Accepted Manuscript

Impaired metabolic profileis a predictor of capillary rarefaction in a population of hypertensive and normotensive individuals

Areti Triantafyllou, Panagiota Anyfanti, Georgios Triantafyllou, Xenophon Zabulis, Spyros Aslanidis, Stella Douma

PII: S1933-1711(16)30276-5

DOI: 10.1016/j.jash.2016.04.007

Reference: JASH 905

To appear in: *Journal of the American Society of Hypertension*

Received Date: 11 February 2016

Revised Date: 23 March 2016

Accepted Date: 17 April 2016

Please cite this article as: Triantafyllou A, Anyfanti P, Triantafyllou G, Zabulis X, Aslanidis S, Douma S, Impaired metabolic profileis a predictor of capillary rarefaction in a population of hypertensive and normotensive individuals, *Journal of the American Society of Hypertension* (2016), doi: 10.1016/j.jash.2016.04.007.

Impaired metabolic profileis a predictor of capillary rarefaction in a population of hypertensive and normotensive individuals

Areti Triantafyllou[1], Panagiota Anyfanti[1], Georgios Triantafyllou[1], Xenophon Zabulis[2], Spyros Aslanidis[3], Stella Douma[1]

[1]3rd Department of Internal Medicine, Papageorgiou Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece

[2]Institute of Computer Science, Foundation for Research and Technology–Hellas, Heraklion, Greece

[3]2nd Propedeutic Department of Internal Medicine, Hippokration Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece

Short title: Predictions of capillary rarefaction in hypertension

Conflicts of interest: The authors declare they have no conflicts of interest.

Financial disclosures: None.

Corresponding author

Panagiota Anyfanti, MD

Papageorgiou General Hospital

21 Ring Road Nea Efkarpia

22 56429, Thessaloniki, Greece

23 Tel: +30 2310 991 533

24 Fax: +30 2310 512 378

25 e-mail address: panyfan@hotmail.com

26

Abstract

Capillary rarefaction is typically encountered in essential hypertension, yet identification of factors interfering with this phenomenon remains substantially underinvestigated. We examined whether components of metabolic profile (dyslipidemia, insulin resistance), inflammatory (high sensitivity C-reactive protein, hsCRP) and angiogenic (vascular endothelial growth factor, VEGF) factors are implicated in this phenomenon in a population of newly-diagnosed, never-treated hypertensive patients and normotensive controls. Nailfold capillary density was estimated with nail fold capillaroscopy using specifically designed software. A total of 159individuals, 93 hypertensives and 66 normotensives, were included. Nailfold capillary density was lower among hypertensives compared to normotensives (146.4±31.0 vs 155.4±26.9 respectively, p=0.047). In the total population, capillary density significantly correlated with HDL (r=0.232, p=0.003), HDL/LDL ratio (r=0.175, p=0.025), age (r=0.236, p=0.003), but neither with VEGF or hsCRP. An inverse association was found with body mass index (r=–0.174, p=0.029), insulin levels (r=–0.200, p=0.018) and HOMA-IR (r=–0.223, p=0.009). In the separate analysis for the hypertensive population, sex (p=0.014) and HOMA-IR (p=0.011) were identified as significant predictors of capillary rarefaction after adjustment for other factors. On the contrary, only HDL levels (p=0.036) predicted capillary density in the multiple regression model for the normotensive population. Different aspects of impaired metabolic profile, i.e. insulin resistance and low HDL levels, but not angiogenic or inflammatory markers, appear to be independently associated with capillary rarefaction in hypertensive and normotensive individuals.

Keywords: capillary rarefaction, nailfold capillaroscopy, hypertension, metabolic profile, insulin resistance, dyslipidemia

Introduction

Cardiovascular diseases are thriving and the need for identification of early, easily applicable indices of subclinical microvascular organ damage, before the progression to overt cardiovascular disease, is urgent. The microvasculature is subject to a series of functional and morphological changes in cardiovascular diseases, with capillary rarefaction representing a typical and consistent finding (1-3). Dermal capillaries represent an ‘open’ and representative window for thein vivo study of human microcirculation that can be directly, repetitively and easily visualized by non-invasive techniques such as nailfold capillaroscopy (4). Nailfold capillaroscopy has been used as an estimate of the microvascular status in patients with cardiovascular diseases, and particularly in the field of hypertension. Of all the morphologic alterations that have been described in hypertensive patients by use of nailfold capillaroscopy, capillary rarefaction (i.e., decreased capillary density per visual field) has been recognized as an early characteristic feature of the disease, even in ‘naïve’ hypertensive patients (newly-diagnosed, never-treated, free from any other comorbidities) (5, 6).

