
Skin Grafting Impairs Postsynaptic Cutaneous Vasodilator and Sweating Responses

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This study tested the hypothesis that postsynaptic cutaneous vascular responses to endothelial-dependent and -independent vasodilators, as well as sweat gland function, are impaired in split-thickness grafted skin 5 to 9 months after surgery. Intradermal microdialysis membranes were placed in grafted and adjacent control skin, thereby allowing local delivery of the endothelial-dependent vasodilator, acetylcholine (ACh; 1×10^{-7} to 1×10^{-1} M at 10-fold increments) and the endothelial-independent nitric oxide donor, sodium nitroprusside (SNP; 5×10^{-8} to 5×10^{-2} M at 10-fold increments). Skin blood flow and sweat rate were simultaneously assessed over the semipermeable portion of the membrane. Cutaneous vascular conductance (CVC) was calculated from the ratio of laser Doppler-derived skin blood flow to mean arterial blood pressure. Δ CVC responses from baseline to these drugs were modeled via nonlinear regression curve fitting to identify the dose of ACh and SNP causing 50% of the maximal vasodilator response (EC_{50}). A rightward shift in the CVC dose response curve for ACh was observed in grafted ($EC_{50} = -2.61 \pm 0.44 \log M$) compared to adjacent control skin ($EC_{50} = -3.34 \pm 0.46 \log M$; $P = .003$), whereas the mean EC_{50} for SNP was similar between grafted ($EC_{50} = -4.21 \pm 0.94 \log M$) and adjacent control skin ($EC_{50} = -3.87 \pm 0.65 \log M$; $P = 0.332$). Only minimal sweating to exogenous ACh was observed in grafted skin whereas normal sweating was observed in control skin. Increased EC_{50} and decreased maximal CVC responses to the exogenous administration of ACh suggest impairment of endothelial-dependent cutaneous vasodilator responses in grafted skin 5 to 9 months after surgery. Greatly attenuated sweating responses to ACh suggests either abnormal or an absence of functional sweat glands in the grafted skin. (J Burn Care Res 2007;28:435–441)

Increases in skin blood flow and sweating are critical responses for humans to appropriately regulate internal temperature during exercise and/or hyperthermic exposure. If these heat-dissipating mechanisms are absent, exercise can increase internal temperature to

unsafe levels within 10 minutes.¹ Wounds such as burns seriously damage the skin, requiring, in many cases, excising of the damaged tissue and subsequent skin grafting. The excising process leaves little or no dermal tissue at the injured site. It is this dermal layer that contains sweat glands and a rich vascular network, both of which are vital for thermoregulation. Although some degree of revascularization occurs in grafted skin, little is known regarding the consequences of skin grafting with respect to the control of skin blood flow and sweating in the grafted tissue.

Split-thickness skin grafts, 5 to 9 months after surgery, have impaired reflex cutaneous vasodilation and sweating in response to indirect whole-body heating.² In addition, grafted skin had reduced ability to maximally vasodilate in response to a local heating stimulus.² Taken together, these impairments indicate that grafted skin has a greatly attenuated capability to con-

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tribute to thermoregulation. However, the specific mechanisms responsible for the observed impairments in cutaneous vasodilation and sweating in grafted skin are unknown, but may be related to altered postsynaptic function (ie, decreased sensitivity to vasodilator neurotransmitters and cofactors).

The primary aim of this investigation was to determine whether altered postsynaptic function contributes to attenuated cutaneous blood flow and sweating responses observed during indirect whole-body heating in grafted skin 5 to 9 months after surgery.² To address this question, we tested the hypothesis that endothelial-dependent and -independent vasodilation, as well as sweat gland function, are impaired in grafted skin compared with adjacent control skin in response to exogenous administration of the endothelial-dependent vasodilator, acetylcholine, and the endothelial-independent nitric oxide donor, sodium nitroprusside.

METHODS

Human Subjects

Twelve individuals (six men, six women) who had undergone split-thickness autograft application after tangential excision to viable fat within the previous 5 to 9 months participated in this study. Patients with shallow and deep dermal excisions were not enrolled. The mean age, height, and weight of the subjects were 32.7 ± 2.8 years, 168.0 ± 2.0 cm, and 84.8 ± 4.6 kg, respectively (mean \pm SEM). Protocols were approved by the Institutional Review Board at the University of Texas Southwestern Medical Center at Dallas and Presbyterian Hospital of Dallas and subjects provided their informed consent. Subjects were not taking any medications that would affect cutaneous vasodilatory or sweating responses. Subjects refrained from caffeine, alcohol, and exercise 24 hours before the study.

