

## Supplementary text

### Skin ultrasound protocol

Skin ultrasound examination was performed using a Siemens ACUSON S2000 Ultrasound System HELX Evolution. Participants rested for 15 minutes in a supine position before ultrasound. During the examination, each participant was kept in supine and relaxed position with a pillow under the head. The probe was positioned perpendicular to the skin surface, in a longitudinal orientation, with the middle point of the probe footprint placed at the precise locations described by Moore *et al.* (18). A gel interface was used (about 0.5 cm layer), to minimise probe pressure and avoid artefactual changes in the skin. Ultrasound measures were taken and reported according to the WSF Recommendations for skin ultrasound studies in SSc (16).

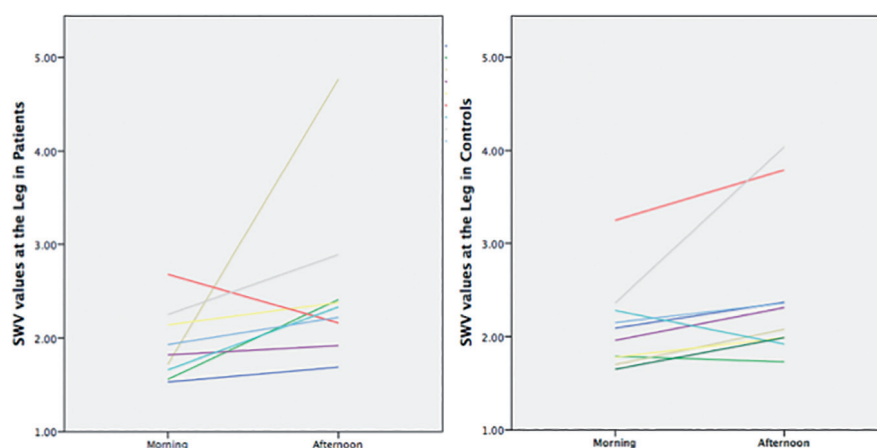
### Dermal thickness

Dermal thickness was measured in ultrasound B-mode, with an 18 MHz, 57 mm wide, linear probe. To be accepted, every image was required to demonstrate: i) adequate depiction of epidermis, dermis, and subcutis, with distinct and parallel interfaces between them, and ii) a gel film layer on the skin surface, to guarantee that no excessive pressure was being applied. Qualified images were collected and stored for later analyses.

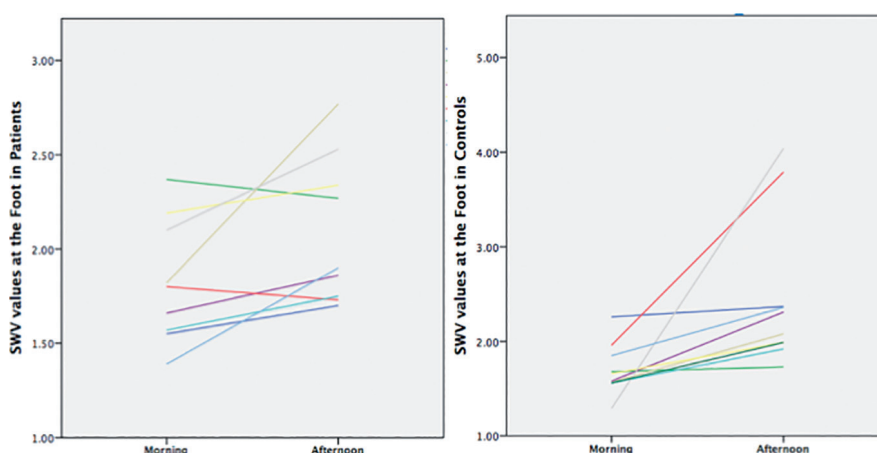
Ultrasound dermal thickness was determined on each single image, through a dedicated image viewer (DICOM®), using an electronic calliper to measure the distance between the upper epidermis-dermis interface and the lower dermis-subcutis interface (18). Separate measures were taken at the left, and right and in the middle of each image, and the average of these three values was considered for statistical analysis. All measures were expressed in millimetres and rounded to centesimals. During image analysis, all raters were blinded for the participant's demographic characteristics.

