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Vascular Actions of Angiotensin 1–7 in the Human Microcirculation – Novel Role for Telomerase

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Abstract

Objective—This study examined vascular actions of angiotensin 1–7 (ANG 1–7) in human atrial and adipose arterioles.

Approach and Results—The endothelial-derived hyperpolarizing factor of flow mediated dilation (FMD) switches from anti-proliferative nitric oxide (NO) to pro-atherosclerotic hydrogen peroxide (H_2O_2) in arterioles from humans with coronary artery disease (CAD). Given the known vasoprotective properties of ANG 1–7, we tested the hypothesis that overnight ANG 1–7 treatment restores the NO-component of FMD in arterioles from CAD patients. Endothelial telomerase activity is essential for preserving the NO-component of vasodilation in the human microcirculation, thus we also tested whether telomerase activity was necessary for ANG 1–7 mediated vasoprotection by treating separate arterioles with ANG 1–7 ± the telomerase inhibitor BIBR-1532. ANG 1–7 dilated arterioles from patients without CAD, whereas dilation was significantly reduced in arterioles from CAD patients. In atrial arterioles from CAD patients incubated with ANG 1–7 overnight, the NO synthase inhibitor L-NAME abolished FMD while the H_2O_2 scavenger PEG catalase had no effect. Conversely, in vessels incubated with ANG 1–7 + BIBR-1532, L-NAME had no effect on FMD but PEG catalase abolished dilation. In cultured human coronary artery endothelial cells, ANG 1–7 significantly increased telomerase activity.

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These results indicate that ANG 1–7 dilates human microvessels, and dilation is abrogated in the presence of CAD. Further, ANG 1–7 treatment is sufficient to restore the NO component of FMD in arterioles from CAD patients in a telomerase-dependent fashion.

Conclusion—ANG 1–7 exerts vasoprotection in the human microvasculature via modulation of telomerase activity.

Keywords

Angiotensin 1–7; Vascular; Telomerase; Vasodilation; Coronary Artery Disease

Introduction

The vasoactive peptide angiotensin 1–7 (ANG 1–7) has been widely studied over the past two decades. Largely the protective actions of ANG 1–7 oppose the pro-oxidative, proliferative, and pressor actions of angiotensin II (ANG II), making it an attractive target for cardioprotective therapies. ANG 1–7 has been demonstrated to be a potent vasodilator in multiple vascular beds; however the majority of these studies have been performed in rodents. The vasodilator properties of ANG 1–7 have scarcely been described in humans, and those reports that do exist are conflicting. For example, Sasaki et al. have shown that intra-arterial infusion of ANG 1–7 significantly increases forearm blood flow in healthy subjects and that the response is blunted in hypertensive subjects;[1] while Wilsdorf et al conversely showed that intra-brachial ANG 1–7 infusion had no effect on forearm blood flow and also had no potentiating effect on bradykinin-induced vasodilation.[2] In this study, we first sought to determine if ANG 1–7 causes vasodilation in the human coronary and peripheral microcirculations, and if this response is altered in patients with coronary artery disease (CAD).

We have previously demonstrated that human atrial and adipose arterioles from subjects with CAD invoke a compensatory vasodilator pathway in response to increased intraluminal flow (flow mediated dilation; FMD), and that this pathway releases endothelial-derived hydrogen peroxide (H_2O_2) rather than nitric oxide (NO) as the vasoactive mediator.[3–6] The long-term consequences of invoking a vasodilator pathway which repeatedly generates H_2O_2, a molecule which favors a proliferative and oxidative state in the endothelium, vs. NO, a molecule which is anti-proliferative and anti-inflammatory, remains unknown. However, it could be surmised that long term exposure to H_2O_2 in the vascular milieu exacerbates an already damaged vascular endothelium, thus it may be beneficial to restore NO-mediated vasodilation to promote vascular homeostasis. Previous studies have shown plasma ANG 1–7 levels positively correlate with brachial artery FMD in human subjects[7], and recent findings by Zhang et al. demonstrated that treating renal arterioles from diabetic patients with ANG 1–7 overnight restores acetylcholine mediated dilation.[8] In light of these findings, we also tested the hypothesis that exposing human atrial vessels from subjects with CAD to ANG 1–7 would restore the NO-component of the FMD response.

Telomerase is a ribonucleoprotein that has been classically described as the protein responsible for elongating telomeres at the ends of chromosomes and is a regulator of cellular senescence. However, emerging evidence indicates that telomerase also has non-

nuclear roles, which include protecting against mitochondrial dysfunction.[9, 10] Recently, telomerase activity has been shown to be critically involved in maintaining the NO-component of vasodilation in atrial and adipose microvessels from humans with CAD.[11] While maintaining telomerase activity may be vital to preserving the NO component of FMD in the human microcirculation, the regulation of telomerase in the human vasculature is not well understood. Pro-atherosclerotic factors such as ANG II have been shown to down-regulate telomerase activity in a number of cell types, including the endothelium, by mechanisms directly related to ROS accumulation.[12–14] Conversely, the effect of ANG 1–7 on telomerase activity has not been described. Since ANG 1–7 largely opposes the effects of ANG II, and in light of the recent finding that telomerase activity is essential in maintaining NO-mediated dilation,[11] we hypothesized that ANG 1–7 exerts vasoprotective actions in the human microcirculation by increasing vascular telomerase activity.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Adipose and atrial resistance arterioles were obtained from 34 subjects with a clinical diagnosis of CAD and 16 subjects without known CAD. The age, sex, body mass index (BMI), location of adipose tissue, and underlying conditions of the subjects are shown in Table 1. There was no age difference between the subjects in the CAD vs. the non-CAD group (66±1.7 years vs. 64±3.5 years, respectively; $P>0.05$; t-test). A total of 45 vessels were used for analysis of vasodilation in this study. The vessels had an average internal diameter of 166±8.8 μm and were constricted to 40±1.3 % of their maximal diameter prior to assessing vasodilation. The size of vessels used between CAD and non-CAD tissues was not different, nor was the percent constriction ($P>0.05$; t-test).