Protocol 1: Endothelial-Dependent Vasodilation and Sweating

We inserted two intradermal microdialysis probes, consisting of two reinforced sections of polyimide tubing connected by a 1-cm dialysis membrane (Bioanalytical Systems, West Lafayette, IN), into unanesthetized grafted and adjacent control skin (Table 1) by advancing a 25-gauge needle 15 to 20 mm through the dermal layer, followed by threading the microdialysis probe through the lumen of the needle and withdrawing the needle. Microdialysis probes were perfused with lactated Ringer solution (Baxter, Deer-

Table 1. Location of split-thickness skin graft and skin donor site

Subject			
No.	Sex	Graft Site	Donor Site
1	Female	Left forearm	Right thigh
2	Male	Right forearm	Right calf
3	Male	Left thigh	Left thigh
4	Male	Right forearm	Right thigh
5	Female	Right hand/wrist	Right thigh
6	Female	Right forearm	Right thigh
7	Female	Left calf	Right thigh
8	Female	Left calf	Right thigh
9	Male	Right forearm	Right thigh
10	Male	Left forearm	Right thigh
11	Female	Right arm	Right thigh
12	Male	Left hand/wrist	Left thigh

field, IL) at a rate of $2 \mu\text{l}/\text{min}$ via a perfusion pump (Harvard Apparatus, Holliston, MA) while hyperemia associated with insertion trauma subsided (a minimum of 90 minutes). A specially designed humidity chamber, having a small window (10×5 mm, ie, surface area of 0.5 cm^2), was placed over each microdialysis probe such that sweating could be assessed directly over the semipermeable portion of each microdialysis membrane.

Sweat rate was assessed by the ventilated-capsule method with compressed nitrogen as the perfusion gas delivered at a rate of $300 \text{ ml}/\text{min}$. Humidity of the effluent gas was measured via a humidity-temperature probe (model HMP 35E, Vaisala, Woburn, MA) positioned 1 m from the capsule on the skin. The humidity and temperature probe was connected to a humidity data processor (HMT38, Vaisala, Woburn, MA) that calculated absolute humidity from the measurement of relative humidity and temperature. An integrating laser-Doppler flowmetry probe (model PF413, Perimed, Sweden) housed within the sweat chamber permitted simultaneous assessment of skin blood flow and sweat rate from the same location directly over each microdialysis membrane. Throughout the protocol, heart rate was obtained from an electrocardiogram (Agilent, Palo Alto, CA) and arterial blood pressure was measured from the upper arm via electro-sphygmomanometry (SunTech, Raleigh, NC).

At each site, dose-response curves for both skin blood flow and sweating were assessed upon administration of increasing doses of acetylcholine ($1 \times 10^{-7} \text{ M}$ to $1 \times 10^{-1} \text{ M}$ at 10-fold increments). Each dose was administered for 5 min at a perfusion rate of

2 $\mu\text{l}/\text{min}$. Arterial blood pressure was measured during the final minute of each dose.

Protocol 2: Endothelial-Independent Vasodilation

Two intradermal microdialysis probes were inserted into grafted skin and adjacent control skin in a similar manner as described previously. Subjects were instrumented as noted previously with the exception of humidity chambers. An integrating laser Doppler flowmetry probe (model PF413, Perimed, Sweden) was placed over each microdialysis probe such that skin blood flow (model PF4000, Perimed, Sweden) could be assessed directly over the semi-permeable portion of each microdialysis membrane.

At each site, dose-response curves were obtained upon the administration of increasing doses of the endothelial-independent vasodilator sodium nitroprusside (5×10^{-8} M to 5×10^{-2} M at 10-fold increments), with each dose being delivered for 5 min at a perfusion rate of 2 $\mu\text{l}/\text{min}$. Skin blood flow was continuously measured directly above each microdialysis membrane during drug administration. Arterial blood pressure was obtained during the final minute of each dose.

