19th Scientific Annual Meeting of the International Society of Vascular Health ang Ageing (ISVH)

HRVATSKO DRUŠTVO ZA ZDRAVO VASKULARNO STARENJE

HRVATSKA LIGA ZA HIPERTENZIJU

Workshop:

Endothelial function

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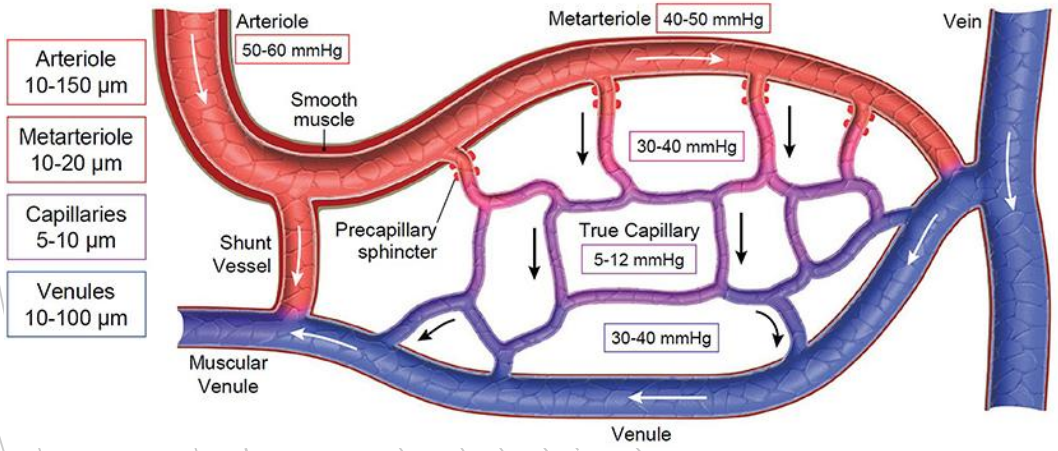
The microcirculation



- The microcirculation refers to arteries with the smallest resistance (less than ~150 mm in diameter), arterioles, capillaries, and venules
- *capillary network is essential for nutrient and gas exchanges between blood and tissue*
- *arterioles are prominently implicated in blood flow regulation*
- The mechanisms involved:
  - arteriolar myogenic response
  - flow-induced vasodilation (in response to wall shear stress)
  - and metabolic and neural control (sympathetic activation, calcitonin gene-related peptide (CGRP) and substance P)

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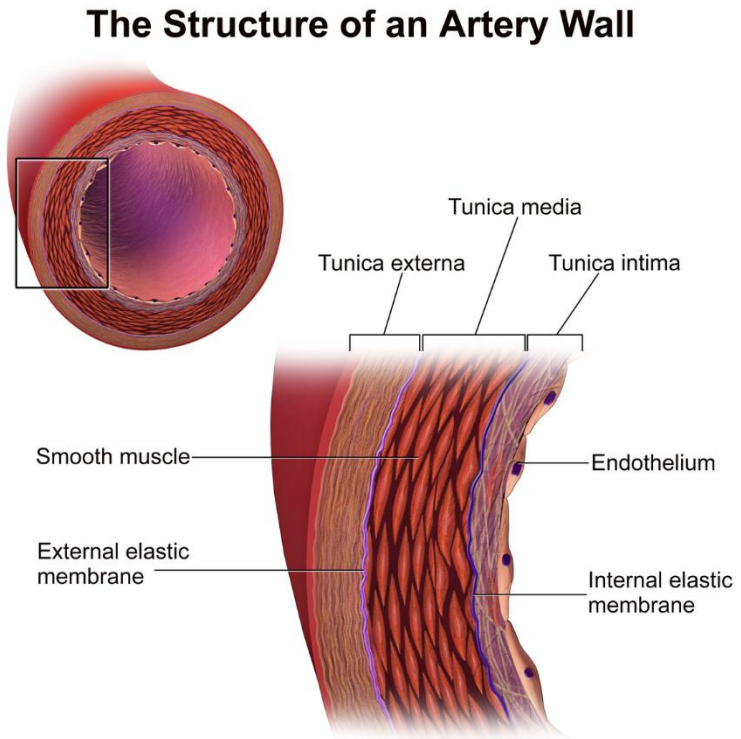
Fluid Therapy and the Microcirculation in Health and Critical Illness
Edward S. Cooper1* and Deborah C. Silverstein2

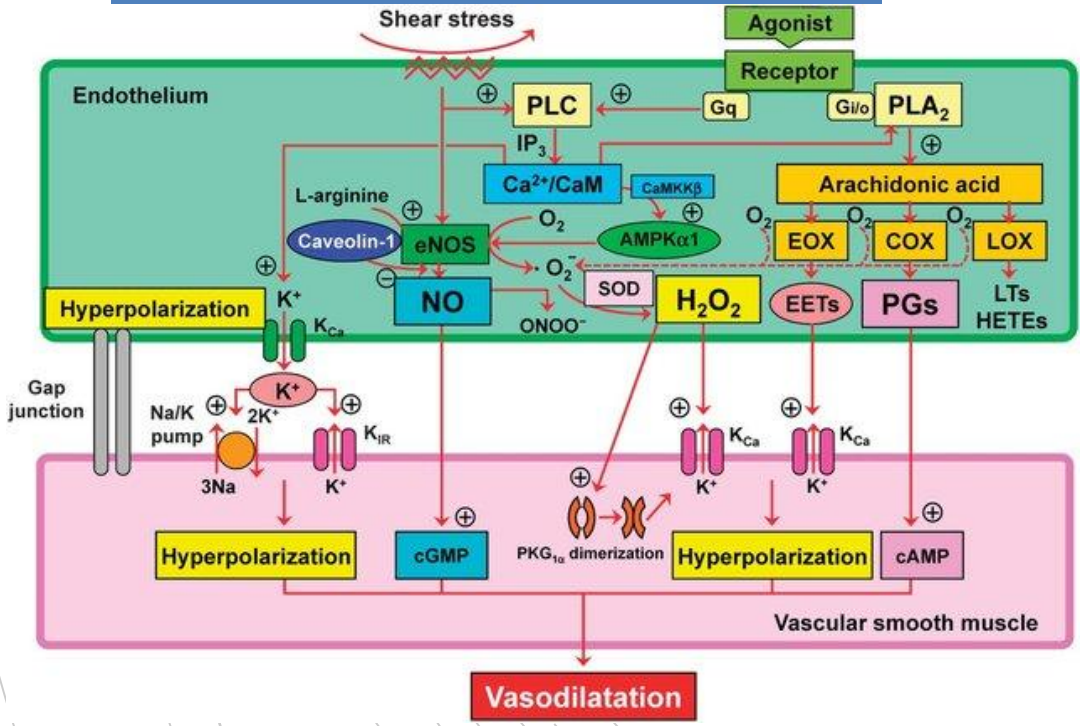
Endothelium

- **Vascular smooth muscle cells (VSMCs)** - common effectors
- **Endothelium** - releases mediators involved in relaxation and contraction



