Published in final edited form as:

Clin Sci (Lond). 2019 January 15; 133(1): 55–74. doi:10.1042/CS20171550.

Fetal programming and the angiotensin-(1–7) axis: a review of the experimental and clinical data

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Abstract

Hypertension is the primary risk factor for cardiovascular disease that constitutes a serious worldwide health concern and a significant healthcare burden. As the majority of hypertension has an unknown etiology, considerable research efforts in both experimental models and human cohorts has focused on the premise that alterations in the fetal and perinatal environment are key factors in the development of hypertension in children and adults. The exact mechanisms of how fetal programming events increase the risk of hypertension and cardiovascular disease are not fully elaborated; however, the focus on alterations in the biochemical components and functional aspects of the renin–angiotensin (Ang) system (RAS) has predominated, particularly activation of the Ang-converting enzyme (ACE)-Ang II-Ang type 1 receptor (AT₁R) axis. The emerging view of alternative pathways within the RAS that may functionally antagonize the Ang II axis raise the possibility that programming events also target the non-classical components of the RAS as an additional mechanism contributing to the development and progression of hypertension. In the current review, we evaluate the potential role of the ACE2-Ang-(1–7)-Mas receptor (MasR) axis of the RAS in fetal programming events and cardiovascular and renal dysfunction. Specifically, the

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Competing interests

The authors declare that there are no competing interests associated with the manuscript.

review examines the impact of fetal programming on the Ang-(1–7) axis within the circulation, kidney, and brain such that the loss of Ang-(1–7) expression or tone, contributes to the chronic dysregulation of blood pressure (BP) and cardiometabolic disease in the offspring, as well as the influence of sex on potential programming of this pathway.

Introduction

Cardiovascular disease including its primary risk factor hypertension continues to constitute a serious worldwide health concern that accounts for an increasing burden on our healthcare system [1]. The majority of hypertensive patients exhibit an undefined etiology for a sustained increase in blood pressure (BP) and they often remain inadequately treated or incompletely responsive to current therapeutic approaches [2]. Hypertension may exacerbate other pathologies including diabetes mellitus, dyslipidemia, obesity, and vascular diseases that further complicate effective therapeutic treatments. Moreover, with the advent of the new 2017 American Heart Association/American College of Cardiology guidelines that set lower thresholds for defining elevated BP and hypertension [3], a larger proportion of the United States population (70–100 million) may have hypertension and cardiovascular and renal disease that will require clinical monitoring and therapeutic intervention [4,5]. In lieu of this major global health issue, the elucidation of the underlying mechanisms that contribute to hypertension and the development of new therapies remains a critical clinical goal.

A concentrated research effort in both experimental animals and human cohorts has focused on the premise that alterations in the fetal and perinatal environment contribute to the development of hypertension and other cardiometabolic pathologies in adults and even during childhood [6,7]. Although the underlying insult in the fetal origin of cardiovascular disease was originally thought to reflect maternal undernutrition and intrauterine growth restriction/low birth weight, additional biological affronts to the fetal environment include preeclampsia, preterm birth, hypoxia, stress, and exogenous corticosteroid administration [8–13]. The exact mechanisms underlying how fetal programming events increase the risk of hypertension and cardiovascular disease are not fully elaborated; however, the focus on alterations in the biochemical components and functional aspects of the renin–angiotensin (Ang) system (RAS) is predominant in this area of research [9–13].

In parallel with the development of the fetal origins of cardiovascular disease concept, our conceptualization of the RAS has undergone significant revision over the same time period. The long-standing view of the RAS was a classic endocrine system involving Ang-converting enzyme (ACE)-dependent formation of the peptide Ang II from the decapeptide Ang I and subsequent recognition that the Ang II type 1 receptor (AT₁R) initiates various central and peripheral signaling events to regulate BP. The emerging view of alternative pathways within the RAS that may functionally antagonize the Ang II axis clearly reflects identification of the des-[Phe⁸]-Ang II or Ang-(1–7) axis in the circulation and various tissues [14–16]. The elaboration of biochemical components that comprise the ‘Ang-(1–7) axis’ is now firmly established with the identification of distinct receptors for Ang-(1–7) including the G-protein-coupled Mas receptor (MasR) and Mas-related receptor (Mrg-D)

that may account for the majority, but not all actions of Ang-(1–7) [17], selective receptor antagonists and agonists, an ACE peptidase (ACE2) that catalyzes the processing of Ang II to Ang-(1–7), and the recognition of various signaling pathways distinct from that of Ang II and AT₁R [14–16].

The Ang II axis of the RAS is recognized as a key pathway in the cardiovascular pathology of fetal programming events; however, the impact of fetal programming on the counterbalancing Ang-(1–7) axis should be considered as well. In the current review, we evaluate the potential role of the ACE2-Ang-(1–7)-MasR axis in fetal programming events and cardiovascular dysfunction. Specifically, the review focuses on the impact of fetal programming on the Ang-(1–7) axis within the circulation, kidney, and brain of the offspring that may contribute to the chronic dysregulation of BP and cardiometabolic disease in the adult, and the influence that sex may confer on these relationships, in both experimental animals and in patients, particularly those born preterm (Figure 1).

Ang-(1–7) formation

Ang II-independent pathways

Both Ang II and Ang-(1–7) are ultimately derived from the precursor protein angiotensinogen, a 50-kDa glycosylated protein that is primarily synthesized and secreted by the liver, but other tissues including the brain, kidney, pancreas, adipose, and vasculature express the precursor that may constitute a local tissue RAS [15]. Renin initiates the RAS cascade by hydrolyzing the amino-terminal portion angiotensinogen to form Ang I, which is rapidly cleaved by ACE to form the bioactive peptide Ang II (Figure 2). Since circulating levels of both Ang I and Ang-(1–7) are markedly increased following ACE inhibitor treatment, our early studies focussed on identifying Ang II-independent pathways for the generation of Ang-(1–7) [15].

Indeed, we and others identified the endopeptidase neprilysin as contributing to the circulating levels of Ang-(1–7) in animals chronically treated with ACE inhibitors [15] (Figure 2). Neprilysin cleaves the Pro⁷–Phe⁸ bond of Ang I to directly form Ang-(1–7). Domenig et al. [18] recently showed that renal Ang-(1–7) was significantly reduced in mice chronically treated with the neprilysin inhibitor LBQ657, but *increased* in ACE2-deficient mice; these studies utilized a robust ultra-HPLC-mass spectroscopy (UHPLC-MS) approach to quantitate Ang-(1–7) and nine other Angs in the whole kidney. Velez et al. also applied MS analysis to the processing of exogenous Ang I in isolated mouse podocytes and human glomerular endothelial cells to reveal the predominant conversion of Ang I into Ang-(1–7) by neprilysin [19]. Moreover, despite >80% depletion of renal Ang II levels in ACE knockout mice, renal Ang-(1–7) content was not diminished which further supports an Ang II-independent pathway for the generation of Ang-(1–7) within the kidney [20]. Other endopeptidases may potentially contribute to the tissue or cellular levels of Ang-(1–7) that include thimet oligopeptidase (TOP) and prolyl oligopeptidase (POP). Both TOP and POP cleave the Pro⁷–Phe⁸ bond of Ang I, but the extent that TOP or POP contribute to endogenous levels of Ang-(1–7) is not currently known [15].

