Impaired transient vasodilation and increased vasoconstriction to digital local cooling in primary Raynaud’s phenomenon

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Roustit M, Blaise S, Millet C, Cracowski JL. Impaired transient vasodilation and increased vasoconstriction to digital local cooling in primary Raynaud’s phenomenon. Am J Physiol Heart Circ Physiol 301: H324–H330, 2011.—Raynaud’s phenomenon (RP) is defined as episodic ischemia of the extremities in response to cold. Although the structure of skin capillaries is normal in primary RP, some data suggest impairment of microvascular function. We aimed at testing whether digital skin blood flow was lower in RP than in controls while cooling locally. We further evaluated the contribution of sensory nerves in the response. We recruited 21 patients with primary RP and 20 healthy volunteers matched on age and gender. After a 10-min baseline at 33°C, skin temperature was cooled at 15 or 24°C during 30 min on the forearm and the finger while monitoring perfusion with a custom-design laser Doppler flowmetry probe. Perfusion was also assessed after topical anesthesia. Blood flow was expressed as cutaneous vascular conductance (CVC). Data were subsequently expressed as area above the curve (AAC0–30) of the percentage decrease from baseline CVC (%BL). CVC on the dorsum of the finger was lower in RP patients compared with controls at 15°C (AAC0–30 were 106,237.2 and 69,544.3%BL·s, respectively; \( P = 0.02 \)) and at 24°C (AAC0–30 were 86,915 and 57,598%BL·s, respectively; \( P = 0.04 \)) whereas we observed no significant difference on the finger pad and the forearm. Topical anesthesia increased CVC in patients with RP (\( P = 0.05 \)), whereas it did not affect reactivity in controls (\( P = 0.86 \)). Our study shows exaggerated skin microvascular vasoconstriction to local cooling on the dorsum of the finger in primary RP compared with controls. Part of this abnormal response in primary RP depends on sensitive nerves.

Several features of primary RP pathophysiology that could explain decreased vasodilation have been suggested, including a loss of calcitonin gene-related peptide (CGRP) containing nerve fibers in the digits (5) or increased endothelin-1-dependent vasoconstriction in RP (15). Another mechanism could involve postjunctional \( \alpha_{2C} \)-adrenoreceptors, which are clustered distally, whereby increasing translocation of \( \alpha_{2C} \)-adrenoreceptors from cytosol to cell surface occurs during cooling, thus enhancing contraction (7). In vitro, Furspan et al. (13) showed increased cooling-induced \( \alpha_{2C} \)-adrenorenergic constriction of arterioles isolated from patients with primary RP compared with controls, suppressed by protein tyrosine kinase inhibitors. The role of the RhoA/Rho kinase (ROCK) pathway in mediating vasoconstriction after local cooling in healthy subjects has been confirmed in vivo (31). Indeed, cold-induced activation of ROCK may initiate constriction, mediated by both \( \alpha_{2C} \)-adrenoreceptors (through their mobilization from the Golgi apparatus to the vascular smooth muscle plasma membrane) and direct calcium sensitization (10). Therefore, an impaired signal transduction pathway could be involved in the pathophysiology of RP (10), but this remains to be confirmed.

Local cooling of the skin induces an initial vasoconstriction followed by a transient vasoconstriction and, finally, a prolonged vasoconstriction (18). The initial vasoconstriction would be mainly dependent on norepinephrine and mediated by the ROCK pathway (by translocating \( \alpha_{2C} \)-adrenoreceptors; Ref. 31), whereas the prolonged vasoconstriction probably involves both the ROCK and the nitric oxide (NO) pathways (18). On the other hand, sensory nerves could play a role in the transient vasoconstriction, which is less well understood (18). Such transient vasoconstriction is more obvious when the cooling is rapid (35).

Assessment of the microvascular response to cooling in RP is of interest so as to better understand its pathophysiology. However, it is challenging as most tests (i.e., cold pressure test, systemic cooling) induce a tremendous increase in the sympathetic response, overshadowing the local reactivity. Therefore, to study microvascular reactivity due to local cooling in RP, we developed a local cooling test (26) using a cooling laser Doppler flowmetry (LDF) probe inspired by the device made by Johnson et al. (19). This test was reproducible, well tolerated, and did not induce distant sympathetic activation of the skin of healthy subjects (26).

