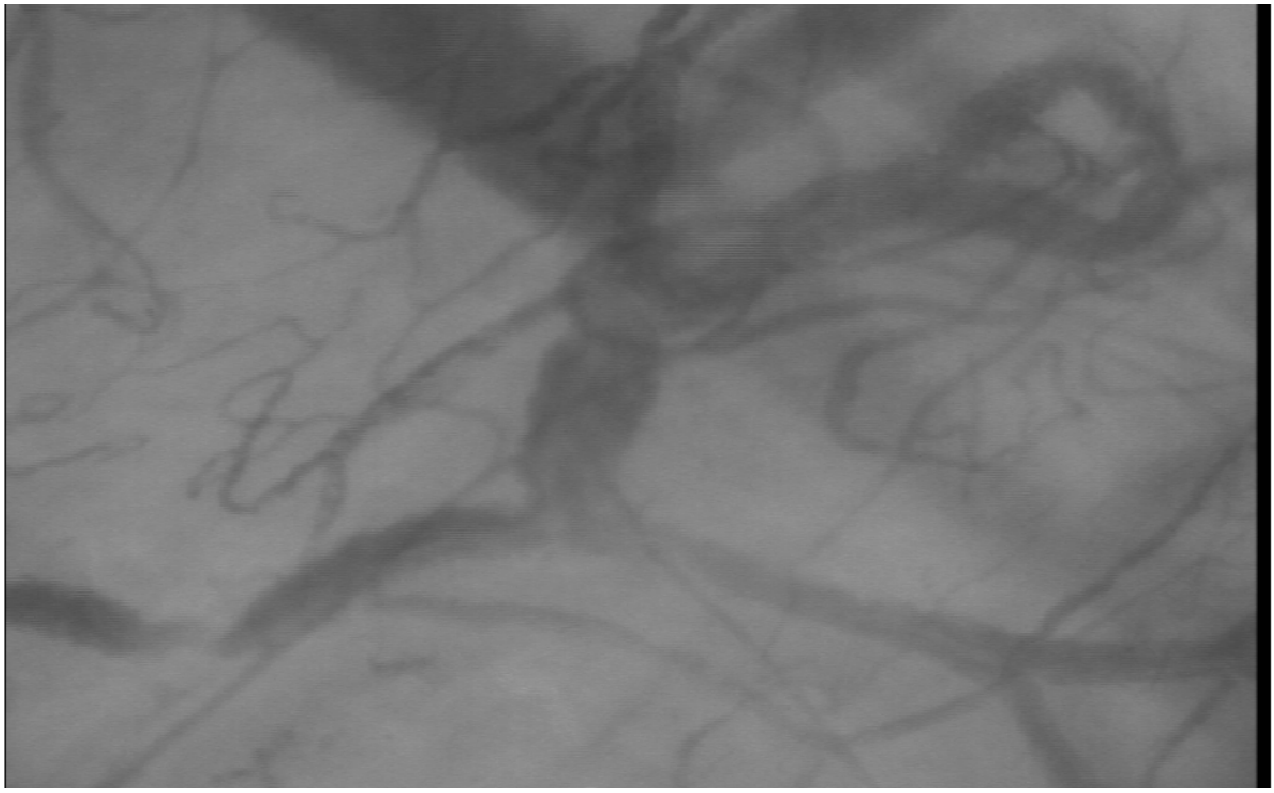
Supplemental data 1

Oxidative stress

Plasma malondialdehyde (MDA) concentrations were determined by high liquid performance chromatography (HPLC) using a previously described method (reference 57), with some adaptations. The derivatization method used thiobarbituric acid as agent. This method measures the total content of MDA in the sample. Five microliters of sample were applied to a Omnispher C18 HPLC column (internal diameter: 3 µm, column length: 10 cm; Agilent Technologies, Middelburg, The Netherlands) operated at 20°C. The starting eluent consisted of 1% acetic acid (pH 4.5), followed by linear gradient or isocratic elution with methanol at the indicated concentration: 0% (0.5 min), 80% (11 min), 80% (13 min) and 0% (30 min). The flow rate was 0.8 mL per minute. The chromatographic system consisted of an Ultimate 3000 pump, autosampler, RS variable wavelength detector and column compartment (Dionex Corporation, Sunnyvale, USA).

Supplemental data 2

Sidestream darkfield imaging of the sublingual microvasculature



Supplemental data 3

Imaging of the microcirculation using Sidestream Darkfield microscopy
Reproducibility data:
Reproducibility data was acquired by performing SDF imaging on two separate days (V01 and V02) in 16 male volunteers. Participants did not smoke, did not use any medication, and were free from any illness, including overt cardiovascular disease. All experiments were performed after an overnight fast. Group averages (SD) for RBCW, PBR and DPerf are RBCW_V01: 10.09 (1.23) µm, RBCW_V02: 10.05 (1.06) µm; PBR_V01: 2.72 (0.59) µm, PBR_V02: 2.59 (0.50) µm; DPerf_V01: 16.01 (1.19) µm, DPerf_V02: 15.67 (1.38) µm.