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Sustained Impairments in Cutaneous Vasodilation and Sweating in Grafted Skin Following Long-Term Recovery

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Abstract

We previously identified impaired cutaneous vasodilation and sweating in grafted skin 5 to 9 months postsurgery. The aim of this investigation was to test the hypothesis that cutaneous vasodilation, but not sweating, is restored as the graft heals. Skin blood flow and sweat rate were assessed from grafted skin and adjacent noninjured skin in three groups of subjects: 5 to 9 months postsurgery (n = 13), 2 to 3 years postsurgery (n = 13), and 4 to 8 years postsurgery (n = 13) during three separate protocols: 1) whole-body heating and cooling, 2) local administration of vasoactive drugs, and 3) local heating and cooling. Cutaneous vasodilation and sweating during whole-body heating were significantly lower ($P < .001$) in grafted skin when compared with noninjured skin across all groups and demonstrated no improvements with recovery time postsurgery. Maximal endothelial-dependent (acetylcholine) and endothelial-independent (sodium nitroprusside) cutaneous vasodilation remained attenuated ($P < .001$) in grafted skin up to 4 to 8 years postsurgery, indicating postsynaptic impairments. In grafted skin, cutaneous vasoconstriction during whole-body and local cooling was preserved, whereas vasodilation to local heating was impaired, regardless of the duration postsurgery. Split-thickness skin grafts have impaired cutaneous vasodilation and sweating up to 4 to 8 years postsurgery, thereby limiting the capability of this skin's contribution to thermoregulation during a heats stress. In contrast, grafted skin has preserved vasoconstrictor capacity.

The control of skin blood flow in humans occurs through two distinct sympathetic neural pathways. The first pathway involves a sympathetic vasoconstrictor system, whereas the second pathway modulates the skin blood flow through a nonadrenergic sympathetic active vasodilator system.^{1,2} Appropriate vasoconstriction in split-thickness grafted skin 5 to 9 months postsurgery during cold stress suggests reinnervation and restoration of autonomic control of the vasoconstrictor pathway of the cutaneous vasculature.³ Despite this observation, split-thickness skin grafts 5 to 9 months postsurgery have impairments in cutaneous

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vasodilation and sweating during heat stress when compared with that of healthy, uninjured skin.⁴ Altered postsynaptic function 5 to 9 months postsurgery likely contributes to these observed impairments in cutaneous vasodilator and sweating responses in grafted skin.⁵ Because increases in skin blood flow and sweating are critical responses for humans to appropriately regulate internal temperature during exercise and/or hyperthermic exposure, attenuation of cutaneous vasodilation, and sweating in grafted skin could impair thermoregulatory responses to heat stress and possibly account for the higher rectal temperatures observed in some burned individuals during a thermal challenge.^{6–8}

Despite impairments relative to healthy noninjured skin, some neural control of the cutaneous vasculature was restored in grafted skin 5 to 9 months after the initial injury and subsequent grafting surgery.⁴ This observation leaves open the possibility that function could be further restored with longer recovery times after surgery. Relatively little is known regarding the long-term effects of skin grafting on autonomic control of cutaneous vasodilation and sweating of the grafted area. Few studies have investigated the long-term effects of skin grafting on efferent thermoregulatory responses.^{7–12} For example, Freund et al¹⁰ reported impaired forearm blood flow responses to indirect whole-body heating in grafted skin in one subject 5 years postsurgery. Sweat function also remains diminished in mature split-thickness grafts.^{7,10,12,13} This latter response is thought to be attributable to a combination of the initial injury destroying sweat glands at the recipient tissue and/or a lack of sweat glands in the harvested tissue in most split-thickness grafts.¹³ Limited information can be drawn from these early studies because of small sample sizes, lack of control for postgraft surgery duration, and methodological concerns, resulting in an inability to separate cutaneous blood flow responses between grafted and adjacent noninjured skin.

The primary aim of the present investigation was to test the hypothesis that cutaneous vasodilator responses in grafted skin to indirect whole-body heating previously shown to be impaired 5 to 9 months postsurgery would improve after longer periods of recovery postsurgery, whereas sweating responses would remain impaired. A secondary aim of this investigation was to examine the mechanisms of the hypothesized improvements in cutaneous vasodilation in grafted skin after longer periods of recovery postsurgery. Specifically, it was hypothesized that postsynaptic vasodilator responses to exogenous administration of the endothelial-dependent vasodilator, acetylcholine, and the endothelial-independent nitric oxide donor, sodium nitroprusside, will increase as the skin graft matures. A final aim of the investigation was to test the hypothesis that cutaneous vasoconstriction during whole-body and local cooling remains preserved in grafted skin after long-term recovery.³ These objectives were accomplished using a cross-sectional assessment of the control of skin blood flow and sweating in split-thickness skin grafts in three separate groups at three time points of recovery postsurgery.

