

## ***chapter 2 – vasomotion and capillary recruitment***

*Insulin-induced changes in microvascular vasomotion and capillary recruitment are associated in humans*

*Microcirculation 21: 380-387, 2014*

Michiel P de Boer

Rick I Meijer

John Newman

Coen D A Stehouwer

Etto C Eringa

Yvo M Smulders

Erik H. Serné

## **Abstract**

Insulin-induced capillary recruitment is considered a significant regulator of overall insulin-stimulated glucose uptake. Insulin's action to recruit capillaries has been hypothesized to involve insulin-induced changes in vasomotion. Data directly linking vasomotion to capillary perfusion, however, are presently lacking.

We therefore investigated whether insulin's actions on capillary recruitment and vasomotion were interrelated in a group of healthy individuals. We further assessed the role of capillary recruitment in the association between vasomotion and insulin-mediated glucose uptake. Changes in vasomotion and capillary density were determined by laser Doppler flowmetry (LDF) and capillary videomicroscopy in skin, respectively, before and during a hyperinsulinemic euglycemic clamp in 19 healthy volunteers. Insulin-induced increase in the neurogenic vasomotion domain was positively related to insulin-augmented capillary recruitment ( $r=0.51$ ,  $P=0.04$ ), and both parameters were related to insulin-mediated glucose uptake ( $r=0.47$ ,  $P=0.06$  and  $r=0.73$ ,  $P=0.001$ , respectively). The change in insulin-augmented capillary recruitment could, at least statistically, largely explain the association between the neurogenic domain and insulin-mediated glucose uptake. Insulin-induced changes in vasomotion and capillary recruitment are associated in healthy volunteers. These data suggest that insulin's action to recruit capillaries may in part involve action on the neurogenic vasomotion domain, thereby enhancing capillary perfusion and glucose uptake.

## Introduction

Vasomotion, a rhythmic change in vascular diameter, is a typical feature of microvascular networks (1, 2). Whereas the physiological significance of vasomotion in microcirculatory flow remains unclear (2), hypotheses have been put forward suggesting its effects on capillary exchange of substances between blood and tissues (3, 4). In fact, theoretical modeling has demonstrated that vasomotion may strongly influence capillary exchange (5). Based on this assumption, insulin has been proposed to alter arteriolar vasomotion with a resultant increase in the capillary exchange surface termed 'capillary recruitment' (6, 7, 8). Insulin-induced capillary recruitment is considered a significant determinant of overall insulin-mediated glucose uptake, because it controls the access of insulin and glucose to muscle interstitium. Insulin delivery to skeletal muscle interstitium is a rate-limiting step in insulin-stimulated glucose uptake by skeletal muscle, and is slower in insulin-resistant subjects than in healthy subjects (9).

Vasomotion causes a rhythmic variation in blood flow (flow motion), which can be detected by laser Doppler flowmetry (10). Vasomotion activity in the microvascular bed can, therefore, be explored by analysis of the component frequencies of the laser Doppler signal using spectral analyses such as Fast-Fourier analysis or a Wavelet transform (7, 8, 11, 12, 13). Distinct periodic oscillations in the laser Doppler signal have been attributed to, consecutively, the heart beat (spectral peaks at 0.4-1.6 Hz), respiration (0.15–0.4 Hz), myogenic activity in the vessel wall (0.06–0.15 Hz), neurogenic activity (0.02–0.06 Hz), and endothelial activity (0.01–0.02 Hz) (12). Using these analyses, human studies have suggested that systemic hyperinsulinemia affects microvascular vasomotion by increasing endothelial and neurogenic activity in skin and muscle (7, 8, 11), and that it is particularly the contribution of endothelial and neurogenic activity to microvascular vasomotion which is impaired in obese, insulin-resistant individuals (14). In rats, insulin acts to increase the myogenic component of vasomotor activity in muscle. Moreover, both muscle microvascular flow and myogenic activity were depressed in the  $\alpha$ -methyl serotonin-induced acute insulin resistant state (13). Although these studies suggest that insulin's recruitment of microvascular flow, and subsequent glucose uptake in skeletal muscle, may in part involve action on vasomotion, a direct link between changes in vasomotion and insulin-induced microvascular recruitment has yet to be demonstrated.

The aim of the present study, therefore, was to investigate whether (changes in) microvascular vasomotion and (changes in) capillary recruitment are associated in a group of healthy individuals. We further assessed whether changes in vasomotion were associated with insulin-mediated glucose uptake and whether capillary recruitment could explain part of this association.

## Methods

### Subjects

Nineteen healthy volunteers participated in this study (table 1). They were recruited through local advertisements. None had a history of cardiovascular disease, all were non-diabetic (15) 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 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

Measurements were conducted in a fasting state on an outpatient basis in a quiet, temperature-controlled room ( $23.0 \pm 1.0^\circ\text{C}$ ) after 30 minutes of acclimatization. Subjects had abstained from caffeine, alcohol and meals overnight. The microvascular measurements (laserDoppler/vasomotion and capillaroscopy) were performed simultaneously in the supine position for both the baseline microvascular measurement, as well as the

microvascular measurement during hyperinsulinemia (figure 1). The hyperinsulinemic euglycemic clamp was chosen as the intervention. It utilizes high physiological insulin levels as seen in obese subject and microvascular measurements can be performed during a metabolic steady state. Similar (or in obese subject even higher) endogenous levels of insulin are reached for a comparable duration during a meal-test (16). However, this is accompanied by the secretion or increase of a myriad of vasoactive substances such as incretins and free fatty acids, hindering the investigation of insulin-specific effects on the microvasculature (16). Due to technical problems one videomicroscopy measurement and one laserDoppler registration failed on separate occasions.