Data and Statistical Analyses

For both protocols, data were continuously acquired at a sampling rate of 50 Hz using a data collection system (Biopac System, Santa Barbara, CA). Skin blood flow is reported in arbitrary units (au), given that the area sampled by these probes is unknown. One-minute-averaged responses were calculated at the end of each dose. Cutaneous vascular conductance (CVC) was calculated from the ratio of laser Doppler-derived skin blood flow to mean arterial blood pressure. CVC data, expressed as a change from baseline (ΔCVC), was mathematically modeled via non-linear regression curve fitting (GraphPad, San Diego, CA). The minimum and maximum ΔCVC at both grafted and adjacent control skin were generated from individual dose response curves for both acetylcholine (Protocol 1) and sodium nitroprusside (Protocol 2). The effective concentration causing 50% of the maximal response (EC_{50}) also was calculated from nonlinear regression modeling. This parameter was used as an index of the drug responsiveness.³ Mathematical modeling was unable to generate sweating dose response curves because of minimal sweating responses at all doses of acetylcholine in grafted skin.

Student's paired *t*-tests were used to compare minimum and maximum ΔCVC responses, as well as EC_{50} , between grafted and adjacent control skin for both protocols. Student's paired *t*-tests also were

used to compare differences in sweat rate from normothermic baseline between grafted and adjacent control skin at the highest dose of acetylcholine. Statistical significance was accepted at $P < .05$. All data are presented as mean \pm SEM.

RESULTS

Endothelial-Dependent Vasodilation

Baseline CVC was similar between the graft site (0.42 ± 0.09 au/mm Hg) and adjacent control skin (0.37 ± 0.09 au/mm Hg; $P = .63$). Dose-response curve modeling for acetylcholine had high goodness of fit in both control (mean $R^2 = 0.96 \pm 0.01$) and grafted skin (mean $R^2 = 0.88 \pm 0.05$; Figure 1). Minimum ΔCVC dose-response curve parameters were similar between grafted skin and adjacent control skin (Table 2). Maximum ΔCVC dose-response curve parameters were significantly lower in grafted skin (0.61 ± 0.09 au/mm Hg) compared with adjacent control skin (1.34 ± 0.15 au/mm Hg; $P < .001$; Table 2). The EC_{50} was significantly greater in grafted skin (-2.61 ± 0.15 log M) compared with adjacent control skin (-3.59 ± 0.19 log M; $P = .003$; Table 2 and Figure 2), indicating a rightward shift in the dose-response curve (ie, a higher dose of acetylcholine was needed to cause similar vasodilator responses) in grafted skin (Figure 1).

Endothelial-Independent Vasodilation

Baseline CVC was significantly greater in grafted skin (0.55 ± 0.09 au/mm Hg) compared with adjacent control skin (0.27 ± 0.04 au/mm Hg; $P = .016$). Dose-response curve modeling for sodium nitroprusside had high goodness of fit in both control skin

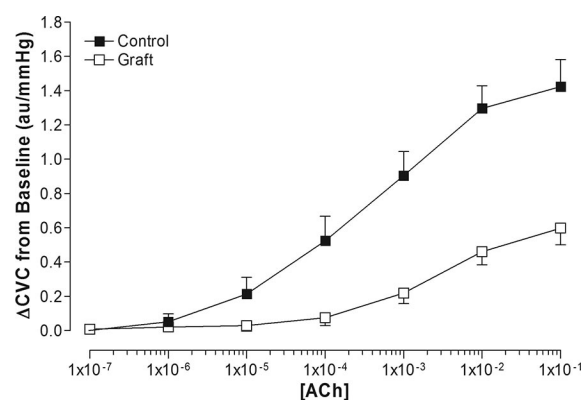


Figure 1. Nonmodeled dose-response relationship of cutaneous vascular conductance (ΔCVC from baseline) to acetylcholine (ACh) administration in grafted skin (graft) and adjacent control skin (control).

