chapter 3 – microvascular recruitment in skin and muscle

Insulin-induced microvascular recruitment in skin and muscle are related and both are associated with whole body glucose uptake

Microcirculation 19: 494-500, 2012

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Abstract

Insulin-induced capillary recruitment is considered a determinant of insulin-mediated glucose uptake. Insulin action on the microvasculature has been assessed in skin, however, there is concern as to whether the vascular responses observed in skin reflect those in muscle. We hypothesized that insulin-induced capillary recruitment in skin would correlate with microvascular recruitment in muscle in a group of subjects displaying a wide variation in insulin sensitivity. Capillary recruitment in skin was assessed using capillary videomicroscopy, and skeletal muscle microvascular recruitment (i.e. increase in microvascular blood volume) was studied using contrast-enhanced ultrasonography (CEU) in healthy volunteers (n = 18, mean age 30.6 ± 11.1 years). Both microvascular measurements were performed during saline infusion, and during a hyperinsulinemic euglycemic clamp. During hyperinsulinemia, capillary recruitment in skin was augmented from 58.1±18.2 to 81.0±23.9% ($p<0.0001$). Hyperinsulinemia increased microvascular blood volume in muscle from 7.00 (2.66 – 17.67) to 10.06 (2.70 – 41.81) units ($p = 0.003$). Insulin's vascular effect in skin and muscle were correlated ($r=0.57$). Insulin's microvascular effects in skin and muscle showed comparable strong correlations with insulin-mediated glucose uptake; $r=0.73$ and $r=0.68$, respectively. In conclusion: insulin-augmented capillary recruitment in skin parallels insulin-mediated microvascular recruitment in muscle and both are related to insulin-mediated glucose uptake.

Introduction

Evidence suggests that insulin delivery to skeletal muscle interstitium is the rate-limiting step in insulin-stimulated muscle glucose uptake, and that delayed delivery contributes to insulin resistance (1,2,3). It has been proposed that insulin promotes its own access to muscle interstitium by effects on the microcirculation (4,5,6,7). Specifically, it has been suggested that insulin, by relaxation of pre-capillary arterioles and effects on microvascular vasomotion, recruits capillaries to expand the endothelial surface available for transportation of insulin, glucose and other nutrients (8,9).

The study of insulin's microvascular actions has long-time been hampered by the perceived lack of appropriate techniques for its study in humans. Because of its easy accessibility, the skin microvasculature has been studied in a variety of conditions (10). At present, many efforts to examine the microvascular action of insulin have come from work examining skin microvasculature (4). The skin is an insulin-sensitive organ (11) and the only site available in humans to directly and non-invasively assess capillary density as well as vasomotion, i.e. the spontaneous rhythmic change of arteriolar diameter (online supplement). Using capillary videomicroscopy and laser Doppler fluxmetry, we previously reported that, in healthy individuals, systemic hyperinsulinemia increases the number of erythrocyte-perfused capillaries and influences vasomotion (12). Moreover, blunted insulin-mediated capillary recruitment is associated with impaired insulin-mediated glucose uptake observed in obesity (13) and during elevated plasma FFA concentrations (14). These studies provided evidence that insulin regulates the microvasculature and, specifically, can expand the surface area available for nutrient exchange. However, concern has been expressed as to whether the vascular responses observed in skin reflect those in muscle (4,5,15,16). Whereas the effects of insulin on vasomotion measured in skin (12) can be reproduced in muscle (17), it is unclear whether insulin-induced capillary recruitment in skin is paralleled by capillary recruitment in muscle. Capillary recruitment cannot be directly assessed in muscle, but contrast-enhanced ultrasound (CEU) (which provides a measure of microvascular volume) has been used successfully to examine the effects of insulin (18) and insulin resistance (19,20) on human muscle microvasculature. To enhance our understanding and interpretation of the skin microvascular studies, the first objective of this study is to assess whether insulin's microvascular actions in skin are correlated to those in muscle. Secondly, we aimed to assess the association between insulin's microvascular action in muscle and insulin-mediated glucose uptake. As yet, a significant association between insulin-mediated glucose uptake and microvascular recruitment has only been reported in human skin, but not human skeletal muscle.