METHODS

Human Subjects

Individuals who had undergone split-thickness autograft application (nominal thickness <0.012 in) after tangential excision to viable fat with no visible dermis were recruited to participate in this study and placed into the following three groups according to their time postsurgery: 1) 5 to 9 months postsurgery, 2) 2 to 3 years postsurgery, and 3) 4 to 8 years postsurgery (see Table 1 for descriptive information of subject groups). Individuals in all groups had grafts covering less than 20% of their total body surface area. Graft locations were on the extremities (upper and lower) where there was an adjacent noninjured skin that showed no evidence of damage from the injury or surgery. Evaluation sites in both grafted skin and adjacent noninjured skin were selected carefully to avoid visible large blood vessels (ie, surface

veins), surface irregularities, and areas of scarring with the exception of the consistent pattern associated with meshing in grafted skin.

This study consisted of three separate protocols and required multiple visits to the laboratory. The data presented generally represent 13 subjects per group. However, some subjects did not complete the entire battery of tests, resulting in 9 to 12 subjects per group for some protocols. The number of subjects participating in each protocol is indicated. Data from the 5 to 9 months group have been previously published.^{3–5} These data are included to allow for cross-sectional comparisons to evaluate the effects of recovery postsurgery on the assessed responses.

Participants provided informed written consent before testing. All protocols were approved by the Institutional Review Board at the University of Texas Southwestern Medical Center at Dallas and Presbyterian Hospital of Dallas and were conducted in accordance with the Declaration of Helsinki principles. Subjects were not taking any medications that would affect cutaneous vasodilatory or sweating responses. No subjects reported a history of heat exhaustion or heat stroke. Subjects refrained from caffeine, alcohol, and exercise for 24 hours before each protocol in the study.

Protocol 1: Whole-Body Heating and Cooling

Subjects—Thirteen individuals (seven men, six women) in the 5 to 9 months postsurgery group, 13 individuals (11 men, two women) in the 2 to 3 years postsurgery group, and 13 individuals (seven men, six women) in the 4 to 8 years postsurgery group participated in the whole-body heating and cooling protocol. In addition, 11 individuals (five men, six women) in the 5 to 9 months postsurgery group, nine individuals (seven men, two women) in the 2 to 3 years postsurgery group, and 10 individuals (five men, five women) in the 4 to 8 years postsurgery group returned a minimum of 48 hours after completing protocol 1 and repeated the whole-body heating protocol to assess responses from the donor site.

Instrumentation—Heart rate was obtained from an electrocardiogram (Agilent, Palo Alto, CA) with the signal interfaced with a cardi tachometer (CWE, Ardmore, PA). Arterial blood pressure was measured from the brachial artery via electrosp ygmomanometry (SunTech, Raleigh, NC). Internal temperature was indexed from an ingestible pill telemetry system (HQ, Inc., Palmetto, FL). The telemetry pill correlates well with other methods of internal temperature measurement such as esophageal and rectal temperatures.¹⁴ Mean skin temperature was measured via the weighted average of six thermocouples attached to the skin.¹⁵

Skin blood flux was measured continuously from integrating laser-Doppler flowmetry probes (model PF413, Perimed, Sweden), each housed in a 3-cm diameter heater element (Perimed, Sweden) placed on the grafted skin and the adjacent noninjured skin. This technique permits continuous measurements of skin blood flow over a relatively small area (~0.28 cm²). Laser-Doppler flowmetry is accepted widely as the standard for measurement of cutaneous blood flow, independent of muscle blood flow, and it has been shown to correlate in a linear fashion with flow measured using established techniques including plethysmography and isotope clearance.^{16–19}

Sweat rate was measured using capacitance hygrometry by perfusing 100% nitrogen at a flow rate of 300 ml/min through a ventilated capsule (surface area = 2.83 cm²) placed on grafted skin and adjacent noninjured skin. Humidity of the effluent gas was measured via humidity-temperature probes (model HMP 35E, Vaisala, Woburn, MA) positioned 1 m from the capsule on the skin. Each humidity-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.