#### Skin microvascular measurements (laser Doppler)

Skin temperature was registered continuously and was above 28°C at the start of all microvascular measurements. Skin blood flow was measured in conventional perfusion units (PU) by means of a laser Doppler system (Periflux 5010, Perimed, Stockholm, Sweden). Microvascular measurements were performed with one thermostatic laser Doppler probe (PF 457, Perimed, Stockholm, Sweden) positioned at the dorsal side of the wrist of the left hand (17).

#### Vasomotion

Wavelet analysis of LDF signals with a minimum of 30 minutes (with a sampling frequency of 32 Hz resulting in approximately 58.000 data points) in length was conducted to assess the frequency spectrum between 0.01 and 1.6 Hz as described above (18).

Wavelet analysis was performed using the wavelet toolbox in Matlab (7.8.0.347; The Mathworks, Inc., Natick, MA, USA), as described earlier (13). Scales were chosen for a resulting frequency range from 0.01 to 1.6 Hz. To eliminate edge effects, the first and last 2000 samples were removed from the resulting wavelet transform (19). The relative amplitude was calculated for each of the five frequency bands by dividing the average amplitude within a band by the average amplitude of the entire spectrum. This normalisation takes into account the variation in the LDF signal strength between subjects and/or within subjects during an intervention (18, 19, 20).

#### Skin microvascular measurements (capillary videomicroscopy)

Nailfold capillary studies were performed as described in detail elsewhere (21). Briefly, 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 (visible flowmotion under baseline conditions). Capillary density after postocclusive reactive hyperemia (PRH) was determined by counting all visible capillaries after four minutes of arterial occlusion. 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  $3.4\pm 2.0\%$  and  $3.9\pm 1.6\%$  respectively, as determined in 10 subjects on separate days (21).

#### Insulin sensitivity

Insulin sensitivity was assessed by the hyperinsulinemic, euglycemic clamp technique (22). Briefly, insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) was infused in a primed (0.4 U ml<sup>-1</sup>) continuous manner at a rate of 1 mU·kg·min<sup>-1</sup>. 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 analyser (YSI, Yellow Springs, USA). 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 Analysis

All variables were first checked for normality of distribution. Data are presented as mean  $\pm$  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. Correlation analyses (Spearman's Rho for non-normally distributed data) were used to investigate correlations between (changes in) microvascular function and insulin sensitivity. In addition, multivariate analysis was performed to investigate whether the association between vasomotion and insulin sensitivity remained when allowing for capillary recruitment. A two-tailed P-value of  $<0.05$  was considered significant. All analyses were performed using the statistical software package SPSS (version 18.0, SPSS, Inc., Chicago, USA).

### Results

#### Characteristics of the study group

Baseline characteristics of the intervention group are shown in [table 1](#). The participants demonstrated a wide variation in insulin sensitivity ranging from 3.6 mg/kg lean weight/minute to 18.2 mg/kg lean weight/minute.

#### Insulin increases laser Doppler flux in skin

[Table 2](#) shows skin microvascular measurements before and during hyperinsulinemia. The median laser Doppler flux during the 30-minute recording increased significantly during hyperinsulinemia, from 8.4 PU (range 5-16) to 11.2 PU (range 5-66,  $p < 0.01$ ).

#### Insulin affects normalized laser Doppler vasomotion amplitudes in skin

[Figure 2](#) shows normalized skin laser Doppler vasomotion amplitudes before and during hyperinsulinemia. All vasomotion domains demonstrated a significant change from baseline during hyperinsulinemia, but none of the changes were significantly associated with the increase in laser Doppler flux during hyperinsulinemia.

#### Insulin increases capillary recruitment in skin

Capillary recruitment in skin, as measured by capillary videomicroscopy, increased significantly during hyperinsulinemia ( $58.1 \pm 17.2\%$  vs.  $81.4 \pm 22.6\%$ ,  $p < 0.001$ ) ([table 2](#)).

insulin-induced increase in amplitude of the normalized neurogenic vasomotion domain correlates with insulin-augmented capillary recruitment in skin and insulin-mediated glucose uptake

The insulin-induced change in the amplitude of the normalized neurogenic vasomotion domain was correlated with insulin-augmented capillary recruitment (i.e. change in capillary recruitment from baseline to hyperinsulinemia) ( $r = 0.51$ ,  $p = 0.04$ ) ([table 3](#) and [figure 3](#)) and insulin-mediated glucose uptake ( $r = 0.47$ ,  $p = 0.06$ ) ([Table 3](#)).