ANG 1–7 vasodilation is mediated via the Mas receptor in the human microcirculation

ANG 1–7 dilated both human adipose and atrial microvessels from subjects without CAD in a dose-dependent manner (Figure 1). Importantly, previous studies have shown human plasma concentrations of ANG 1–7 to be in the range of 1–50 pmol/L, thus the concentrations of ANG 1–7 which caused measurable vasodilation were physiological.[15–18] Dilation to ANG 1–7 was also mediated by Mas receptor activation as pre-incubation with the Mas receptor antagonist A779 significantly reduced dilation in both vascular beds. Vasodilation to the direct smooth muscle activator papaverine (10^{-4} mol/L) was unaffected by the presence of A779 (≥95% dilation in all groups, $P>0.05$ between groups; t-test; data not shown).

ANG 1–7 mediated vasodilation is reduced in microvessels from subjects with CAD

As shown in Figure 2, ANG 1–7 mediated vasodilation was significantly lower in atrial and adipose microvessels from subjects with CAD compared to subjects without CAD. These findings are consistent with studies performed in rodent models of cardiovascular disease which show reduced ANG 1–7 mediated vasodilation in the aorta of spontaneously

hypertensive rats[19] and in the coronary arteries from a rat model of cardiac hypertrophy[20]. The difference in ANG 1–7 mediated dilation in microvessels from CAD and non-CAD subjects is likely not due to reduced Mas receptor density, as the receptor is robustly expressed in both the endothelial and vascular smooth muscle cell layer of vessels from both CAD and non-CAD subjects (Supplemental Figure I). Dilation to papaverine was unaffected by CAD (≥95% dilation in both CAD and non CAD vessels, $P>0.05$; t-test; data not shown).

ANG 1–7 prevents flow-induced mitochondrial H_2O_2 generation in atrial vessels from CAD subjects

To measure mitochondrial H_2O_2 generation, the fluorescent dye MitoPY1 was used. It has previously been shown that mitochondrial H_2O_2 is the primary mediator of FMD in adipose and coronary arterioles from subjects with CAD.[3, 5, 6, 11, 21] This was confirmed in Figure 3 because when flow was induced through the lumen of vehicle-treated, cannulated coronary arterioles from subjects with CAD, a significant increase in MitoPY1 fluorescence was observed. Conversely, the increase in MitoPY1 fluorescence was attenuated in vessels treated with ANG 1–7 overnight or vehicle-treated vessels that had the H_2O_2 scavenger PEG catalase added to the organ bath. To confirm the specificity of MitoPY1 for mitochondrial ROS, the mitochondrial electron transport chain uncoupler antimycin A caused a time-dependent increase in MitoPY1 fluorescence in separate vessels (Supplemental Figure III).

ANG 1–7 restores physiological NO-mediated vasodilation in a telomerase-dependent manner

Our group has previously shown that the magnitude of FMD in adipose and atrial arterioles from subjects with CAD is similar compared to subjects without CAD, however the mediator of vasodilation switches from NO to H_2O_2 during CAD.[3–6] This finding was confirmed in Figure 4A, as PEG Catalase inhibited FMD in atrial vessels from patients with CAD, while L-NAME had a potentiating effect on the magnitude of FMD. The effect of L-NAME to potentiate dilation is not altogether unexpected and may be explained as eNOS-derived NO is a physiological regulator of mitochondrial oxygen consumption and ROS formation in endothelial cells.[22–24] The FMD response in atrial arterioles from CAD subjects requires mitochondrial H_2O_2 as the vasoactive mediator of dilation, thus in the absence of NO, shear-induced mitochondrial ROS formation may increase more than when physiological levels of NO are present, resulting in potentiated dilation as observed in Figure 4A.

As shown in Figure 4B, overnight incubation with ANG 1–7 (10^{-9} mol/L, the approximate ED_{50} of ANG 1–7 in human microvessels; Figure 1) restored the NO component of FMD as evidenced by the observation that L-NAME abrogated vasodilation while the H_2O_2 scavenger PEG-catalase had no effect. The effect of ANG 1–7 to restore the NO-component of dilation was abolished when the arterioles were co-incubated with the specific telomerase inhibitor BIBR-1532 (Figure 4C), indicating that the protective effects of ANG 1–7 require telomerase activity. Incubation with BIBR-1532 alone had no effect on the mediator of FMD as PEG-catalase significantly reduced FMD (Figure 4D), similar to untreated atrial vessels from CAD patients. BIBR-1532 is the most specific pharmacological inhibitor of human TERT currently available (reviewed by Phatak and Burger),[25] and cytotoxicity in TERT-

treated cells has not been observed using the dose reported here.[26] Further, BIBR-1532 does not directly affect eNOS mRNA, protein expression, or nitric oxide levels in human endothelial cells,[11] or have an acute effect on mitochondrial ROS in human microvessels (Supplemental Figure III), thus the effects of BIBR-1532 on the mechanism of FMD are most likely due to its telomerase-inhibiting effects and not off-target effects which neither reduce eNOS expression/activity and acutely increase mitochondrial ROS.

Effects of ANG 1–7 on endothelial TERT expression and telomerase activity

As shown in Figure 5A, TERT mRNA is significantly increased in cultured human coronary artery endothelial cells (HCAECs) treated overnight with ANG 1–7 (16–20 hours, 10^{-9} mol/L). ANG 1–7 treatment also caused a significant increase in telomerase activity in cultured HCAECs as assessed and quantified by modified TRAP assay using ddPCR (Figure 5B). ANG 1–7 treatment also caused a significant increase in TERT protein expression as assessed by Western Blot analysis (Figure 5C). Together these data indicate that ANG 1–7 increases telomerase activity by increasing transcription and translation of TERT, the catalytic subunit of telomerase.