Protocol—Individuals were dressed in a tube-lined suit (Med-Eng, Ottawa, Canada) that permitted the control of skin and core temperature by changing the temperature of water perfusing the suit. The perfusion suit covered the entire body with the exception of the head, hands, feet, and instrumented area. Because instrumented areas were not in contact with the suit, observed changes in skin blood flow and sweating associated with the whole-body heat stress were not due to the effects of locally heating the skin, but rather were an autonomic response associated with the integration of internal and skin temperatures in the thermoregulatory centers primarily located in the hypothalamus. Data were collected with the subject in the supine position. Baseline measurements were obtained while perfusing the suit with 34 °C water. After normothermic data collection, a whole-body cold stress was performed by perfusing 5 °C water through the suit for 3 minutes. On completion of this cold stress, a whole-body heat stress immediately ensued by perfusing 46 °C water through the suit until internal temperature increased ~0.8 °C. On completion of the heat stress, the temperature of the water perfusing the suit was returned to 34 °C, and the sites where skin blood flow was monitored were then locally heated by increasing local skin temperature to 42 °C via the heating elements housing the laser-Doppler flow probes. This local temperature was held constant for 30 minutes to elicit maximal cutaneous vasodilation.²⁰

To evaluate responses at donor sites, subjects returned to the laboratory on a separate day and were instrumented in the same manner as described earlier with the exception that integrating laser-Doppler flowmetry probes, local heaters, and sweat capsules were placed on donor skin sites and adjacent noninjured skin. This protocol was performed exactly as specified earlier.

Protocol 2: Exogenous Drug Delivery via Microdialysis

Subjects—Twelve individuals (six men, six women) in the 5 to 9 months postsurgery group, 12 individuals (10 men, two women) in the 2 to 3 years postsurgery group, and 12 individuals (six men, six women) in the 4 to 8 years postsurgery group participated in the first phase of this protocol (endothelial-dependent vasodilation and sweating responses to exogenous acetylcholine). One male subject in the 2 to 3 years postsurgery group did not participate in the second phase of this protocol (endothelial-independent vasodilation to exogenous sodium nitroprusside).

Endothelial-Dependent Vasodilation and Sweating (Acetylcholine)—Two intradermal microdialysis probes, consisting of two reinforced sections of polyimide tubing connected by a 1-cm dialysis membrane (Bioanalytical Systems, West Lafayette, IN), were inserted into grafted and adjacent noninjured skin. This was accomplished by advancing a 25-gauge needle 15–20 mm through the dermal layer without anesthesia, followed by threading the microdialysis probe through the lumen of the needle and withdrawing the needle. Microdialysis probes were perfused with lactated Ringers solution (Baxter, Deerfield, IL) at a rate of 2 μ l/min via a perfusion pump (Harvard Apparatus, Holliston, MA), whereas hyperemia associated with insertion trauma subsided (a minimum of 90 minutes). A specially designed humidity chamber, with a small window (10 $\times$ 5 mm, ie, surface area of 0.5 cm²), was placed over each microdialysis probe such that sweating could be assessed directly over the semi-permeable portion of each microdialysis membrane. Sweat rate was assessed by the ventilated-capsule method as described previously. An integrating laser-Doppler flowmetry probe was housed within the sweat chamber, thus simultaneous assessment of skin blood flow and sweat rate from the same location directly over each microdialysis membrane was obtained. Throughout the protocol, heart rate and arterial blood pressure were measured as described previously.

At each site, dose-response curves for both skin blood flow and sweating were assessed on administration of increasing doses of acetylcholine (1×10^{-7} to 1×10^{-1} M at 10-fold

increments). Acetylcholine was administered at a rate of 2 μ l/min for 5 minutes per dose. Arterial blood pressure was measured during the final minute of each dose.

Endothelial-Independent Vasodilation (Sodium Nitroprusside)—Two intradermal microdialysis probes were inserted into grafted skin and adjacent control skin in the same manner as described earlier and were instrumented as outlined earlier with the exception of the humidity chambers. An integrating laser-Doppler flowmetry probe was placed over each microdialysis probe such that skin blood flow was assessed directly over the semi-permeable portion of each microdialysis membrane.

At each site, dose-response curves were obtained on administration of increasing doses of the endothelial-independent vasodilator sodium nitroprusside (5×10^{-8} to 5×10^{-2} M at 10-fold increments). Each dose of sodium nitroprusside was delivered for 5 minutes at a perfusion rate of 2 μ L/min. Arterial blood pressure was obtained during the final minute of each dose.

Protocol 3: Local Cooling

Subjects—Thirteen individuals (seven men, six women) in the 5 to 9 months postsurgery group, nine individuals (eight men, one woman) in the 2 to 3 years postsurgery group, and 12 individuals (six men, six women) in the 4 to 8 years postsurgery group participated in the local cooling protocol.

