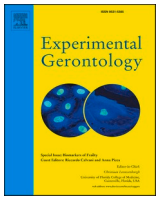
Experimental Gerontology 192 (2024) 112457

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero



Endothelial function in healthy centenarians living in the Madonie's district (Italy)

Carola Buscemi [a,b,1], Cristiana Randazzo [b,c,1], Anna Maria Barile [b,c,*], Roberta Caruso [b], Piero Colombrita [b], Martina Lombardo [b], Piergiorgio Lo Verde [b], Natalia Sottile [b], Mario Barbagallo [b,d], Silvio Buscemi [b,c]

[a] Unit of Internal Medicine, "V. Cervello Hospital", Palermo, Italy
[b] Dipartimento di Promozione della Salute, Materno-Infantile, Medicina Interna e Specialistica di Eccellenza (PROMISE), University of Palermo, Palermo, Italy
[c] Unit of Clinical Nutrition, Obesity and Metabolic Diseases, University Hospital Policlinico "P. Giaccone", Palermo, Italy
[d] Geriatric Unit, University Hospital Policlinico "P. Giaccone", Palermo, Italy

ARTICLE INFO

Section Editor: Marzetti Emanuele

Keywords:
Aging
Atherosclerosis
Centenarians
Endothelial function
Flow-mediated dilation

ABSTRACT

Background: Endothelial function declines with age and plays a critical role in cardiovascular health. Therefore, investigating endothelial function in successful aging models, such as centenarians, is of interest. Flow-mediated dilation (FMD) of the brachial artery is the gold standard for measuring endothelial function in vivo in humans. Therefore, we investigated, for the first time, the FMD of the brachial artery in a group of healthy centenarians.
Methods: Selected as part of the ABCD project (nutrition, cardiovascular wellness, and diabetes) centenarians (aged ≥100 years) living in the municipalities of Madonie (Palermo, Italy) were compared with a younger (aged <65 years) sex-matched control group from the ABCD general cohort. FMD of the brachial artery was measured in all participants using a real-time computed video analysis system for B-mode ultrasound images. Body composition (bioimpedance), carotid intima-media thickness (IMT), and ankle-brachial index (ABI) were also measured.
Results: Eleven participants (males 36.4 %; age: 101 ± 1 years) out of 28 healthy centenarians successfully cooperated with the FMD test procedures, which require remaining with the upper limb immobile for approximately 10 min. This subgroup was compared with a control group of 76 healthy and younger individuals (males 36.8 %; aged: 41 ± 14 years; $P < 0.001$). Centenarians exhibited better endothelial function than the control group (FMD: 12.1 ± 4.3 vs 8.6 ± 5.3 %; $P < 0.05$). The carotid IMT was higher in the centenarian group than in the control group (0.89 ± 0.09 vs 0.56 ± 0.18 mm; $P < 0.001$), whereas the ABI was comparable between the two groups.
Conclusions: This small group of centenarians demonstrated an unusually favorable endothelial function, which may contribute to their unique aging profile. Further research is needed to determine whether FMD is a valid prognostic marker for successful aging.

1. Introduction

Aging constitutes a worldwide public health issue, prompting a keen interest in investigating models of successful aging, with centenarians serving as noteworthy exemplars. This special population can evade or survive age-related diseases, including cardiovascular diseases, cancer, and diabetes. Endothelial function plays a critical role in atherosclerosis (Davignon and Ganz, 2004) and aging (Brandes et al., 2005). Endothelial dysfunction is an essential requisite for atherosclerosis, resulting from an imbalance between oxidant and antioxidant factors due to genetic, nutritional and environmental causes (Seals et al., 2011). In vivo and in vitro (Bloom et al., 2023; Kalies et al., 2024) studies demonstrated that endothelial cells undergo senescence-related processes that may be mitigated by genetic and environmental factors. Therefore,

* Corresponding author at: Unit of Clinical Nutrition, Obesity and Metabolic Diseases, University Hospital Policlinico "P. Giaccone", Piazza delle Cliniche, 2, 90127 Palermo, Italy.
E-mail addresses: annamaria.barile@unipa.it (A.M. Barile), silvio.buscemi@unipa.it (S. Buscemi).
[1] Carola Buscemi and Cristiana Randazzo contributed equally to the study.

https://doi.org/10.1016/j.exger.2024.112457
Received 1 April 2024; Received in revised form 27 April 2024; Accepted 8 May 2024

healthy aging is expected to be associated with good endothelial function, particularly in centenarians and ultracentenarians. However, to date, no study has investigated endothelial function in centenarians. Flow-mediated dilation (FMD) of the brachial artery is the gold-standard test for measuring endothelial function in vivo in humans (Celermajer et al., 1992). Therefore, we investigated, for the first time, FMD in a group of healthy centenarians living in Madonie, a district in Palermo, Italy, with a high prevalence of centenarians.

