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From pregnancy to cardiovascular disease: lessons from relaxin-deficient animals to understanding relaxin actions in the vascular system

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Abstract:

Early maternal vascular adaptations to pregnancy are predominantly driven by changes in vascular tone, reactivity and remodelling. Failure of the maternal systemic vasculature to adapt sufficiently can lead to serious complications of pregnancy. The hormone relaxin is widely recognised for its contribution to the essential renal and systemic haemodynamic adaptations in early pregnancy through direct actions on the maternal vasculature. Studies in relaxin gene knockout mice revealed that endogenous relaxin is not only a 'pregnancy hormone' but has pleiotropic actions in various tissues in males and non-pregnant females. There is strong interest in relaxin's actions in the vasculature and its utility in the treatment of vascular diseases. Relaxin treatment in rodents for 2-5 days or acute intravenous injection enhances endothelium-dependent relaxation and decreases myogenic tone in resistance arteries. These vascular actions are prolonged, even in the absence of circulating relaxin, and are underpinned by the production of endothelium-derived relaxing factors including nitric oxide, endothelium-derived hyperpolarization and prostacyclin. Relaxin is also capable of remodelling the vascular wall in a variety of blood vessels in disease conditions. Lessons learned in pregnancy research have aided studies investigating the potential therapeutic potential of relaxin in cardiovascular disease.

Keywords: serelaxin, vasoprotective, pregnancy, remodelling

Overview:

Relaxin is traditionally considered a hormone of pregnancy. It was first discovered when serum from pregnant guinea pigs injected into non-pregnant guinea pigs caused relaxation of the inter-pubic ligament[1]. Numerous studies show that relaxin plays a major role in the remodelling of the female reproductive tract in pregnancy and parturition, especially in rodents and pigs[2]. Relaxin is predominantly produced by the corpus luteum and placenta, with the highest circulating levels in pregnancy. But it is now recognised for having a broad range of actions across the body in non-pregnant females and males. Notably, relaxin stimulates tissue remodelling in the heart, kidneys, lung, liver, skin and vasculature. This

review will discuss the effects of relaxin on tissue remodelling with particular attention to the reproductive tract and vasculature. It will also focus on relaxin's heterogeneous effects in different types of vasculature. We will then discuss the role of endogenous relaxin in normal physiological processes, and finally compare the effects of relaxin treatment in both healthy and diseased animals.

Relaxin remodels the reproductive tract in pregnancy:

Pregnancy induces a multitude of changes in maternal physiology, particularly in the reproductive tract, including the uterus, cervix, vagina, pubic ligament and nipples[2]. Relaxin mediates several pregnancy adaptations in these tissues, with well-established effects on the extracellular matrix (ECM) and collagen turnover. Removal of relaxin during mid to late pregnancy in rodents (days 12-22, term = 22 days) with a monoclonal antibody against relaxin (MCA1) neutralizes circulating endogenous relaxin and reverses its adaptive effects in pregnancy. MCA1-treated rats have increased collagen fibre density and reduced epithelial cells in the cervix and vagina[3]. Furthermore, vaginal growth is stunted in MCA1-treated rats (reduced vaginal weight, length, diameter and DNA content) compared with controls[4]. Pregnant relaxin gene knockout (*Rln*$^{-/-}$) mice exhibit abnormal ECM remodelling throughout the reproductive tract. The pubic ligament fails to lengthen in *Rln*$^{-/-}$ mice, and is associated with more dense and less organised collagen[5]. Pregnant *Rln*$^{-/-}$ mice also exhibit increased vaginal collagen content and density[5, 6], and a higher incidence of dystocia during parturition[7]. The dystocia is predominantly driven by abnormal remodelling of the cervix in late pregnancy (increased collagen density without changes to total collagen content). Concentrations of the glycosaminoglycan hyaluronan in the cervix are reduced in *Rln*$^{-/-}$ mice on day 18.5 of pregnancy, as is hyaluronan synthase and aquaporin 3 expression[8]. Thus, relaxin appears to reduce collagen density in the mouse cervix by promoting water recruitment into the ECM (by stimulating hyaluronan synthesis and aquaporin 3 expression), rather than solely acting on collagen synthesis/degradation. Moreover, relaxin clearly has a vital role in the remodelling of the reproductive tract in late pregnancy and early parturition.