In a group of normotensive and glucose-tolerant subjects showing a wide range in insulin sensitivity, the current study examined the effects of physiological hyperinsulinemia on skin capillary density and muscle microvascular volume as assessed by capillary videomicroscopy and CEU, respectively. Relationships between insulin's metabolic and vascular actions in skin and muscle were also studied.

Methods

Subjects

Eighteen healthy volunteers participated in this study (table1). They were recruited through local advertisements. None had a history of cardiovascular disease, all were non-diabetic (21) and normotensive (<140/<90 mmHg) as determined by triplicate office blood pressure measurement. Participants were of Caucasian origin and non-smokers. No medication was used during 4 weeks leading up to and on the day of study. The experiments were performed at the clinical research unit, in a quiet temperature-controlled room.

The study protocol was approved by the local Ethics Committee and in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Study design

All individuals underwent the study protocol as shown in figure 1. Measurements were conducted in a fasting state on an outpatient basis in a quiet, temperature-controlled room (23.0±1.0°C) after 30 minutes of acclimatization. Subjects had abstained from caffeine, alcohol and meals overnight. The microvascular measurements (skin and muscle) were performed in the supine position and in random order for both the baseline microvascular measurement, as well as the microvascular measurement during hyperinsulinemia.

Measurements

Skin microvascular measurements (capillary videomicroscopy)

Nailfold capillary studies were performed as described previously (22). Nailfold capillaries in the dorsal skin of the third finger of the left hand were visualized by a capillary microscope (Zeiss), linked to a television camera (Philips LDH 070/20). A 3.2x objective (Zeiss 3.2/0.07) was used with a total system magnification of 99x. The number of perfused capillaries was counted off-line by an experienced investigator (M.P.d.B.) from a videotape. Capillary density at baseline was defined as the number of capillaries per square millimeter which were continuously perfused for 15 seconds during an observation-period of 30 seconds. During this observation period, some capillaries are continuously perfused with erythrocytes, whereas others are only intermittently perfused. Intermittently perfused capillaries are considered an important functional reserve that can be recruited during situations of increased metabolic demand and hyperinsulinemia (12). Since the assessment of the cumulative open-time of the intermittently perfused capillaries is difficult and laborious, we have used the difference between capillary density at baseline and postocclusive reactive hyperemia (PRH) after four minutes of arterial occlusion as a measure of this functional reserve (12,23). PRH closely approximates counting all capillaries which are perfused for any amount of time during a three minute observation. Both approaches demonstrate an increase in capillary density during hyperinsulinemia (12). Capillary recruitment was calculated as the relative increase in capillary density from the continuously filled fraction to capillary density after PRH. Insulin-augmented capillary recruitment (percentage-points) was defined as the increase in capillary recruitment during hyperinsulinemia from capillary recruitment during saline infusion. The day-to-day coefficients of variation (CV) of continuously perfused capillary density and peak capillary density were 3.4±2.0% and 3.9±1.6% respectively, as determined in 10 subjects on separate days.

Skeletal muscle microvascular measurements (contrast enhanced ultrasonography)

Contrast enhanced ultrasound experiments were performed with a Siemens-Acuson Sequoia 512 with a 17L5 transducer (Siemens-Acuson, Mountain View, CA, USA). The flexor muscles of the right forearm were imaged with the volunteer in the supine position. Microbubbles (SonoVue®, Bracco, Milan, Italy) were infused continuously at a rate of 2.5 ml/minute for four minutes up to a total volume of 10 ml. When a systemic steady state microbubble concentration was achieved (2 minutes), 3 real-time inflow curves of >25 seconds each were performed after destruction of the microbubbles in the ultrasound beam with a high Mechanical Index (1.4).

The microbubble inflow curves were analyzed off-line using the Image Processing toolbox in Matlab (Mathworks, Natick, MA). The mean video-intensity during the first half second was subtracted to correct for background noise and large vessels in the region of interest (ROI) which was drawn in the skeletal muscle. Mean video-intensities in the ROI were normalized for the video-intensity in a large vessel to correct for differences in systemic bubble-concentrations, generating measurements comparable within and between subjects. The derived video-intensities were then fitted to the exponential function: $y=A(1-e^{-\beta(t-0.5)})$, (y represents video-intensity, A is the theoretical plateau video-intensity after an infinite amount of time and represents the microvascular blood volume (MBV), β is the microvascular flow velocity (MFV), which represents vascular resistance and t is the time after the start of the

inflow-curve; t is subtracted with half a second because of the correction for background noise and larger vessels (25).