Discussion

There are four novel findings in this study. First, we show that ANG 1–7 is a potent vasodilator in both the human coronary and peripheral microcirculations, and that this response is dependent on the Mas receptor. Second, ANG 1–7 exerts cardioprotective effects by restoring the NO-component of FMD in coronary microvessels from humans with CAD. These vessels typically invoke compensatory H_2O_2-mediated vasodilation in response to increased flow.[3–6] The observed changes induced by ANG 1–7 are endothelial in nature as smooth muscle function (tested by papaverine) was not altered. Third, the protective actions of ANG 1–7 on the human endothelium require telomerase activity, as the protective effects of ANG 1–7 are completely inhibited in the presence of BIBR-1532, a specific inhibitor of telomerase activity. Lastly, the mechanism of action of ANG 1–7 appears to be via increased telomerase activity, which is induced (at least in part) via transcriptional activation of TERT, the catalytic subunit of telomerase.

Our understanding of the role of the renin-angiotensin system (RAS) in cardiovascular homeostasis is continually evolving, but the general consensus is the vasoprotective ACE2 – ANG 1–7 – Mas receptor axis opposes many of the actions of the classically defined ACE – ANG II – angiotensin type 1 receptor axis (Figure 6). Much of the data which examines the beneficial cardiovascular actions of the ANG 1–7 – Mas receptor axis comes from animal models, with a limited number of studies evaluating the direct actions of ANG 1–7 in humans. An important observation made by Zisman et al. demonstrated a putative role for ANG 1–7 in the human heart as intracoronary infusion of ANG I (a precursor for both ANG II and ANG 1–7) resulted in a marked increase in ANG 1–7.[27] Another important observation from that study was that ANG 1–7 generation from ANG I was dependent on ANG II as a substrate, as ANG 1–7 formation was suppressed by co-administration of enalaprilat, an ACE inhibitor. While that study didn’t examine a functional role for ANG 1–

7, it importantly showed that formation of ANG 1–7 also results in a net reduction of ANG II in the human heart.

ACE inhibitors are a front line therapeutic to combat high blood pressure and congestive heart failure, and much of the blood pressure lowering and cardioprotective effects of ACE inhibitors are attributed to their ability to lower plasma ANG II levels. Interestingly, a number of studies have shown that ACE inhibitors also dramatically increase plasma ANG 1–7 levels (between 5- and 25-fold) in both animals[28–31] and humans[32]. To what degree the beneficial effects of ACE inhibitors can be attributed to their ANG II lowering effects vs. their ability to increase ANG 1–7 levels remains to be determined. Interestingly, in hypertensive humans treated with captopril, subjects who had the largest increases in plasma ANG 1–7 concentrations also had the greatest reductions in diastolic blood pressure.[32] Our results show clear evidence that ANG 1–7 both potently dilates the human coronary microcirculation and exerts a chronic vasoprotective role by promoting NO-mediated vasodilation during disease. Taken together with the recent study by Zhang et al. which showed that exogenous ANG 1–7 treatment restores vasodilation in renal arterioles from diabetic patients,[8] it is conceivable that increased ANG 1–7 levels in the plasma during ACE inhibitor therapy may significantly contribute to their cardiovascular benefit.

ANG 1–7 signaling has been shown to be primarily mediated via the Mas receptor; however numerous studies have shown that ANG 1–7 can also signal through the AT_2 receptor,[33–35] most likely through transactivation of the receptor as the binding affinity of ANG 1–7 to the AT_2 receptor is low.[36, 37] Furthermore, it has been also shown that while ANG 1–7 has an even lower binding affinity for the AT_1 receptor,[36] some effects of ANG 1–7 can be mediated through a binding site which is losartan-sensitive, suggesting ANG 1–7 may also signal via the ACE – ANG II – AT_1 receptor axis under certain conditions.[38, 39] The contributions of the AT_1 and AT_2 receptors to ANG 1–7-mediated vasodilation were not assessed in this study, thus we cannot say with certainty that these receptor subtypes do not partially contribute to ANG 1–7 mediated signaling in the human microvasculature. A779, a specific Mas-receptor antagonist, inhibited vasodilation >50% in both atrial and adipose arterioles however, indicating that the Mas receptor is primarily responsible for effecting the actions of ANG 1–7 in these vascular beds.

Our findings that ANG 1–7 both exerts vasoprotection in a telomerase-dependent manner and directly increases telomerase activity in the human endothelium, coupled with the recent observation that telomerase activity is necessary to maintain NO-mediated dilation in the human microcirculation,[11] suggests that the ACE2 – ANG 1–7 – Mas receptor axis of the RAS may be critically involved in promoting NO-mediated dilation in the human heart via modulation of telomerase activity. The direct mechanism by which ANG 1–7 modulates telomerase activity remains to be elucidated, but it is important to note that NO activates endothelial cell telomerase activity,[40, 41] and increased telomerase activity elevates NO production and eNOS expression,[11] suggesting a potential feed-forward mechanism. Thus, studies aimed at elucidating the direct role of ANG 1–7-mediated NO generation on modulating telomerase activity would be valuable.

If telomerase sits at a critical junction to regulate vascular health,[11, 42] it is likely that ANG 1–7 is one of many positive regulators of endothelial cell telomerase activity. For example, peroxisome proliferator-activated receptor-γ (PPAR-γ) activators (thiazolidinediones, TZDs) are well studied agents which increase endothelial nitric oxide,[43, 44] improve vascular function in humans,[45, 46] and prevent atherosclerotic disease progression.[47] While TZDs suppress telomerase expression and activity in proliferating cell types,[48, 49] the opposite is observed in endothelial cells,[50] which are non-proliferating cells. In support of this, *ex vivo* rosiglitazone treatment is also sufficient to restore NO-mediated vasodilation in CAD adipose arterioles in a BIBR-1532-inhibitable manner (Supplemental Figure IV). There is also evidence that chronic ANG 1–7 treatment increases PPAR-γ expression in spontaneously hypertensive rats,[51] thus it is possible that PPAR-γ may serve as a downstream signaling effector through which ANG 1–7 modulates endothelial cell telomerase activity.

As our data indicates, the increase in endothelial cell telomerase activity is due to a transcriptional increase in TERT, the catalytic subunit of telomerase. There are several regulatory layers which modulate TERT protein expression and activity, including transcriptional,[52, 53] post-transcriptional,[54] and epigenetic[55] modifiers. Complete understanding of these pathways is still not defined (reviewed by Akincilar, Unal and Tergaonkar)[56]. Future studies to decipher these complex interactions and their contribution to the development of cardiovascular disease are needed to completely understand how ANG 1–7, via TERT, contributes to the regulation of vascular tone in health and disease.

