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Hypertension and retinal microvascular dysfunction (HyperVasc): protocol of a randomised controlled exercise trial in patients with hypertension

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ABSTRACT

Introduction Hypertension is a global healthcare burden that affects the structure and function of the macrocirculation and microcirculation and induces disease-specific end-organ damage. Vascular biomarkers are essential to timely diagnose this end-organ damage to improve cardiovascular (CV) risk stratification and medical decision making. Exercise therapy is an effective means to improve vascular health and reduce overall CV risk. However, it is still not clear whether high-intensity interval training (HIIT) is recommendable for patients with hypertension to reduce blood pressure, increase cardiorespiratory fitness and ameliorate vascular health.

Methods and analysis The 'Hypertension and retinal microvascular dysfunction' trial will investigate macrovascular and microvascular impairments in hypertensive patients compared with healthy controls to investigate hypertension-induced end-organ damage by using gold-standard methods as well as newly developed unique retinal microvascular biomarkers. In addition, this trial will investigate the reversibility of retinal end-organ damage by assessing the effects of an 8-week supervised and walking based HIIT on blood pressure, cardiorespiratory fitness as well as macrovascular and microvascular health, compared with a control group following standard physical activity recommendations. Primary outcome will be the arteriolar-to-venular diameter ratio. Secondary outcomes will be arteriolar and venular diameters as well as the flicker-light-induced dilation. Further outcomes will be other retinal microvascular biomarkers, flow-mediated dilation of the brachial artery as well as blood pressure, cardiorespiratory fitness, microalbuminuria, hypertensive retinopathy and classical CV risk markers. Analysis of variance and analysis of covariance will be used to investigate group differences between healthy controls and hypertensive patients and training effects in hypertensive patients, respectively.

Ethics and dissemination The Ethics Committee of Northwestern and Central Switzerland approved this study (EKNZ-2021-00086). All participants will give informed consent.

Trial registration number NCT04763005.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The Hypertension and retinal microvascular dysfunction trial will investigate, for the first time, hypertension-related macrovascular and microvascular impairments by using newly developed non-invasive techniques of retinal microvascular phenotyping.
⇒ The results of this study will (1) improve the understanding of microvascular impairments in hypertensive patients, (2) improve the knowledge of exercise-induced macrovascular and microvascular remodelling, and (3) highlight the potential of retinal vessel imaging for cardiovascular risk stratification and therapy monitoring in patients with hypertension to improve medical decision making in a personalised medicine approach.
⇒ Generalisation of the results to other patients' cohorts will only be possible with reserve.

INTRODUCTION

Arterial hypertension is a growing global healthcare burden. The number of hypertensive patients is predicted to increase globally to 60% by 2025.[1] The prevalence in Europe is thought to be about 30%–45%, with increasing blood pressure (BP) levels at higher age.[2] About 40% of all annual deaths in Europe are directly related to hypertension-induced cardiovascular (CV) disease.[3]

Hypertension affects the structure and function of the macro- and microcirculation and leads to advanced vascular ageing. A timely diagnosis of dysfunction beyond vascular ageing allows for improved estimation of individual CV risk and more specific clinical decision making in primary and secondary CV prevention.[4] The European Society of Cardiology Working Groups on Atherosclerosis and Vascular Biology, Aorta and Peripheral Vascular Diseases, Coronary Pathophysiology and Microcirculation, and Thrombosis have recently discussed several

vascular biomarkers to detect endothelial function or dysfunction.[5] The authors discussed advantages and disadvantages of macrovascular and microvascular assessments and highlighted the potential of retinal vessel imaging as non-invasive technique to quantify microvascular endothelial function. However, they criticised the lack of normative data and standard operating procedures for this method. We have addressed this research gap and recently published normative data and standard operating procedures for static (SVA) and dynamic retinal vessel analysis (DVA).[6] Nevertheless, more clinical studies with reference values for different cohorts as well as follow-up assessments are needed to quantify benefits and the added value for CV risk stratification and potential treatment monitoring.[5]