Insulin sensitivity

Insulin sensitivity was assessed by the hyperinsulinemic, euglycemic clamp technique (24). Briefly, insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) was infused in a primed (0.4 U ml-1) continuous manner at a rate of 1 mU·kg·min-1. Euglycemia (5 mmol/L) was maintained by adjusting the rate of a 20% glucose infusion based on plasma glucose measurements performed at 5-10 minute intervals using an YSI 2300 STAT Plus analyzer (YSI, Yellow Springs, OH). Whole body glucose uptake (M-value) was calculated from the glucose infusion rate during steady state of the clamp and expressed per kilogram of (lean) body weight.

Anthropometrics

Lean muscle mass was determined by bioelectrical impedance analysis (BF906, Maltron, Rayleigh, UK).

Statistical analyses

All variables were first checked for normality of distribution. Data are presented as mean ± SD, or median and range when applicable. A paired samples t-test (Wilcoxon signed rank test for non-normally distributed data) was used to compare measurements during saline and insulin infusion. Pearson's correlation analyses were used to investigate correlations between (changes in) microvascular function in the different vascular beds and insulin sensitivity. A two-tailed P-value of <0.05 was considered significant. All analyses were performed using the statistical software package SPSS version 15.0.

Results

Characteristics of the study group

Baseline characteristics of the study group are shown in table 1. 18 subjects were included in this study, of which 3 were men. The age of the study population averaged 30.6 years (range 18 to 54 years). BMI on the study-day ranged between 19.9 and 39.4 kg/m2. Metabolic study results are reported in table 2. The participants demonstrated a wide variation in insulin sensitivity ranging from 3.6 mg/kg lean weight/minute to 18 mg/kg lean weight/minute. Unfortunately, one videomicroscopy measurement and one CEU measurement failed in two separate subjects due to unexpected technical problems.

Insulin increases capillary recruitment in skin

Table 2 and figure 2 show skin microvascular measurements before and during hyperinsulinemia. The number of continuously perfused capillaries did not change during hyperinsulinemia (40.8±8.3 vs. 40.3±8.6 per mm2). Capillary density of continuously and intermittently perfused capillaries, as assessed during peak reactive hyperemia, increased significantly during hyperinsulinemia (64.8±16.8 vs. 72.9±17.5 per mm2, $p < 0.0001$). Consequently, capillary recruitment increased significantly during hyperinsulinemia (58.1±18.2% vs. 81.0±23.9%, $p < 0.0001$).

Insulin increases microvascular blood volume in muscle

Table 2 and figure 3 show microvascular measurements in muscle before and during hyperinsulinemia. The video-intensity of the CEU signal at plateau was significantly

increased during hyperinsulinemia (7.00 (2.66 – 17.67) vs. 10.06 (2.70 – 41.81) $p = 0.003$), indicating an increase in MBV, two subjects even exhibited a derecruitment of their microvascular blood volume. MFV, the rate at which the microbubbles reappear in the region of interest, was not significantly different between the saline infusion and

hyperinsulinemia (0.076 (0.012 - 0.347) vs. 0.120 (0.046 – 0.244), p = 0.266). The product of MBV x MFV; a measure of microvascular flow, increased significantly during hyperinsulinemia from 0.47 (0.06 – 2.70) to 1.22 (0.45 – 5.03), p = 0.006

The change in microvascular blood volume in muscle is correlated with insulin-augmented capillary recruitment in skin

As shown in figure 4, the insulin-induced change in MBV is correlated with the insulin-augmented capillary recruitment in skin (r = 0.57, p = 0.02).

Insulin-augmented capillary recruitment in skin is associated with whole body glucose disposal

Figure 5 shows the association between augmentation of skin capillary recruitment by insulin and whole body glucose disposal (M-value). Insulin-induced augmentation of capillary recruitment showed a positive association with the insulin-mediated glucose disposal rate (r = 0.73, p = 0.001).

Insulin-induced increase in microvascular blood volume in muscle is associated with whole body glucose disposal rate

Figure 6 shows the association between the increase in MBV by insulin and whole body glucose disposal rate (M-value) (r = 0.68, p = 0.003). Microvascular blood flow (MBV * MFV) did not show an association with insulin sensitivity.

