



ENDOTHELIAL FIBRINOLYTIC CAPACITY IS IMPAIRED IN HIV-1-INFECTED MEN

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ABSTRACT

Background: Rates of atherosclerotic lesion development, myocardial infarction and restenosis are higher in HIV-1-seropositive adults compared with the general population. The mechanisms responsible for this heightened cardiovascular risk are not fully understood. The capacity of the endothelium to release tissue-type plasminogen activator (t-PA), the primary activator of the fibrinolytic system, is a key endogenous defense mechanism against intravascular fibrin deposition and thrombosis. We tested the hypotheses that: 1) the capacity of the vascular endothelium to release t-PA is reduced in HIV-1-seropositive treatment naïve compared with HIV-1-seronegative adults; and 2) the decrease in endothelial t-PA release in HIV-1 infected adults is due, in part, to increased oxidative stress. Methods: 10 HIV-1-seronegative (age: 36±3 yr) and 7 HIV-1-seropositive treatment naïve (34±2 yr) men were studied. All subjects were free of overt metabolic and cardiovascular disease. Net endothelial release of t-PA was determined, in vivo, in response to intrabrachial infusions of bradykinin (BK: 125-500 ng/min) and sodium nitroprusside (SNP: 2.0-8.0 mcg/min). BK was selected to stimulate endothelial t-PA release due to its effectiveness at eliciting a local and rapid response. SNP was required to establish that any observed differences in t-PA release to BK were not due to increased blood flow related shear stress. To determine the effects of oxidative stress on endothelial t-PA release, the BK and SNP dose response curves were repeated with a co-infusion of the antioxidant vitamin C (24 mg/min). Group differences were determined by repeated measures ANOVA. Results: Net endothelial t-PA release was significantly lower (~40%; P<0.05) in the HIV-1-seropositive (from -0.14±1.2 to 47.2±10.9 ng/100 mL tissue/min) compared with seronegative (from -0.43±0.9 to 75.4±11.0 ng/100 mL tissue/min) men. Vitamin C markedly increased (~30%) t-PA release in response to BK in the HIV-1-seropositive (-1.4±0.5 to 60.4±9.1 ng/100 mL tissue/min) but not seronegative (-1.3±0.9 to 73.4±11.0 ng/100 mL tissue/min) men. There was no effect of SNP on t-PA release in either group. Conclusions: The capacity of the endothelium to release t-PA is blunted in untreated HIV-1-seropositive compared with seronegative men and this dysfunction is due, in part, to oxidative stress. Diminished endothelial fibrinolytic capacity may contribute to the increased risk of atherothrombotic events in HIV-1-infected adults.

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BACKGROUND

- HIV-1 infected individuals demonstrate a two- to four-fold greater incidence of cardiovascular disease and atherothrombotic events compared with the general population.
- Impaired endothelial fibrinolytic capacity, a hallmark characteristic of endothelial dysfunction, is thought to be an underlying event in the pathogenesis of atherosclerotic vascular disease.
- It has been suggested that impaired endothelial fibrinolytic capacity may contribute to increased cardiovascular risk in HIV-1-seropositive adults.
- However, this postulate is based on plasma fibrinolytic markers. Importantly, it is the ability of the endothelium to release tissue-type plasminogen activator (t-PA) and not circulating plasma concentrations that determines endogenous thrombolytic capacity.
- Currently there is no information on the influence of untreated HIV-1 infection on the capacity of the endothelium to release t-PA.
- Accordingly, the experimental aim of this ongoing study is to determine whether endothelial regulation of fibrinolysis is impaired in HIV-1-infected adult humans; and, if so, whether oxidative stress contributes to this endotheliopathy.

HYPOTHESES

The capacity of the vascular endothelium to release t-PA is reduced in HIV-1-seropositive treatment naïve compared with HIV-1 seronegative adults. Furthermore, the decrease in endothelial t-PA release in HIV-1 infected adults is due, in part, to increased oxidative stress.