Ang II-dependent pathways

A pathway for Ang-(1–7) formation directly from Ang II was essentially established with the identification of the ACE homolog ACE2 and subsequent studies that revealed Ang II was the preferred substrate rather than Ang I or other bioactive peptides [21,22] (Figure 2). In contrast with ACE, ACE2 is a mono-carboxypeptidase that cleaves the Pro⁷–Phe⁸ bond of Ang II to form Ang-(1–7); ACE2 is expressed throughout the body in both membrane-bound and soluble forms that are catalytically active [15]. Chronic administration of soluble ACE2 attenuated cardiac and renal injury in diabetic mice that was associated with reduced tissue Ang II but higher renal and cardiac levels of Ang-(1–7) [23,24]. Tikellis et al. [25] found that the renal content of Ang-(1–7) was lower in ACE2^{-/-} mice and this reduction was comparable with chronic treatment of wild-type mice with an ACE inhibitor. Lavrentyev et al. [26] show that ACE2 siRNA treatment reduced the cellular levels of Ang-(1–7) in rat aortic smooth muscle cells. Mompeón et al. [27] recently demonstrated that estradiol increased the cellular levels of Ang-(1–7) that associated with both higher ACE and ACE2 expression and activity via activation of the estrogen receptor- α (ER α) in human umbilical vein endothelial cells. The present study suggests that intracellular Ang-(1–7) expression is dependent in part on Ang II generation by ACE and subsequent conversion by ACE2; however, whether Ang-(1–7) generation reflects intracellular processing or that extracellular conversion of Ang II into Ang-(1–7) leads to the subsequent uptake of Ang-(1–7) by the MasR is not currently known [15]. The contribution of ACE2 compared with endopeptidases such as neprilysin to the generation of Ang-(1–7) may reflect different tissue or cellular (intracellular) compartments, as well as the underlying pathological conditions.

Ang-(1–7) degradation

Endogenous expression of peptide hormones is balanced by both forming and degrading peptidases [28]. We identified ACE as a key degrading peptidase of Ang-(1–7) in the circulation, lung, kidney, and cerebrospinal fluid (CSF) [15]. ACE acting as a dipeptidyl carboxypeptidase efficiently cleaves the Ile⁵–His⁶ bond of Ang-(1–7) to form Ang-(1–5) and the dipeptide His–Pro [15] (Figure 2). We could not demonstrate the generation of Ang-(1–7) in the circulation following Ang I administration in WKY and SHR rats unless they were pretreated with an ACE inhibitor that likely reflects the greater abundance of vascular ACE to neprilysin [15]. Similarly, ACE inhibition was necessary to show accumulation of Ang-(1–7) from Ang I (neprilysin) or Ang II (ACE2) in isolated proximal tubules (PTs) of sheep [29]. In sheep CSF, ACE hydrolyzed Ang-(1–7) to Ang-(1–5); however, an additional activity distinct from ACE accounted for greater metabolism of Ang-(1–7) [30,31]. Cruz-Diaz et al. [32] subsequently purified and identified this activity as dipeptidyl aminopeptidase 3 (DPP3). This peptidase cleaves two residues from the N-terminus of peptides between four and eight residues in length. DPP3 is primarily a cytosolic peptidase, but soluble forms are present in the blood, urine, and CSF [15]. The peptidase initially cleaves the Arg²–Val³ bond of Ang-(1–7) to form Ang-(3–7) and Asp–Arg, then rapidly hydrolyzes the Tyr⁴–Ile⁵ bond of Ang-(3–7) to Ang-(5–7) and the dipeptide Val–Tyr [32] (Figure 2). We also identified JMV390 as a potent inhibitor of DPP3-dependent hydrolysis of Ang-(1–7) (IC₅₀ < 1 nM) in screening various metallopeptidase inhibitors that may attenuate DPP3 activity [32]. Low-dose treatment tended to increase the intracellular levels

of Ang-(1–7) in human HK-2 renal cells; at higher doses, JMV390 inhibits the metalloendopeptidases neprilysin and TOP that form Ang-(1–7), thus, a more targeted approach is required to discern an endogenous role of DPP3 in the metabolism of Ang-(1–7) [32].

Ang-(1–7) receptors and signaling pathways

Key contributions to the concept of an Ang-(1–7) axis functionally distinct from the Ang II pathway were the identification of the Mas protein as the putative receptor for Ang-(1–7) and the synthesis of selective receptor agonists and antagonists [33–36]. The identification of a unique Ang-(1–7) receptor also accelerated studies to identify the signaling pathways of the peptide. Heitsch et al. [37] first reported that Ang-(1–7) directly stimulates nitric oxide (NO) in bovine endothelial cells that likely contribute to the vascular effects of Ang-(1–7); the NO response was subsequently confirmed in human cells that also identified the role of PI3 kinase and Akt in NO stimulation by the peptide [38]. Under certain conditions, stimulation of NO by Ang-(1–7) may involve downstream interactions with the bradykinin B₂ receptor, AT₁R, or AT₂R that suggest a complex interaction of Ang-(1–7)-MasR with other receptor systems [16,17]. As one example, the potent vasorelaxation of the rat renal artery by Ang-(1–7) [ED₅₀ = 3 nM] was abolished by the MasR antagonists [D-Ala⁷]-Ang-(1–7) and [D-Pro⁷]-Ang-(1–7) [39]. However, Ang-(1–7) vasorelaxation was also blocked by AT₁R (losartan), AT₂R (PD123319), and B₂ receptor (HOE 140) antagonists [39]. Indeed, these data confirm earlier findings by Heitsch et al. [37] that multiple receptor antagonists attenuated NO generation by Ang-(1–7) in endothelial cells. It is possible that Ang-(1–7) may recognize endothelial AT₁Rs to stimulate NO release, but not the smooth muscle AT₁R that predominate in the overall vasoconstrictor response to Ang II [39]. Ang-(1–7) is reported to act as a biased agonist at the AT₁R to promote β -arrestin stimulation, albeit higher concentrations of Ang-(1–7) were required (300 nM) to evoke this response [40]. In regard to the AT₂ receptor, activation of the Ang II receptor subtype is linked to the release of bradykinin and stimulation of the B₂R-NO response. Moreover, Steckelings et al. recently showed that MasR-AT₂R heterodimerization was required for the actions of Ang-(1–7) to induce the CX3C chemokine receptor in astrocytes, although the contribution of the B₂R pathway in the present study was not assessed [41]. Ang-(1–7) or its derivative [Ala¹]-Ang-(1–7) (alamandine) also recognizes the Mrg-D receptor which appears to share similar functional properties to the MasR [16,42]. The identity of the receptor(s) that mediate the actions of Ang-(1–7) receptor is not clearly resolved at this time and may reflect the particular tissue, species, and pathological conditions. Different Ang-(1–7) receptors may also reflect other signaling pathways invoked by the Ang-(1–7) axis that include the stimulation of vasoactive prostaglandins, regulation of cellular phosphatases, a beneficial influence on redox balance, and a recent report on improved telomerase expression [14–16,42–44].