Discussion

The present study demonstrates that insulin-augmented capillary recruitment in skin is paralleled by insulin-mediated microvascular recruitment in muscle. Microvascular recruitment in both tissues were mutually correlated and showed an association with insulin-mediated whole body glucose uptake.

The ability to investigate microvascular structure and function is important in improving our understanding of pathophysiological processes in many areas of cardiometabolic disease (25). Because of their accessibility, cutaneous microvessels are suitable for mechanistic studies on vascular function and more specifically on function of nutritive capillaries. This unique property prompted us to examine the cutaneous microcirculation in order to investigate the microvascular actions of insulin which are proposed to control the delivery of insulin to muscle and thereby regulate skeletal muscle glucose uptake (12,13). Indirect evidence suggesting that the vascular responses observed in skin indeed might reflect those in muscle is provided by the fact that the effects of obesity and free fatty acids on insulin-induced capillary recruitment in skin (13,14) can be reproduced in the microcirculation of human muscle (19,20). However, direct evidence that regulation of skin microvascular perfusion represents what is occurring in muscle was previously lacking.

The present study is the first to demonstrate concurrent insulin-augmented microvascular recruitment in skin and skeletal muscle. Both were mutually correlated and strongly associated with whole body glucose uptake. The association between insulin-augmented capillary recruitment in skin and insulin-mediated microvascular recruitment in muscle, however, was only modest. Theoretically, this may be due to differences in function of the microcirculation in skin and muscle: the vasculature of skin is highly specialized for processes such as heat exchange. However, heat dissipation is regulated mainly by arterio-venous shunts rather than capillaries, which serve a nutritive function. As opposed to LaserDoppler flowmetry which, due to its average depth of penetration, mainly measures flux in the subpapillary plexus, capillary videomicroscopy specifically examines capillaries (26). Alternatively, the modest association between insulin-augmented capillary recruitment in skin and insulin-mediated microvascular recruitment in muscle may be explained by important differences in measurement principles. Capillary videomicroscopy is a technique that allows direct visualization of erythrocyte-perfused

capillaries in skin. During capillary videomicroscopy, some capillaries seem to be continuously filled with erythrocytes, whereas others are only intermittently perfused, depending on pre-capillary arteriolar tone.

Contrast-enhanced ultrasonography is an imaging tool which enables the assessment of the microcirculation in skeletal muscle (18). It utilizes gas-filled microbubbles that are inert, remain entirely within the vascular space, and possess an intravascular rheology similar to that of red blood cells. During an intravenous infusion of these microbubbles and attainment of a steady state, the microbubbles are destroyed with high energy ultrasound and the rate of microbubble replenishment within the ultrasound beam is measured, which represents mean flow velocity in the ROI (MFV). When the beam is fully replenished, the ultrasound signal represents relative blood volume within the beam, which translates to the volume of blood within skeletal muscle (MBV). The latter signal will not only include capillaries but also microbubbles-filled arterioles and venules. Therefore, it may not be surprising that both techniques used to measure microvascular recruitment only demonstrate a modest relationship, because different levels of the microvasculature are being examined.

We assessed PRH with the capillary videomicroscope and not with CEU, because of methodological considerations. PRH makes it possible to easily and effectively quantify the intermittently perfused capillaries in videomicroscopy. With real-time CEU, intermittently (contrast-) perfused capillaries contribute towards average video-intensity at any time, obviating the need for PRH.

The effect size of insulin-induced microvascular recruitment is much larger in muscle than in skin. This may be caused by the aforementioned methodological aspects of the two techniques, or be the result of a genuine physiological difference. Although one is not able to directly assess capillary perfusion with CEU in skeletal muscle, the observation that extra capillaries are recruited in skin during hyperinsulinemia suggests that the increase in muscle-MBV can be attributed to a similar process i.e. an increase in the number of perfused microvessels, and not the increase in diameter of already perfused microvessels. Two of our volunteers exhibited a derecruitment of MBV during hyperinsulinemia. As insulin has both a vasodilator (through NO) as well as a vasoconstrictor effect (though endothelin-1) (9), the vasoconstrictor pathway presumably prevailed in these subjects. Vasoconstriction of microvessels in skeletal muscle leads to a decrease in MBV.