METHODS

Subjects

- 17 Adult Men
 - 10 HIV-1-Seronegative [age: 22-52 yrs]
 - 7 HIV-1-Seropositive treatment naïve [age: 28-43 yrs]

Inclusion Criteria

- normotensive
- non-diabetic
- non-medicated
- sedentary
- normolipidemic
- free of overt cardiovascular disease

HIV-1-infected adults:

- Seropositive for the HIV-1 virus for a minimum of one year. Mean duration of infection: 26.5±5.3 months.
- CD4+ T cell counts > 350 cells/mm. Mean cell number: 747±100 cells/ μ L.
- No history of antiretroviral therapy.

Screening and Testing Procedures

- Medical history with physical examination and graded exercise testing
- Fasting blood chemistries
- Body composition (dual energy X-ray absorptiometry)

Assessment of Blood Flow and Endothelial t-PA Release In Vivo

- Brachial artery and deep antecubital vein catheterized
- Forearm blood flow (FBF) measured by plethysmography in response to intra-arterial:
 - Bradykinin (BK): 12.5, 25.0 and 50.0 ng/100 mL tissue/min
 - Sodium nitroprusside (SNP): 1.0, 2.0, and 4.0 μ g/100 mL tissue/min
 - Vitamin C (Vit C): 24 mg/min for 5 minutes
 - BK + SNP dose-responses were repeated with the co-administration of Vit C
- Arterial and venous blood samples were collected simultaneously at baseline and after each drug dose (BK, SNP, Vit C, BK + Vit C, SNP + Vit C)
 - Plasma concentrations of t-PA antigen were measured by ELISA.
- Net endothelial release (or uptake) of t-PA and PAI-1 at each time point was calculated as follows:

$$\text{Net Release} = (C_V - C_A) \times (\text{FBF} \times \left[\frac{101 - \text{Hematocrit}}{100} \right])$$

$(C_V - C_A)$ = arteriovenous concentration gradient
 C_V = venous concentration C_A = arterial concentration

STATISTICAL ANALYSIS

Group differences in subject characteristics and area under the curve data were determined by one-way analysis of variance (ANOVA). Changes in net release of t-PA antigen across the forearm in response to BK, SNP, and BK + Vit C and SNP were determined by repeated measures ANOVA. The main effect of Vit C was determined by two-way repeated measures ANOVA. All data are presented as mean \pm SEM. Statistical significance was set *a priori* at P < 0.05.