2. Methods

2.1. Study population

Centenarians (aged ≥100 years) residing in the municipalities of the Madonie area (1728 km^2 with 61.500 inhabitants) near Palermo (Italy) were contacted, through municipal administrations, including their caregivers. Fifty centenarians were identified (estimated prevalence of centenarians: 1/1230 residents); of these, 28 consented to be examined in their homes between 19th and 30th of July 2021. A team of researchers, comprising doctors and dieticians, performed all evaluations. This project was approved by the Palermo 1 Ethics Committee (Ref. # 6/2021) and is part of the ABCD project (Nutrition, Cardiovascular Wellness, and Diabetes; ISRCTN15840340). The centenarian group was compared with healthy individuals (those without diabetes, known cardiovascular diseases except for hypertension, tumors, or known chronic diseases were excluded), younger (< 65 years old), and sex-matched controls extracted from the ABCD general cohort (Buscemi et al., 2018). All participants and their caregivers were interviewed by one of the investigators. Clinical history and general blood examinations (serum concentrations of glucose, total cholesterol, high-density lipoprotein cholesterol, triglycerides, uric acid, and creatinine) were obtained from all participants.

Height and body weight were measured with participants lightly dressed and without shoes (SECA; Birmingham, UK). Body mass index (BMI) was calculated as body weight (kg)/height^2 (m^2). Waist circumference, measured at the umbilicus, and served as an indirect index of visceral adiposity. Systolic and diastolic arterial blood pressures (two measurements obtained at 5-min intervals in a seated position) and heart rate (Omron M6; Omron Healthcare Co., Matsusaka, Mie, Japan) were measured according to standardized procedures. Body composition, in terms of fat mass and fat-free mass, was estimated using Bioelectrical Impedance Analysis (BIA; BIA-101 Anniversary; Akern, Firenze, Italy), following the manufacturer's equations, as previously described (Buscemi et al., 1998).

2.2. Flow mediated dilation

FMD of the brachial artery was assessed using a high-resolution ultrasound linear probe (10 MHz, Cx50 Philips, US), as described earlier (Buscemi et al., 2013). A sphygmomanometer, cuffed at 220–250 mmHg 2 cm below the antecubital fossa, induce reactive hyperemia by occluding the artery for 300 s. Real-time computed video analysis of B-mode ultrasound images (FMD Studio; Institute of Physiology CNR; Pisa, Italy) recorded variations in brachial artery diameter. The baseline vessel size was determined as the mean of measurements obtained during the first minute. FMD was calculated as the maximum percentage increase in brachial artery diameter over baseline. All FMD tests were performed by the same operator, and the intra-observer coefficient of variation for FMD was 2.9 % in our laboratory.

2.3. Ankle brachial index

The ankle-brachial index (ABI), serving as a measure of peripheral arterial obstructive disease and a biomarker associated with mortality risk as per recent guidelines (Aboyans et al., 2012), was calculated (normal values 0.90–1.40). The ABI was obtained using an automated system (iHealth Pro; Paris, France).

2.4. Carotid intima-media thickness (IMT)

Images of the right and left extracranial carotid artery walls were captured in various projections using a high-resolution ultrasonographic 10-MHz linear array probe (10 MHz, Cx50 Philips, US). The end-diastolic IMT of the far wall of both common carotid arteries was measured, as previously described (Buscemi et al., 2009). Mean values from both sides were considered in the calculations.

2.5. Statistical analysis

Data are reported as the means ± SD for continuous variables and as percentages for categorical variables. The Student's *t*-test for unpaired data was employed to compare continuous variables between groups. Differences in categorical variables were analyzed using the χ^2 test. Pearson's correlation coefficients were calculated to explore the associations between FMD and other continuous variables included in the study. Statistical significance was set at P value<0.05. All analyses were performed using Systat software (Windows version 13.0; San Jose, CA, USA).