### Skin stiffness

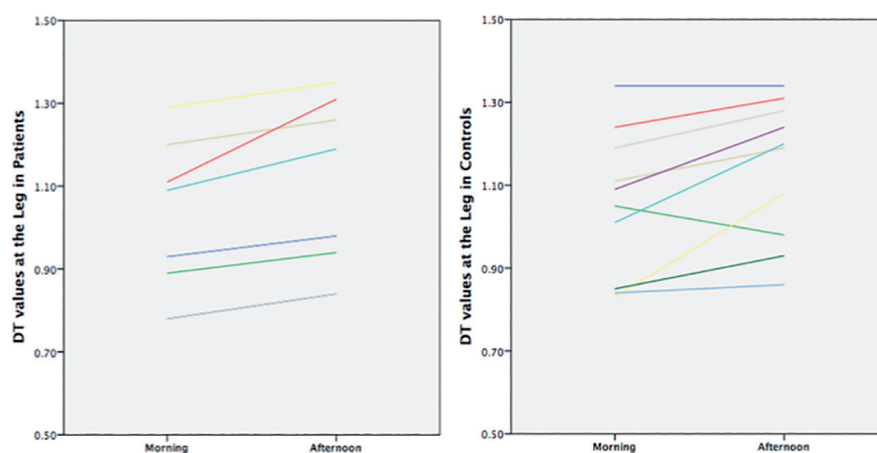
Skin stiffness was evaluated through shear-wave elastography using virtual



**Supplementary Fig. S1.** Skin stiffness (*i.e.*, shear-wave velocity values) measured at the leg, in the morning and in the afternoon in SSc and controls.



**Supplementary Fig. S2.** Skin stiffness (*i.e.*, shear-wave velocity values), measured at the foot, in the morning and in the afternoon in SSc and controls.



**Supplementary Fig. S3.** Dermal thickness, measured at the leg, in the morning and in the afternoon in SSc and controls.

touch image quantification (VTIQ), with a 9 MHz, 40 mm wide, linear probe. The VTIQ output simultaneously displays a color-coded tissue stiffness map as well as shear-wave

velocity values (in m/s, up to 10 m/s) in distinct sampling gates of interest within each image. Higher shear-wave velocity values indicate greater tissue stiffness.

The probe was positioned as described above. The sonographer placed three sampling gates (2x2mm) over the epidermis and dermis, at the left, centre and right of each image, and shear-wave velocity values were recorded. The average of these three measures

was used for statistical analysis. Skin stiffness was expressed in meters per second (m/s) and all measures were given in centesimals.

In the four studies, all B-mode ultrasound images were acquired and then stored for later analyses. During the

image analysis, the raters were blinded for the participant clinical characteristics. In shear-wave elastography, image acquisition and analysis were performed simultaneously, in each session of ultrasound evaluation.

**Supplementary Table S1.** Skin ultrasound measures, dermal thickness, and skin stiffness, in the five skin sites evaluated, at cold (16-17°C) versus warm room temperature (22-24°C).

	SSc patients (n=10) <sup>a</sup>				Controls (n=10)			
	hot	cold	Diff. (%)	p	hot	cold	Diff. (%)	
<b>Dermal thickness (mm)</b>								
Forearm	0.89 (0.13)	0.8 (0.11)	5.44	0.167	0.82 (0.12)	0.83 (0.09)	-1.82	0.476
Hand	0.76 (0.06)	0.76 (0.05)	6.22	0.434	0.64 (0.07)	0.63 (0.07)	2.24	0.050
Finger	0.81 (0.10)	0.82 (0.11)	1.52	0.150	0.73 (0.15)	0.72 (0.17)	-0.77	0.906
Leg	1.03 (0.28)	1.05 (0.25)	2.99	0.261	1.11 (0.25)	0.98 (0.22)	9.99	0.314
Foot	0.85 (0.16)	0.83 (0.16)	2.55	0.531	0.70 (0.06)	0.68 (0.08)	2.38	0.677
<b>Skin stiffness (m/s)</b>								
Forearm	1.38 (0.31)	1.38 (0.30)	0.50	0.683	1.45 (0.10)	1.53 (0.27)	-3.70	0.593
Hand	1.70 (0.37)	1.62 (0.19)	4.32	0.513	1.60 (0.19)	1.67 (0.30)	-3.18	0.333
Finger	2.19 (0.55)	2.09 (0.62)	5.42	0.241	1.94 (0.49)	1.87 (0.21)	2.88	0.959
Leg	2.16 (0.52)	2.06 (0.32)	4.22	0.541	1.72 (0.27)	1.72 (0.33)	2.60	0.646
Foot	1.87 (0.52)	1.63 (0.35)	15.89	0.358	1.58 (0.28)	1.44 (0.22)	8.42	0.075

<sup>a</sup>Four patients only tolerated 5 minutes of acclimatization in cold temperature because they developed exacerbation of Raynaud and/or digital ulcers.