Instrumentation—Subjects were instrumented in the same manner as described earlier for heart rate and arterial blood pressure. Skin blood flow was measured continuously from both grafted and adjacent control skin using integrating laser-Doppler flowmetry probes, each housed in a custom-designed Peltier heating/cooling device. A thermocouple was placed between the skin and the Peltier device allowing for precise control and recording of local skin temperature at grafted skin and adjacent noninjured skin.

Protocol—Local skin temperature was increased initially to 39 °C over a period of 10 minutes and then maintained constant for 15 minutes to reduce the influence of tonic vasoconstrictor tone.²¹ Local skin temperature was then decreased to 19 °C in 5 °C decrements (ie, to 34, 29, 24, 19 °C). For each local temperature, local skin temperature was decreased over the first 10 minutes to the desired temperature and was then maintained at that temperature for 5 minutes.

Data and Statistical Analyses

For all protocols, data were acquired continuously at a sampling rate of 50 Hz using a data acquisition system (Biopac System, Santa Barbara, CA). One-minute-averaged responses were calculated at the final minute of normothermic baseline, whole-body cooling, and whole-body heating. Cutaneous vascular conductance (CVC) was calculated from the ratio of laser-Doppler derived skin blood flow to mean arterial blood pressure. Because of the heterogeneity of skin blood flow, coupled with the inability of laser-Doppler flowmetry to provide an absolute blood flow (eg, in ml or volume tissue or min), laser Doppler flow measurements are expressed usually as a percentage of maximum. However, because of the potential for differences in maximal skin blood flows during local heating, as well as differences in baseline skin blood flow in grafted sites and adjacent noninjured skin between groups, data were presented only as a change from baseline in absolute CVC units (au/mm Hg) to minimize possible effects of baseline and maximal skin blood flow differences in group comparisons.

For the microdialysis protocols, one-minute-averaged responses were calculated at the end of each dose. CVC data, expressed as a change from baseline (Δ CVC), were modeled mathematically via nonlinear regression curve fitting (GraphPad, San Diego, CA). Maximum Δ CVC responses at both grafted and adjacent control skin were generated from individual dose

response curves for both acetylcholine and sodium nitroprusside. The effective concentration causing 50% of the maximal response (EC_{50}) was also calculated from nonlinear regression modeling. This parameter was used as an index of the cutaneous responsiveness to a given drug.²² Mathematical modeling was unable to generate sweating dose response curves because of minimal sweating responses at all doses of acetylcholine in grafted skin.

For the local cooling protocol, one-minute-averaged responses were calculated during the final minute of each local temperature stage. However, the absolute change in CVC (ie, ΔCVC ; au/mm Hg) during local cooling between local skin temperatures of 39 and 19 °C was used to address the specific hypothesis.

Cutaneous vascular and sweating responses for each protocol were analyzed via a mixed-model two-way analysis of variance with main factors of skin site (graft and adjacent noninjured) and recovery time postsurgery. Statistical significance was accepted at $P < .05$. All data are presented as mean $\pm$ SEM.

RESULTS

Protocol 1: Whole-Body Heating

Preheat stress CVC was not different between grafted skin compared with adjacent noninjured skin ($P = .64$ main effect of skin site; Table 2). However, the interaction between skin site and recovery time postsurgery approached significance ($P = .09$), indicating potential differences in baseline CVC between grafted sites with recovery (Table 2). CVC was elevated initially and then reduced in the more mature grafts, which could be physiologically meaningful despite lack of statistical significance.

Indirect whole-body heating significantly increased ($P < .001$) internal temperature in all three groups with no difference in the magnitude or temperature obtained between groups ($P = .69$). There was a significant main effect between skin sites for CVC ($P < .001$), suggesting that the increases in absolute CVC (au/mm Hg) from normothermic baseline caused by the heat stress were greater at the noninjured sites relative to grafted sites (Figure 1). There was an absence of an interaction ($P = .15$) for absolute CVC between skin sites and recovery time postsurgery, suggesting that cutaneous vasodilation remains impaired up to 4 to 8 years postsurgery (Figure 1).

The magnitude of cutaneous vasodilation to local heating was significantly less ($P < .001$) at grafted sites (skin site main effect) relative to the adjacent noninjured sites (Figure 2). However, the degree of attenuation at the grafted site was unaffected by the recovery time postsurgery ($P = .15$ for the interaction).

Sweating responses were significantly less ($P < .001$) at grafted sites (skin site main effect) relative to the adjacent control sites (Figure 3), consistent with previously observed data 5 to 9 months postsurgery. In addition, the sweating responses at grafted sites were unaffected by the recovery time postsurgery ($P = .27$ for the interaction).

