and hemodynamic variables before and during hyperinsulinemic clamp and time-volume control studies

Variable	$t = -60$		$t = 120$ min	
	Insulin study	Control study	Insulin study	Control study
Plasma insulin (pmol/l)	55 ± 12	56 ± 20	409 ± 61†	48 ± 11‡
Blood glucose (mmol/l)	4.6 ± 0.3	4.9 ± 0.3	4.6 ± 0.4	4.8 ± 0.3
Heart rate (bpm)	56 ± 6	57 ± 8	63 ± 11	56 ± 11
Systolic blood pressure (mmHg)	127 ± 10	125 ± 11	124 ± 10	123 ± 11
Mean arterial pressure (mmHg)	87 ± 6	85 ± 9	84 ± 8*	84 ± 10
Diastolic blood pressure (mmHg)	66 ± 6	65 ± 9	63 ± 8*	64 ± 10
Leg blood flow (ml · min^{-1} · dl^{-1})	2.8 ± 1.6	2.9 ± 1.1	4.7 ± 2.7*	2.4 ± 1.0§

Data are means ± SD. *$P < 0.05$, †$P < 0.001$ vs. $t = -60$; ‡$P < 0.05$, §$P < 0.01$ for change during insulin study versus change during control study.

15-s and 3-min count) increased significantly (37.7 ± 5.4 vs. 41.1 ± 5.0 [$P < 0.001$] and 52.4 ± 5.8 vs. 57.1 ± 5.8 per mm^2 [$P < 0.01$], respectively). Peak capillary density during PRH also increased significantly during the insulin study (53.0 ± 6.3 vs. 58.1 ± 6.1; $P < 0.01$). In contrast, during the control study, baseline capillary density (both 15-s and 3-min count) decreased significantly (37.9 ± 5.4 vs. 34.2 ± 6.8 [$P < 0.05$] and 52.5 ± 5.4 vs. 47.6 ± 5.3 per mm^2 [$P < 0.01$], respectively), and peak capillary density decreased nonsignificantly (53.1 ± 6.5 vs. 52.3 ± 5.5). The observed increase in capillary numbers during the insulin study was significantly different from that observed during the control study ($P < 0.01$). Skin temperature was not significantly different during the experiments (32.7 ± 0.9°C).

Endothelium-(in)dependent vasodilatation of skin microcirculation. Results are shown in Table 3 and Fig. 3. The increase in baseline skin perfusion during hyperinsulinemia was significantly different from the decrease in baseline perfusion observed during the control study (4.5 ± 4.9 vs. –1.0 ± 2.6 arbitrary perfusion unit [PU] [$P < 0.05$] for the baseline measurements preceding the acetylcholine procedure, and 1.7 ± 3.8 vs. –3.1 ± 3.1 PU [$P = 0.05$] for the baseline measurement before the sodium nitroprusside procedure). In addition, hyperinsulinemia significantly increased the vasodilatation induced by both acetylcholine and sodium nitroprusside. In contrast, the vehicle responses were unaltered during hyperinsulinemia (absolute changes in microcirculatory flow before and during hyperinsulinemia: 0.9 ± 8.9 vs. 0.6 ± 4.5 PU [$P = 0.9$] and 6.9 ± 14.1 vs. 14.3 ± 24.6 [$P = 0.4$] during iontophoretically applied acetylcholine and sodium nitroprusside vehicle, respectively). After subtraction of the vehicle responses from the drug responses, hyperinsulinemia still resulted in an augmentation of the vasodilatation caused by acetylcholine (139.9 ± 14.7 vs. 210.3 ± 30.5 PU; $P < 0.05$) and sodium nitroprusside (103.2 ± 11.8 vs. 133.5 ± 10.4 PU; $P < 0.05$). Comparison of the total dose-response curves with ANOVA for repeated measures demonstrated no significant differences between the insulin and control study at $t = -30$ for both acetylcholine and sodium nitroprusside (Fig. 3, left panel). In contrast, the same analysis at $t = 150$ demonstrated significant differences between the insulin and control study ($F = 8.108$, $P < 0.05$ and $F = 6.949$, $P < 0.05$ for acetylcholine and sodium nitroprusside, respectively) (Fig. 3, right panel).

