BASES Physiological Testing Guidelines

Chapter title: The assessment of peripheral blood flow and vascular function.

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1. Introduction

Whilst exercise-induced cardio-protection can be partly attributed to improvements in traditional cardiovascular risk factors, the magnitude of effect does not fully explain the risk reduction seen in cardiovascular outcomes and all-cause mortality (1). Peripheral vascular dysfunction represents a precursor of atherosclerosis (2), subsequently leading to the development and progression of cardiovascular disease (3). For this reason, measurement of peripheral vascular dysfunction shows strong predictive capacity for future coronary vascular events (4). Moreover, the potent cardioprotective effects of regular exercise training are, at least partly, explained through improvement in vascular function (5, 6).

The improvements in vascular function that are observed with chronic exercise training appear to be mediated through elevations in hemodynamic stimuli (e.g. blood flow). This makes peripheral vascular function and blood flow central features in the protection against cardiovascular events. The assessment of peripheral blood flow and vascular function is therefore an important area of investigation. These procedures allow insight into the development of cardiovascular disease, but also contribute to better understanding of the detrimental effects of modern lifestyle behaviours, such as reduced physical activity, increased sedentary behaviour, and poor diet. These measurements of vascular function and blood flow also provide a promising avenue for early risk identification, but also to evaluate the cardioprotective effects of exercise training. This chapter provides an overview of testing guidelines for common, non-invasive assessments of vascular physiology in humans. This overview is categorised based on techniques evaluating first conduit arteries (large, elastic vessels that maintain high pressure blood flow) and then resistance arteries (small diameter vessels in the microcirculation that constitute major sites of vascular resistance).

2. Conduit arteries

2.1 Ultrasonography

Given the size of peripheral conduit arteries, typically varying between 2 and 10mm (7), ultrasound can be used to visualise these arteries and to evaluate blood velocity. This allows for the evaluation of resting blood flow and vessel structure, but also the assessment of functional characteristics such as flow-mediated dilation (FMD) and carotid artery reactivity

(CAR). Ultrasound measures of vascular function typically use high-resolution duplex ultrasound with a 10-12-MHz probe, as most arteries of interest are relatively superficial (~2-5cm depth). Duplex ultrasound provides a two-dimensional image of the vessel diameter (B-mode), combined with determination of blood flow velocity (Doppler; Figure 1) (8). A sonographer will optimise ultrasound parameters to achieve a satisfactory image of the artery, from which, the ultrasound probe's position should be maintained for the remainder of the protocol. Training of the sonographer is important to guarantee high-quality output (9). For research purposes, post-test analysis of the artery diameter and blood flow velocity is recommended to be performed using custom-designed edge-detection and wall-tracking software that is largely independent of investigator bias (10).

Blood flow. Ultrasonography can be used to examine conduit artery diameter and blood velocity, which can be used to calculate blood flow through a conduit artery. These procedures are also used to calculate shear stress or shear rate, the frictional force of blood on the arterial wall. Shear stress represents a highly relevant area in science, which plays an important role in inducing changes to the arterial wall, typically via endothelial cell signal transduction (5). Shear stress is the tangential force of blood flow on the endothelial surface of the artery. The magnitude of shear stress (τ) in straight vessels can be estimated as being directly proportional to the viscosity of blood (μ) and inversely proportional to the third power of the inner radius of the vessel (R), with flow rate represented as Q (11, 12).

$$\tau = 4\mu Q/R^3$$

In vivo observations indicate that fluctuations in blood flow or shear stress play critical roles in vascular homeostasis and remodeling, and consequently improved vascular health (13, 14). For example, at the onset of acute exercise, mean blood flow and shear stress drastically increase in the active limbs (15, 16), whilst decreases are seen in the inactive limbs (5). The distinct pattern of shear stress have important consequences for acute and chronic adaptations in conduit artery structure (17). This highlights the importance to assess conduit artery blood flow and shear stress, both under resting and during various conditions that cause alterations in blood flow and shear stress (e.g. exercise).

2.2 Vascular function

Ultrasound can also be used to examine the function of conduit arteries, which is typically performed by examining the change in conduit artery diameter in response to a physiologically relevant stimulus (e.g. shear stress). Here, we discuss two procedures that can be used to evaluate peripheral (flow-mediated dilation; FMD) and central (carotid artery reactivity; CAR) conduit artery vascular function (Figure 1).

