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## TITLE PAGE

Local heating-induced cutaneous vasodilation in reinnervated and noninnervated deep inferior epigastric perforator flaps

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**Tables and figures**

- Tables: 4
- Figures: 4

1 **ABSTRACT (323 words)**

2 **Introduction** Cutaneous vascular reactivity to local heating in free flaps has not been  
3 characterized. We aimed to assess local heating-induced cutaneous vasodilation in  
4 reinnervated and noninnervated deep inferior epigastric perforator (DIEP) flaps.

5 **Methods** We conducted a cross-sectional study of 21 female patients with an  
6 uncomplicated unilateral delayed DIEP breast reconstruction at least two years after  
7 surgery. DIEP flaps and contralateral breasts were subjected to direct local heating, and  
8 skin blood flow was assessed using laser-Doppler flowmetry. To evaluate sensory-nerve-  
9 fiber function, touch perception thresholds were assessed using a 20-piece Touch-test™  
10 Sensory Evaluator, and cutaneous warm detection and heat pain thresholds were  
11 measured using a TSA-II device.

12 **Results** Of the participants, 10 had a reinnervated DIEP flap with a single coapted nerve  
13 (mean flap weight, 610 ± 296 g) and 11 had a noninnervated DIEP flap (mean flap  
14 weight, 613 ± 169 g). Mean age was 58 ± 11 years, mean follow-up time was 5 ± 1  
15 years, and mean BMI was 24 ± 3 kg/m<sup>2</sup>. DIEP flaps exhibited significantly weaker  
16 cutaneous vasodilation in response to local heating than contralateral breasts (median  
17 peak skin blood flow, 59 [25<sup>th</sup>-75<sup>th</sup> percentile, 36 – 71] a.u. for DIEP flaps *versus* 94 [74  
18 – 141] a.u. for contralateral breasts;  $p < 0.001$ ). The magnitude of the response was  
19 similar between reinnervated and noninnervated flaps (median peak skin blood flow, 55  
20 [25<sup>th</sup>-75<sup>th</sup> percentile, 39 – 68] a.u. for reinnervated DIEP flaps *versus* 66 [36 – 77] a.u.  
21 for noninnervated DIEP flaps;  $p = 0.75$ ). Of participants with reinnervated DIEP flaps,  
22 90% perceived heat pain below the 50°C safety threshold, as compared to 36% of  
23 participants with noninnervated DIEP flaps (two-tailed  $p = 0.02$ ).

24 **Conclusion** Our results suggest that free flap transfer causes longstanding impairment,  
25 yet not complete abolition, of both the sensory nerve-mediated and nitric oxide-  
26 dependent local heating-induced cutaneous vasodilatory systems. We found no statistical  
27 evidence that flap reinnervation improves the ability to raise skin blood flow in response  
28 to local heating.

29 **Keywords:** local heating; skin blood flow; vasodilation; breast reconstruction; nerve  
30 coaptation

31 **INTRODUCTION (358 words)**

32 The deep inferior epigastric perforator (DIEP) flap consists of skin and subcutaneous  
33 tissue harvested from the lower abdomen along with its blood supply. The DIEP flap is  
34 ideally suited for breast reconstruction after mastectomy, yielding high levels of patient-  
35 reported aesthetic outcomes.<sup>1,2</sup> Nevertheless, sensory outcomes are often poor due to  
36 nerve disruption with flap harvest. Patients mourn reduced or absent sensation in their  
37 reconstructed breast whilst dressing, taking a shower, and in intimate settings.<sup>3</sup>  
38 Protective sensation too is often lost, as is illustrated by reports of burns to breast  
39 reconstruction flaps induced by heat exposure (*e.g.*, sun, shower, and hot water bottles)  
40 up to five years after surgery.<sup>4-6</sup> These burns might ensue from lost ability to register  
41 heat, but sparing of adjacent skin indicate that the flap struggles to actively protect itself  
42 against heat, too.

43 Healthy human skin eliminates heat through vasodilation. Whole body passive heating  
44 elevates core temperature, which evokes an increase in skin blood flow mediated  
45 predominantly by sympathetic cholinergic nerves.<sup>7</sup> Klasson and colleagues observed  
46 normal cutaneous vasodilation in response to whole body heating in DIEP flaps at a  
47 median of 1.3 years after surgery.<sup>8</sup> Direct local heating of hairy skin elicits cutaneous  
48 vasodilation in the heated area only, a response largely dependent on sensory nerve  
49 endings and nitric oxide.<sup>9-11</sup> Whether DIEP flaps (or indeed any free flap) exhibit an  
50 adequate cutaneous vasodilator response to local heating has not been examined.

51 Surgical coaptation of a sensory nerve supplying the DIEP flap to a sensory nerve at the  
52 mastectomy site is thought to help restore sensory supply by guiding axonal  
53 regeneration, a premise supported by enhanced sensory detection ability observed in  
54 reinnervated flaps.<sup>12</sup> We hypothesized that as part of the cutaneous vasodilator response  
55 to local heating is sensory nerve-mediated, the response would be superior in  
56 reinnervated flaps, where neural continuity has been partially restored.

57 The study aims were to examine cutaneous vasodilation in response to local heating in  
58 DIEP flaps, and to evaluate whether flap reinnervation might enhance the response. We  
59 also explored which factors might explain variation in the magnitude of the response. We  
60 compared vasodilation results against sensory detection thresholds to infer cutaneous  
61 nerve fiber function.