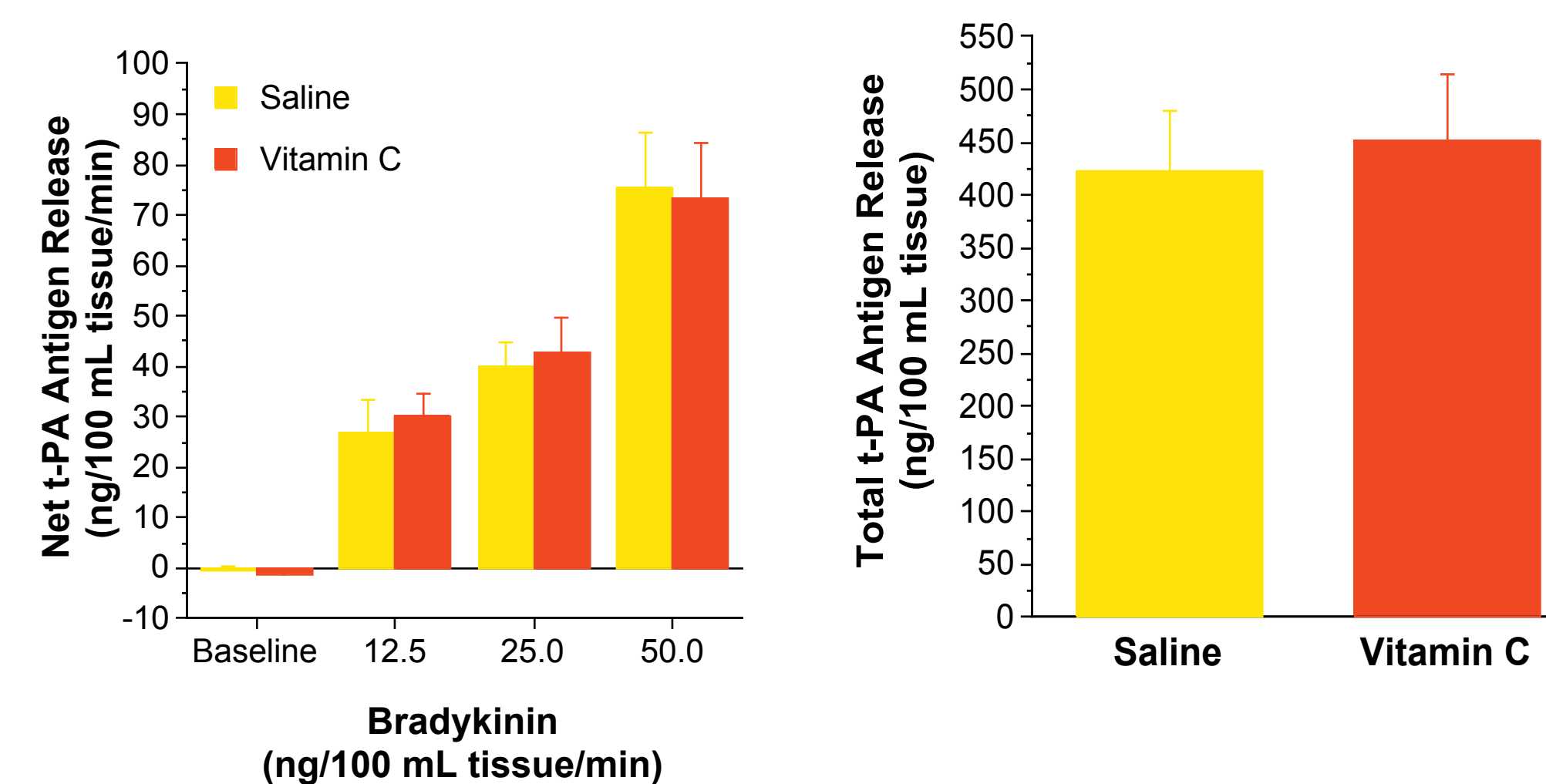
RESULTS

I. Subject characteristics

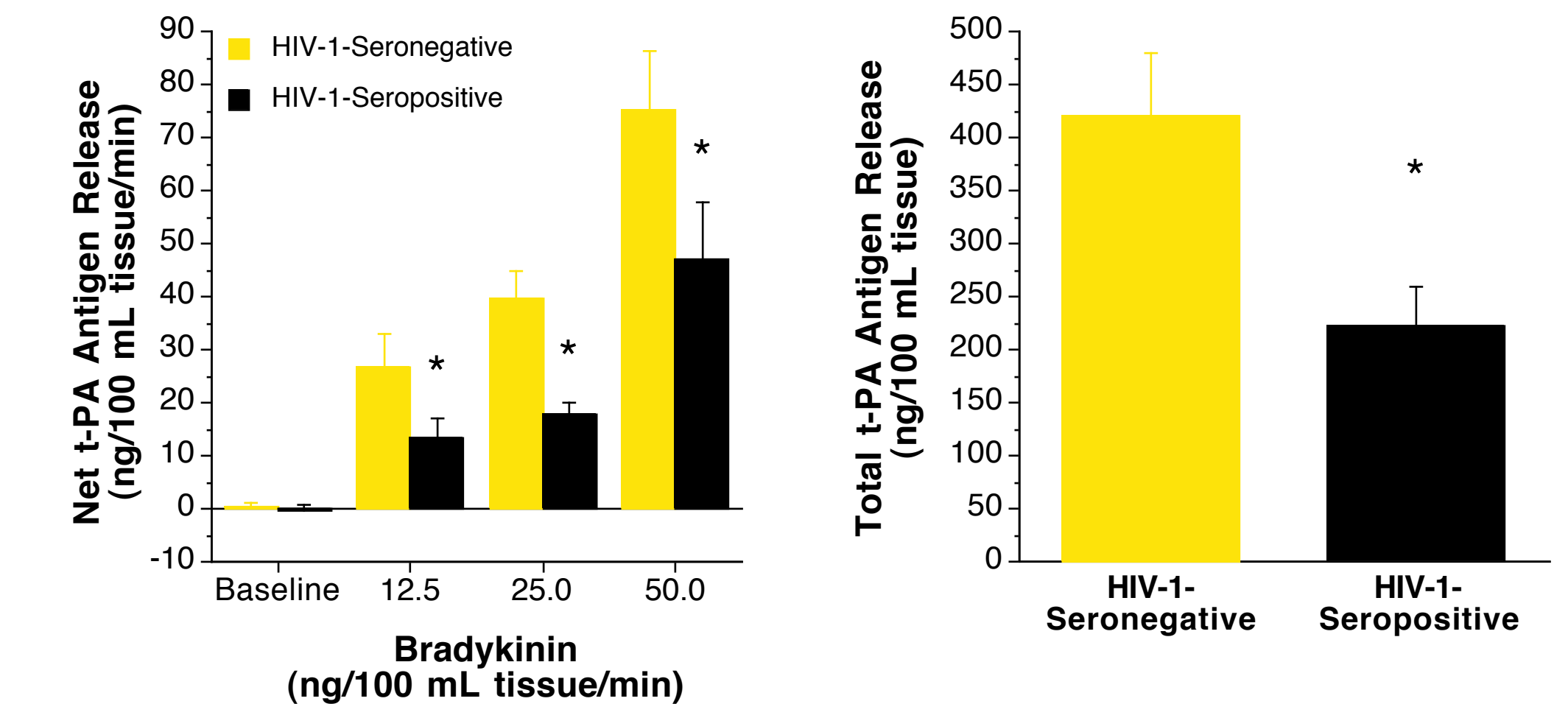
Variable	HIV-1-Seronegative (n=10)	HIV-1-Seropositive (n=7)
Age, yr	36 \pm 3	34 \pm 2
BMI, kg/m ²	26 \pm 2.1	25.7 \pm 0.9
Body mass, kg	80.9 \pm 4.7	76.1 \pm 6.1
Body fat, %	19.4 \pm 2.1	24.8 \pm 4.3
Waist circumference, cm	86.6 \pm 2.8	87.3 \pm 4.5
Systolic BP, mmHg	119 \pm 3	118 \pm 4
Diastolic BP, mmHg	72 \pm 3	78 \pm 3
Total cholesterol, mmol/L	4.2 \pm 0.3	4.6 \pm 0.5
LDL-cholesterol, mmol/L	2.7 \pm 0.2	2.8 \pm 0.4
HDL-cholesterol, mmol/L	1.1 \pm 0.1	0.9 \pm 0.1
Triglycerides, mmol/L	1.0 \pm 0.1	2.0 \pm 0.4*
Glucose, mmol/L	4.7 \pm 0.1	5.1 \pm 0.2
Insulin, pmol/L	38.2 \pm 0.6	70.1 \pm 18.1
HOMA-IR	0.9 \pm 0.2	2.4 \pm 0.6*

Values are mean \pm SEM; *P < 0.05 vs. HIV-1-seropositive.

