Am J Physiol Regul Integr Comp Physiol 314: R753–R760, 2018.
First published February 7, 2018; doi:10.1152/ajpregu.00276.2017.

REVIEW | *Model Systems for the Study of Integrative Physiology: The Rebirth of Translational Biology*

Peptide hormone relaxin: from bench to bedside

Maria Jelinic,[1] Sarah A. Marshall,[1] Dennis Stewart,[2] Elaine Unemori,[3] Laura J. Parry,[1] and Chen Huei Leo[1,4]

[1]*School of BioSciences, The University of Melbourne, Parkville, Victoria, Australia;* [2]*Molecular Medicine Research Institute, Sunnyvale, California;* [3]*Oakland, California; and* [4]*Science and Maths Cluster, Singapore University of Technology and Design, Singapore*

Submitted 14 July 2017; accepted in final form 5 February 2018

Jelinic M, Marshall SA, Stewart D, Unemori E, Parry LJ, Leo CH. Peptide hormone relaxin: from bench to bedside. *Am J Physiol Regul Integr Comp Physiol* 314: R753–R760, 2018. First published February 7, 2018; doi:10.1152/ajpregu.00276.2017.—The peptide hormone relaxin has numerous roles both within and independent of pregnancy and is often thought of as a "pleiotropic hormone." Relaxin targets several tissues throughout the body, and has many functions associated with extracellular matrix remodeling and the vasculature. This review considers the potential therapeutic applications of relaxin in cervical ripening, in vitro fertilization, preeclampsia, acute heart failure, ischemia-reperfusion, and cirrhosis. We first outline the animal models used in preclinical studies to progress relaxin into clinical trials and then discuss the findings from these studies. In many cases, the positive outcomes from preclinical animal studies were not replicated in human clinical trials. Therefore, the focus of this review is to evaluate the various animal models used to develop relaxin as a potential therapeutic and consider the limitations that must be addressed in future studies. These include the use of human relaxin in animals, duration of relaxin treatment, and the appropriateness of the clinical conditions being considered for relaxin therapy.

animal models of disease; relaxin; therapeutic

INTRODUCTION

The peptide hormone relaxin was discovered in 1926 by Frederick Hisaw, who observed that injection of serum from pregnant guinea pigs caused the pubic symphysis of virgin guinea pigs to "relax" (30). Numerous roles have been attributed to relaxin, both during and independent of pregnancy. In fact, relaxin treatment results in biological responses in several very different organ systems, many of which are associated with extracellular matrix remodeling. This review is not about the pleiotropic functions of relaxin as this has been reviewed extensively previously (5, 24, 70); instead, it is an overview of the animal models used to investigate the potential for relaxin as a therapeutic in several human diseases. Specifically, this review discusses if the failure to replicate the many beneficial effects of relaxin in clinical trials could be, in part, due to the animal model used in preclinical studies. It also considers the limitations of animal models that should be addressed in future preclinical studies.

Address for reprint requests and other correspondence: C. H. Leo, Science and Maths Cluster, Singapore Univ. of Technology and Design, 8 Somapah Rd., Singapore 487372 (e-mail: chenhuei_leo@sutd.edu.sg).

EARLY CLINICAL TRIALS IN THE CERVIX

Cervical ripening is a key process during labor. It involves softening of the cervix (loss of structural integrity and tensile strength) through extensive extracellular matrix remodeling (73). This process is essential for cervical dilation; impaired cervical ripening results in prolonged labor that can lead to further complications. Based on studies in pigs, cows, and rodents (50, 66), early clinical trials used porcine relaxin preparations to assess the possibility that relaxin could be a cervical ripening agent (68). One of these demonstrated in a randomized double-blind placebo-controlled trial that porcine relaxin (2 mg in a viscous gel) instilled in the cervical canal on the evening before the surgical induction of labor improved the mean cervical score compared with the placebo treatment (38). These data provided the proof of concept that relaxin treatment could induce labor through actions on the cervix.