Apart from measuring directly at the site of interest, CEU has additional benefits over capillary-videomicroscopy, in theory one could simultaneously study the (micro-)vasculature in other organs, as the microbubbles are distributed in a steady state throughout the entire body. Nevertheless, capillary-videomicroscopy is more apt to study microvascular function in large epidemiological studies due to its non-invasive character and lower cost.

Perspectives

These data suggest that the cutaneous microcirculation is a representative vascular bed to examine insulin's actions on the microcirculation. Insulin-mediated microvascular recruitment in muscle and insulin-augmented recruitment in skin are related and both are associated with insulin-mediated glucose uptake, supporting the functional coupling between insulin's microvascular and metabolic actions.

Tables

table 1 - characteristics of the study group

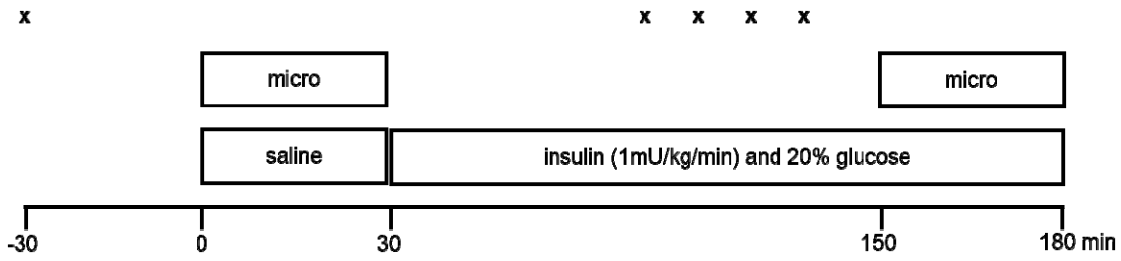
Characteristic	mean±SD or median (range)
n (males)	18 (3)
age, y	30.6±11.1
waist (m)	0.85±0.13
waist-to-hip ratio	0.83±0.07
body mass index, $kg \cdot m^{-2}$	25.7±5.6
systolic blood pressure, mm Hg	118±12
diastolic blood pressure, mm Hg	71±7
heart rate, bpm	64±12
fasting plasma glucose, mmol/L	4.9±0.4
fasting plasma insulin, pmol/L	32.7 (13.4 – 86.4)
fasting HDL-cholesterol, mmol/L	1.4±0.4
fasting LDL-cholesterol, mmol/L	2.4±0.7
fasting serum triglycerides, mmol/L	0.8 (0.5 – 2.8)
fasting serum FFA, mmol/L	0.56±0.20

table 2 – metabolic and microvascular results

Characteristic	mean±SD or median (range)
metabolic measurements	
steady state plasma insulin clamp, pmol/L	515.5±95.2
M-value, insulin sensitivity, mg· lean $kg^{-1} \cdot min^{-1}$	10.7±3.7
skin microvascular measurements	
saline continuously perfused capillaries, n/mm^2	40.8±8.3
saline total capillary density, after PRH, n/mm^2	64.8±16.8
saline capillary recruitment, %	58.1±18.2
insulin, baseline continuously perfused capillaries, n/mm^2	40.3±8.6
insulin, total capillary density, after PRH, n/mm^2	72.9±17.5
insulin, capillary recruitment, %	81.0±23.9
insulin-augmented capillary recruitment, %-points	15.3 (0.4 – 51.1)
muscle microvascular measurements	
saline, MBV, video intensity	7.00 (2.66 – 17.67)
saline, MFV, 1/sec	0.076 (0.012 - 0.347)
insulin, MBV, video intensity	10.06 (2.70 – 41.81)
insulin, MFV, 1/sec	0.120 (0.046 – 0.244),
delta MBV, %	78±72