Traditionally, capillary rarefaction in both experimental and clinical studies of hypertension is perceived as a consequence or even a cause of altered hemodynamics (4). However, other pathophysiological pathways in the cause-effect links between hypertension and capillary rarefactionmay be implicated. A postulated mechanism of hypertension-related capillary rarefaction involves the low-grade inflammatory response, which may contribute to the structural changes of the arterial wall and subsequent capillary loss(7). In addition, impaired angiogenesis may characterize arterial hypertension, indicated by an imbalance of pro-angiogenic, including vascular endothelial growth factor (VEGF), versus anti-angiogenic factors (8). Microvascular

rarefaction is a hallmark in patients treated with anti-VEGF molecules, implying a crucial role of VEGF in maintaining normal structural andfunctional microvasculature in humans (9).

Although the clinical significance of hypertension-relatedcapillary rarefactionis currently undergoing thorough investigation, the field appears uncharted when it comes to the potential impact of lipid metabolismon skin capillary density of hypertensive patients.Dyslipidemia is a major and well-established cardiovascular risk factors (10) and a very frequent comorbidity in hypertensive patients (11). Whether and to which extent it exerts similar to high blood pressure effects on capillary density remains unknown. In addition, while evidence suggests that insulin resistance is associated with microvascular abnormalities detected with nailfold capillaroscopy in patients with metabolic syndrome (12, 13), data are scarce when it comes to healthy individuals and newly diagnosed hypertensivepatients.

Therefore, the aim of the present study was to identify predictors of capillary rarefaction in a population of newly-diagnosed, never-treated, otherwise healthy hypertensive patients and healthy individuals, focusing on metabolic disturbances (dyslipidemia, insulin resistance). In addition, we investigated whether angiogenic (VEGF levels) and inflammatory (high sensitivity C-reactive protein, hsCRP) factors might contribute to decreased capillary density in our population.

Materials and Methods

Study population

Consecutive newly-diagnosed, never-treated, otherwise healthy patients with hypertension and/or dyslipidemia attending the hypertension outpatient clinic of our departmentwere included in the study. Healthy individuals attending their regular check-up appointments and healthy volunteers from the local community comprised the control group. A thorough medical history recording, physical examination, blood pressuremeasurement, routine laboratory testing, and nailfold capillaroscopy was performed to all participants. Exclusion criteria were a) secondary causes of hypertension, b) known chronic or familial diseases (including previously diagnosed hypertension), and c) regular medication use for any reason. All participants gave their written informed consent prior to participation. The study was conducted in accordance with the principles of Helsinki declaration and was approved by our hospital's ethics committee.

Blood pressure measurement

Office blood pressure was measuredin both arms using a validated oscillometric device (Microlife Exact BP, Microlife AG, Widnau, Switzerland) after 10 minutes rest..Office BP was recorded as the mean of the second and third value of three consecutive measurements with a two minute interval in the arm with the higher BP.

Blood samples and analyses

Blood was obtained between 9 am and 12 pm by venipuncture in resting conditions after overnight fasting. Total cholesterol (TC), triglycerides (TG),LDL- and HDL-cholesterol were measured by conventional enzymatic method (Olympus AU560, Hamburg, Germany).Dyslipidemia was defined as TC≥240 mg/dL and/or LDL≥160 mg/dL, HDL<40mg/dLTG≥200 mg/dL(14).

Insulin levels were estimated with immunoradiometric assay (IRMA). Insulin resistance was evaluated with the homeostasis model assessment-insulin resistance (HOMA-IR) index, based on the equation HOMA-IR=fasting glucose (mmol/l)*fasting insulin (μU/ml)/22.5(15).