Capillary recruitment statistically explains the association between the change in the amplitude of the normalized neurogenic vasomotion domain and insulin-mediated glucose uptake

An increase in amplitude of the normalized neurogenic vasomotion domain of 1 was associated with an increase in glucose disposal of 6.09 milligram of glucose per kilogram lean body weight per minute (model 1, 95%-CI: 10.16 to 14.29;  $p = 0.06$ ). In model 2, after adjustment for insulin-augmented capillary recruitment, the regression coefficient of the association between the change in amplitude of the normalized neurogenic vasomotion domain and insulin-mediated glucose disposal decreased by 86% (0.88, 95%-CI: -4.71 to 6.46;  $P = 0.741$ ) (Table 4).

## Discussion

The present study in humans demonstrates, for the first time, an association between insulin-induced changes in vasomotion and the number of perfused capillaries, corroborating the paradigm that insulin regulates its own delivery and that of glucose to the interstitium via effects on vasomotion. Hyperinsulinemia significantly changed the normalized amplitude of the neurogenic vasomotion domain as well as the number of perfused capillaries. An insulin-induced increase in the normalized amplitude of the neurogenic vasomotion domain was positively related to an insulin-induced augmentation in capillary recruitment. Both changes were positively associated with insulin-mediated glucose uptake. Capillary recruitment could, at least statistically, explain most of the association between the change in vasomotion and insulin-mediated glucose uptake.

The present study shows that subjects with the highest insulin-induced increase in the normalized amplitude of the neurogenic vasomotion domain demonstrated, in parallel, the highest increase in insulin-induced capillary recruitment. This finding is consistent with results from earlier studies analyzing vasomotion in different metabolic phenotypes. In earlier studies, we demonstrated that, at baseline, the amplitudes of the neurogenic and endothelial domains were lower in obese women compared to lean women (14). Moreover, neurogenic activity in skin increased in lean subjects, but decreased in the obese subjects after a mixed meal (23), a stimulus which has been shown to induce microvascular recruitment in muscle (24). Although significant, the effect size in change of the endothelial vasomotion domain is smaller than reported earlier in lean subjects (7, 8). This is probably explained by the heterogeneous population of the present study with a large range in BMI and insulin-sensitivity. Although this greatly improves the study's power to detect relationships between changes in vasomotion and capillary recruitment, however, estimates of the measured effects of insulin on vasomotion amplitude within the whole group may be averaged out between the phenotypic extremes. Other studies demonstrated insulin to affect yet a third distinct vasomotion domain. Local hyperinsulinemia during cathodal iontophoresis of insulin seems to increase myogenic activity (25). Rat muscle studies also showed the main increase in vasomotion output due to insulin to be of myogenic origin (13). We could confirm the increase in the normalized myogenic vasomotion domain during hyperinsulinemia in our population. Since the latter study used the anesthetized rat model, some of the differences with the results in this study may be explained by the presence of the anesthetic which has been shown to affect vasomotion (26). The lower three frequency bands in particular were diminished in laser Doppler fluxmetry signals in skin during anesthesia (27).

Important new findings in the present study were the associations between the insulin-induced change in the neurogenic vasomotion domain and insulin-induced capillary recruitment, as well as insulin-mediated glucose uptake. The association between the change in vasomotion and insulin-mediated glucose uptake could, at least statistically, be explained by the increase in the number of perfused capillaries. Accordingly, we propose that the insulin-induced increase in capillary density that has been detected previously (7, 28) is at least in part due to a change in the neurogenic vasomotion domain at terminal arterioles, resulting in increased 'open-time' and preferential flow towards downstream capillary beds. In muscle, this has the potential to increase delivery of both insulin and glucose to the interstitium and subsequently delivery to the myocytes (9). A relationship between changes in vasomotion and insulin-mediated glucose uptake has been described earlier (11). In that study, the change in the endothelial domain during hyperinsulinemia was associated with metabolic insulin sensitivity.

Nevertheless, the relatively short laser Doppler registration length used for vasomotion analysis in that study could be a limitation to detect changes in the lower frequency domains, e.g. neurogenic domain and endothelial domain. The registration (and subsequent inclusion for analysis) of less than 10 arteriolar contractions within a distinct frequency domain impairs the accuracy of the spectral analysis for that frequency domain. (30, 31, [supplemental figure 1](#) – comparison signal length and vasomotion output).

The 0.02 to 0.06 Hz frequency domain is thought to be of neurogenic origin after studies, using local or ganglionic nerve blockade and sympathectomy, have shown a decrease of its contribution to the total vasomotion amplitude (29, 30). Any increase in sympathetic nervous system (SNS) activity is commonly assumed to lead to a vasoconstrictive state, characterized by a reduced capillary recruitment, an increase in blood pressure and insulin resistance. However, opposite results have been reported in healthy subjects in whom insulin increased SNS activity (as assessed through microneurographic sympathetic nerve activity to skeletal muscle (MSNA)), yet also led to vasodilation (32). As a possible explanation, Agapitov et al. demonstrated a dissociation between elevated SNS activity and normal sympathetic vascular tone (as assessed via adrenergic receptor blockade by phentolamine) in normotensive obese (33). Such a dissociation between parameters of SNS activity would fit the positive relationship between an insulin-induced increase in amplitude of the neurogenic domain (ie increased SNS activity) and insulin-augmented capillary recruitment (after vasodilatation of precapillary arterioles) in the present study, as all subjects were normotensive.