Skin microcirculatory vasomotion. Table 4 summarizes the energy densities within each frequency interval and the calculated total energy density within the interval 0.01–1.6 Hz. Hyperinsulinemia increased the total spectral energy density of the blood flow signal. Compared to the control study, insulin significantly ($P < 0.05$) increased the relative energy contribution of the frequency synchronized with the heart rate (from 0.4 to 1.6 Hz). In addition, the relative contribution of spectral components around 0.01–0.02 Hz, thought to result from local endothelial activity, was also significantly increased ($P < 0.05$).

Study 2.

Insulin iontophoresis. Both insulin and the control substance increased skin blood flow significantly (33.2 ±

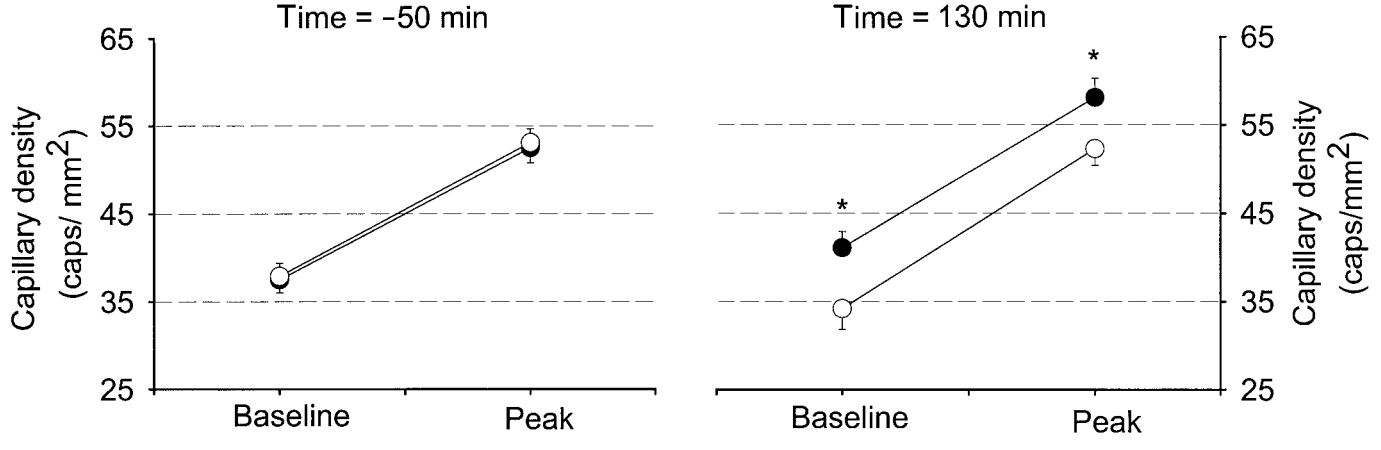

FIG. 2. Capillary densities before (baseline) and during postocclusive reactive hyperemia (peak) at $t = -50$ and 130 min during the hyperinsulinemic clamp study (●) and time-volume control study (○). *$P < 0.05$ vs. control study. The results are expressed as means ± SE.

TABLE 3
Endothelium-dependent and -independent vasodilatation of skin microcirculation before and during hyperinsulinemic clamp and time-volume control studies

	$t = -30$ min		$t = 150$ min	
	Insulin study	Control study	Insulin study	Control study
ACh-mediated vasodilatation				
Skin temperature (°C)	31.0 ± 0.9	31.6 ± 0.7	31.2 ± 0.7	31.2 ± 0.7
Baseline skin perfusion (PU)	18.8 ± 5.2	18.6 ± 3.6	23.7 ± 7.8*	17.5 ± 4.3‡
Plateau after ACh (PU)	159.6 ± 45.7	162 ± 36.0	234.2 ± 84.7*	140.2 ± 31.3‡
ACh-mediated vasodilatation (%)	780 ± 304	811 ± 319	989 ± 495*	722 ± 206‡
SNP-mediated vasodilatation				
Skin temperature (°C)	31.2 ± 1.0	31.4 ± 0.8	31.3 ± 0.7	31.6 ± 0.9
Baseline skin perfusion (PU)	18.7 ± 6.9	20.8 ± 4.7	20.4 ± 7.0	17.7 ± 4.7*
Plateau after SNP (PU)	128.8 ± 30.9	143.1 ± 22.6	168.3 ± 33.2†	123.8 ± 25.7†‡
SNP-mediated vasodilatation (%)	629 ± 186	611 ± 155	788 ± 276†	618 ± 159‡

