**HIGHLIGHTED TOPIC | Mechanisms and Modulators of Temperature Regulation**

Thermal provocation to evaluate microvascular reactivity in human skin

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Minson CT. Thermal provocation to evaluate microvascular reactivity in human skin. J Appl Physiol 109: 1239–1246, 2010. First published May 27, 2010; doi:10.1152/japplphysiol.00414.2010.—With increased interest in predictive medicine, development of a relatively noninvasive technique that can improve prediction of major clinical outcomes has gained considerable attention. Current tests that are the target of critical evaluation, such as flow-mediated vasodilation of the brachial artery and pulse-wave velocity, are specific to the larger conduit vessels. However, evidence is mounting that functional changes in the microcirculation may be an early sign of globalized microvascular dysfunction. Thus, development of a test of microvascular reactivity that could be used to evaluate cardiovascular risk or response to treatment is an exciting area of innovation. This mini-review is focused on tests of microvascular reactivity to thermal stimuli in the cutaneous circulation. The skin may prove to be an ideal site for evaluation of microvascular dysfunction due to its ease of access and growing evidence that changes in skin vascular reactivity may precede overt clinical signs of disease. Evaluation of the skin blood flow response to locally applied heat has already demonstrated prophylactic utility, and the response to local cooling holds promise in patients in whom cutaneous disorders are present. Whether either of these tests can be used to predict cardiovascular morbidity or mortality in a clinical setting requires further evaluation.

**Endothelial; heat; hyperemia; cutaneous; cold**

The need to measure vascular reactivity in the skin spans basic research on the nature of skin blood flow, identification of cardiovascular and metabolic disease risk in patients, measurement and evaluation of disease progression or treatment, and evaluation of microvascular or endothelial dysfunction in cutaneous disorders. The skin is an ideal site for evaluation of microvascular reactivity because it is easily accessible, and stimuli can be applied with non- or minimally invasive approaches. It has been suggested that the cutaneous circulation can serve as a model for generalized microvascular dysfunction and can be used to predict cardiovascular morbidity or mortality in a clinical setting. The Framingham Risk Score. The goal of this mini-review is to provide a general overview of cutaneous microvascular reactivity, with an emphasis on tests employing thermal provocation, to highlight what is known about the mechanisms underlying the tests and to review recent literature on the use of the tests to predict microvascular dysfunction and progression.

**Tests of Endothelial and Microvascular Function**

The term “endothelial dysfunction” was established in the mid-eighties following the major breakthrough by Furchgott and Zawadzki (27) that acetylcholine requires the presence of endothelial cells to relax the underlying vascular smooth muscle. Today, endothelial function typically refers to the ability of the endothelium to release any number of different compounds able to induce a direct relaxation of smooth muscle cells within the vascular wall. To determine whether endothelial “dysfunction” is present, this endothelium-dependent vasomotor response is typically compared with the effect of an exogenous vasoactive donor [such as nitric oxide (NO) via sodium nitroprusside], which acts directly on smooth muscle cells. Thus a comparison between the endothelium-dependent and endothelium-independent responses can be made.

Even before NO was identified as the factor released by acetylcholine, it had been observed in humans and even earlier in animals that endothelium-dependent relaxation of coronary arteries was impaired in atherosclerotic patients (60). It was subsequently suggested that this endothelial dysfunction could...
be an early marker of atherosclerosis (41). Since that time, there has been a profound interest in trying to develop a test of endothelial function in humans that could provide predictive value of cardiovascular risk. The most extensively used of the various tests currently available is flow-mediated vasodilation (FMD) of the brachial artery by Doppler ultrasound, which is a noninvasive, indirect method to evaluate endothelial dysfunction (18). Growing evidence demonstrates FMD responses are significantly correlated with coronary artery function (3, 82) and even provide independent prognostic value when added to traditional measures of cardiovascular risk (19, 75). That said, endothelial testing by FMD is not without controversy, is dependent on trained personnel, requires substantial equipment, and is specific to the larger conduit vessels (9, 73, 74).