**Supplementary Table S2.** Skin ultrasound measures, dermal thickness, and skin stiffness, evaluated in the five Rodnan skin sites, at menstrual (day 2-4) and the ovulatory/early luteal (day 14-18) phases.

	SSc patients (n=10) <sup>a</sup>				Controls (n=10) <sup>b</sup>			
	menstrual	ovulatory/lut	Diff. (%)	p	menstrual	ovulatory/lut	Diff. (%)	
<b>Dermal thickness (mm)</b>								
Forearm	1.00 (0.21)	0.95 (0.18)	-5.30	0.056	0.91 (0.16)	0.88 (0.16)	-3.65	0.322
Hand	0.77 (0.12)	0.77 (0.16)	-1.39	0.384	0.73 (0.16)	0.71 (0.12)	0.11	0.259
Finger	0.83 (0.24)	0.81 (0.21)	4.04	0.678	0.65 (0.12)	0.65 (0.10)	0.21	0.944
Leg	1.17 (0.24)	1.24 (0.32)	-3.04	0.444	1.16 (0.12)	1.12 (0.14)	-2.44	0.553
Foot	0.77 (0.13)	0.80 (0.13)	1.17	0.109	0.74 (0.11)	0.79 (0.18)	10.14	0.261
<b>Skin stiffness (m/s)</b>								
Forearm	1.54 (0.26)	1.56 (0.36)	4.32	0.635	1.44 (0.14)	1.34 (0.11)	8.05	0.074
Hand	1.83 (0.18)	1.71 (0.34)	-4.34	0.484	1.66 (0.23)	1.58 (0.17)	5.61	0.646
Finger	2.19 (0.29)	2.07 (0.28)	-5.47	0.086	1.83 (0.12)	1.84 (0.20)	0.40	1.000
Leg	1.83 (0.36)	2.58 (1.15)	33.95	0.066	1.89 (0.69)	2.04 (0.56)	6.36	0.169
Foot	1.90 (0.28)	1.99 (0.44)	5.80	0.484	1.70 (0.26)	1.79 (0.24)	4.69	0.333

<sup>a</sup>Four SSc patients and <sup>b</sup>five controls were taking contraceptive (oral or intrauterine device).

Skin ultrasound measures evaluated at the leg and foot at menstrual day and ovulatory/early luteal day are represented in Figure S4 and S5.

**Supplementary Table S3.** Inter- and intra-rater reliability of ultrasound dermal thickness measures in the 20 controls and in 20 SSc patients – intraclass correlation coefficients (95% confidence interval).

