HIGHLIGHTED TOPIC | Mechanisms and Modulators of Temperature Regulation

Peripheral mechanisms of thermoregulatory control of skin blood flow in aged humans

Lacy A. Holowatz¹ and W. Larry Kenney¹,²

¹Department of Kinesiology and ²Graduate Physiology Program, The Pennsylvania State University, Noll Laboratory, University Park, Pennsylvania

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Holowatz LA, Kenney WL. Peripheral mechanisms of thermoregulatory control of skin blood flow in aged humans. J Appl Physiol 109: 1538–1544, 2010. First published April 22, 2010; doi:10.1152/japplphysiol.00338.2010.—Human skin blood flow is controlled via dual innervation from the sympathetic nervous system. Reflex cutaneous vasoconstriction and vasodilation are both impaired with primary aging, rendering the aged more vulnerable to hypothermia and cardiovascular complications from heat-related illness. Age-related alterations in the thermoregulatory control of skin blood flow occur at multiple points along the efferent arm of the reflex, including 1) diminished sympathetic outflow, 2) altered presynaptic neurotransmitter synthesis, 3) reduced vascular responsiveness, and 4) impairments in downstream (endothelial and vascular smooth muscle) second-messenger signaling. This mechanistic review highlights some of the recent findings in the area of aging and the thermoregulatory control of skin blood flow.

HUMAN SKIN BLOOD FLOW (SkBF) is reflexly controlled by two distinct branches of the sympathetic nervous system, including an adrenergic vasoconstrictor branch and a cholinergic active vasodilator branch. With primary human aging, even in the absence of overt pathology, reflex cutaneous vasoconstriction and vasodilation are attenuated (Fig. 1). This impaired cutaneous vascular response is apparent, even when subjects are matched for fitness level (maximum O₂ uptake) (37), body composition (10, 11, 38), acclimation status (1), and hydration status (41), suggesting that this is a primary aging phenomenon. On average, healthy aged (60–90 yr) humans display a 25–50% attenuation in SkBF compared with 18- to 30-yr-old adults, rendering older men and women more susceptible to both cold- and heat-related illness (23).

The influence of primary human aging on the neurovascular mechanisms mediating reflex cutaneous vasodilation and vasoconstriction have been thoroughly reviewed elsewhere (30, 40). The purpose of this Highlighted Topic review is to concisely update the literature with recent findings in the field. Furthermore, the impact of pharmacological therapies common to older individuals are discussed. Other modifiers of reflex cutaneous vascular control mechanisms are covered in detail by Charkoudian in a separate review in this Highlighted Topics series (7).

AGING AND REFLEX VASOCONSTRICION

Reflex cutaneous vasoconstriction is an early and sustained response to whole body cold exposure, limiting convective heat loss to the environment. This thermoregulatory response is markedly impaired in aged skin (8, 11, 20, 22, 71). Attenuated reflex vasoconstriction contributes to an inability to maintain body core temperature (Tc) during exposure to even mild cold stress (22°C) in aged humans (11). Compromised vasoconstriction in aged skin is due to impaired mechanisms at multiple points along the efferent arm of the sympathetic reflex, including 1) depressed autonomic skin sympathetic nerve activity (SSNA); 2) alterations in axonal synthesis and packaging of norepinephrine (NE) and putative cotransmitters, including neuropeptide Y and adenosine triphosphate (ATP); 3) reduced end-organ vascular responsiveness to these adrenergic neurotransmitters; and 4) alterations in downstream second-messenger signaling in the cutaneous vascular smooth muscle (Fig. 2).

Sympathetic Autonomic Dysfunction

Sympathetic efferent outflow in response to a cold stimulus is decreased with aging. When integrated SSNA was recorded in young (18–29 yr), middle-aged (38–51 yr), and elderly subjects (65–81 yr), the cold-induced increase in vasomotor nerve activity (bursts/min) was depressed by ~60% in the elderly group compared with the other two groups (22). Thus the sympathetic efferent signal for cold-induced cutaneous vasoconstriction is significantly weaker in aged skin.

Impaired Neurotransmitter Synthesis/Packaging/Release

In addition to the attenuated sympathetic stimulation of perivascular nerves and subsequent release of sympathetic transmitters, the relative contributions of these transmitters is also impaired with aging (Fig. 2). Reflex constriction in young...
skin is mediated by both NE (60%) and cotransmitters (40%) (65, 66); however, the cotransmitter portion of constriction is abolished in aged skin (71). Thus older subjects rely entirely on impaired NE pathways to mediate the reflex vascular response to cold (71). The decrements in NE- and cotransmitter-mediated constriction are due, in part, to age-related decreases in transmitter synthesis and/or release (9, 15, 20).