VEGF and hs-CRP were used as markers of angiogenesis (16) and vascular inflammation (17) respectively.Both were measured in patient's serum by the use of immunoenzymatic ELISA method (Quantikine® Human VEGF Immunoassay kit, DVE00, R&D systems, Inc Minneapolis USA, and high sensitive CRP ELISA kit, IBL, Hamburg, Germany).

Nailfold capillaroscopy

Nailfold capillary density was estimated with nailfold capillaroscopy (DS Medica, Milan Italy, x200 magnification). Images of the distant phalanx were taken after mineral oil placement to improve image quality, where the capillaries could be seen in transverse section. The subjects were seated with the hand supported. All procedures were conducted in the same temperature-controlled room. In order to determine the capillary number/visual field, a semiautomated software was developed in collaboration with the Foundation for Research and Technology-Hellas(18). The qualitatively best two pictures of each participant were analyzed using previously describedmethodology by a grader blind to the subject's identity, blood pressure and dyslipidemia status (6).

Statistical analysis

Analysis was performed using the Statistical Package for Social Sciences (SPSS) 22.Student t or Mann Whitney test was used to estimate differences between mean

values of two groups. Analysis of qualitative variables was made with Chi Square test. Correlation coefficients were calculated with Pearson and Spearman rank tests. Multivariate linear regression analysis (enter method) was used to examine which of the covariates can independently predict capillary rarefaction (numbers of capillaries per visual field). A probability value of $p \leq 0.05$ was considered statistically significant.

Results

A total of 159individuals, 93 hypertensives and 66 normotensives aged 43±12 years, were included in this study. Baseline characteristics of the study are presented in Table 1.Patients with hypertensionexhibited significantly lower mean nailfold capillary number/visual field compared to individuals with normal blood pressure (146.4±31.0 vs155.4±26.9respectively, p=0.047).

In the univariate analysis for the total population, capillary density significantly correlated with age (r=0.236, p=0.003), BMI (r= –0.174, p=0.029), HDL (r=0.232, p=0.003),HDL/LDL ratio (r=0.175, p=0.025), insulin levels (r=–0.200, p=0.018and HOMA-IR (r=–0.223, p=0.009). No significant associations were observed between capillary density and either systolic/diastolic blood pressure (SBP/DBP), HDL, LDL, triglycerides, VEGF, hsCRP or aldosterone levels (p>0.05 for all).In the linear regression analysis for the total population (Table 2a), only age (p=0.025) and sex (p=0.002) remained significant predictors of capillary density. However, when we repeated the regression analysis separately for the hypertensive and normotensive population, sex (p=0.014) and HOMA-IR (p=0.011) were identified as significant predictors of capillary rarefaction among hypertensive patients (Table

178 2b), even after adjustment for the same factors (age, BMI, SBP, HDL). On the
179 contrary, only HDL levels (p=0.036) significantly affected capillary density in the
180 normotensive population in the multiple regression model (Table 2c).

181 Since an adverse metabolic profile appeared to affect capillary rarefaction in
182 our population subgroups, we further stratified our population according to their
183 serum lipid levels to those with newly diagnosed dyslipidemia and those with normal
184 lipid profile. Capillary density did not differ between hypertensive patients with
185 normal and elevated lipid levels (150.9±31.6 vs 141.7±30.0, p=0.157). On the
186 contrary, individuals with normal blood pressure and lipid levels exhibited
187 significantly higher capillary density compared to normotensive individuals with
188 dyslipidemia (160.3±26,4 vs 140.5±23.5, p=0.008).

189

190 **Discussion**

191 Over the previous years, accumulatingdata support that capillary rarefaction,either
192 alone or as a component of diffusemicrovascular damage, may denote higher
193 individual cardiovascular risk(6, 19).In this concept,identification of factors
194 associated with capillary rarefaction is not only important for an in-depth
195 understanding of the underlying pathophysiological mechanisms, but may help
196 efficiently predict this relatively early phenomenon in the course of essential
197 hypertension and lead towards the development of protective therapies and
198 subsequent treatments. For this purpose, we included a variety of factors potentially
199 interfering with capillary density in a meticulously selected population of newly-
200 diagnosed, never-treated, otherwise healthy patients with hypertension and healthy
201 individuals. The results of our study point towards an adverse metabolic profile as a

significant contributor to capillary rarefaction. In addition, different aspects of impaired metabolic profile appear to be implicated in capillary rarefaction in normotensive and hypertensive individuals.