Study Limitations

It cannot be said with certainty if the difference in the magnitude of ANG 1–7 mediated dilation in vessels from CAD and non-CAD subjects is due to the presence of CAD itself, or a combination of cardiovascular risk factors which contributed to the onset of the disease. For example, of the CAD subjects, 27 had hypertension, which is known to reduce ANG 1–7 mediated dilation in animals.[57] To determine which cardiovascular diseases reduce ANG 1–7 mediated dilation in the human microvasculature, larger, prospective studies would need to be undertaken. Similarity, a larger study would need to be performed to determine if age, sex, or adipose location affects microvascular dilation to ANG 1–7.

We also did not test the time-course needed for ANG 1–7 to restore NO-mediated vasodilation in this study. Faria-Silva and colleagues showed in conscious Wistar rats that infusion of either ANG 1–7 or the Mas-receptor agonist AVE0991 for as little as 30 minutes is sufficient to improve endothelial function in a NO-dependent manner.[58] Overnight treatment with ANG 1–7 was chosen for our *ex-vivo* studies to allow for sufficient time for transcription and translation of proteins which could be modulated by ANG 1–7 (such as TERT). While a full examination of these mechanisms is beyond the scope of this study, it is important to note that treatment with ANG 1–7 for as little as 16–20 hours was sufficient to reverse a phenotype which took years to decades to develop – i.e. CAD and the associated switch in vasodilator mechanism which favors H_2O_2.

Due to the limited size of the human microvessels used in this study (each vessel has an approximate wet weight of 10 μg) we cannot accurately measure telomerase activity in the

vessels. As a surrogate, we demonstrated that ANG 1–7 increases both TERT mRNA and protein expression, as well as telomerase activity in cultured HCAECs. Because of the inability to accurately measure telomerase activity in human arterioles, we are also unable to say with certainty that telomerase activity was inhibited by BIBR-1532; however, the dose which was used (10 μM) is more than 100-fold greater than the reported EC_{50} by Damm and colleagues (93 nM).[59] It is also important to note that BIBR-1532 was used at doses up to 50 μM in that study and no cytotoxic effects were observed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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List of Abbreviations

ACE	Angiotensin Converting Enzyme
ANG	Angiotensin
AT1	Angiotensin Type 1 Receptor
BIBR-1532	2-[[(2E)-3-(2-Naphthalenyl)-1-oxo-2-butenyl1-yl]amino]benzoic acid
BMI	Body Mass Index
BSA	Bovine Serum Albumin
CAD	Coronary Artery Disease
cDNA	Complimentary Deoxyribonucleic Acid
ddPCR	Droplet Digital Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
eNOS	Endothelial Nitric Oxide Synthase
FMD	Flow Mediated Dilation
H_2O_2	Hydrogen Peroxide
HCAEC	Human Coronary Artery Endothelial Cells
HPLC	High Performance Liquid Chromatography
L-NAME	NG-Nitro-L-arginine methyl ester

MitoPY1	Mitochondria peroxy yellow 1
NEP	Neutral Endopeptidase
NO	Nitric Oxide
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
PPAR-γ	Peroxisome Proliferator-Activated Receptor-γ
RCF	Relative Centrifugal Force
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RT-qPCR	Reverse Transcription Polymerase Chain Reaction
TERT	Catalytic Subunit of Telomerase
TRAP	Telomeric Repeat Amplification Protocol
TZD	Thiazolidinediones

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Highlights

- This study is the first to show that ANG 1–7 causes vasodilation in the human coronary and peripheral microcirculations and promotes NO-mediated dilation in coronary arterioles from subjects with CAD.
- These effects are mediated by increasing telomerase activity.
- Based on its high vasodilator potency and attenuation with disease, these findings indicate that ANG 1–7 may have an important physiological role in human cardiovascular pathophysiology. Thus, pharmacological targeting of the ACE2 – ANG 1–7 – Mas receptor axis of the RAS may promote NO-mediated vasodilation during cardiovascular disease.

