Favorable Vascular Actions of Angiotensin-(1–7) in Human Obesity

Francesca Schinzari, Manfredi Tesauro, Augusto Veneziani, Nadia Mores, Nicola Di Daniele, Carmine Cardillo

See Editorial Commentary, pp 68–69

Abstract—Obese patients have vascular dysfunction related to impaired insulin-stimulated vasodilation and increased endothelin-1–mediated vasoconstriction. In contrast to the harmful vascular actions of angiotensin (Ang) II, the angiotensin-converting enzyme 2 product Ang-(1–7) has shown to exert cardiovascular and metabolic benefits in experimental models through stimulation of the Mas receptor. We, therefore, examined the effects of exogenous Ang-(1–7) on vasodilator tone and endothelin-1–dependent vasoconstriction in obese patients. Intra-arterial infusion of Ang-(1–7) (10 nmol/min) resulted in significant increase in unstimulated forearm flow (P=0.03), an effect that was not affected by the Mas receptor antagonist A779 (10 nmol/min; P>0.05). In the absence of hyperinsulinemia, however, forearm flow responses to graded doses of acetylcholine and sodium nitroprusside were not different during Ang-(1–7) administration compared with saline (both P>0.05). During infusion of regular insulin (0.15 mU/kg per minute), by contrast, endothelium-dependent vasodilator response to acetylcholine was significantly enhanced by Ang-(1–7) (P=0.04 versus saline), whereas endothelium-independent response to sodium nitroprusside was not modified (P=0.91). Finally, Ang-(1–7) decreased the vasodilator response to endothelin A receptor blockade (BQ-123; 10 nmol/min) compared with saline (6±1% versus 93±17%; P<0.001); nitric oxide inhibition by L-*N*-monomethylarginine (4 µmol/min) during concurrent endothelin A antagonism resulted in similar vasoconstriction in the absence or presence of Ang-(1–7 Ang-(1–7) (P=0.69). Our findings indicate that in obese patients Ang-(1–7) has favorable effects not only to improve insulin-stimulated endothelium-dependent vasodilation but also to blunt endothelin-1–dependent vasoconstrictor tone. These findings provide support for targeting Ang-(1–7) to counteract the hemodynamic abnormalities of human obesity. **(*Hypertension*. 2018;71:185-191. DOI: 10.1161/HYPERTENSIONAHA.117.10280.)** ● **Online Data Supplement**

Key Words: angiotensin ■ endothelin-1 ■ endothelium ■ insulin ■ obesity

Obesity, in particular abdominal fat accumulation, drives an increased cardiometabolic risk, as indicated by the higher frequency of cardiovascular sequelae and type 2 diabetes mellitus observed in these patients.[1] Insulin resistance plays a key role in both the metabolic and cardiovascular consequences of obesity,[2] being an impaired insulin-stimulated skeletal muscle microvascular perfusion the common mechanistic background.[3] This concept is supported by the observation showing that insulin-mediated physiological enhancement of vasodilator responses to a variety of stimuli is impaired in obesity.[4] In conjunction with this defective vasodilator capacity, increased vasoconstrictor tone predominantly related to enhanced endothelin-1 (ET-1) activity contributes to the vascular dysfunction of these patients.[5–7]

Activation of the renin–angiotensin system seems critically involved in obesity-related vascular dysfunction, given that upregulation of angiotensin (Ang) II results in increased oxidative stress and inflammation,[8,9] insulin resistance,[10] and activation of ET-1–dependent vasoconstriction.[11] Angiotensin-converting enzyme (ACE) 2 is a homolog of ACE that converts Ang II into Ang-(1–7).[12] The latter peptide, originally believed to be an inactive metabolite of the renin–angiotensin system, has been subsequently identified, together with its G protein–coupled receptor Mas, as a natural counter-regulatory axis of the renin–angiotensin system.[13] Thus, even though not all the biological effects of Ang-(1–7) may be attributable to direct antagonism of Ang II,[14] the ACE2/Ang-(1–7)/Mas axis blunts the detrimental effects of ACE/Ang II/angiotensin type 1 receptor activation in a variety of conditions, such as systemic[15] and pulmonary[16] arterial hypertension, cardiac hypertrophy,[17,18] and heart failure.[19]

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From the Policlinico A. Gemelli, Rome, Italy (F.S., A.V., N.M., C.C.); Department of Internal Medicine, University of Tor Vergata, Rome, Italy (M.T., N.D.D.); and Departments of Surgery (A.V.), Pharmacology (N.M.), and Internal Medicine (C.C.), Catholic University, Rome, Italy.

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Correspondence to Carmine Cardillo, Istituto di Patologia Speciale Medica e Semeiotica Medica, Università Cattolica del Sacro Cuore, Largo Gemelli 8, 00168 Rome, Italy. E-mail carmine.cardillo@unicatt.it

Tissue-specific expression of the biologically active molecules of the ACE2/Ang-(1–7)/Mas system has been reported in perivascular adipose tissue, especially in aortic, mesenteric, and epicardial perivascular adipose tissue.[20,21] Importantly, downregulation of Ang-(1–7) signaling in obese perivascular adipose tissue has been evoked as a critical element of adipose tissue inflammation, decreased vasodilator reactivity[22] and cardiac dysfunction.[23] In addition to its cardiovascular benefits, Ang-(1–7) is also provided of favorable actions on glucose metabolism, improving insulin resistance and hypertension in high-fructose–fed rats.[24] Moreover, Ang-(1–7) improves pancreatic β-cell function in mice, increasing insulin secretion by Mas-dependent signaling.[25] Conversely, ACE2 deficiency reduces β-cell mass and impairs β-cell proliferation in another mouse model of obesity.[26]

In view of these cardiovascular and metabolic actions, therefore, we hypothesized that Ang-(1–7) might beneficially impact obesity-related vascular dysfunction. To this purpose, the present study was designed to assess the effects of Ang-(1–7) on basal flow and agonist-induced vasodilator responses, in the absence or presence of hyperinsulinemia, in patients with central obesity. Because of evidence showing that Ang-(1–7) may interfere with vasoconstrictor signaling molecules,[27] we also investigated its interactions with the ET-1 system in these patients.

Methods

Study Subjects

White patients with central obesity (waist circumference ≥102 cm for males or ≥88 cm for females), without or with the metabolic syndrome (defined according to the National Cholesterol Education Program's Adult Treatment Panel III),[28] but with no history or current evidence of cardiovascular disease (coronary artery disease, cerebrovascular or peripheral occlusive arterial disease, coagulopathy, vasculitis) or any other systemic condition, were recruited for this study. In patients taking antihypertensive or lipid-lowering drugs, treatment was discontinued for at least 1 week before the vascular studies. During this time, blood pressure was repeatedly measured and, when needed, treatments were restarted with the exclusion of the patient from the study. None of the participants was a smoker, and all participants were asked to refrain from drinking alcohol and beverages containing caffeine for at least 24 hours before the study. None of the participants was engaged in programs of regular physical activity. Because of the potential effect of sex hormones on the ET-1 system,[29] all female patients were studied within the first week from the beginning of their menstrual cycle. The study protocol was approved by the institutional review boards, and all participants gave written informed consent before their participation in the study.

Protocols

Each study consisted of infusions of drugs into the brachial artery and measurement of forearm blood flow (FBF) by means of strain-gauge venous occlusion plethysmography, according to a procedure previously reported[7] (Figures in the online-only Data Supplement).