Ang-(1–7) programming–experimental studies

Circulation

We have extensively studied an experimental model of fetal programming in which pregnant sheep are administered the glucocorticoid agonist betamethasone in the third trimester (80th day of gestation; 0.17 mg/kg) and the offspring are delivered at full term at day 145 [14]. The dose and timing of betamethasone exposure were designed to parallel glucocorticoids treatment of pregnant women at risk of delivering prematurely [45]. The clinical relevance of this model is that approximately 100000 individuals are exposed to exogenous glucocorticoids *in utero* each year in the United States to promote fetal lung development prior to premature birth [45]. In betamethasone-exposed (BMX) sheep, both female and male offspring exhibit reduced nephron number, altered autonomic function by 2 months of age, and a sustained increase in BP by 6 months of age, as well as the development of cardiometabolic disorders [14,46]. Importantly, we find no differences in the overall increase in BP or attenuation of baroreflex between female and male adult BMX sheep; however, the cellular mechanisms or signaling pathways that contribute to the BMX phenotype may significantly differ amongst females and males [14].

In the circulation of BMX sheep, ACE2 activity was reduced by approximately 50% while ACE activity tended to increase (~40%) in comparison with adult controls (Figure 3A). The ratio of ACE to ACE2 activity in the circulation was more highly correlated to BP in control and BMX sheep than either enzyme alone (**inset**) [47]. Circulating levels of Ang II and Ang-(1–7) were not significantly changed in the BMX adult sheep (*data not shown*); however, the ratio of plasma Ang II to Ang-(1–7) was significantly higher in BMX sheep as compared with the unexposed sheep suggesting that BMX-induced programming may differentially impact the RAS yielding greater Ang II but reduced Ang-(1–7) expression or tone (Figure 3B). In contrast with our studies in BMX sheep, Yu et al. [48] reported reduced plasma levels of both Ang II and Ang-(1–7) in male adult offspring of dexamethasone-treated pregnant rats (14–20 days of gestation; 0.1 mg/kg). However, plasma Ang-(1–7) levels in the present study were *30-fold higher* than Ang II (1200 compared with 40 pg/ml; 1400 compared with 40 pM), and the specificity of their Ang-(1–7) measurements by ELISA and/or treatment of the plasma samples is a concern [48]. We note that circulating ACE2 activity was comparable with ACE activity in the sheep as both peptidases were assayed under identical enzymatic conditions using Ang II and Ang I as substrates, respectively (Figure 3A). However, serum ACE2 was markedly lower than ACE in normotensive Lewis or hypertensive female and male mRen2.Lewis rats [49]. Although serum ACE2 activity was higher in the hypertensive males and increased in both female and male diabetic mRen2.Lewis rats, ACE remained the predominant activity in serum that would strongly favor Ang II generation (and Ang-(1–7) degradation) [49]. The functional significance of comparable ACE2 and ACE activities in the circulation of sheep is presently unclear. The sheep ACE2 isoform may be more susceptible to sheddase activity to release the soluble form of the peptidase, or ACE2 may play a greater role in the formation of Ang-(1–7) in the circulation of sheep, particularly as circulating neprilysin activity in either unexposed or BMX sheep was undetectable (Figure 3A).

Kidney

The kidney is a key target of programming events in various experimental models and programming may stimulate the Ang II axis to enhance vasoconstriction and the reabsorption of Na^+ to increase BP [10,12,50,51]. In contrast with the pressor and anti-natriuretic actions of Ang II, Ang-(1–7) promotes vasorelaxation and the excretion of Na^+ to reduce blood volume and pressure as well as antagonize Ang II-AT₁R signaling within the kidney [16,52]. Moreover, down-regulation of the ACE2-Ang-(1–7)-MasR axis by fetal programming events may facilitate renal Ang II tone, inflammation and oxidative stress to enhance Na^+ reabsorption particularly in males [52]. Indeed, the male BMX sheep excrete less Na^+ than that of the unexposed males following an acute intravenous Na^+ load [53–55]. Lithium excretion was also attenuated in BMX male sheep and suggests that programming events may influence PT handling of Na^+ [53]. Remarkably, the natriuretic response to exogenous Ang-(1–7) was essentially absent from the male BMX sheep while the Ang II response was exacerbated [53]. In contrast with the male sheep, females excreted more Na^+ , and the Ang-(1–7) natriuretic response was preserved in the female BMX sheep [53]. The attenuated Ang-(1–7) response in BMX males was not due to enhanced ACE-dependent degradation of the peptide as renal ACE activity was comparable in isolated tubules of unexposed and BMX sheep [47]. However, ACE2 activity and protein expression were significantly reduced (50 and 75%, respectively) in the BMX male, while neprilysin was unchanged [47]. Urinary levels of ACE2 were also reduced in the BMX group which suggests that enhanced shedding of the peptidase does not account for the reduced renal content of the peptidase [47]. Tain et al. [56] reported a trend for reduced renal ACE2 expression in a fructose-fed rat model of programming, and that administration of the soluble epoxide hydrolase inhibitor 12-[[[tricyclo[3.3.1.1.3,7]dec-1-ylamino)carbonyl]amino]-dodecanoic acid (AUDA) reversed the decline in ACE2 expression. ACE2 mRNA levels were also reduced in the kidneys of adult female rats exposed to corticosterone *in utero*; however, renal Ang-(1–7) tended to *increase* which may confer a renoprotective effect in this model [57]. The mechanism for reduced ACE2 and enhanced levels of Ang-(1–7) in the kidney was not addressed in the latter study, but renal endopeptidases may contribute to higher Ang-(1–7) content in this model. Moreover, since ACE2 is subject to post-transcriptional regulation, the measure of mRNA levels is not sufficient to fully characterize the activity of this key metabolism pathway [15]. Riviere et al. [58] found no change in renal ACE2 activity in adult male offspring of undernutrition-programmed rats, but pulmonary ACE2 activity increased two-fold which again may serve as a protective response to promote higher levels of Ang-(1–7). In contrast, Goyal et al. [59] demonstrated reduced pulmonary ACE2 in female but not male offspring of protein-restricted mice that correlated with higher BP in the females. Although the disparate changes in ACE2 expression may reflect different programming models in these studies, programming events that target a reduction in this enzymatic focal point of the RAS would likely promote a higher ratio of Ang II to Ang-(1–7) within the kidney that may facilitate an increase in vascular tone and Na^+ reabsorption contributing to an increase in BP.