Localisation of relaxin receptors: relaxin also targets the cardiovascular system

Relaxin and relaxin-related peptides bind to a family of G-protein coupled receptors known as relaxin family peptide receptors (RXFPs)[9]. There are four RXFPs (1-4) with RXFP1 the cognate receptor for relaxin. RXFP1 has been localised in a number of non-reproductive organs, including the central nervous system, gastrointestinal tract and cardiovascular

system[10]. The discovery of relaxin and the expression of RXFP1 in non-reproductive tissues completely transformed relaxin research, initiating a plethora of studies independent of pregnancy[11]. Relaxin also alters ECM turnover in various non-reproductive tissues in healthy animals including the cardiovascular system[12].

Early studies demonstrated highly specific relaxin binding sites in rat atria[13]. RXFP1 has subsequently been localised throughout the vasculature, including the aorta, vena cava, small renal, mesenteric, external iliac and uterine vessels[14-16]. Specifically, RXFP1 is localised to both endothelial and vascular smooth muscle cells (VSMCs), although the relative level of expression in these parts of the arterial wall differs throughout the vasculature[15]. The localisation of immuno-reactive relaxin in small renal arteries suggests the presence of a local relaxin ligand-receptor system within the vasculature[17]. Moreover, the cardiovascular system, and in particular the vasculature, is a target of relaxin action. This is further supported by relaxin's effects on haemodynamics. Relaxin treatment in conscious normotensive and hypertensive male and non-pregnant female rats increases cardiac output and global arterial compliance and reduces systemic vascular resistance (SVR) without affecting mean arterial pressure[18, 19]. These effects are dose-dependent with a biphasic profile. Continuous infusion of low-dose (4µg/h) relaxin in rats increases cardiac output and reduces steady and pulsatile arterial loads. These effects are not observed when animals are treated with higher doses of relaxin (50µg/h)[20]. Following this work on the dose-dependent effects of relaxin, subsequent vascular studies in rats (many of which are discussed later in this review) predominantly used an *in vivo* dose of 4µg/h (equivalent of 13.33µg/h/kg in some studies) which yields concentrations of circulating relaxin similar to those measured on gestational days 12–14 in pregnant rats (~40-80ng/ml)[2]. Relaxin treatment also improves renal function, increasing renal plasma flow, glomerular filtration rate and plasma volume and osmolality[21]. These changes to haemodynamics and renal function are largely attributed to the vascular actions of relaxin. The remainder of this review will focus on the vascular effects of relaxin – both during and independent of pregnancy.

The vascular role of relaxin in pregnancy

Two different animal models of relaxin deficiency have contributed significantly to our current understanding of the actions of endogenous relaxin (studies summarised in Table 1). The first model involves treatment with MCA1 to neutralise circulating endogenous relaxin[22, 23]. As MCA1 only neutralises circulating relaxin, this technique is limited to pregnancy - the