Different non-invasive techniques to investigate the macro- and microvascular structure and function exist. Flow-mediated dilatation (FMD) is considered to be the gold standard to quantify endothelial function in the macrocirculation.[7] Blunted FMD has been shown to be predictive for CV events[8] and all-cause mortality.[9] Each unit decrease of FMD was associated with 16% higher risk to develop hypertension.[10] Impaired endothelial function leads to the development of atherosclerosis by increasing arterial wall thickness and plaque formation.[11 12] Together with markers of arterial stiffness and central haemodynamics, FMD is a frequently used vascular biomarker for the evaluation of vascular dysfunction in the macrocirculation. However, the method has not yet been implemented in clinical routine mainly due to a remaining high interobserver and day-to-day variability.

Microvascular organ damage can be investigated by performing the SVA or DVA, which are both non-invasive techniques and valid biomarkers of vascular health and CV risk.[6] Narrower retinal arteriolar diameter equivalents (CRAE), wider venular diameter equivalents (CRVE) and a lower arteriolar-to-venular diameter ratio (AVR) have been associated with a higher risk of stroke,[13–15] coronary artery disease[16] and CV mortality.[17 18] Retinal arteriolar narrowing is predictive for the development of hypertension and is associated with the severity of hypertension.[19–21]

The method of DVA has the potential to directly and non-invasively investigate microvascular endothelial function by measuring flicker light-induced dilatation (FID) over time. FID is negatively associated with CV risk factors such as age,[22 23] increased body mass,[24 25] BP[26] or cholesterol[27] as well as manifest CV diseases such as diabetes[28] or heart failure.[29] Impaired FID seems to be predictive for non-fatal and fatal CV disease events in high risk cohorts.[30 31] Every SD decrease in FID reduced all-cause mortality by up to 35% in end-stage renal disease patients. The reclassification rate of additional FID was 27% compared with standard care in this 3 years follow-up study.[30]

We have recently established normative data and recommendations for standard operating procedures for SVA and DVA.[6] In addition, we developed two new methods to improve non-invasive phenotyping in the retinal microcirculation, which have not yet been applied in patients with hypertension.[32 33] The first new approach allows for the investigation of the wall-to-lumen ratio in retinal arterioles and venules.[32] By inducing an acute standardised BP rise using a defined hand-grip exercise, we are able to assess the retinal myogenic constriction in addition to and as a counter regulatory mechanism of FID.[33]

Lifestyle interventions such as exercise, salt-reduced diet or alcohol reduction can have a significant impact in lowering BP and may have additional health benefits beyond their BP impact.[34] We have recently shown that physical activity (PA) and exercise improve retinal microvascular health in healthy children and adults, as well as CV risk patients.[35] In addition, regular PA is effective in reducing BP and evidence suggests that PA can reduce the risk of developing de novo hypertension.[36] A meta-analysis of 13 prospective cohort studies including more than 135 000 participants confirmed a 19% risk reduction of developing hypertension in individuals with high versus low PA.[37] PA and high cardiorespiratory fitness (CRF) seem to be beneficial even in hypertensive patients. A study on the combined effects of BP and PA on CV mortality revealed health benefits for individuals with high versus no PA, independent of their BP levels.[38] Especially, high CRF seems to protect against vascular ageing.[39–41] Hypertensive patients with high CRF levels demonstrated a lower prevalence of carotid atherosclerosis. High-intensity interval training (HIIT) is an effective method to increase CRF and seems to have superior health benefits compared with moderate-continuous training in healthy[42] and diseased populations.[43 44] Costa *et al* showed no differences between HIIT and moderate-continuous training interventions on resting BP. However, HIIT showed higher improvements in CRF compared with moderate-continuous training with potential for long-term health benefits for patients.[45] The European Association of Preventive Cardiology and the Council on Hypertension of the European Society of Cardiology (ESC) have recently summarised the evidence of exercise in the prevention and treatment of arterial hypertension.[46] Endurance training was the first exercise priority for hypertensive patients with an expected BP lowering effect of −4.5 to −7.4 mm Hg, followed by a combination of endurance and resistance training (-5.3 to −5.6 mm Hg), isometric (-5.1 to −5.2 mm Hg) and dynamic resistance training (-2.3 to −2.4 mm Hg). However, the authors noted that the evidence for HIIT as exercise therapy to reduce BP is scarce. Therefore, the Hypertension and retinal microvascular dysfunction (HyperVasc) study will investigate whether HIIT is a suitable exercise therapy for hypertensive patients to reduce BP, increase CRF and improve subclinical retinal microvascular end-organ damage indicating overall vascular risk reduction.