Data are means ± SD. ACh = acetylcholine; SNP = sodium nitroprusside. *$P < 0.05$, †$P < 0.01$ vs. $t = -30$; ‡$P < 0.05$ for change during insulin study versus change during control study.

7.9 to 59.7 ± 26.7 PU [$P < 0.01$] and 35.0 ± 13.5 to 46.7 ± 20.4 [$P < 0.05$], respectively) (Fig. 4). The absolute increase observed during iontophoresis of insulin was significantly larger than that observed during iontophoresis of the control substance (26.5 ± 25.3 vs. 11.7 ± 13.4 PU; $P < 0.05$; ANOVA for repeated measures $F = 6.115$, $P < 0.05$). The relative increase from baseline was significantly higher during iontophoresis of insulin compared to the control substance (82.8 ± 85.7 vs. 34.4 ± 42.5%; $P < 0.05$; ANOVA for repeated measures $F = 7.715$, $P < 0.05$). Systemic glucose levels did not change during the iontophoresis of insulin (4.4 ± 0.4 vs. 4.5 ± 0.5 mmol/l; $P = 0.5$) or control substance (4.6 ± 0.5 vs. 4.7 ± 0.6 mmol/l; $P = 0.4$).

DISCUSSION

Results from recent studies (4–8) have suggested that insulin-mediated changes in muscle perfusion can modulate insulin-mediated glucose uptake. However, the puta-