This latter point is important, as most studies aimed at investigating endothelial dysfunction have focused on conduit arteries as a surrogate endpoint to coronary artery disease. However, a growing body of evidence suggests the microcirculation may be the initial site for endothelial damage in subjects at risk of cardiovascular disease (13). Furthermore, endothelial alterations may appear earlier in resistance arteries than in conduit arteries in some diseases (1, 42) and may precede large artery stiffening (40, 81). Generalized microvascular dysfunction has been identified as a crucial step in complications associated with pathophysiological conditions associated with diabetes, hypertension, coronary artery disease, peripheral artery disease, essential hypertension, renal failure, hypercholesterolemia, systemic sclerosis, and aging (42).

Taking the above into consideration, it is not surprising that brachial FMD does not correlate well with intrabrachial arterial infusions of acetylcholine or other tests of microvascular function in the resistance arteries, demonstrating they are measuring different aspects of vascular function (25, 42, 69, 72). An intra-arterial infusion of acetylcholine into the forearm requires a relatively high degree of invasiveness, limiting its utility as routine measurement for use in large-scale clinical trials. Thus a test of microvascular function in resistance arteries that does not require the same level of invasiveness as intra-arterial infusions of vasoactive agents is needed. The skin may prove to be the ideal site for these measurements.

ANATOMIC CONSIDERATIONS FOR TESTS OF VASCULAR REACTIVITY IN THE SKIN

The blood vessels in most areas of the skin are similar to those in other vascular beds, with blood vessels composed of vascular smooth muscle and an endothelium that releases a host of vasodilator and vasoconstrictor agents, such as NO, prostanoids, and endothelium-derived hyperpolarizing and constricting factors. Most of the body surface area is covered with “hairy” or nonacral (also called nonglabrous) skin, whereas the skin of the fingers, lips, ears, palms, and plantar aspects of the feet is acral or glabrous skin. Blood vessels in glabrous skin are primarily innervated by sympathetic adrenergic nerves and contain a high proportion of arteriovenous anastomoses, whereas blood vessels in the nonglaborous skin contain both adrenergic and sympathetic cholinergic nerves, both of which are involved in temperature regulation. Both skin types are richly populated by sensory nerves that respond to thermal, chemical, and mechanical stimuli. These nerves serve to provide feedback to the central nervous system, but also release local neuropeptides and other vasoactive agents that influence cutaneous vascular tone. Due to the structural and neurovascular regulatory differences in acral vs. nonacral skin, regulation of cutaneous vascular tone in these areas can differ, as well as the susceptibility of these areas to microvascular dysfunction. For example, in the finger pad, the reactive hyperemic response following vascular occlusion is ~60% dependent on NO (71), whereas the skin on the forearm does not display a profound NO-dependent component to this same stimuli (95, 100). Recognizing the difference in types of skin is important, as tests of vascular reactivity are evaluating different aspects of a disease process, depending on the location where the tests are performed (11).

TECHNICAL CONSIDERATIONS FOR PERFORMING TESTS OF MICROVASCULAR REACTIVITY IN THE SKIN

Study of microvascular reactivity in the skin has most commonly been performed in humans using single-point laser Doppler flowmetry, with evaluation of the skin blood flow response as the outcome variable of interest. The use of laser Doppler to record skin blood flow is relatively straightforward, but has some inherent strengths and limitations, depending on the methods used, as discussed in greater detail elsewhere (17, 45, 98, 99). Pertinent to tests of microvascular reactivity in the skin, there is a relatively wide heterogeneity in the level of skin blood flow from one site to the next, even across the forearm (45, 49), resulting in a lack of standardization in the assessment of microvascular function reported in the literature (for reviews on this topic see Refs. 17, 89). It is, therefore, recommended to normalize the measurement at a given site to a characteristic for that site, such as baseline or maximal vasodilation. However, which characteristic to use in specific circumstances is the subject of some debate. In general, responses are normalized to maximal skin blood flow for studies in which a given perturbation will result in vasodilatation. This can be achieved with local skin heating to at least 42°C or by administration of sodium nitroprusside, administered by iontophoresis or microdialysis. Alternatively, with local skin cooling, the practice is to normalize to baseline. However, this does present some documented challenges as well (17, 30, 76, 77). A suggestion in this case may be to perform baseline measurements at a specific clamped local temperature, such as 33°C.