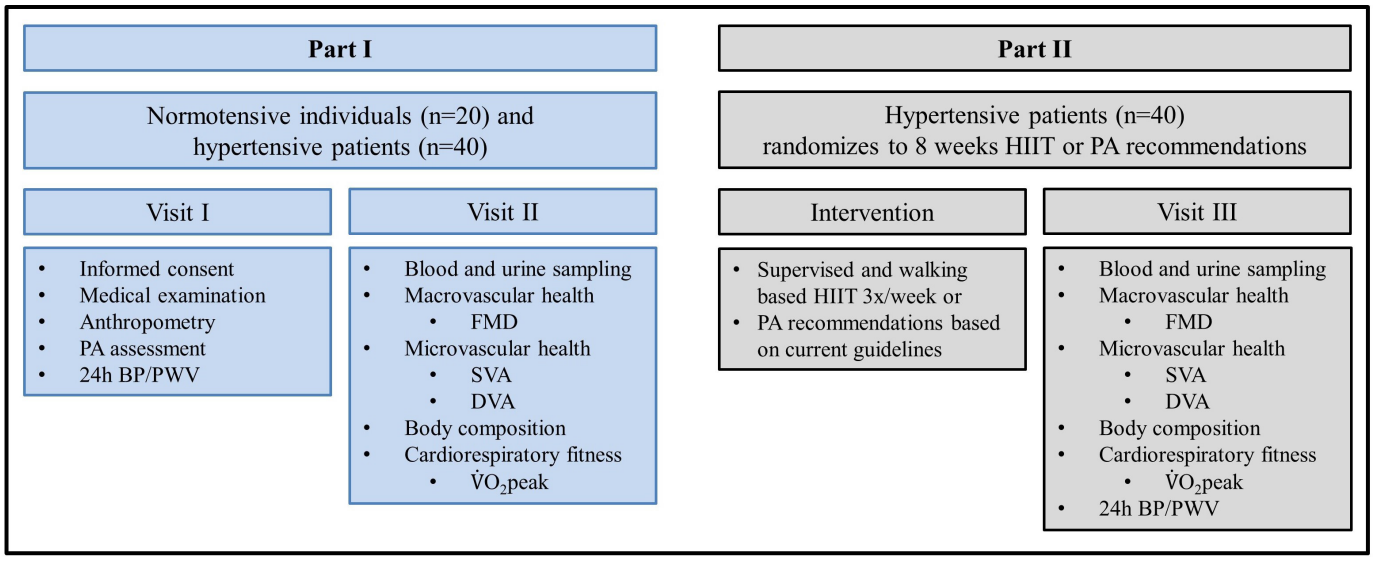

Figure 1 Study design of the HyperVasc trial. BP, blood pressure; DVA, dynamic retinal vessel analysis; FMD, flow-mediated dilatation; HIIT, high-intensity interval training; HyperVasc, Hypertension and retinal microvascular dysfunction; PA, physical activity; PWV, pulse wave velocity; SVA, static retinal vessel analysis; $\dot{V}O_2$peak, peak oxygen uptake.