Immunofluorescent studies revealed expression of the MasR in the PT, as well as other tubule elements including the thick ascending limb of Henle and medullary collecting duct of the sheep kidney [14]. Consistent with earlier studies in isolated rabbit PTs and perfused

rat PT segments, Ang-(1–7) potentially inhibits Na⁺ uptake in primary PT cells purified from unexposed (Control) male and female sheep (Figure 4A) [60–62]. Similar to the intact BMX animal, Ang-(1–7) failed to inhibit Na⁺ uptake in primary PT cells from male, but not female BMX sheep (Figure 4B). The attenuated Ang-(1–7) response may reflect, in part, the reduced expression of the MasR in male BMX sheep [62]. However, the NO donor SNAP and the non-hydrolysable cGMP analog 8-bromo-cGMP also failed to inhibit Na⁺ uptake in male BMX PT cells; the SNAP and cGMP responses in the female BMX PT cells remained intact (Figure 4B). Importantly, these data suggest that the signaling pathway *downstream* from the Ang-(1–7)-MasR-NO axis is also impaired in the kidney of the BMX males. cGMP is an important cofactor for several kinases including protein kinase G (PKG) which may directly phosphorylate the Na⁺/H⁺ exchanger 3 (NHE3) on multiple residues to attenuate activity and/or trafficking to the apical membrane of epithelial cells [63,64]. Indeed, overall NHE3 protein expression was higher in the PT cells from male BMX sheep as compared with controls and may contribute, in part, to enhanced Na⁺ uptake [54].

Preliminary findings also reveal that the MAP kinase (MAPK) pathway is chronically stimulated in the PTs of adult male BMX sheep. Isolated PT cells from adult male BMX sheep at 1 year of age exhibit higher expression of both phospho-ERK1/2 (p-ERK1/2) and p-p38 than non-exposed animals (Supplementary Figure 1A,B, respectively) [65]. Chronic MAPK stimulation is also evident in the dorsal medulla of adult male BMX sheep, a key cardiovascular center in the brain involved in autonomic flow and baroreflex sensitivity [66] —see following section on ‘Brain RAS’. Gallagher et al. [67] demonstrate that an activated MAPK pathway contributes to the down-regulation of ACE2 and that Ang-(1–7) treatment preserves ACE2 in response to Ang II or endothelin treatment. Thus, programming events that stimulate MAPK pathway may contribute to down-regulation of ACE2 in multiple tissues. Since Ang-(1–7) stimulates cellular phosphatases that may inactivate MAPK, we also assessed protein expression of the dual specificity phosphatase 1 isoform (DUSP1) in isolated PTs. DUSP1 expression was similar in control and BMX PTs (Supplementary Figure 1C). The increase in MAPK within the PT of BMX sheep does not appear to reflect an attenuated expression of DUSP1; however, the loss of Ang-(1–7) tone may fail to antagonize the Ang II-AT₁R axis within the kidney upstream of MAPK that could facilitate an increase in Na⁺ uptake. Although the signaling pathways for Ang-(1–7) in the PT and other nephron segments are far from resolved, our studies suggest that the Ang-(1–7) axis is a target of fetal programming events following glucocorticoid exposure that may contribute to alterations in Na⁺ handling in males. The female sheep appear resistant to BMX programming effects on the renal Ang-(1–7) axis and Na⁺ handling; however, the exact nature of this protective mechanism is unknown and whether this effect wanes in older animals with reduced estrogen tone awaits further study.

Brain

The brain RAS, particularly activation of the Ang II axis, is increasingly associated with inflammation, hypertension, and altered autonomic function [68,69]. Few studies to date have investigated the influence of fetal programming events on the brain RAS and, in particular, the role of the Ang-(1–7) axis in the brain is virtually unknown. In the BMX sheep, baroreflex sensitivity which regulates the control of heart rate is markedly attenuated

in both females and males as early as 6 weeks of age; the decline in the baroreflex is apparent well before the increase in BP that occurs at 6 months [70]. Preliminary studies reveal that chronic intraventricular infusion of Ang-(1–7) at 5 months of age abolishes the increase in BP and improves various indices of autonomic function in both female and male BMX sheep [71]. The central effects of Ang-(1–7) infusion in the BMX sheep were associated with a reduction in both p-ERK1/2 and the oxidative stress marker 4-hydroxynonenal (4HNE) in the dorsal medulla of the male BMX sheep; however, p-ERK1/2 and 4HNE expression were not changed in females despite the improved autonomic function and reduction in BP [71]. These data again suggest sex differences in the regulatory signaling pathways that may be linked to alterations in the Ang-(1–7) axis due to fetal programming events.

The functional effects of fetal programming on the central Ang-(1–7) axis are supported by alterations in the biochemical components within the dorsal medulla and CSF compartments. We documented renin activity and angiotensinogen protein expression in the sheep dorsal medulla, and that medullary angiotensinogen was higher in male BMX sheep as compared with the unexposed sheep (Figure 5A) [72]. These data support a local RAS system in the brain, particularly the dorsal medulla that appears to be an important target of programming events to influence BP [68,69,73]. In the brain medullary tissue, the peptide ratios of Ang II to Ang I and Ang II to Ang-(1–7) were significantly higher in BMX sheep as compared with controls (Figure 5B) [72]. Similar to our findings in the kidney, immunoblot analysis revealed lower expression of the MasR protein in the dorsal medulla of BMX sheep as compared with the controls at 0.5 and 1.8 years of age (Figure 5C) [72]. Preliminary studies also reveal that ACE2 protein expression was markedly reduced (~90%) in the brain medulla of adult BMX sheep which may contribute to the higher peptide ratios of Ang II/Ang I and Ang II/Ang-(1–7) in this region (Figure 5D) [74]. The effects of programming events on other peptidases including ACE, neprilysin, and TOP in the brain medulla are the focus of ongoing studies. Furthermore, endogenous Ang-(1–7) content was positively correlated to MasR expression in the BMX and control sheep in keeping with the concept that the peptide may have a positive impact on its own receptor [15,16,73]. This may explain the efficacy of Ang-(1–7) treatment to lower BP and restore autonomic function despite the reduction in MasR expression in the brain of BMX sheep [72,73]. Preliminary studies find that MasR expression tended to increase in the dorsomedial medulla of male BMX sheep following ICV Ang-(1–7) infusion (Supplementary Figure 2A). Our preliminary data also reveal increased tissue levels of the scavenging enzyme superoxide dismutase 1 (SOD1) in the male BMX sheep following ICV Ang-(1–7) which may contribute to reduced levels of oxidative stress (4HNE) and the correction of autonomic function in these animals (Supplementary Figure 2B) [71].