Assessment of the Effect of Ang-(1–7) on Unstimulated Forearm Flow: Role of the MAS Receptor (Protocol 1)

To ascertain the effects of Ang-(1–7) on unstimulated FBF and the possible role of the MAS receptor, 5 patients with central obesity were recruited. After the forearm was instrumented, saline was given intra-arterially for 15 minutes, at which point baseline FBF was recorded and infusion of Ang-(1–7) (Bachem AG, Weil am Rhein, Germany) was started at the dose of 10 nmol/min. This dose had been previously shown effective to induce vasodilation in the forearm circulation of healthy subjects.[30] Ang-(1–7) was given for 20 minutes, FBF was measured 3× at 5-minute intervals during the last 10 minutes of infusion. Then, while maintaining constant the administration of Ang-(1–7), infusion of the MAS receptor blocker A779 (Sigma-Aldrich, St. Louis, MO), at the dose of 10 nmol/min, was started. This dose had been recently shown able to block the vasomotor response to Ang-(1–7) in in vitro vascular preparations of human arteries.[31] Combined infusion of Ang-(1–7) and A779 was given for 20 minutes, and FBF was measured again as before.

Assessment of the Effects of Ang-(1–7) on Vascular Reactivity in the Absence of Hyperinsulinemia (Protocol 2)

To determine the effects of Ang-(1–7) on nitric oxide (NO)–dependent vasodilator responses in the absence of hyperinsulinemia, 6 patients with central obesity were enrolled. After the forearm was instrumented and baseline blood samples were collected, saline was infused intra-arterially for 15 minutes, basal FBF was measured, and dose–response curves to the endothelium-dependent vasodilator acetylcholine (acetylcholine chloride, Sigma-Aldrich) and the exogenous NO donor sodium nitroprusside (SNP; Malesci, Florence, Italy) were obtained as previously reported.[4] The sequence of acetylcholine and SNP infusion was randomized to avoid bias related to the order of these procedures. Then, after a 30-minute period to allow its return to baseline, FBF was measured again and an intra-arterial infusion of Ang-(1–7) (Bachem AG, Weil am Rhein, Germany), at the dose of 10 nmol/min, was started. After 20 minutes, unstimulated FBF was reassessed and the dose–response curves to acetylcholine and SNP were repeated as before.

Assessment of the Effects of Ang-(1–7) on Vascular Tone and Reactivity in the Presence of Hyperinsulinemia (Protocol 3)

To assess whether the presence of hyperinsulinemia might affect the action of Ang-(1–7) on endothelium-dependent and -independent vascular reactivity, 5 participants were enrolled. After the forearm was instrumented and baseline blood samples were collected, saline was given intra-arterially for 15 minutes, at which point an infusion of regular insulin (Humulin; Eli Lilly, Indianapolis, IN) at 0.15 mU/kg per minute was started in the same line. To avoid any confounding effect related to changes in glycemia, plasma glucose levels were determined periodically during insulin administration, and an infusion of 20% dextrose into a contralateral arm vein was adjusted to maintain glucose levels at values similar to baseline; the doses of glucose needed to maintain glycemic levels were generally small in all participants. After 45 minutes of insulin infusion, venous blood samples were again collected from the instrumented arm for insulin measurement, basal FBF was measured, and dose–response curves to acetylcholine and SNP were obtained as in protocol 1. Then, after a 30-minute period to allow FBF return to baseline, intra-arterial infusion of Ang-(1–7) was started. After 20 minutes, baseline FBF was reassessed and the dose–response curves to acetylcholine and SNP were repeated as detailed above.

Assessment of the Effects of Ang-(1–7) on Vascular Responses to ET_A Receptor Blockade: Role of NO (Protocol 4)

To investigate the effects of exogenous Ang-(1–7) on the ET-1 system and on the NO pathway, 8 additional patients were recruited for a study using selective endothelin A (ET_A) receptor blockade and NO synthase inhibition. To this purpose, after the forearm was instrumented, saline was given for 15 minutes and baseline FBF was measured; at which point, an infusion of BQ-123 (Bachem), a selective antagonist of ET_A receptors, was started at the dose of 10 nmol/min for 60 minutes, and FBF was measured every 10

minutes. Then, while maintaining constant the administration of BQ-123, infusion of L-NMMA (L-*N*-monomethylarginine; 4 µmol/min) was superimposed for 15 minutes and FBF was measured again at the end of this period. Afterward, after a 15 minutes resting period to allow FBF return to baseline, infusion of Ang-(1–7) was started for 20 minutes and FBF was reassessed. Then, while maintaining Ang-(1–7) infusion, BQ-123 and L-NMMA infusions were repeated as before.

Analytic Procedures

Glucose was determined in duplicate by the glucose oxidase method on a glucose analyzer (Beckman Instruments, Fullerton, CA). Insulin plasma concentrations were determined by electrochemiluminescent immunoassay (Roche Diagnostics, Mannheim, Germany).

Statistical Analysis

Group comparisons were performed by 1-way ANOVA. Within group analyses were performed by paired *t* test, 1-way, and 2-way ANOVA for repeated measures, as appropriate. The Holm–Sidak test was used for post hoc comparisons when needed. All calculated probability values are 2-tailed, and a $P<0.05$ was considered statistically significant. All group data are reported as mean±SEM.

Results

Among 24 patients with central obesity participating in this investigation (Table), 6 (25%) were affected by the metabolic syndrome. During vascular studies, blood pressure and heart rate did not change significantly after infusion of any of the substances used, thus indicating that their effects were limited to the infused forearm and did not extend to the systemic circulation. In patients participating in studies with hyperinsulinemia, forearm insulin plasma levels were 13±2 µU/mL at baseline and rose to 179±38 µU/mL after intra-arterial infusion of insulin ($P<0.001$ versus baseline).

Effects of Ang-(1–7) on Unstimulated FBF and Role of the MAS Receptor

Administration of exogenous Ang-(1–7) resulted in a significant vasodilator response (23±5% increase of FBF from baseline); during infusion of Ang-(1–7), however, blockade of MAS receptor by A779 did not result in any significant change in FBF compared with Ang-(1–7) alone ($P=0.86$; Figure 1).

Table. Clinical Characteristics of the Study Population

Variable	Protocol 1 (n=5)	Protocol 2 (n=6)	Protocol 3 (n=5)	Protocol 4 (n=8)	*P* Value
Sex, M/F	3/2	4/2	2/3	4/4	
Age, y	45±3	46±3	42±3	41±4	0.49
BMI, kg/m^2	33±3	32±2	38±1	37±2	0.11
Waist, cm	117±8	112±6	121±5	120±5	0.20
MAP, mm Hg	97±2	102±3	103±6	95±2	0.09
Glucose, mmol/L	5.1±0.2	5.1±0.2	4.9±0.2	5.1±0.2	0.91
Total cholesterol, mmol/L	6.0±0.3	5.7±0.3	5.2±0.5	5.2±0.4	0.45
HDL cholesterol, mmol/L	1.3±0.2	1.2±0.1	1.3±0.2	1.3±0.1	0.95
Triglycerides, mmol/L	1.9±0.6	1.2±0.1	1.7±0.3	1.4±0.2	0.19
Insulin, µU/mL	15±2	12±2	19±4	17±6	0.24

Data are expressed as mean±SEM. Comparisons were performed by 1-way ANOVA. BMI indicates body mass index; F, female; HDL, high-density lipoprotein; M, male; and MAP, mean arterial pressure.