While the approach to scale responses to a maximal vasodilatation by heating the skin to 42–44°C is acceptable in healthy subjects and in mechanistically driven, carefully controlled studies, it should not be extrapolated in patient populations or in longitudinal studies without further consideration. For example, if the intent is to determine whether a decreased skin blood flow response to a specific stimuli is the result of a functional abnormality or a structural defect, it is not reasonable to compare maximal red blood cell flux values obtained with laser Doppler between subjects or across skin sites due to the high site-to-site and individual variability (17, 45). To determine whether maximal skin blood flow is actually reduced, one could measure absolute forearm vascular changes to whole arm heating in a water spray device using plethysmography, as pictured in Fig. 1 (44, 83, 84). Using this approach, the majority of blood flow above resting levels is directed to the skin, providing a measurement of absolute blood flow. This approach requires additional training and
One approach to overcome the spatial heterogeneity problem is to use laser Doppler imaging, which applies the same physical principle as laser Doppler flowmetry. In imaging, the laser beam is reflected by a computer-driven mirror to progressively scan the area of interest. Although laser Doppler imaging provides an integrated index over a larger cutaneous surface than afforded with single-point laser Doppler flowmetry, thereby decreasing spatial variability, it is lacking in temporal aspects because it is not possible to record continuously from the area of interest. It is, therefore, possible to miss key time points in the pattern of responses in the skin to thermal provocation. This is important as the timing and pattern of responses provide insight into the potential mechanism of microvascular function or dysfunction with thermal provocation, as discussed below.

A relatively new approach to measure skin blood flow that holds promise for tests of microvascular reactivity is speckle contrast imaging (12, 21), which has been used to assess skin blood flow in a limited number of patients with microvascular disease (68). Laser speckle contrast imaging is an optical image technique that has been developed for imaging in vivo blood flow dynamics and vascular structure with high spatial and temporal resolution. Coherent light detected by a camera is time integrated over the exposure duration to yield an image with a speckle pattern of variable amplitude, dependent on motion and other parameters (88). The full-field spatiotemporal characteristics of microcirculation is then recorded in real time. Unfortunately, there are insufficient data at this time to know whether laser speckle contrast imaging will be an advantage over more traditional measures of skin blood flow with laser Doppler approaches.

EVALUATING MICROVASCULAR REACTIVITY IN THE SKIN

Acetylcholine iontophoresis is the most commonly used approach to study microvascular function in the skin and has been expertly reviewed elsewhere (89). However, there are some limitations to the approach that need to be recognized, and since it is the most widely used test, a brief discussion here is germane to the thermal tests of microvascular reactivity that are the focus of this mini-review.

The use of acetylcholine delivered by iontophoresis as a specific test of endothelial function per se is debatable. This method drives acetylcholine through the interstitium surrounding the blood vessels, which is quite different compared with delivery by arterial infusion. When delivered through the lumen of a blood vessel, the drug is in contact with the endothelium first and foremost, whereas, when delivered outside the vessel, this is not the case. Acetylcholine is known to have a number of different effects in skin on blood vessels and the surrounding nerves, leading to the release of nonendothelium-derived substances, including neuropeptides (7, 20). For example, acetylcholine induces an axon reflex that participates in the increase of skin blood flow (7). With iontophoresis, the anodal current on its own is known to cause a current-induced hyperemia (7, 22, 23). Thus, when acetylcholine is delivered to the skin by iontophoresis, the vasodilator response is not specifically endothelium dependent. Furthermore, the specific mechanisms by which acetylcholine causes vasodilation in the skin is not fully understood. Inhibition of NO synthase has only a modest effect on the cutaneous vasodilation to acetylcholine, with a substantial prostanoid-dependent component and a component that is not sensitive to combined NO and COX inhibition (36, 50). An observed reduction in the vasodilator response to acetylcholine is not attributable to a specific mechanism. The thermal tests discussed below also have some inherent limitations, but may provide additional insight into specific pathologies or provide an alternative index of globalized microvascular dysfunction. For a more comprehensive treatment on the mechanisms of the local thermal control of the skin, the reader is referred to the mini-review by Johnson and Kellogg as part of this series (43).