Methods and analysis

Study design

The HyperVasc study consists of two parts. Part I is designed as cross-sectional study, part II is designed as randomised controlled trial. Twenty healthy and normotensive controls and 40 hypertensive patients are included in part I to investigate group differences in BP, CRF and the hypertension-induced macrovascular and microvascular end-organ damage. Part I is essential especially for the new methods of retinal vessel imaging to be able to analyse hypertension-induced vascular maladaptation compared with healthy controls. In part II, hypertensive patients are randomised following their baseline assessment into a HIIT group (n=20) or a control group with standard PA recommendations (n=20) to investigate the exercise effects on BP, CRF as well as the macrovascular and microvascular phenotype (figure 1). In addition to vascular health, extensive phenotyping including classical CV risk markers is performed. Phenotyping details are described below. The phenotyping as well as the training intervention will take place at the Department of Sport, Exercise and Health (DSBG), Basel, Switzerland. The study is planned and conducted in accordance with the Declaration of Helsinki.[47] All participants have to sign a written informed consent, prior to the first assessment. The Ethics Committee of Northwestern and Central Switzerland approved this study (EKNZ-2021-00086). This study was registered on the official registry platform for research with humans in Switzerland (kofam: NCT04763005) directly after we received ethical approval. The first patient contacted us directly after the study was published on kofam. A few days later we also registered our study on ClinicalTrials.gov: NCT04763005 (February 2022). Due to SARS-nCOV-2 pandemic, all assessments are conducted under consideration of the hygiene recommendations developed by the Task-Force of the University of Basel based on the guidelines of the National Ministry of Health (BAG).

Inclusion and exclusion criteria

Men and women previously diagnosed with hypertension, receiving drug treatment for arterial hypertension and controlled BP, hypertension grade I is accepted (study threshold:≤159/99 mm Hg) as well as normotensive healthy controls (study threshold: ≤129/84 mm Hg) between 40 and 70 years of age are recruited via advertisements in local newspapers. For BP categorisation, patients are measured on two separate days according to the current 2018 hypertension guidelines from the ESC and the European Society of Hypertension.[48] Exclusion criteria for both groups are any CV medication (except for antihypertensive medication in the hypertensive group), history of CV, pulmonary or chronic inflammatory disease, active smoking status, body mass index ≥30 kg/m^2, macular degeneration, glaucoma or any chronic eye disease, exercise-limiting orthopaedic problems, high intraocular pressure (IOP) (≥20 mm Hg) or changes in antihypertensive medication during the intervention period.

Anthropometry, BP, PA and fitness

Anthropometric data are measured in the morning under fasting conditions. Height and waist circumference are measured by use of standard procedures. The Inbody 720R (JP Global Markets, Germany) is used to obtain body mass, body mass index, lean body mass and body fat.[49] Blood samples are drawn by venepuncture of the cubital fossa of the right or left arm by trained staff. Urine samples are taken to measure microalbuminuria in all participants. Blood and urine samples are centrifuged and stored at −80° for further analysis

after data acquisition has been completed successfully. BP is measured over 24 hours using the Mobil-O-Graph 24 hours pulse wave reflection monitor device (I.E.M. GmbH, Aachen, Germany) with integrated ARCSolver software. This device measures the peripheral and central haemodynamics as well as the 24 hours pulse wave velocity (PWV) every 20 min during the day and every 60 min during the night. IOP is measured with the ICare PRO (Tiolat Oy, Helsinki, Finland) rebound tonometer.

Self-reported PA levels are analysed using the Freiburg Questionnaire of Physical Activity.[50] Physical fitness is measured with an individualised bicycle ramp protocol as previously described[51] using the Cortex Metalyzer R 3B metabolic test system (Cortex Biophysik, Leipzig, Germany) to analyse circulatory and ventilatory parameters including peak oxygen uptake (VO_2peak) and maximal heart rate (HRmax). Individual exhaustion is achieved when participants reach the previously defined respiratory exchange ratio cut-off value of 1.10 for participants between 40 and 59 years of age and 1.06 for participants between 60 and 69 years of age.[52] The individual ramp protocol is repeated on a separate day if individual exhaustion is not achieved.

Macrovascular and microvascular assessments

All vascular assessments are performed in the morning under fasting conditions. Participants are asked to avoid alcohol and exercise 24 hours prior the appointment and any food or caffeine intake at the day of the appointment. Only unsweetened water and medication intake is allowed. Investigators are blinded for the patients' characteristics and group allocation in the vascular assessments at baseline and during image analysis of vascular parameters at the end of the study.