Recombinant human relaxin became available in the 1990s, which was an important advance in furthering the clinical potential of this hormone. The idea that relaxin could supplement or replace current treatment regimens (prostaglandin E_2) to prepare the cervix for delivery was revisited. There were two clinical trials in the 1990s that used 1–4 mg recombinant human relaxin in a methylcellulose gel applied to the posterior

vaginal fornix the evening before induction. Women who did not spontaneously go into labor within 15 h of gel application were given the standard induction protocol. Both studies reported that relaxin treatment had no effect as a cervical ripening agent before induction of labor at term (7, 14). A key limitation in these clinical trials was the assumption that if relaxin was applied in a methylcellulose gel in the vagina, the peptide would effectively enter the circulation to reach receptors on the cervix. As serum levels of relaxin (0.4–0.5 ng/ml) did not increase after topical application of relaxin, it was assumed this was not the case (14). This limitation was addressed in the most recent clinical trial that investigated relaxin as a cervical ripening drug (NCT00259103). Pregnant (40+ wk) women planned for induction were treated with recombinant human relaxin intravenously for 24 h. Although the study demonstrated relaxin was well tolerated by women in late pregnancy, it reported that supraphysiological serum levels of relaxin (8–13 ng/ml) did not ripen the cervix in women (81).

An important consideration as to why the effects of relaxin treatment on the cervix in animals have not been replicated in human clinical trials might be explained by the marked differences in the pregnancy physiologies in animals compared with humans. First, there are striking differences in the site of steroid hormone (estrogen and progesterone) production. For example, in rodents, progesterone synthesis by the corpus luteum is essential until term, whereas in humans, progesterone synthesis is largely a function of the placenta from 6 to 8 wk of gestation (41). In effect, the corpus luteum is not required to maintain pregnancy in humans. Serum relaxin concentrations also vary between species. In humans, serum relaxin levels increase before embryonic implantation and peak in the first trimester in pregnant women (53). In contrast, circulating relaxin is first detected midpregnancy in most other species examined, with a peripartum surge in mice, rats, and pigs (66). This surge likely contributes to cervical ripening as well as a loosening of the interpubic ligament. This substantial increase in relaxin concentrations at the end of pregnancy does not occur in humans or other primates (66); this could be due to the diminished role of the corpus luteum in human pregnancy. Therefore, relaxin treatment in the form of a 24-h exogenous peripartum surge in late pregnant women may not have had any effect on the cervix in the clinical trial because unlike many other species, endogenous human relaxin may play a limited role in peripartum cervical remodeling. Alternatively, the 24-h infusion protocol may not have been sufficient to initiate extracellular matrix remodeling. Future clinical trials may have to consider longer durations of relaxin treatment, mindful of the potential off-target side effects of a longer relaxin treatment.

IN VITRO FERTILIZATION: A NEW USE FOR RELAXIN?

Despite the significant advances in fundamental knowledge of conception and early pregnancy physiology, the harsh reality is that approximately half of in vitro fertilization (IVF) treatment cycles are unsuccessful (3). This is a major concern for people wanting to conceive using IVF because treatments are expensive and time consuming. Therefore, improving the outcomes of pregnancies through IVF continues to be a major focus in biomedical research. One strategy is to enhance the maternal uterine environment to improve implantation rates (60). Deficiency in vascular endothelial growth factor-induced angiogenesis is thought to contribute to impaired endometrial receptivity in women with recurrent implantation failure following IVF (13). Interestingly, women with a history of recurrent miscarriage present with low levels of circulating relaxin compared with those without a history of miscarriage (4). Furthermore, early preclinical studies in nonhuman primates concluded that relaxin treatment could play an important role in IVF to stimulate endometrial angiogenesis in early pregnancy and improve the likelihood of implantation (28, 29).