https://en.wikipedia.org/wiki/Tunica_media

Endothelium



- **Endothelium-dependent relaxation and conctriction** - involves different pathways

- (1) Nitric oxide (NO)
- (2) Metabolites of arachidonic acid that signal via cyclooxygenases, lipoxygenases and cytochrome P450 pathways
- (3) Peptides like endothelin, urotensin, C-type natriuretic peptide (CNP), adrenomedullin, adenosine, purines, reactive oxygen species and others

Shimokawa H, Godo S. Diverse Functions of Endothelial NO Synthases System: NO and EDH. J Cardiovasc Pharmacol. 2016 May;67(5):361-6. doi: 10.1097/FJC.0000000000000348.

Microvascular dysfunction

- In both physiological (aging) and pathological conditions, with special relevance in cardiovascular disease
  - hypertension
  - increased reactive oxygen species (ROS)
  - diabetes

- These examples highlight the potential relevance of early detection of microvascular dysfunction
- In most cases **direct study of the target microvascular bed is technically challenging** (e.g., the coronary microcirculation)
- **!!! the need for appropriate surrogate markers**

The skin microcirculation as a model of generalized microvascular endothelial function

- Owing to its accessibility, the **cutaneous microcirculation** has been suggested as a model of generalized microvascular function
- This implies an association between microvascular dysfunction in the skin and in other vascular beds
- *In the past 10 years, it has been shown that skin microvascular reactivity is abnormal in a variety of cardiovascular diseases*.
- <u>the skin circulation is an accessible vascular bed for which dysfunction correlates with markers of cardiovascular disease</u>

Non-invasive methods to assess skin microvascular function

- **Videocapillaroscopy** - direct observation of skin capillaries with a microscope

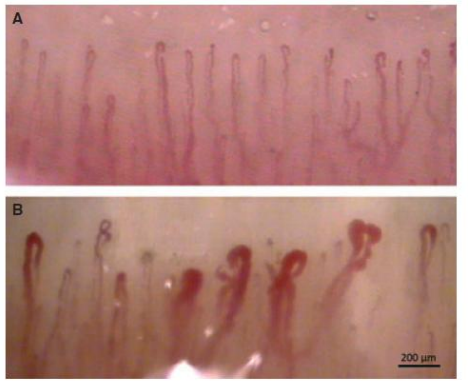
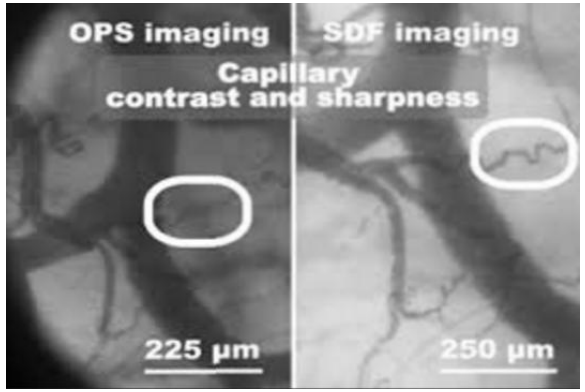


Figure 1. Representative images of nailfold videocapillaroscopy (NVC) with a magnification ×100. (**A**) Normal pattern showing homogenous distribution of capillary loops. (**B**) Pattern observed in a patient with systemic sclerosis, showing disorganized enlarged/giant capillaries.

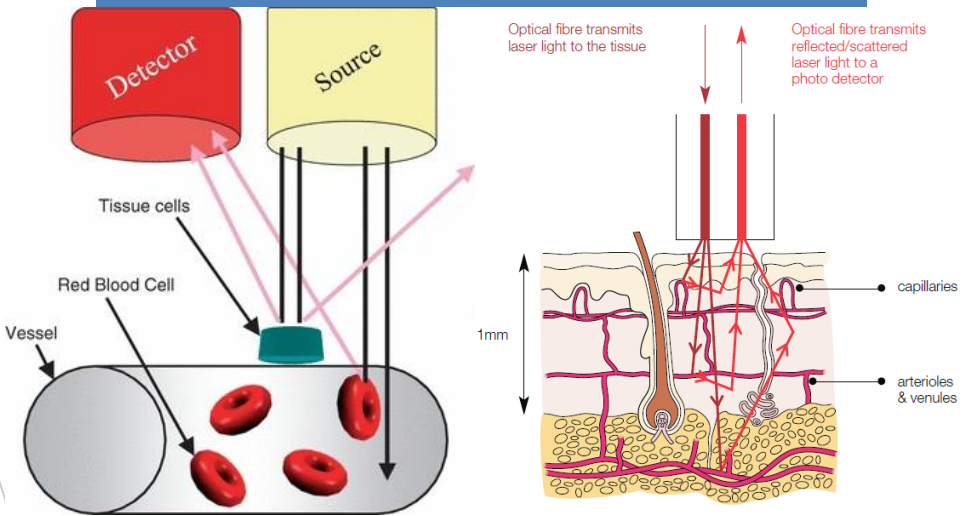
- **Orthogonal polarization spectral imaging** and **sidestream dark-field imaging** - images of the microcirculation with a high level of contrast on organs covered by a thin epithelial layer



→ **these techniques do not allow easy mechanistic studies of the pathways underlying microvascular dysfunction**

- **Laser Doppler techniques** or the recently developed laser speckle contrast imaging (LSCI) can yield accurate quantification of relative changes in skin perfusion.