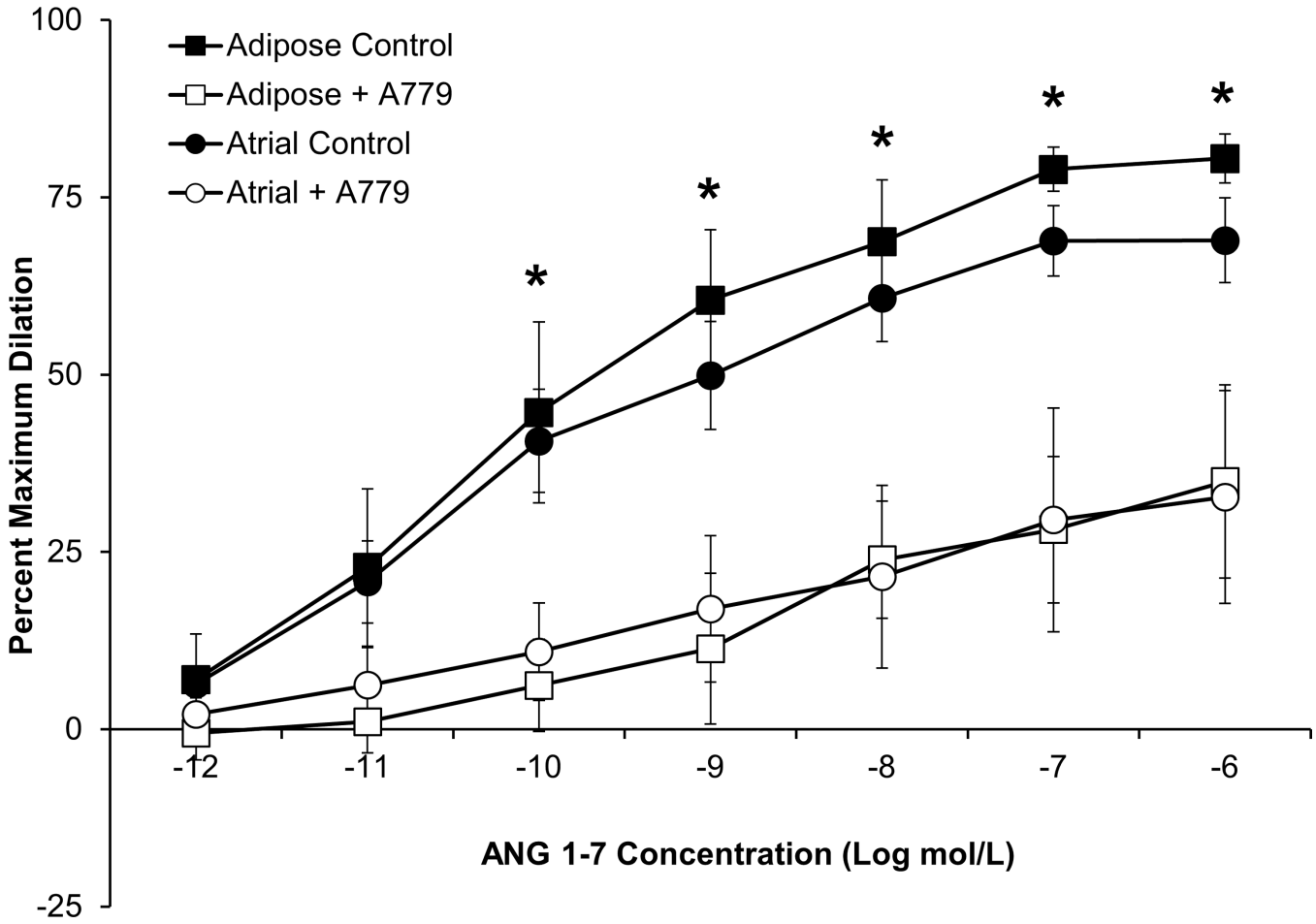

Figure 1.

ANG 1–7 dilated atrial and adipose arterioles from human subjects without CAD. The Mas receptor antagonist A779 (10^{-5} mol/L) significantly reduced the vasodilator response to ANG 1–7 in both vessel types. n=5 atrial, n=4 adipose. *P<0.05 vs. A779 treated. A779 vessels were paired with the control responses. n=number of vessels, each from a different individual.

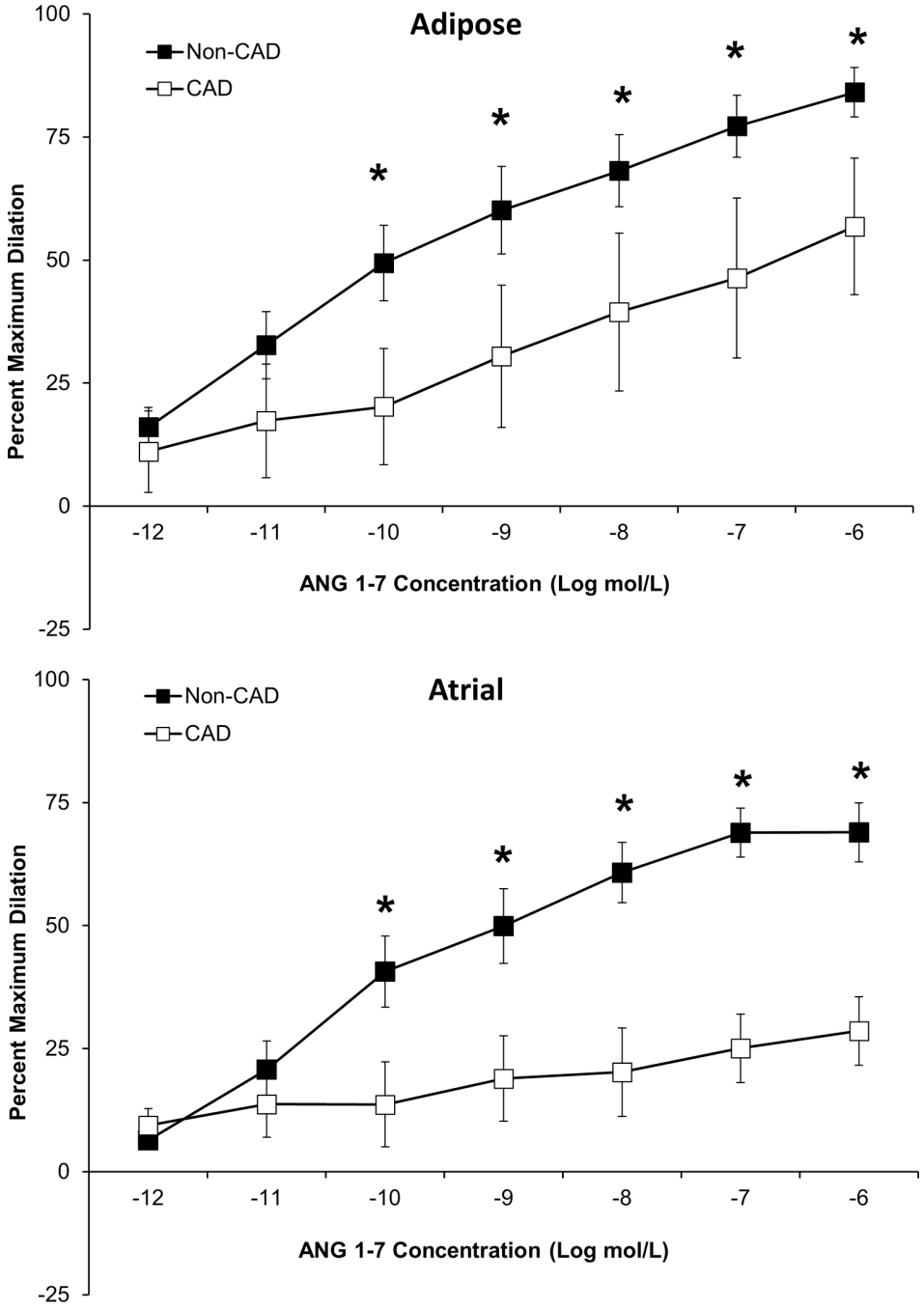

Figure 2.