## 62 **MATERIALS & METHODS (1138 words)**

### 63 **Design**

64 We performed a cross-sectional study. Study participants were recruited between April  
65 2021 and December 2021. Experiments were conducted in July 2021 and January 2022  
66 at Maastricht University Medical Center; Maastricht, the Netherlands.

### 67 **Participants**

68 The protocol was approved by the institutional ethics committee. Participants gave  
69 informed consent.<sup>13</sup> We included female patients with an uncomplicated unilateral  
70 delayed DIEP breast reconstruction at least two years after surgery to allow peripheral  
71 nerves within the flap time to regenerate.<sup>14</sup> Exclusion criteria were a history of cancer in  
72 the contralateral breast, absence of a DIEP skin island, and conditions known to impair  
73 peripheral nerve regeneration (*i.e.*, post-reconstruction radiotherapy<sup>15</sup>, active smoking<sup>16</sup>,  
74 and diabetes mellitus<sup>17</sup>). Peripheral neuropathy, prevalent in the study population, was  
75 not an exclusion criterium but a covariable to ensure a large enough pool of eligible  
76 patients.

### 77 **Surgical technique**

78 Unilateral, delayed DIEP flap breast reconstructions were performed according to the  
79 conventional technique.<sup>18</sup> DIEP flaps were reinnervated with a single coapted nerve upon  
80 patient request. A sensory branch of the 10th to 12th intercostal nerve was dissected as  
81 a donor nerve, and the anterior cutaneous branch of the second or third intercostal nerve  
82 as recipient nerve.<sup>19</sup> After completing vascular anastomoses and flap inset, direct end-to-  
83 end coaptation was performed using epineural sutures and fibrin glue. No nerve conduits  
84 or nerve grafts were used.

### 85 **Variables**

86 In healthy humans, local heating of hairy skin induces a biphasic response.<sup>10</sup> Our primary  
87 outcome was the rapid increase in skin blood flow within the first 3 to 5 minutes of local  
88 heating, a reaction that relies predominantly on local activity of sensory nerve endings.



89 Our secondary outcome was the slower vasodilation phase that attains a plateau in  
90 response to sustained local heating, a reaction mediated mainly by nitric oxide.<sup>9,10</sup>  
91 Additional outcomes were cutaneous mechanical detection, warm detection, and heat  
92 pain thresholds as surrogates of sensory nerve function. Owing to combinations of factors  
93 including patient preference, technical feasibility, and surgeon perseverance, not all DIEP  
94 flaps were reinnervated at the time of reconstruction. The independent variable of  
95 interest was measurement site (*i.e.*, contralateral breasts, reinnervated DIEP flaps, and  
96 noninnervated DIEP flaps). Observers were blinded to DIEP innervation status;  
97 participants were not. Data extracted from medical records comprised age, body mass  
98 index, menopausal status, history of smoking, peripheral neuropathy, hypertension,  
99 hypercholesterolemia, anti-cancer treatments, flap type, flap weight, ischemia time,  
100 follow-up time (*i.e.*, the interval between breast reconstruction and experiment), and  
101 contralateral breast symmetrization.

#### 102 **Local heating-induced cutaneous vasodilatation**

103 Skin blood flow in response to direct local heating was monitored using laser-Doppler  
104 flowmetry (PeriFlux System 6000; Perimed, Stockholm, Sweden). We tested participants'  
105 DIEP flaps and contralateral breasts (figure 1). One site per flap or breast was tested;  
106 scars were avoided. After 10 minutes of supine acclimatization in a quiet, constant-  
107 temperature room, data collection started with a one-minute normothermic baseline  
108 followed by 20 minutes of local heating at 42°C (457-PI probe; Perimed, Stockholm,  
109 Sweden). Skin temperature was measured before onset of local heating; control skin  
110 blood flow was continuously recorded 3 to 5 cm away from the heated area (440 probe;  
111 Perimed, Stockholm, Sweden). Blood flow recordings were analyzed in arbitrary units  
112 (a.u.). Before the protocol, core temperature was measured with an infrared ear  
113 thermometer (ThermoScan Pro 6000; Braun, Meluncheon, Germany). Systolic and  
114 diastolic blood pressures were measured using a Gamma XXL LF sphygmomanometer  
115 (Heine, Germany) directly before and after the protocol.

#### 116 **Cutaneous quantitative sensory testing**

117 Touch perception thresholds were assessed using a 20-piece Touch-test™ Sensory  
118 Evaluator (North Coast Medical, Inc., Gilroy, CA, USA). Monofilaments were applied three  
119 times in an ascending fashion.<sup>14,20</sup> An affirmative response was recorded when  
120 participants felt the stimulus at least one out of three times and could accurately locate  
121 it. Scores were converted into target stress (g/mm<sup>2</sup>). Cutaneous warm detection and  
122 heat pain thresholds were measured using a TSA-II device (Medoc, Ramat-Yishai, Israel)  
123 with a 9.0 cm<sup>2</sup> Peltier-type thermode. Participants were asked to abort a thermal  
124 stimulus increasing from 32°C to 50°C at a rate of 1°C/s when perceived. Warm  
125 detection and heat pain thresholds were both tested three times; for each series, the  
126 mean was recorded as the final value. If a warm detection or heat pain threshold was not  
127 reached within the safety limit, 50°C was entered for analysis.