3. Results

3.1. Physical and clinical characteristics

Eleven (males 36.4 %) of the 28 healthy centenarians were able to cooperate with the FMD test procedures, which require remaining with the upper limb immobile for approximately 10 min. The control group comprised 76 adults (males 36.8 %). The physical and clinical characteristics of the centenarian and healthy younger control groups are presented in Table 1. Specifically, centenarians exhibited a higher prevalence of hypertension and the use of diuretics, ACE-inhibitors, angiotensin receptor blockers, and statins. Additionally, centenarians had higher serum creatinine and uric acid concentrations.

3.2. Cardiovascular characteristics and endothelial function

The FMD was significantly higher in the centenarian group than in the control group, and centenarians also showed higher carotid IMT values. No significant correlation was observed between FMD and the continuous variables included in the study in the centenarian group (data not shown). Results are detailed in Table 2 and Fig. 1.

4. Discussion

This study demonstrated that the endothelial function of centenarians, measured as the FMD of the brachial artery, was significantly higher than that of a healthy control group younger than about 60 years. This result is noteworthy because FMD is expected to decrease with age (Yavuz et al., 2008; Skaug et al., 2013; Königstein et al., 2021; Königstein et al., 2022). Understanding endothelial function is crucial for comprehending longevity and predicting cardiovascular events. Consistent with our findings, healthy centenarians have been shown to exhibit a low degree of oxidative stress (Paolisso et al., 1998), a well-known factor that may induce endothelial dysfunction (Donato et al., 2018). Therefore, the surprisingly high endothelial function observed, likely influenced by favorable genetic (Gardener et al., 2011; Lu et al., 2023) and lifestyle (Man et al., 2020) factors, may be a protective characteristic contributing to centenarian age. We observed roughly normal age carotid IMT values; moreover, ABI values did not differ from those of the control group, indicating good vascular health, which plays a critical role in aging (Franceschi and Bonafè, 2003). However, certain clinical traits in the centenarians in our study seemed to conflict with their excellent endothelial function. The carotid IMT of our centenarians

Table 1
Physical and clinical characteristics of the centenarian and healthy younger control groups from the ABCD general cohort.

	Centenarians	Controls	P[a]
Males/Females	4/7	28/48	
Age (years)	101 ± 1 (100–105)	41 ± 14 (18–64)	< 0.001
Hypertension (%)	63.6	36.8	< 0.01
Use of (%):			
Beta-blockers	18.2	21.7	< 0.05
Diuretics	63.6	11.8	< 0.001
ACE/AR blockers	54.5	17.1	< 0.005
ca-channel blockers	9.1	11.8	0.09
Statins	18.2	6.6	< 0.001
Body weight (kg)	60.6 ± 10.3 (43.0–74.7)	82.0 ± 21.8 (42.0–145.5)	< 0.005
Body mass index (kg/m^2)	27.5 ± 4.8 (17.9–32.7)	28.7 ± 9.5 (22.2–47.7)	0.68
Fat mass (%)	32.4 ± 8.3 (20.0–46.1)	34.2 ± 10.2 (13.9–53.0)	0.58
Fat-free mass (kg)	40.4 ± 4.7 (33.9–46.5)	53.6 ± 13.5 (35.1–101.9)	< 0.005
Waist circumference (cm)	104.1 ± 6.9 (93.0–113.0)	99.5 ± 21.2 (65.0–153.0)	0.48
Blood concentrations of (mg/dl):			
Glucose	81 ± 7 (73–91)	92 ± 13 (55–119)	< 0.01
Cholesterol	181 ± 31 (124–222)	196 ± 45 (118–255)	0.29
HDL-cholesterol	54 ± 23 (38–90)	55 ± 17 (32–102)	0.86
Triglycerides	118 ± 48 (72–168)	106 ± 55 (38–292)	0.49
Uric acid	6.2 ± 1.5 (3.8–8.0)	4.6 ± 1.2 (2.5–7.3)	< 0.001
Creatinine	1.4 ± 0.8 (0.8–3.0)	0.8 ± 0.2 (0.3–1.2)	< 0.001

Mean ± SD.
[a] Unpaired Student's *t*-test or χ^2 when appropriate.

