Dietary copper supplementation has previously been shown to significantly reduce the formation of atherosclerotic lesions and improve vascular function in the cholesterol-fed rabbit. The present study was undertaken to elucidate the biological effect of copper supplementation on arterial function in a non-atherosclerotic model. Adult C57/BL6 mice were fed a normal mouse chow diet and their drinking water contained either penicillamine (2.3 mg/day); a low copper (copper acetate 12 μg/day) or a high copper supplement (copper acetate 240 μg/day) for 6 weeks. Control mice were killed by cervical dislocation and the thoracic aorta was isolated and mounted in organ baths. Aortic rings were pre-contracted with 0.1 μM phenylephrine and relaxed with acetylcholine, calcium ionophore A23187, or sodium nitroprusside (SNP) allowing cumulative relaxant concentration response curves to be constructed. Maximal relaxation of the aorta in response to acetylcholine was increased from 61.4±5.2% in the control group and 74.5±8.4% in the low copper group to 90.2±3.5% in the high copper supplemented group (P<0.05). The low copper supplemented diet had no effect in enhancing relaxation and copper deficiency (penicillamine) impaired relaxation to acetylcholine (55.8%). However, there was no change in potency of EC50. Furthermore, there was no significant difference in the relaxation to A23187 or to SNP in any of the dietary groups. These data suggest that copper may be required to preserve endothelial-mediated vasodilation and that high dietary copper supplements improve vascular function. Copper may directly interact with the muscarinic receptor or at a point in the pathway between receptor stimulation and the subsequent increase in intracellular calcium. This may have a protective effect in the development of atherosclerotic lesions, by preserving the normal function of the endothelium.

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**M31**

**FIRST DEMONSTRATION IN HUMANS OF SYSTEMIC NEUTRAL ENDOPEPTIDASE AND ENDOTHELIN CONVERTING ENZYME INHIBITION USING A NEW, ORALLY ACTIVE, DUAL METALLOPROTEASE INHIBITOR, SLV 306.**

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**Introduction:** Simultaneous manipulation of several endogenous neurohumoral systems is a new and promising therapeutic approach. SLV 306, through its active metabolite KC 12615, has dual neutral endopeptidase (NEP) and endothelin converting enzyme (ECE) inhibiting activity in vitro. The aim of this study was to demonstrate these actions in human vascular tissue.

**Methods:** 13 healthy males, studied on 4 occasions, were randomised to placebo or one of 3 doses of SLV 306. Each visit involved, taking a single dose of SLV or placebo, supine rest (3 hours) and infusions of 8 and 12 pmol/kg/min of big ET-1 (20 minutes each). Systolic and diastolic blood pressure (SBP, DBP), ANP, big ET and its cleavage product through the action of ECE on big ET-1, endothelin-1 (ET-1), were measured over 4 hours.

**Results:** Mean peak changes in SBP (mmHg) after the second big ET-1 infusion were: 19.4 (placebo), 16.5 (dose 1), 14.6 (dose 2), 12.9 (dose 3) (P<0.05). The respective changes in DBP were 16.2, 14.3, 12.0 and 11.4 (P<0.05). The mean, placebo corrected, peptide changes following the second big ET-1 infusion for doses 1, 2 and 3 were: ANP: 7, 11, 15 pg/mL (p<0.01), big ET-1: 33, 89, 121 fmol/mL (p<0.01) and ET-1: 0.95, 0.56, 0.02 fmol/mL. ET-1 levels did not increase as would have been expected from a pure NEP-inhibitor, whereas big ET levels were increased dose dependently in the SLV 306 groups compared to placebo, indicating that the breakdown of big ET was inhibited. SLV 306 was well tolerated.

**Conclusions:** This is the first demonstration in humans of both systemic ECE and NEP inhibition. SLV 306, the lead orally active agent in this new class of combined metalloprotease inhibitors, may have therapeutic potential in hypertension and heart failure.