LOCAL THERMAL HYPEREMIA AS A TEST OF VASCULAR REACTIVITY

The skin blood flow response to the standard local heating protocol most commonly used as a test of thermal hyperemia is mediated by at least two independent phases: an initial peak in cutaneous blood flow during the first 10 min, followed by a plateau after 20–30 min of warming (17, 66). The initial rapid phase is predominantly mediated by local sensory nerves and can be significantly attenuated in the presence of local anesthesia (Fig. 1) (4, 66), but not by proximal neural blockade or muscarinic receptors blockade (48, 66). In contrast, the 20- to 30-min plateau is mediated predominantly by NO (47, 66), with recent evidence suggesting the NO is generated from the endothelial NO synthase isoform (49), although this is not a universal finding (80). In either case, NO synthase inhibition does not fully suppress the plateau phase, suggesting other vasodilators may be involved. Neither prostanooids nor histamine seems to play a role (28, 29, 64, 96). Recent studies have demonstrated the complexity and integrated nature of the cutaneous response to local heating, and there is now evidence for an involvement of the sympathetic neurotransmitters noradrenaline and neuropeptide Y in both the initial peak and sustained plateau phases (32). How these sympathetic neuro-

Fig. 1. Photograph of the arm spray device in which maximal skin blood flow (SKBF) can be measured. A: the device is attached to a temperature-controlled water bath that circulates water into the cylindrical device. Water is applied to the forearm via numerous spray nozzles. B: the upper cuff for venous occlusion plethysmography. C: the distal cuff to occlude blood flow to the hand. D: temperature gauge within the water spray.
transmitters may interact with the sensory neurotransmitters and NO is an area of exciting exploration. The challenge, of course, is that it is not possible to determine the underlying cause of the dysfunction in conditions in which the thermal hyperemic response is diminished without pharmacological intervention.

Recently, two different studies examined the reproducibility of the thermal hyperemic response, an important assessment for determining its potential as a clinical tool. In the first study, Agarwal and colleagues (2) compared day-to-day thermal hyperemia to other tests of vascular responsiveness (reactive hyperemia and acetylcholine iontophoresis) on the forearm and reported that the reactive hyperemic response was the most reproducible of the tests, with a coefficient of variation of 9.3% when expressed as a change in perfusion from baseline, but not when expressed as percent change from baseline values (2, 37). Unfortunately, they did not perform a maximal heating stimulus to scale responses to maximal blood flow. Roustit et al. (76) recently examined the reproducibility of local thermal hyperemia on both the finger pad and the forearm. These authors reported that reproducibility of the test on the finger pad was considered acceptable, but the forearm displayed greater interday variability, which the authors contributed to the greater spatial variability of capillary density in the skin of the forearm compared with the finger. Reproducibility of the initial peak response on the forearm, when expressed as a percentage of maximal blood flow, was also considered good and has been used by this group to measure skin neurovascular function in patients with systemic sclerosis (78). In this latter study, the authors suggest that thermal hyperemia could be monitored as a clinical test for neurovascular function.

Microvascular reactivity has been investigated using thermal hyperemia in a number of different pathological conditions in recent years. Consistent data show that thermal hyperemia is impaired in diabetes (16, 52, 93, 94), with advanced age (36, 62, 67), in smokers (24), and in renal failure patients (55, 79). Furthermore, thermal hyperemia has been used as a clinical surrogate marker in various diseases, such as Raynaud’s phenomenon and systemic sclerosis (10, 68, 78). Patients with low-flow postural tachycardia syndrome exhibit exclusively a decreased plateau (65), while those with chronic spinal cord injury exhibited a decreased axon reflex (70, 91).