ANG 1–7 dilated atrial (n=5) and adipose (n=8) arterioles from human subjects without CAD while the response was significantly abrogated in arterioles from subjects with CAD. CAD atrial n=4 and CAD adipose n=5. n=number of vessels, each from a different individual. *P<0.05 vs. CAD.

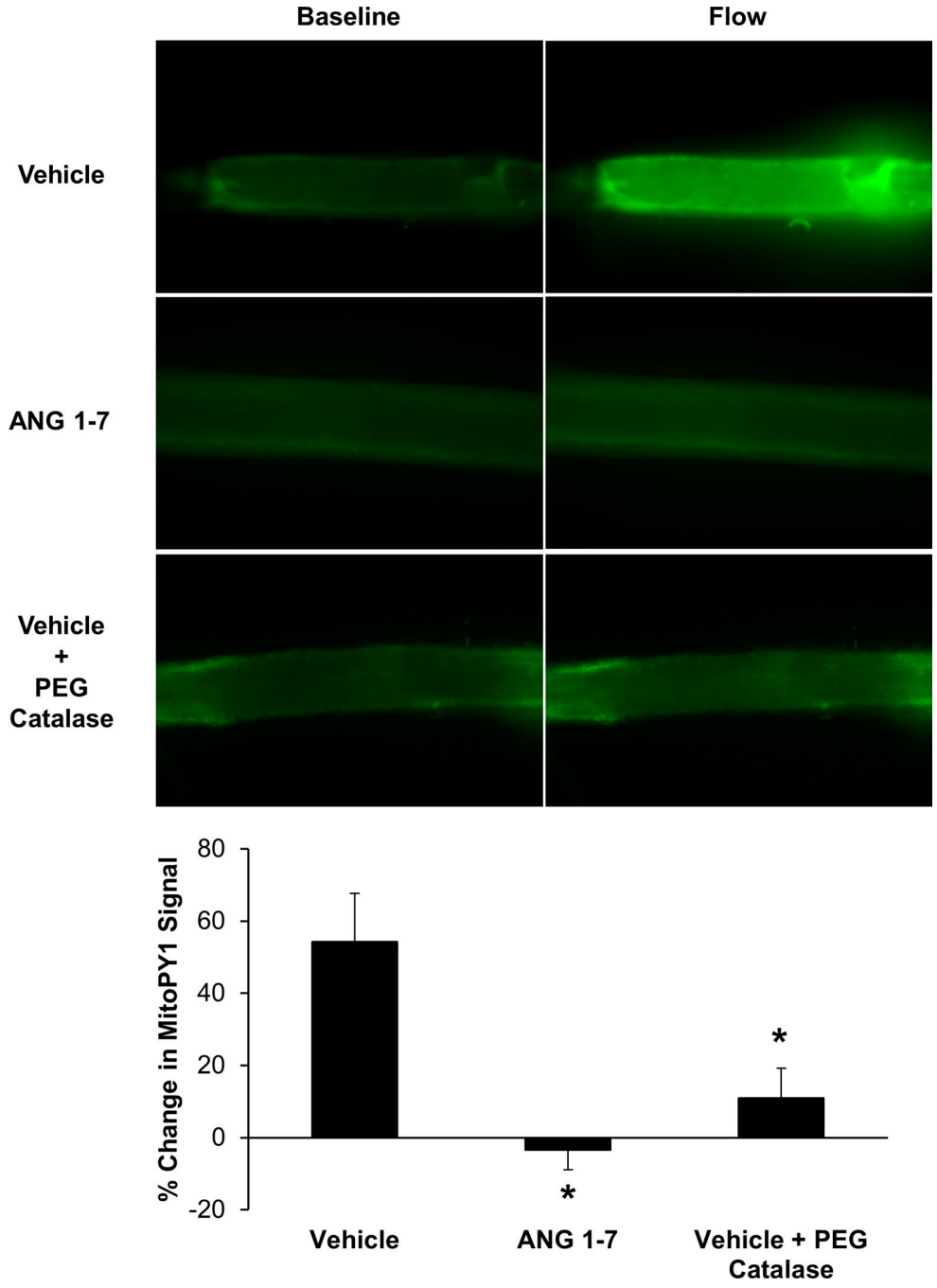

Figure 3.

(A) Representative images of cannulated human atrial arterioles from subjects with CAD with MitoPY1, a fluorescent probe specific for mitochondrial H_2O_2, in the lumen before and after intraluminal flow was induced. **(B)** Vehicle-treated atrial vessels from CAD subjects (n=7) showed a significant increase in MitoPY1 fluorescence after flow compared to arterioles that were treated with ANG 1–7 overnight (n=5) or vehicle treated vessels which had PEG-catalase added to the organ bath (n=5). n=number of vessels, each from a different individual. *P<0.05 vs. Vehicle.