Table 2
Cardiovascular characteristics and endothelial function of the centenarian and healthy younger control groups from the ABCD general cohort.

	Centenarians	Controls	P[a]
Blood pressure (mmHg):			
Systolic	139 ± 27 (109–175)	125 ± 22 (112–165)	0.06
Diastolic	68 ± 15 (41–84)	80 ± 11 (50–110)	< 0.005
FMD (%)	12.1 ± 4.3 (8.0–21.7)	8.6 ± 5.3 (3.2–28.9)	< 0.05
Carotid IMT (mm)	0.89 ± 0.09 (0.80–1.65)	0.56 ± 0.18 (0.35–1.28)	< 0.001
ABI (average)	1.03 ± 0.27 (0.80–1.65)	1.11 ± 0.17 (0.70–1.65)	0.18

Mean ± SD; ABI, ankle-brachial index; FMD, flow-mediated dilation; IMT, intima-media thickness.
[a] Unpaired Student's *t*-test.

was on average about 0.3 mm higher than that of the 60 years younger control group. Also, the prevalence of hypertension was significantly higher in centenarians than in controls. There is a great debate on the controversial role of c-IMT as predictor of cardiovascular events and as expression of atherosclerosis (Kim and Youn, 2017; Li et al., 2021). In fact, the IMT results from the sum of intimal plus sub-intimal thickness and the thickness of the media layer. However, it has also been shown that carotid IMT is associated not only with endothelial function but also with age and blood pressure. Therefore, changes in intima thickness might be related to aging and early atherosclerosis, while hypertension is probably responsible of the remodeling of the muscular media layer (Kim and Youn, 2017; Li et al., 2021). Consequently, despite excellent

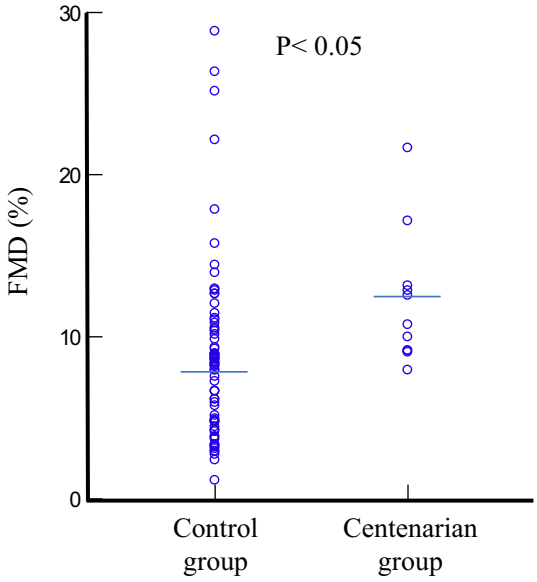


Fig. 1. Individual values of flow-mediated dilation (FMD) in both the centenarian and control groups, with the short perpendicular line indicating the mean value for each group.

endothelial function, age- and hypertension-related factors could explain the slightly but significantly higher carotid IMT observed in centenarians. Furthermore, hypertension is a common age-related disorder, and the prevalence found in our cohort was very similar to that reported in a large Portuguese cohort of centenarians (64.4 %) (Pereira da Silva et al., 2019). A possible explanation is that centenarians often have well-controlled blood pressure or low-grade hypertension, and this condition may not be an obstacle to reaching the age of ≥100 years (Pereira da Silva et al., 2020). In line with this hypothesis, the systolic and diastolic blood pressure values of our centenarians were not significantly higher than those of the control group, possibly due, at least in part, to the higher use of diuretics and ACEi/ARBs. Another seemingly contradictory finding is the presence of reduced renal function in centenarians, as indicated by higher serum creatinine concentrations than those in the control group. Additionally, centenarians exhibit higher serum concentrations of uric acid, potentially due to their reduced renal function and unfavorable cardiovascular consequences (Taher et al., 2019). A progressive reduction in renal function is known to occur concomitantly with aging, with a glomerular filtration rate (GFR) decline of 0.75 ml/min/year (Lindeman et al., 1985). This rate of GFR decline may explain the absolute difference in GFR values observed between the centenarians and the control group, using the predictive equations of the National Kidney Foundation (Levey and Stevens, 2010), although not validated for centenarians, to estimate their approximate GFR values from serum creatinine. Coppolino et al. observed that centenarians may have a preserved ability to mobilize endothelial progenitor cells, improving renal microvascular repair and preventing GFR decline with age (Coppolino et al., 2007).

