A humanised neutralising antibody to VEGF-A_{165}b induces collateral formation in obese and diabetic models of peripheral arterial disease.

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Introduction
Lack of efficient collateralisation during vascular occlusion leads to tissue ischemia in coronary, peripheral vascular disease. Patients with peripheral arterial disease have elevated levels of the anti-angiogenic isoform of VEGF (VEGF-A_{165}b), which correlates with poorer outcomes. In experimental animals, VEGF-A_{165}b neutralisation improves blood flow and collateralisation after femoral or coronary artery ligation. We tested the hypothesis that a humanised, potent, high affinity, neutralising antibody to VEGF-A_{165}b could induce collateralisation in rodent models of diabetic peripheral ischemia.

Methods
The efficacy of a humanised anti-VEGF-A_{165}b antibody in vitro was tested using endothelial cell migration assays and in vivo using the hindlimb ischemia (HLI) model in C57Bl6 mice fed a high fat high sucrose (HFHS) diet, and in Zucker Diabetic Fatty rats. Mouse and rat tissues were stained for arterioles with smooth muscle actin and for endothelial cells with isolectin B4 and vessel density determined by counting.

Results
The humanised anti-VEGF-A_{165}b antibody had an in vitro affinity of 600pM as measured by biolayer interferometry. 0.3µg/ml of this clone reversed the inhibitory effect of recombinant human VEGF-A_{165}b (31±4%, N=6 of VEGF-A_{165}a alone) on migration of endothelial cells to 40ng/ml rhVEGF-A_{165}a (89±9% N=3). HLI in HFHS mice impaired blood flow after 28 days in IgG treated mice (70±7.7% of pre-ischemic blood flow, N=6), which was restored to normal (93.3±5.5, $p<0.05$, N=8) by intraperitoneal injection of 1mg/kg HC4LC2. HC4LC2 resulted in a 29±4% increase in blood vessels compared with a 21±13% decrease in IgG treated animals. Arterioles increased by 12±5% in the anti-VEGF-A_{165}b treated animals compared with a 39±6% decrease in the IgG treated, compared with the contralateral side. Blood flow recovery after HLI was also seen in ZDF rats treated with HC4LC2 (106±16% of pre-ischemic flow, N=5) compared with IgG treated animals (76±.9%, N=5, $p<0.05$, two-way ANOVA, all values mean ±SEM). Arterioles were increased by 35±3% in the anti-VEGF-A_{165}b treated but decreased by 13±8% in the IgG treated controls.

Conclusion
These results indicate that HC4LC2 is a humanised, high affinity neutralising antibody to VEGF-A_{165}b that could have therapeutic potential for patients with cardiovascular disease, by enhancing collateral formation.