Laser Doppler



- Laser Doppler techniques provide *an index of skin perfusion* by measuring the Doppler shift induced by coherent monochromatic light scattering by moving red blood cells
- The signal is quantified as the product of average red blood cell velocity and concentration
- Because *it does not provide an exact measure of flow (ml/min),* it is often referred to as **flux**
- A linear relationship between flux and actual flow has been demonstrated
- Two distinct tools are used to assess perfusion:

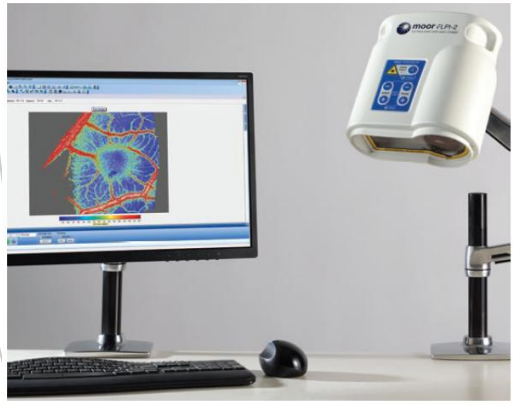
Laser Doppler flowmetry (LDF)

moorVMS-LDF – single and dual channel modules with MemoryChip probes.

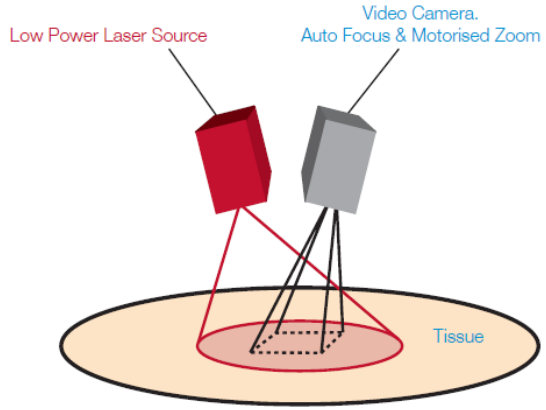
- Assesses blood flow over a small volume (1 mm3 or smaller) with a high sampling frequency (often 32 Hz)
- Single-point LDF (comprising one transmitting and one receiving optical fiber) is accurate at quantifying **fast changes in skin blood flux**
- The regional heterogeneity of skin perfusion due to the skin anatomy leads to spatial variability → the relatively poor reproducibility of the technique
- The use of integrating probes (made of seven or eight collecting fibers) decreases spatial variability and improves reproducibility by averaging the signal from different scattering volumes

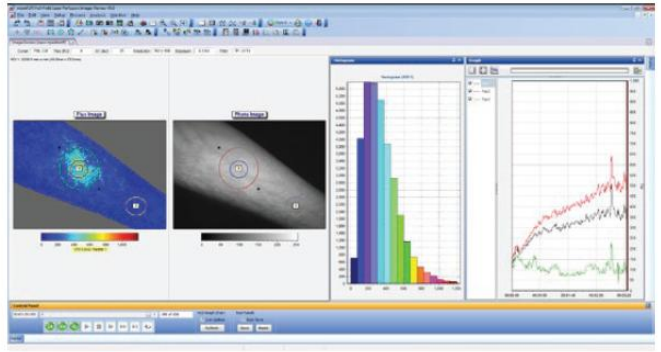
Laser Doppler Imaging (LDI)

- The laser beam is emitted at a certain distance above the skin surface and reflected by a computer-driven mirror to progressively scan the area of interest
- Providing 2D images mapping the cutaneous perfusion
- <u>LDI has lower spatial variability compared to LDF</u>
- It is much slower (a few minutes may be required to capture one image)
- <u>Rapid changes in skin blood flow over large areas are thus more difficult to record</u>



moorFLPI-2 – desktop setup.





Forearm – wheel, flare and axon reflex due to capsaicin injection. Scan area approximately 15 x 20cm.

Reactivity tests

- Laser techniques provide relative signals
- Microvessels are therefore stimulated to assess their reactivity

- **Mechanical stimuli** (occlusion of an artery)
- **Thermal provocation** (heating or cooling)
- **Electrical stimuli**
- **Local administration of pharmacological agents**

- All these tests vary in different aspects such as their reproducibility or ease of implementation, as well as the physiological pathways they explore

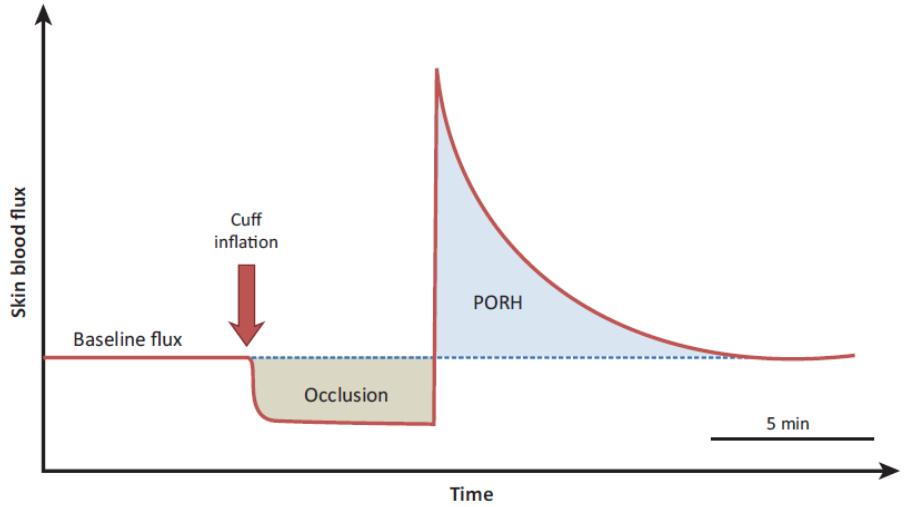
Post-occlusive reactive hyperemia (PORH)

- Analogy with a standard method to assess endothelial function in conduit vessels
- Arterial occlusion has been proposed as a test of endothelial microvascular function
- **Post-occlusive reactive hyperemia (PORH)** - a transient increase in cutaneous flux in human skin observed immediately after occlusion