Ovariectomized macaque and rhesus monkeys treated with progesterone and estrogen are often used to investigate aspects of the human menstrual cycle and early pregnancy (28). Additional treatment of these monkeys with relaxin enhanced uterine growth, endometrial lymphocyte number, endometrial vascularization (22, 28), and decidualization (22). Notably, relaxin increased the proliferation of endothelial cells in endometrial blood vessels and the number of arterioles in the endometrium (28). An IVF study in the macaque showed that 21 days of recombinant human relaxin infusion (8 $\mu g \cdot kg^{-1} \cdot day^{-1}$) during the peri-implantation period improved the uterine environment for implantation (increased endometrial thickness at *days 7* and *28* and increased implantation bleeding compared with *day 0*), resulting in a slightly higher implantation rate (29). However, the overall rates of successful pregnancy were high in both relaxin- and vehicle-treated groups and reflect the drawbacks of using healthy macaques to study infertility (29). In addition, the normal endogenous levels of relaxin that would have already been circulating in these monkeys could have been a confounding factor. Nevertheless, some of the findings in nonhuman primates have been replicated in nonpregnant women. Continuous subcutaneous recombinant human relaxin treatment (25 and 100 $\mu g \cdot kg^{-1} \cdot day^{-1}$) for 24 wk resulted in a marked increase in the proportion of women experiencing menometrorrhagia (irregular prolonged or excessive uterine bleeding), consistent with increased endometrial vascularization (76). Human cell studies indicate that relaxin treatment modulates expression of vascular endothelial growth factor (54, 76) and numerous other angiogenesis-related genes in endometrial stromal cells (42). Moreover, increased in vitro relaxin secretion by human granulosa cell culture in IVF is also predictive of implantation success (69). However, further work is needed to realize full potential of relaxin as a therapeutic in the context of IVF.

Progress in this area of research has been considerably hindered by the lack of an animal model that accurately represents infertility in women, specifically with regard to impaired endometrial receptivity. One scientifically reasonable approach would be to use ovariectomized macaques, similar to those reported in the study of Dallenbach-Hellweg et al. (22), to investigate whether or not relaxin administration could improve implantation rates of embryos fertilized and transferred in a controlled IVF setting as described by Hayes et al. (29). Unfortunately, such a study would be extremely expensive and logistically and perhaps ethically challenging, which is why so much research is conducted in rodents. The dissimilarity in early pregnancy relaxin profiles between humans and rodents suggests that certain relaxin effects on tissue targets during the peri-implantation period in primates may not be observed in rodent models. In fact, providing relaxin to mice early in pregnancy when it is usually not present resulted in

resorption of embryos due to its inhibition of myometrial activity, which is essential to normal embryo spacing in that species (68). This reinforces the importance of selecting the right animal model to investigate specific relaxin physiologies and disease conditions.

MORE THAN JUST A HORMONE OF PREGNANCY

During normal pregnancy, remarkable changes occur to the maternal cardiovascular system, including an increase in stroke volume (SV), cardiac output (CO), and glomerular filtration rate with a concomitant decrease in systemic vascular resistance (SVR) and mean arterial pressure (1, 16, 39, 40). Multiple studies in rodents demonstrate that relaxin plays an essential role in modulating these profound changes (19).

Relaxin treatment in nonpregnant animals mimics many of the cardiovascular changes that occur in pregnancy. These effects can be replicated in healthy male and nonpregnant female rodents. Briefly, chronic relaxin treatment increases CO, SV, heart rate, renal blood flow, and glomerular filtration rate while decreasing SVR (20). Acute intravenous treatment (2–4 h) with recombinant human relaxin increased CO, SV, and heart rate and reduced SVR in an angiotensin II-induced rat hypertension model, but a longer treatment duration (24 h) was required to see similar effects in spontaneously hypertensive rats (23), highlighting the importance of selection of animal model. A hemodynamic study conducted in patients with acute heart failure demonstrated a rapid (2 h) relaxin-induced decrease in SVR and blood pressure, a trend toward increased cardiac index, and no effect on heart rate (56). Although the hemodynamic studies in both rodents and humans, along with observations made in other clinical trials, support the conclusion that relaxin can cause rapid vasodilation (74), the differences highlighted in the hemodynamic studies reflect the limitations of using a single animal model to try to understand human pharmacology of relaxin. The vascular effects of relaxin, both with regard to vasodilation and its ability to reduce vascular stiffness, have been researched extensively (34), and it became apparent that relaxin only had effects in certain vascular beds (renal, mesenteric, aorta) and not others (femoral, middle cerebral artery) (31). Furthermore, discrepancies occurred between studies in which different regimens of relaxin treatment or different strains, ages, and genders of rodents were used. For example, 3 and 5 days of subcutaneous relaxin treatment reduced mesenteric artery stiffness in male Wistar rats (33, 37) but not in female Wistar Hannover rats (77). More recent work demonstrated that 2 and 10 days of relaxin treatment in male Wistar rats also had no effect on mesenteric artery stiffness (31, 32). The discrepancies between studies raise two important questions: *1*) which experimental model in healthy animals best mimics the human, and *2*) is it relevant to treat rodents (and other animals) with recombinant human relaxin? As this is the most readily available form of relaxin, it is the most commonly used in experiments, but it is important to consider species specificity, as well as potential issues with immunogenicity when administering human relaxin to animals (59). Regardless, a major limitation of relaxin treatment studies in healthy animals is that they bear little relevance to human disease.