In particular, we showed for the first time a significant association between nailfold capillary density and HDL cholesterol in normotensive, healthy individuals. On the contrary, no association was observed between capillary rarefaction andthe rest lipid fractions. This finding could be attributed to some of the non-classic, "pleiotropic", atheroprotective actions of HDL on the vasculature, which have been tested in both human and experimental studies. Some of these mechanisms involve the downregulation of cytokine-induced expression of cell adhesion molecules (CAMs), the inhibition of cellular adhesion molecules expression and complement activation, and the increase in prostacyclin release, endothelial nitric oxide synthase (eNOS) expression and activation and subsequent NO release and bioavailability (20-22). These vasoprotective effects of HDL mightcontribute to the preservation of capillary density in individuals with no underlying clinical pathology, but this hypothesis warrants further testing in the appropriate settings.

Interestingly, our study also shows that patients with dyslipidemia exhibit decreased nailfold capillary density. Very few studies have focused on this relationship; in a previous study with a small and diverse population (9 elderly treated and 9 elderly untreated individuals, 15 elderly and 25 young controls)differences in morphological and functional dermal capillary features, but not in capillary density, were observed (23). Available indirect data appear in agreement with our results, based on the observed improvement of functional microcirculatory indices after treatment with either fluvastatin (24) or fenofibrate(25).

Among hypertensive patients, on the other hand,insulin resistance was identified as a significant prognostic factor of capillary rarefaction.This novel finding seems to not only confirm but further extend previous observations in smaller groups of patients with metabolic syndrome (12, 13). In both studies, patients exhibited lower functional dermal capillary density compared to controls. In the first study of 36 elderly patients, a direct inverse association of capillary density with insulin resistance was demonstrated (13). In the second study of 36 relatively young patients, insulin resistance was not associated with capillary density, but with other indices of functional and morphological abnormalities detected by nailfold videocapillaroscopy (12). These studies added fuel to previous observations regarding the pathophysiological background of the reported association. Consistent with the vasodilatory effects of insulin, systemic hyperinsulinemia in skin induces recruitment of capillaries and a rapid increase in total skin blood flow in healthy, normoglycemic individuals (26). Treatment of normoglycemic subjects with metabolic syndromewith an insulin-sensitizing agent significantly improves functional dermal capillary density (27). On the other hand, microcirculatory abnormalities have been hypothesized as a cause of insulin resistance (28), and reduced capillary blood flow may precipitate insulin resistance in muscle and other organs (29, 30). Our study reveals that this association is present not only in individuals with clinically evident metabolic impairment as part of the metabolic syndrome, but in individuals with newly diagnosed, never-treated hypertension as well. Therefore, it could be hypothesized that the intercorrelation between high blood pressure and capillary rarefaction is not entirely straightforward, at least to the extent that is generally perceived, but insulin resistance may in fact represent a significant intermediate component in the mutually

reinforcing triad between capillary rarefaction, metabolic disturbances, and hypertension.

Silent, low-grade inflammation of the vasculature has been proposed as a precursor of essential hypertension and a critical contributor to the development of associated micro- and macrovascular damage (7). Higher levels of CRP were associated with both insulin resistance and skin nailfold capillary density in a population of 295 relatively young individuals without hypertension or diabetes (31) . However, we failed to demonstrate an association between hsCRP and capillary density, either in our total populationor in the study subgroups. The smaller number of participants in our study, or the different composition of the population of the previous study (45% African- or Asian American, 63% females, with alcohol consumption reported by 77%) might account for the divergent results. Either way, further studies are needed to establish whether and to what extent inflammatory markers correlate with structural microvascular abnormalities independently of other factors and in the presence or absence of comorbid conditions modulating the systemicinflammatory milieu.