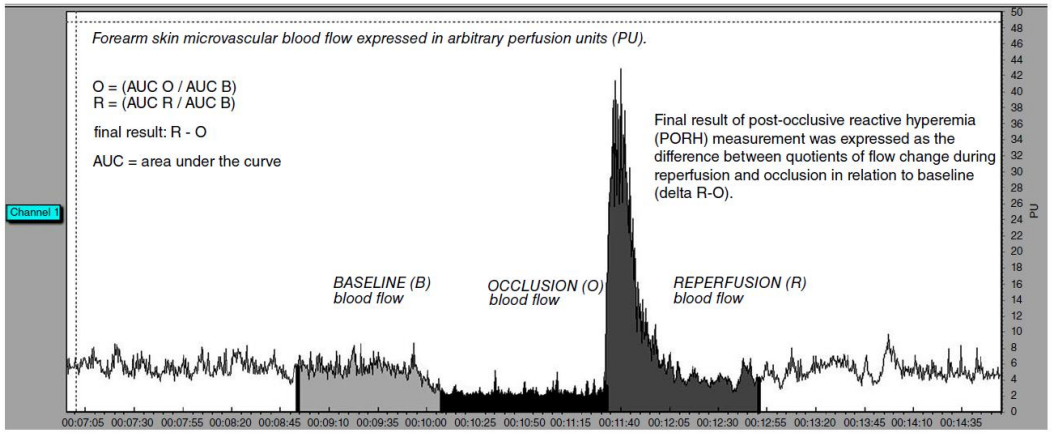
- Test is usually performed by placing a cuff on the upper arm while recording flux on the forearm or the finger (the distance between the cuff and the measurement site influences the time to peak)
- The cuff is inflated to above systolic pressure for a few minutes
- The typical occlusion time is 5 min, but times of 1–15 min have been used
- There is a linear correlation between the period of ischemia and hyperemia

Post-occlusive reactive hyperemia (PORH)

- The parameter quantified is the absolute peak value or the peak value expressed as a function of baseline
- The area under the curve also takes into account the time course of the response (i.e., until the return to baseline flux)



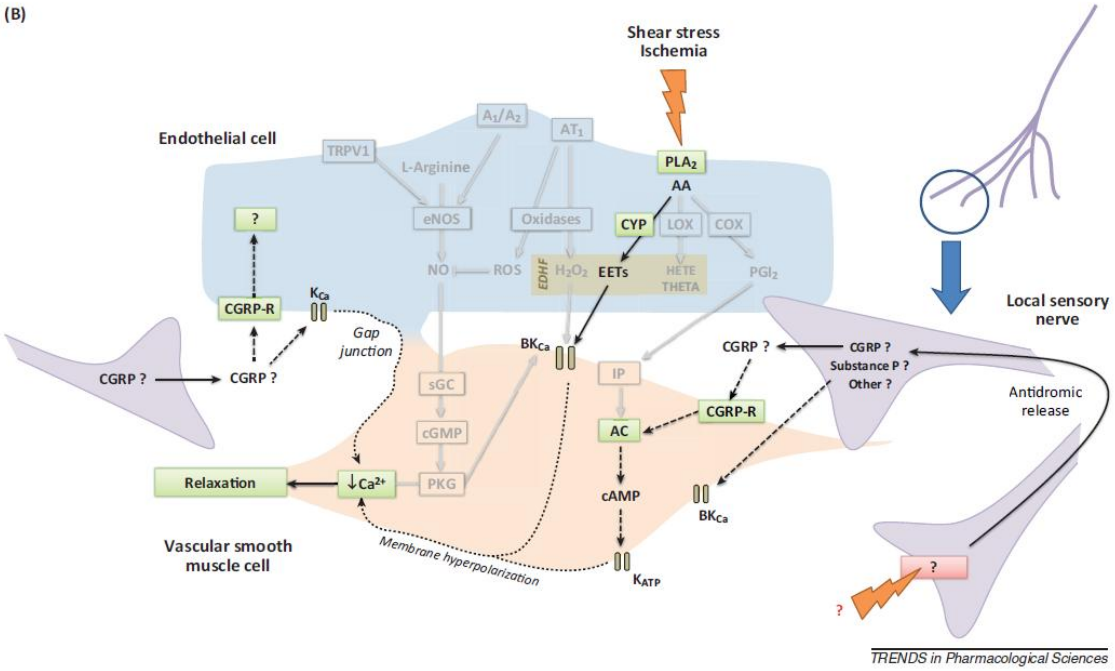
Roustit M, Cracowski JL. Assessment of endothelial and neurovascular function in human skin microcirculation. Trends Pharmacol Sci. 2013 Jul;34(7):373-84. doi: 10.1016/j.tips.2013.05.007.



Cavka A, Cosic A, Jukic I, Jelakovic B, Lombard JH, Phillips SA, Seric V, Mihaljevic I, Drenjancevic I. The role of cyclo-oxygenase-1 in high-salt diet-induced microvascular dysfunction in humans. J Physiol. 2015 Dec 15;593(24):5313-24. doi: 10.1113/JP271631.

Figure 1. LDF measurement of skin microvascular blood flow
Microcirculatory blood flow in a given time was expressed in arbitrary perfusion units and determined by software calculating the area under the curve (AUC) during baseline flow, occlusion and reperfusion (AUC is denoted by the shaded portions of the trace). Because the flow does not reach the value of zero even when perfusion is absent, flow values are expressed as a quotient of a standard comparator – baseline flow. The final result was expressed as the difference between the quotients of flow change during reperfusion and occlusion in relation to baseline (ΔR-O). Source: original trace, Laboratory for Vascular Physiology, Department of Physiology and Immunology, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek).