No differences in skin blood flow ($P = .54$ main effect of skin site) or sweat rate ($P = .52$ main effect of skin site) were observed between donor sites and adjacent noninjured skin at 2 to 3 years and 4 to 8 years postsurgery. These results are consistent with previous findings at 5 to 9 months postsurgery indicating donor sites maintain the ability to appropriately vasodilate and sweat in response to heat stress.⁴

Protocol 2: Exogenous Drug Delivery via Microdialysis

Endothelial-Dependent Vasodilation—Dose-response curve modeling for acetylcholine had high “goodness of fit” in both noninjured and grafted skin for all three groups (mean $\pm$ SEM for all groups combined: graft = 0.93 ± 0.02 ; control = 0.96 ± 0.01). The maximum increase in CVC generated from acetylcholine dose-response curve modeling was significantly lower in grafted skin compared with noninjured skin (skin site main effect $P < .001$; Table 3). These attenuated vasodilator responses at the grafted site were unaffected by recovery time postsurgery ($P = .74$ for the interaction; Table 3).

The EC₅₀ of the dose response curve was significantly greater in grafted skin compared with adjacent control skin (skin site main effect $P < .001$; Table 3), 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. The interaction between skin site and recovery time postsurgery approached significance ($P = .08$). These findings, taken together, suggest that endothelial-dependent vasodilation in grafted skin may show some, albeit very small, improvements over time that could be physiologically meaningful despite lack of statistical significance.

Endothelial-Independent Vasodilation—Dose-response curve modeling for sodium nitroprusside had high goodness of fit in both adjacent and grafted skin for all three groups (mean $\pm$ SEM for all groups combined: graft = 0.95 ± 0.01 ; control = 0.97 ± 0.01). The maximum increase in CVC calculated from the sodium nitroprusside dose-response curve modeling was similar between grafted skin and adjacent control skin (skin site main effect $P = .17$; Table 4). These maximum responses were not affected by the time postsurgery ($P = .78$ for the interaction; Table 4).

No main (skin site) or interaction ($P = .21$) effects were observed in EC₅₀ responses during sodium nitroprusside administration, thereby indicating responsiveness to exogenous nitric oxide is similar between grafted and adjacent noninjured skin regardless of the time postsurgery (Table 4).

Sweat Gland Function With Acetylcholine Administration—Dose-response curves using nonlinear mathematical modeling could not be generated for sweating because of minimal sweating responses at all doses of acetylcholine in grafted skin in all groups. The highest dose of acetylcholine (1×10^{-1} M) elicited a significantly larger increase in sweat rate of noninjured skin compared with an absence of or minimal sweating in grafted skin (skin site main effect $P < .001$; Figure 4). Maximum sweating responses to acetylcholine did not improve at the grafted site over time postsurgery ($P = .41$ for the interaction; Figure 4).

Protocol 3: Whole Body and Local Cooling

Indirect whole-body cooling significantly decreased ($P < .001$) mean skin temperature in all three groups. No main (skin site or recovery time postsurgery) or interaction ($P = .40$) effects for CVC were observed during whole-body cooling (Figure 5). Similarly, no main or interaction ($P = .34$) effects were observed for CVC responses to local cooling between grafted sites and adjacent noninjured skin (Figure 6). These findings indicate that cutaneous vasoconstriction during whole-body and local cooling is preserved as early as 5 to 9 months postsurgery and remains preserved up to 4 to 8 years postsurgery.³

DISCUSSION

The primary finding of this investigation is that cutaneous vasodilatory responsiveness to heat stress did not improve with recovery over time postsurgery. Contrary to the proposed