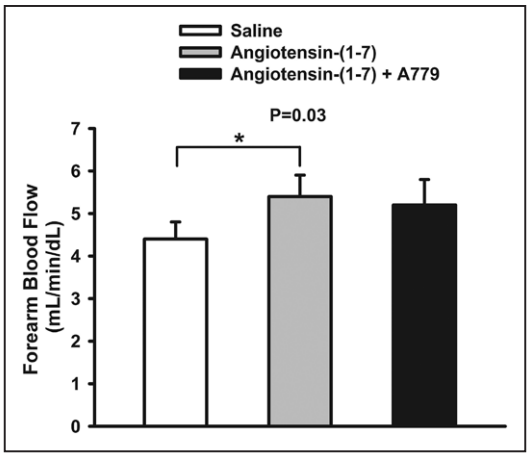

Figure 1. Forearm flow values (protocol 1) at baseline, after infusion of angiotensin (Ang)-(1–7) alone, and after the concomitant infusion of Ang-(1–7) and the Mas receptor blocker A779. The *P* value refers to the comparison of vascular responses under different conditions by 1-way ANOVA for repeated measures. All values are means±SEM. **P*<0.05 at the Holm–Sidak post hoc test for multiple comparisons.

Effects of Ang-(1–7) on Vasodilator Reactivity in the Absence of Hyperinsulinemia

During saline administration, the infusion of escalating doses of acetylcholine and SNP resulted in a progressive increase in FBF from baseline ($P<0.001$ for both drugs). Ang-(1–7) infusion, however, did not result in any significant changes in the vasodilator responses to acetylcholine and SNP (Figure 2).

Effects of Ang-(1–7) on Vasodilator Reactivity in the Presence of Hyperinsulinemia

During insulin infusion, administration of escalating doses of acetylcholine and SNP resulted in a progressive increase in FBF from baseline ($P<0.001$ for both drugs). When the acetylcholine and SNP curves were repeated after Ang-(1–7)

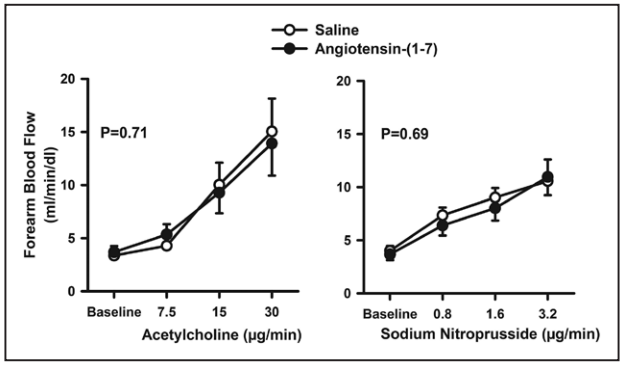

Figure 2. Forearm flow responses (protocol 2) to intra-arterial infusion of acetylcholine (**left**) and sodium nitroprusside (**right**) during the concomitant infusion of saline (○) or angiotensin (Ang)-(1–7) (●). The *P* values refer to the comparisons of vascular responses under the different conditions by 2-way ANOVA for repeated measures. All values are means±SEM.

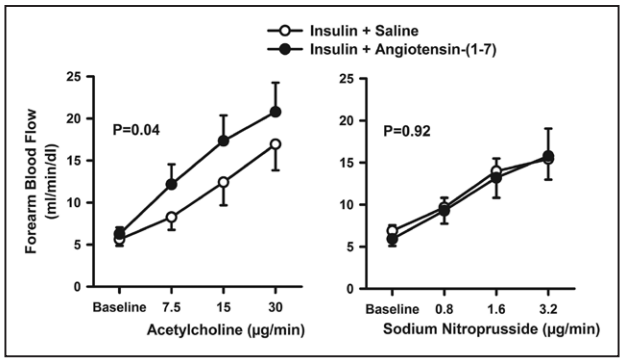

Figure 3. Forearm flow responses (protocol 3) to intra-arterial infusion of acetylcholine (**left**) and sodium nitroprusside (**right**) during the concomitant infusion of insulin alone (○) or insulin and angiotensin (Ang)-(1–7) (●). The *P* values refer to the comparisons of vascular responses under the different conditions by 2-way ANOVA for repeated measures. All values are means±SEM.

had been added on top of the insulin infusion, the vasodilator responses to acetylcholine was significantly enhanced; the response to SNP, by contrast, was not significantly modified during Ang-(1–7) coadministration compared with insulin infusion alone (Figure 3).

Effects of Ang-(1–7) on Vascular Responses to ET_A Receptor Blockade and NO Synthase Inhibition

During saline, ET_A receptor antagonism by BQ-123 resulted in a marked increase in FBF (Figure 4, left). Administration of Ang-(1–7) resulted in a significant vasodilation; during infusion of Ang-(1–7), however, BQ-123 did not result in further increase of FBF (Figure 4, left). To account for the baseline imbalance in flow resulting from Ang-(1–7)–mediated vasodilation, we also compared the vasodilator effect of ET_A receptor blockade as a percent change[32] to observe that is was significantly greater during saline than during Ang-(1–7) administration (93±17% versus 6±5%; $P>0.001$).

Infusion of L-NMMA during the concurrent blockade of ET_A receptors was associated with a significant decrease in FBF during either saline or Ang-(1–7) (Figure 4); the degree of this vasoconstriction was not different between the 2 circumstances (37±7% versus 32±5%; $P=0.69$).

Discussion

In our study, infusion of Ang-(1–7) increased unstimulated forearm flow, thereby indicating that the vasodilator effect of the peptide is preserved in the forearm vasculature of obese patients. Thus, this finding is in keeping with a previous observation of Ueda et al,[27] who had reported a significant vasodilator response to Ang-(1–7) compared with placebo in the forearm circulation of healthy subjects. Similarly, Sasaki et al[30] had observed a NO-independent vasodilator response to Ang-(1–7) in both healthy subjects and hypertensive patients. Interestingly, Durand et al[31] have recently tested the effects of Ang-(1–7) in human microvessels harvested from disparate adipose tissue depots (mesenteric, omental, pericardial, perirectal) of patients with coronary artery disease or a variety of other conditions, including hypertension, hyperlipidemia, diabetes mellitus, and atrial fibrillation. They observed that Ang-(1–7) dilates the adipose arterioles of patients without, but not with, coronary artery disease, an effect that was significantly reduced by the Mas receptor antagonist A779. At odds with those findings, however, in our study administration of A779 did not significantly modify forearm flow during infusion of Ang-(1–7), to suggest that the vasodilator response to the peptide was not mediated by Mas-activated signaling. The reasons for these discrepancies are not entirely clear, but likely reasons include differences in study methodologies, vascular districts examined, and clinical characteristics of the patients. Possible explanations for the Mas-independent vasodilation elicited by Ang-(1–7) in our patients involve a potential vasodepressor effect of Ang-(1–7) via angiotensin II type 2 receptor[33]; downregulation of angiotensin II type 1 receptor,[34] possibly because of internalization as a consequence of biased agonism by Ang-(1–7),[35] should also be taken into account.

In spite of its action to reduce basal vascular tone, in our patients, Ang-(1–7) did not improve NO-mediated vasodilator responses to acetylcholine and SNP in the absence of hyperinsulinemia. Previous studies have suggested a role for Ang-(1–7) to improve endothelial function in normotensive[36] and high-salt diet–fed[37] rats, as well as in apolipoprotein-deficient mice.[38] Similarly, ACE2 overexpression[15] or activation[39] promotes beneficial effects on endothelial function in other experimental models. Of note, prolonged treatment