Post-occlusive reactive hyperemia (PORH)



- Among **mediators contributing to PORH**, the involvement of sensory nerves has been described as a major contributor to both the peak and time course

- *inhibition of NO production does not alter PORH in the skin*
- *interestingly, COX inhibition unmasks the NO dependence of PORH*
- *results are conflicting concerning the implication of prostaglandins, but there is no strong evidence that prostanoids participate in PORH*
- *BKCa inhibition suggests that EDHF play a major role in PORH; more specifically, fluconazole, a CYP 2C9 and 2C19 inhibitor, inhibits skin PORH*

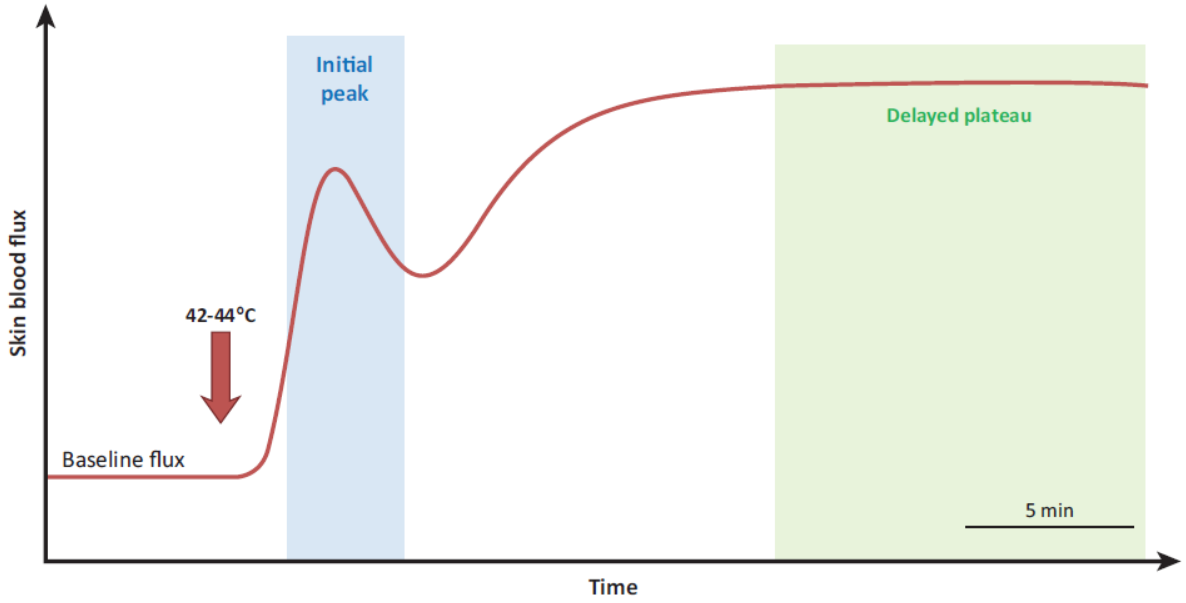
- Sensory nerves and CYP metabolites (putatively EETs) are the major contributors to skin PORH

Roustit M, Cracowski JL. Assessment of endothelial and neurovascular function in human skin microcirculation. Trends Pharmacol Sci. 2013 Jul;34(7):373-84. doi: 10.1016/j.tips.2013.05.007.

Post-occlusive reactive hyperemia (PORH)

- *Skin PORH is impaired in **patients with cardiovascular risk** and is increased by statin or antihypertensive therapy*
- *It has recently been shown that skin PORH is an independent marker of atherosclerotic damage in **patients with type 1 diabetes***
- *Our research group repeatedly shown impaired PORH following short-term **high-salt diet** in healthy individuals*
- ***N-3 PUFAs supplementation** in a for of functional food enhances PORH in healthy individuals (sedentary and athletes) and cardiovascular patients*

Thermal challenges



- The central role of the cutaneous microcirculation in human thermoregulation is characterized by extreme reactivity to thermal challenges
- Local heating of the skin induces **local thermal hyperemia (LTH)** characterized by a biphasic rise in skin blood flow
- A rapid initial peak is observed within 2–3 min after the onset of heating, which mostly depends on a local sensory nerve axon reflex
- The initial peak is followed by a brief nadir and then by a prolonged plateau after 20–30 min of heating

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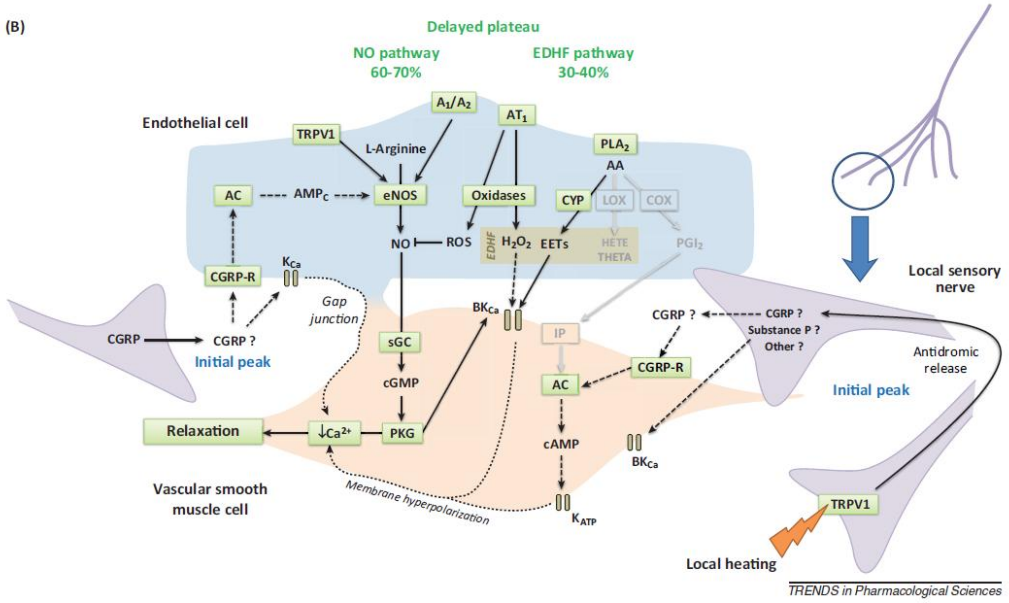
Thermal challenges