Another interesting point in our study is the observed lack of association between VEGF and capillary density in our population. Most data regarding the role of VEGF in hypertension have been extrapolated from studies in patients with various types of cancer receiving VEGF inhibitors, who subsequently developed secondary hypertension. A significant association between capillary rarefaction and administration of VEGF inhibitors has been previously observed in patients with metastatic colorectal cancer (32) as well as advanced solid tumors (33), suggesting a possible pathophysiological link. However, such results should be addressed with circumspection in the field of essential hypertension, which is an entirely distinct

clinical entity. Surprisingly, data regarding VEGF and capillary rarefaction in essential hypertension are scarce. Our study failed to demonstrate an association between VEGF levels and capillary density. Even though VEGF has been proposed an additional modulator of endothelium depended-vascular tone even in normal individuals (16), normaladult vasculature is widely regarded as largely independentof VEGF for survival, stability, and normal function, unliketumor vessels, for which VEGF is a survival factor(34). In addition, contrary to a previous study by Marek-Trzonkowska et al(8), we did not observe any differences in VEGF levels between hypertensive and normotensive individuals. However, only untreated patients with a perceived relatively short duration of hypertension were included in our study, whereas the previous study included patients treated under antihypertensive treatment for a median periodof 6.5 years. Further studies are needed to elucidate the role of VEGF in the field of essential hypertension and in terms of capillary rarefaction.

Finally, it should be noted that a capillary rarefaction seemed to be decreasing with age in our population. Even though this association was not confirmed in the separate analyses for the hypertensive and the normotensive population, it remained significant in the multivariate regression model. There are few data suggesting that capillary rarefaction is more pronounced with increasing age, but they mostly emerge from other vascular beds (i.e., skeletal muscle) (35).Surprisingly, previous studies on dermal capillary rarefaction have either not explored whether an association exists with age(12, 36),or failed to identify such an association (37).In the light of the above, we believe that our finding should be addressed with circumspection, until further studies establish whether an association exists between skin capillary rarefaction and age, and towards which direction.

Overall, our study attempted to identify potential factors implicated in capillary rarefaction in hypertensive and normotensive individuals, showing that insulin resistance in hypertensivesand low levels of HDL in normotensives, but not VEGF or hsCRP, are independent predictors of decreased capillary density. Our study has certain limitations, including the recording of nailfold capillaroscopy measurements in the resting condition only. Thismethodology accounts for structural rarefaction only and not functional rarefaction, which is typically assessed by applying modest forearm pressure (venous occlusion) as a maneuver to reveal some dormant or under-perfused capillaries for the maximization of capillary density. Althoughthe reduction in capillary density in hypertensionhas beenprimarily attributed to the structural (anatomic) absence of capillaries rather than functional nonperfusion (36), functional capillary rarefaction is also important since it is considered to precede irreversible structural rarefaction (38). Furthermore, given the associative nature of our results, it cannot be deducted whether metabolic disturbances trigger microvascular abnormalities, or vice versa. However, the direction of this association might not be so important in clinical terms, where it should rather serve as a reminder of the early identification and proper management of metabolic disturbances not only in hypertensive patients, but also in asymptomatic individuals without clinically evident cardiovascular diseases.

Highlights

- Insulin resistance predicts capillary rarefaction among hypertensive patients
- Low HDL levels are associated with capillary rarefaction in normotensive subjects
- Neither VEGF nor hsCRP correlated with capillary density in our population

References

1. Nazzaro P, Schirosi G, Mezzapesa D, Petruzzellis M, Pascazio L, Serio G, et al. Effect of clustering of metabolic syndrome factors on capillary and erebrovascular impairment. European journal of internal medicine. 2013;24(2):183-8.

2. Thang OH, Serne EH, Grooteman MP, Smulders YM, ter Wee PM, Tangelder GJ, et al. Capillary rarefaction in advanced chronic kidney disease is associated with high phosphorus and bicarbonate levels. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2011;26(11):3529-36.

3. Tibirica E, Souza EG, De Lorenzo A, Oliveira GM. Reduced systemic microvascular density and reactivity in individuals with early onset coronary artery disease. Microvascular research. 2015;97:105-8.

4. Triantafyllou A, Anyfanti P, Pyrpasopoulou A, Triantafyllou G, Aslanidis S, Douma S. Capillary rarefaction as an index for the microvascular assessment of hypertensive patients. Current hypertension reports. 2015;17(5):543.