Table 2. Mean cutaneous vascular conductance (Δ CVC from baseline) dose-response curve parameters to the exogenous administration of acetylcholine

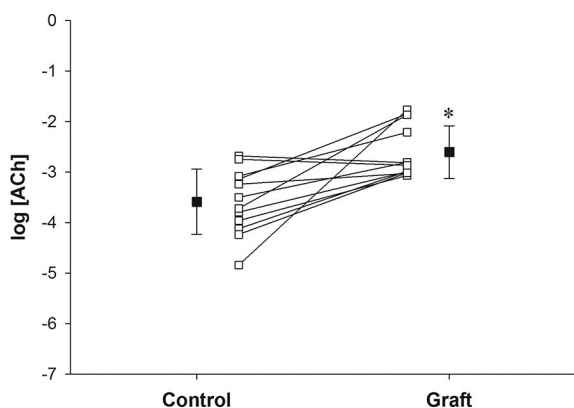
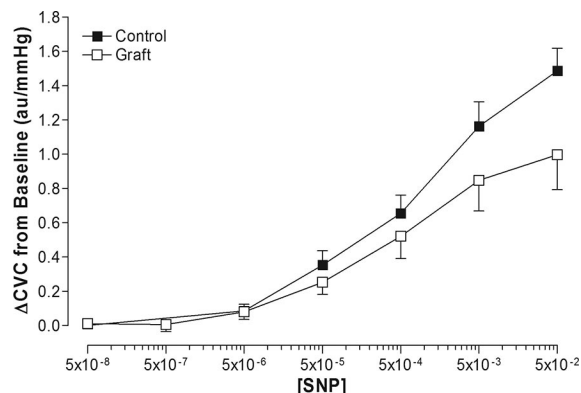
Acetylcholine Response	Skin Type		P Value (2-tailed paired <i>t</i> -test)
	Control	Graft	
Minimum Δ CVC (au/mm Hg)	0.04 \pm 0.03	0.02 \pm 0.02	.499
Maximum Δ CVC (au/mm Hg)	1.34 \pm 0.15	0.61 \pm 0.09	<.001
EC ₅₀ (log M)	-3.59 \pm 0.19	-2.61 \pm 0.15	.003

These values depict the minimum and maximum CVC response, as well as the effective concentration causing 50% of the maximum response (EC₅₀) to increasing concentrations of acetylcholine.

(mean $R^2 = 0.97 \pm 0.03$) and grafted skin (mean $R^2 = 0.95 \pm 0.06$; Figure 3). Minimum Δ CVC dose-response parameters were similar between grafted skin and adjacent control skin (Table 3). Maximal Δ CVC tended to be lower in grafted skin (0.99 ± 0.20 au/mm Hg) compared to adjacent control skin (1.45 ± 0.12 au/mm Hg; $P = .098$; Table 3). No differences were observed in the EC₅₀ of grafted skin compared to adjacent control skin (Table 3, Figure 4).

Sweat Gland Function

Nonlinear mathematical modeling was unable to generate dose-response curves for sweating due to minimal sweating responses at all doses of acetylcholine in grafted skin. The highest dose of acetylcholine (1×10^{-1} M) elicited a significantly large increase in ASR in control skin (0.81 ± 0.23 mg/cm²/min)

**Figure 2.** Mean responses \pm SEM (■) and individual responses (□ with lines) for the effective concentration of acetylcholine (ACh) that yields 50% of the maximal response (EC₅₀) in grafted skin (graft) and adjacent control skin (control). *Difference from control ($P < .05$).**Figure 3.** Nonmodeled dose-response relationship of cutaneous vascular conductance (Δ CVC from baseline) to sodium nitroprusside (SNP) administration in grafted skin (graft) and adjacent control skin (control).

compared to an absence of sweating in grafted skin (Figure 5).

DISCUSSION

The primary finding of this investigation demonstrates that vasodilator responsiveness to exogenous agents is altered in grafted skin. Endothelial-dependent cutaneous vasodilation, as assessed via the administration of acetylcholine, is reduced in grafted skin 5 to 9 months after surgery compared with adjacent control skin. In addition, the absence of appreciable sweating to the administration of acetylcholine in grafted skin suggests either abnormal or an absence of functional sweat glands in grafted skin. Furthermore, maximal endothelial-independent cutaneous vasodilation to exogenous nitric oxide administration tends ($P = .098$) to be less in grafted compared with control skin

Table 3. Mean cutaneous vascular conductance (Δ CVC from baseline) dose-response curve parameters to the exogenous administration of sodium nitroprusside

Sodium Nitroprusside Response	Skin Type		P Value (2-tailed paired <i>t</i> -test)
	Control	Graft	
Minimum Δ CVC (au/mm Hg)	0.05 \pm 0.01	0.04 \pm 0.04	0.772
Maximum Δ CVC (au/mm Hg)	1.45 \pm 0.12	0.99 \pm 0.20	0.098
EC ₅₀ (log M)	-3.87 \pm 0.19	-4.21 \pm 0.27	0.332

These values depict the minimum and maximum CVC response, as well as the effective concentration causing 50% of the maximum response (EC₅₀) to increasing concentrations of sodium nitroprusside.