To the best of our knowledge, this is the first study to measure FMD in centenarians. In addition, our study stands out for the commendable effort to register all centenarians residing in a wide area, ensuring a homogeneous sample investigated within a short timeframe and during the same season, thereby minimizing potential biases. Nevertheless, this study has inherent limitations that warrant acknowledgement. First, the inclusion of only a small number of centenarians is a constraint. Performing the FMD test in centenarians is challenging, requiring their collaboration for ≥15 min to sustain absolute immobility. This aspect introduces a potential bias, considering that participants unable to collaborate might have poor endothelial function. The difficulty in recruiting a large number of centenarians is also attributable to the

necessity of conducting the test at their homes due to the impracticality of reaching the hospital laboratory, often located up to 2 h away by car. Furthermore, it was not possible for us to select a relatively healthy control group aged between 65 and 99 years. However, as endothelial function is known to degline with age, if we had included a control group aged 65 to 99, we would have probably found an even more significant difference in favor of centenarians. Another notable limitation is the intrinsic organization constraints imposed by the distance from our laboratory, preventing more comprehensive blood evaluations, such as antioxidant activities or cytokine levels, which could have contributed to the FMD results.

In conclusion, our study reveals remarkably favorable endothelial function in our centenarian group, potentially attributable to these challenges. However, our data are preliminary and further investigation is required. As endothelial function is intricately linked to aging and represents the culmination of all etiopathogenic factors associated with atherosclerosis and cardiovascular diseases, further research is imperative to determine whether FMD serves as a valid prognostic marker for successful aging.

CRediT authorship contribution statement

Carola Buscemi: Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Cristiana Randazzo:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Anna Maria Barile:** Writing – original draft, Methodology, Data curation. **Roberta Caruso:** Visualization, Investigation, Data curation. **Piero Colombrita:** Visualization, Software, Data curation. **Martina Lombardo:** Validation, Investigation, Data curation. **Piergiorgio Lo Verde:** Investigation, Formal analysis, Data curation. **Natalia Sottile:** Investigation, Formal analysis, Data curation. **Mario Barbagallo:** Writing – review & editing, Conceptualization. **Silvio Buscemi:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Data availability

The authors do not have permission to share data.

Acknowledgments

We express our sincere gratitude to the centenarians and their families for their generous participation in this study. We cannot exclude the fact that this generosity and availability to others contributed to their reaching such a remarkable age. We also thank the mayors and administrations of the Madonie municipalities (Campofelice di Roccella, Castelbuono, Castellana Sicula, Collesano, Ganci, Geraci, Gratteri, Isnello, Lascari, Polizzi Generosa, San Mauro Castelverde, Scillato) for their precious and intense collaboration. We are witnesses to the affection and attention they pay to these special fellow citizens. This attention from local administrations probably contributes to maintaining good endothelial function and becoming a centenarian.

Financial disclosure

This research received no external funds.

References

Aboyans, V., Criqui, M.H., Abraham, P., Allison, M.A., Creager, M.A., Diehm, C., Fowkes, F.G., Hiatt, W.R., Jönsson, B., Lacroix, P., Marin, B., McDermott, M.M., Norgren, L., Pande, R.L., Preux, P.M., Stoffers, H.E., Treat-Jacobson, D., American Heart Association Council on Peripheral Vascular Disease, Council on Epidemiology and Prevention, Council on Clinical Cardiology, Council on Cardiovascular Radiology and Intervention, Council on Cardiovascular Surgery and Anesthesia, 2012. Measurement and interpretation of the ankle-brachial index: a scientific statement from the American Heart Association. Circulation 126 (24), 2890–2909. https://doi.org/10.1161/CIR.0b013e318276fbcb.

Bloom, S.I., Islam, M.T., Lesniewski, L.A., Donato, A.J., 2023. Mechanisms and consequences of endothelial cell senescence. Nat. Rev. Cardiol. 20 (1), 38–51. https://doi.org/10.1038/s41569-022-00739-0.

Brandes, R.P., Fleming, I., Busse, R., 2005. Endothelial aging. Cardiovasc. Res. 66 (2), 286–294. https://doi.org/10.1016/j.cardiores.2004.12.027.