only physiological condition in which there are detectable levels of relaxin in the circulation. MCA1 inhibits the pregnancy-induced increase in global arterial compliance and decrease in SVR[23, 24], and stimulates inward eutrophic remodelling to increase uterine artery stiffness without altering collagen composition[16]. The second model of relaxin deficiency is the *Rln*$^{-/-}$ mouse. Systolic blood pressure (measured by telemetry) is increased in pregnant *Rln*$^{-/-}$ mice during mid - late pregnancy[28]. *Rln*$^{-/-}$ mice also have compromised vascular function at this time-point. Pregnant 8-month-old *Rln*$^{-/-}$ mice have stiffer uterine arteries, which is associated with reduced elastin and matrix metalloproteinase (MMP) -2, -10 and -4 gene expression compared with wild-type mice. Furthermore, relaxin treatment in *Rln*$^{-/-}$ reverses this abnormal vascular phenotype[25]. The adaptation of the maternal systemic vasculature is also compromised in pregnant *Rln*$^{-/-}$ mice. Mesenteric arteries from pregnant *Rln*$^{-/-}$ mice do not exhibit the normal pregnancy-associated attenuation of angiotensin II-mediated vasoconstriction. This vascular dysregulation is associated with reduced VSMC-derived vasodilator prostanoids[26]. Additionally, the normal reduction in uterine artery myogenic tone that occurs in pregnancy failed to occur in arteries from *Rln*$^{-/-}$ mice. The maintenance of raised myogenic tone in uterine arteries during pregnancy likely contribute to reduced uteroplacental perfusion and fetal growth restriction that is consistently observed in this model[27]. These studies demonstrate that relaxin deficiency (whether it be global or circulating) adversely affects vascular function and structure in late pregnant rodents, and highlight that endogenous relaxin has an integral role in the normal vascular adaptations to pregnancy.

The vasoprotective properties of endogenous relaxin in males

A key benefit of the *Rln*$^{-/-}$ mouse model is the ability to investigate the role of endogenous relaxin in non-pregnant females and male mice. As mentioned previously, RXFP1 is present in a variety of blood vessels[14, 16, 17]. Hence, it is not surprising that there are altered vascular phenotypes in non-pregnant *Rln*$^{-/-}$ mice. In 4-6 month old male *Rln*$^{-/-}$ mice, myogenic tone is increased and passive compliance is reduced in the small renal arteries[17]. These arteries also exhibit inward geometric remodelling, reduced VSMC density and increased total collagen content compared to *Rln*$^{+/+}$ mice[30], suggesting that endogenous relaxin is important in the homeostasis of normal small renal artery remodelling.

Mesenteric arteries are also a target for endogenous relaxin. Passive volume compliance is reduced in the mesenteric and external iliac arteries of 3-12 month old *Rln*$^{-/-}$ mice[31, 32].

Conversely passive mechanical wall properties of arteries from older *Rln*$^{-/-}$ mice (18-23 month old) are more comparable to their wild-type counterparts, suggesting that the effects of endogenous relaxin on the vasculature are more prominent in younger animals, and that relaxin only plays a minor role in vascular ageing[32]. Mesenteric arteries of *Rln*$^{-/-}$ mice also exhibit altered vascular reactivity. Specifically, sensitivity to the vasoconstrictors, such as the α_1-adrenoceptor agonist phenylephrine and the thromboxane mimetic U46619, are increased in an endothelium-dependent fashion in *Rln*$^{-/-}$ mice due to the impairment of nitric oxide (NO) and vasodilator prostanoid pathways[31]. Furthermore, vasoconstrictor prostanoid pathways are upregulated in mesenteric arteries from *Rln*$^{-/-}$ mice, reducing vasodilation evoked by the endothelium-dependent agonist, acetylcholine (ACh)[31].

The aorta is also a target of endogenous relaxin action; however, the effects are not as prominent as in the resistance vasculature. *Rln*$^{-/-}$ mice have increased superoxide production, which is independent of superoxide dismutase or nicotinamide adenine dinucleotide phosphate oxidase protein expression in the aorta. Despite increased oxidative stress, endothelium-dependent relaxation of the aorta is comparable between *Rln*$^{+/+}$ and *Rln*$^{-/-}$ mice. This is associated with reduced basal NO synthase (NOS) activity and total endothelial NOS (eNOS) protein. Interestingly, eNOS phosphorylation is increased suggesting a compensatory upregulation of eNOS activation to maintain endothelial function[14]. In summary, endogenous relaxin regulates the vascular system, particularly in the mesenteric and renal arteries during pregnancy, to promote a vasodilatory phenotype and reduce arterial wall stiffness. As the vascular phenotypes caused by relaxin deficiency are similar to those of diseased arteries, we suggest that relaxin plays an important vasoprotective role if normal physiological function is compromised.