Placenta

There is evidence for a placental Ang-(1–7) system and that the tissue components may be altered by programming events [75–80]. Stettner et al. [78] reported that Ang-(1–7) was the primary product of Ang I metabolism in the human placenta using HPLC separation and MS analysis, although the peptidases responsible for Ang-(1–7) formation were not identified in their study. ACE2 protein expression was significantly reduced in the placenta of both

female and male protein-restricted rats while ACE was unchanged; however, the placental contents of Ang-(1–7) or Ang II were not determined [79]. Ghadhanafar et al. [80] found both ACE2 and Ang-(1–7) expression were reduced in the placenta of dexamethasone-exposed rats that associated with low birth weight of the offspring; however, protein expression of the MasR and neprilysin were significantly *increased* in this model. Since the activity levels of ACE2 and neprilysin in the formation of Ang-(1–7) were not assessed, it is unclear whether lower ACE2 or higher neprilysin would have a greater impact on Ang-(1–7) levels in the placenta. Moreover, the placental expression of Ang-(1–7) in the Ghadhanafar et al. [80] study was detected by Western blot at a molecular size of 34 kDa. As the native size of Ang-(1–7) is 40-fold less at 0.89 kDa and peptide levels typically in the fmol (pg) per gram range are below immunoblot detection limits, the identity of placenta Ang-(1–7) in this study is highly questionable [80]. Yamalayeve et al. [75] utilized an extensive tissue extraction method and distinct RIAs to demonstrate that placental levels of Ang II were 2.5-fold higher than Ang-(1–7); however, Ang II and Ang-(1–7) levels were not different in the placentas of control and preeclamptic rats. Although the AT₁R was the predominant receptor in the placenta of both groups (>95% binding) in the present study, the proportion of MasR binding in the placenta was also comparable between control and preeclamptic animals [75]. Finally, Bharadwaj et al. [77] using an identical biochemical approach with the previous study reported 3-fold higher Ang II content in the placenta of ACE2 null mice that exhibit impaired gestational weight gain and fetal growth restriction (Figure 6). Higher placental Ang II in the ACE2 transgenic mice would be expected with the loss of this metabolism pathway; however, there was no change in either Ang-(1–7) content or neprilysin activity and these data may imply that neprilysin is the predominant pathway for the generation of Ang-(1–7) in the placenta (Figure 6). Moreover, the augmented levels of placenta Ang II may contribute to the fetal phenotype of this transgenic model [77].

Premature birth

Premature birth and other adverse perinatal events are associated with an increased risk of cardiovascular disease later in life that is detectable during childhood [6,81–83]. Evidence from experimental and clinical studies suggest that numerous organ systems are affected and thus contribute to the development of cardiovascular disease, including alterations to the vasculature and heart, the kidneys, and the brain [84]. Individuals with perinatal risk factors such as preterm birth, very low birth weight, intrauterine growth restriction, or maternal preeclampsia have higher BP and an increased risk of hypertension later in life, even during childhood, adolescence, and young adulthood [85–88]. Preterm birth and intrauterine growth restriction are associated with dyslipidemia, impaired endothelial function, and atherosclerosis during childhood, adolescence, and later adulthood [89–92]. Preterm birth is also associated with increased left and right ventricular mass, altered biventricular geometry, and heart failure in children and young adults [93–95]. Preterm birth and intrauterine growth restriction influence renal function and increase the risk for chronic kidney disease which contributes to the elevated risk for hypertension [96,97]. Finally, preterm birth and extremely low birth weight are associated with abnormal autonomic function that is present in infants and persists into adulthood and likely further contributes to cardiovascular disease development [98,99].

The effects of glucocorticoid exposure combined with prematurity on the development of cardiovascular disease remains equivocal in young adults. Several studies report no effects while others suggest that exposed individuals are at increased cardiovascular risk [100,101]. The reasons for this discrepancy are unclear, but likely reflect the heterogeneity in the various perinatal programming events including the underlying reasons for an infant's premature birth. Our data suggest that subpopulations including African-Americans, female sex, and those exposed to maternal hypertensive pregnancy all may be more susceptible to this particular programming event. Indeed, the analysis of Ang-(1–7) levels may be clinically useful to risk-stratify these individuals [102–104]. Moreover, genetic polymorphisms in ACE2 that are reported to associate with hypertension and cardiovascular diseases may potentially contribute to the altered cardiovascular phenotype of the exposed offspring [105,106]. The emerging evidence in humans supports the findings in experimental studies that early life events may induce long-lasting effects on the Ang-(1–7) axis. Thus, Ang-(1–7) programming may serve as a mechanism underlying the development of hypertension and cardiovascular disease in at-risk patients.

Ang-(1–7) programming–human studies

Circulation

In regard to the maternal RAS, Merrill et al. [107] reported lower plasma levels of Ang-(1–7) in pregnant women with preeclampsia as compared with normotensive pregnant women. Circulating levels of Ang-(1–7) increased 60% from 17 to 27 pM in normal pregnancy, but levels were blunted to 13 pM in preeclamptic women [107]. Higher circulating Ang-(1–7) may potentially facilitate the marked increase in vasodilatory tone and vascular remodeling that occurs during pregnancy [107]. Velloso et al. [108] also reported a similar reduction in plasma levels of Ang-(1–7) (19 compared with 25 pM) in preeclamptic women as compared with those with a healthy pregnancy. Moreover, lower Ang-(1–7) levels were associated with the deletion/deletion (DD) ACE genotype and higher serum ACE activity which may facilitate greater metabolism of Ang-(1–7) [108]. Studies by Chen et al. [109] revealed that maternal plasma Ang-(1–7) levels were lower in pregnant women with premature birth and reduced Ang-(1–7) may constitute an independent risk factor for premature birth. These investigators also show higher plasma aldosterone and plasma renin activity in pregnant women with gestational diabetes [110]. Indeed, Nogueira et al. [111] reported that pregnant women with gestational diabetes exhibited lower plasma levels of Ang-(1–7) compared with healthy pregnancy (19 compared with 48 pM), as well as a higher Ang II to Ang-(1–7) ratio. Since Ang-(1–7) is known to improve metabolic function [16], a deficit in Ang-(1–7) in addition to greater Ang II tone may potentially contribute to impaired glycemic control during pregnancy or possibly in the affected offspring.