One recently explored mechanism underlying age-related impairments in prejunctional neurotransmitter synthesis is the bioavailability of tetrahydrobiopterin (BH₄). BH₄ is critical for optimal reflex vasoconstrictor function because it is required for the hydroxylation of tyrosine to L-3,4-dihydroxyphenylalanine by tyrosine hydroxylase (TH) in the prejunctional biosynthesis of catecholamines, as well as a powerful reductant that maintains TH in a reduced, active form (Fig. 2) (21, 59). However, both de novo synthesis and recycling pathways for BH₄ are particularly vulnerable to oxidation, and, therefore, BH₄ bioavailability decreases with increased oxidant stress. Because human aging is associated with a systemic net increase in reactive oxygen species (ROS) and reactive nitrogen species due to both increased production and decreased clearance (12, 44), a decrease in BH₄ bioavailability may result. When BH₄ is locally delivered to the cutaneous vasculature (by intradermal microdialysis), both physiologically (whole body cooling) and pharmacologically induced (tyramine) vasoconstriction are enhanced in aged skin (46). Furthermore, enhancing substrate availability by localized supplementation with tyrosine also augmented cutaneous vasoconstriction, with no additive effect of combining BH₄ and tyrosine (47). These data suggest that optimal TH function is required to fully express the cutaneous vasoconstrictor response, and that reduced functional substrate and cofactor for TH contributes to the attenuated vasoconstriction in aged skin.

**Blunted α-Adrenergic Receptor Responsiveness**

In addition to a muted sympathetic signal and reduced NE (and functionally absent cotransmitter) synthesis and release, the end-organ vasoconstrictor response is also attenuated in aged skin (20, 43, 45, 69–71). NE-mediated constriction is significantly impaired in aged skin, with elderly subjects exhibiting a blunted constrictor response to both physiological and maximal doses of NE (70, 71). Evidence from other vascular aging models suggests that neuromodulatory Y-mediated vasoconstriction undergoes a similar reduction (45), and ATP-mediated constriction is profoundly depressed (43). To date, the in vivo human data indicate that aged skin relies entirely on attenuated NE-mediated vasoconstriction to induce reflex vasoconstriction.

**Second-messenger Signaling**

The final potential site of age-related alteration in reflex vasoconstriction is the second-messenger signaling pathways that couple adrenoreceptor activation with the vascular smooth muscle contraction. Recently, the relative contribution of Rho kinase (ROCK)-dependent pathways in reflex cutaneous vasoconstriction has been explored. ROCK can be stimulated by NE and/or, in the case of localized cooling of the skin, by mitochondrial generation of superoxide (Fig. 2) (2, 3). Once activated, ROCK elicits vasoconstriction through two distinct mechanisms, including 1) inhibition of myosin light chain phosphatase, thereby maintaining myosin light chain phosphorylation without additional calcium (Ca²⁺) influx (Ca²⁺ sensitization); and 2) inducing the translocation of α₂c-receptors from the Golgi apparatus to the cell membrane. In aged human skin, inhibition of ROCK significantly attenuated the reflex vasoconstrictor response and vasoconstriction induced by exogenous administration of NE (48). These data suggest that older skin relies on ROCK-dependent pathways (~50% of the total vasoconstrictor response) to a greater extent than their younger counterparts. Interestingly, this alteration in second-messenger signaling may be due to globalized pathological changes in microvascular function induced by aged-related increases in oxidant stress.

Cumulatively, these findings suggest that the age-associated decrement in thermoregulatory reflex vasoconstriction is attributable to several factors: 1) reduced efferent sympathetic signal, 2) reduced sympathetic synthesis and release of NE through decreased BH₄ and tyrosine bioavailability, 3) a functional loss of sympathetic cotransmitters function, 4) a significant loss of end-organ sensitivity to NE, and 5) alterations in second-messenger signaling, including a greater dependence on ROCK-mediated pathways (see Fig. 2).
AGING AND REFLEX VASODILATION