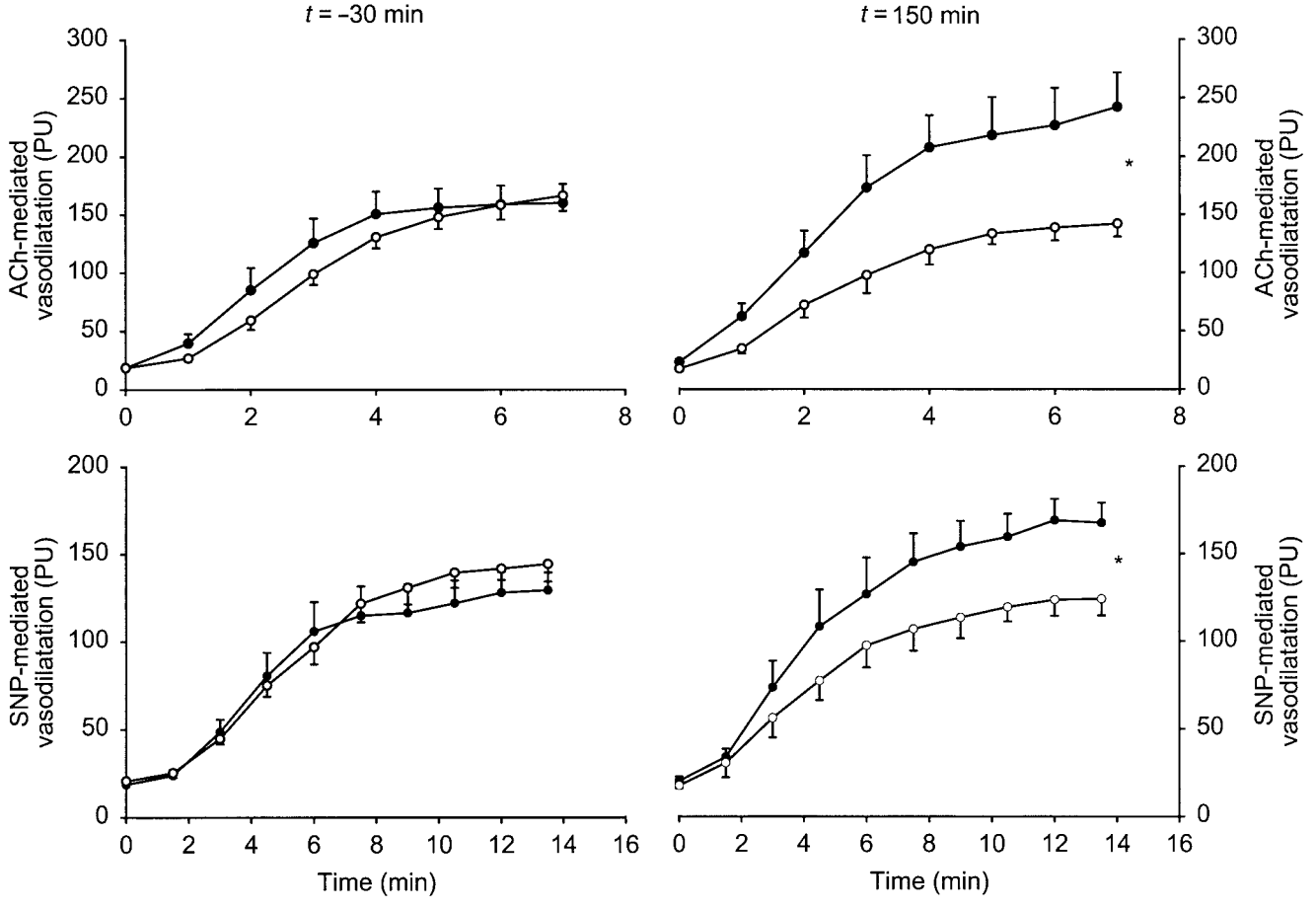

FIG. 3. Blood flow responses to iontophoresis of acetylcholine and sodium nitroprusside at $t = -30$ and 150 min during the insulin study (●) and control study (○). *$P < 0.05$ vs. control study. The results are expressed as means ± SE

TABLE 4
Energy densities of skin microcirculatory blood flow within each of the five frequency intervals before and during hyperinsulinemic clamp and time-volume control studies

	$t = -50$ min		$t = 130$ min	
Frequency domain	Insulin study	Control study	Insulin study	Control study
0.01–0.02 Hz	0.49 (0.11–1.30)	0.53 (0.51–1.38)	1.54† (0.44–2.49)	0.59‡ (0.22–1.17)
0.02–0.06 Hz	1.45 (0.51–2.72)	1.33 (0.54–3.25)	1.47 (0.28–4.74)	0.96 (0.41–2.37)
0.06–0.15 Hz	2.87 (2.11–4.99)	2.60 (0.42–4.62)	2.25 (1.37–7.04)	2.22 (0.21–4.68)
0.15–0.40 Hz	0.64 (0.50–3.12)	0.77 (0.17–3.71)	0.75 (0.46–1.33)	0.73 (0.17–2.65)
0.40–1.60 Hz	2.68 (1.47–5.95)	2.47 (1.22–6.66)	6.26† (2.24–21.20)	2.33‡ (0.50–4.44)
Total energy density (0.01–1.60 Hz)	7.74 (6.02–16.59)	9.01 (4.83–16.48)	14.05* (6.01–32.70)	8.03§ (2.24–11.49)

Data are median values (range). Values shown are energy densities within each of the five frequency domains and the total energy density within the 0.01- to 1.6-Hz interval obtained with Fourier analysis. *$P < 0.05$, †$P < 0.01$ vs. $t = -50$; ‡$P < 0.05$, §$P < 0.01$ for change during insulin study vs. change during control study.

tive effects of insulin on microcirculation that permit such modulation have not been studied. To address this issue, we examined the effects of systemic physiological hyperinsulinemia on skin microcirculatory function, the only site available in humans to directly and noninvasively assess microcirculatory dynamics. In addition, the effects of locally administered insulin on skin blood flow were assessed. We report four novel observations. First, hyperinsulinemia can induce recruitment of capillaries in skin. Second, the vasodilatation of skin microcirculation induced by both acetylcholine, an endothelium-dependent vasodilator, and sodium nitroprusside, an endothelium-independent vasodilator, is augmented by hyperinsulinemia. Third, hyperinsulinemia influences vasomotion—that is, oscillations observed in skin microcirculatory blood flow. Specifically, the relative contribution to total skin blood flow of the spectral components thought to result from heart rate frequency and endothelial activity was increased by hyperinsulinemia. Fourth, insulin induces an increase in microcirculatory blood flow, independent of its systemic actions.