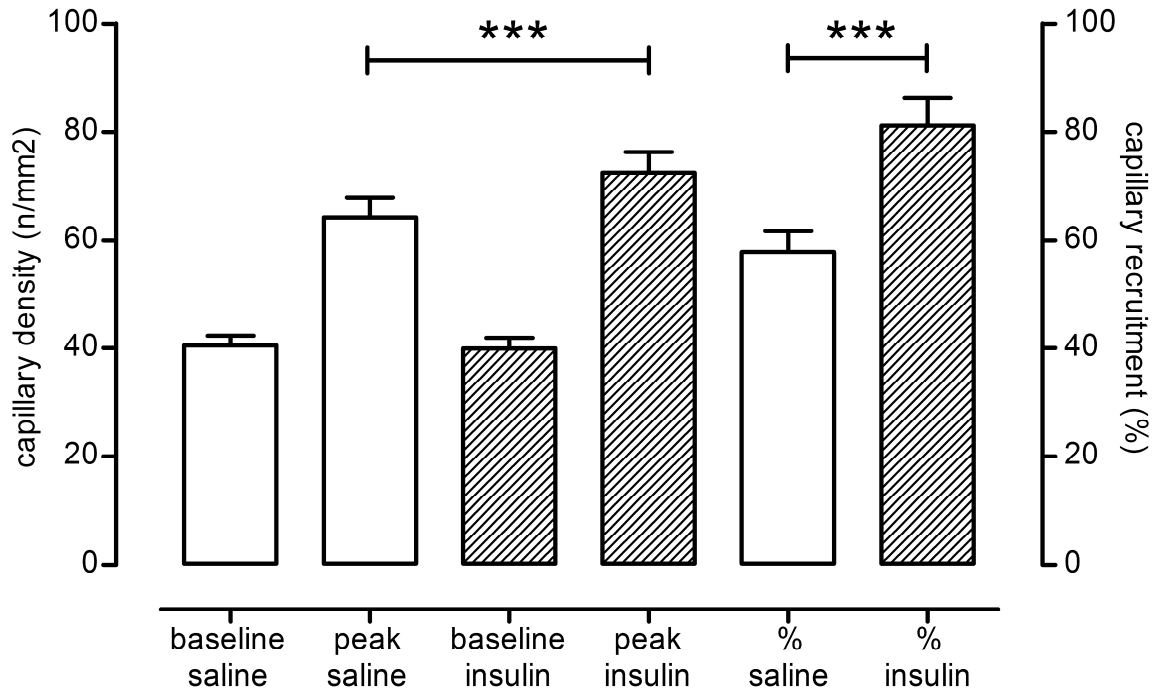
Figures

figure 1



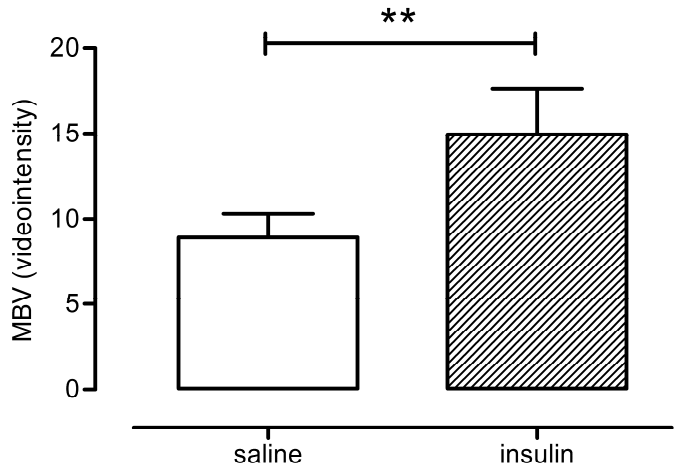
Design of the study. Micro indicates video-microscopy and LDF; x, blood samples for fasting measurements and insulin concentrations.