Small renal arteries - identifying mechanisms of relaxin action:

Relaxin's beneficial effects on haemodynamics (increased renal plasma flow and glomerular filtration rate)[21] are underpinned by an endothelium-dependent reduction in myogenic reactivity and tone in the small renal arteries[33]. Specifically, relaxin increases ET_B receptor activation, in turn increasing eNOS activity, and NO production, reducing both vascular tone and myogenic reactivity. This pathway is associated with the up-regulation of vascular MMP-2 and MMP-9 activity which stimulates the cleavage of big endothelin (ET) -1 to ET_{1-32}[34]. This large compilation of work by Conrad and colleagues clearly demonstrates that relaxin

treatment reduces vascular tone and modulates vascular ECM remodelling in the small renal artery.

The vascular actions of relaxin also extend to modifications of passive mechanical wall properties through changes in the vascular ECM. Continuous subcutaneous relaxin infusion in rats and mice increases arterial compliance in small renal arteries[18, 30]. In mice administered relaxin, the increase in small renal artery compliance is mediated by both geometric (outward) and compositional remodelling. Relaxin treatment also increases VSMC density and decreases total collagen content without altering pro-MMP-2 and MMP-9 to alter the composition of the vessel wall[30]. Conversely, short-term subcutaneous relaxin treatment (4-6h) in non-pregnant female rats increases the activity of pro-MMP-9 and MMP-9[35], while a longer treatment (5-day) upregulates pro-MMP-2 and MMP-2[36]. These increases in MMP-2 and MMP-9 activity are strongly associated with relaxin's effects in reducing myogenic tone in the small renal artery, but could also be responsible for the observed reductions in collagen content[35]. The effects of relaxin in other vascular beds are far less established, with many conflicting results published to date[37].

Mesenteric arteries: enhanced endothelium-dependent relaxation

Over the past decade mesenteric arteries have also been established as a key target of relaxin action[38]. The effects of relaxin vary depending on the duration and mode of treatment. Following bolus intravenous (IV) injection, relaxin enhances bradykinin (BK)-mediated endothelium-dependent relaxation in rat mesenteric arteries 3h and 24h after injection. This enhanced BK-mediated relaxation is associated with an upregulation of endothelium-derived hyperpolarization (EDH) at 3h, and an upregulation of prostaglandin (PGI_2) and inducible NOS at 24h. Vasoconstriction to endothelin-1 (ET-1) is also suppressed at 3h but not 24h after relaxin injection[39]. This suppression of ET-1-mediated contraction is dependent on endothelium-derived NO whereby basal NOS activity and Akt phosphorylation are upregulated. Therefore, relaxin injection rapidly upregulates endothelium-derived NO to antagonise ET-1-mediated contraction in the mesenteric arteries. Relaxin has a short half-life of approximately 10 minutes[40], so unsurprisingly only low concentrations (~2ng/ml) of circulating relaxin plasma levels were detected 3h after a single relaxin injection and it was not detectable 24h post-injection. This was one of the first studies to demonstrate sustained vasodilator actions of relaxin after it has been metabolised. This study also established that the mechanisms of relaxin action vary at different time-points post-bolus injection[39].

Extended relaxin treatment via subcutaneous relaxin infusion for 5 days enhances flow-mediated relaxation and reduces myogenic reactivity in rat mesenteric arteries, predominantly via NO[33, 41-43]. Intravenous (IV) relaxin infusion for 2 and 3 days also enhances BK-mediated relaxation in mesenteric arteries, albeit via different mechanisms at the two time-points. At 2 days, relaxin upregulates BK-mediated relaxation via NO. This increase in NO also alters the underlying contribution of NO to the regulation of myogenic tone (i.e. tone in the presence of L-NAME), without altering overall tone in the mesenteric artery[44]. Conversely, after 3 days of relaxin infusion, basal NOS activity, eNOS phosphorylation and eNOS protein expression are reduced in mesenteric arteries, even though BK-mediated relaxation remains enhanced. At 3 days, relaxin increases BK-mediated relaxation via cycloxygenase-2-derived PGI_2[45]. These findings suggest that during prolonged periods of relaxin infusion, a switch occurs from NO to PGI_2 between days 2-3 to maintain the enhanced endothelial vasodilator function (specifically BK-mediated relaxation).