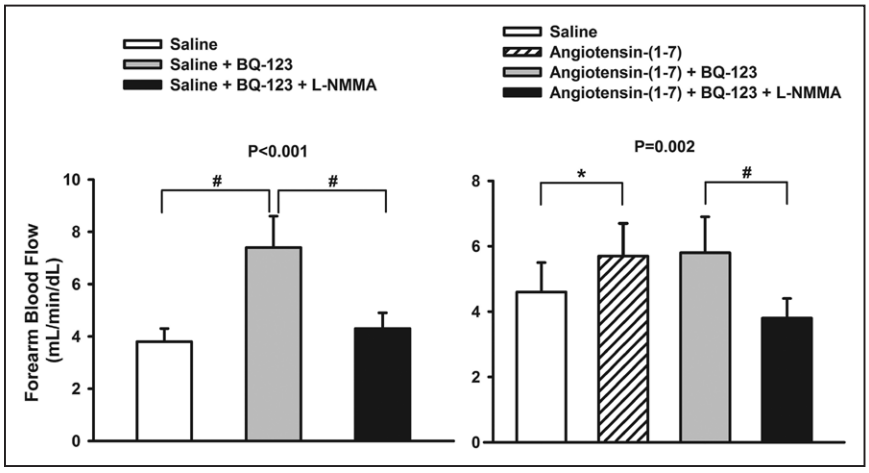

Figure 4. Forearm flow values (protocol 4) during blockade of endothelin A receptors, followed by nitric oxide synthase inhibition, during the concomitant infusion of saline (**left**) or Ang-(1–7) (**right**). The *P* values refer to the comparisons of vascular responses under different conditions by 1-way ANOVA for repeated measures. All values are means±SEM. #$P<0.001$ at the Holm–Sidak post hoc test for multiple comparisons. L-NMMA indicates L-*N*-monomethylarginine.

with Ang-(1–7) is able to improve endothelial function also in a mouse model of diet-induced obesity.[40] Human studies looking at the effect of Ang-(1–7) on endothelium-dependent vasodilator response to bradykinin, however, have yielded conflicting results. Thus, Ueda et al[41] have reported potentiation of bradykinin-induced vasodilation in the forearm resistance vessels of healthy subjects. In keeping with our current observations, however, Wilsdorf et al[42] did not observe any change of bradykinin-induced vasodilator response during infusion of Ang-(1–7). Differences in the given doses of Ang-(1–7), in the agonist used to elicit endothelium-dependent vasodilation, as well as in the characteristics of the participants, might have accounted for those discrepancies in clinical studies.

In our patients, the vasodilator response to acetylcholine, but not to SNP, was potentiated by Ang-(1–7) in the presence of hyperinsulinemia. This finding, therefore, suggests a specific action of Ang-(1–7) to improve the facilitatory role of insulin on endothelium-dependent vasodilator reactivity. Such an effect would be in keeping with previous observations demonstrating that Ang-(1–7) acts to enhance insulin sensitivity in a variety of animal models. Thus, Ang-(1–7) has proven able to recruit muscle microvasculature, hence improving insulin's metabolic action, through binding with the Mas receptor.[43] Moreover, Ang-(1–7) enhances glucose disposal in the skeletal muscle of high-fat–fed mice and restores glucose tolerance independently of blood pressure changes by increasing the translocation of glucose transporter 4 to the sarcolemma.[44] It is conceivable, therefore, that the insulin-sensitizing effect of Ang-(1–7) might extend to the vascular endothelium, hence restoring the capacity of insulin to act as a catalyst for the vasodilator stimuli to facilitate the delivery of substrates to peripheral tissues. In addition to a direct effect of Ang-(1–7) to stimulate the PI3K (phosphatidylinositide 3-kinase)/Akt signaling pathway, inhibition of the negative cross-talk between the Ang II and the insulin signaling or reduction of oxidative stress via NADPH oxidase inhibition are other likely mechanisms involved in the favorable outcome on insulin-stimulated endothelial function.[45] Our previous observations of improved vascular insulin sensitivity after reduction of oxidative stress in patients with similar clinical characteristics[46,47] lend more credit to the latter hypothesis.

In our group of obese patients, the vasodilation induced by ET_A antagonism was lower during Ang-(1–7) than during saline, thereby suggesting that ET-1–mediated vasoconstriction was indeed decreased by the infusion of the peptide. A modulator of the constrictor forces within blood vessels is the L-arginine/NO pathway, provided that, in addition to its direct action to relax smooth muscle cells, NO also inhibits the production of vasoconstrictor substances.[48] In view of previous evidence that Ang-(1–7) may increase NO bioavailability,[49] we tested the effects of Ang-(1–7) on NO activity by use of L-NMMA. We observed that, during blockade of ET_A receptors, the magnitude of vasoconstriction after NO synthase inhibition was similar in the absence and the presence of Ang-(1–7). These findings suggest that changes in NO activity were not a driving force behind the vascular effects of Ang-(1–7) observed under those circumstances, in keeping with previous data showing that the vasodilator effect of Ang-(1–7) in the human forearm circulation is independent of activation of the NO pathway.[30] The mechanism underlying the favorable effect of Ang-(1–7) to reduce ET-1 activity, therefore, remains unclear, but it must be considered that, being that our study was performed in the human intact circulation, an inherent limitation of its methodology is the difficulty to ascertain precisely all the molecular mechanisms involved.

Perspectives

Despite some weakness, including the absence of lean controls and the limited number of patients recruited, our investigation clearly indicates that, in patients with obesity, Ang-(1–7) enhances insulin-stimulated endothelium-dependent vasodilator responsiveness and reduces ET-1–dependent vasoconstrictor tone. These results look promising for their potential translational relevance, a view strengthened by the results of a study showing positive effects of recombinant human ACE2 in patients with heart failure.[50] Because of these promises, therefore, ACE2/Ang-(1–7) axis seems worth of further investigation as a potential target for cardiovascular prevention in obesity.

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Disclosures

None.

References

1. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F; American Heart Association; National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*. 2005;112:2735–2752. doi: 10.1161/CIRCULATIONAHA.105.169404.
2. Kahn BB, Flier JS. Obesity and insulin resistance. *J Clin Invest*. 2000;106:473–481. doi: 10.1172/JCI10842.
3. Mather KJ, Steinberg HO, Baron AD. Insulin resistance in the vasculature. *J Clin Invest*. 2013;123:1003–1004. doi: 10.1172/JCI67166.
4. Schinzari F, Tesauro M, Rovella V, Galli A, Mores N, Porzio O, Lauro D, Cardillo C. Generalized impairment of vasodilator reactivity during hyperinsulinemia in patients with obesity-related metabolic syndrome. *Am J Physiol Endocrinol Metab*. 2010;299:E947–E952. doi: 10.1152/ajpendo.00426.2010.
5. Tesauro M, Schinzari F, Rovella V, Di Daniele N, Lauro D, Mores N, Veneziani A, Cardillo C. Ghrelin restores the endothelin 1/nitric oxide balance in patients with obesity-related metabolic syndrome. *Hypertension*. 2009;54:995–1000. doi: 10.1161/HYPERTENSIONAHA.109.137729.
6. Schinzari F, Tesauro M, Rovella V, Di Daniele N, Mores N, Veneziani A, Cardillo C. Leptin stimulates both endothelin-1 and nitric oxide activity in lean subjects but not in patients with obesity-related metabolic syndrome. *J Clin Endocrinol Metab*. 2013;98:1235–1241. doi: 10.1210/jc.2012-3424.
7. Schinzari F, Iantorno M, Campia U, Mores N, Rovella V, Tesauro M, Di Daniele N, Cardillo C. Vasodilator responses and endothelin-dependent vasoconstriction in metabolically healthy obesity and the metabolic syndrome. *Am J Physiol Endocrinol Metab*. 2015;309:E787–E792. doi: 10.1152/ajpendo.00278.2015.