We have to address some potential limitations of the present study. The sample size is relatively small. The wide range in insulin sensitivity however does improve the statistical power of the study. Furthermore, most of our subjects are female which makes the results less generalizable. In a recent study in a different cohort of 94 subjects (46 male and 48 female) we did not find a difference in vasomotion output between men and women under baseline conditions (chapter 7). We have previously shown vasomotion as assessed via laserDoppler to be similar throughout the different phases of the menstrual cycle (34). We did not perform a control saline study (to control for changes in intravascular volume during the insulin and glucose infusion) in the present study as we have shown this not to affect vasomotion in previous studies (7, 8). Central to our hypothesis is that insulin's capacity to recruit capillary surface area, so called capillary recruitment, leads to increased interstitial insulin concentrations. A recent study (35) provided direct experimental evidence to support this hypothesis. The term capillary recruitment, however, has become the topic of a vigorous debate (36, 37, 38). In our opinion and irrespective of how insulin recruits capillary surface area, be it de novo capillary recruitment defined as non-perfused capillaries becoming perfused, or longitudinal recruitment defined as recruitment of capillary surface area along the length of previously flowing capillaries, or a combination of both, insulin increases microvascular perfusion which enhances insulin delivery and glucose uptake. Interestingly, the concept of insulin-induced changes in vasomotion can very easily be reconciled with the concept of longitudinal capillary recruitment. It suggests temporal heterogeneity in microvascular perfusion which can be influenced by insulin. Although skeletal muscle is the site of interest when studying insulin's vascular and metabolic effects, data in rats suggest that skin is an insulin-sensitive organ explaining approximately 30% of glucose disposal during an euglycemic, hyperinsulinemic clamp (39). Moreover, we recently found skin and skeletal muscle microvascular parameters (as assessed via videomicroscopy of capillaries in skin and contrast enhanced ultrasonography in muscle (CEUS)) to be a highly correlated to each other during a hyperinsulinemic euglycemic clamp (21). Finally, Rattigan et al recently reported flowmotion/vasomotion to be apparent in skeletal muscle using CEUS (40). Therefore, the observed correlation between changes in vasomotion and capillary recruitment in skin are likely to be representative of microvascular physiology in other vascular beds such as skeletal muscle (21, 41).

In conclusion, these data suggest that insulin's action to recruit capillaries involves stimulation of the sympathetic nervous system which enhances arteriolar vasomotion resulting in increased capillary perfusion and glucose uptake.

## Tables

Table 1 - characteristics of the study population

| characteristic  | mean±SD or median (range) |
|---|---------------------------|
| <i>n</i> (males)  | 19 (4)                    |
| age, y  | 30.5±10.5                 |
| body mass index, kg·m <sup>-2</sup>                                       | 25.6±5.3                  |
| systolic blood pressure, mm Hg  | 118±12                    |
| diastolic blood pressure, mm Hg   | 71±7                      |
| fasting plasma glucose, mmol/L  | 4.9±0.4                   |
| fasting plasma insulin, pmol/L  | 33.0 (13.4 - 86.4)        |
| M-value, insulin sensitivity, mg·lean kg <sup>-1</sup> ·min <sup>-1</sup> | 10.8±3.5                  |
| fasting HDL-cholesterol, mmol/L   | 1.4±0.3                   |
| 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                 |

Data are presented as mean±SD or median (range). M-value/insulin sensitivity indicates glucose infusion rate during a hyperinsulinemic, euglycemic clamp.

Table 2 - microvascular parameters of the study population

| characteristic                                      | mean±SD or median (range) |
|---|---------------------------|
| <i>laser Doppler</i>                                |                           |
| saline average, PU                                  | 8.4 (5 - 16)              |
| insulin average, PU                                 | 11.2 (5 - 66)**           |
| <i>capillary videomicroscope</i>                    |                           |
| saline, capillary density, n/mm <sup>2</sup>        | 40.8±7.9                  |
| saline, capillary density, peak, n/mm <sup>2</sup>  | 64.8±15.9                 |
| saline, capillary recruitment, %                    | 58.1±17.2                 |
| insulin, capillary density, n/mm <sup>2</sup>       | 40.4±8.1                  |
| insulin, capillary density, peak, n/mm <sup>2</sup> | 73.2±16.5***              |
| insulin, capillary recruitment, %                   | 81.4±22.6***              |
| insulin-augmented capillary recruitment, %-points   | 16.4 (0.4 – 51.1)         |

Data are presented as mean±SD (range). Saline indicates measurements during a saline (control) infusion. Insulin indicates measurements during the steady state of a hyperinsulinemic, euglycemic clamp. PU indicates (arbitrary) perfusion units. \*\* p<0.01, \*\*\* p<0.001 compared to saline