We hypothesized that skin microvascular response to local cooling would be exaggerated in patients with primary RP compared with controls. The primary objective of this study was therefore to compare cutaneous vascular conductance (CVC) in patients with primary RP and matched controls by using a 30-min local cooling test on the dorsum of the finger and the finger pad. In addition, we aimed at assessing which phase would be affected in primary RP (i.e., increased initial...
vasoconstriction, impaired transient vasodilation, and/or exaggerated prolonged vasoconstriction). As transient vasodilation is mediated through sensory nerves, we also compared conductance after local anesthesia. Finally, we tested the safety of our device in patients with primary RP.

METHODS

Study population. All the participants enrolled in this study were recruited through local newspaper advertisements and included between February 2009 and February 2010. All subjects were 18 yr of age or older. Patients with primary RP were diagnosed according to the criteria of LeRoy and Medsger (20). Subjects taking calcium-channel blockers were instructed to stop medication 1 wk before enrolment in the study.

Noninclusion criteria included cigarette smoking and any associated chronic disease. Additional noninclusion criteria for patients with RP included abnormal capillaroscopic pattern (8). Antinuclear autoantibodies were assessed for all participants. In case of positive antinuclear autoantibodies (>80 U/mL), specific autoantibodies against topoisomerase I (Scl–70) or centromere-associated proteins were sought. Positive autoantibodies against topoisomerase I (Scl–70) or centromere-associated proteins were a noninclusion criterion.

The investigation conforms with the principles outlined in the Declaration of Helsinki. Grenoble Institutional Review Board approval was obtained on August 2, 2008, and each subject gave written informed consent before participation.

Study design. All subjects were fasted and all experiments were performed in a temperature-controlled room (24 ± 1°C). After clinical examination, the participants remained supine for the whole duration of the experiments, with forearms resting at heart level. Blood pressure was recorded continuously by using digital photoplethysmography (Nexfin monitor; Bmye B.V., Amsterdam, The Netherlands) during skin blood flow measurements. Before recording started, the arm was immobilized with a vacuum cushion to ensure stable positioning (Fig. 1).

Five skin sites were randomly chosen on the fingers between the index, the middle, and the ring finger. Among them, two sites were chosen on the finger pad (sites 1 and 2, on 2 distinct fingers of the left hand), and three on the dorsum of the finger [middle phalanx, 1 on the left hand (site 3) and two on distinct fingers of the right hand (sites 4 and 5)]. For patients with primary RP, all sites were chosen on fingers affected by RP. Maricq color charts (22) were used to confirm the diagnosis of RP among the index, the middle, and the ring finger and to specify its topography. When the three fingers were equally affected by RP, two of them were randomly chosen. Another site (site 6) was selected on the ventral side of the left upper forearm, more than 5 cm from the elbow and the prominence of the wrist, avoiding visible veins. Local cooling started after a 30-min resting period for acclimation.

Local cooling and skin blood flow measurements. Cutaneous blood flow was assessed by LDF (Periflux System 5000; Perimed, Järfålla, Sweden) by using custom-designed LDF cooling probes (Probe 413–317; Perimed; Ref. 26). Risk analysis was performed and was fully compatible with human use.

Baseline skin temperature was maintained at 33°C, and blood flow was recorded over 10 min. We standardized baseline skin temperature because we had previously observed a tendency to lower baseline skin blood flow in patients with primary RP compared with healthy controls (28, 30). Afterwards, local cooling tests were sequentially performed as follows: sequence 1: local temperature was decreased to 15°C (sites 1 and 6); sequence 2: cooling at 24°C (sites 2 and 3); and sequence 3: cooling at 15°C (sites 4 and 5). This design allowed study of microvascular reactivity at both 15 and 24°C on the finger pad and on the dorsum of the finger. On the forearm, the temperature was decreased to 15°C only. All the tests were performed on distinct skin sites, and the reproducibility of the test has been previously demonstrated (26).