hypothesis, cutaneous vasodilation in grafted skin remained greatly attenuated relative to adjacent control nongrafted skin. Attenuated increases in cutaneous vasodilation in grafted skin could potentially be due to diminished sympathetic neural function (ie, inappropriate or absence of required sympathetic innervation and/or decreased neurotransmitter release). Another possibility is altered postsynaptic function (ie, decreased sensitivity to vasodilator neurotransmitters), which may also contribute to an attenuation of cutaneous vasodilation in grafted skin during heating. Although the primary neurotransmitter(s) responsible for cutaneous active vasodilation is likely a peptide coreleased with acetylcholine from sympathetic cholinergic nerves, which peptide that remains unknown.^{23–27} Thus, tests were not performed to evaluate the effects of skin grafting on responsiveness to all potential neurotransmitter(s) responsible for active cutaneous vasodilation. Nevertheless, a component of the elevation in skin blood flow to a whole-body heat stress is nitric oxide dependent,²⁸ and thus we sought to identify whether at least an element of the attenuated vasodilator responses of grafted skin could be due to altered endothelial-dependent and independent cutaneous vasodilation. Maximal vasodilator responsiveness was attenuated significantly in grafted skin after acetylcholine administration. The EC₅₀ associated with acetylcholine administration was also significantly shifted to the right (ie, reduced sensitivity to the drug) in grafted skin compared with adjacent noninjured skin. However, maximal vasodilator responsiveness and EC₅₀ were not impaired after administration of sodium nitroprusside at grafted sites compared with noninjured skin. Taken together, these data suggest that skin grafting results in endothelial dysfunction rather than impaired responsiveness to nitric oxide. Endothelial dysfunction may impair not only acetylcholine responsiveness but also responsiveness of other vasodilatory neurotransmitters and substances involved in cutaneous vasodilation including vasoactive intestinal peptide, calcitonin gene-related peptide, histamine, and prostaglandins.^{23,27,29–32} Further research is warranted to understand the potential effects of skin grafting on these vasodilatory mechanisms.

Increases in CVC were observed during local heating (ie, directly heating the skin where blood flow is measured) in both grafted and adjacent uninjured skin. However, the magnitude of this increase was significantly less in grafted skin when compared with adjacent noninjured skin and demonstrated no improvements with recovery. Maximal vasodilatory responses after sustained local heating occur primarily through endothelial-dependent nitric oxide release.^{33–36} Attenuated vasodilator responses to sustained local heating in grafted skin could, therefore, be due to alterations in nitric oxide release, presumably from endothelial sources based on the decreased sensitivity to the endothelial-dependent vasodilator acetylcholine discussed earlier. Alterations in nitric oxide release could also partially explain impaired cutaneous active vasodilator responses during whole-body heating, given that ~30% of cutaneous vasodilation during indirect whole-body heating is also nitric oxide dependent.²⁸ However, such an effect alone would be unlikely to completely explain differences in CVC between sites during indirect whole-body heating as most subjects had impaired cutaneous vasodilation in grafted skin that was greater than 30% between sites.

In addition to sustained impairments in cutaneous vasodilation in grafted skin, sweating during indirect whole-body heating was impaired significantly in grafted skin across all groups. This finding is consistent with previous studies documenting diminished sweating from split-thickness grafts.^{7,9,11,12} However, three subjects in the 2 to 3 years and two subjects in the 4 to 8 years postsurgery groups exhibited some degree of sweating in the grafted skin during whole-body heating, although the magnitude of sweating at these sites was less than at the control sites. These observations are similar to those of Ponten,¹² who reported sweating in grafted skin on one patient, suggesting the potential for small improvements in sweating in grafted skin. Based solely on the observations from the whole-body heating protocol, it is unknown whether reduced sweating responses were due to an absence of functional sweat glands or due to disrupted innervation of the sweat gland. To address this question, sweating

responses in grafted skin were evaluated in a dose-dependent manner using exogenous acetylcholine to stimulate sweating.^{37,38} The intent was to model these data via non-linear regression techniques to identify the EC₅₀ of the response. However, this modeling could not be performed because sweating to exogenous acetylcholine at the skin graft sites was absent, regardless of the dose or graft maturity.⁵ Grafted sites with observed sweating responses during whole-body heating responded to acetylcholine administration but with minimal sweating. These data suggest that impaired sweating responsiveness in grafted skin in the majority of graft patients is likely due to an absence of functional sweat glands secondary to the injury and subsequent excising of burned skin, which disrupt and/or remove sweat glands at the recipient tissue coupled with an absence of sweat glands in the split-thickness graft. It is unclear why a small number of subjects exhibited sweating during whole-body heating and acetylcholine administration and warrants further investigation.

Cutaneous vasoconstriction during whole-body and local cooling seems to be preserved in grafted skin regardless of duration postsurgery. Whole-body cooling causes an increase in cutaneous sympathetic vasoconstrictor nerve activity, which leads to the release of norepinephrine, along with vasoconstrictor cotransmitters, causing subsequent cutaneous vasoconstriction.^{39–44} Therefore, grafted skin must have functional adrenergic nerves, functional α -adrenergic receptors on the cutaneous vasculature, and normal smooth muscle responses to α -adrenergic receptor stimulation to vasoconstrict in response to indirect whole-body cooling. In addition to adrenergic mechanisms, local cooling induced vasoconstriction involves nonadrenergic mechanisms (nitric oxide pathways and sensory nerves).^{45–48} Collectively, the findings from whole-body and local cooling protocols are suggestive of selective reinnervation of the cutaneous vaso-constrictor system in grafted skin. However, careful inspection of the whole-body and local cooling data suggest potential differences could exist between grafted sites and noninjured healthy skin 4 to 8 years postsurgery despite the absence of statistical significance from the analysis of variance results, suggesting potential impairments in cutaneous vasoconstriction. Because of the cross-sectional design used in this study, variation between subjects likely contributed to the absence of statistical significance of these responses in this group. Further research using a longitudinal design, with the same subjects tested over time, may provide more insight into vasoconstrictor function and temperature regulation during hypothermic challenges in grafted skin.