Table 3. Correlation analysis of changes in vasomotion amplitude, insulin-augmented capillary recruitment and insulin sensitivity in 19 subjects

| characteristic | change cardiac domain         |       | change respiratory domain |      | change myogenic domain |      | change neurogenic domain |      | change endothelial domain |       | insulin augmented recruitment |       |
|----------------|-------------------------------|-------|---------------------------|------|------------------------|------|--------------------------|------|---------------------------|-------|-------------------------------|-------|
|                | r                             | p     | r                         | p    | r                      | p    | r                        | p    | r                         | p     | r                             | p     |
|                | insulin augmented recruitment | -0.15 | 0.57                      | 0.09 | 0.72                   | 0.20 | 0.43                     | 0.51 | 0.04                      | -0.18 | 0.49                          | -     |
| M-value        | -0.13                         | 0.61  | -0.38                     | 0.12 | 0.03                   | 0.91 | 0.47                     | 0.06 | 0.04                      | 0.87  | 0.73                          | 0.001 |

Change indicates change in normalized amplitude as assessed by wavelet spectral analysis. M-value (insulin sensitivity) indicates glucose infusion rate per kilogram body weight per minute during a hyperinsulinemic, euglycemic clamp.

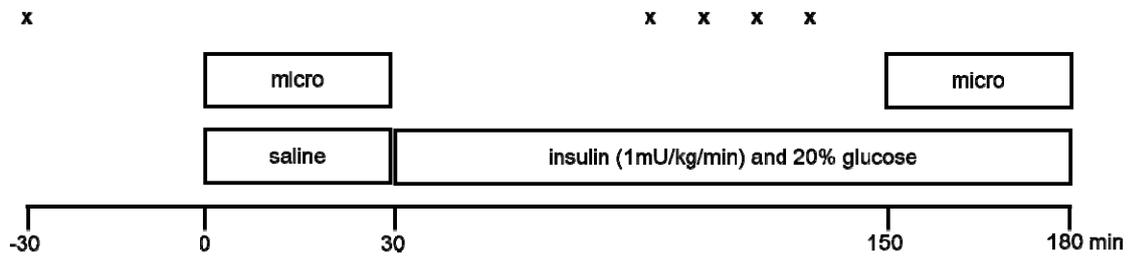
Table 4. Regression analysis with insulin-mediated glucose uptake as dependent variable in 19 subjects.

| Model                         | B    | 95%-CI         | standardized beta | P-value |
|-------------------------------|------|----------------|-------------------|---------|
| 1.                            |      |                |                   |         |
| change neurogenic domain      | 6.09 | 10.16 to 14.29 | 0.47              | 0.06    |
| 2.                            |      |                |                   |         |
| change neurogenic domain      | 0.88 | -4.71 to 6.46  | 0.07              | 0.741   |
| insulin augmented recruitment | 0.17 | 0.07 to 0.27   | 0.74              | 0.002   |

Model 1: bivariate with change in amplitude of the normalized neurogenic vasomotion domain as the independent variable. Model 2: multivariate; as model 1 with the addition of insulin augmented recruitment.