Macrocirculation

FMD is measured with a semiautomatic and ECG-guided high resolution B-mode ultrasound system (UNEX EF 38G, UNEX, Nagoya, Japan) after 15 min of rest in a supine position. Measurement takes place in a dark, quiet and temperature-controlled room. The arm of the participants is abducted in a 90° angle in a relaxed position. A 10 MHz H-shaped probe is used to measure the right brachial artery on a short axis and long axis. The device continuously corrects the probe position during the whole procedure to generate the image with the highest quality. A cuff placed at the forearm of the participants (5–10 cm distal to the probe and 1–2 cm proximal to the cubital fossa) increases cuff pressure for 5 min 50 mmHg above the resting BP, measured after 15 min of rest in a supine position. Rest diameter of the right brachial artery is measured before cuff-inflation for 10 s. In addition, the diameter of the brachial artery is measured continuously during the last 60 s of the inflation period and during 3 min after cuff deflation to analyse sheer stress induced vascular response.

The 24-hour PWV is analysed every 20 min during the day and every 60 min during the night with the Mobil-O-Graph, in combination with the BP measurement, as described above.

Microcirculation

Conventional eye drops (Tropicamide 0.5%) are used to dilate the pupil of the right eye of each participant. The left eye is used in cases of local eye disease on the right eye. Images of the eye background with the optic disc in the centre using the Dynamic Vessel Analyser (IMEDOS Systems, Jena, Germany) and a fundus camera (450 FF; Carl Zeiss, Jena, Germany) are taken to assess retinal vessel diameters. Standard operating procedures are used to analyse CRAE, CRVE and AVR as described previously[6] based on three high-quality images with an angle of 50°. The retinal vessel microstructure, including the retinal vessel wall and the wall-to-lumen ratio is analysed based on three images with an angle of 20° and a green filter as described previously.[32] The oxygen saturation of the retinal microcirculation is analysed based on two images with a specific oxygen filter.[53] A senior ophthalmologist rates the presence and severity of hypertensive retinopathy based on one high-definition image of each participant.

Retinal endothelial function is analysed with the same camera by measuring arteriolar and venular diameters over time with two protocols. The first protocol investigates neurovascular coupling using FID as a marker of retinal, and therefore, cerebrovascular endothelial function. A detailed method description can be found elsewhere.[6] The second protocol investigates the myogenic constriction of the retinal microcirculation, also known as the Bayliss effect. Participants are asked to perform a standardised handgrip exercise to increase their BP, which results in a myogenic constriction of the smooth muscles of the vessels. To standardise the BP increase, the Leonardo Mechanograph GF device (Novotec Medical, Pforzheim, Germany) is used to test the grip strength one repetition maximum (1RM) of the left hand. The best value of three attempts is taken as guidance for the handgrip exercise. The BP is controlled beat-to-beat during the whole procedure by using the Finapres (Finapres Medical Systems B.V., Enschede, Netherlands) device on the middle finger of the right hand. The setup has been described in detail previously.[33] The second protocol starts with a 50 s rest phase. This rest phase is used to calculate the baseline diameter. After 50 s of rest the participants are asked to press the Leonardo Mechanograph GF device with the left hand for 30 s with 30% of their 1RM. The produced power is controlled with the Leonardo Mechanography BAS V.4.4 software. An acoustic signal is implemented to help the participants stay at 30% of their 1RM. A variance of 2% is tolerated. After 30 s at 30% 1RM, participants are verbally motivated to press as hard as possible for another 30 s (all-out phase) to reach a peak BP increase. A subsequent rest phase of 80 s is implemented to investigate the vessel response after myogenic constriction. The vessel diameters of two arteriolar and venular vessel segments are measured continuously during the whole procedure.