Flow-mediated dilation (FMD). As introduced by Celermajer in 1992 (18), peripheral vascular function can be assessed with the flow-mediated dilation (FMD) technique. Using ultrasound, brachial or femoral FMD relates to the evaluation of the artery diameter in response to a 5-minute period of ischaemia, typically induced by inflating a pneumatic cuff around the forearm or the thigh (distal from the imaged artery) to supra-systolic levels. The artery is typically recorded for a 1-minute baseline period, followed by a 5-minute epoch of cuff occlusion (ischaemia), and then a 3-minute period of post-cuff release (reperfusion). It is important to note that substantial between-laboratory variation is present in the FMD protocol, which impairs reproducibility and comparability between studies. Therefore, it is essential to follow expert-consensus guidelines in the performance of the FMD (19). Following these guidelines, studies have demonstrated that the brachial artery FMD likely represents an endothelium-dependent, largely nitric oxide-mediated dilation of conduit arteries (19). Moreover, this non-invasive method has been validated against coronary endothelial function (20) and independently predicts cardiac events in subjects with cardiovascular disease and even in asymptomatic individuals (4). Specifically, each one percent increase in FMD has been associated with an 8-15 % decreased risk of cardiovascular events and mortality (21-24). This demonstrates the clinical relevance of using the FMD technique in (pre)clinical studies.

Carotid artery reactivity (CAR). More recently, a procedure has been introduced to evaluate central artery vascular function, which adopts activation of the sympathetic nervous system to subsequently evaluate the carotid artery diameter response. Sympathetic nervous system activation is an important and clinically relevant prognostic stimulus, which can be used to evaluate coronary artery function (25). For example, the cold pressor test (placing one hand in ice slush), is a potent sympathetic stimulus resulting in coronary artery vasodilation in healthy individuals, yet a marked vasoconstriction in people with cardiovascular disease (26,

27). Although this test of coronary artery vascular function holds independent prognostic value, the invasive and technical nature of coronary angiography prohibits large-scale clinical use. Interestingly, similar to coronary arteries (28), carotid artery dilation occurs in healthy participants, whereas high-risk participants demonstrate a vasoconstrictive response during cold pressure test-induced sympathetic stimulation (29). Based on this remarkable observation, ultrasound can be used to examine carotid artery reactivity (CAR) in response to sympathetic stimulation using the cold pressor test (30, 31). Following 10-minutes of supine rest, carotid artery diameter and blood flow velocity are recorded using ultrasound sonography. The participant then immerses their left hand (up to the wrist) in ice slush (≤4°C) for 3 minutes. During which, the ultrasound probe position should be maintained, and the participant instructed to remain still, not hyperventilate, or talk unless necessary (19, 31). Finally, as the sympathetic stimulus (ice slush) can cause hyperventilation (32), this should be controlled for by monitoring gaseous exchange when feasible. Previous work has found strong correlation between coronary and carotid artery responses to the cold pressor test (33). Moreover, the CAR-test independently predicts adverse clinical events in patients with peripheral arterial disease (34). Finally, it was recently demonstrated that carotid vasoconstriction in response to sympathetic stimulation can be reversed following exercise training (29). This highlights the utility of this novel test of central vascular function in (pre)clinical studies.

Structure. Arterial structure, including vessel diameter and wall thickness, are key variables to measure both independently and in conjunction with vascular function (Figure 1). For example, resting diameter is inversely associated with shear rate and directly related to peak flow (35). As such, baseline artery diameter is inversely associated with FMD response, with smaller arteries producing a larger functional stimuli (and higher FMD) (7). It is therefore essential to consider potential differences in artery size when comparing FMD results between participants. It is also important to measure the peak diameter responses in order to exclude the impact of vasodilators and/or constrictors that influence resting vessel diameter (19).