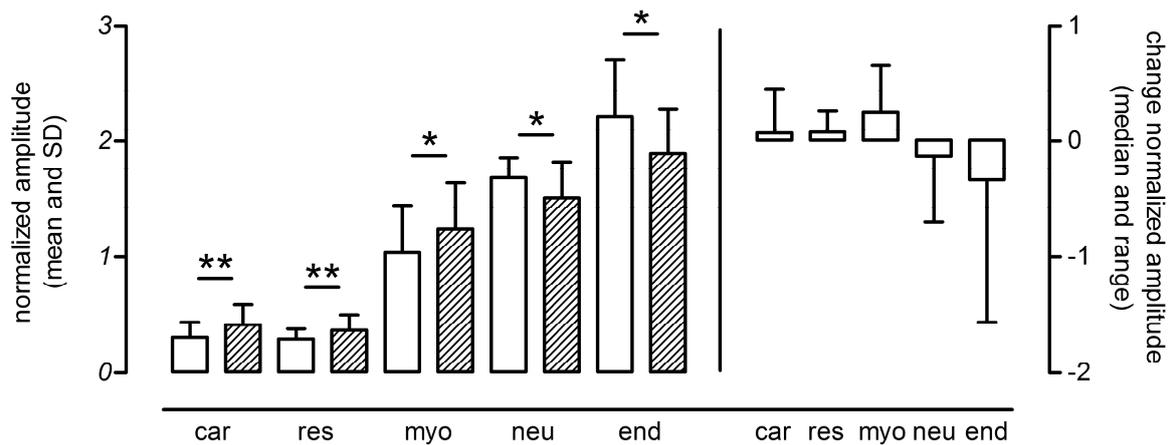
## Figures

figure 1



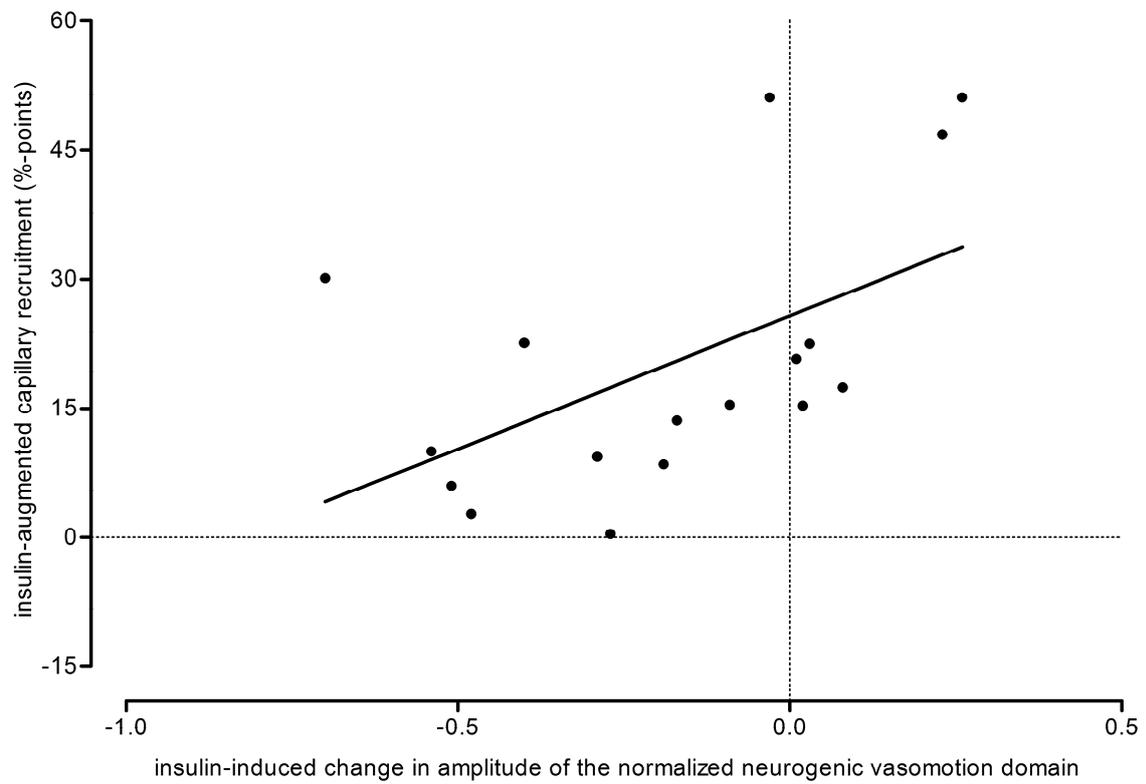
Design of the study. Micro indicates video-microscopy and LDF; x, blood samples for fasting measurements and insulin concentrations.

figure 2



Laser Doppler vasomotion output amplitudes. Relative amplitudes are normalized to the average amplitude of the whole spectrum; car indicates frequency spectrum attributed to heart beat, res indicates frequency spectrum attributed to respiration, myo indicates frequency spectrum attributed to vascular smooth muscle cells, neu indicates frequency spectrum attributed to SNS activity, and end indicates frequency spectrum attributed to endothelial activity. White bars during saline infusion, hatched bars during hyperinsulinemia. \*p < 0.05 compared to saline, \*\*p < 0.01 compared to saline.

figure 3



Association between insulin-augmented capillary recruitment in skin insulin-induced change in and amplitude of the normalized neurogenic vasomotion domain;  $n = 17$ ;  $r = 0.51$ ,  $p = 0.04$ .