### 128 **Nerve fiber integrity**

129 We histologically examined dermal and epidermal small sensory nerve fibers in a DIEP  
130 skin island excised as a secondary procedure. The tissue originated from a 39-year-old  
131 patient who had unilateral breast cancer. She was treated with neoadjuvant  
132 chemotherapy, trastuzumab, mastectomy, and tamoxifen. Sixteen months before skin  
133 island excision, she had an uncomplicated delayed bipediced DIEP breast reconstruction  
134 with dual nerve coaptation. The DIEP flap weighted 895 grams. The patient never  
135 smoked and was healthy apart from breast cancer. Mechanical detection, warm  
136 detection, and heat pain thresholds were obtained before surgery; skin blood flow in  
137 response to local heating could not be assessed because the laser-Doppler device was  
138 unavailable. Immediately after excision, the skin island was fixed in 4% buffered  
139 formaldehyde solution and stored at 4°C for 24 hours. After rinsing in PBS RNase free  
140 solution, the sample was immersed in 10% sucrose for 24 hours at 4°C. The next day,  
141 the tissue was immersed in isopentane for ~30-60 seconds and stored at -80°C. Sections  
142 30 µm thick were prepared using Leica CM3050 S Cryostat and stored at -80°C. After  
143 two hours of curing at room temperature, sections were saturated with 5% goat serum,  
144 2% BSA, and 0.1% Tween-20 for one hour. Sections were incubated overnight in a

145 humidified chamber at 4°C with primary rabbit antibody (1:100) against human protein  
146 gene product 9.5 (PGP9.5; ab27053), to identify dermal and epidermal neuronal  
147 structures. They were then washed with 1X-PBS and incubated for 45 minutes with goat  
148 anti-rabbit Alexa-546 (1:1000; A-11035) at room temperature in a dark chamber. After  
149 washing, sections were mounted with Fluoroshield™ with DAPI medium (F6057) to  
150 visualize cell nuclei, and examined using a Nikon Ti-E Fluorescence Motorized microscope  
151 under 40× magnification.

## 152 **Data analysis**

153 Participants and DIEP flaps were characterized using counts and percentages for  
154 categorical variables, means and standard deviations for normally distributed variables  
155 (Shapiro-Wilk  $p \geq 0.01$ ), and medians and 25<sup>th</sup> and 75<sup>th</sup> percentiles for other variables.  
156 The paired-samples Wilcoxon Signed Rank Test was used for comparisons between  
157 contralateral breasts and DIEP flaps. Normally distributed data were compared between  
158 reinnervated and noninnervated flaps using the independent samples t-test, and other  
159 data using the Mann-Whitney  $U$  test. Data were compared between contralateral breasts,  
160 reinnervated DIEP flaps, and noninnervated DIEP flaps using one-way analysis of  
161 variance for dependent samples followed by Tukey's multiple-comparison test. Factors  
162 that might explain variation in peak and plateau skin blood flow were identified using  
163 univariable linear regression analyses. Spearman correlation coefficients ( $r_s$ ) were used to  
164 estimate associations between variables. All statistical analyses were performed using R  
165 version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria), with  $\alpha = 0.05$   
166 unless otherwise stated.

167 **RESULTS (518 words)**

168 **Participants**

169 Of 661 patients who underwent a DIEP breast reconstruction between January 2010 and  
170 July 2019, 101 were eligible for inclusion, 51 of whom had a reinnervated flap. Twenty-  
171 one patients agreed to participate, 10 of whom had a reinnervated flap (mean flap  
172 weight, 610 ± 296 g) and 11 of whom had a noninnervated flap (mean flap weight, 613  
173 ± 169 g). Mean age was 58 ± 11 years, mean follow-up time was 5 ± 1 years, and mean  
174 BMI was 24 ± 3 kg/m<sup>2</sup>. No statistically significant differences in baseline characteristics  
175 between outcome groups were observed (table 1). All but one participant were  
176 postmenopausal; none took hormone replacement therapy. Four patients with  
177 reinnervated flaps had contralateral breast symmetrization (40%) as compared to two  
178 patients with noninnervated flaps (18%). Data completeness was 100%.

179 **Local heating-induced cutaneous vasodilation**

180 In both contralateral breasts and DIEP flaps, local heating to 42°C evoked a biphasic  
181 increase in skin blood flow, characterized by an initial peak followed by slower  
182 vasodilatation attaining a plateau (figure 2A-C). DIEP flaps exhibited weaker cutaneous  
183 vasodilation in response to local heating than contralateral breasts (median peak skin  
184 blood flow, 59 [25<sup>th</sup>–75<sup>th</sup> percentile, 36 – 71] a.u. for DIEP flaps *versus* 94 [74 – 141]  
185 a.u. for contralateral breasts;  $p < 0.001$ ; table 2). Areas under the curve were  
186 significantly greater in contralateral breasts than in DIEP flaps (figure 2D). Peak and  
187 plateau skin blood flow did not differ between reinnervated and noninnervated DIEP flaps  
188 (median peak skin blood flow, 55 [25<sup>th</sup>–75<sup>th</sup> percentile, 39 – 68] a.u. for reinnervated  
189 DIEP flaps *versus* 66 [36 – 77] a.u. for noninnervated DIEP flaps;  $p = 0.75$ ). Univariable  
190 linear regression analysis showed that chemotherapy was inversely associated with peak  
191 and plateau skin blood flow in both contralateral breasts and DIEP flaps (table 3).