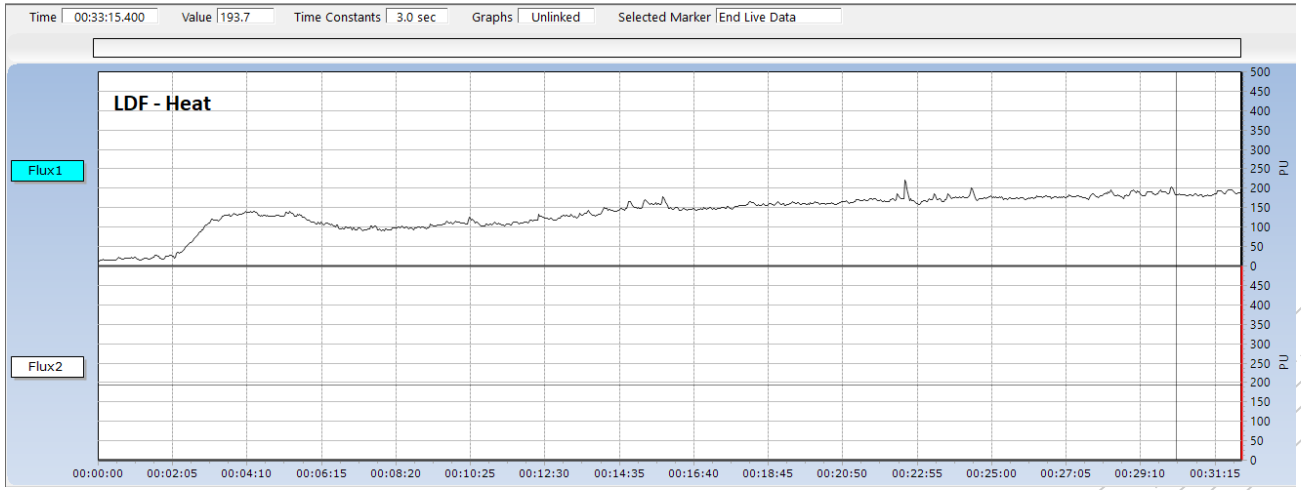


- **LTH-mediating mechanisms:**
- The late plateau depends predominantly on **endothelial factors**, among which **NO accounts** for approximately two-thirds of the response
- NOS inhibition does not completely abolish the plateau phase, so other contributors must be involved
- Although the COX pathway does not seem to be involved in the response, **EDHF is involved in the plateau phase**, half of which is **EET-dependent**
- Combined blockade of NO and EDHF pathways almost completely abolishes plateau hyperemia, with a synergistic effect, suggesting reciprocal upregulation between these two pathways

Roustit M, Cracowski JL. Assessment of endothelial and neurovascular function in human skin microcirculation. Trends Pharmacol Sci. 2013 Jul;34(7):373-84. doi: 10.1016/j.tips.2013.05.007.

Thermal challenges

- Commercially available LDF probes with built-in metallic heaters make this test particularly easy to implement
- Although different heating protocols have been proposed, a temperature of 42–44 °C is usually recommended because it induces the biphasic response without being painful
- Heating protocols should last at least 30 min to observe the plateau, although it should be noted that beyond 45–50 min a slow reversal towards baseline is observed on the forearm (referred to as the die away phenomenon)



Thermal challenges

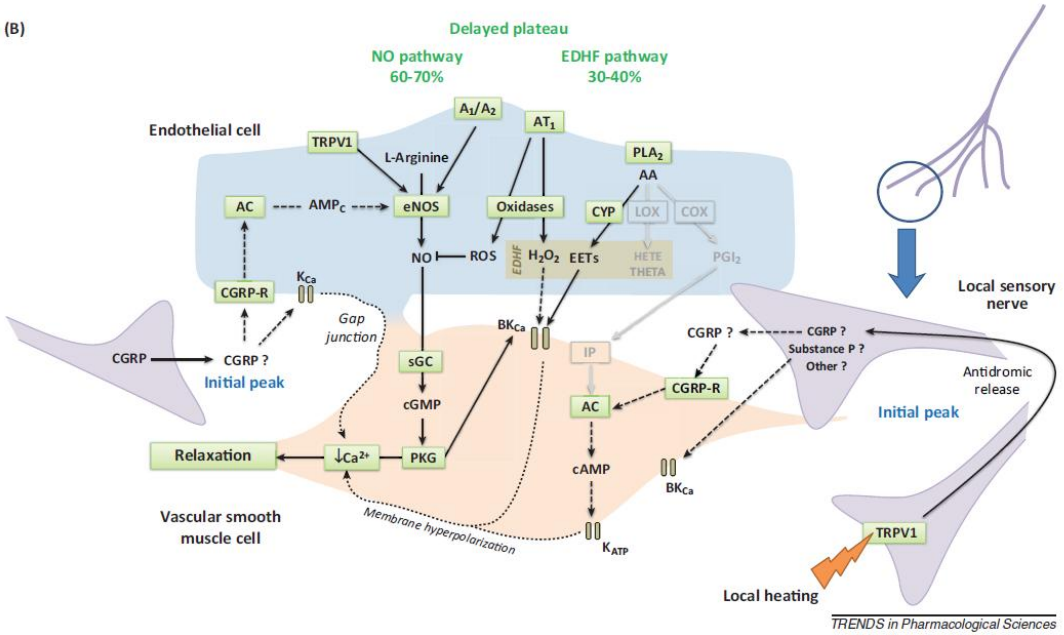
Research and Clinical Application:

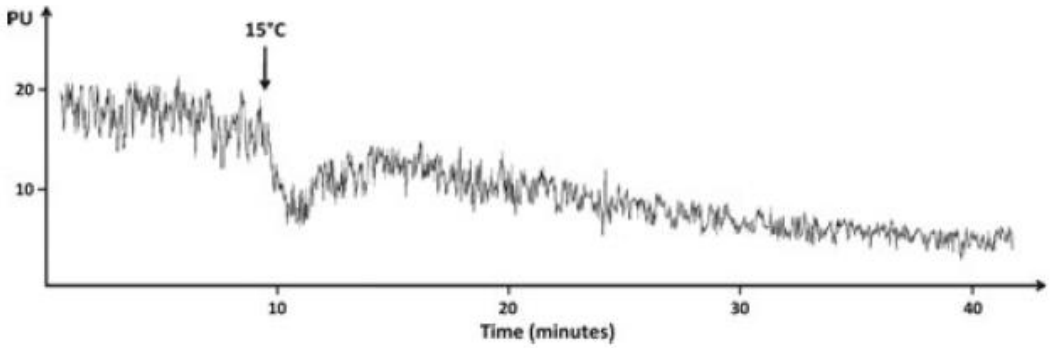
- *Recent applications of local heating coupled with LDF showed decreased cutaneous endothelial vasodilation in patients with hypertension and in women with polycystic ovary syndrome*