5. Serne EH, Gans RO, ter Maaten JC, Tangelder GJ, Donker AJ, Stehouwer CD. Impaired skin capillary recruitment in essential hypertension is caused by both functional and structural capillary rarefaction. Hypertension. 2001;38(2):238-42.

6. Triantafyllou A, Anyfanti P, Zabulis X, Gavriilaki E, Karamaounas P, Gkaliagkousi E, et al. Accumulation of microvascular target organ damage in newly diagnosed hypertensive patients. Journal of the American Society of Hypertension : JASH. 2014;8(8):542-9.

7. Pauletto P, Rattazzi M. Inflammation and hypertension: the search for a link. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2006;21(4):850-3.

8. Marek-Trzonkowska N, Kwieczynska A, Reiwer-Gostomska M, Kolinski T, Molisz A, Siebert J. Arterial Hypertension Is Characterized by Imbalance of Pro-Angiogenic versus Anti-Angiogenic Factors. PloS one. 2015;10(5):e0126190.

9. Mourad JJ, Levy BI. Mechanisms of antiangiogenic-induced arterial hypertension. Current hypertension reports. 2011;13(4):289-93.

10. Reiner Z, Catapano AL, De Backer G, Graham I, Taskinen MR, Wiklund O, et al. ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). European heart journal. 2011;32(14):1769-818.

11. Martell-Claros N, Galgo-Nafria A. Cardiovascular risk profile of young hypertensive patients: the OPENJOVEN study. European journal of preventive cardiology. 2012;19(3):534-40.

12. Czernichow S, Greenfield JR, Galan P, Jellouli F, Safar ME, Blacher J, et al. Macrovascular and microvascular dysfunction in the metabolic syndrome. Hypertension research : official journal of the Japanese Society of Hypertension. 2010;33(4):293-7.

13. Kraemer-Aguiar LG, Laflor CM, Bouskela E. Skin microcirculatory dysfunction is already present in normoglycemic subjects with metabolic syndrome. Metabolism: clinical and experimental. 2008;57(12):1740-6.

14. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of

High Blood Cholesterol In Adults (Adult Treatment Panel III). Jama. 2001;285(19):2486-97.

15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

16. Thijs AM, van Herpen CM, Sweep FC, Geurts-Moespot A, Smits P, van der Graaf WT, et al. Role of endogenous vascular endothelial growth factor in endothelium-dependent vasodilation in humans. Hypertension. 2013;61(5):1060-5.

17. Lazich I, Sarafidis P, de Guzman E, Patel A, Oliva R, Bakris G. Effects of combining simvastatin with rosiglitazone on inflammation, oxidant stress and ambulatory blood pressure in patients with the metabolic syndrome: the SIROCO study. Diabetes, obesity & metabolism. 2012;14(2):181-6.

18. Karamaounas P ZX. Capillaroscope2011; Institute of Computer Science-FORTH: Heraklion Greece. Available from: users.ics.forth.gr/~zabulis/2011.TR417_Capillaroscope.pdf.

19. Debbabi H, Uzan L, Mourad JJ, Safar M, Levy BI, Tibirica E. Increased skin capillary density in treated essential hypertensive patients. American journal of hypertension. 2006;19(5):477-83.

20. Gomaraschi M, Ossoli A, Pozzi S, Nilsson P, Cefalu AB, Averna M, et al. eNOS activation by HDL is impaired in genetic CETP deficiency. PloS one. 2014;9(5):e95925.

21. Hamilton KK, Zhao J, Sims PJ. Interaction between apolipoproteins A-I and A-II and the membrane attack complex of complement. Affinity of the apoproteins for polymeric C9. The Journal of biological chemistry. 1993;268(5):3632-8.

22. Xiong SL, Liu X, Yi GH. High-density lipoprotein induces cyclooxygenase-2 expression and prostaglandin I-2 release in endothelial cells through sphingosine kinase-2. Molecular and cellular biochemistry. 2014;389(1-2):197-207.

23. Lopes FG, Bottino DA, Oliveira FJ, Mecenas AS, Clapauch R, Bouskela E. In elderly women moderate hypercholesterolemia is associated to endothelial and microcirculatory impairments. Microvascular research. 2013;85:99-103.