192 **Cutaneous quantitative sensory testing**

193 Compared with contralateral breasts, cutaneous mechanical detection, warm detection,  
194 and heat pain thresholds were elevated in reinnervated DIEP flaps and further elevated in  
195 noninnervated DIEP flaps (figure 3). Significantly more participants with reinnervated  
196 DIEP flaps felt heat pain below the 50°C safety limit (table 4). As heat pain threshold and  
197 peak skin blood flow both rely on functional small unmyelinated heat-sensitive C-fibers<sup>21</sup>,  
198 we expected an inverse correlation between these variables; however, this was neither  
199 the case in contralateral breasts ( $r_s = 0.11$ , 95% CI = -0.38 – 0.55;  $p = 0.63$ ) nor in  
200 DIEP flaps ( $r_s = 0.17$ , 95% CI = -0.22 – 0.54;  $p = 0.46$ ). Mechanical detection threshold  
201 and peak skin blood flow were uncorrelated in contralateral breasts ( $r_s = 0.04$ , 95% CI =  
202 -0.41 – 0.49;  $p = 0.88$ ) and in DIEP flaps ( $r_s = 0.20$ , 95% CI = -0.27 – 0.60;  $p = 0.39$ ).

203 **Nerve fiber integrity**

204 The mechanical detection threshold of the examined reinnervated skin island was 68.2  
205 g/mm<sup>2</sup>; neither warmth nor heat were perceived below the 50°C safety limit. In healthy  
206 abdominal control skin of a 40-year-old woman, we observed small sensory nerve fibers  
207 mainly in the dermis, but also in the epidermis (figure 4A). Conversely, the reinnervated  
208 DIEP skin island showed a complete loss of dermal and epidermal small sensory nerve  
209 fibers (figure 4B).

210 **DISCUSSION (969 words)**

211 DIEP flaps three to seven years after surgery exhibited classic biphasic cutaneous  
212 vasodilation in response to local heating. However, peak and plateau skin blood flow in  
213 DIEP flaps were significantly lower than in contralateral breasts. These findings indicate  
214 that free flap transfer causes longstanding impairment, yet not complete abolition, of  
215 both the sensory nerve-mediated and nitric oxide-dependent local heating-induced  
216 cutaneous vasodilatory systems. This has not been demonstrated before.

217 In healthy human skin, small unmyelinated heat-sensitive C-fibers respond to local  
218 heating by releasing neurotransmitters that cause a rapid increase in skin blood flow.<sup>10,21</sup>  
219 Above a certain threshold, the same C-fibers transmit an action potential to the brain,  
220 resulting in conscious heat pain perception. As such, the observed attenuated peak skin  
221 blood flow in response to local heating (figure 2B-C) and elevated heat pain threshold  
222 (figure 3C) both provide evidence for C-fiber dysfunction in DIEP flaps. Elevated  
223 mechanical and warm detection thresholds (figure 3A and 3B) indicate additional  
224 dysfunction of large myelinated A $\beta$ -fibers and small thinly myelinated A $\delta$ -fibers,  
225 respectively.<sup>22</sup> These findings resonate with clinical manifestations of sensory dysfunction  
226 in DIEP flaps described in the introduction.

227 Nine participants (43%) had a history of small fiber neuropathy. This condition damages  
228 peripheral autonomic and somatic small sensory nerve fibers, and has been associated  
229 with diminished sensory nerve-mediated cutaneous vasodilation in response to local  
230 heating.<sup>23</sup> Absence of an inverse association between small fiber neuropathy and peak  
231 skin blood flow in our sample might be due to heterogeneous neuropathy etiology and  
232 duration.

233 Not only peak but also plateau skin blood flow in response to local heating was  
234 significantly weaker in DIEP flaps than in contralateral breasts. This latter phase is  
235 primarily (though not exclusively) mediated by nitric oxide, meaning that free flap  
236 transfer might alter nitric oxide production, vascular responsiveness to nitric oxide, or

237 both. Although still under debate, the plateau vasodilator phase probably requires intact  
238 sympathetic adrenergic function for its full expression.<sup>11</sup> The constrained plateau phase in  
239 DIEP flaps could therefore be a symptom of cutaneous sympathetic denervation.

240 Prevailing theory indeed attributes pathological burns in breast reconstruction flaps to  
241 sympathetic dysfunction.<sup>4-6</sup> Contrary to this theory, Klasson and colleagues found normal  
242 cutaneous sympathetic nerve-mediated vasodilation in response to whole body heating in  
243 DIEP flaps.<sup>8</sup> Also, our experiments were conducted at unheated skin temperatures where  
244 adrenergic vasoconstrictor activity maintains resting cutaneous vascular tone (~32°C).  
245 Unaltered baseline skin blood flow in DIEP flaps (table 2) refutes attenuated  
246 vasoconstrictor tone and, by extension, sympathetic denervation.<sup>10,24</sup> Which exact  
247 mechanisms impair the cutaneous plateau vasodilator response to local heating in DIEP  
248 flaps could be clarified by studying effects of pharmacologic inhibitors.

249 Significantly more participants with reinnervated DIEP flaps were able to feel heat pain  
250 below the 50°C safety limit than participants with noninnervated flaps (90% *versus* 36%;  
251 table 4). The fact that the histologically examined tissue showed no signs of C-fiber  
252 regeneration (*i.e.*, no small sensory nerve fibers identified upon histological examination  
253 [figure 4B] and no heat pain perception below 50°C) despite dual nerve coaptation might  
254 lie with the relatively short follow-up time of 1.3 years.