RELAXIN TREATMENT IN ACUTE HEART FAILURE

The numerous studies that demonstrated cardioprotective effects of relaxin gave rise to the development of relaxin as a potential therapeutic in cardiovascular disease and led to large scale clinical trials for the use of relaxin in acute heart failure (25). The phase II clinical trial and first phase III trial (RELAX-AHF-1) yielded promising results and indicated that continuous intravenous recombinant human relaxin treatment ($30\ \mu g \cdot kg^{-1} \cdot day^{-1}$) for just 48 h improved one of the primary dyspnoea end points in the short term and reduced all-cause mortality at 180 days in acute heart failure patients with normal-to-elevated blood pressure (72). Relaxin also improved renal function as measured by a number of clinical assessments (48). However, there were questions raised around secondary end points of cardiovascular death and readmission to hospital for heart failure so these findings were not sufficient for immediate Food and Drug Administration approval on a single phase III trial. Therefore, a second phase III study (RELAX-AHF-2) was conducted (NCT01870778).

Unlike the earlier phase III trial, early reports indicate that relaxin did not reduce cardiovascular death through *day 180* or reduce worsening heart failure in the second phase III trial (71). Attempts to understand the underlying basis for observations in the acute heart failure clinical trials have been limited due to the complex pathophysiology of the syndrome and perhaps the naiveté of drug (47), as well as to the lack of a robust animal model of this disease. Some might argue that relaxin, a molecule with a short half-life and associated limitations on clinically feasible treatment duration, is better suited to treating disorders of acute onset and recovery, instead of attempting to alter an end point remote from the time relaxin was actually administered. The 48-h intravenous infusion utilized in the acute heart failure trials has been shown to rapidly improve hemodynamics (59), consistent with observations in some preclinical models (23), suggesting that relaxin may be better suited to therapeutic indications in which such rapid modulation is clinically meaningful.

Because there are few robust animal models of acute cardiovascular disorders, the majority of relaxin research (and demonstrated efficacy) has been performed in animal models of chronic cardiovascular diseases. These models should be evaluated with regard to their applicability to human disease, and in concert, investments in developing stable small molecule RXFP1 agonists and/or relaxin analogs with an extended half-life should be considered. The majority of animal studies that have revealed cardioprotective effects of relaxin have used treatment durations that exceed 48 h (25, 35). Treatment durations longer than 48 h have not been explored clinically in heart failure due to the fact that relaxin must be administered parenterally and continuously, which results in a relatively rapid rise and stable elevation in serum relaxin levels. The inability of the peptide to be taken orally (as it is broken down during digestion and not absorbed into the circulation) may also be a key limitation in the therapeutic development of relaxin. Oral medications are theoretically more easily tolerated by patients and physicians and are often far cheaper to administer. The therapeutic potential of relaxin would likely increase significantly if this obstacle could be overcome.

The effects of relaxin treatment in chronic cardiovascular diseases (and associated risk factors) have been studied in a

number of animal models. These include models of hypertension (23, 64, 82), obesity (78), aging (77), and diabetes (51). Although relaxin has beneficial effects on the vasculature in many of these models (34), there are discrepancies between studies, which are summarized in Table 1. These discrepancies have likely impeded the development of relaxin as a therapeutic in cardiovascular disease.