24. Haak E, Abletshauser C, Weber S, Goedicke C, Martin N, Hermanns N, et al. Fluvastatin therapy improves microcirculation in patients with hyperlipidaemia. Atherosclerosis. 2001;155(2):395-401.

25. Haak T, Haak E, Kusterer K, Weber A, Kohleisen M, Usadel KH. Fenofibrate improves microcirculation in patients with hyperlipidemia. European journal of medical research. 1998;3(1-2):50-4.

26. Serne EH, RG IJ, Gans RO, Nijveldt R, De Vries G, Evertz R, et al. Direct evidence for insulin-induced capillary recruitment in skin of healthy subjects during physiological hyperinsulinemia. Diabetes. 2002;51(5):1515-22.

27. Kraemer de Aguiar LG, Laflor CM, Bahia L, Villela NR, Wiernsperger N, Bottino DA, et al. Metformin improves skin capillary reactivity in normoglycaemic subjects with the metabolic syndrome. Diabetic medicine : a journal of the British Diabetic Association. 2007;24(3):272-9.

28. Wiernsperger N, Nivoit P, De Aguiar LG, Bouskela E. Microcirculation and the metabolic syndrome. Microcirculation (New York, NY : 1994). 2007;14(4-5):403-38.

29. Jonk AM, Houben AJ, de Jongh RT, Serne EH, Schaper NC, Stehouwer CD. Microvascular dysfunction in obesity: a potential mechanism in the pathogenesis of

obesity-associated insulin resistance and hypertension. Physiology (Bethesda, Md). 2007;22:252-60.

30. Serne EH, de Jongh RT, Eringa EC, RG IJ, Stehouwer CD. Microvascular dysfunction: a potential pathophysiological role in the metabolic syndrome. Hypertension. 2007;50(1):204-11.

31. Cheng C, Daskalakis C. Association of Adipokines with Insulin Resistance, Microvascular Dysfunction, and Endothelial Dysfunction in Healthy Young Adults. Mediators of inflammation. 2015;2015:594039.

32. Mourad JJ, des Guetz G, Debbabi H, Levy BI. Blood pressure rise following angiogenesis inhibition by bevacizumab. A crucial role for microcirculation. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2008;19(5):927-34.

33. Steeghs N, Gelderblom H, Roodt JO, Christensen O, Rajagopalan P, Hovens M, et al. Hypertension and rarefaction during treatment with telatinib, a small molecule angiogenesis inhibitor. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008;14(11):3470-6.

34. Longo R, Sarmiento R, Fanelli M, Capaccetti B, Gattuso D, Gasparini G. Anti-angiogenic therapy: rationale, challenges and clinical studies. Angiogenesis. 2002;5(4):237-56.

35. Groen BB, Hamer HM, Snijders T, van Kranenburg J, Frijns D, Vink H, et al. Skeletal muscle capillary density and microvascular function are compromised with aging and type 2 diabetes. Journal of applied physiology (Bethesda, Md : 1985). 2014;116(8):998-1005.

36. Antonios TF, Singer DR, Markandu ND, Mortimer PS, MacGregor GA. Structural skin capillary rarefaction in essential hypertension. Hypertension. 1999;33(4):998-1001.

37. Prasad A, Dunnill GS, Mortimer PS, MacGregor GA. Capillary rarefaction in the forearm skin in essential hypertension. Journal of hypertension. 1995;13(2):265-8.

38. Cheng C, Diamond JJ, Falkner B. Functional capillary rarefaction in mild blood pressure elevation. Clinical and translational science. 2008;1(1):75-9.