Our data demonstrated that hyperinsulinemia is associated with recruitment of capillaries in human skin. This insulin-dependent capillary recruitment is associated with the number of capillaries recruited during postocclusive reactive hyperemia without insulin infusion ($r = 0.71$; $P < 0.05$), a measure of capillary recruitment that has been shown to be related to insulin-mediated whole-body glucose uptake (10,11) and to be decreased in insulin-resistant hypertensive subjects (11). Interestingly, during hyperinsulinemia, the number of capillaries perfused during postocclusive reactive hyperemia was similar to the maximal number of capillaries that can be observed during venous congestion (16), a method to expose a maximal number of nonperfused capillaries (16,21). This suggests that hyperinsulinemia facilitates recruitment of a maximal number of available capillaries.

Because the intermittent character of capillary flow and capillary recruitment seem, in part, to be controlled by precapillary vasodilatation and vasomotion (22), it is of interest to assess the effects of insulin on these variables. In the present study, the increase in baseline skin microcirculatory blood flow during hyperinsulinemia, in parallel with that in total leg blood flow, was significantly different from the decrease in baseline skin microcirculatory blood flow observed during the control study. Therefore, consistent with results from most (23,24), but not all (25), studies, hyperinsulinemia induced vasodilatation at the microcirculatory level in skin. Moreover, hyperinsulinemia increased the vasodilatation of skin microcirculation induced by both acetylcholine, an endothelium-dependent vasodilator, and sodium nitroprusside, an endothelium-independent vasodilator, whereas the nonspecific microcirculatory reactivity to the drug vehicles was unaltered. The insulin-induced augmentation of endothelium-dependent vasodilatation in skin microcirculation is in agreement with similar findings at the level of the resistance vessels, primarily in muscle (26), but the increase in endothelium-independent vasodilatation is not (26). Nevertheless, the increase in the effects of sodium nitroprusside, a nitric oxide donor, is consistent with recent findings showing that insulin increases the nitric oxide–stimulated production of cyclic guanosine monophosphate in vascular smooth muscle cells, regardless of whether the nitric oxide was derived from vascular smooth muscle cell–inducible nitric oxide synthase or from an exogenous source (27). Cyclic guanosine monophosphate attenuates the contractile agonist-induced intracellular calcium transient in vascular smooth muscle cells and affects contractile protein function, thereby decreasing vascular smooth muscle cell contraction (27) and favoring vasodilatation. How these changes relate to

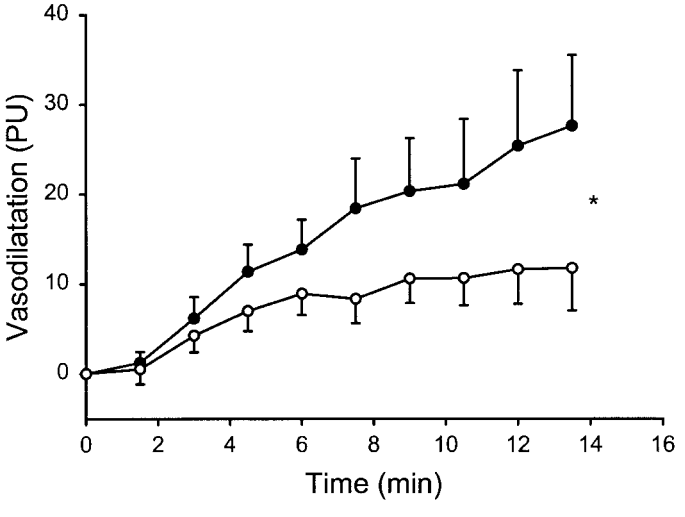

FIG. 4. Normalized skin blood flow (PU) during iontophoresis of insulin (●) and a control substance (○). *$P < 0.05$ vs. control study. The results are expressed as means ± SE.

capillary recruitment, blood flow redistribution, and glucose uptake remains to be determined.