In addition to the evaluation of the resting and peak diameter, ultrasound can also be used to examine the intima-media thickness (IMT). IMT, measured via ultrasound as the distance

between the lumen-intima and media-adventitia interfaces, is a marker of subclinical atherosclerosis and can be measured in multiple arteries, most commonly the common carotid artery. In the evaluation of conduit artery IMT, it is important to use automated wall-tracking software to measure the far wall of the vessel as this improves reproducibility (36, 37). Following automated calibration of vessel diameter (a pixel-density algorithm can automatically identify the angle-corrected near and far wall e-lines for every pixel column for diameter assessment), the same algorithm can be used to identify the far wall media-adventitia interface, allowing for IMT interpretation on every frame selected (38). Regardless of target vessel, IMT is associated with risk of atherosclerosis (39) and carotid IMT is associated with increased risk of cerebral events (40). Carotid IMT may also precede and predict future cardiovascular events (41, 42), though, does not seem to improve conventional risk prediction models (43, 44). Importantly, exercise training can decrease arterial wall thickness in healthy asymptomatic individuals and those with cardiovascular disease that present with already increased arterial wall thickness (39). This highlights the utility of measuring vascular structure in (pre)clinical studies.

2.3 Arterial stiffness - pulse wave velocity (PWV)

Despite the various possible sites of measurement and devices used, pulse wave velocity (PWV) is typically determined by measuring the velocity of waveforms at two different locations and the time delay (or transit time) measured between the two waveforms. The transit time is therefore the time the waveform takes over a known distance. A variety of different waveforms can be used including pressure, distension, and doppler (45). The distance covered by the waveforms is typically measured by the surface distance between the two recording sites:

PWV = Distance (metres) / time (seconds)

Typically, the higher the PWV, the higher the arterial stiffness and consequent risk of cardiovascular disease. The most common devices for assessing PWV include applanation tonometry (i.e. SphygmoCor or PulsePen devices), Piezoelectric mechanotransducer (i.e. Complior or Aortic devices), and cuff-based oscillometry (i.e. Arteriograph or Mobil-O-Graph devices).

PWV has been developed as a feasible and clinically important direct marker of arterial stiffness, able to independently predict cardiovascular events and mortality risk (46). Indeed, aortic PWV (aPWV) improves the prediction of cardiovascular events beyond more traditional risk factors (45, 47). Although carotid-femoral pulse wave velocity (cfPWV) has been defined as the gold standard for determining arterial stiffness (48), a number of other sites have been validated as useful surrogates, including brachial-ankle, cardiac-ankle, and finger-toe PWV (49). Nonetheless, PWV (regardless of measurement site) is an important and independent marker of cardiovascular risk, and useful in (pre)clinical studies.