8. Nguyen Dinh Cat A, Montezano AC, Burger D, Touyz RM. Angiotensin II, NADPH oxidase, and redox signaling in the vasculature. *Antioxid Redox Signal*. 2013;19:1110–1120. doi: 10.1089/ars.2012.4641.
9. Youn JY, Siu KL, Lob HE, Itani H, Harrison DG, Cai H. Role of vascular oxidative stress in obesity and metabolic syndrome. *Diabetes*. 2014;63:2344–2355. doi: 10.2337/db13-0719.
10. Folli F, Kahn CR, Hansen H, Bouchie JL, Feener EP. Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. A potential role for serine phosphorylation in insulin/angiotensin II crosstalk. *J Clin Invest*. 1997;100:2158–2169. doi: 10.1172/JCI119752.
11. Moreau P, d'Uscio LV, Shaw S, Takase H, Barton M, Lüscher TF. Angiotensin II increases tissue endothelin and induces vascular hypertrophy: reversal by ET(A)-receptor antagonist. *Circulation*. 1997;96:1593–1597.
12. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res*. 2000;87:E1–E9.
13. Santos RA, Simoes e Silva AC, Maric C, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci U S A*. 2003;100:8258–8263. doi: 10.1073/pnas.1432869100.
14. Nicklin SA. A novel mechanism of action for angiotensin-(1-7) via the angiotensin type 1 receptor. *Hypertension*. 2016;68:1342–1343. doi: 10.1161/HYPERTENSIONAHA.116.08215.
15. Rentzsch B, Todiras M, Iliescu R, Popova E, Campos LA, Oliveira ML, Baltatu OC, Santos RA, Bader M. Transgenic angiotensin-converting enzyme 2 overexpression in vessels of SHRSP rats reduces blood pressure and improves endothelial function. *Hypertension*. 2008;52:967–973. doi: 10.1161/HYPERTENSIONAHA.108.114322.
16. Dai H, Jiang L, Xiao Z, Guang X. ACE2-angiotensin-(1-7)-Mas axis might be a promising therapeutic target for pulmonary arterial hypertension. *Nat Rev Cardiol*. 2015;12:374. doi: 10.1038/nrcardio.2015.6-c1.
17. Gomes ER, Lara AA, Almeida PW, Guimarães D, Resende RR, Campagnole-Santos MJ, Bader M, Santos RA, Guatimosim S. Angiotensin-(1-7) prevents cardiomyocyte pathological remodeling through a nitric oxide/guanosine 3',5'-cyclic monophosphate-dependent pathway. *Hypertension*. 2010;55:153–160. doi: 10.1161/HYPERTENSIONAHA.109.143255.
18. Santiago NM, Guimarães PS, Sirvente RA, Oliveira LA, Irigoyen MC, Santos RA, Campagnole-Santos MJ. Lifetime overproduction of circulating Angiotensin-(1-7) attenuates deoxycorticosterone acetate-salt hypertension-induced cardiac dysfunction and remodeling. *Hypertension*. 2010;55:889–896. doi: 10.1161/HYPERTENSIONAHA.110.149815.
19. Patel VB, Zhong JC, Grant MB, Oudit GY. Role of the ACE2/angiotensin 1-7 axis of the renin-angiotensin system in heart failure. *Circ Res*. 2016;118:1313–1326. doi: 10.1161/CIRCRESAHA.116.307708.
20. Lee RM, Lu C, Su LY, Gao YJ. Endothelium-dependent relaxation factor released by perivascular adipose tissue. *J Hypertens*. 2009;27:782–790. doi: 10.1097/HJH.0b013e328324ed86.
21. Patel VB, Basu R, Oudit GY. ACE2/Ang 1-7 axis: a critical regulator of epicardial adipose tissue inflammation and cardiac dysfunction in obesity. *Adipocyte*. 2016;5:306–311. doi: 10.1080/21623945.2015.1131881.
22. Xia N, Li H. The role of perivascular adipose tissue in obesity-induced vascular dysfunction. *Br J Pharmacol*. 2017;174:3425–3442. doi: 10.1111/bph.13650.
23. Patel VB, Mori J, McLean BA, Basu R, Das SK, Ramprasath T, Parajuli N, Penninger JM, Grant MB, Lopaschuk GD, Oudit GY. ACE2 deficiency worsens epicardial adipose tissue inflammation and cardiac dysfunction in response to diet-induced obesity. *Diabetes*. 2016;65:85–95. doi: 10.2337/db15-0399.
24. Giani JF, Mayer MA, Muñoz MC, Silberman EA, Höcht C, Taira CA, Gironacci MM, Turyn D, Dominici FP. Chronic infusion of angiotensin-(1-7) improves insulin resistance and hypertension induced by a high-fructose diet in rats. *Am J Physiol Endocrinol Metab*. 2009;296:E262–E271. doi: 10.1152/ajpendo.90678.2008.
25. Sahr A, Wolke C, Maczewsky J, Krippeit-Drews P, Tetzner A, Drews G, Venz S, Gürtler S, van den Brandt J, Berg S, Döring P, Dombrowski F, Walther T, Lendeckel U. The angiotensin-(1-7)/Mas axis improves pancreatic β-cell function *in vitro* and in vivo. *Endocrinology*. 2016;157:4677–4690. doi: 10.1210/en.2016-1247.
26. Shoemaker R, Yiannikouris F, Thatcher S, Cassis L. ACE2 deficiency reduces β-cell mass and impairs β-cell proliferation in obese C57BL/6 mice. *Am J Physiol Endocrinol Metab*. 2015;309:E621–E631. doi: 10.1152/ajpendo.00054.2015.
27. Ueda S, Masumori-Maemoto S, Ashino K, Nagahara T, Gotoh E, Umemura S, Ishii M. Angiotensin-(1-7) attenuates vasoconstriction evoked by angiotensin II but not by noradrenaline in man. *Hypertension*. 2000;35:998–1001.
28. Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C, American Heart Association, National Heart, Lung, and Blood Institute. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation*. 2004;109:433–438.
29. Tostes RC, Fortes ZB, Callera GE, Montezano AC, Touyz RM, Webb RC, Carvalho MH. Endothelin, sex and hypertension. *Clin Sci (Lond)*. 2008;114:85–97. doi: 10.1042/CS20070169.
30. Sasaki S, Higashi Y, Nakagawa K, Matsuura H, Kajiyama G, Oshima T. Effects of angiotensin-(1-7) on forearm circulation in normotensive subjects and patients with essential hypertension. *Hypertension*. 2001;38:90–94.
31. Durand MJ, Zinkevich NS, Riedel M, Gutterman DD, Nasci VL, Salato VK, Hijjawi JB, Reuben CF, North PE, Beyer AM. Vascular actions of angiotensin 1-7 in the human microcirculation: novel role for telomerase. *Arterioscler Thromb Vasc Biol*. 2016;36:1254–1262. doi: 10.1161/ATVBAHA.116.307518.
32. Wilkinson IB, Webb DJ. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *Br J Clin Pharmacol*. 2001;52:631–646.
33. Walters PE, Gaspari TA, Widdop RE. Angiotensin-(1-7) acts as a vasodepressor agent via angiotensin II type 2 receptors in conscious rats. *Hypertension*. 2005;45:960–966. doi: 10.1161/01.HYP.0000160325.59323.b8.
34. Clark MA, Diz DI, Tallant EA. Angiotensin-(1-7) downregulates the angiotensin II type 1 receptor in vascular smooth muscle cells. *Hypertension*. 2001;37:1141–1146.
35. Galandrin S, Denis C, Boularan C, Marie J, M'Kadmi C, Pilette C, Dubroca C, Nicaise Y, Seguelas MH, N'Guyen D, Banères JL, Pathak A, Sénard JM, Galés C. Cardioprotective angiotensin-(1-7) peptide acts as a natural-biased ligand at the angiotensin II type 1 receptor. *Hypertension*. 2016;68:1365–1374. doi: 10.1161/HYPERTENSIONAHA.116.08118.
36. Faria-Silva R, Duarte FV, Santos RA. Short-term angiotensin(1-7) receptor MAS stimulation improves endothelial function in normotensive rats. *Hypertension*. 2005;46:948–952. doi: 10.1161/01.HYP.0000174594.17052.33.
37. Raffai G, Lombard JH. Angiotensin-(1-7) selectively induces relaxation and modulates endothelium-dependent dilation in mesenteric arteries of salt-fed rats. *J Vasc Res*. 2016;53:105–118. doi: 10.1159/000448714.
38. Tesanovic S, Vinh A, Gaspari TA, Casley D, Widdop RE. Vasoprotective and atheroprotective effects of angiotensin (1-7) in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2010;30:1606–1613. doi: 10.1161/ATVBAHA.110.204453.
39. Fraga-Silva RA, Costa-Fraga FP, Murça TM, et al. Angiotensin-converting enzyme 2 activation improves endothelial function. *Hypertension*. 2013;61:1233–1238. doi: 10.1161/HYPERTENSIONAHA.111.00627.
40. Beyer AM, Guo DF, Rahmouni K. Prolonged treatment with angiotensin 1-7 improves endothelial function in diet-induced obesity. *J Hypertens*. 2013;31:730–738. doi: 10.1097/HJH.0b013e32835ecbe5.
41. Ueda S, Masumori-Maemoto S, Wada A, Ishii M, Brosnihan KB, Umemura S. Angiotensin(1-7) potentiates bradykinin-induced vasodilatation in man. *J Hypertens*. 2001;19:2001–2009.
42. Wilsdorf T, Gainer JV, Murphey LJ, Vaughan DE, Brown NJ. Angiotensin-(1-7) does not affect vasodilator or TPA responses to bradykinin in human forearm. *Hypertension*. 2001;37:1136–1140.
43. Fu Z, Zhao L, Aylor KW, Carey RM, Barrett EJ, Liu Z. Angiotensin-(1-7) recruits muscle microvasculature and enhances insulin's metabolic action via mas receptor. *Hypertension*. 2014;63:1219–1227. doi: 10.1161/HYPERTENSIONAHA.113.03025.
44. Williams IM, Otero YF, Bracy DP, Wasserman DH, Biaggioni I, Arnold AC. Chronic angiotensin-(1-7) improves insulin sensitivity in high-fat fed mice independent of blood pressure. *Hypertension*. 2016;67:983–991. doi: 10.1161/HYPERTENSIONAHA.115.06935.
45. Dominici FP, Burghi V, Muñoz MC, Giani JF. Modulation of the action of insulin by angiotensin-(1-7). *Clin Sci (Lond)*. 2014;126:613–630. doi: 10.1042/CS20130333.
46. Tesauro M, Schinzari F, Rovella V, Melina D, Mores N, Barini A, Mettimano M, Lauro D, Iantorno M, Quon MJ, Cardillo C. Tumor necrosis factor-alpha antagonism improves vasodilation during hyperinsulinemia in metabolic syndrome. *Diabetes Care*. 2008;31:1439–1441. doi: 10.2337/dc08-0219.