255 Various confounding factors may have influenced the magnitude of the vasodilator  
256 response to local heating. Exploratory analyses showed an inverse association between  
257 chemotherapy and peak and plateau skin blood flow in contralateral breasts and DIEP  
258 flaps (table 3). This finding corresponds to impaired nitric oxide-related cutaneous  
259 vasodilation in response to acetylcholine iontophoresis reported in patients treated with  
260 various chemotherapy regimens.<sup>25,26</sup> Because of the protective effect of endogenous  
261 estrogen on endothelial function<sup>27</sup>, menopausal status, hormone replacement therapy,  
262 and endocrine anticancer therapy are potentially relevant parameters. The study sample  
263 showed little to no variation regarding menopausal status and hormone replacement  
264 therapy. However, 11 participants (52%) received endocrine therapy, which, depending

265 on the mechanism of action, may either improve or reduce endothelial function.  
266 Tamoxifen and related compounds competitively antagonize estrogen receptors and can  
267 increase serum estrogen levels in postmenopausal women<sup>28</sup>, with potential protective  
268 effects on endothelial function.<sup>29,30</sup> By contrast, aromatase inhibitors inhibit estrogen  
269 production, reducing levels of endogenous estrogen and impairing endothelial  
270 function.<sup>31,32</sup> In our study, chemotherapy likely confounded estimates of association  
271 between tamoxifen and the magnitude of the vasodilator response. Lastly, advanced age  
272 has been associated with significantly reduced peak and plateau skin blood flow in  
273 response to local heating.<sup>33,34</sup> Absence of an inverse association between age and  
274 microvascular function in this study might be attributable to the participants' narrow  
275 range of age.

276 Several study limitations should be considered. Contralateral breasts are flawed controls  
277 of abdomen-derived DIEP flaps. Although similar innervation densities have been  
278 reported for female breast and abdominal skin<sup>35</sup>, there could be important functional and  
279 structural differences. For instance, a prior study reported significantly more pronounced  
280 nitric oxide-dependent cutaneous vasodilation in response to local heating in the  
281 abdomen than in the chest area.<sup>36</sup> Six of our study participants underwent surgical  
282 procedures on their contralateral breast, which may have affected cutaneous nerve  
283 integrity and microvascular function. For these reasons, we were unable to eliminate  
284 potential patient-level confounding by expressing values as ratio to the contralateral  
285 breast. Another study limitation is that blood flow recordings were not normalized to  
286 maximal or baseline skin blood flow. Maximal values were not obtained, and baseline skin  
287 blood flow was variable meaning that correction would have distorted rather than  
288 rectified raw flux data. As site-to-site variability is perhaps the most important drawback  
289 of the laser-Doppler technique<sup>37</sup>, skin blood flow results should be interpreted with  
290 caution and warrant follow-up study with adequate normalization.

291 The evidence derived from this study is too premature for informing clinical  
292 recommendations regarding the effects of flap reinnervation on vasodilatory function.



293 The fact that we found no evidence for a better vasodilatory response to local heating in  
294 reinnervated flaps does not mean that no such relationship exists. Further research is  
295 needed to verify and build on our research findings before they can have wider  
296 implications for clinical practice.

297 **CONCLUSION (87 words)**

298 This study suggests that long-term consequences of nerve disruption inherent to free flap  
299 transfer extends beyond loss of sensory detection ability: we found significant  
300 impairment of both the sensory nerve-mediated and nitric oxide-dependent local heating-  
301 induced cutaneous vasodilatory systems that protect the skin from trauma. We found no  
302 clear evidence that flap reinnervation enhances the skin's ability to mount an adequate  
303 vasodilatory response to local heating. Nonetheless, significantly lower sensory  
304 thresholds in reinnervated DIEP flaps suggest that reinnervation may provide a  
305 meaningful degree of sensory nerve regeneration.