Patients enrolled in this study had injuries that required grafting over less than 20% of their body surface area. The impact of impaired cutaneous vasodilation and sweating in grafts covering such a small area may be minimal with respect to whole-body thermoregulation as healthy skin should be able to compensate for the impaired thermoregulatory capacity of the grafted skin. However, based on the present findings, the risk of impaired thermoregulation may become greater in individuals with grafts covering a larger portion of the body surface area. Consistent with this thought, previous studies have reported adults with skin burns covering large portions of their body surface area may be at a higher risk for a heat-related injury (eg, heat exhaustion or stroke) when compared with unburned individuals.^{8,49} However, these observations are controversial as others have not observed impaired thermoregulatory responses in subjects with large body surface areas of burned or grafted skin.⁵⁰

CONCLUSION

In summary, split-thickness skin grafts have impaired cutaneous vasodilation and sweating responsiveness to indirect whole-body heating up to 4 to 8 years postsurgery. In addition, grafted skin has reduced maximal vasodilatory responsiveness to a local heating stimulus. Impairments in endothelial-dependent vasodilation may contribute to attenuated cutaneous vasodilation in grafted skin, whereas other factors yet to be identified (eg, possible disruption of reinnervation of cutaneous active vasodilator nerves) likely have a more prominent role.

The obtained data indicate that diminished sweating responses in grafted skin are due to an absence of functional sweat glands. Attenuated capabilities to dissipate heat via cutaneous vasodilation and sweating from grafted skin does not recover upward to 4 to 8 years after graft surgery, raising the possibility that individuals with a significant amount of body surface area of grafted skin are at an increased risk of a heat-related injury. Conversely, preserved vasoconstrictor responses to both indirect whole-body cooling and local cooling, regardless of graft maturity, suggest sustained capability to regulate internal temperature via cutaneous vasoconstriction during cold exposure.

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Figure 1.

Increases in cutaneous vascular conductance (ΔCVC) from normothermic baseline during indirect whole-body heating in grafted (graft) and adjacent noninjured (control) skin in groups 5 to 9 months postsurgery ($n = 13$), 2 to 3 years postsurgery ($n = 13$), and 4 to 8 years postsurgery ($n = 13$). Values are expressed as means $\pm$ SEM. Significant main effect for skin site demonstrates attenuated vasodilator responses to indirect whole-body heating at the grafted sites regardless of the duration post-surgery ($P < 0.001$).

Figure 2.

Increases in cutaneous vascular conductance (CVC) expressed in absolute units during local heating in grafted (graft) and adjacent noninjured (control) skin in groups 5 to 9 months postsurgery ($n = 13$), 2 to 3 years postsurgery ($n = 13$), and 4 to 8 years postsurgery ($n = 13$). Values are expressed as means $\pm$ SEM. Significant main effect for skin site demonstrates attenuated vasodilator responses to local heating at the grafted sites regardless of the duration postsurgery ($P < 0.001$).

Figure 3.

Changes in sweat rate (ΔSR) from normothermic baseline during whole-body heating from grafted (graft) and adjacent noninjured (control) skin in groups 5 to 9 months postsurgery ($n = 13$), 2 to 3 years postsurgery ($n = 13$), and 4 to 8 years postsurgery ($n = 13$). Values are expressed as means $\pm$ SEM. Significant main effect for skin site demonstrates attenuated sweating responses to whole-body heating at the grafted sites regardless of the duration postsurgery ($P < 0.001$).

Figure 4.

Maximal sweat rate (Δ SR) from baseline after administration of 1×10^{-1} M acetylcholine in grafted (graft) and adjacent noninjured (control) skin in groups 5 to 9 months postsurgery ($n = 12$), 2 to 3 years postsurgery ($n = 12$), and 4 to 8 years postsurgery ($n = 12$). Values are expressed as means $\pm$ SEM. Significant main effect for skin site demonstrates attenuated sweating responses to exogenous administration of acetylcholine at the grafted sites regardless of the duration postsurgery ($P < 0.001$).

Figure 5.