In sequence 3, local cooling was performed after topical anesthesia (site 5) to assess whether local digital neurovascular control was affected in patients with primary RP. Before the local cooling was started, 2 g of lidocaine/prilocaine cream (5-g tubes containing 125 mg lidocaine and 125 mg prilocaine; Anesderm, Pierre Fabre, Boulogne, France) was placed on site 5 over a 1-cm² skin surface. Subsequently, an occlusive transparent dressing was placed over the cream to enhance cutaneous diffusion. No cream was placed on the other sites. After 1 h, lidocaine/prilocaine cream was removed with a cotton swab and the cooling probe was positioned on site 5. The anesthetized skin area was larger than the size of the local cooling probe.

Data analysis. Data were digitized, stored on a computer, and analyzed off-line with signal processing software (Perisoft 2.5.5; Perimed). Skin blood flow was expressed as CVC (flux in millivolts divided by mean arterial pressure). Expressing data as conductance is more of a physiological approach, as it takes into account differences and variations in blood pressure (24). To take into account baseline (BL) flux variations, data were subsequently expressed as a percentage decrease from baseline CVC, as previously described (19). Baseline CVC was averaged over 5 min just before cooling onset. Then, a minute-by-minute analysis of CVC was performed to assess the kinetics of the response (CVC was averaged over 20 s, providing 3 points/min), and data were expressed as area above the curve over the 30-min cooling period (AACC_{0–30}, in %BL/s) as the primary outcome. We subsequently analyzed the three phases of the response: 1) initial vasoconstriction: CVC was averaged over 1 min around the lowest flux value within the first 3 min; 2) transient vasodilation: CVC was averaged over 1 min around the highest flux value within the first 15 min; and 3) late prolonged vasoconstriction: CVC was averaged over the last 3 min of the measurement.

Statistical analysis. Quantitative data are expressed as the mean (SD). Qualitative data are expressed as number and percentage. Repeated-measures ANOVA were used to compare evolution of CVC over time between the two groups. Mauchly’s test of sphericity was used to assess equality of variance in the data. When significant (i.e., inequality of variance cannot be excluded), Greenhouse-Geisser adjustment was used. We tested the effect of the phase, of the group, as well as the interaction between phase and group. To compare the difference in phases between the two groups, we performed a post hoc analysis of covariance as previously described (33). Paired t-tests

Fig. 1. Single-point laser Doppler probes (A) with a liquid cooling system made of silicon tubes (B). C: probes were fixed with double-sided tape. D: a vacuum cushion was used to decrease artifacts associated with arm movements.
Two-sided significance tests were used throughout. *P* values of <0.05 were considered significant.

**RESULTS**

**Population characteristics.** The demographic and clinical characteristics of the 41 participants enrolled in the study are listed in Table 1. One of the participants initially included in the control group was diagnosed with primary RP and was therefore switched to the other group, thus explaining the difference in sample size between the two groups. Mean serum creatinine was 70.4 (10.3) μmol/l, glycemia was 4.5 (0.8) mmol/l, and total cholesterol was 1.9 (0.4) g/l. There was no significant difference between the groups. Three participants (2 patients with primary RP and 1 control) had positive antinuclear antibodies. None of them had positive autoantibodies against topoisomerase I (Scl-70) or centromere-associated proteins; clinical examination and anamnesis did not argue for a connective-tissue disease. The nailfold videocapillaroscopy pattern was normal in all subjects.

Seven women were taking oral contraceptives (3 with primary RP and 4 in the control group). Women were matched in terms of hormonal status (menopause and hormonal phase) between controls and patients with primary RP.

**Baseline CVC.** Before cooling at 15°C, CVC on the finger pad of participants with primary RP and controls was 13.6 (9.9) and 10.86 (8.2) mV/mmHg, respectively (*P* = 0.36). On the dorsum of the finger, baseline CVC was 4.39 (3.6) in primary RP patients compared with 3.06 (1.51) mV/mmHg in controls (*P* = 0.14). On the forearm, baseline CVC at 33°C was 0.95 (0.5) in participants with primary RP and 0.64 (0.3) mV/mmHg in controls (*P* = 0.03).