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## TABLES

**Table 1.** Participant characteristics, by outcome group

|  | Reinnervated<br>DIEP flaps | Noninnervated<br>DIEP flaps |
|--|----------------------------|-----------------------------|
| No. of participants  | 10                         | 11                          |
| Mean age at time of experiment, yr   | 55 ± 9                     | 60 ± 12                     |
| Mean body mass index, kg/m <sup>2</sup>  | 24 ± 2                     | 25 ± 4                      |
| Menopausal status, no. (%)   |                            |                             |
| Premenopausal  | 0                          | 1 (9)                       |
| Postmenopausal   | 10 (100)                   | 11 (91)                     |
| Ex-smoker, no. (%)   |                            |                             |
| No   | 5 (50)                     | 5 (45)                      |
| Yes  | 5 (50)                     | 6 (55)                      |
| Small fiber neuropathy   |                            |                             |
| No, no. (%)  | 7 (70)                     | 5 (45)                      |
| Yes, no. (%) <sup>a</sup>  | 3 (30)                     | 6 (55)                      |
| Time since symptom onset, median (25 <sup>th</sup> –75 <sup>th</sup> percentile), yr | 6 (5 – 7)                  | 27 (18 – 35)                |
| Hypertension, no. (%)  |                            |                             |
| No   | 8 (80)                     | 8 (73)                      |
| Yes  | 2 (20)                     | 3 (27)                      |
| Hypercholesterolemia, no. (%)  |                            |                             |
| No   | 10 (100)                   | 11 (91)                     |
| Yes  | 0                          | 1 (9)                       |
| Indication for mastectomy, no. (%)   |                            |                             |
| Invasive breast cancer   | 10 (100)                   | 9 (82)                      |
| Ductal carcinoma in situ   | 0                          | 2 (18)                      |
| Chemotherapy, no. (%)  |                            |                             |
| No   | 2 (20)                     | 4 (36)                      |
| Yes <sup>b</sup>   | 8 (80)                     | 7 (64)                      |
| Trastuzumab, no. (%)   |                            |                             |
| No   | 8 (80)                     | 10 (91)                     |
| Yes  | 2 (20)                     | 1 (9)                       |
| Endocrine therapy, no. (%)   |                            |                             |
| None   | 3 (30)                     | 7 (64)                      |
| Tamoxifen  | 6 (60)                     | 3 (27)                      |
| Anastrozole  | 1 (10)                     | 1 (9)                       |
| Pre-reconstruction radiotherapy, no. (%)   |                            |                             |
| No   | 6 (60)                     | 8 (73)                      |
| Yes  | 4 (40)                     | 3 (27)                      |
| Flap type, no. (%)   |                            |                             |
| Unipedicled DIEP flap  | 5 (50)                     | 8 (73)                      |
| Bipedicled or stacked DIEP flap  | 5 (50)                     | 3 (27)                      |
| Recipient nerve used for coaptation  |                            |                             |
| Anterior cutaneous branch of the second intercostal nerve                            | 4 (40)                     | -                           |
| Anterior cutaneous branch of the third intercostal nerve                             | 6 (60)                     | -                           |
| Mean flap weight, g <sup>c</sup>   | 610 ± 296                  | 613 ± 169                   |
| Mean ischemia time, min  | 43 ± 8                     | 47 ± 13                     |
| Mean follow-up time, yr  | 5 ± 1                      | 5 ± 1                       |
| Contralateral breast symmetrization, no. (%)   |                            |                             |
| No   | 6 (60)                     | 9 (82)                      |
| Yes <sup>d</sup>   | 4 (40)                     | 2 (18)                      |
| Mean core temperature before experiment, °C  | 36.6 ± 0.2                 | 36.6 ± 0.2                  |
| Mean systolic blood pressure, mmHg   |                            |                             |
| Before experiment  | 138 ± 25                   | 131 ± 17                    |
| After experiment   | 143 ± 26                   | 137 ± 20                    |
| Mean diastolic blood pressure, mmHg  |                            |                             |
| Before experiment  | 92 ± 17                    | 89 ± 12                     |
| After experiment   | 94 ± 21                    | 93 ± 12                     |

Note: Plus–minus values are means  $\pm$  SD.

Abbreviation: DIEP, deep inferior epigastric perforator.

<sup>a</sup> Related to chemotherapy ( $n = 5$ ), sarcoidosis, Crohn's disease, Lyme disease, or vitamin B6 intoxication.

<sup>b</sup> *I.e.*, doxorubicin, cyclophosphamide, paclitaxel, and docetaxel, in various combinations.

<sup>c</sup> For breast reconstructions involving two stacked flaps, only the heaviest flap was considered.

<sup>d</sup> *I.e.*, breast reduction ( $n = 5$ ) and breast augmentation ( $n = 1$ ).

**Table 2.** Skin blood flow in response to local heating

|   | <b>Contralateral<br/>breasts</b> | <b>Reinnervated<br/>DIEP flaps</b> | <b>Noninnervated<br/>DIEP flaps</b> | <b><i>p</i></b> |
|---|----------------------------------|------------------------------------|-------------------------------------|-----------------|
| No. of breasts/flaps  | 21                               | 10                                 | 11                                  |                 |
| Mean skin temperature, °C   | 31.7 ± 1.4                       | 32.0 ± 0.8                         | 32.0 ± 1.0                          | 0.66            |
| Median baseline skin blood flow, (25 <sup>th</sup> -75 <sup>th</sup> percentile), a.u.                      | 23 (15 - 38)                     | 20 (13 - 35)                       | 22 (14 - 26)                        | 0.41            |
| Mean onset of peak skin blood flow, min   | 2.8 ± 0.7                        | 2.8 ± 0.5                          | 3.0 ± 0.5                           | 0.88            |
| Median peak skin blood flow, (25 <sup>th</sup> -75 <sup>th</sup> percentile), a.u.                          | 94 (74 - 141)                    | 55 (39 - 68)                       | 66 (36 - 77)                        | 0.002           |
| Median plateau skin blood flow, (25 <sup>th</sup> -75 <sup>th</sup> percentile), a.u.                       | 145 (112 - 234)                  | 71 (63 - 101)                      | 86 (57 - 142)                       | 0.004           |
| Median area under the curve, (25 <sup>th</sup> -75 <sup>th</sup> percentile), (x10 <sup>3</sup> a.u. x min) | 126 (109 - 213)                  | 82 (51 - 120)                      | 81 (54 - 103)                       | 0.003           |

Note: Plus-minus values are means ± SD. Baseline skin blood flow was calculated as average skin blood flow over a one-minute normothermic period. Abbreviations: DIEP, deep inferior epigastric perforator; a.u., arbitrary units.