- *In hypercholesterolemic patients, statin therapy restores the decreased endothelium-dependent plateau*

Thermal challenges

- Several **local cooling** methods coupled with LDF have been described and some induce a reproducible triphasic response
- The initial rapid vasoconstriction is followed by transient vasodilation and, finally, prolonged vasoconstriction
- The initial vasoconstriction is mediated by activation of the RhoA–Rho kinase pathway, enhancing the translocation of a2c-adrenoceptors to the cell surface
- The prolonged vasoconstriction probably involves both the RhoA–Rho kinase pathway and inhibition of NO
- It is likely that the transient vasodilation is mostly mediated by sensory nerves, but this remains unclear





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Thermal challenges

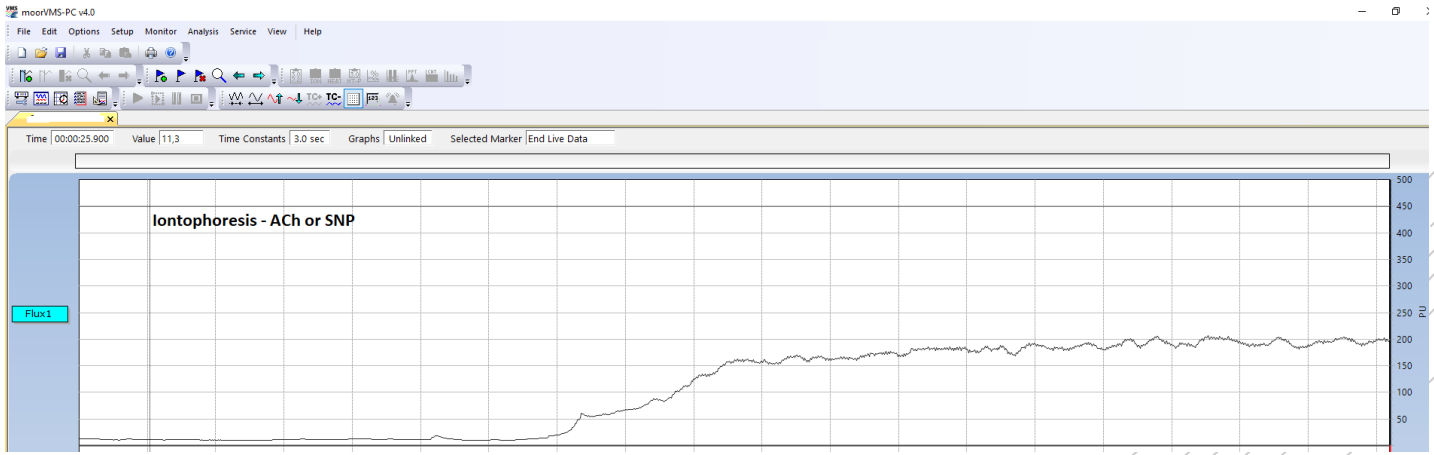
- **Research and Clinical Application:**

- *Abnormal microvascular reactivity to local cooling has been shown in Raynaud's phenomenon (RP), justifying the recent use of this test to assess the effect of drugs in such patients*

Iontophoresis of acetylcholine (Ach) and sodium nitroprusside (SNP)

- **Iontophoresis** has been widely used as a non-invasive pharmacological tool
- The main iontophoresis applications in reactivity testing involve Ach and SNP, both widely used to assess microvascular endothelium-dependent and independent vasodilation, respectively.

- The exact mechanisms by which iontophoresis of Ach induces vasodilation of skin microvessels remain debated
- *COX-dependent pathway seems likely, although data are conflicting*
- *In the same way, the involvement of NO is controversial*



Iontophoresis of acetylcholine (Ach) and sodium nitroprusside (SNP)

- Iontophoresis of SNP consists of exogenous delivery of NO to SMCs, and has therefore been used as an endothelium-independent reactivity test

- One of the issues with iontophoresis is non-specific, current-induced vasodilation (CIV) → this confounding effect is difficult to control

- Research and Clinical Application:
- *Endothelium-dependent and -independent vasodilation assessed with iontophoresis of Ach and SNP, respectively, is impaired in patients with coronary artery disease, in nondiabetic women with angina, in patients with type 2 diabetes and obstructive sleep apnea*

Reactivity tests (summary)

Stimulus and technique	Laser Doppler flowmetry
Mechanical	
Arterial occlusion (PORH)	++ Well-established validity, easy to implement, fair to poor reproducibility (improved when normalizing skin temperature)
Thermal	
Local heating (LTH)	++ Well-established validity, easy to implement, fair to poor reproducibility (improved when normalizing skin temperature and/or using integrated probes)
Local cooling	++ Well-established validity, difficult to implement (requires custom-made devices), acceptable reproducibility
Pharmacological	
Anodal iontophoresis of acetylcholine	+ Conflicting data, heterogeneity in methods, easy to implement, fair reproducibility, (interference with CIV)
Cathodal iontophoresis of sodium nitroprusside (SNP)	+ Easy to implement, heterogeneity in methods, poor reproducibility, interference with CIV
Time–frequency analysis	+ Easy to perform (on baseline flux), heterogeneity in methods, unknown reproducibility

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Methodological issues

Box 2. Methodological issues

There are several sources of variability that investigators should be aware of when assessing cutaneous microvascular reactivity. When possible, the following parameters must be controlled and reported in publications:

Characteristics of the population

(i) Age: although aging does not affect resting blood flow, response to thermal challenges and to PIV is impaired in the elderly [53,100].
(ii) Gender and oral contraceptives: the influence of variations in female hormone levels across the menstrual cycle on skin blood flux has led to conflicting conclusions. Recent work suggests a modest impact on LTH and PORH [101]. Consequently, the phase of the menstrual cycle and the use of oral contraceptives should be taken into account in clinical studies.
(iii) Stress: it has been reported that mental stress decreases the baseline skin blood flux [23]. Measurements should therefore be performed in a quiet environment.
(iv) Diseases and vasoactive drugs: the skin microcirculation is impaired in several diseases (see text for details). Such diseases, as well as the use of vasoactive drugs, should therefore be carefully explored.
(v) Participants should be asked to refrain from smoking cigarettes [102], drinking coffee, and consuming food prior to microvascular exploration.