figure 2



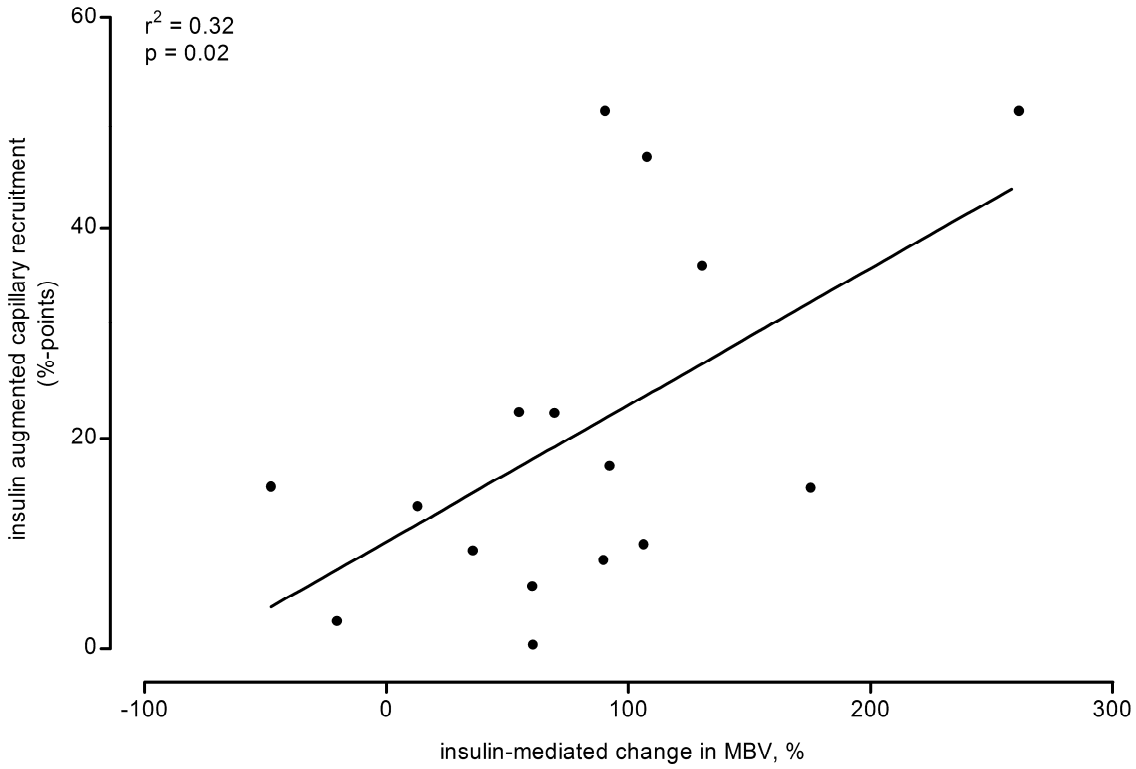
Skin microvascular measurements before and during hyperinsulinemia. Cont., continuously perfused capillaries; total, total capillary density after PRH; %, capillary recruitment. ***p < 0.0001 insulin vs. saline.

figure 3



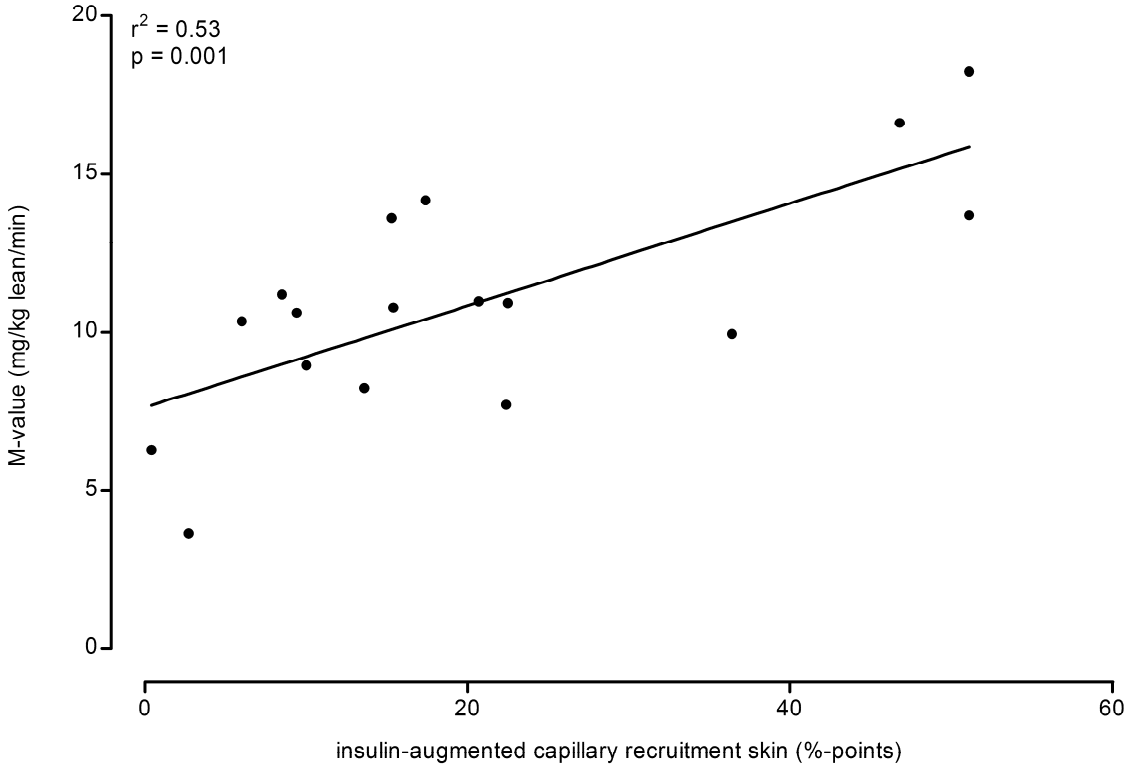
Muscle microvascular measurements before and during hyperinsulinemia, median and range. **p < 0.01 saline vs. insulin.