There is consensus that relaxin treatment produces effects in the mesenteric artery to enhance agonist-induced endothelium-dependent relaxation[38]. However, there are conflicting data regarding an effect of relaxin on vascular passive mechanical wall properties in the mesenteric artery[37]. Subcutaneous relaxin treatment for 3 days in Long-Evans non-pregnant female rats and for 5 days in male Wistar rats increases mesenteric artery compliance[15, 46]. Conversely, a 5-day relaxin treatment failed to alter passive mechanical wall properties of young (10-12 week old) or old (40-46 week old) non-pregnant female Wistar Hannover rats[41]. Furthermore, IV relaxin treatment for 2 days has no effect on mesenteric artery passive mechanics in male Wistar rats[44]. It is yet to be demonstrated if these differences reported for the effects of relaxin treatment on passive mechanical wall properties are strain or sex-dependent.

Region-dependent effects of relaxin

ET-1 binds to ET_A and ET_B receptors in VSMCs to induce vasoconstriction, but can also induce vasodilation by acting on endothelial ET_B receptors[47]. Cell culture studies of human umbilical vein endothelial cells and VSMCs reveal that relaxin treatment (supraphysiological concentrations of 1nmol/L) increases ET_B but not ET_A receptor gene expression only in the endothelial cells[48]. In bovine pulmonary artery endothelial cells, relaxin treatment (5nmol/L)

inhibits angiotensin II-stimulated ET-1 secretion by increasing ET_B receptor expression, an effect abolished by selective blockade of ET_B receptors[49]. Taken together, these data illustrate relaxin's ability to selectively upregulate ET_B receptors on endothelial cells and enhance vasodilation and ET-1 clearance. The effect of relaxin in altering ET_B receptor expression may be region-specific, as relaxin treatment in non-pregnant female rats did not alter ET_B receptor expression in small renal arteries[50].

Intravenous relaxin infusion (for 2 days) enhances ACh-mediated relaxation in the rat aorta via a mechanism involving NO (this was associated with increased eNOS expression). Interestingly, ACh-mediated relaxation is not augmented after 3 days of continuous IV relaxin infusion in the rat aorta[45], suggesting that shorter durations of relaxin treatment may be more beneficial in the aorta. This also highlights the importance of treatment duration when considering the vascular effects of relaxin.

In pressurised brain parenchymal arterioles, relaxin infusion for 10 days suppressed myogenic tone via a mechanism involving intermediate-conductance calcium-activated potassium (IK_{Ca}) channels, suggesting that relaxin upregulates EDH in this vascular bed[51]. *In vitro* relaxin treatment (10^{-12}-10^{-5}M) in hamster skeletal muscle arterioles causes direct rapid vasodilation which is inhibited by non-selective blockade of Ca^{2+}-activated K^+ channels[52]. Similarly, *in vitro* relaxin treatment (3-100ng/ml; maximal serum levels of relaxin in humans are ~1ng/ml[2]) also induces rapid dilation of rodent small renal and human subcutaneous arteries via PI3 kinase and NO[53]. However, placebo or time controls were not performed in the studies on human blood vessels, wherein sustained agonist-induced contraction can be difficult to maintain (unpublished observations). Thus, a drop in tension development during testing could be misinterpreted as relaxation.

In vivo relaxin infusion (3 days) does not alter myogenic tone in rat mesenteric veins[46] nor does it affect ACh or BK-mediated relaxation in mesenteric and external iliac veins[15]. In fact, no studies to date have shown relaxin to affect vasodilation in intact veins. Nevertheless, cell culture experiments revealed that increasing concentrations of relaxin (10^{-11}-10^{-6}M) stimulate cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) accumulation in human umbilical vein endothelial cells although, in a bell-shaped fashion[54]. These findings imply that the sensitivity of the relaxin signalling pathway is reduced in veins

compared with arteries and that supraphysiological levels of circulating relaxin may be required to induce changes in venous function *in vivo*.