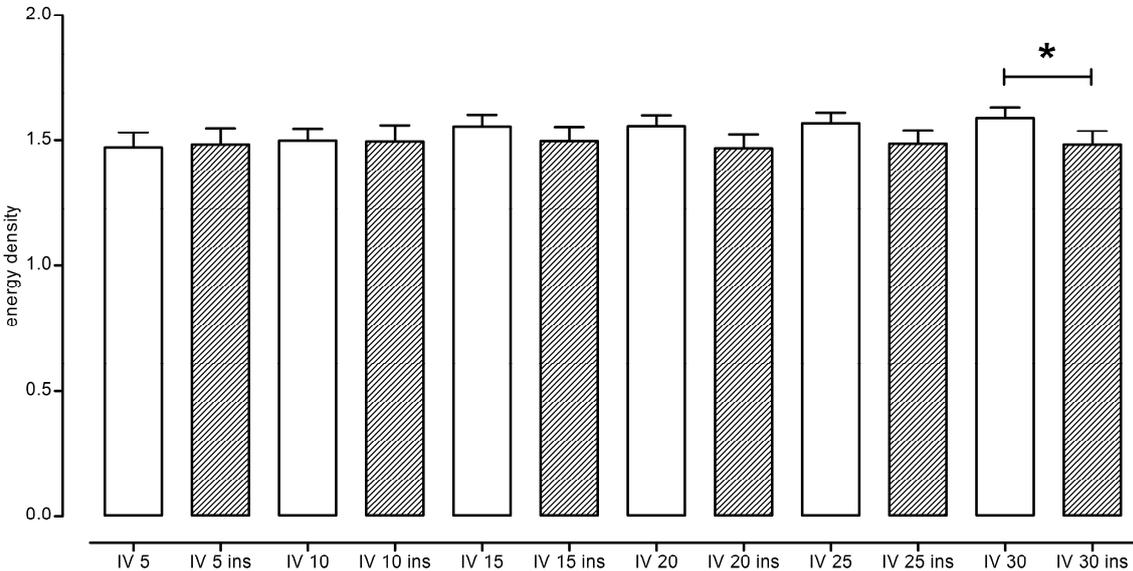
## References

- 1 Rucker M, Strobel O, Vollmar B, Roesken F, Menger MD. Vasomotion in critically perfused muscle protects adjacent tissues from capillary perfusion failure. *Am J Physiol Heart Circ Physiol* 279: H550-H558, 2000.
- 2 Pradhan RK, Chakravarthy VS. Informational dynamics of vasomotion in microvascular networks: a review. *Acta Physiol (Oxf)* 201: 193-218, 2011.
- 3 Sakurai T, Terui N. Effects of sympathetically induced vasomotion on tissue-capillary fluid exchange. *Am J Physiol Heart Circ Physiol* 291: H1761-H1767, 2006.
- 4 Tsai AG, Intaglietta M. Evidence of flowmotion induced changes in local tissue oxygenation. *Int J Microcirc Clin Exp* 12: 75-88, 1993.
- 5 Intaglietta M, Gross JF. Vasomotion, tissue fluid flow and the formation of lymph. *Int J Microcirc Clin Exp* 1: 55-65, 1982.
- 6 Baron AD. Hemodynamic actions of insulin. *Am J Physiol* 267: E187-E202, 1994.
- 7 Serné EH, IJzerman RG, Gans RO, Nijveldt R, De Vries G, Evertz R, Donker AJ, Stehouwer CD. Direct evidence for insulin-induced capillary recruitment in skin of healthy subjects during physiological hyperinsulinemia. *Diabetes* 51: 1515-1522, 2002.
- 8 de Jongh RT, Clark AD, IJzerman RG, Serné EH, de Vries G, Stehouwer CD. Physiological hyperinsulinemia increases intramuscular microvascular reactive hyperaemia and vasomotion in healthy volunteers. *Diabetologia* 47: 978-986, 2004.
- 9 Barrett EJ, Eggleston EM, Inyard AC, Wang H, Li G, Chai W, Liu Z. The vascular actions of insulin control its delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action. *Diabetologia* 52: 752-764, 2009.
- 10 Colantuoni A, Bertuglia S, Intaglietta M. Microvascular vasomotion: origin of laser Doppler flux motion. *Int J Microcirc Clin Exp* 14: 151-158, 1994.
- 11 Clough GF, L'Esperance V, Turzyniecka M, Walter L, Chipperfield AJ, Gamble J, Krentz AJ, Byrne CD. Functional dilator capacity is independently associated with insulin sensitivity and age in central obesity and is not improved by high dose statin treatment. *Microcirculation*. 18:74-84, 2011.
- 12 Kvernmo HD, Stefanovska A, Kirkeboen KA, Kvernebo K. Oscillations in the human cutaneous blood perfusion signal modified by endothelium-dependent and endothelium-independent vasodilators. *Microvasc Res* 57: 298-309, 1999.
- 13 Newman JM, Dwyer RM, St-Pierre P, Richards SM, Clark MG, Rattigan S. Decreased microvascular vasomotion and myogenic response in rat skeletal muscle in association with acute insulin resistance. *J Physiol* 587: 2579-2588, 2009.
- 14 deJongh RT, Serné EH, IJzerman RG, Jørstad HT, Stehouwer CD. Impaired local microvascular vasodilatory effects of insulin and reduced skin microvascular vasomotion in obese women. *Microvasc Res* 75: 256-262, 2008.
- 15 ADA. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 31: S55-S59, 2011.
- 16 van Genugten RE, Serné EH, Heymans MW, van Raalte DH, Diamant M. Postprandial microvascular function deteriorates in parallel with gradual worsening of insulin sensitivity and glucose tolerance in men with the metabolic syndrome or type 2 diabetes. *Diabetologia* 56: 583-587, 2013.
- 17 Ketel IJ, Stehouwer CD, Serné EH, Korsen TJ, Hompes PG, Smulders YM, de Jongh RT, Homburg R, Lambalk CB. Obese but not normal-weight women with polycystic ovary syndrome are characterized by metabolic and microvascular insulin resistance. *J Clin Endocrinol Metab* 93: 3365-3372, 2008.
- 18 Stefanovska A, Bracic 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.
- 19 Bracic M, Stefanovska A. Wavelet-based analysis of human blood-flow dynamics. *Bull Math Biol* 60: 919-935, 1998.
- 20 Graaff R, Morales F, Smit AJ, de Jong ED, de Mul FF, Rakhorst G. Normalization of vasomotion in laser Doppler perfusion monitoring. *Conf Proc IEEE Eng Med Biol Soc* 4076-4079, 2007.
- 21 Meijer RI, de Boer MP, Groen MR, Eringa EC, Rattigan S, Barrett EJ, Smulders YM, Serné EH. Insulin-induced microvascular recruitment in skin and muscle are related and both are associated with whole body glucose uptake. *Microcirculation* 19: 494-500, 2012.
- 22 DeFronzo RA, Tobin JD and Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237: E214-E223, 1979.
- 23 De Boer MP, Meijer RI, Wijnstok NJ, Jonk AM, Houben AJ, Stehouwer CD, Smulders YM, Eringa EC, Serné EH. Microvascular dysfunction: a potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension. *Microcirculation* 19: 5-18, 2012.