Baseline CVC on the finger pad before cooling at 24°C was 11.8 (10.4) mV/mmHg in participants with primary RP and controls was 10.1 (7.2) mV/mmHg in controls (*P* = 0.55). On the dorsum of the finger, it was 3.52 (2.9) mV/mmHg in primary RP patients compared with 1.96 (1.9) mV/mmHg in controls (*P* = 0.07).

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**Table 1. Demographic and clinical characteristics of patients with primary RP and controls**

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 20)</th>
<th>Primary RP (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>42.8 (16.2)</td>
<td>43 (18.5)</td>
</tr>
<tr>
<td>Female</td>
<td>15 (75)</td>
<td>16 (76)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>22.6 (3.4)</td>
<td>21.1 (2.3)</td>
</tr>
<tr>
<td>Blood pressure (MAP), mmHg</td>
<td>94.3 (10.2)</td>
<td>98.3 (20.5)</td>
</tr>
<tr>
<td>RP: duration, yr</td>
<td>NA</td>
<td>14.2 (11.3)</td>
</tr>
<tr>
<td>RP: number of fingers involved</td>
<td>NA</td>
<td>7.9 (1.6)</td>
</tr>
<tr>
<td>RP: thumb involved</td>
<td>NA</td>
<td>5 (24)</td>
</tr>
<tr>
<td>RP: other locations (feet, ears, or nose)</td>
<td>NA</td>
<td>3 (14)</td>
</tr>
</tbody>
</table>

Quantitative data are expressed as mean (SD). Qualitative data (female, thumb involvement, and other locations) are expressed as number (percentage). RP, Raynaud’s phenomenon. MAP, mean arterial pressure; NA, not applicable.
Microvascular reactivity to local cooling. Qualitatively, we observed a similar pattern of skin blood flow during local cooling on the fingers of healthy subjects (Fig. 2) to that described on the forearm, i.e., initial vasoconstriction, followed by transient vasodilation and prolonged vasoconstriction (Fig. 3).

On the dorsum of the finger, overall vasoconstriction (AAC_{0–30}) was more pronounced in patients with RP than in controls, both at 15 and 24°C (Table 2). At 15°C, the interaction between group and phase is not significant, suggesting that the difference affected in the same way all the phases of the response (Table 2). However, the interaction between group and phase is significant at 24°C, suggesting that both groups behave differently over time. Post hoc analysis showed that the difference between groups is significant for transient vasodilation (Table 2).

Vasoconstriction while cooling at 15°C on the forearm was similar between controls and patients with RP (Table 3; Fig. 3).

Effect of lidocaine/prilocaine on microvascular reactivity during local cooling at 15°C. Local anesthesia with lidocaine/prilocaine cream did not affect microvascular reactivity to local cooling on the dorsum of the finger in controls (Table 4; Fig. 4). However, in patients with primary RP, pretreatment with lidocaine/prilocaine suppressed the exaggerated vasoconstriction of the initial phase of the vasomotor response during cooling locally at 15°C, with AUC values similar to those of controls (Table 4; Fig. 4).

Safety. None of the subjects complained of pain associated with the local cooling and local cooling did not induce any symptoms of RP among the patients. No adverse effects were observed requiring notification to the safety committee. Blood pressure did not change significantly during the measurements.

DISCUSSION

The present study shows decreased microvascular perfusion in response to local cooling on the dorsum of the finger of patients with primary RP compared with controls, using an original local cooling test. Both transient vasodilation and prolonged vasoconstriction were affected when cooling down to 15°C. Moreover, local anesthesia suppressed the increased vasoconstriction during the initial phase seen in patients with RP.