In studies of programming events in the offspring, Franco et al. [112] found reduced circulating Ang-(1–7) that negatively associated with BP in the low birth weight children at 10 years of age while Ang II and ACE activity positively correlated to pressure. However, plasma levels of Ang-(1–7) and Ang II in this study were 40–80 nM which are >1000-fold higher than the expected plasma values for either peptide (10–50 pM), and raise particular concern on the appropriate handling of the blood samples and the specificity of their peptide

analysis by direct HPLC-UV absorption [15]. Chen et al. [109] has subsequently shown that plasma Ang-(1–7) levels obtained from umbilical cord blood were ~40% lower in neonates born preterm compared with term-born and that fetal Ang-(1–7) may also be a risk factor for prematurity. Fetal plasma levels of Ang-(1–7) in the term group were 11-fold higher than Ang II (900 compared with 80 pM) as assessed by separate ELISAs and a similar concentration range for Ang-(1–7) and Ang II was reported for the maternal circulation [109]. Moreover, this group has recently reported that the fetal but not maternal ACE2 polymorphism rs2074192 associated with lower birth weight [113]. We have examined the effects of perinatal programming on future cardiovascular health in a prospective cohort of individuals born preterm with very low birth weight compared with a term-born control cohort and show that alterations in the Ang-(1–7) pathway persist into adolescence and young adulthood. At the age of 14 years, adolescents born preterm had reduced plasma Ang-(1–7) levels and a higher plasma ratio of Ang II to Ang-(1–7) as compared with their term-born peers; importantly, these effects were greater in female adolescents and in subjects with obesity (Figure 7A,B, respectively) [102]. In this cohort, the circulating levels of Ang-(1–7) and Ang II were quantified in the low pM range (5–30 pM) with a 2–3 fold higher Ang II to Ang-(1–7) ratio [102]. We also find that amongst the preterm-born adolescents, antenatal glucocorticoid exposure was associated with increased plasma Ang-(1–7) and a decreased plasma ratio of Ang II to Ang-(1–7), especially in black adolescents [104]. The reason for this apparent discrepancy is unclear but higher levels of Ang-(1–7) may serve as a compensatory systemic mechanism to counteract local tissue-level reductions in Ang-(1–7) and augmented Ang II as evident in several pathologic states [49,114].

Our preterm birth cohort, compared with those born at term, had persistently higher BP and a greater rate of high BP as compared with the term birth cohort both at 14 and 19 years of age [102,115]. In this case, the higher BP was associated with an increased ratio of plasma Ang II to Ang-(1–7) [116]. We have begun to identify specific mechanisms through which programmed changes in the Ang-(1–7) pathway may contribute to cardiovascular disease. Uric acid, a byproduct of purine metabolism, is associated with increased BP and development of hypertension [117]. We reported that serum uric acid is higher in our preterm at age 14 as compared with the term group [117,118]. Moreover, the higher serum uric acid content was associated with lower plasma Ang-(1–7) and a higher ratio of Ang II to Ang-(1–7) in our cohort of preterms at 14 years of age [115]. Uric acid stimulates expression of components of the classical RAS including ACE, angiotensinogen, and Ang II by increased oxidative stress and activated MAPK in endothelial, vascular smooth muscle, and fat cells [119–121]; however, the impact on the ACE2-Ang-(1–7)-MasR axis is currently unknown. Our clinical data suggest that perinatal programming of the circulating Ang-(1–7) axis may lead to hypertension and cardiovascular disease and that this may in part be related to alterations in the levels of serum uric acid.

Kidney

We have evidence that perinatal programming is associated with long-term alterations in the Ang-(1–7) pathway in the kidneys. Amongst patients born preterm, antenatal glucocorticoid exposure was associated with an increased urinary ratio of Ang II to Ang-(1–7) [104]. In addition to our findings of renal RAS alterations, preterm-born adolescents had reduced

renal function compared with those born at term (preterm-born compared with term-born estimated glomerular filtration rate: 126.2 compared with 134.3 ml/min/1.73 m², $P<0.05$), an effect that was greater in female adolescents and in subjects with obesity [122].

We have identified potential mechanisms through which programmed renal Ang-(1–7) pathway abnormalities may contribute to cardiovascular disease. The BP-lowering effect of renal Ang-(1–7) may be blunted in individuals born preterm. Higher urinary Ang-(1–7) levels evident at the age of 14 years were associated with lower BP at 14 years (Figure 8A,B), as well as predicted lower BP at 19 years (Figure 8C,D) in term-born subjects but not preterm-born subjects [115]. At age 19 years, lower urinary Ang-(1–7) was also associated with reduced urinary sodium in the preterm birth cohort only [115]. The anti-ageing protein α -klotho is associated with reduced BP and protects against cardiovascular disease [123,124]. We recently reported that at age 19 years, suppressed levels of urinary Ang-(1–7) were associated with reduced urinary α -klotho and increased serum uric acid in the preterm birth group only [115,125]. Thus, we have emerging clinical evidence that perinatal programming may induce long-lasting effects on the renal Ang-(1–7) pathway which contributes to hypertension and cardiovascular disease through interactions with renal sodium handling, α -klotho, and uric acid.

Brain

Consistent with other clinical studies, our preterm birth cohort exhibits abnormal autonomic function including altered heart rate variability (HRV) and reduced baroreflex sensitivity [126–128]. Impaired autonomic control in children born preterm may reflect differences in maturation of the two branches of the autonomic nervous system (ANS). Schneider et al. [129] find that HRV during the fetal life is differentially influenced by the sympathetic and parasympathetic systems. During prenatal life sympathetic activity is predominant while parasympathetic activity rapidly increases between 25 and 32 weeks of gestation [129]. Further longitudinal monitoring of fetal HRV provided additional support to the concept of increasing sympathetic activity from late second trimester into early third trimester associated by a higher variability in parasympathetic modulation [130]. This is consistent with previous reports that maturation of primitive neurons in the parasympathetic brain stem nuclei reaches their maximal pace at 30 weeks of gestational age and the vagal component of ANS is stable past week 32 of gestation [131,132]. Mulkey et al. [133] recently reported that infants born preterm with normal neuroimaging at 39 weeks of age exhibited a depressed ANS tone (HRV) in both sympathetic and parasympathetic indices as compared with term born infants at a similar age. Moreover, infants born preterm at 29 weeks exhibited a greater low frequency to high frequency ratio (LF/HF), an index of sympathovagal balance and reduced HF measures of HRV assessed by frequency domain analysis as compared with age corrected term controls at 2–3 months of age [134]. In addition to reduced Ang-(1–7), higher serum uric acid was associated with impaired baroreflex sensitivity in young adults born preterm but not those born term [127]. Indeed, this is consistent with the BMX sheep where the exposed animals exhibited a higher LF/HF ratio and lower HRV and baroreflex sensitivity that is indicative of reduced parasympathetic tone [70,135]. Moreover, chronic intracerebroventricular administration of Ang-(1–7) in the BMX sheep at 5 months of age improved the parasympathetic component of HRV and baroreflex sensitivity and reduced the