In arteries from vascular beds other than the kidney and mesentery, relaxin treatment has subtle or no remodelling effects in healthy animals. For example, relaxin increases wall thickness and inner diameter of brain parenchymal arterioles, indicative of outward remodelling, without affecting passive compliance in non-pregnant female rats[55]. The effects of relaxin on vascular passive mechanical properties appear to be region-specific, as relaxin infusion ($\geq$5 days) has no effect on the external iliac[30] and middle cerebral arteries[55], or mesenteric veins[15, 46]. Moreover, there are key differences in experimental design between vascular remodelling studies including gender, animal strain, age and duration of treatment. These factors could contribute to the heterogeneity in the effects of relaxin on vascular remodelling in different vascular beds. These must to be taken into consideration when exploring the effects of relaxin treatment on vascular remodelling, and when comparing findings from different studies.

Vascular effects of relaxin in disease

Relaxin therapy has protective effects in multiple organs[56], and many of the vascular actions of relaxin determined in healthy animals oppose the changes that are associated with disease. Therefore, it is hypothesised that the vasoprotective effects of relaxin treatment would potentially be greater in the setting of disease. Relaxin treatment for 20h in acute heart failure patients improves renal function and reduces SVR, an effect which is sustained for at least 4h post-infusion, likely in the absence of circulating relaxin[57]. The mechanisms underpinning these persistent vascular effects of relaxin are not known.

The vascular remodelling that occurs with disease and ageing is commonly associated with endothelial dysfunction, increased vascular stiffness, hypertrophic remodelling (vascular wall thickening), and increased collagen content[58-60]. Relaxin infusion (14-day) with a 7-day post-treatment period in 17-month-old spontaneously hypertensive rats (SHRs) increases vessel diameter and elastin content and reduces collagen content in the aorta while enhancing passive circumferential compliance in the carotid artery[61]. Similarly, relaxin infusion (14-day) in younger (14-16 week old) female SHRs reverses the hypertension-induced inward remodelling to increase passive distensibility in brain parenchymal arterioles[51]. A shorter relaxin infusion of 5 days does not promote vascular remodelling or alter passive mechanical

wall properties in mesenteric arteries of obese, aged (10-12 months old) or SHRs[41-43]. It is undetermined whether or not a longer relaxin treatment period (> 5 days) would alter mesenteric artery passive mechanics.

A series of studies by van Drongelen *et al*. report that the vasodilator effects of relaxin are blunted in aging and disease[41-43]. In healthy, young (10-12-week-old) female normotensive rats, relaxin (5-day infusion) increases flow-mediated vasodilatation (an assessment of endothelial function) via NO and reduces myogenic reactivity in mesenteric arteries. These vasodilator responses are absent in mesenteric arteries from aged (40-46 weeks old) and obese female rats[41, 42]. Furthermore, relaxin had no effect on myogenic reactivity in mesenteric arteries from 10-12 week old female SHRs, but it did improve flow-mediated NO-dependent vasodilation[43]. It is important to note that neither ageing, obesity nor hypertension are associated with decreased flow-mediated vasodilation or enhanced myogenic reactivity in these studies[41-43], suggesting that endothelial dysfunction was minimal in these models.