- 24 Keske MA, Clerk LH, Price WJ, Jahn LA, Barrett EJ. Obesity blunts microvascular recruitment in human forearm muscle after a mixed meal. *Diabetes Care* 32: 1672-1677, 2009.
- 25 Rossi M, Maurizio S, Carpi A. Skin blood flow motion response to insulin iontophoresis in normal subjects. *Microvasc Res* 70: 17-22, 2005.
- 26 Nilsson H, Aalkjaer C. Vasomotion: mechanisms and physiological importance. *Mol Interv* 3: 79-89, 2003.
- 27 Landsverk SA, Kvandal P, Bernjak A, Stefanovska A, Kirkeboen KA. The effects of general anesthesia on human skin microcirculation evaluated by wavelet transform. *Anesth Analg* 105: 1012-1019, 2007.
- 28 Rattigan S, Clark MG, Barrett EJ. Hemodynamic actions of insulin in rat skeletal muscle: evidence for capillary recruitment. *Diabetes* 46: 1381-1388, 1997.
- 29 Kastrop J, Bülow J, Lassen NA. Vasomotion in human skin before and after local heating recorded with laser Doppler flowmetry. A method for induction of vasomotion. *Int J Microcirc Clin Exp* 8: 205-215, 1989.
- 30 Söderström T, Stefanovska A, Veber M, Svensson H. Involvement of sympathetic nerve activity in skin blood flow oscillations in humans. *Am J Physiol Heart Circ Physiol* 284: H1638-H1646, 2003.
- 31 Stefanovska A, Sheppard LW, Stankovski T, McClintock PV. Reproducibility of LDF blood flow measurements: dynamical characterization versus averaging. *Microvasc Res* 82: 274-276, 2011.
- 32 Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J Clin Invest* 87: 2246-2252, 1991.
- 33 Agapitov AV, Correia ML, Sinkey CA, Haynes WG. Dissociation between sympathetic nerve traffic and sympathetically mediated vascular tone in normotensive human obesity. *Hypertension* 52: 687-695, 2008.
- 34 Ketel IJ, Stehouwer CD, Serné EH, Poel DM, Groot L, Kager C, Hompes PG, Homburg R, Twisk JW, Smulders YM, Lambalk CB. Microvascular function has no menstrual-cycle-dependent variation in healthy ovulatory women. *Microcirculation* 16: 714-724, 2009.
- 35 Kubota T, Kubota N, Kumagai H, Yamaguchi S, Kozono H, Takahashi T, Inoue M, Itoh S, Takamoto I, Sasako T, Kumagai K, Kawai T, Hashimoto S, Kobayashi T, Sato M, Tokuyama K, Nishimura S, Tsunoda M, Ide T, Murakami K, Yamazaki T, Ezaki O, Kawamura K, Masuda H, Moroi M, Sugi K, Oike Y, Shimokawa H, Yanagihara N, Tsutsui M, Terauchi Y, Tobe K, Nagai R, Kamata K, Inoue K, Kodama T, Ueki K, Kadowaki T. Impaired insulin signaling in endothelial cells reduces insulin-induced glucose uptake by skeletal muscle. *Cell Metab* 13: 294-307, 2011.
- 36 Clark MG, Rattigan S, Barrett EJ, Vincent MA. Point: There is capillary recruitment in active skeletal muscle during exercise. *J Appl Physiol* 104: 889-891, 2008.
- 37 Poole DC, Brown MD, Hudlicka O. Counterpoint: There is not capillary recruitment in active skeletal muscle during exercise. *J Appl Physiol* 104: 891-893, 2008.
- 38 Poole DC, Copp SW, Ferguson SK, Musch TI. Skeletal muscle capillary function: contemporary observations and novel hypotheses. *Exp Physiol* 98: 1645-1658, 2013.
- 39 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.
- 40 Rattigan S, Bradley E, Richards S, Keske M. Detection of microvascular flow motion within skeletal muscle by real-time contrast-enhanced ultrasound (RT-CEU) and laser Doppler flowmetry (LDF). *Diabetes* 62(supplement 1): A509, 2013.
- 41 Holowatz LA, Thompson-Torgerson CS and Kenney WL. The human cutaneous circulation as a model of generalized microvascular function. *J Appl Physiol* 105: 370-372, 2008.

Supplemental material

figure 1



The effect of increasing laser Doppler signal lengths on energy density output for the normalized neurogenic domain after wavelet analysis. Shown are separate wavelet analyses on 5, 10, 15, 20, 25, and 30 minutes of laser Doppler signal during saline (white) and insulin infusion (gray). \*p < 0.05.