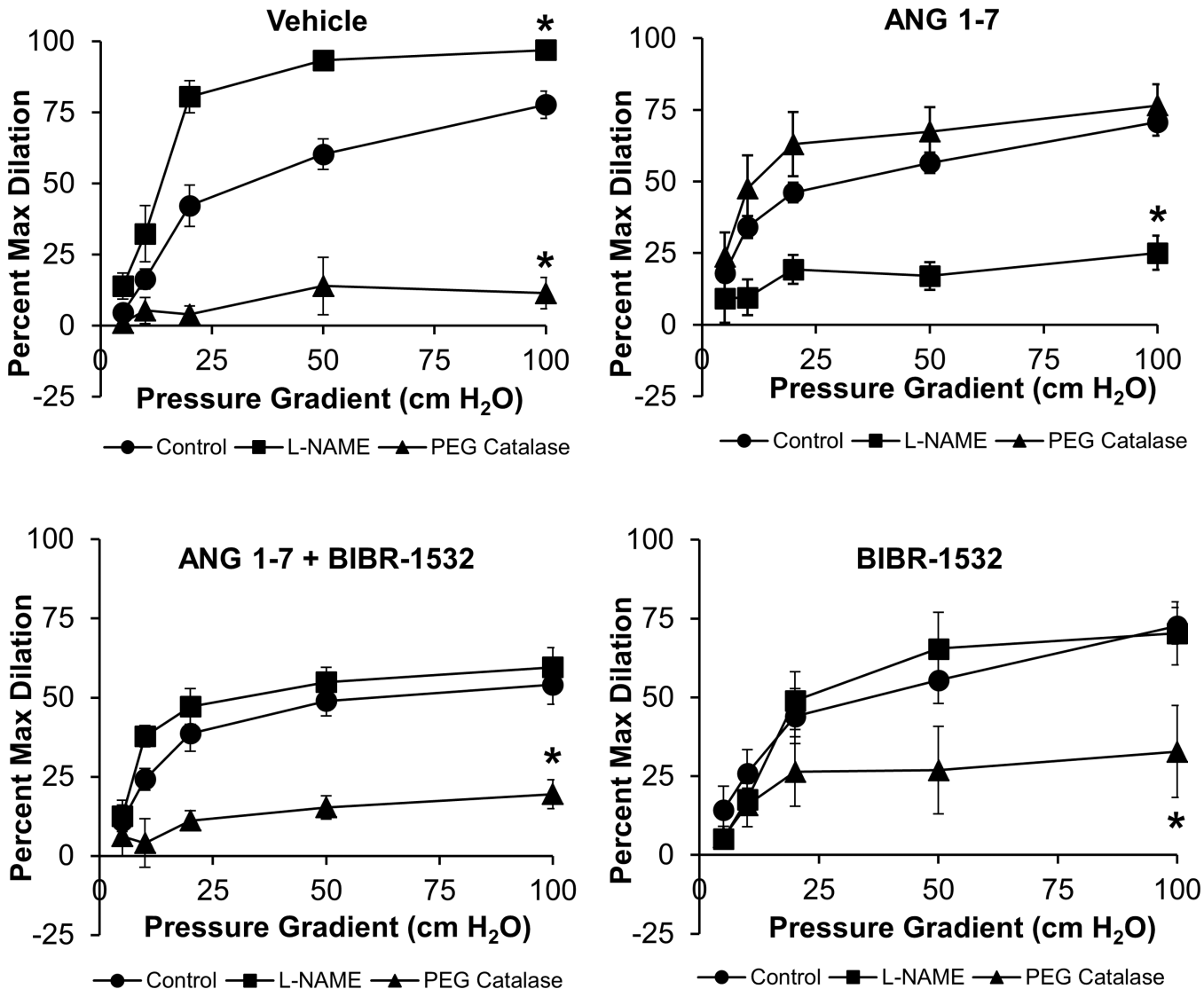

Figure 4.

(A) In human atrial vessels from CAD subjects incubated overnight in cell culture media + PBS vehicle, PEG-catalase significantly reduced FMD while L-NAME potentiated dilation, indicating that the primary mediator of vasodilation was H_2O_2. **(B)** Overnight treatment with ANG 1–7 (10^{-9} mol/L) restored the NO-component of FMD in atrial arterioles from subjects with CAD as L-NAME significantly reduced dilation while PEG catalase had no effect. **(C)** ANG 1–7 no longer restored the NO-component of dilation when vessels were co-treated with the telomerase inhibitor BIBR-1532 (10^{-5} mol/L) as L-NAME had no effect on dilation while PEG catalase significantly reduced dilation. **(D)** BIBR-1532 treatment alone had no effect on the mediator of FMD as PEG-catalase reduced FMD similarly to untreated vessels. n=4–6, all groups. n=number of vessels, each from a different individual. *P<0.05 vs. Control.

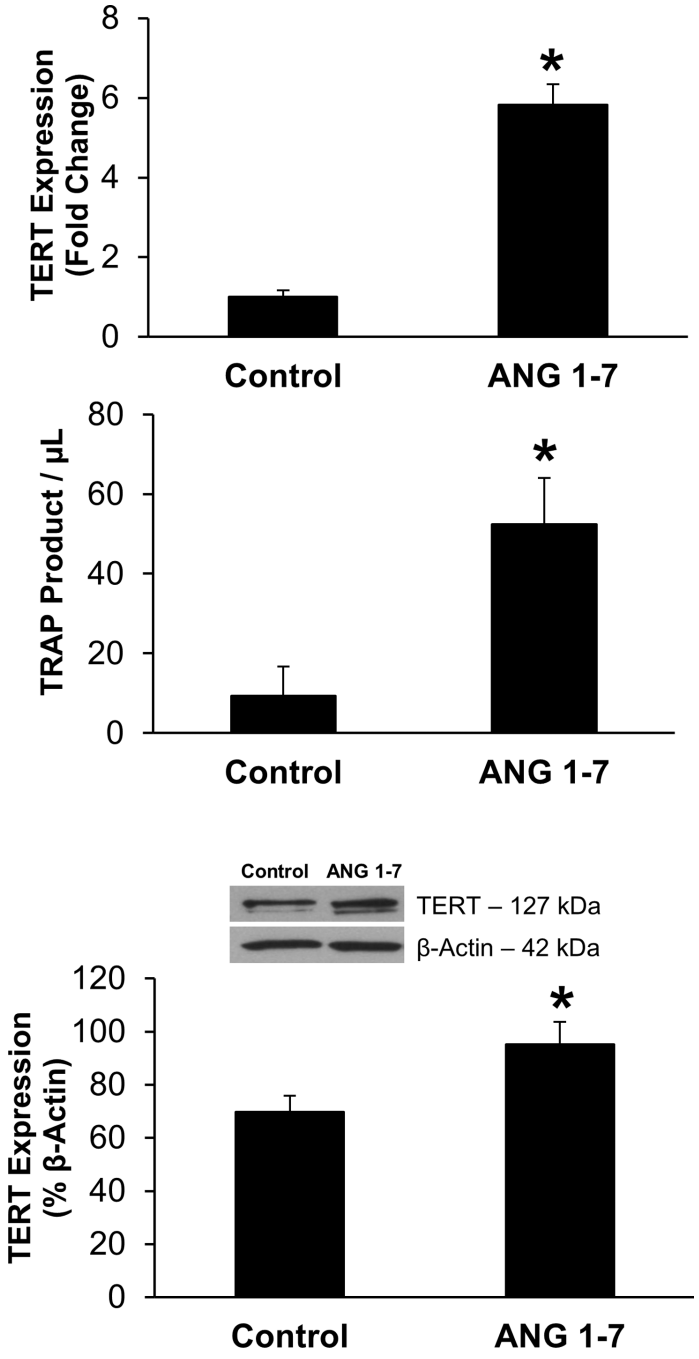

Figure 5.