	Controls, n=20		SSc Patients, n=20	
	Inter-	Intra-	Inter-	Intra-
Face	0.84 (0.61 to 0.93)	0.89 (0.76 to 0.95)	0.90 (0.77 to 0.96)	0.88 (0.70 to 0.96)
Chest	0.82 (0.57 to 0.93)	0.88 (0.74 to 0.95)	0.89 (0.74 to 0.95)	0.85 (0.55 to 0.94)
Abdomen	0.87 (0.69 to 0.95)	0.94 (0.86 to 0.97)	0.89 (0.74 to 0.95)	0.95 (0.88 to 0.98)
Upper arm right	0.86 (0.64 to 0.94)	0.89 (0.75 to 0.95)	0.89 (0.73 to 0.95)	0.92 (0.76 to 0.96)
Upper arm left	0.89 (0.73 to 0.96)	0.88 (0.69 to 0.95)	0.85 (0.62 to 0.94)	0.91 (0.77 to 0.97)
Forearm right	0.88 (0.68 to 0.95)	0.91 (0.79 to 0.96)	0.87 (0.66 to 0.95)	0.94 (0.81 to 0.98)
Forearm left	0.92 (0.77 to 0.96)	0.88 (0.69 to 0.95)	0.88 (0.72 to 0.95)	0.95 (0.79 to 0.98)
Hand right	0.82 (0.58 to 0.92)	0.90 (0.77 to 0.96)	0.92 (0.79 to 0.97)	0.93 (0.83 to 0.97)
Hand left	0.85 (0.71 to 0.92)	0.89 (0.69 to 0.95)	0.95 (0.88 to 0.98)	0.91 (0.76 to 0.96)
Finger right	0.96 (0.89 to 0.98)	0.83 (0.61 to 0.93)	0.96 (0.88 to 0.98)	0.89 (0.66 to 0.96)
Finger left	0.93 (0.82 to 0.97)	0.87 (0.65 to 0.94)	0.92 (0.77 to 0.97)	0.87 (0.66 to 0.95)
Thigh right	0.93 (0.81 to 0.97)	0.89 (0.71 to 0.95)	0.91 (0.76 to 0.97)	0.88 (0.70 to 0.95)
Thigh left	0.92 (0.79 to 0.97)	0.85 (0.60 to 0.95)	0.91 (0.77 to 0.96)	0.84 (0.59 to 0.93)
Leg right	0.85 (0.60 to 0.94)	0.90 (0.77 to 0.96)	0.88 (0.68 to 0.95)	0.86 (0.63 to 0.94)
Leg left	0.91 (0.74 to 0.96)	0.92 (0.61 to 0.97)	0.91 (0.77 to 0.96)	0.86 (0.40 to 0.96)
Foot right	0.89 (0.71 to 0.95)	0.90 (0.78 to 0.96)	0.86 (0.71 to 0.93)	0.89 (0.74 to 0.96)
Foot left	0.88 (0.69 to 0.96)	0.88 (0.71 to 0.95)	0.86 (0.63 to 0.94)	0.88 (0.72 to 0.95)

**Supplementary Table S4.** Inter- and intra-observer reliability of the skin stiffness in the 20 controls and 20 patients – intraclass correlation coefficients (95% confidence interval).

	Controls, n=20		SSc Patients, n=20	
	Inter-	Intra-	Inter-	Intra-
Face	0.84 (0.61 to 0.94)	0.83 (0.63 to 0.93)	0.84 (0.57 to 0.94)	0.83 (0.63 to 0.93)
Chest	0.90 (0.75 to 0.96)	0.87 (0.70 to 0.94)	0.75 (0.40 to 0.90)	0.87 (0.70 to 0.94)
Abdomen	0.83 (0.60 to 0.92)	0.89 (0.74 to 0.96)	0.79 (0.50 to 0.92)	0.89 (0.74 to 0.96)
Upper arm right	0.89 (0.76 to 0.95)	0.88 (0.68 to 0.95)	0.82 (0.52 to 0.93)	0.88 (0.68 to 0.95)
Upper arm left	0.89 (0.75 to 0.95)	0.88 (0.69 to 0.95)	0.79 (0.46 to 0.93)	0.83 (0.54 to 0.93)
Forearm right	0.84 (0.62 to 0.93)	0.88 (0.70 to 0.95)	0.84 (0.62 to 0.93)	0.88 (0.70 to 0.95)
Forearm left	0.77 (0.50 to 0.90)	0.95 (0.88 to 0.98)	0.87 (0.69 to 0.95)	0.78 (0.40 to 0.92)
Hand right	0.88 (0.67 to 0.95)	0.89 (0.74 to 0.95)	0.88 (0.67 to 0.95)	0.89 (0.74 to 0.95)
Hand left	0.88 (0.76 to 0.94)	0.98 (0.95 to 0.99)	0.79 (0.44 to 0.92)	0.87 (0.70 to 0.95)
Finger right	0.97 (0.93 to 0.99)	0.88 (0.72 to 0.96)	0.79 (0.50 to 0.93)	0.88 (0.72 to 0.96)
Finger left	0.98 (0.97 to 0.99)	0.98 (0.95 to 0.98)	0.83 (0.62 to 0.93)	0.99 (0.97 to 0.99)
Thigh right	0.88 (0.72 to 0.95)	0.86 (0.70 to 0.94)	0.88 (0.72 to 0.95)	0.86 (0.70 to 0.94)
Thigh left	0.93 (0.82 to 0.97)	0.91 (0.76 to 0.96)	0.77 (0.40 to 0.91)	0.85 (0.65 to 0.94)
Leg right	0.93 (0.81 to 0.97)	0.96 (0.91 to 0.98)	0.88 (0.71 to 0.95)	0.96 (0.91 to 0.98)
Leg left	0.93 (0.82 to 0.97)	0.94 (0.86 to 0.97)	0.79 (0.52 to 0.92)	0.85 (0.64 to 0.94)
Foot right	0.84 (0.63 to 0.93)	0.94 (0.87 to 0.98)	0.78 (0.40 to 0.92)	0.94 (0.87 to 0.98)
Foot left	0.82 (0.57 to 0.92)	0.92 (0.80 to 0.97)	0.77 (0.47 to 0.91)	0.95 (0.89 to 0.98)