Relaxin treatment is vasoprotective in robust models of endothelial dysfunction. In streptozotocin-induced diabetic mice, relaxin treatment (14-day) reverses endothelial dysfunction (reduced sensitivity to ACh) in the aorta and mesenteric arteries by upregulating NO-mediated relaxation[62]. Relaxin also reverses endothelial dysfunction in mesenteric arteries, by downregulating the role of vasoconstrictor prostanoids. Glyceryl nitrate (GTN) is frequently used to treat acute heart failure patients. However, prolonged GTN use induces tolerance, largely due to increased oxidative stress. A combination of relaxin infusion (3-day) with low-dose GTN attenuates the development of GTN-induced tolerance in the rat aorta by reducing superoxide production[63]. In 18-week old female SHRs, EDH-mediated relaxation in brain parenchymal arterioles is reduced and a 14-day relaxin infusion reverses this phenotype [51]. Relaxin treatment (28-day) reduces oxidative stress and atherosclerotic plaque development while enhancing endothelium-dependent relaxation in the aortae from apolipoprotein E-deficient mice fed a high-fat, cholesterol-rich diet[64]. Overall, relaxin appears to consistently enhance vasodilation and reduce oxidative stress in both the aorta and mesenteric arteries in models of vascular disease.

In vitro studies have also been used to examine the effects of relaxin in the setting of vascular dysfunction. Incubation of rat aorta with the pro-inflammatory cytokine, tumour necrosis factor (TNF)-α for 48h increases oxidative stress and impairs ACh-mediated relaxation. This

is associated with decreased total eNOS expression and increased eNOS phosphorylation reducing overall eNOS activity. Relaxin co-incubation for 48h reduces oxidative stress and prevents eNOS phosphorylation in a phosphoinositide 3-kinase (PI3K) -dependent manner and attenuates arginase II expression. This in turn stimulates an overall increase in eNOS activity and improves ACh-mediated endothelium-dependent relaxation[65]. In human umbilical vein endothelial cells and aortic VSMCs, TNF-α incubation also increases vascular cell adhesion molecule-1 and monocyte chemotactic protein-1 expression, which are early markers of vascular inflammation. Relaxin co-incubation abolishes this pro-inflammatory effect in a dose-dependent manner[66]. Collectively, these studies demonstrate that relaxin co-incubation prevents TNF-α-induced oxidative stress, vascular inflammation and endothelial dysfunction. While these findings are promising, it is yet to be established whether relaxin incubation would restore artery function in those already damaged by TNF-α (both *in vivo* and *in vitro*).

Conclusions:

Relaxin, both endogenous and via exogenous treatment, has protective effects in a variety of tissues (Figure 1). Exogenous relaxin treatment rapidly enhances endothelium-dependent vasorelaxation and decreases myogenic reactivity in a variety of vascular beds. This vascular response to relaxin is prolonged even in the absence of circulating relaxin and is regulated by the production of endothelium-derived relaxing factors including NO, EDH and PGI_2 (at least in mesenteric arteries). But relaxin has a broader range of biological actions including anti-oxidant effects, suggesting it acts as a vasoprotective molecule limiting the damage inflicted by disease. Relaxin can also remodel the vasculature and improve arterial passive mechanics, particularly under disease conditions. Thus, relaxin is clearly differentiated from other 'vasodilator' agents and has the potential to be used as a therapeutic in cardiovascular diseases either alone or in combination with standard therapy.

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Figure Legends

Figure 1. Pleiotropic actions of relaxin.

Both endogenous and exogenous relaxin have comparable effects in the heart and cardiovascular system, kidneys, vasculature and female reproductive tract of healthy animals. BP = blood pressure, CO = cardiac output, CVS = cardiovascular system, EDH = endothelium-derived hyperpolarisation, GAC = global arterial compliance, GFR = glomerular filtration rate, HR = heart rate, MMP = metalloproteinase, NO = nitric oxide, PGs = prostaglandins, SV = stroke volume, SVR = systemic vascular resistance.