Systemic hyperinsulinemia also influenced the rhythmic fluctuations (so-called vasomotion) of skin microcirculatory blood flow. Compared to basal skin microcirculatory blood flow, systemic hyperinsulinemia increased the total spectral energy density of the blood flow signal, the relative energy contributions in the 0.01- to 0.02-Hz interval, in which local endothelial activity is thought to be manifested (12), and the relative energy contributions in the 0.4- to 1.6-Hz interval, which is synchronous with the heart rate (12). The increased relative contribution of the spectral components resulting from the heart rate, a central control mechanism of microcirculatory flow, is consistent with microcirculatory vasodilatation, which facilitates the transmission of a more upstream signal to the microcirculation. The increased relative contribution of local endothelial activity to the total peripheral blood flow may imply that insulin modulates microvascular flow locally via an endothelium-dependent mechanism. How these changes in vasomotion relate to capillary recruitment or blood flow redistribution also remains to be determined.

It should be recognized that the intermittent character of capillary flow and capillary flow distribution is determined not only by the precapillary arteriolar network, but also by the characteristics of the capillary network itself (28,29). This may explain why experimental studies have demonstrated that insulin-mediated glucose uptake in skeletal muscle can be influenced by changes in capillary perfusion, even if total flow to the muscle remains constant (5–7).

Because part of the observed effects of systemic hyperinsulinemia on skin microcirculatory function may be secondary to other systemic effects of insulin (1,2), we assessed whether insulin was able to influence skin blood flow directly. Although iontophoresis of insulin did not induce systemic effects, it did induce a rapid increase in skin microcirculatory blood flow. Nevertheless, it may not be obvious that insulin, applied topically through iontophoresis, penetrates the skin and evokes a blood flow response. The charge of regular insulin is a function of pH. Above its isoelectric point (pKa 5.3), regular insulin is negatively charged (20). The pH of the diluting medium for soluble insulin is ~7.4. The actual range of pHs encountered as insulin progresses into the human skin is 5.4–7.3 (20,30). Therefore, regular insulin is predominantly negatively charged in skin and can be delivered by cathodal iontophoresis. A recent study in humans using cathodal iontophoresis demonstrated that insulin iontophoresis does influence skin blood flow (18), but no definite conclusions could be reached because a control study was not performed. In the present study, the vascular response to iontophoresed insulin was significantly different from that caused by an appropriate control substance, suggesting that insulin was able to penetrate the skin and reach the microcirculation. Whether this vascular response is attributable to an insulin receptor−mediated action is unknown. Nevertheless, the 48% increase in skin microcirculatory blood flow is comparable in magnitude to the increase in total limb blood flow observed during systemic hyperinsulinemia (9).

Although muscle is considered the main peripheral site of insulin-mediated glucose uptake (31) and insulin-mediated vasodilatation (32), comparable metabolic (33,34) and vascular effects (23,24,35) of systemic insulin infusion can be demonstrated in skin. In patients with diabetes, it has been demonstrated that during systemic insulin administration, in parallel with metabolic improvement, there is a redistribution of skin blood flow that favors the nutritive microcirculation (23,35). However, those studies (23,35) lacked a control group and therefore were not able to discriminate between direct effects on skin blood flow of insulin versus those of improved diabetic control. Skin microvascular vasodilator capacity is also associated with insulin's metabolic (10,11,36,37) and vascular actions (11) in skeletal muscle. Moreover, influences on skin blood flow have been described during systemic infusion of C-peptide (38). Therefore, it appears reasonable to investigate skin to learn more about the potential effects of insulin on the microcirculation. Nevertheless, it should be stated that findings concerning skin microcirculation in humans should be extrapolated to muscle with caution.