Knowledge about the mechanisms underlying the microvascular response to local cooling has greatly improved in the past few years, especially through the work by the Johnson, Kel-

Table 2. Digital skin microvascular reactivity to local cooling to 15 and 24°C in participants with primary RP and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Primary RP</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group</td>
<td>Phase</td>
<td>Interaction</td>
</tr>
<tr>
<td>Finger pad 15°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall (AAC_{0–30})</td>
<td>117.550 (39.226)</td>
<td>124.594 (32.193)</td>
<td>0.45</td>
</tr>
<tr>
<td>Initial VC</td>
<td>−63.50 (24.8)</td>
<td>−66.44 (18.5)</td>
<td>0.4</td>
</tr>
<tr>
<td>Transient VD</td>
<td>−30.73 (41.5)</td>
<td>−45.12 (29.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prolonged VC</td>
<td>−78.38 (21)</td>
<td>−80.94 (18.6)</td>
<td>0.62</td>
</tr>
<tr>
<td>Dorsum 15°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall (AAC_{0–30})</td>
<td>69.544 (66.481)</td>
<td>106.237 (33.068)</td>
<td>0.02</td>
</tr>
<tr>
<td>Initial VC</td>
<td>−52.46 (19.6)</td>
<td>−62.23 (14.4)</td>
<td>0.04</td>
</tr>
<tr>
<td>Transient VD</td>
<td>−18.63 (47.4)</td>
<td>−44.15 (44.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prolonged VC</td>
<td>−49.70 (39.1)</td>
<td>−70.09 (17.8)</td>
<td>0.35</td>
</tr>
<tr>
<td>Finger pad 24°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall (AAC_{0–30})</td>
<td>90.558 (49.477)</td>
<td>105.483 (33.439)</td>
<td>0.19</td>
</tr>
<tr>
<td>Initial VC</td>
<td>−64.43 (20.8)</td>
<td>−65.08 (18.2)</td>
<td>0.08</td>
</tr>
<tr>
<td>Transient VD</td>
<td>−4.19 (82.9)</td>
<td>−50.88 (26.8)*</td>
<td>0.001</td>
</tr>
<tr>
<td>Prolonged VC</td>
<td>−65.59 (23.1)</td>
<td>−68.77 (19.3)</td>
<td>0.05</td>
</tr>
<tr>
<td>Dorsum 24°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall (AAC_{0–30})</td>
<td>57.598 (59.045)</td>
<td>86.915 (27.301)</td>
<td>0.04</td>
</tr>
<tr>
<td>Initial VC</td>
<td>−50.49 (17.9)</td>
<td>−50.60 (17.1)</td>
<td>0.15</td>
</tr>
<tr>
<td>Transient VD</td>
<td>2.21 (111)</td>
<td>−39.31 (19.6)*</td>
<td>0.02</td>
</tr>
<tr>
<td>Prolonged VC</td>
<td>−43.81 (30.5)</td>
<td>−54.19 (19.5)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data are expressed as area above the curve of CVC decrease from baseline (in %BL/s) over the 30-min cooling (AAC_{0–30}). Each phase of the response, i.e., initial vasoconstriction (VC), transient vasodilation (VD), and prolonged VC, is expressed as CVC decrease from baseline (in %BL). Data were analyzed with repeated-measures ANOVA between groups over time. *P < 0.05 vs. controls, post hoc analysis of covariance.
logg, Flavahan, and Kenney groups (18, 31). Briefly, direct cooling of the skin (on the forearm) first induces an initial vasoconstriction followed by a transient vasodilation, within the first 10–15 min after cooling onset, followed by prolonged vasoconstriction. This involves inhibition of the NO system, postsynaptic upregulation of α2C-adrenoceptors through the ROCK pathway and cold-sensitive afferents (18).

In the present work, we did not observe any difference in the initial vasoconstriction after patients with primary RP and controls. On the contrary, Lütolf et al. (21) showed significant difference in digital skin blood flux recorded with single-point LDF while cooling locally. However, the cooling protocols were different as they used a stream of CO2 at −10°C on the nailfold during 60 s.

By using a simple local cooling test with a custom-designed LDF probe inspired from the work of these groups, we observed increased long-term vasoconstriction on the dorsum of the finger at 15°C in participants with primary RP compared with controls. This could support the involvement of the ROCK pathway in the pathophysiology of primary RP. Indeed, this would be consistent with previous findings that showed, in vitro, increased cooling-induced α2-adrenergic constriction of arterioles isolated from patients with primary RP compared with controls, reversed by protein tyrosine kinase inhibitors (13). There is a similar tendency at 24°C, but it does not reach significance, probably because of a lack of power in our study. The potential involvement of the ROCK pathway remains to be explored in a pharmacological study using ROCK inhibitors in patients with primary RP. Moreover, the involvement of ROCK, NO, and sensory nerves in skin microvascular reactivity to local cooling has been studied on the forearm; we therefore extrapolate that similar mechanisms are involved on the finger, which is to be confirmed.

