Association of *in vivo* endothelium-dependent forearm skin blood flow response with *ex vivo* relaxation of small arteries from subcutaneous adipose tissue from human participants

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We are able to assess human microvascular endothelial responsiveness to pharmacological agents using both *in vivo* and *ex vivo* techniques. For example, agents can be delivered to the dermis and the resulting microvascular response monitored by laser Doppler techniques. For more mechanistic studies, small resistance arteries can be isolated from subcutaneous adipose tissue (SAT) and their responsiveness examined by pressure myography. However, to the best of our knowledge, it has not been examined whether responsiveness is similar between these two vascular beds.

The aim of this study was to examine whether there is an association between endothelial responsiveness to acetylcholine, a known vasodilator, in these two different peripheral vascular beds in humans.

21 human subjects (9 individuals without diabetes and 12 with type 2 diabetes) underwent dermal microinjection of 10 µl acetylcholine (1% ACh [10 mg/ml]; Miphtel; Farmigea S.p.A., Italy) or saline (0.9% w/v) (injection trauma control) to the volar forearm. Skin perfusion response was assessed by laser Doppler perfusion imaging at baseline and for 10 minutes post-microinjection. Results were expressed as stabilised response (SR; mean perfusion (voltage) of the last 2.5 min scanning period). Small arteries were isolated from a SAT biopsy (abdominal or gluteal) from the same individuals, for pressure myography. The isolated arteries (264 – 374 µm outer diameter range) were pressurised to 70 mmHg and pre-constricted (80%) with noradrenaline prior to relaxation with ACh. Results are expressed as maximal relaxation responses to 1µM acetylcholine chloride (ACh; expressed as % of pre-constriction).

Across the whole cohort (67% male, age mean (SD): 68 ± 6 years, BMI 30 ± 4m^2/kg), microinjection of ACh induced a substantial and sustained increase in skin blood flow in all subjects compared to saline (saline SR: perfusion range 0.49 – 0.90 V, median 0.69; ACh SR: range 1.39 – 2.85, median 1.80; $p < 0.0001$, Mann Whitney U test). Maximal relaxation to ACh by isolated small arteries had an 8 – 97% response range, median 69.2%. Maximal relaxation to ACh correlated positively with the ACh-induced increase in skin blood flow ($p=0.03$, $r_s = 0.473$; Spearman's correlation).

Endothelium-dependent relaxation responses to ACh in isolated resistance arteries from SAT correspond with endothelium-dependent skin microvascular blood flow responses in humans. This relationship supports the concept that measuring microvascular endothelial function in the human forearm by changes in skin blood flow is a suitable model for endothelial function of other peripheral microvascular beds, including those accessible only by invasive methods.