

Response to comment on: Semi-automatic assessment of skin capillary density: Proof of principle and validation

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Letter to the Editor

Response to comment on: Semi-automatic assessment of skin capillary density: Proof of principle and validation


We thank Dr. Neubauer-Geryk and co-workers for their comments concerning our recent article on the description of a semi-automatic technique for the assessment of skin capillary density (CapiAna) (Gronenschild et al., 2013). In their letter, three important issues were raised (Neubauer-Geryk et al.,).

First, Neubauer et al. mention that measuring changes in skin capillary density only partly addresses the phenomenon of skin microvascular reactivity. We fully agree with this issue. Nevertheless, the variables we used, i.e., hyperemic (functional) capillary recruitment and venous congestion, are thought to reflect functional and structural capillary density, respectively (Antonios et al., 1999; Serne et al., 2001). Indeed, skin capillaries in the resting state are thought to work on a 'rota system' (i.e., some are temporarily perfused, whereas others are temporarily shut down), and capillaries intermittently perfused in the resting state seem to be an important functional reserve that can be recruited during post-occlusive reactive hyperemia (PORH) (Antonios et al., 1999; Serne et al., 2001). In addition, venous congestion increases venous back pressure, which allows passive trapping of red blood cells in non-perfused and intermittently perfused capillaries, thereby enhancing the visualization of capillaries filled with red blood cells (i.e., allows visualization of the maximal number of skin capillaries) (Antonios et al., 1999; Serne et al., 2001). Thus, regardless of the issue that skin capillary density only partly addresses the phenomenon of skin microvascular reactivity, ample evidence is available indicating that hyperemic (functional) capillary recruitment and venous congestion are functional and structural, and therefore physiologically relevant, measures of the skin microcirculation, respectively.

Second, Neubauer et al. point out that the capillary densities as measured with CapiAna and the manual counting procedure were statistically different. We found, however, a borderline significant ($P = 0.06$) difference in baseline capillary density derived with CapiAna as compared to the manual counting procedure ($4.5 [-0.3; 9.3]$ capillaries/mm²). In addition, the capillary densities during hyperemic (functional) capillary recruitment and venous congestion did not differ significantly between CapiAna and the manual counting procedure. Next, analyzing agreement with Bland–Altman analysis is the most appropriate way to compare two methods (Bland and Altman, 1986). With a Bland–Altman analysis, we showed a mean difference of 2.0 capillaries/mm² between the methods with acceptable limits of agreement ($-13.5; 18.4$ capillaries/mm²). More importantly, we found no evidence for systematic errors between CapiAna and the manual counting procedure over the range of capillary densities studied. Thus, these results indicate that CapiAna can be used for a wide range of capillary densities.

Third, Neubauer et al. raise questions about the duration of ischemia and, additionally, about differences in skin capillary density between a

recovery time of 5 min (used in our study) as compared to the "typical" 10 min (Antonios et al., 1999). For the assessment of arterial occlusion, a miniature cuff was inflated to suprasystolic levels for 4 min, as described in materials and methods section (subsection "skin capillaroscopy"; page 193). The commonly used occlusion of 4 min (Jonk et al., 2010; Serne et al., 2001) is expected to cause vasodilatation through both the mechanism of myogenic vasodilatation and metabolic vasodilatory stimuli (Johnson et al., 1976). For logistic reasons (i.e., the use of an extensive phenotyping approach of the Maastricht Study (Schram et al., 2014)), we used a recovery time of 5 min between PORH and venous congestion. Unfortunately, we do not have data on possible differences in skin capillary density between recovery times of 5 min as compared to 10 min. Nevertheless, in line with previous literature (Antonios et al., 1999; Jonk et al., 2010; Serne et al., 2001), we clearly demonstrated that the maximal number of capillaries observed with venous congestion (95.2 ± 24.4 capillaries/mm²) exceeds that observed with PORH (87.4 ± 21.9 capillaries/mm²). Thus, these observations support the conclusion that the recovery time between conditions used in our study was sufficient.

In conclusion, assessment of skin microvascular reactivity by capillaroscopy, using different stimuli, may be an important diagnostic tool for the evaluation of skin microcirculation (Jonk et al., 2010; Neubauer-Geryk et al., 2013, 2014; Serne et al., 2001). In addition, CapiAna is in agreement with the manual counting procedure, has a better reproducibility as compared to the manual counting procedure and is time-saving. As a result, CapiAna facilitates the assessment of functional and structural skin capillary density, and thus the investigation of the skin microcirculation in health and disease.

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