In summary, our data provided the first direct evidence for insulin-dependent capillary recruitment in human skin. In addition, systemic physiological hyperinsulinemia induces, in parallel with the increase in total leg blood flow (mainly muscle), an increase in total skin microcirculatory blood flow, and augments nitric oxide–mediated vasodilatation in skin microcirculation. It increases the relative contribution of local endothelial activity to skin microcirculatory flow. Moreover, insulin, locally administered and independent of its systemic actions, induces vasodilatation in skin microcirculation. These findings offer a potential physiological framework for further study of the functional coupling between insulin's metabolic and vascular actions.

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REFERENCES

1. Yki-Järvinen H, Utriainen T: Insulin-induced vasodilatation: physiology or pharmacology? *Diabetologia* 41:369–379, 1998
2. Baron AD: Cardiovascular actions of insulin in humans: implications for insulin sensitivity and vascular tone. In *Clinical Endocrinology and Metabolism*. Ferrannini E., Ed. London, Bailliere's, Tindan & Cox, 1993, p. 961–987
3. Cleland SJ, Petrie JR, Ueda S, Elliott HL, Connell JM: Insulin-mediated vasodilation and glucose uptake are functionally linked in humans. *Hypertension* 33:554–558, 1999
4. Baron AD, Tarshoby M, Hook G, Lazaridis EN, Cronin J, Johnson A, Steinberg HO: Interaction between insulin sensitivity and muscle perfusion on glucose uptake in human skeletal muscle: evidence for capillary recruitment. *Diabetes* 49:768–774, 2000
5. Clark MG, Colquhoun EQ, Rattigan S, Dora KA, Eldershaw TP, Hall JL, Ye J: Vascular and endocrine control of muscle metabolism. *Am J Physiol* 268:E797–E812, 1995
6. Rattigan S, Clark MG, Barrett EJ: Hemodynamic actions of insulin in rat skeletal muscle: evidence for capillary recruitment. *Diabetes* 46:1381–1388, 1997
7. Rattigan S, Clark MG, Barrett EJ: Acute vasoconstriction-induced insulin resistance in rat muscle in vivo. *Diabetes* 48:564–569, 1999