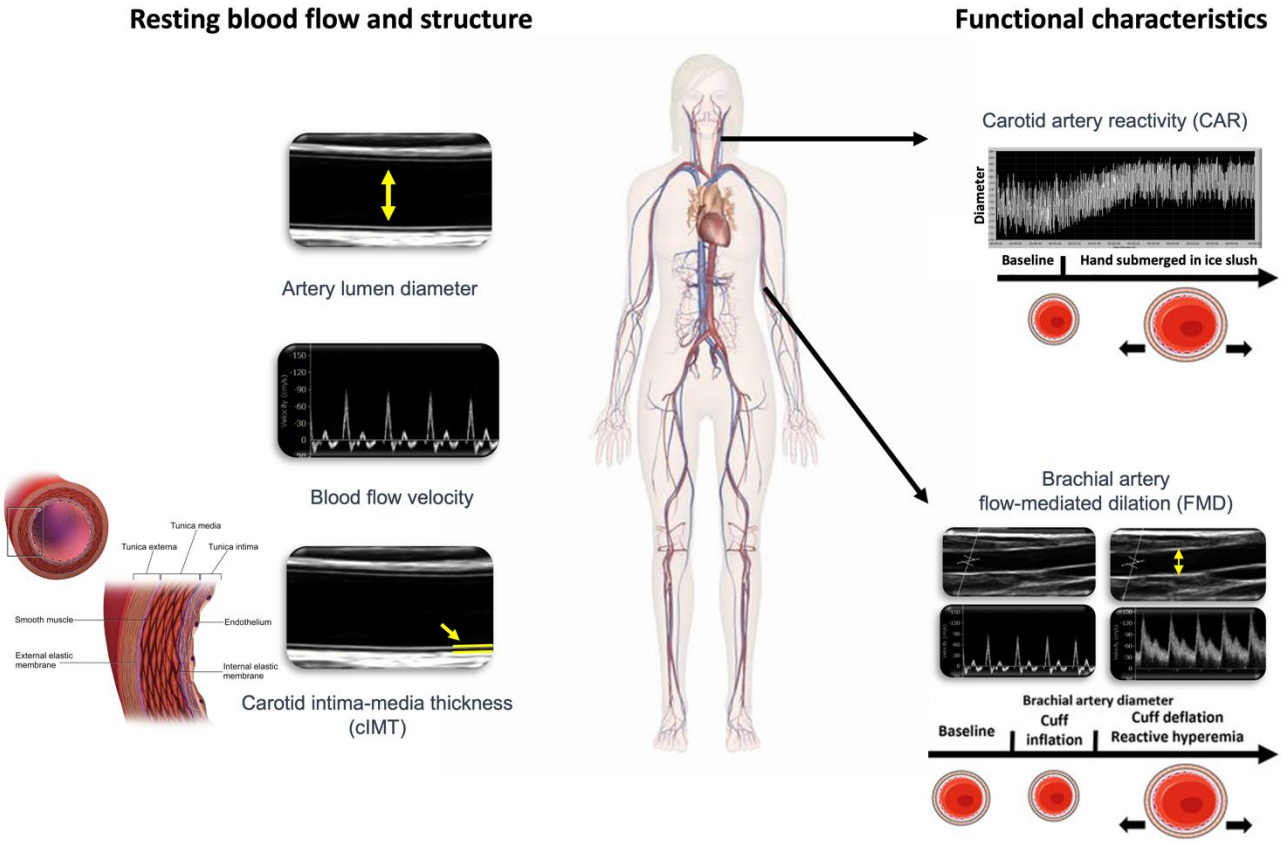

Figure 1. Illustration of ultrasound measurement of resting blood flow and vessel structure (left) and vascular function (FMD and CAR; right) in healthy participants.

3. Resistance arteries

3.1 Blood flow (plethysmography)

Introduced in 1909, venous occlusion plethysmography is a non-invasive and low-cost tool that can be used to assess blood flow and reactive hyperaemia in the forearm, calf, and thigh (50-52). The central principle is that when venous outflow from an extremity is blocked, typically performed by inflating a cuff to sub-diastolic levels, any immediate increase in volume reflects the rate of arterial inflow (i.e. blood flow). Since conception, venous occlusion plethysmography has been used to examine resting blood flow, but also study the acute effects of various physiological stimuli (e.g. sympathetic activation) and/or pharmacological stimuli (e.g. endothelium-dependent and independent dilators/constrictors) (53-59). These studies contribute to a better understanding of the (patho)physiology of resistance artery vascular function within and between patient subgroups.

In brief, venous outflow from the target limb is interrupted by a cuff inflated above venous pressure and below arterial diastolic pressure (~40-60 mmHg), proximal to the area of interest (typically lasting ~8 heart beats). The target limb is positioned at the level of the heart to ensure adequate venous emptying during cuff deflation (lasting ~8 heart beats). When examining the forearm, the hands are usually excluded from the circulation during measurements by initial rapid inflation of smaller cuffs, placed around the wrist to supra-systolic pressure (~220 mmHg) (60). The wrist-cuffs must be inflated at least 60s before starting measurements of flow to allow forearm blood flow to stabilize (61). Upon cuff inflation, limb volume immediately changes. These changes in volume can be measured using mercury-in-silastic strain gauges. Peripheral limb blood flow, determined via venous occlusion plethysmography, is then usually expressed as ml blood per 100 ml of forearm volume per minute.

3.2 Vascular function and structure (plethysmography + pharmacology)

Venous occlusion plethysmography is most frequently used to assess changes in resistance artery blood flow when combined with physiological or pharmacological stimuli (Figure 2). For example, maximal blood flow can be examined after a prolonged (5-10 minutes) ischaemic exercise, induced by combining handgrip exercise with inflating a blood pressure cuff to supra-systolic pressure. Peak blood flow values can then be used to examine resistance artery structure. By maximally dilating resistance arteries through this ischaemic stimulus,

peak blood flow reflects the structure of the resistance artery vascular bed. Furthermore, vascular function can be examined using venous occlusion plethysmography through evaluation of blood flow changes in response to vasoactive drugs, typically administered intra-arterially via a canular under local anaesthesia. For example, infusion of powerful vasoconstrictors (endothelin 1 (58)), endothelium-dependent dilators (acetylcholine (62)), endothelium-independent dilators (glyceryl trinitrate (63)), or selective blockers (e.g., L-NG-Monomethyl-arginine (64)), have been used to varying degrees to explore vascular function. An important advantage to this technique is the ability to investigate local effects of selected vasoactive substances, without invoking concomitant systemic effects. To control for potential systemic effects, evaluation of venous occlusion plethysmography is typically performed on both forearms, where one forearm is used to locally infuse vasoactive substances and the contra-lateral arm is used to evaluate and control for potential systemic effects (including effects affecting blood pressure).