**Table 3.** Univariable linear regression analysis of factors associated with skin blood flow in response to local heating

|                                     | Contralateral breasts |         |          |         |          |          | DIEP flaps |         |          |         |         |          |
|-------------------------------------|-----------------------|---------|----------|---------|----------|----------|------------|---------|----------|---------|---------|----------|
|                                     | Peak                  |         |          | Plateau |          |          | Peak       |         |          | Plateau |         |          |
|                                     | $\beta$               | 95% CI  | <i>p</i> | $\beta$ | 95% CI   | <i>p</i> | $\beta$    | 95% CI  | <i>p</i> | $\beta$ | 95% CI  | <i>p</i> |
| Univariable analysis                |                       |         |          |         |          |          |            |         |          |         |         |          |
| Flap reinnervation                  |                       |         |          |         |          |          | 4          | -26, 35 | 0.77     | -9      | -56, 39 | 0.71     |
| Follow-up time, yr                  |                       |         |          |         |          |          | 1          | -12, 13 | 0.94     | 5       | 15, 24  | 0.62     |
| Chemotherapy                        | -40                   | -38, 3  | 0.07     | -32     | -114, 50 | 0.42     | -14        | -47, 20 | 0.40     | -45     | -93, 3  | 0.06     |
| Tamoxifen                           | -37                   | -77, 2  | 0.06     | -13     | -89, 62  | 0.71     | -5         | -36, 26 | 0.74     | -23     | -70, 23 | 0.30     |
| Hypertension                        | 21                    | -28, 71 | 0.37     | -13     | -101, 75 | 0.76     | -3         | -40, 33 | 0.85     | 8       | -47, 64 | 0.76     |
| Ex-smoker                           | -13                   | -56, 29 | 0.52     | -26     | -100, 48 | 0.48     | -2         | -33, 29 | 0.90     | 6       | -41, 54 | 0.78     |
| Small fiber neuropathy              | 5                     | -38, 48 | 0.81     | 32      | -42, 106 | 0.38     | 7          | -24, 38 | 0.63     | -1      | -49, 46 | 0.95     |
| Age at time of experiment, yr       | 2                     | -0, 4   | 0.07     | -0      | -4, 3    | 0.92     | 0          | -1, 2   | 0.54     | 1       | -1, 3   | 0.44     |
| Contralateral breast symmetrization | -33                   | -78, 11 | 0.14     | -26     | -108, 57 | 0.52     |            |         |          |         |         |          |

Abbreviations : DIEP, deep inferior epigastric perforator; CI, confidence interval.



**Table 4.** Warm detection and heat pain thresholds, by outcome group

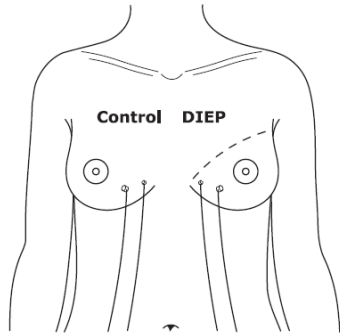
|                     | <b>Reinnervated<br/>DIEP flaps</b> | <b>Noninnervated<br/>DIEP flaps</b> |
|---------------------|------------------------------------|-------------------------------------|
| No. of participants | 10                                 | 11                                  |
| WDT, no. (%)        |                                    |                                     |
| ≤ 50°C              | 6 (60)                             | 3 (27)                              |
| > 50°C              | 4 (40)                             | 8 (73)                              |
| HPT, no. (%)*       |                                    |                                     |
| ≤ 50°C              | 9 (90)                             | 4 (36)                              |
| > 50°C              | 1 (10)                             | 7 (64)                              |

Abbreviations: DIEP, deep inferior epigastric perforator; WDT, warm detection threshold; HPT, heat pain threshold.

\*  $p < 0.05$ , by Fisher's exact test.