A more striking observation is the difference in the transient vasodilation between the two groups. Indeed, in most cases such vasodilation was blunted in patients with RP compared with controls. Although the mechanisms underlying the transient vasodilation are not fully understood, it is limited by intact sensory nerves (16). This is interesting as in healthy subjects, local anesthesia does not affect transient vasodilation to local cooling, whereas it is unmasked by the blockade of norepinephrine release or adrenergic receptors (18). These results are consistent with the previously suggested impaired cold-induced digital vasodilation in primary RP, indirectly assessed with skin temperature (17). More surprisingly, cold-induced vasodilation was also blunted or decreased on the forearm of five out of six patients with primary RP (3), which we did not observe in the present study. However, experimental conditions were different from ours as the authors used a 2-min cooling at 4–6°C and recorded skin blood flux before and after cooling.

In primary RP, several neural abnormalities have been described. Bunker et al. showed the loss of CGRP containing nerve fibers (5), with decreased skin blood flow response to CGRP when fingers were exposed to cold (using an environmental chamber; Ref. 4). In the present study, the use of local anesthesia on the dorsum of the finger partially restored the transient vasodilation in participants with primary RP when cooling locally at 15°C. These results suggest an abnormal

<table>
<thead>
<tr>
<th>Group</th>
<th>Without Lidocaine/Prilocaine Cream</th>
<th>With Lidocaine/Prilocaine Cream</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls Overall (AAC0–30)</td>
<td>69.544 (66.481)</td>
<td>72.821 (60.380)</td>
<td>0.86</td>
</tr>
<tr>
<td>Initial VC</td>
<td>−52.46 (19.6)</td>
<td>−50.23 (26.7)</td>
<td>0.82</td>
</tr>
<tr>
<td>Transient VD</td>
<td>−18.63 (47.4)</td>
<td>−21.15 (50.3)</td>
<td>0.71</td>
</tr>
<tr>
<td>Prolonged VC</td>
<td>−49.70 (39.1)</td>
<td>−52.74 (30.4)</td>
<td>0.76</td>
</tr>
<tr>
<td>Primary RP Overall (AAC0–30)</td>
<td>106.237 (33.068)*</td>
<td>82.569 (56.880)</td>
<td>0.05</td>
</tr>
<tr>
<td>Initial VC</td>
<td>−62.23 (14.4)</td>
<td>−48.46 (26.2)</td>
<td>0.13</td>
</tr>
<tr>
<td>Transient VD</td>
<td>−44.15 (44.3)</td>
<td>−27.95 (44.7)</td>
<td>0.04</td>
</tr>
<tr>
<td>Prolonged VC</td>
<td>−70.09 (17.8)</td>
<td>−61.43 (21.3)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Data are expressed as area above the curve of CVC decrease from baseline (in %BL) over the 30-min cooling (AAC0–30). Each phase of the response, i.e., initial VC, transient VD, and prolonged VC, is expressed as CVC decrease from baseline (in %BL). Paired-t-tests were used to analyze the effect of lidocaine/prilocaine in each participant. *P < 0.02 vs. controls, repeated-measures ANOVA.