47. Tesauro M, Schinzari F, Adamo A, Rovella V, Martini F, Mores N, Barini A, Pitocco D, Ghirlanda G, Lauro D, Campia U, Cardillo C. Effects of GLP-1 on forearm vasodilator function and glucose disposal during hyperinsulinemia in the metabolic syndrome. *Diabetes Care*. 2013;36:683–689. doi: 10.2337/dc12-0763.
48. Flowers MA, Wang Y, Stewart RJ, Patel B, Marsden PA. Reciprocal regulation of endothelin-1 and endothelial constitutive NOS in proliferating endothelial cells. *Am J Physiol*. 1995;269(6 pt 2):H1988–H1997.
49. Yang G, Chu PL, Rump LC, Le TH, Stegbauer J. ACE2 and the homolog collectrin in the modulation of nitric oxide and oxidative stress in blood pressure homeostasis and vascular injury. *Antioxid Redox Signal*. 2017;26:645–659. doi: 10.1089/ars.2016.6950.
50. Basu R, Poglitsch M, Yogasundaram H, Thomas J, Rowe BH, Oudit GY. Roles of angiotensin peptides and recombinant human ACE2 in heart failure. *J Am Coll Cardiol*. 2017;69:805–819. doi: 10.1016/j.jacc.2016.11.064.

Novelty and Significance

What Is New?

- We report for the first time the effects of exogenous angiotensin (Ang)-(1–7) on vasodilator reactivity and vasoconstrictor tone in patients with central obesity.

What Is Relevant?

- Ang-(1–7) is provided of beneficial actions not only on the cardiovascular system but also on insulin resistance and glucose metabolism. Consequently, upregulation of the angiotensin-converting enzyme 2/Ang-(1–7) axis may offer therapeutic advantages on the hemodynamic and metabolic abnormalities associated with obesity.

Summary

We have observed that, in patients with central obesity, Ang-(1–7) has favorable effects not only to improve insulin-stimulated endothelium-dependent vasodilation but also to blunt endothelin-1–dependent vasoconstriction. These results, therefore, make the angiotensin-converting enzyme 2/Ang-(1–7) axis a potential target for vascular prevention in an insulin resistant state like obesity.

Favorable Vascular Actions of Angiotensin-(1–7) in Human Obesity

Francesca Schinzari, Manfredi Tesauro, Augusto Veneziani, Nadia Mores, Nicola Di Daniele and Carmine Cardillo

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STUDY PROTOCOLS

Favorable vascular actions of angiotensin-(1-7) in human obesity

Francesca Schinzari, MD[1]; Manfredi Tesauro, MD[2]; Augusto Veneziani, MD[1,3]; Nadia Mores, MD[1,4]; Nicola Di Daniele, MD[2]; Carmine Cardillo, MD[1,5]

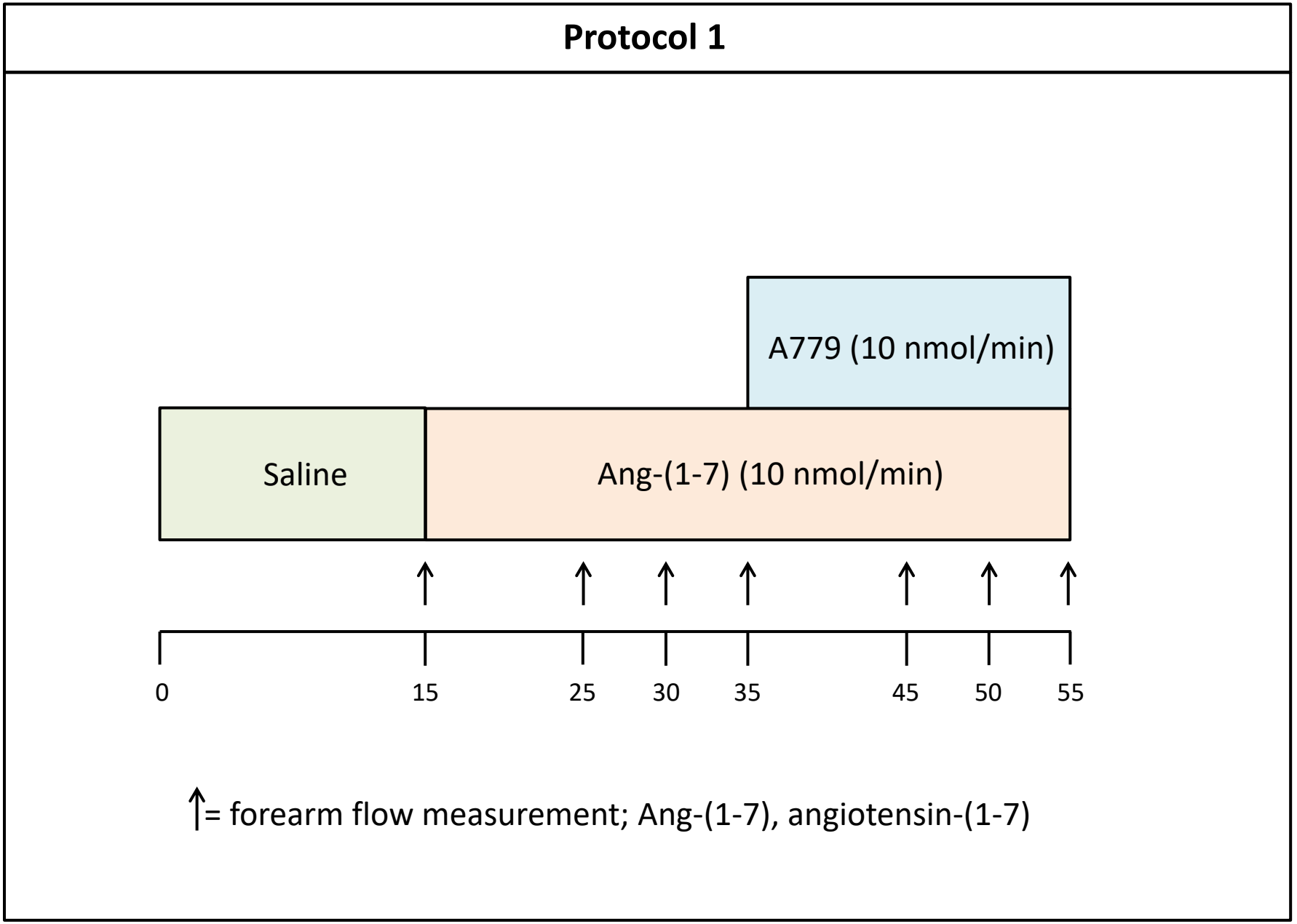
Running title: Angiotensin-(1-7) and vascular function in obesity