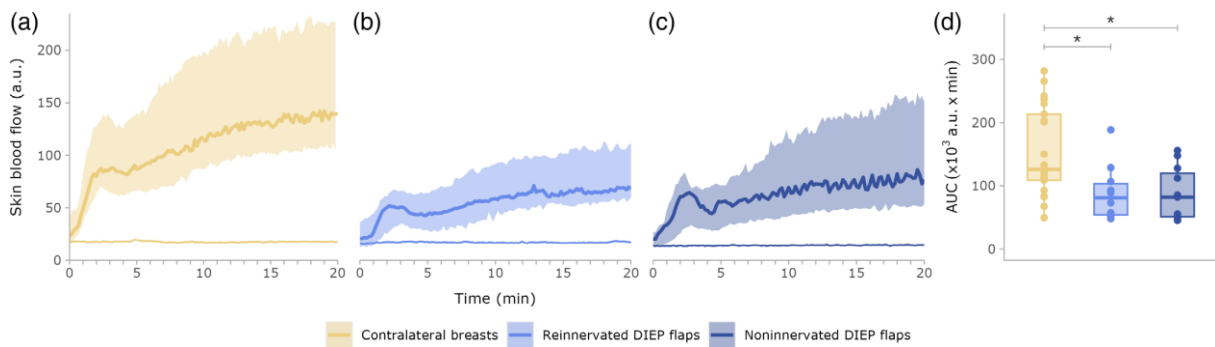
## FIGURE LEGENDS

**Figure 1.** Experimental setup for local heating-induced cutaneous vasodilatation



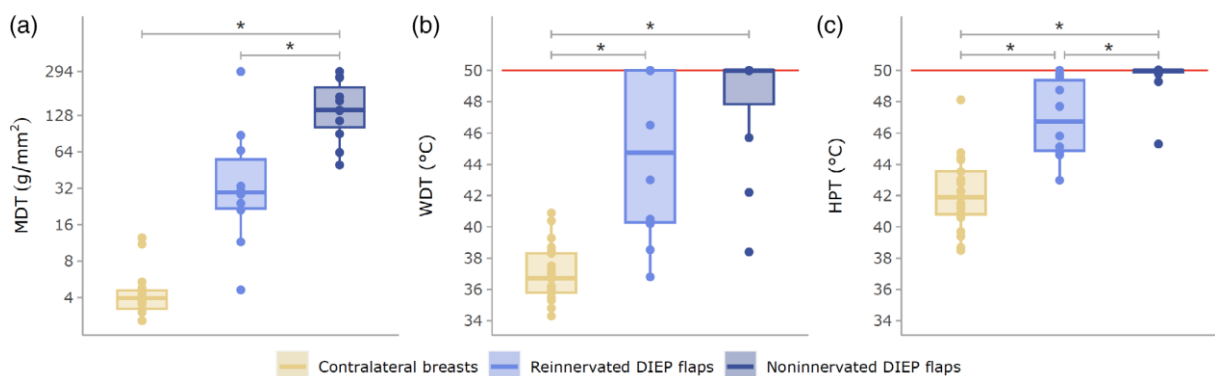
Skin blood flow was monitored using laser-Doppler flowmetry (PeriFlux System 6000; Perimed, Stockholm, Sweden). Medially on both the DIEP flap and contralateral breast, we simultaneously measured skin blood flow in response to local heating at 42°C using a 457-PI probe (Perimed, Stockholm, Sweden), and control skin blood flow using a 440 probe (Perimed, Stockholm, Sweden).

**Figure 2.** (A-C) Skin blood flow in response to local heating, (D) area under the curve



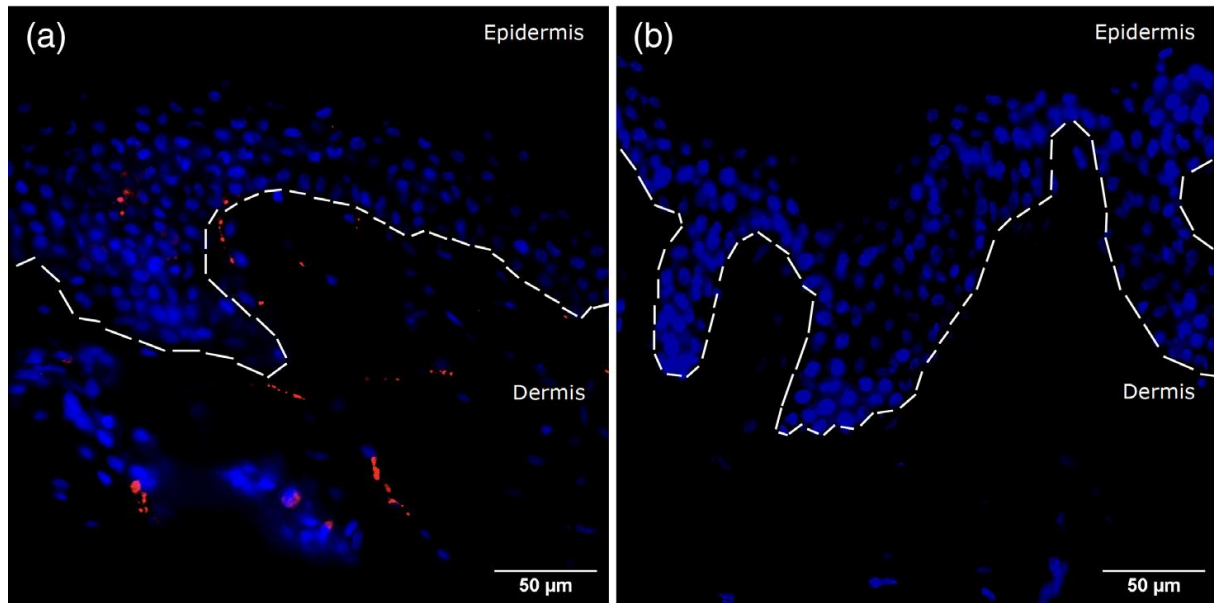
(A-C) Thick colored lines represent median skin blood flow in response to local heating for 21 contralateral breasts (A), 10 reinnervated deep inferior epigastric perforator (DIEP) flaps (B), and 11 noninnervated DIEP flaps (C). Shaded areas represent interquartile spans. Thin colored lines represent mean control skin blood flow. (D) Horizontal lines inside the boxes represent median area under the curve (AUC). Shaded areas represent interquartile spans. a.u., arbitrary units. \*  $p < 0.05$ .

**Figure 3.** (A) Mechanical detection, (B) warm detection, (C) and heat pain thresholds



Horizontal lines inside the boxes represent median mechanical detection thresholds (MDT; *A*), warm detection thresholds (WDT; *B*), and heat pain thresholds (HPT; *C*) for 21 contralateral breasts, 10 reinnervated deep inferior epigastric perforator (DIEP) flaps, and 11 noninnervated DIEP flaps. Shaded areas represent interquartile spans. Red lines represent the 50°C safety limit. \*  $p < 0.05$ .

**Figure 4.** Structural integrity of dermal and epidermal small sensory nerve fibers (*A*) in abdominal control skin, but not (*B*) in a reinnervated DIEP skin island



Dermal/epidermal small sensory nerves (PGP9.5; red) and cell nuclei (DAPI; blue) under 40× magnification. The white dashed lines indicate the dermal-epidermal junction.