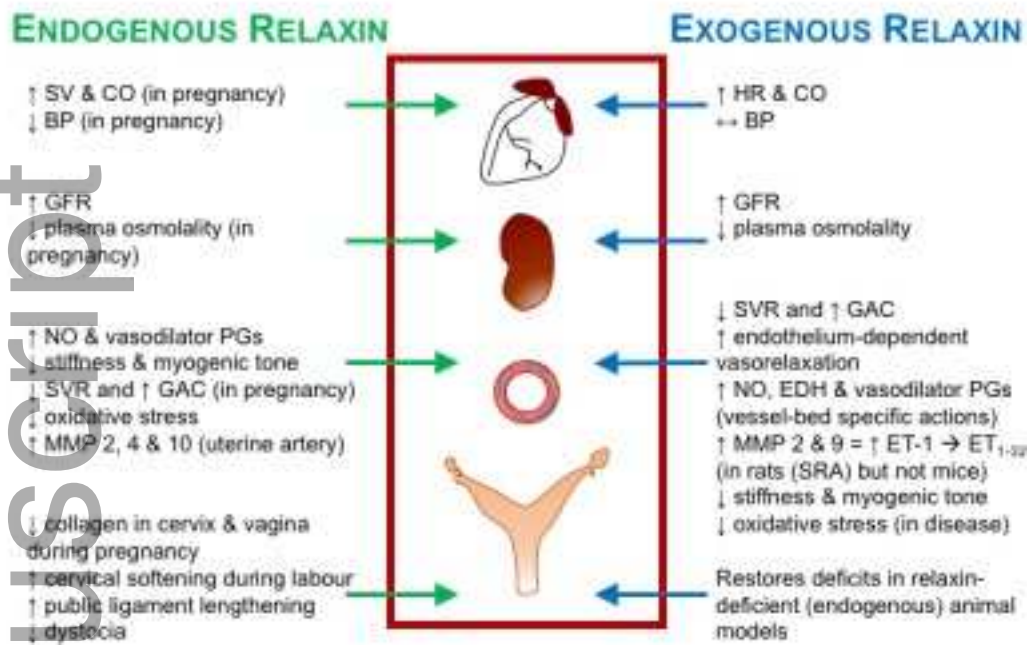
Table 1. Summary of rodent studies investigating the vascular role of endogenous relaxin in pregnancy

A summary of the vascular phenotypes during pregnancy of various animal models of endogenous relaxin deficiency. Animal strain, age and mode of relaxin removal vary between studies on different areas of the vasculature.

Animal model	Animal/ strain	Age (weeks)	Effect of endogenous relaxin removal
MCA1 (GD8 – 15)	Sprague Dawley	12-14	**GD 11-15:** ↓ CO, SV & global arterial compliance; ↑ SVR, plasma osmolality; ↔ heart rate & MAP **GD 15:** ↔ MA remodelling
MCA1 (GD8 – 14)	Sprague Dawley	13-18	**GD 11 &14:** ↓ glomerular filtration rate **GD 12-14:** ↑ small renal artery myogenic tone
MCA1 (GD17 – 20)	Wistar Kyoto	12	**GD 20:** inward remodelling, ↑ stiffness (uterine artery)
$Rln^{-/-}$ mice	C57BL6	20	**GD 17.5:** ↔ uterine artery stiffness
		24	**GD 17.5:** ↑ uterine artery stiffness
$Rln^{-/-}$ mice	C57BL6	12-20	**GD 12.5:** ↔ ANG II-mediated vasoconstriction (MA) **GD 17.5:** ↑ ANG II-mediated vasoconstriction (MA)
$Rln^{-/-}$ mice	C57BL6	12-16	**GD 17.5:** ↔ plasma osmolality, MAP, SBP & DBP
		20-28	**GD 17.5:** ↔ plasma osmolality, SBP, DBP & MAP
			GD 8-14: ↑ SBP & MAP (DBP only increased GD 9-10)
$Rln^{-/-}$ mice	C57BL6	12-16	**GD 17:** ↑ SBP & DBP; ↔ MAP
			GD 19: ↑ heart rate, SBP, DBP & MAP

ANG II = angiotensin II, CO = cardiac output, DBP = diastolic blood pressure, GD = gestational day, MA = mesenteric artery, MAP = mean arterial pressure, MCA1 = monoclonal antibody against relaxin, $Rln^{-/-}$ = relaxin gene knockout, SBP = systolic blood pressure, SV = stroke volume, SVR = systemic vascular resistance.

Figure 1.



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