8. Bonadonna RC, Saccomani MP, Del Prato S, Bonora E, DeFronzo RA, Cobelli C: Role of tissue-specific blood flow and tissue recruitment in insulin-mediated glucose uptake of human skeletal muscle. *Circulation* 98:234–241, 1998
9. Cleland SJ, Petrie JR, Ueda S, Elliott HL, Connell JMC: Insulin as a vascular hormone: implications for the pathophysiology of cardiovascular disease. *Clin Exp Pharmacol Physiol* 25:175–184, 1998
10. Serné EH, Stehouwer CD, Ter Maaten JC, Ter Wee PM, Donker AJ, Gans ROB: Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. *Circulation* 99:896–902, 1999
11. Serné EH, Gans ROB, Ter Maaten JC, Ter Wee PM, Donker AJM, Stehouwer CDA: Capillary recruitment is impaired in essential hypertension and relates to insulin's metabolic and vascular actions. *Cardiovasc Res* 49:161–168, 2001
12. Stefanovska A, Braciè M, Kvernmo HD: Wavelet analysis of oscillations in the peripheral blood circulation measured by laser Doppler technique. *IEEE Trans Biomed Eng* 46:1230–1239, 1999
13. Harris MI, Eastman RC, Cowie CC, Flegal KM, Eberhardt MS: Comparison of diabetes diagnostic categories in the U.S. population according to the 1997 American Diabetes Association and 1980–1985 World Health Organization diagnostic criteria. *Diabetes Care* 20:1859–1862, 1997
14. Ferrannini E, Mari A: How to measure insulin sensitivity. *J Hypertens* 16:895–906, 1998
15. ter Maaten JC, Voorburg A, de Vries PM, ter Wee PM, Donker AJ, Gans RO: Relationship between insulin's haemodynamic effects and insulin-mediated glucose uptake. *Eur J Clin Invest* 28:279–284, 1998
16. Serné EH, Gans ROB, ter Maaten JC, Tangelder GJ, Donker AJM, Stehouwer CDA: Impaired skin capillary recruitment in essential hypertension is caused by both functional and structural capillary rarefaction. *Hypertension* 38:238–242, 2001
17. Coffman JD: Effects of endothelium-derived nitric oxide on skin and digital blood flow in humans. *Am J Physiol* 267:H2087–H2090, 1994
18. Delaney CA, Murchie KJ, Westerman RA, de Courten MP: Rapid actions of insulin on sensory nerve function. *Neuroreport* 9:2775–2779, 1998
19. Kari B: Control of blood glucose levels in alloxan-diabetic rabbits by iontophoresis of insulin. *Diabetes* 35:217–221, 1986
20. Sage BH. Insulin iontophoresis (Review). *Pharm Biotechnol* 10:319–341, 1997
21. Antonios TF, Rattray FE, Singer DR, Markandu ND, Mortimer PS, MacGregor GA: Maximization of skin capillaries during intravital video-microscopy in essential hypertension: comparison between venous congestion, reactive hyperaemia and core heat load tests. *Clin Sci* 97:523–528, 1999
22. Renkin EM: Control of microcirculation and blood-tissue exchange. In *Handbook of Physiology: The Cardiovascular System IV*. Berne RM, Sperelakis N, Geiger SE, Eds. Baltimore, MD, Williams & Wilkins, 1979, p. 627–687
23. Tooke JE, Lins PE, Ostergren J, Adamson U, Fagrell B: The effects of intravenous insulin infusion on skin microcirculatory flow in type 1 diabetes. *Int J Microcirc Clin Exp* 4:69–83, 1985
24. Tuominen JA, Eriksson JG, Koivisto VA: Acute administration of metoprolol and enalaprilat reduces insulin-stimulated thermogenesis and skin blood flow. *J Intern Med* 239:399–406, 1996
25. Utriainen T, Malmström R, Mäkimatilla S, Yki-Järvinen H: 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
26. Taddei S, Virdis A, Mattei P, Natali A, Ferrannini E, Salvetti A: Effect of insulin on acetylcholine-induced vasodilation in normotensive subjects and patients with essential hypertension. *Circulation* 92:2911–2918, 1995
27. Kahn AM, Allen JC, Seidel CL, Song T: Protein kinase C mediates insulin-inhibited Ca2+ transport and contraction of vascular smooth muscle. *Am J Hypertens* 13:383–388, 2000
28. Clark MG, Rattigan S, Newman JM, Eldershaw TP: Vascular control of nutrient delivery by flow redistribution within muscle: implications for exercise and post-exercise muscle metabolism. *Int J Sports Med* 19:391–400, 1998
29. Johnson PC: Active and passive determinants of capillary density: a historical perspective. *Int J Microcirc Clin Exp* 15:218–222, 1995
30. Braun-Falco O, Korting HC: Der normale pH-Wert der menschlichen Haut. *Der Hautarzt* 37:126–129, 1986
31. Baron AD, Brechtel G, Wallace P, Edelman SV: Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. *Am J Physiol* 255:E769–E774, 1988
32. Baron AD, Brechtel G: Insulin differentially regulates systemic and skeletal muscle vascular resistance. *Am J Physiol* 265:E61–E67, 1993
33. Lang CH: Rates and tissue sites of noninsulin- and insulin-mediated glucose uptake in diabetic rats. *Proc Soc Exp Biol Med* 199:81–87, 1992
34. Lang CH, Dobrescu C, Bagby GJ: Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. *Endocrinology* 130:43–52, 1992
35. Tymms DJ, Tooke JE: The effect of continuous subcutaneous insulin infusion (CSII) on microvascular blood flow in diabetes mellitus. *Int J Microcirc Clin Exp* 7:347–356, 1988
36. Jaap AJ, Shore AC, Tooke JE: Relationship of insulin resistance to microvascular dysfunction in subjects with fasting hyperglycaemia. *Diabetologia* 40:238–243, 1997
37. Caballero AE, Arora S, Saouaf R, Lim SC, Smakowski P, Park JY, King GL, Logerfo FW, Horton ES, Veves A: Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. *Diabetes* 48:1856–1862, 1999
38. Forst T, Kunt T, Pohlmann T, Goitom K, Engelbach M, Beyer J, Pfutzner A: Biological activity of C-peptide on the skin microcirculation in patients with insulin-dependent diabetes mellitus. *J Clin Invest* 101:2036–2041, 1998