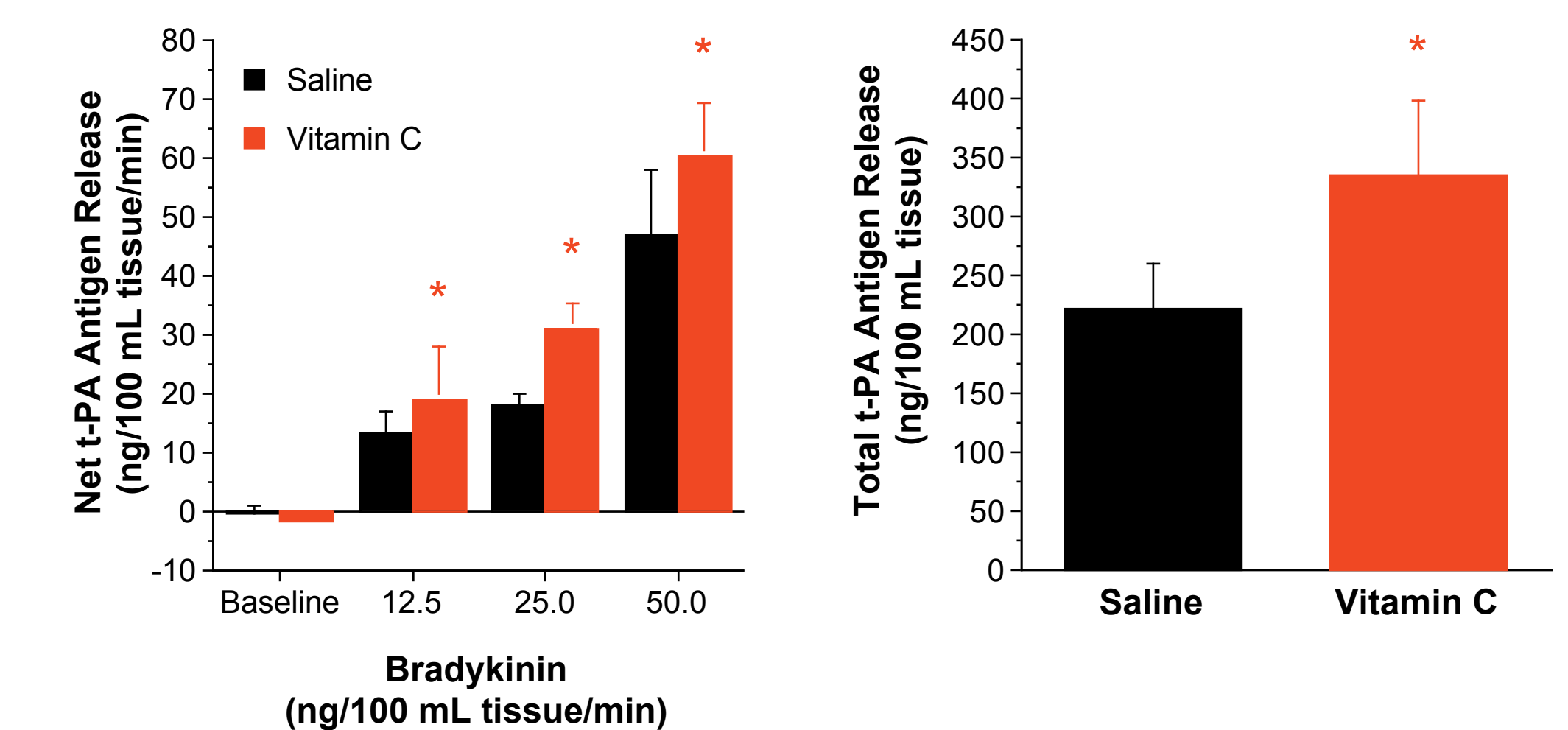
III. Acute vitamin C administration did not significantly increase endothelial t-PA release to BK in HIV-1-seronegative men.



II. Net release rate and total t-PA released (area under the curve) in response to BK was ~40% lower in the HIV-1-seropositive treatment naïve compared with HIV-1-seronegative men. There was no effect of SNP on t-PA release in either group.



IV. In contrast to the HIV-1-seronegative men, acute vitamin C administration significantly increased (~30%) endothelial t-PA release to BK in HIV-1-seropositive men. *P < 0.05 vs HIV-1



CONCLUSIONS

These results indicate that the capacity of the endothelium to release t-PA is blunted in HIV-1-seropositive treatment naïve men compared with HIV-1-seronegative men. This is due, in part, to oxidative stress. Diminished endothelial fibrinolytic capacity may contribute to the increased risk of atherothrombotic events in HIV-1-infected adults.