[1]Policlinico A. Gemelli, Rome, Italy; Department of [2]Internal Medicine, University of Tor Vergata, Rome, Italy; Departments of [3]Surgery, [4]Pharmacology, and [5]Internal Medicine, Catholic University, Rome, Italy;

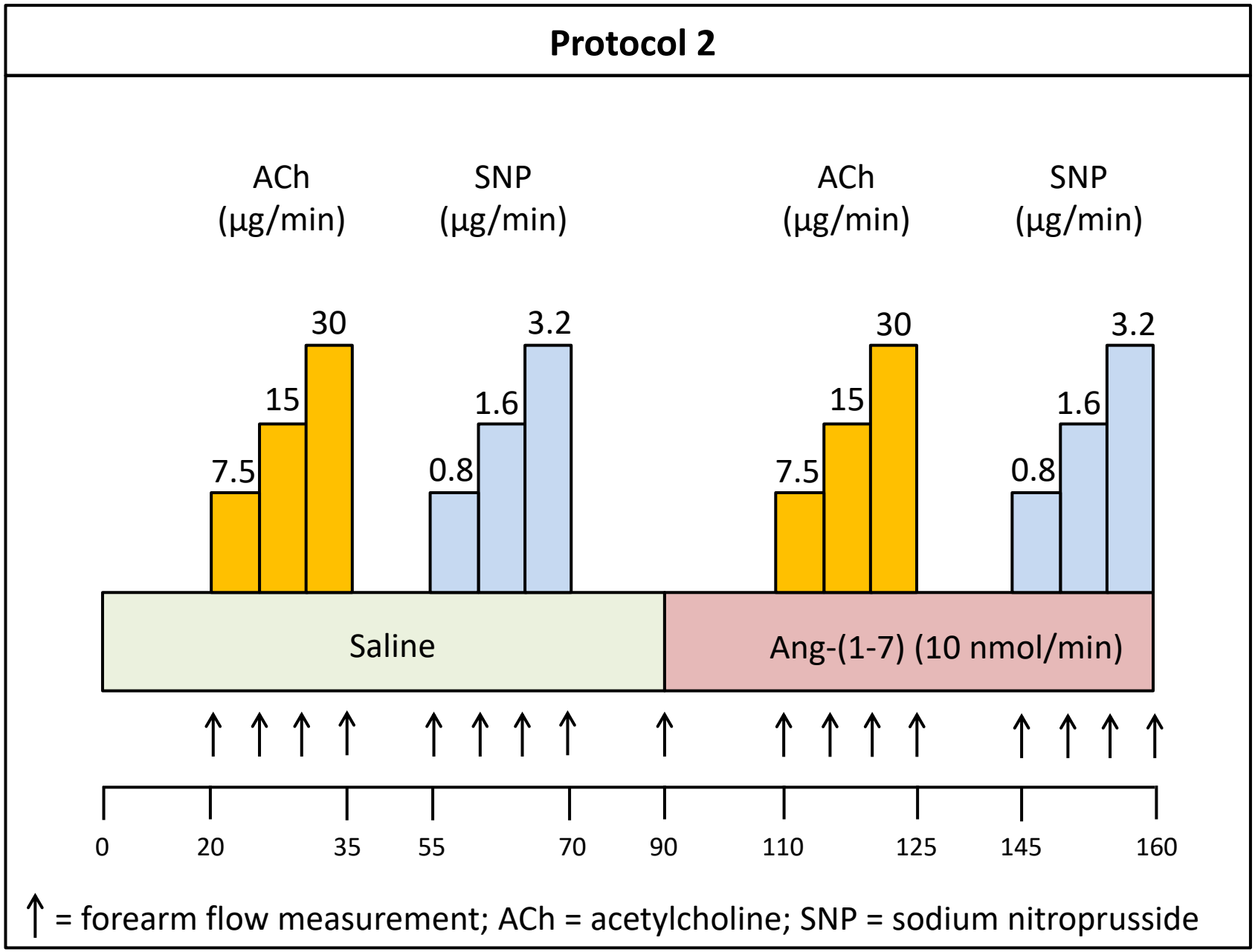
Correspondence:

Carmine Cardillo, MD
Istituto di Patologia Speciale Medica e Semeiotica Medica
Università Cattolica del Sacro Cuore
Largo Gemelli 8, 00168 Rome, Italy
Tel +39 (06) 3503-4432
Fax +39 (06) 3015-7232
E-mail carmine.cardillo@unicatt.it