LF/HF ratio [135]. Overall, these data suggest that modulation of the RAS, particularly attenuation of the Ang-(1–7) axis may contribute to a sustained impairment in autonomic function in those born preterm, and that uric acid may have an influence on Ang-(1–7) tone.

Conclusion

Cardiovascular disease is the leading cause of morbidity and mortality worldwide, and hypertension is the leading risk factor for cardiovascular disease and the largest contributor to mortality. The early life environment is emerging as a key window for programming of many biological processes by perinatal events that increase an individual's lifetime risk for developing hypertension and cardiovascular disease. Opportunities may exist for altering this adverse trajectory, particularly in childhood and early adulthood. The counter-regulatory axis of the RAS, the Ang-(1–7) pathway, may play a crucial role in preventing and attenuating cardiovascular and renal disease. Experimental studies have suggested that perinatal programming events lead to chronic alterations in the Ang-(1–7) axis, particularly targeting ACE2 and the MasR within the kidney and brain, thus providing a potential mechanism for the development of hypertension and cardiovascular disease. Emerging clinical evidence from a longitudinal cohort of young adults born preterm strongly supports this experimental data implicating long-lasting perinatal programming effects on the Ang-(1–7) axis in the development of hypertension and cardiovascular disease in humans characterized by persistent alterations in Ang-(1–7) in the circulation and the kidney during adolescence and young adulthood that are associated with abnormal renal sodium handling. Additional targets may include the anti-ageing protein α -klotho and hypertension-promoting uric acid that may differentially impact the two major axes of the RAS. Further work is clearly needed to determine causal relationships amongst perinatal programming events and Ang-(1–7) pathway abnormalities and their ability to predict the development of hypertension and cardiovascular disease. In this regard, the appropriate approaches in sample handling and peptide analysis are imperative for the unequivocal evaluation of programming events and the Ang-(1–7) axis [15]. Moreover, novel therapies may be identified that focus on up-regulating the Ang-(1–7) and α -klotho systems and reducing uric acid, in order to prevent or treat perinatal programmed hypertension and cardiovascular disease. For example, further research is warranted as to whether exogenous treatment with orally active and stable Ang-(1–7) agonists or allopurinol (an inhibitor of uric acid production) are effective [136,137]. The molecular mechanisms of programming for the targeted down-regulation of the ACE2-Ang-(1–7)-MasR-NO axis in the brain, kidney, and other issues also require elucidation as this may impact future therapeutic approaches to target possible epigenetic alterations that may influence the functional Ang-(1–7) axis in the children of programmed adults. Although there is substantial evidence for epigenetic alterations of various targets in programming events [138–140], to our knowledge there are no published studies regarding epigenetic changes in the components of the Ang-(1–7) axis including the MasR, ACE2, and neprilysin. Finally, while both males and females are afflicted by prematurity in their increased risk of cardiovascular disease, experimental and clinical data reveal that the molecular and biochemical mechanisms underlying the impaired autonomics and hemodynamics likely differ. Thus, therapeutic approaches targeted

appropriately to these sex differences may offer the best opportunity to mitigate the progression of cardiovascular disease in both women and men.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding

This work was supported in part by the Wake Forest University Health Sciences [grant number CTSI UL1TR001420]; the Cardiovascular Sciences Center [grant number CVSC-830114]; the Hypertension and Vascular Research Center; the National Institutes of Health [grant numbers HD-047584, NS-105212-01, HD-008843, HD-084227]; the American Heart Association [grant numbers AHA-151521, AHA-18TPA34170522]; and the Farley Hudson Foundation.

Abbreviations

ACE	angiotensin-converting enzyme
Akt	Protein Kinase B
Ang	angiotensin
ANS	autonomic nervous system
AT₁R	Ang II type 1 receptor
BMX	betamethasone-exposed
BP	blood pressure
CSF	cerebrospinal fluid
DPP3	dipeptidyl aminopeptidase 3
DUSP1	dual specificity phosphatase 1 isoform
ERK	Extracellular Regulated Kinase
HRV	heart rate variability
ICV	Intracerebroventricular
LF/HF	low frequency to high frequency ratio
MAP	Mean Arterial Pressure
MAPK	MAP kinase
MasR	Mas receptor
Mrg-D	Mas-related receptor
NHE3	Na ⁺ /H ⁺ exchanger 3
PI3K	Phosphoinositol 3 Kinase

POP	prolyl oligopeptidase
PT	proximal tubule
RAS	renin–Ang system
SHR	Spontaneously Hypertensive rat
SNAP	S-Nitroso-N-acetyl-Penicillamine
TOP	thimet oligopeptidase
WKY	Wistar-Kyoto normotensive rat
4HNE	4-hydroxynonenal

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Fetal Programming Events

ACE2-Ang-(1-7)-MasR-NO Axis

Circulation

(-) ACE2
(+) ACE/ACE2
(+) Ang II/Ang7

Kidney

(-) MasR
(-) ACE2
(-) NO
(-) Na⁺
(+) MAPK

Brain

(-) MasR
(-) ACE2
(-) BRS
(+) MAPK
(-) Ang7
(+) Ang II/Ang7

Figure 1. Programming events may contribute to the development of hypertension and cardiovascular disease by attenuating the ACE2-Ang-(1-7)-MasR-NO axis
Fetal programming targets components of the Ang-(1-7) axis in the circulation, kidney, and brain that may contribute to the chronic dysregulation of BP and autonomic function leading to hypertension and cardiovascular disease. Based on a model of antenatal glucocorticoid exposure in sheep and a cohort of young adults born preterm, our studies suggest that programming reduces ACE2 (-) and enhances the ratio of ACE to ACE2 (+) and the ratio of Ang II to Ang-(1-7) (+) in the circulation and brain. Programming events also reduce expression of the MasR (-) in brain and kidney that is associated with reduced Na⁺ excretion, lower nitric oxide (NO) tone, and higher MAPK stimulation as well as attenuated baroreflex sensitivity (BRS).

