Insulin treatment triggers oxidative stress in the vascular wall of patients with atherosclerosis, independently of diabetes or systemic insulin resistance: The protective role of DPP-IV inhibition

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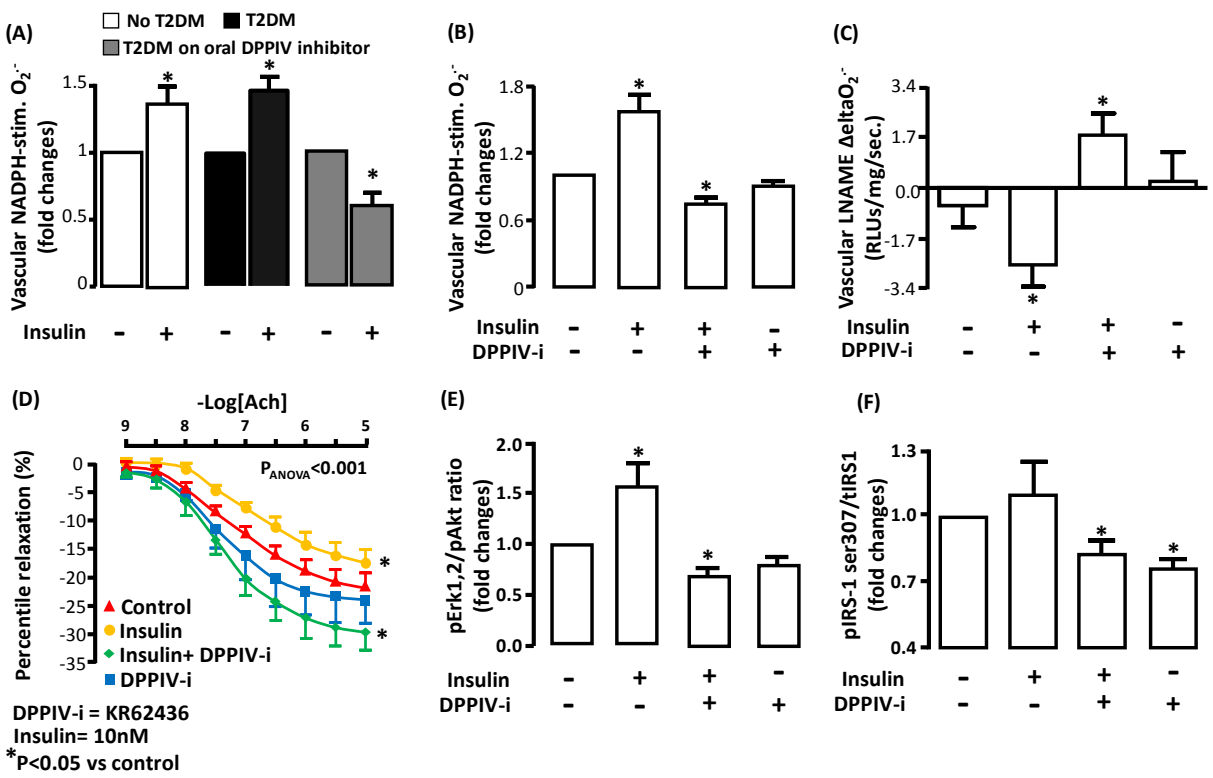
Background: Insulin may have protective roles in vascular cells, but its downstream signalling and effects on human vessels in patients with atherosclerosis are unknown. Dipeptidyl peptidase IV (DPP-IV) inhibitors are a class of insulin-sensitising agents that may regulate vascular responses to insulin.

Methods: We recruited 72 patients undergoing coronary bypass surgery. Segments of internal mammary arteries (IMA) and saphenous veins (SV) were collected and incubated with insulin glargine active metabolite M1 (insulin), insulin degludec (DEG) and human insulin (HI) (1-100nM as stated), with or without pre-incubation with KR62436 at 70μM (a chemical DPP-IV inhibitor). Vascular superoxide ($O_2^{\cdot-}$) was quantified by lucigenin chemiluminescence, where NADPH 100μM stimulation and LNAME 100μM inhibition were used to evaluate the NADPH-oxidases activity and endothelial nitric oxide (eNOS) coupling respectively. Nitric oxide bioavailability was evaluated by quantifying the vasorelaxations to acetylcholine.

Results: Insulin increased NADPH oxidases-derived $O_2^{\cdot-}$ in both IMA and SV from patients with or without diabetes, an effect reversed in vascular segments from diabetic patients pre-treated with oral DPP-IV inhibitor (A). Pre-incubation of human vessels with KR62436 (DPPIV-i), also reversed the effect of insulin on vascular $O_2^{\cdot-}$, supressing NADPH-oxidases activity and improving eNOS coupling in both SV (B and C respectively) and IMA (not shown), leading to improved endothelial function (D). This was a class effect, replicated using DEG and HI (data not shown). While insulin induced activation of vascular MAPK (Erk1&2), pre-treatment with a DPP-IV inhibitor modified downstream insulin signalling and leading to activation of Akt (E). DPP-IV inhibition reduced insulin receptor substrate 1 (IRS1) phosphorylation at Ser307, a site linked to molecular insulin resistance (F).

Conclusions: We demonstrate for the first time that insulin induces vascular oxidative stress and endothelial dysfunction in vascular segments derived from patients with atherosclerosis, independently of systemic insulin resistance. This may be one of the mechanisms underlying the clinical inability of insulin treatment to improve cardiovascular outcomes in patients with moderately elevated blood glucose. Pre-treatment with a DPP-IV inhibitor (orally or ex vivo) restores local insulin sensitivity modulating the vascular responses to insulin. These findings suggest that vascular sensitisation may be a crucial objective in the treatment of diabetic patients in secondary prevention.


(A)
No T2DM
T2DM
T2DM on oral DPPIV inhibitor
Vascular NADPH-stim. $O_2^{\cdot-}$ (fold changes)
Insulin
(B)
Vascular NADPH-stim. $O_2^{\cdot-}$ (fold changes)
Insulin
DPPIV-i
(C)
Vascular LNAME Δelta$O_2^{\cdot-}$ (RLUs/mg/sec.)
Insulin
DPPIV-i
(D)
-Log[Ach]
Percentile relaxation (%)
P_{ANOVA}<0.001
Control
Insulin
Insulin+ DPPIV-i
DPPIV-i
DPPIV-i = KR62436
Insulin= 10nM
*P<0.05 vs control
(E)
pErk1,2/pAkt ratio (fold changes)
Insulin
DPPIV-i
(F)
pIRS-1 ser307/tIRS1 (fold changes)
Insulin
DPPIV-i