Human aging in the absence of overt pathology is also associated with attenuated cutaneous vasodilation during thermal stress (37). The age-related reduction in SkBF during hyperthermia appears to be of peripheral origin due to decreased sensitivity of the active vasodilator system (39). Similar to vasoconstrictor mechanisms (71), impairments in the mechanisms mediating reflex vasodilation occur at multiple points along the efferent arm of this sympathetic reflex. These impairments include 1) an attenuated sympathetic neural drive during heating (22), 2) a reduced contribution of sympathetic cholinergic cotransmitters (24), and 3) alterations in downstream signaling in both endothelial and vascular smooth muscle cells (24, 25). However, because the precise identities of the cholinergic cotransmitter(s) mediating reflex vasodilation remain elusive, studies examining age-related changes to date have primarily focused on downstream signaling, specifically nitric oxide (NO)- and cyclooxygenase (COX)-mediated pathways. Both of these pathways have been important molecular targets from a vascular aging and health perspective and have significant potential for the development of successful intervention strategies. Finally, recent evidence suggests a novel role for platelet activation in reflex vasodilation that is significantly inhibited by commonly prescribed anti-platelet agents (26).

Sympathetic Dysfunction

Efferent sympathetic outflow in response to a mild heat stress stimulus is likely reduced with aging, but this decrement is not as well defined or characterized as that associated with cold stress. Grassi et al. (22) recorded SSNA during mild heat stress and found that the aged (65–81 yr) had an attenuated reduction in the integrated neurogram signal (47). These data are difficult to interpret, because single-unit nerve signals were...
not obtained. The integrated multiunit neurogram is a combination of vasoconstrictor, vasodilator, piloerector, and sudomotor fibers. Furthermore, the subjects were only mildly heated during this study. Nevertheless, the aged subjects did display an altered SSNA response to heating, suggesting some degree of sympathetic vasodilator dysfunction.

**Acetylcholine**

The functional contribution of cholinergic cotransmitter(s) to reflex vasodilation is absent in aged skin until \( T_c \) is significantly elevated (\( \Delta T_c \geq 0.9^\circ C \)) (24). Acetylcholine released from sympathetic cholinergic nerves is capable of modulating the initial rise in SkBF in the early phase of active vasodilation (63), and aged subjects display reduced SkBF in this \( T_c \) range. Studies using exogenous administration of acetylcholine (at a concentration that induced an increase in SkBF similar in magnitude to that observed during the initial phase of reflex vasodilation) indicate that vascular sensitivity at this concentration is not decreased in aged skin, but the relative contributions of the downstream second-messenger systems are altered. Specifically, COX-dependent vasodilation is attenuated, and endothelium-derived hyperpolarization factor-dependent vasodilation is augmented (33), suggesting that impaired COX-dependent second-messenger signaling may contribute to reduced reflex vasodilation in aged cutaneous vasculature.

**COX-dependent Mechanisms**

In young healthy humans, COX-derived vasodilators contribute to reflex vasodilation independent of NO (51). In contrast, with healthy aging, acute inhibition of COX does not alter the SkBF response during hyperthermia, suggesting that COX-derived vasodilators do not contribute to reflex vasodilation (25). There are several possible explanations for this finding, involving differential synthesis of COX-derived vasoactive products and vascular smooth muscle receptor activation with aging. First, COX is capable of synthesizing several vasodilator prostanoids (PGI\(_2\), PGE, PGF, PGD) and vasoconstrictor (thromboxane) substances, and, with vascular aging, there is a shift toward the latter, resulting in a proconstrictor state. Second, aged humans display reduced vascular responsiveness to vasodilator prostanoids through alterations in vascular smooth muscle receptor activation (54). Prostacyclin can act as either a vasodilator or a vasoconstrictor, depending on specific vascular smooth muscle receptor activation, where, in general, thromboxane prostanoid (TP) receptor activation induces vasoconstriction and inositol phosphatase (IP) receptor activation induces vasodilation (Fig. 3) (17). With aging, as well as with several documented vascular pathologies, the expression of these vascular smooth muscle receptors shifts to increased TP and reduced IP receptors, respectively (17, 34, 53, 68). Further research utilizing specific thromboxane synthase and TP and IP receptor antagonists are needed to fully elucidate these mechanisms.