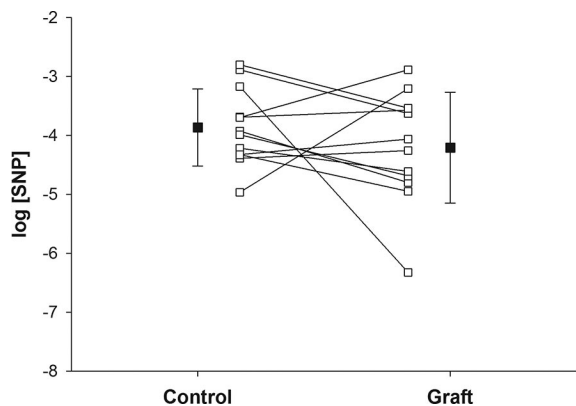


Figure 4. Mean responses \pm SEM (■) and individual responses (□ with lines) for the effective concentration of sodium nitroprusside (SNP) that yields 50% of the maximal response (EC_{50}) in grafted skin (graft) and adjacent control skin (control).

at 5 to 9 months after surgery despite no observed differences in sensitivity (EC_{50}) to nitric oxide. Given these findings, altered postsynaptic function is a possible mechanism for previously observed impairments in vasodilation and sweating in grafted skin during indirect whole-body heating and local heating.²

The active cutaneous vasodilator system mediates 85% to 95% of the increase in skin blood flow in nonglabrous skin during whole-body heating.^{4,5} Cutaneous active vasodilation is initiated by the release of neurotransmitters from sympathetic cholinergic nerves following increases in internal temperature. Acetylcholine is one of the neurotransmitters released from sympathetic cholinergic nerves and causes endothelial-dependent release of vasoactive factors, including nitric oxide, prostanoids, and endothelial-

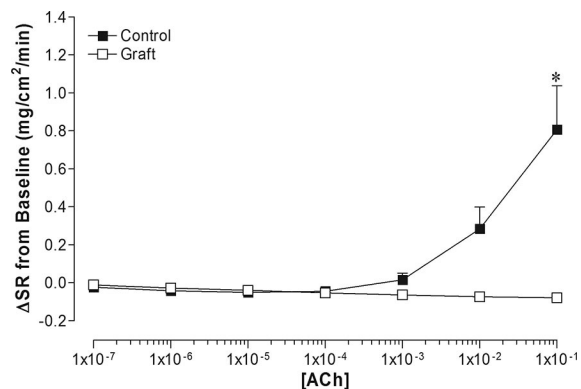


Figure 5. Dose-response relationship of sweat rate (ΔSR from baseline) to acetylcholine (ACh) administration in grafted skin (Graft) and adjacent control skin (control). *Difference from control ($P < .05$).

derived hyperpolarizing factor.^{6,7} Attenuated increases in CVC, accompanied with an elevated EC_{50} during acetylcholine administration, indicate an impairment of endothelial-dependent cutaneous vasodilator responses in grafted skin compared with adjacent control skin. The exact mechanism leading to this impaired response has yet to be identified.

Because nitric oxide is released from the endothelium during the administration of acetylcholine, altered nitric oxide-mediated vasodilation could account for impaired endothelial-dependent vasodilation. Nitric oxide is a potent vasodilator in many tissues, including skin. In the current study, the sensitivity to nitric oxide was similar (ie, no differences in EC_{50} for sodium nitroprusside) between grafted skin and adjacent control skin. However, maximum CVC responses tended to be different between sites ($P = .098$). These observations indicate that the vascular smooth muscle in grafted skin is responsive to exogenous nitric oxide, but maximal nitric oxide-mediated vasodilation may be altered in grafted skin 5 to 9 months after surgery. This tendency for attenuated maximal vasodilation could explain impaired vasodilation during local heating,² as cutaneous vasodilation during sustained local heating is primarily nitric oxide dependent.^{8,9} Impairments in nitric oxide-mediated vasodilation could also partially explain attenuated cutaneous blood flow responses in grafted skin during indirect whole-body heating,² as approximately 30% of cutaneous vasodilation during whole-body heating is nitric oxide dependent.^{10,11}