One of the most provocative studies to date using thermal hyperemia evaluated parameters of the thermal hyperemic response in end-stage renal patients (55) in both cross-sectional and longitudinal designs. The high-risk population was characterized by a markedly diminished local thermal hyperemic response, evident at all measured data points: the initial heat peak, nadir, and second heat peak (see Fig. 2). The most robust measurement of the thermal hyperemic response was the area under the curve of the 30-min heating. These authors reported that patients who had abnormal thermal responses showed increased cardiovascular mortality, despite similar Cardiorisk and Framingham Risk Assessments to those without impaired thermal responses. This suggests thermal hyperemia as a test of microvascular reactivity may improve cardiovascular risk prediction through incorporation with the risk assessments. Importantly, a more common test of vascular reactivity, reactive hyperemia following occlusive blood flow restriction, was not found to correlate with cardiovascular risk in this study, although it has been shown to be inversely correlated to the severity of cardiovascular risk exposure in a large female population (92) and to be enhanced by statins (8) in hypercholesterolemic patients. Like the thermal hyperemic response, reactive hyperemia represents a complex microvascular response, but, unlike thermal hyperemia, it is independent of NO (95, 100). Rather, there seems to be significant involvement of the local sensory nerves and an EDHF pathway via large-conductance Ca$^{2+}$-activated K channels (59). Thus the reactive hyperemic and thermal hyperemic responses may be measuring different aspects of microvascular function and, when combined in studies or clinical trials, may provide insight into mechanism of dysfunction.

**LOCAL COOLING AS A TEST OF VASCULAR REACTIVITY**

Compared with thermal hyperemia, the cutaneous response to local cooling has received much less attention, with the majority of studies focused on cutaneous disorders in which a microvascular dysfunction is present. However, with recent advances in our understanding of this surprisingly complex response, local cooling may demonstrate utility as a tool to investigate different aspects of microvascular reactivity than are currently in use. Local cooling has been used to evaluate

![Fig. 2. Representative tracing of the SkBF response to local heating to 42°C, followed by maximal heating to 43.5°C. The initial rapid rise relies predominantly on the local sensory nerves and nitric oxide. The second rise leads to a plateau that predominantly dependent on nitric oxide.](http://jap.physiology.org/Downloaded from 10.220.33.6 on October 30, 2017)
vascular responsiveness in familial hypertension (63), differences between men and women (14), with aging (56, 57, 86, 87), and in patients with Raynaud’s phenomenon (26, 61).

Investigating the mechanisms that underlie the cutaneous response to local cooling has been an area of much recent activity. A typical pattern of the response to local cooling is displayed in Fig. 3, but, like the response to local heating, the rate of change in temperature, and perhaps even the extent of temperature change, will impact the pattern and the underlying mechanisms involved (31, 46). In general, the response is characterized by an initial decrease in skin blood flow, followed by a transient vasodilation, and a secondary progressive vasoconstriction. There is a clear and well-established adrenergic nerve component involving norepinephrine-mediated vasoconstriction throughout the cooling stimulus (33, 46). This is thought to mediate the initial decrease in skin blood flow at the initiation of cooling. However, the complexity of the mechanisms that mediate this response was demonstrated by the Flavahan laboratory (5, 6, 15), in which they found that local cooling of skin blood vessels in vitro stimulates mitochondrial production of reactive oxygen species, which then acts on Rho kinase to cause the translocation of \( \alpha_2 \)-receptors to the plasma membrane. Norepinephrine released from adrenergic nerves then acts on the upregulated receptors to overcome an overall reduced neurotransmitter release associated with the local cooling stimulus, resulting in net cutaneous vasoconstriction. Thompson-Torgerson and colleagues (85) confirmed this finding in vivo by applying a Rho kinase inhibitor alone and in combination with postsynaptic blockade of \( \alpha_1,2 \)-receptors. The early transient vasodilation often observed with local cooling is unexplained at the present time, but it is not obviously linked to sensory nerve function. However, sensory nerves typically associated with vasodilation appear to play a counterintuitive role in the response, in which blockade of these nerves unmask an underlying vasodilator response (33, 46). A role for NO in the local cooling response has also been demonstrated, in which prolonged cooling inhibits NO synthase (34, 97).