454 **Table 1.Baseline characteristics of the study population.**

	Overall	Hypertensives	Normotensives	P value
Number of participants (M/F)	159 (106/53)	93 (72/21)	66 (34/32)	0.001
Smoking (%)	34.2	23.4	10.8	NS
LDL > 160 (%)	16.4	12.6	3.8	0.049
Dyslipidemia (%)	23.3	17	6.3	NS
Age (years)	43.1 ±12.0	44.2±11.8	41.5 ± 12.2	NS
BMI (kg/m^2)	27.1±4.1	27.7±3.9	26.2±4.2	0.025
Visit SBP (mmHg)	138±17.9	149.2± 13.2	122.2 ± 10.1	<0,001
Visit DBP (mmHg)	87.7 ±12.5	94.5 ± 10.4	78 ± 8.2	<0,001
Heart rate (bpm)	69.2 ±9.9	70.8±10.7	66.9±8.2	0.01
Total cholesterole (mg/dL)	197.8±39.6	203.8±42.4	189.4±33.9	0.024
Triglycerides (mg/dL)	98 (72-138)	97 (73-148)	103 (69.25-132.5)	NS
LDL (mg/dL)	126.3 ±34.5	132.8 ±34.6	117.1±32.4	0.004
HDL (mg/dL)	48.2±11.7	47.1 ± 12.3	49.8± 10.8	NS
Capillaries/visual field	150.19 ±29.6	146.4 ± 31.0	155.5 ±26.9	0.047
HDL/LDL ratio	0.38 (0.30-0.5)	0.34 (0.28-0.46)	0.44(0.35-0.53)	0.001
HOMA-IR	1.75 (1.16-3)	1.76(1.17-3.13)	1.75(1.05-2.73)	NS
Insulin (µU/ml)	7.9 (5.56-12)	8 (5.65-12.5)	7.7 (5.35-11.85)	NS
VEGF(pg/ml)	301.7(205.23-444.48)	295 (200.79-424.86)	310 (213.8-490)	NS
hsCRP (µg/ml)	1.08 (0.16-2.74)	1.32(0.23-3.13)	0.71(0.08-2.24)	NS
Glucose (mg/dL)	90 (83-100)	93.2 (85-101)	87 (81-94.25)	0.017
Uric acid (mg/dL)	5.1 ± 1.44	5.29±1.46	4.88 ±1.4	NS

PRA (ng/ml*hour)	0.62 (0.21-1.4)	0.51 (0.21-1.4)	0.84(0.21-1.49)	NS
Aldosterone (ng/dL)	11.8±6.4	12.7 ± 6.43	7.6 (5.4-13.7)	0.013

NS: non-significant, M: male, F: female, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, bpm: beats per minute, HDL: high density lipoprotein, LDL: low density lipoprotein, HOMA-IR: homeostatic model assessment- insulin resistance, VEGF: vascular-endothelial growth factor, hsCRP: high-sensitivity C-reactive protein, PRA: plasma renin activity

Data are presented as mean±standard deviation for normally distributed variables and as median (Q1-Q3) for non-normally distributed variables.

455

456

457 **Table 2. Multiple regression models (a) for the total population, (b) for the hypertensive**

458 **population and (c) for the normotensive population.**

459

Multiple linear regression models of capillary density												
	(a) Total Population (R^2=0.217, adjusted R^2 = 0.180, p<0.001)				(b) Hypertensive Population (R^2=0.215, adjusted R^2 = 0.151, p=0.005)				(c) Normotensive Population (R^2=0.352, adjusted R^2 = 0.271, p=0.001)			
			CI 95%				CI 95%				CI 95%	
	Unst. C	P	LB	UB	Unst. C	P	LB	UB	Unst. C	P	LB	UB
Constant	143.85	<0.001	90.924	196.776	161.53	0.001	69.186	253.875	102.799	0.029	10.969	194.628
Age (years)	0.486	0.025	0.063	0.909	0.434	0.163	-0.18	1.048	0.453	0.113	-0.112	1.019
BMI (kg/m^2)	-0.91	0.175	-2.231	0.411	-0.462	0.619	-2.304	1.381	-0.865	0.353	-2.72	0.99
Sex	18.039	0.002	6.667	29.41	21.728	0.014	4.547	38.909	11.536	0.124	-3.288	26.36
SBP (mmHg)	-0.085	0.547	-0.365	0.194	-0.094	0.718	-0.612	0.423	0.005	0.988	-0.646	0.659
HDL (mg/dL)	0.028	0.905	-0.429	0.484	-0.414	0.167	-1.004	0.177	0.795	0.036	0.053	1.538
HOMA-IR	-13.386	0.108	-29.728	2.957	-6.357	0.011	-11.214	-1.499	-3.131	0.75	-22.808	16.545
BMI: body mass index, SBP: systolic blood pressure, HDL: high density lipoprotein, HOMA-IR: homeostatic model assessment- insulin resistance												

460

461