Fig. 4. Mean (SE) CVC (expressed as a percentage decrease from baseline CVC) in participants with primary RP while cooling to 15°C on the dorsum of the finger with (plain line) and without (dash line) lidocaine/prilocaine pretreatment.
neural response in primary RP depending on cold-sensitive nerves. The nonspecific effect of lidocaine/prilocaine does not permit to explain the exact mechanism of the transient vasodilation, but available evidence suggests that sensory nerves play a key role in transient vasodilation (16). Of interest, when using local thermal hyperemia as an integrated test to study microvascular function, the initial axon-reflex vasodilator response to heating (which is thought to be mediated through CGRP and substance P; Ref. 23) is not affected in primary RP (1, 29). Therefore, the mechanism through which microvascular dysfunction to local cooling is mediated in primary RP differs from that involved during local thermal hyperemia. Indeed, response to local cooling and heating are triggered by different transient receptor potential proteins (TRPs). In response to local warming, the response is temperature dependently mediated by TRPV4 (27–34°C), TRPV3 (33–39°C), and TRPV1 (43°C), the latter being responsible for heat nociception. In contrast, local cooling mostly activates TRPM8 (23–28°C) while TRPA1 (17°C) is mostly responsible of cold nociception (32).

It is interesting that we observed no difference in CVC between participants with RP and controls when cooling to 15°C on the finger pad. This is probably due to the marked vasoconstriction observed in both groups, suggesting that local cooling to 15°C on the finger pad induces maximal vasoconstriction both in controls and in participants with primary RP. There are little published data concerning digital skin blood flow of patients with primary RP while cooling locally, but another group (12) came to similar conclusions when cooling down to 8°C on the finger pad, comparing their results to the same protocol previously performed in healthy controls. In the same way, Jobe et al. (17) observed little difference in skin temperature between primary RP patients and controls when immersing the finger in a 5°C water bath, whereas differences were more pronounced at higher temperatures. Another potential explanation is that the TRP activated during local cooling differs between 24 and 15°C, the latter being putatively TRPA1 dependent.

The originality of the test used in this study is that it cools locally, close to the area where blood flux is measured. We have previously shown that this local cooling test does not induce a systemic cutaneous vascular response (26), unlike cold water immersion, which is the most common cooling test because of its relative ease of use (11, 34). Our method therefore allows study of microvascular response with limited systemic sympathetic interference.

The choice of baseline skin temperature raises a methodological issue. Indeed, patients with primary and secondary RP have lower baseline CVC compared with controls (4, 14, 28). As temperature plays a key role in baseline flux, standardizing baseline skin temperature when performing microvascular reactivity improves reproducibility, especially when expressing data as a function of baseline (26, 27). Therefore, in the present study, we decided to set baseline temperature to skin thermoneutrality (i.e., 33°C), as previously described (23). We observed a slightly higher baseline CVC in participants with primary RP than in controls. However, these data should be considered with caution due to the high variability of baseline flux on the forearm when recording with a single-point LDF probe (27). Moreover, the comparable profile of microvascular response to local cooling on the forearm between the two groups strengthens the argument that data should be expressed as a function of baseline while cooling locally, as already suggested by previous work (26).

Moreover, basal cutaneous blood flow has been shown to be lower in the fingers of young women than in young men, which appears to be due to a basal increase in sympathetic tone (6). This may reflect the influence of estrogen in the prevalence of RP, higher in women than in men, and decreasing in postmenopausal women. Of interest, a recent study (9) has shown that estrogen can increase the expression of α2-adrenoreceptors and also increased α2-adrenoreceptor-mediated constriction during exposure to cold. In the present study, we were not able to observe any difference between women according to their hormonal status, but the sample size was too small to address this issue. Nonetheless, these findings strengthen the fact that women should be matched in terms of hormonal status when studying skin microvascular function, especially when participants with RP are involved.

In the present work, standardizing baseline skin temperature further suggests that decreased digital CVC in RP patients compared with controls while cooling locally is not due to decreased baseline skin temperature but rather to an abnormal reactivity.

Finally, local cooling did not induce any paroxystic ischemia among the patients, suggesting that microvascular dysfunction in primary RP preexists in the absence of the syndrome.

In conclusion, our study shows with a noninvasive and original test that microvascular reactivity to local cooling is impaired on the dorsum of the finger in primary RP, with exaggerated vasoconstriction compared with controls. The underlying mechanism may involve the ROCK pathway, but this remains to be confirmed. This work further shows that the initial transient vasodilation was blunted on the dorsum of the finger and that part of this abnormal response in primary RP depends on cold-sensitive nerves.

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DISCLOSURES

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

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