Exercise intervention and control condition

Hypertensive patients are randomised to an 8-week HIIT or a control group. The exercise intervention is a supervised and walking-based HIIT (3x/week), starting with a habituation week with an intensity of 75% HRmax. In the following 7 weeks, the participants will perform a HIIT based on the following protocol and with a total duration of 45 min per session (modified from Wisløff *et al*[54]): warm-up for 10 min at 60%–70% HRmax followed by a high-intensity interval consisting of 4×4 min at 80%–95% HRmax with 3 min of active recovery at 60%–70% HRmax and a 10 min cool-down at 60%–70% HRmax. Heart rate will be monitored during training by Polar H7 heart rate sensors combined with Polar M400 watches. Sport scientists motivate the participants during the high intensity intervals and will control the heart rate of each participant during and after every training session. This training programme has previously been used in CV risk patients with a high adherence and without any drop-out related due to the exercise training.[55] The control group will get PA recommendations and exercise training advices based on current guidelines,[56] the CRF test and the PA questionnaire. Participants in the control group document their PA behaviour in a PA diary and get a phone call after 4 weeks to evaluate their well-being.

Data management

Investigators are trained by experts in all assessments. Vascular assessments are performed by one experienced investigator to avoid interobserver variability. Data are stored in a laboratory data base without external access with generated study IDs. Previously selected investigators have access to this database. Only the principal investigator can match names of the participants with study IDs. Data are cleaned and checked for their plausibility at the end of the study. Changes in the database are registered and controlled by an external researcher at the end of the study. This researcher is not involved in planning the study, data acquisition or analysing the data. Auditing during the data acquisition period is not planned but all study entries in the final database will be stored and are replicable at the end of the study. Interim analysis is not planned. Potential drop-outs and their reasons are collected and reported at the end of the study. The study will be stopped immediately for individual patients if any of the following adverse events should occur: adverse CV event such as angina pectoris or major adverse CV event, musculoskeletal adverse events or acute glaucoma incidence. Adverse events will be communicated directly to the ethic committee. Drop-out participants are invited to take part in the follow-up assessment where all assessments from visit III are planned (figure 1). Multiple imputation is used to handle missing data.[57 58]

Randomisation

A blockwise randomisation is done to get equal group sizes in the HIIT and the control group. An independent research assistant draws group allocation from a locked envelope to perform randomisation after baseline assessments. LS is responsible to communicate the enrolment decision to participants. Sex was the only stratification factor during the randomisation process. Participants and sport scientists who supervise the intervention are not blinded for group allocation. Outcome assessors and researchers who analyse data are blinded for group allocation.

Statistical analysis

The primary outcome of this study is the AVR difference between hypertensive patients and normotensive healthy controls in the cross-sectional part (part I) and among hypertensive patients before and after 8 weeks of HIIT compared with the control group (part II). Secondary outcomes are CRAE, CRVE, as well as arteriolar and venular retinal endothelial function. Further outcomes are arteriolar and venular retinal myogenic constriction, retinal wall-to-lumen ratio and retinal oxygen saturation as well as FMD, 24-hour BP, VO2peak and microalbuminuria. Median and IQRs are used to describe patients' characteristics in both parts. Boxplots are used to visualise the primary and secondary outcomes in the cross-sectional and the interventional part. Analysis of variance are used to compare AVR (and secondary outcomes) between patients and healthy peers. For the interventional part, analysis of covariance are calculated to compare AVR (and secondary outcomes) after 8 weeks of intervention between the HIIT and the control group adjusted for the corresponding values at baseline, antihypertensive medication at baseline, age, sex and ΔBP.[59] Intention-to-treat principle is used as primary analysis and per protocol as secondary analysis. The statistical program R (R Foundation for Statistical Computing, Vienna, Austria, V.3.5.0.) will be used for the generation of graphs and for statistical tests with a two-sided CI of 95%.

Sample size calculation

Cross-sectional approach

Based on previous results a mean AVR difference between patients and healthy peers of 0.04 with a SD of 0.04 is expected.[60] To reach a power of 95% with a two-sided significance level of 0.05, a total sample size of 46 participants is needed. To reach the target power in case of missing data, based on insufficient data quality, 20 controls and 40 patients are included in this study.