figure 4



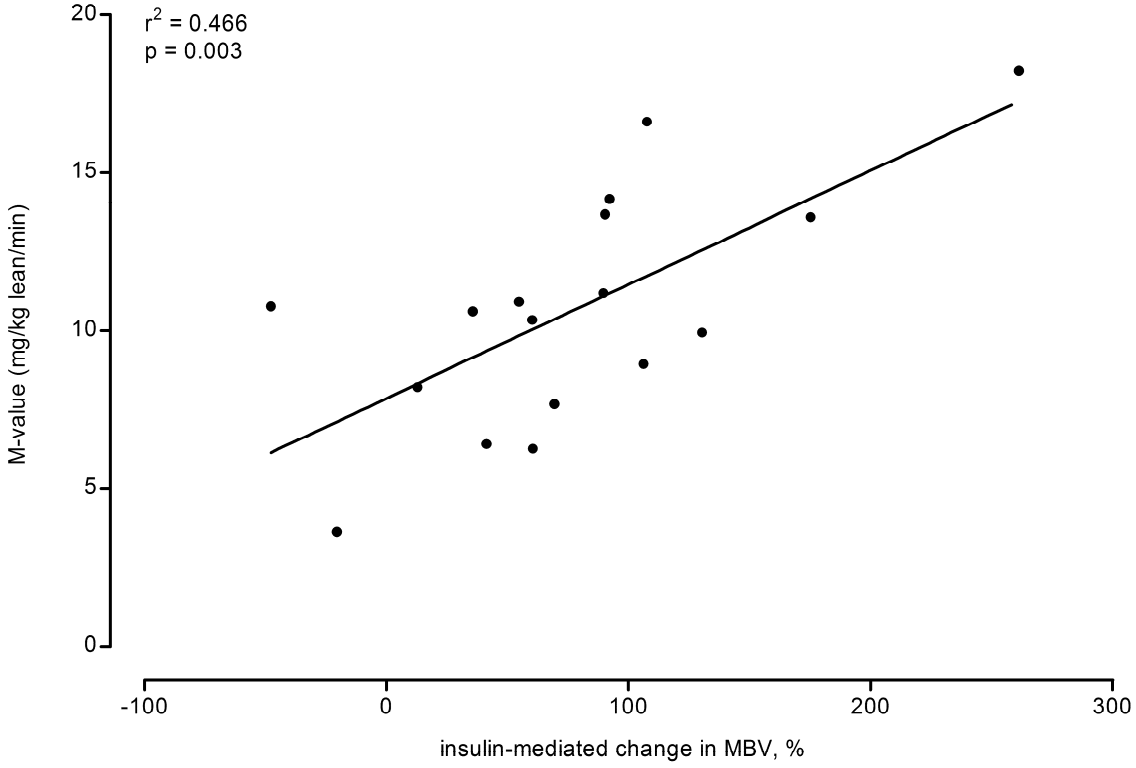
Association between capillary recruitment in skin and muscle microvascular recruitment. n = 16.

figure 5



Association between insulin-augmented capillary recruitment in skin and metabolic insulin sensitivity. n = 17.

figure 6



Association between muscle microvascular recruitment and metabolic insulin sensitivity. n = 17.

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