Buscemi, S., Blunda, G., Maneri, R., Verga, S., 1998. Bioelectrical characteristics of type 1 and type 2 diabetic subjects with reference to body water compartments. Acta Diabetol. 35, 220–223. https://doi.org/10.1007/s005920050135.

Buscemi, S., Verga, S., Batsis, J.A., Cottone, S., Mattina, A., Re, A., Arnone, M., Citarda, S., Cerasola, G., 2009. Intra-renal hemodynamics and carotid intima-media thickness in the metabolic syndrome. Diabetes Res. Clin. Pract. 86 (3), 177–185. https://doi.org/10.1016/j.diabres.2009.09.015.

Buscemi, S., Cosentino, L., Rosafio, G., Morgana, M., Mattina, A., Sprini, D., Verga, S., Rini, G.B., 2013. Effects of hypocaloric diets with different glycemic indexes on endothelial function and glycemic variability in overweight and in obese adult patients at increased cardiovascular risk. Clin. Nutr. 32 (3), 346–352. https://doi.org/10.1016/j.clnu.2012.10.006.

Buscemi, S., Corleo, D., Vasto, S., Buscemi, C., Massenti, M.F., Nuzzo, D., Lucisano, G., Barile, A.M., Rosafio, G., Maniaci, V., Giordano, C., 2018. Factors associated with circulating concentrations of irisin in the general population cohort of the ABCD study. Int. J. Obes. 42 (3), 398–404. https://doi.org/10.1038/ijo.2017.255.

Celermajer, D.S., Sorensen, K.E., Gooch, V.M., Spiegelhalter, D.J., Miller, O.I., Sullivan, I. D., Lloyd, J.K., Deanfield, J.E., 1992. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 340 (8828), 1111–1115. https://doi.org/10.1016/0140-6736(92)93147-f.

Coppolino, G., Basile, G., Bolignano, D., Mauro, V.N., Buemi, M., 2007. Circulating endothelial progenitor cell mobilization in centenarians: a powerful source for extended renal life. J. Am. Geriatr. Soc. 55 (10), 1693–1694. https://doi.org/10.1111/j.1532-5415.2007.01363.x.

Davignon, J., Ganz, P., 2004. Role of endothelial dysfunction in atherosclerosis. Circulation 109 (23 Suppl 1), III27–III32. https://doi.org/10.1161/01.CIR.0000131515.03336.f8.

Donato, A.J., Machin, D.R., Lesniewski, L.A., 2018. Mechanisms of dysfunction in the aging vasculature and role in age-related disease. Circ. Res. 123 (7), 825–848. https://doi.org/10.1161/CIRCRESAHA.118.312563.

Franceschi, C., Bonafè, M., 2003. Centenarians as a model for healthy aging. Biochem. Soc. Trans. 31 (2), 457–461. https://doi.org/10.1042/bst0310457.

Gardener, H., Beecham, A., Cabral, D., Yanuck, D., Slifer, S., Wang, L., Blanton, S.H., Sacco, R.L., Juo, S.H., Rundek, T., 2011. Carotid plaque and candidate genes related to inflammation and endothelial function in Hispanics from northern Manhattan. Stroke 42 (4), 889–896. https://doi.org/10.1161/STROKEAHA.110.591065.

Kalies, K., Knöpp, K., Wurmbrand, L., Korte, L., Dutzmann, J., Pilowski, C., Koch, S., Sedding, D., 2024. Isolation of circulating endothelial cells provides tool to determine endothelial cell senescence in blood samples. Sci. Rep. 14 (1), 4271. https://doi.org/10.1038/s41598-024-54455-5.

Kim, G.H., Youn, H.J., 2017. Is carotid artery ultrasound still useful method for evaluation of atherosclerosis? Korean Circulation Journal 47 (1), 1–8. https://doi.org/10.4070/kcj.2016.0232.

Königstein, K., Wagner, J., Frei, M., Knaier, R., Klenk, C., Carrard, J., Schwarz, A., Hinrichs, T., Schmidt-Trucksäss, A., 2021. Endothelial function of healthy adults from 20 to 91 years of age: prediction of cardiovascular risk by vasoactive range. J. Hypertens. 39 (7), 1361–1369. https://doi.org/10.1097/HJH.0000000000002798.