In addition, venous occlusion plethysmography can be used to differentiate endothelium-dependent from endothelium-independent responses (Figure 2). For example, McVeigh et al. (64) demonstrated *in vivo* for the first time that patients with Type 2 diabetes present with impaired endothelium-dependent and -independent vasoactive responses to acetylcholine and glyceryl trinitrate, respectively. By using L-NG-Monomethyl-arginine to inhibit endothelium-dependent vasodilation via nitric oxide release, the authors were able to investigate endothelium-independent vasodilation in isolation. Impaired endothelial-independent function is associated with structural vascular alterations and alterations in smooth muscle cells (65). It is therefore important to also evaluate endothelial-independent vascular function, though many studies do not, and we therefore know little about non-endothelial vascular adaptations to exercise training. Further investigation will help elucidate the multiple and interconnected pathways underlying upregulated conduit artery function in response to hemodynamic stimuli training in humans (6).

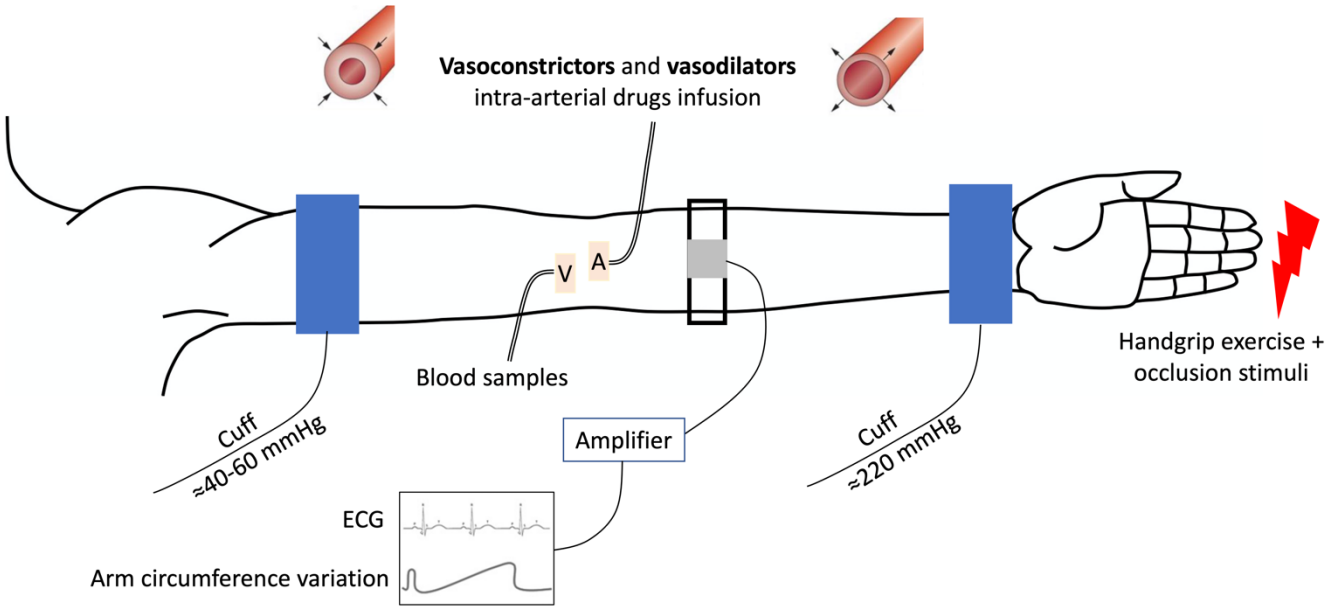

Figure 2. Illustration of the venous occlusion plethysmography technique to determine resistance artery structure and function via evaluation of blood flow changes in response to vasoactive drugs and/or handgrip exercise.

4. Summary

Peripheral and central artery blood flow and vascular function are centrally involved in the development of cardiovascular disease. Thus, the investigation of blood flow and vascular function is of great interest for our understanding of mechanisms, and more clinically, for primary and secondary prevention of cardiovascular disease. The techniques presented in this chapter, therefore, play an integrative role in our understanding of the impact of physical activity and exercise on improving vascular function and health.

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