**NO-dependent Mechanisms**

In young healthy humans, NO contributes significantly to the increase in SkBF during hyperthermia. Due to the reduction in cotransmitter function, the aged rely primarily on impaired NO-dependent mechanisms to increase SkBF during hyperthermia (24). Optimal NO synthase (NOS) function for NO production depends on adequate substrate (L-arginine) and cofactor (BH\(_4\)) availability. The intracellular localization of NOS in relation to the L-arginine subdomains (substrate pools) is also important (72). One potential mechanism limiting L-arginine concentration is also important (72). One potential mechanism limiting L-arginine concentration is also important (72). One potential mechanism limiting L-arginine concentration is also important (72). One potential mechanism limiting L-arginine concentration is also important (72). One potential mechanism limiting L-arginine concentration is also important (72). One potential mechanism limiting L-arginine concentration is also important (72). One potential mechanism limiting L-arginine concentration is also important (72). One potential mechanism limiting L-arginine concentration is also important (72). One potential mechanism limiting L-arginine concentration is also important (72). One potential mechanism limiting L-arginine concentration is also important (72). One potential mechanism limiting L-arginine concentration is also important (72). One potential mechanism limiting L-arginine concentration is also important (72).
arginine for NO synthesis and full expression of reflex cuta-
aneous vasodilation in the aged is through upregulation of
vascular arginase. Arginase is the last enzyme of the urea cycle
and catalyzes the conversion of L-arginine to L-ornithine and
urea, precursors for polyamine and collagen synthesis and thus
vascular smooth muscle remodeling (16, 58). In the vascula-
ture, arginase preferentially utilizes the common NOS sub-
strate, L-arginine, limiting substrate availability for NO syn-
thesis through NOS (Fig. 3). Arginase is upregulated in several
animal models of endothelial dysfunction, including aging,
atherosclerosis, and hypertension (4, 13, 14, 61); however, the
mechanisms underlying the arginase upregulation are pathol-
gy specific. In line with these animal models of vascular dysfunc-
tion, acute arginase inhibition or direct L-arginine supple-
mentation to the cutaneous vasculature significantly aug-
ments reflex vasodilation in aged human skin (31). Interest-
ingly, arginase inhibition or L-arginine supplementation (or
combined) increased SkBF similarly (−45% maximum SkBF),
suggesting that the L-arginine-NOS pathway was saturated (at
maximal NOS activity), and/or the cutaneous vessels were
maximally vasodilated for the given degree of hyperthermic
stress. Collectively, these results suggest that upregulated ar-
ginase limits NO production, and increasing L-arginine avail-
ability for NO synthesis can augment reflex vasodilation in
aged skin (4, 29, 62, 73).

Another potential mechanism for reduced NO bioavailability
with human aging is an increase in oxidant stress contributing
to an increase in NO breakdown. In aged human skin, maximal
cutaneous vasodilation to an exogenous NO donor is attenu-
ated, suggesting either a relative inability of vascular smooth
muscle to respond to NO through cyclic GMP mechanisms, or
that NO may be rapidly degraded by ROS (52), possibly
forming pro-constrictr oxidants (3). Endogenous ROS in-
crease with aging and are capable of readily reacting with NO
to form peroxynitrite (ONOO•−), which limits NO-dependent
vasodilation and may potentially serve as a vasoconstrictor
stimulus (19).

In humans, high-dose nonspecific antioxidant supplementa-
tion (ascorbate) is commonly used to examine effects of
oxidant stress on NO-dependent vasodilation. The advantage
of using ascorbate is that it is water soluble, can be given in
high concentrations in humans, and does not affect NOS
activity. In aged human skin, localized high-dose ascorbate
supplementation modestly augmented vasodilation with mod-
erate increases in body Tc (ΔTc > 0.7°C); however, when
ascorbate supplementation was combined with acute arginase
inhibition, there was a further increase in SkBF (32). In con-
trast, localized ascorbate and/or arginase inhibition do not
alter reflex vasodilation in young subjects. These results sug-
gest that increased oxidant stress and upregulated arginase
activity both limit NO bioavailability in aged skin. Furth-
ermore, these mechanisms may be functionally linked through
the uncoupling of NOS. Under circumstances where L-arginine
and/or essential cofactors BH4 are limited, NOS can uncouple
to produce superoxide instead of NO (19). In this positive
feedback loop, NO reacts with superoxide forming ONOO•−,
which then quickly oxidizes BH4, potentiating of superoxide
production through uncoupled NOS.

While ascorbate is useful in these studies examining simple
oxidant stress NO bioavailability mechanisms, it is nonspecific
and is capable of increasing NO bioavailability by first acting
directly as an antioxidant and secondarily by stabilizing BH4.
Therefore, it is impossible to delineate whether the increase in
NO-dependent vasodilation in these studies was through an
antioxidant effect or through BH4-NOS uncoupling mecha-
nisms. Additional studies examining the relations between
arginase, the localization of L-arginine, and BH4 availability
are necessary to more fully understand how NOS uncoupling
may affect age-related reductions in cutaneous NO-dependent
vasodilation.