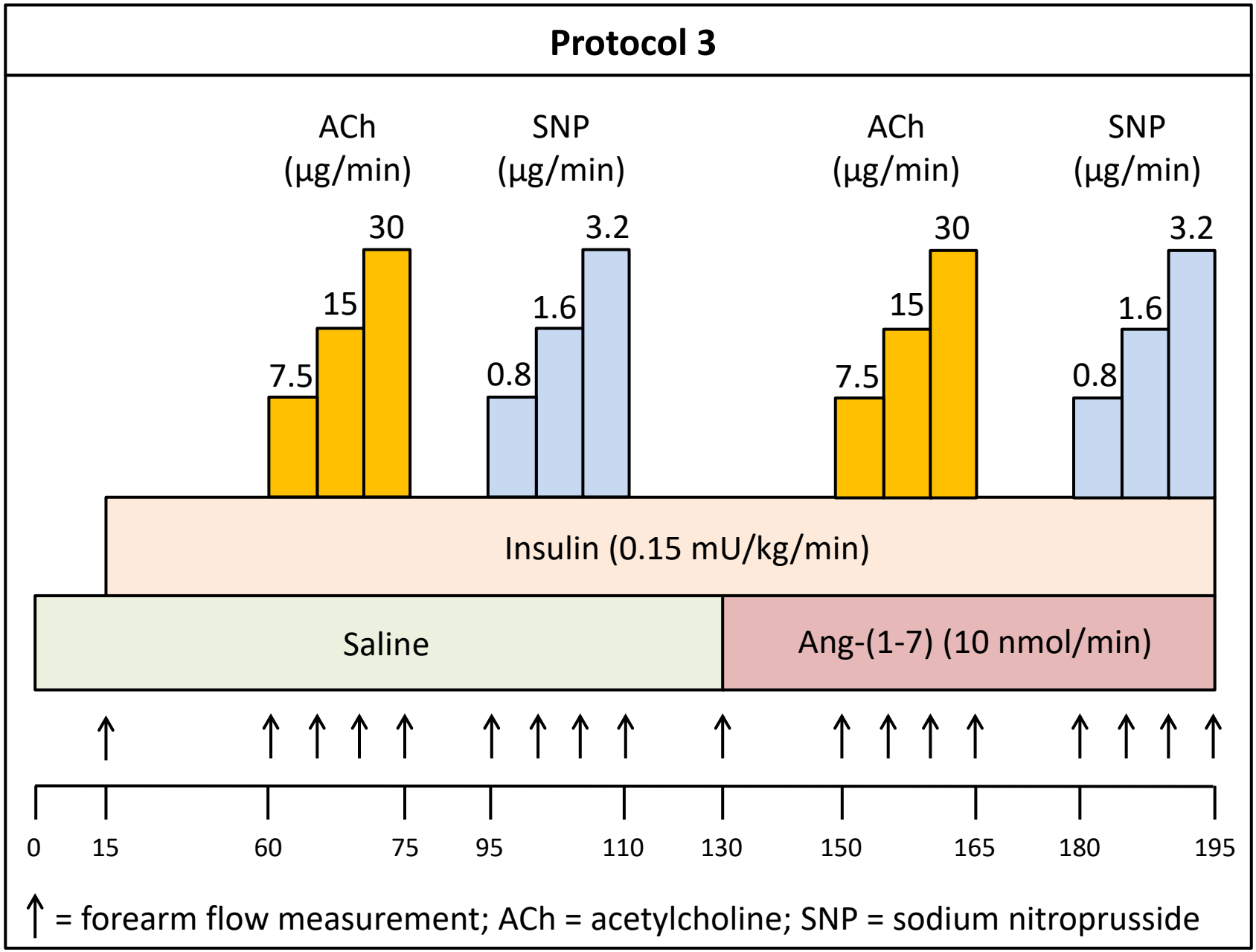
S1


Protocol 1
A779 (10 nmol/min)
Saline
Ang-(1-7) (10 nmol/min)
0
15
25
30
35
45
50
55
↑= forearm flow measurement; Ang-(1-7), angiotensin-(1-7)

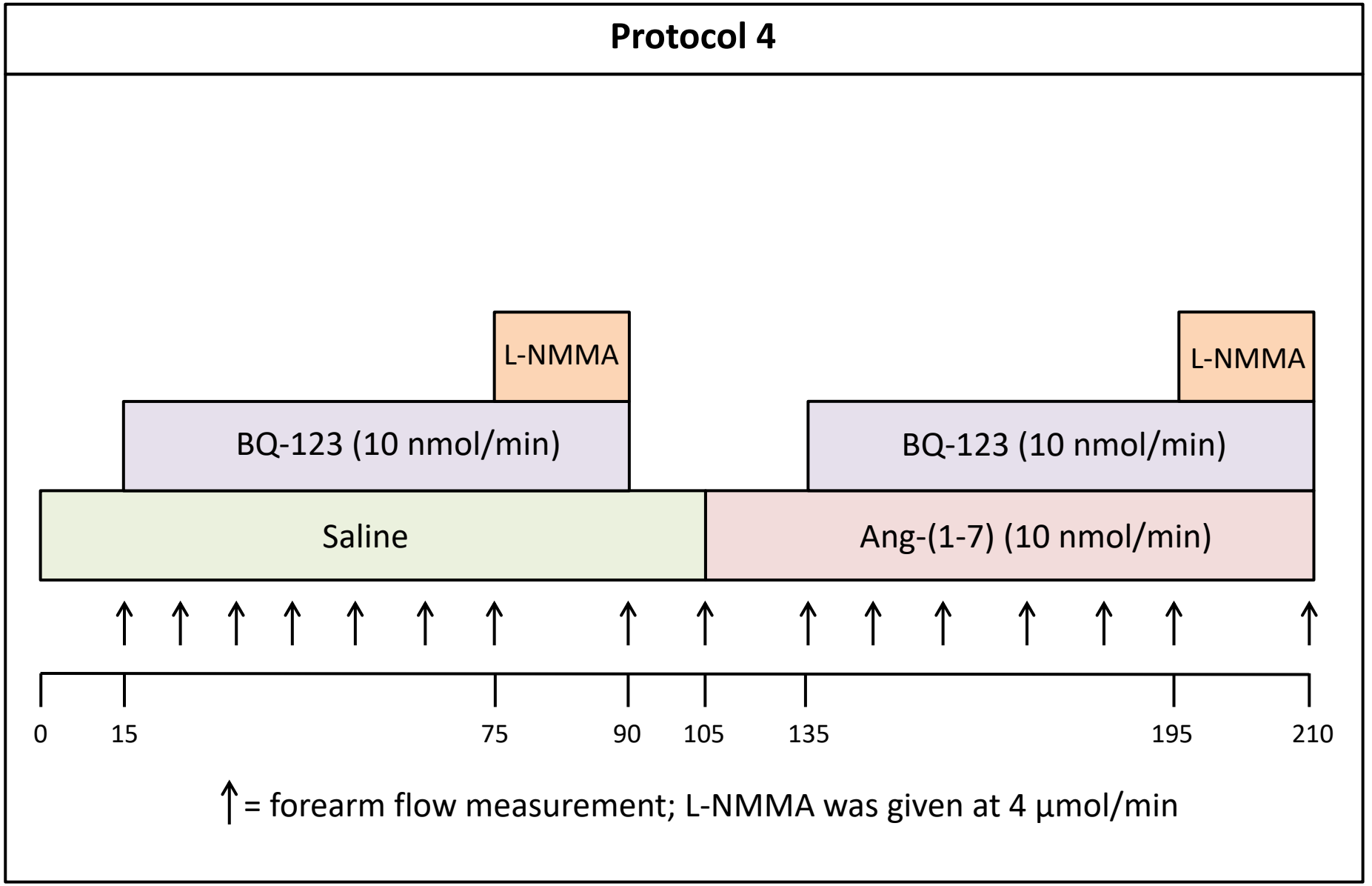
S2


Protocol 2
ACh (µg/min)
SNP (µg/min)
ACh (µg/min)
SNP (µg/min)
7.5
15
30
0.8
1.6
3.2
7.5
15
30
0.8
1.6
3.2
Saline
Ang-(1-7) (10 nmol/min)
0
20
35
55
70
90
110
125
145
160
↑ = forearm flow measurement; ACh = acetylcholine; SNP = sodium nitroprusside

S3


Protocol 3
ACh (µg/min)
SNP (µg/min)
ACh (µg/min)
SNP (µg/min)
7.5
15
30
0.8
1.6
3.2
7.5
15
30
0.8
1.6
3.2
Insulin (0.15 mU/kg/min)
Saline
Ang-(1-7) (10 nmol/min)
0
15
60
75
95
110
130
150
165
180
195
↑ = forearm flow measurement; ACh = acetylcholine; SNP = sodium nitroprusside

S4


Protocol 4
L-NMMA
L-NMMA
BQ-123 (10 nmol/min)
BQ-123 (10 nmol/min)
Saline
Ang-(1-7) (10 nmol/min)
0
15
75
90
105
135
195
210
= forearm flow measurement; L-NMMA was given at 4 µmol/min