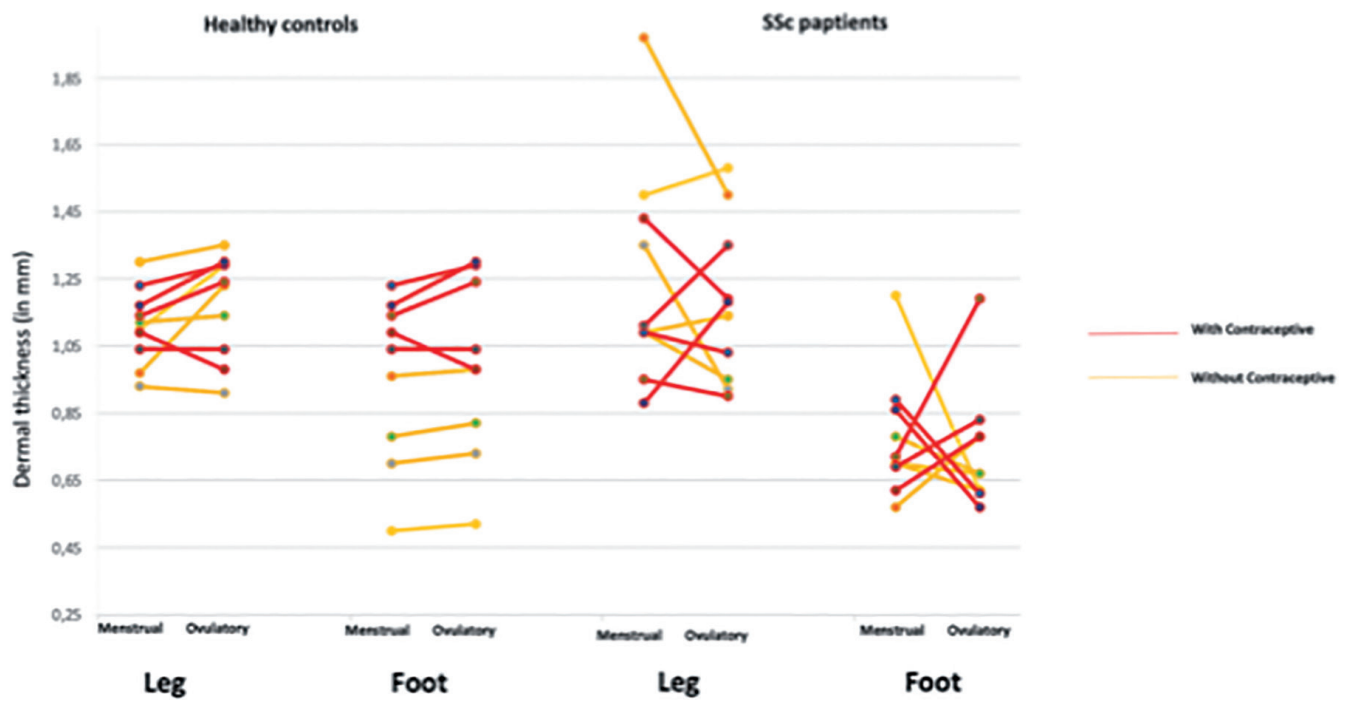


Figure S4

Figure S5

Supplementary Fig. S4 and S5. Dermal thickness, evaluated in the leg and foot, at menstrual (day 2-4) and the ovulatory/early luteal (day 14-18) phases, in menstrual and ovulatory/early luteal phases, in 10 healthy controls and 10 SSc patients.