**Alterations in Vessel Structure**

Maximal cutaneous blood flow decreases linearly as a function
of age (50). This age-associated attenuation is observed in both
absolute forearm maximal cutaneous vascular conductance as-
sessed with venous occlusion plethysmography and laser-Doppler
flowmetry measures of SkBF. The reduction in maximal cutane-
ous vascular conductance with age presumably reflects structural
choices in vessel properties, including, 1) vascular smooth muscle
hypertrophy, and 2) a reduction in capillary density associated
with flattening of the underside of the epidermis (40, 49). While
there are likely many signaling factors stimulating cutaneous
microvascular smooth muscle hypertrophy and inward vessel
remodeling, obvious candidate pathways include arginase (58),
acting through an increase in polyamine and proline synthesis,
ROCK, and vasoconstrictor factors produced through COX (76).
Chronic activation of these pathways induces functional alter-
tations in microvascular anatomy, which likely contributes to
age-related cutaneous vessel structure alterations.

**Platelet Activation**

A series of recent studies in middle-aged humans suggests a
novel role for platelets in reflex vasodilation. This proposed novel
mechanism emerged from the finding that healthy middle-aged
(58 ± 2 yr) humans taking chronic low-dose (81 mg) aspirin
therapy for the primary prevention of atherothrombotic disease
displayed a consistent and significant attenuation in reflex cuta-
aneous vasodilation (27). The subjects in this cross-sectional study
had been taking low-dose aspirin for greater than 1 yr and
displayed a highly attenuated reflex vasodilator response. Platelet
inhibition with low-dose aspirin occurs through acetylation of
COX-1 in the presystemic (portal) circulation (57), which irre-
versibly inhibits the production of COX-1 synthesized products,
including the potent aggregating agent and vasoconstrictor throm-
boxane A2. This inhibition occurs for the life of the platelet (~10
days) because they lack a nucleus and, therefore, do not possess
the genetic machinery to resynthesize COX. In vascular endothel-
ial cells, significantly higher doses of aspirin (600 mg) are
required to fully, but temporarily, inhibit COX which can resyn-
thesize the enzyme in a matter of hours. The most logical mech-
anism by which low-dose acetylsalicylic acid attenuates reflex
vasodilation is that platelets may be activated via COX-1 mecha-
nisms and release vasodilating factors, including NO, ATP,
ADP, and 5-HT (18, 35, 56), all of which have the potential to
stimulate cutaneous vasodilator pathways implicated in reflex
vasodilation.

The hypothesis that platelet activation may contribute to
reflex vasodilation was further explored utilizing a within-
subjects design with two different systemic platelet inhibitors:
low-dose aspirin and the specific platelet ADP (P2Y12) receptor
antagonist, clopidogrel (Plavix). In this study, specific platelet

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**References**

ADP receptor inhibition with clopidogrel significantly attenuated reflex vasodilation to a greater extent than platelet COX-1 inhibition with systemic low-dose aspirin (26). There is in vitro (6, 42) and in vivo (55, 60) evidence for 1) platelet COX-mediated vasodilation in models of neurogenic inflammation, and 2) platelet ADP-receptor-mediated endothelium-dependent vasodilation. Specifically, platelet ADP-receptor stimulation causes the release of dinucleotides stored in platelet dense granule, which potentiates platelet aggregation and induces endothelium-dependent vasodilation (Fig. 3, bottom) (6). The precise mechanism of platelet- vessel wall interactions is complex and not completely understood. However, there is clear evidence for platelets releasing multiple vasodilating factors, some of which are known to contribute to cutaneous reflex vasodilation (5, 36, 51, 74, 75). Further research is necessary to examine the mechanisms of platelet vessel wall interactions underlying this attenuated SkBF response, as well as the potential cardiovascular and thermoregulatory consequences.

PERSPECTIVES

Older aged human skin displays attenuated blood flow responses to both whole body cold and heat stress (24, 31). There are a variety of clinically significant disease states that increase in frequency and severity with aging that may further affect the neurovascular regulation of SkBF. For example, endothelial dysfunction, microvessel wall abnormalities, including inward vessel remodeling, autonomic neuropathy, and/or combinations of these neurovascular impairments are present with atherosclerosis, essential hypertension (28, 29), and diabetes (64, 67), respectively. These modifiers of reflex cutaneous vasodilation and vasoconstriction are discussed in detail in an accompanying highlighted topic (7). In addition to these pathological modifiers, common pharmacological therapies used in the treatment of these conditions may also impair key neurovascular signaling mechanisms involved in blood flow regulation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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