The finding of similar vasodilator responses to sodium nitroprusside in grafted skin, coupled with attenuated vasodilator responses to acetylcholine, suggests that the impaired vasodilator responses to acetylcholine could be the result of either altered endothelial-dependent nitric oxide release and/or altered nonnitric oxide dependent vasodilation associated with the administration of acetylcholine. Regarding the latter, vasodilation caused by the exogenous administration of acetylcholine is not solely mediated by nitric oxide dependent pathways but may involve other pathways, including a prostanoid-dependent pathway, as well as a non-nitric oxide/nonprostanoid-dependent (ie, endothelial-derived hyperpolarizing factor) pathway.¹²⁻¹⁴ Recent findings provide evidence that prostanoids are involved in reflex cutaneous vasodilation during whole body heating.¹³ Thus, reduced endothelial-dependent vasodilation observed in this investigation and impaired reflex vasodilation during whole-body heat stress observed 5 to 9 months after surgery² could be the result of disruptions in these non-nitric oxide pathways, including decreased endothelial production

and release of vasoactive factors (eg, prostanoids and/or endothelial-derived hyperpolarizing factor) and/or decreased receptor sensitivity to these agents. The involvement of these pathways leading to impaired endothelial-dependent vasodilation in grafted skin warrants further investigation.

Impairments in endothelial-dependent vasodilation observed may only partially explain diminished reflex cutaneous vasodilation observed in grafted skin during whole-body and local heating.² The primary neurotransmitter responsible for reflex cutaneous vasodilation during whole-body heating, remains unknown, although it is unlikely to be acetylcholine.^{5,15-17} This unknown neurotransmitter responsible for active vasodilation has been proposed to be coreleased, presumably with acetylcholine, from sympathetic cholinergic nerves.¹⁶ Peptides, including vasoactive intestinal peptide and calcitonin gene-related peptide, also are co-released from sympathetic cholinergic nerves during and may be the neurotransmitters mediating heat stress induced cutaneous vasodilation.¹⁸⁻²¹ Alterations in the release of these vasoactive neurotransmitters and cofactors from sympathetic cholinergic nerves may play a role in impaired active vasodilation of grafted skin. The cutaneous vasculature also could be less responsive (ie, decreased sensitivity, decreased receptor density) to these neural factors after revascularization of the injured skin. Finally, impaired vasodilation observed in grafted skin during a whole-body heat stress² may simply be explained by altered innervation of the cutaneous vasculature (ie, decreased number of nerves innervating cutaneous vessels). The relative role of these potential mechanisms in the impairment of vasodilation of grafted skin warrants further investigation.

Postsynaptic sweating responses to the administration of acetylcholine were absent in grafted skin. This finding is consistent with our findings of impaired sweating responses to indirect whole-body heating in grafted skin.² This observation is also in agreement with previous reports documenting an absence of sweating from split thickness grafts.²²⁻²⁴ However, these previous studies were unable to identify whether the impaired sweating responses was due to nerve disruption or altered postsynaptic responses. The absence of sweating during the exogenous administration of acetylcholine suggests that impaired sweating in this skin is caused by a combination of the initial injury disrupting sweat glands at the recipient tissue, and/or the donor tissue in most split-thickness grafts not containing sweat glands.^{25,26} However, it is unknown whether sweat glands regenerate as the graft matures.

CONCLUSION

Attenuated endothelial-dependent cutaneous vasodilation and a tendency for attenuated endothelial-independent cutaneous vasodilation suggests altered postsynaptic function possibly contributing to impaired cutaneous vasodilator responses in grafted skin 5 to 9 months after surgery. Furthermore, the absence of appreciable sweating in grafted skin suggests either abnormal or an absence of functional sweat glands in grafted skin. These data suggest that split-thickness skin grafts at 5 to 9 months after surgery have reduced capability of contributing to thermoregulation through endothelial-dependent and perhaps nitric oxide-mediated vasodilator responses. Thus, individuals with split-thickness skin grafts may be at an increased risk of heat-related injury if the grafted area covers a large fraction of the skin surface. The long-term consequences of skin grafting with respect to the neural control of skin blood flow and sweating remains unknown.

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