Decreases in cutaneous vascular conductance (ΔCVC) from normothermic baseline during whole-body cooling from grafted (graft) and adjacent noninjured (control) skin in groups 5 to 9 months postsurgery ($n = 13$), 2 to 3 years postsurgery ($n = 13$), and 4 to 8 years postsurgery ($n = 13$). Values are expressed as means $\pm$ SEM.

Figure 6.

Decreases in cutaneous vascular conductance (ΔCVC) during local cooling from a local skin temperature of 39 to 19 °C from grafted (graft) and adjacent noninjured (control) skin in groups 5 to 9 months postsurgery ($n = 13$), 2 to 3 years postsurgery ($n = 9$), and 4 to 8 years postsurgery ($n = 12$). Values are expressed as means $\pm$ SEM.

Table 1

Subject characteristics

	Group		
	5–9 mo	2–3 yr	4–8 yr
Male/female	7/6	11/2	7/6
Age (yr)	33.9 ± 9.8	37.3 ± 8.5	40.0 ± 9.5
Height (cm)	168.6 ± 9.7	180.3 ± 8.5	168.6 ± 12.0
Weight (kg)	84.0 ± 17.1	83.1 ± 14.6	83.5 ± 23.0

Values are expressed as means ± SD.

Table 2

Cutaneous vascular conductance (au/mm Hg) at normothermic baseline before indirect whole-body heating in grafted (graft) and adjacent noninjured (control) skin in groups 5 to 9 months postsurgery group (n = 13), 2 to 3 years postsurgery group (n = 13), and 4 to 8 years postsurgery group (n = 13)

Skin Site	Group			Statistical Analysis
	5–9 mo (n = 13)	2–3 yr (n = 13)	4–8 yr (n = 13)	
Control	0.31 ± 0.04	0.31 ± 0.06	0.30 ± 0.07	Skin site: $P = .64$
Graft	0.53 ± 0.09	0.31 ± 0.14	0.16 ± 0.03	Skin site × group: $P = .09$

Values are expressed as means ± SEM.

Table 3

Modeled maximum cutaneous vascular conductance responses (Δ CVC from baseline) and the effective concentration causing 50% of the maximum response (EC_{50}) generated from acetylcholine dose-response curves in grafted (graft) and adjacent noninjured (control) skin in groups 5 to 9 months postsurgery, 2 to 3 years postsurgery, and 4 to 8 years postsurgery

	Group			Statistical Analysis
	5–9 mo (n = 12)	2–3 yr (n = 12)	4–8 yr (n = 12)	
Maximum (au/mm Hg)				
Control	1.34 $\pm$ 0.15	1.46 $\pm$ 0.21	1.19 $\pm$ 0.19	Skin site: $P < .001$
Graft	0.61 $\pm$ 0.09	0.85 $\pm$ 0.11	0.69 $\pm$ 0.13	Skin site $\times$ group: $P = .74$
EC_{50} (log M drug dose)				
Control	–3.59 $\pm$ 0.19	–3.72 $\pm$ 0.17	–2.89 $\pm$ 0.22	Skin site: $P < .001$
Graft	–2.61 $\pm$ 0.15	–2.99 $\pm$ 0.19	–2.77 $\pm$ 0.24	Skin site $\times$ group: $P = .08$

CVC, cutaneous vascular conductance.

Values are expressed as means $\pm$ SEM.

Table 4

Modeled maximum cutaneous vascular conductance responses (Δ CVC from baseline) and the effective concentration causing 50% of the maximum response (EC_{50}) generated from sodium nitroprusside dose-response curves in grafted (graft) and adjacent noninjured (control) skin in groups 5 to 9 months postsurgery, 2 to 3 years postsurgery, and 4 to 8 years postsurgery

	Group			Statistical Analysis
	5–9 mo (n = 12)	2–3 yr (n = 11)	4–8 yr (n = 12)	
Maximum (au/mm Hg)				
Control	1.46 ± 0.12	1.78 ± 0.21	1.42 ± 0.18	Skin site: <i>P</i> = .17
Graft	0.99 ± 0.20	1.08 ± 0.11	0.81 ± 0.16	Skin site × group: <i>P</i> =.78
EC ₅₀ (log M drug dose)				
Control	−3.87± 0.19	−4.29 ± 0.17	−4.40 ± 0.18	Skin site: <i>P</i> =.65
Graft	−4.21 ± 0.27	−4.13 ± 0.20	−4.00 ± 0.24	Skin site × group: <i>P</i> =.21

CVC, cutaneous vascular conductance.

Values are expressed as means $\pm$ SEM.