As the microvascular responses to local cooling are studied in more patient populations, information regarding how various pathologies impact the pattern of the local cooling response may provide additional insight into the underlying mechanism of the response. For example, changes in the cutaneous microvascular response to local cooling with advanced age have been the focus of a number of studies from the Kenney laboratory (56, 57, 86, 87). This group has found impaired norepinephrine-mediated vasoconstriction (87) and blunted responses to local cooling (86) in the elderly. Importantly, older skin relies on Rho kinase-dependent pathways to a greater extent than the skin of younger subjects (58). It was suggested that this may be due to pathological changes in global microvascular function associated with increased oxidative stress with aging. Consistent with this idea, it has been demonstrated during whole body cooling that local administration of tetrahydrobiopterin, required in the prejunctional biosynthesis of catecholamines, corrects the age-related decline in cutaneous vasoconstriction (56, 57). Whether local cooling can be used as a biological test of oxidative stress or provide prognostic insight into microvascular dysfunction associated with cardiovascular or metabolic disease is not known at the present time.

Before a test of vascular reactivity to local cooling can be used as a clinical tool, studies addressing the reproducibility of the test are needed. Roustit and colleagues (77) recently provided such a study as they evaluated the short-term (same day) and long-term (1 wk) reproducibility of 5- and 30-min local cooling tests from a clamped baseline temperature (33°C) to either 24 or 15°C. These authors found much higher variability in the response to 24°C cooling than to 15°C and recommended the 30-min test as providing the lowest variability. In this study, a cold-induced vasodilation was observed in some subjects at the onset of cooling, as described by others (34, 97). This was not surprising, as they used a high rate of cooling (−16°C/min) known to stimulate this response, a choice based on their interest in developing a test for use in Raynaud’s phenomenon patients, in whom the cold-induced vasodilation may prove to have clinical utility. The rate of cooling and final temperature achieved that would best work for evaluation in other patient groups has not been studied.

**CONCLUSION AND PERSPECTIVES**

With increased interest in predictive medicine, development of a relatively noninvasive technique that can improve prediction of major clinical outcomes above and beyond the Framingham Risk Score has gained considerable attention. The Framingham Risk Score was developed to offer prospective risk assessment for coronary heart risk in men and women who do not have overt coronary disease. In a recent review of studies claiming additional predictive value beyond the Framingham Risk Score, most studies were reported to contain flaws in their design, analyses, and/or reporting (90). If we are to determine whether thermal stimuli tests in the skin can provide prognostic value, more research is needed, and closer scrutiny needs to be

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**Fig. 3.** Representative tracing of the SkBF response to local cooling to 24°C. Data are redrawn from Ref. 75. The initial decrease in SkBF with cooling is followed by a transient vasodilation. This is then followed by a progressive decrease in SkBF with sustained cooling.
applied. At a minimum, a good test of microvascular reactivity should 1) have a sound physiological and mechanistic basis that is well characterized; 2) be reproducible, observer independent, and easily standardized; and 3) provide a demonstrated ability to predict morbidity, mortality, and/or improvement in risk with treatment. If a given test falls short of being able to provide an overall risk evaluation for global microvascular dysfunction, but still provides important insight into microvascular function of the skin as a clinical endpoint, then the test may be useful. Preliminary findings suggest that the local hyperemic response to skin heating may provide prognostic value, but whether or not the test can be reliably used in a clinical setting with the intent to treat has not been demonstrated. Local cooling as a clinical test is still in the very early stages of development and evaluation. Although preliminary findings hold promise, more work needs to be done before thermal tests of microvascular reactivity are ready for clinical prime time.

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