Figure 2. Processing pathways for Ang II and Ang-(1-7)

The precursor angiotensinogen is cleaved by renin to Ang I. Ang I is hydrolyzed by Ang-converting enzyme (ACE) to Ang II which binds to the AT₁R to stimulate reactive oxygen species (ROS), but reduce nitric oxide (NO). Alternatively, Ang I is hydrolyzed by neprilysin directly to Ang-(1-7) or Ang II is cleaved by ACE2 to form Ang-(1-7) that binds to the MasR to stimulate NO and reduce ROS. ACE degrades Ang-(1-7) to Ang-(1-5) while dipeptidyl peptidase 3 (DPP3) hydrolyzes Ang-(1-7) to Ang-(3-7) and then Ang-(3-7) to Ang-(5-7). Note the figure does not depict the binding of Ang II/Ang III or potential interaction of Ang-(1-7) with the AT₂R or bradykinin B₂R. Adapted from Chappell [15].

Figure 3. Betamethasone exposure influences the circulating Ang system in adult male sheep
(A) Circulating ACE2 activity is significantly reduced in antenatal BMX adult male sheep compared with controls; serum ACE activity is significantly higher than ACE2 in adult male BMX but not control male sheep. **Inset:** The ACE to ACE2 activity ratio significantly correlated to mean arterial pressure (MAP) in adult BMX and control male sheep; shown are the correlation coefficient with P-value and regression line with 95% confidence limits. **(B)** The Ang II to Ang-(1-7) ratio is higher in the plasma of adult male BMX sheep compared with controls. Data are means $\pm$ S.E.M. with * P 0.05 by two-way ANOVA. ACE and ACE2 data adapted from Shaltout et al. [47]. Peptide measurements in (B) from plasma of adult male control ($n=6$) and BMX ($n=5$) sheep using separate RIAs for Ang I, Ang II, and Ang-(1-7) with * P 0.05 by unpaired Student's t test (Chappell, unpublished data).

Figure 4. Sodium uptake in isolated proximal tubule cells of female and male controls and BMX sheep

Isolated proximal tubule cells (PTCs) from unexposed (Control) and BMX sheep were maintained in primary cultures for 10 days and the uptake of sodium (Na⁺) was evaluated by Na⁺ green fluorescence. **(A)** Basal Na⁺ uptake was higher in Control male compared with female PTCs. Ang-(1–7) (Ang7, 1 pM) inhibited Na⁺ uptake in Control male and female PTCs. The NO donor SNAP (100 μM) and the stable analog 8-bromo-cGMP (GMP, 1 μM) also inhibited Na⁺ uptake in both Control male and female PTCs. **(B)** Basal Na⁺ uptake was higher in BMX male compared with BMX female PTCs. Treatment with Ang7, SNAP and GMP reduced Na⁺ uptake in BMX female, but failed to reduce Na⁺ in BMX male PTCs.

Data are mean ± S.E.M.; **P* 0.05 compared with males; #*P* 0.05 compared with Basal Females; †*P* 0.05 compared with Basal Males by two-way ANOVA. Adapted from Su et al. [62].

Figure 5. BMX influences the brain Ang system in adult sheep

(A) Dorsomedial medullary expression of angiotensinogen (Aogen) protein is higher in BMX sheep. (B) Peptide ratios of Ang II to Ang I and Ang II to Ang-(1–7) (Ang7) are higher in dorsomedial medulla of BMX sheep. (C) MasR protein expression is lower in dorsomedial medulla of BMX sheep at 0.5 and 1.8 years of age. (D) ACE2 protein expression (120 kDa) is lower in dorsal medulla of BMX sheep. All data are mean $\pm$ S.E.M.; * P 0.05 compared with BMX; ** P 0.01 compared with BMX by unpaired Student's t test. Data in panels (A–C) are adapted from Marshall et al. [30]. Data in (D) are ratio of ACE2 to total protein of the immunoblot as adapted from Hendricks et al. [74].

Figure 6. Placental expression of Angs and neprilysin activity in wild-type and ACE2 knockout mice

Quantitation of Ang I (A), Ang II (B), Ang-(1–7) (C), and neprilysin activity (D) in the placenta of C57Bl/6 (wild-type, WT) and ACE2 knockout (KO) mice. All data are mean $\pm$ S.E.M.; * P 0.05 compared with WT by unpaired Student's t test. Adapted from Bharadwaj et al. [77].

Figure 7. Perinatal programming of the circulatory RAS resulting in reduced Ang-(1-7) is greatest in female adolescents and in adolescents with overweight/obesity at age 14 years
(A) The preterm-term birth difference in plasma Ang-(1-7) at age 14 years is significantly greater in female adolescents (preterm $n=70$, term $n=23$) compared with male adolescents (preterm $n=50$, term $n=20$); $**P = 0.001$ for female preterm-term birth comparison and $*P = 0.05$ for male preterm-term birth comparison. **(B)** The preterm-term birth difference in the ratio of plasma Ang II to Ang-(1-7) is significantly greater in adolescents with overweight/obesity (OWO; preterm $n=42$, term $n=13$; $**P = 0.01$) compared with adolescents with body mass index (BMI) < 85th percentile (preterm $n=78$, term $n=30$; $*P < 0.08$); amongst adolescents born preterm, the ratio of plasma Ang II to Ang-(1-7) is significantly higher in subjects with OWO compared with BMI < 85th percentile ($\#P = 0.001$) but not in term-born subjects. Analysis is between-group comparisons via the Wilcoxon's rank-sum test. Bars = median, large circles = mean, boxes = interquartile ranges, whiskers = $1.5 \times$ IQR. Data are adapted from South et al. [102].

Figure 8. The blood pressure-renal Ang-(1-7) relationship is blunted in patients born preterm as compared with those born at term

(A,B) Urine Ang-(1-7) corrected for urine creatinine (Cr) at 14 years of age inversely correlates with systolic blood pressure (SBP) measured at 14 years of age in Term (solid line, $n=50$; $P=0.07$) but not Preterm adolescents (dashed line, $n=175$; $P=0.98$). (C,D) Urine Ang-(1-7)/Cr at 14 years of age predicts SBP at 19 years of age in Term (solid line, $n=34$; $P=0.004$) but not Preterm young adults (dashed line, $n=139$; $P=0.98$). Urine Ang-(1-7)/Cr is naturally log transformed. Pearson correlation coefficients with corresponding P -values and regression lines with 95% confidence limits are shown (South, unpublished data).