Königstein, K., Wagner, J., Infanger, D., Knaier, R., Nève, G., Klenk, C., Carrard, J., Hinrichs, T., Schmidt-Trucksäss, A., 2022. Cardiorespiratory fitness and endothelial function in aging healthy subjects and patients with cardiovascular disease. Frontiers in Cardiovascular Medicine 28 (9), 870847. https://doi.org/10.3389/fcvm.2022.870847.

Levey, A.S., Stevens, L.A., 2010. Estimating GFR using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation: more accurate GFR estimates, lower CKD prevalence estimates, and better risk predictions. Am. J. Kidney Dis. 55 (4), 622–627. https://doi.org/10.1053/j.ajkd.2010.02.337.

Li, H., Xu, X., Luo, B., Zhang, Y., 2021. The predictive value of carotid ultrasonography with cardiovascular risk factors-a "SPIDER" promoting atherosclerosis. Frontiers in Cardiovascular Medicine 8, 706490. https://doi.org/10.3389/fcvm.2021.706490.

Lindeman, R.D., Tobin, J., Shock, N.W., 1985. Longitudinal studies on the rate of decline in renal function with age. J. Am. Geriatr. Soc. 33 (4), 278–285. https://doi.org/10.1111/j.1532-5415.1985.tb07117.x.

Lu, J., Peng, W., Yi, X., Fan, D., Li, J., Wang, C., Luo, H., Yu, M., 2023. Inflammation and endothelial function-related gene polymorphisms are associated with carotid atherosclerosis-a study of community population in Southwest China. Brain and Behavior 13 (7), e3045. https://doi.org/10.1002/brb3.3045.

Man, A.W.C., Li, H., Xia, N., 2020. Impact of lifestyles (diet and exercise) on vascular health: oxidative stress and endothelial function. Oxidative Med. Cell. Longev. 2020, 1496462. https://doi.org/10.1155/2020/1496462.

Paolisso, G., Tagliamonte, M.R., Rizzo, M.R., Manzella, D., Gambardella, A., Varricchio, M., 1998. Oxidative stress and advancing age: results in healthy centenarians. J. Am. Geriatr. Soc. 46 (7), 833–838. https://doi.org/10.1111/j.1532-5415.1998.tb02716.x.

Pereira da Silva, A., Matos, A., Aguiar, L., Ramos-Marques, N., Ribeiro, R., Gil, Â., Gorjão-Clara, J., Bicho, M., 2019. Hypertension and longevity: role of genetic

polymorphisms in renin-angiotensin-aldosterone system and endothelial nitric oxide synthase. Mol. Cell. Biochem. 455 (1–2), 61–71. https://doi.org/10.1007/s11010-018-3470-1.

Pereira da Silva, A., Costa, M.D.C., Aguiar, L., Matos, A., Gil, Â., Gorjão-Clara, J., Polónia, J., Bicho, M., 2020. Impact on longevity of genetic cardiovascular risk and lifestyle including red meat consumption. Oxidative Med. Cell. Longev. 2020, 1305413. https://doi.org/10.1155/2020/1305413.

Seals, D.R., Jablonski, K.L., Donato, A.J., 2011. Aging and vascular endothelial function in humans. Clin. Sci. 120 (9), 357–375. https://doi.org/10.1042/CS20100476.

Skaug, E.A., Aspenes, S.T., Oldervoll, L., Mørkedal, B., Vatten, L., Wisløff, U., Ellingsen, O., 2013. Age and gender differences of endothelial function in 4739 healthy adults: the HUNT3 fitness study. Eur. J. Prev. Cardiol. 20 (4), 531–540. https://doi.org/10.1177/2047487312444234.

Taher, R., Sara, J.D., Prasad, M., Kolluri, N., Toya, T., Lerman, L.O., Lerman, A., 2019. Elevated serum uric acid is associated with peripheral endothelial dysfunction in women. Atherosclerosis 290, 37–43. https://doi.org/10.1016/j.atherosclerosis.2019.07.013.

Yavuz, B.B., Yavuz, B., Sener, D.D., Cankurtaran, M., Halil, M., Ulger, Z., Nazli, N., Kabakci, G., Aytemir, K., Tokgozoglu, L., Oto, A., Ariogul, S., 2008. Advanced age is associated with endothelial dysfunction in healthy elderly subjects. Gerontology 54 (3), 153–156. https://doi.org/10.1159/000129064.