Interventional approach

An exercise-induced AVR improvement of 0.03 with an SD of 0.05 is expected, based on our previous publication.[55] A total sample size of 32 participants is necessary to reach a power of 95% with a two-sided significance level of 0.05. Due to possible drop-outs 40 participants in total are included to randomise these participants to 20 patients in the control and 20 patients in the exercise intervention condition. We used G*Power software V.3.1.9.2 for the sample size calculation.[61]

Patient and public involvement statement

All methods included in this study have been used in previous trials. Participants' feedback is used to select material and methods. Participants are not involved in the study design. However, participants are asked to take part in the acquisition by communicating the trial with their families and friends. The HIIT is planned to take place at the DSBG. In single cases, the intervention can be performed at home based on participants' availability but controlled by a heart rate sensor and stored on a Polar M400 watch. Participants will be informed about their individual results directly after their visits and about the overall study results at the end of the HyperVasc study. In addition, all participants get detailed and individualised PA and exercise training advices based on the CRF test and the PA questionnaire to optimise their daily PA behaviour.

Time plan

The acquisition started in February 2021 after the ethical approval and is still ongoing. Last patient out is planned for spring 2022. This is the first and only version of the HyperVasc study plan.

Hypotheses and potential impact

The HyperVasc trial will investigate, for the first time, the BP-induced macrovascular and microvascular impairments in detail by using newly developed techniques of retinal microvascular imaging. Primarily, we expect to find a lower AVR in patients with hypertension compared with healthy controls and a more pronounced arteriolar dilatation and venular constriction among further amelioration of the assessed retinal microvascular pathophysiology after 8 weeks of HIIT in patients with hypertension compared with standard PA recommendations. Both hypotheses will be addressed for the secondary and further outcomes. The results of this study will (1) improve the understanding of retinal microvascular impairments in hypertensive patients, (2) enhance the knowledge of exercise-induced macrovascular and microvascular remodelling, and (3) highlight the potential of investigating retinal microvascular end-organ damage for CV risk stratification and therapy monitoring to improve the medical decision making in a personalised medicine approach. Generalisation of the results is limited since we excluded patients with CV comorbidities such as dyslipidaemia or obesity. However, we have previously examined the effects of exercise treatment in patients with multiple CV risk factors including a high prevalence of hypertension.[55]

Ethics and dissemination

The Ethics Committee of Northwestern and Central Switzerland approved this study in February 2021 (EKNZ-2021-00086). Changes in the study protocol will be immediately communicated with the ethic committee. All measurements are non-invasive. Participants are informed verbally about all study procedures, data policy, their right to quit this study without any consequences and signed a written informed consent. An additional written informed consent for stored blood and urine samples is signed (online supplemental file 1). Participants are offered a state-of-the-art CV health check including clinical routine parameters as well as an exercise ECG and non-invasive CV imaging to evaluate their vascular health. All procedures are free of charge and a final medical report is available for each candidate. All participants benefit from the professional recommendations of the PA experts. The intervention group is instructed on how to perform the HIIT exercise. Temporary eye discomfort and slight headaches (in few cases) are potential negative side effects of the mydriaticum and flicker light application. In the unlikely case of persistent discomfort or pain of the eye, the patient is seen by the ophthalmologist in the University Hospital (KG) who is participating in the study.

Data are stored electronically on a database system (SecuTrail) in line with the current ethical and legal requirements. No data that could reveal the participants' identity (eg, name, date of birth, home address) will be recorded in this database. Only the principal investigator (LS) can match the study IDs with the names of each participant. Several scientific publications as well as conference presentations are planned to report the HyperVasc study results to the scientific community. Peer-review publications will be written by investigators involved in this study. No external writers or third parties will be involved in these publications.

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Contributors LS designed the study, wrote the protocol and is responsible for all assessments and the intervention. JG, JC and TH are responsible for clinical examinations and revised the manuscript. CH supports the data acquisition and revised the manuscript. AS-T shares his expertise in macrovascular assessments and revised the manuscript. KG is the ophthalmologist in this study and revised the manuscript. HH designed the study, supports the HyperVasc team with his clinical and academic expertise and revised the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

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