Recording conditions

(i) Technical specifications may induce some variability among studies, and should be reported, e.g. [103]:
- Wavelength;
- Acquisition frequency;
- Laser head to skin distance for imaging (non-contact) techniques; and
- The use of liquid-filled chambers for iontophoresis or local heating (with imaging techniques) modifies the absolute perfusion value [23].

(ii) Environment
- Because of systemic and local thermoregulatory mechanisms, the skin temperature should be kept constant [29].
- Ambient lighting should be kept stable for LSCI [103].
- The use of a vacuum cushion to maintain the hand and forearm as still as possible [23] or subtracting the signal backscattered from an opaque adhesive surface adjacent to the region of interest (especially when the skin surface is moving) [104] are simple methods to reduce movement artifacts.

Data analysis and expression

(i) The regions of interest for imaging techniques must be large enough to limit variability (>10 mm^2 for LSCI [81]).
(ii) The time of interest is the time over which the flux is averaged (for high-frequency acquisition techniques, i.e., LDF and LSCI). It should be carefully chosen according to the performance of the technique and the kinetics of the response [23].
(iii) The way of expressing data can considerably influence reproducibility.

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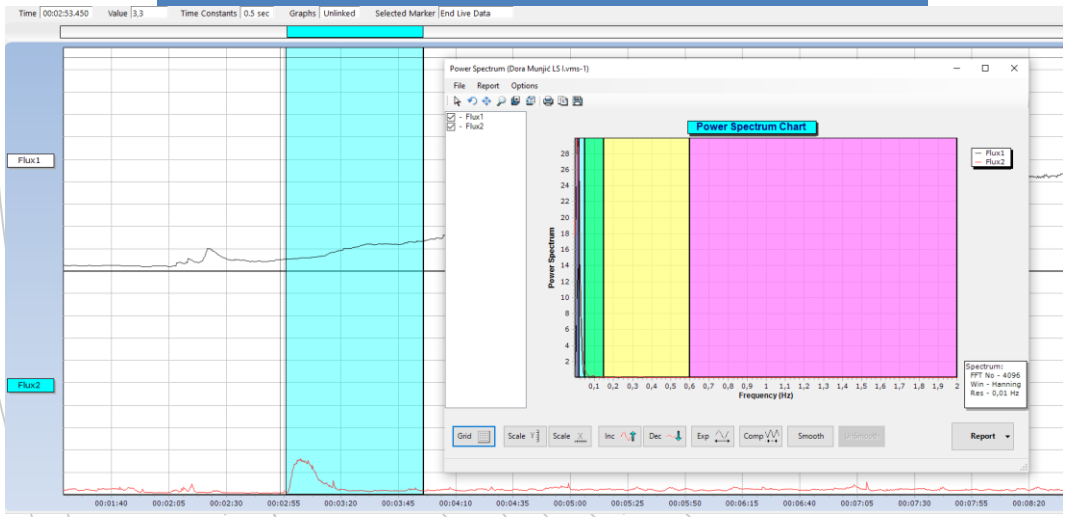
Data expression and reproducibility

- The reactivity tests described above offer many ways to express data
- Commercially available devices provide arbitrary perfusion units (PU; 1 PU=10 mV for laser Doppler
- The results of a reactivity test may be expressed:
  - as raw PU or CVC,
  - as the variation from the baseline flux (e.g., by subtracting baseline, calculating a percentage increase from baseline, or integrating flux over time in terms of area under the curve) or
  - as a percentage of maximal vasodilation
- There is no consensus on the best way to express data.
- Nonetheless, it should be borne in mind that the inter-day reproducibility of most reactivity tests is strongly dependent on the technique used to record skin blood flux and the way the data are expressed

Data expression and reproducibility

- *The use of multi-fiber integrating probes* improves LDF reproducibility
- Other parameters that influence reproducibility include *baseline skin temperature* and *the skin site* studied

Time - frequency analysis



- Periodic oscillations of cutaneous blood flow (so-called flowmotion) can be quantified by spectral analysis of the signal
- Spectral analysis can be performed using the Fourier transform or by the wavelet transform, which takes into account the time component
- Within the frequency spectrum available, five representative frequencies are related to
  - Endothelial activity (0.008–0.02 Hz) – NO-mediated (0.01–0.02 Hz) and non-NO-mediated (0.005–0.0095-Hz)
  - Sympathetic activity (0.02–0.05 Hz),
  - Myogenic activity (0.05–0.15 Hz),
  - Respiratory activity (0.15–0.6 Hz) and
  - Cardiac activity (0.6–2.0 Hz)
- Time–frequency analysis of LDF signal has been studied in a variety of diseases but its use as a surrogate of endothelial function relies on limited evidence

Concluding remarks

- The clinical study of the skin microcirculation is of great interest, either as a model of generalized microvascular function or in diseases in which it is specifically affected
- Among a variety of non-invasive techniques, laser Doppler, when coupled with reactivity tests, offer easy-to-perform and reliable tools to assess endothelial and neurovascular function in the cutaneous circulation.
- Recent advances in our comprehension of the physiological pathways involved in PORH, ionto and in LTH have helped to bring these tests to the foreground

Concluding remarks

- Several key issues remain to be addressed to move forward.
- 1) The generalizability of skin microvascular function to other vascular beds has to be further established in large prospective studies before being used as a surrogate
- 2) The lack of consensus on how to perform reactivity tests is another obstacle to wider use
- 3) Efforts should thus be made to standardize the methods in the future.
- 4) most tests are not specific for a particular pathway, and interactions between underlying mechanisms suggest interactions between the reactivity tests themselves

- Further mechanistic studies in human skin would provide a better understanding of this complex crosstalk.

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