(A) Overnight ANG 1–7 treatment (10^{-9} mol/L) increased TERT mRNA expression in HCAECs compared to untreated cells. n=3 technical replicates performed in triplicate, both groups. **(B)** ANG 1–7 (n=3) increased telomerase activity in HCAECs compared to untreated cells (n=6). Telomerase activity was quantified by measuring telomerase extension product (TRAP) using ddPCR. All experiments were performed in duplicate. **(C)** ANG 1–7 treatment increased TERT expression in HCAECs as evaluated by Western Blot analysis. n=6 both groups. *P<0.05 vs. Control. n=number of technical replicates.

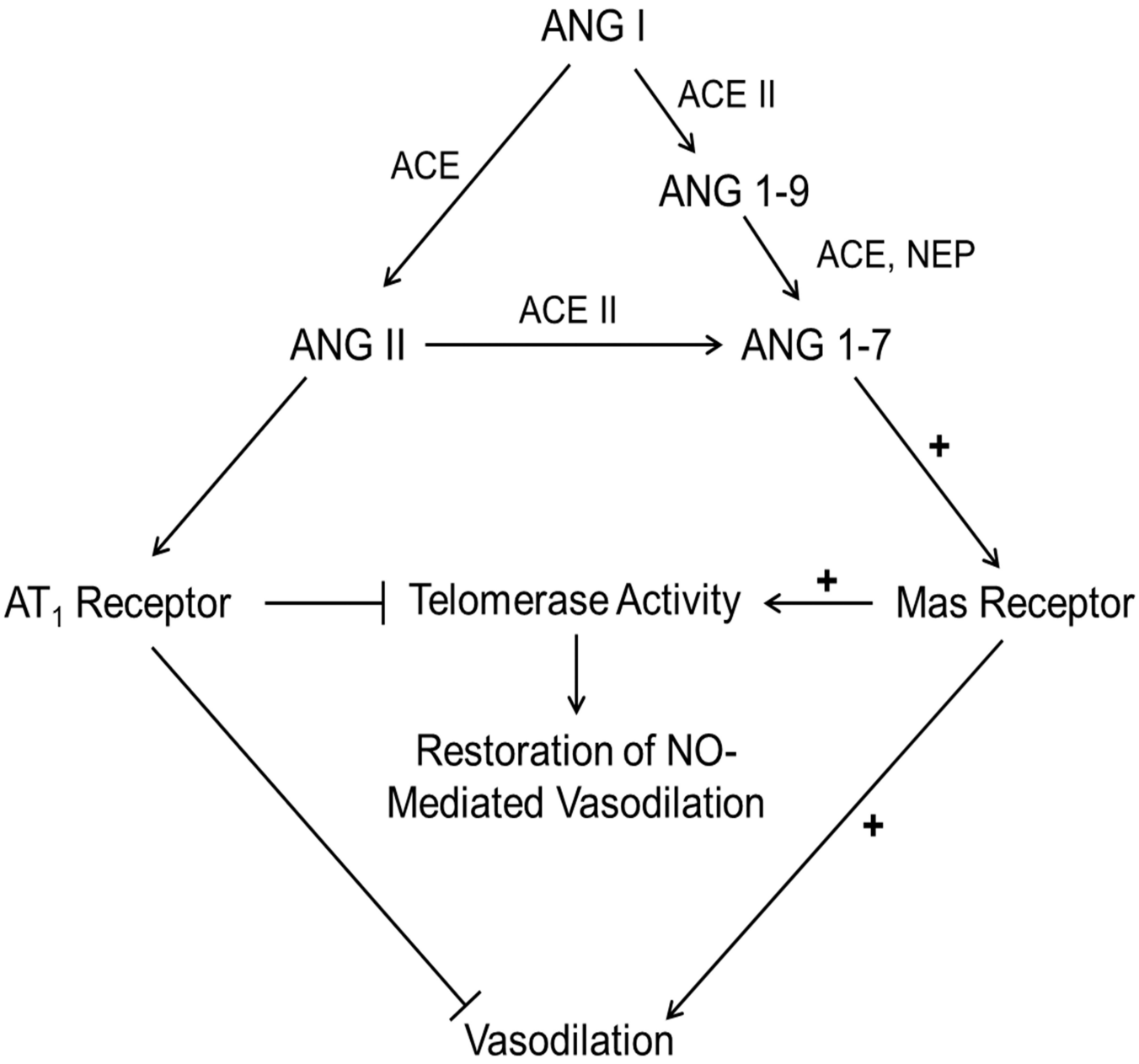

Figure 6.
Proposed vascular actions of ANG 1–7 in the human microvasculature. ACE, angiotensin converting enzyme; ANG, angiotensin; AT1, angiotensin type 1 receptor; NEP, neutral endopeptidase; NO, nitric oxide.

Table 1

Patient demographics.

	Subject Group		
	Non CAD (n=16)	**CAD (n=33)**	**Total (n=49)**
Age	64±3.5	66±1.7	65±2.5
Male/Female	7/9	23/10	30/19
Body Mass Index	30±1.7	31±1.1	31±0.9
Underlying Condition			
Coronary Artery Disease	0	33	33
Hypertension	5	27	32
Valve Disease	4	11	15
Atrial Fibrillation	3	2	5
Peripheral Vascular Disease	0	4	4
Diabetes	4	9	13
Hyperlipidemia	4	9	13
Tobacco Use	2	9	11
Cancer	6	1	7
Bowel Disease	9	2	11
Adipose Depot			
Mesenteric	7	1	8
Omental	3	0	3
Pericardial	2	4	6
Perirectal	0	1	1

n=number of subjects. Age and body mass index are presented as mean ± SEM.