Altered arterial mechanics and vasodilator function in an *in vitro* model of vascular insulin resistance

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Many of the deleterious effects of obesity, diabetes and conditions featuring hyperinsulinemia and insulin resistance are vascular in nature, including impaired arterial dilation, increased arterial stiffness and microvascular rarefaction. Vascular insulin resistance has been characterized by decreased endothelial nitric oxide synthase (eNOS) and increased endothelin activity (De Boer *et al.*, 2012). The aim of this study was to investigate the effects of vascular insulin resistance on endothelium-dependent vasodilation and pressure-induced arterial myogenic tone. A model of *in vitro* insulin resistance was employed, exposing arteries isolated from the rat cremaster muscle to high concentrations of insulin for a short period of time (Yuen *et al.*, 2009).

Male Sprague-Dawley rats (6-8 weeks, ~250 g) were anesthetized (sodium thiopentone, 100 mg/kg i.p.) and the cremaster muscles removed. The main branch of the cremaster muscle artery (1A) was dissected free and mounted in a pressure myograph, with arterial and lumen diameters measured by electronic caliper combined with video microscopy. Arteries were incubated with insulin (0.6 or 60 μg/ml) or vehicle solution (control) for 2h (intra-lumenal exposure only). Protein expression was assayed by Western blot and immunofluorescence techniques.

Arteries maintained at 70 mmHg developed myogenic tone. In vessels incubated with the lower concentration of insulin (0.6 μg/ml), responses to the endothelium-dependent vasodilator acetylcholine (ACh) and the NO donor sodium nitroprusside were enhanced in comparison with control arteries. The contribution of NO to the ACh-induced response was increased compared with control, assessed by sensitivity to combined inhibition of NOS and guanylate cyclase (L-NAME 100 μM and QDQ 10 μM). Vasodilation induced by SKA-31, an activator of intermediate-conductance Ca^{2+}-dependent K^+-channels (IK_{Ca} or IK1) was also increased by this insulin concentration. A higher concentration of insulin (60 μg/ml) did not alter responses to ACh or nitroprusside compared with control, but the contribution of NO to ACh-induced responses was decreased markedly. In contrast, IK1 now accounted for a substantial portion of the response as assessed by the ability of the IK1 inhibitor TRAM-34 (1 μM) to inhibit ACh-induced vasodilation, compared with control. Western blotting and immunofluorescence demonstrated reduced eNOS and increased IK1 expression in these vessels.

Pressure-induced arterial myogenic tone was increased by 60 μg/ml insulin, at 50, 70, 100 and 120 mmHg. Passive arterial diameter (determined in Ca^{2+}-free bathing solution containing 2 mM EGTA) was significantly decreased at 30, 50 and 70 mmHg. These studies demonstrate an *in vitro* model of vascular insulin resistance can mimic many of the observed vascular effects of obesity and Type 2 diabetes, including impaired NO-mediated vasodilation, increased myogenic tone and decreased arterial distensibility.

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