FUTURE OF RELAXIN: ISCHEMIA-REPERFUSION INJURY

The previously mentioned failures to translate findings from animal studies to clinical trials could indicate that the potential of relaxin as a therapeutic is limited. However, relaxin has undeniable cardioprotective effects in animal models and is particularly effective in the vasculature. Furthermore, the potential benefits of relaxin treatment in other acute cardiovascular disorders have not been explored extensively. There are considerable data to support the use of relaxin in ischemia-reperfusion injury (17, 83). Blockage of blood flow as a result of atherosclerosis and thromboembolism leads to ischemia, tissue necrosis, and organ failure. Current therapeutic strategies are aimed at removing these blockages to reestablish blood flow to the previously ischemic area and limit tissue damage. However, reintroduction of blood flow results in another unique form of tissue damage known as reperfusion injury (58). The effects of relaxin in ischemia-reperfusion injury have been interrogated using numerous in vivo and in vitro animal models of myocardial ischemia-reperfusion injury (6, 43). Relaxin has cardioprotective properties in myocardial infarction-reperfusion injury in pigs (52, 55), rats (6, 12), and guinea pigs (43). In the setting of myocardial ischemia-reperfusion injury, relaxin exerts cardioprotective effects by increasing coronary flow to the ischemic region while preventing cellular damage and death. The fact that relaxin has shown beneficial effects in ischemia-reperfusion models in multiple species is promising. However, translation to clinical use would be significantly enhanced if preclinical studies were undertaken to test the effect of relaxin on a background of currently used medical therapy and included clinically relevant end points, such as mortality (8).

The protective effects of relaxin in myocardial ischemia-reperfusion injury prompted numerous studies in rats to investigate the potential of relaxin to protect against ischemia-reperfusion injury in other organs such as kidneys (17, 83), lungs (2), intestines (44), and brain (11). Collectively, these studies demonstrate that relaxin protects these organs by reducing apoptosis, inflammation, and oxidative stress. This is likely mediated by a mechanism involving the nitric oxide pathway. Ischemia-reperfusion injury is a severe unavoidable consequence of organ transplants, particularly in deceased-donor kidney transplants (57, 61).

Ischemia-reperfusion injury complicates transplants in two steps. First, there is immediate damage caused from the reperfusion during transplantation. Second, the injury activates the innate and adaptive immune response, which increases the risk of rejection (26). There is currently a high demand for therapeutics that aid in reducing ischemia-reperfusion injury in kidney transplants, and relaxin may be a potential solution for this. A number of animal models for kidney transplantation (in dogs, rodents, and more recently pigs) are available, and while they do require skilled microsurgical skills, they are possible (65). These models exhibit similar autoimmune responses to those of humans and allow for a greater understanding of the mechanisms of relaxin-action in ischemia-reperfusion injury in the context of kidney transplants.

NEW USES FOR RELAXIN IN PREGNANCY

Relaxin has also recently been considered as a therapeutic in pregnancy-related hypertension, largely because of its vasoprotective functions. A particularly severe form of hypertension in pregnant women is preeclampsia, which presents as the new-onset of high blood pressure after 20 wk of pregnancy, coupled with other symptoms such as proteinuria and fetal growth

Table 1. *Relaxin treatment in rat models of cardiovascular disease and metabolic disorders*

Rat Strain	Disease Model	Sex	Age/Size	Relaxin Treatment	Effect of Relaxin	Reference
WKY/SHR	Essential hypertension	F	13–15 wk	2-day rat RLX (75 ng/h) sc	↓MAP; mesenteric artery: ↓ vasopressin- and norepinephrine-induced vasoconstriction; portal vein: no effect	45
Long-Evans	ANG II induced hypertension	F	12–14 wk	6-h rhRLX (4 μg/h) iv	↑CO, ↑AC_g, ↓SVR, ↔MAP	23
WKY/SHR	Essential hypertension	M	12–15 wk	6-h rhRLX (4 μg/h) iv	No effects	23
				7-day rhRLX (4 μg/h) sc	↑CO, ↑AC_g, ↓SVR, ↔MAP	
WKY/SHR	Essential hypertension	M	17 mo	14-day rhRLX (4 μg/h) sc + 7-day washout period	↔MAP, SVR, and AC_g Carotid: ↑distensibility, outward remodeling	82
Sprague-Dawley	ANG II induced hypertension	M	400–500 g	7-day rhRLX (4 μg/h) sc	↓MAP, ↓proteinuria	64
				28-day rhRLX (4 μg/h) sc	↓MAP, ↓proteinuria	63
Sprague-Dawley	L-NAME-induced hypertension	M	400–500 g	7-day rhRLX (4 μg/h) sc	No effect on MAP or proteinuria	64
Wistar Hannover	Aging (40–46 wk)	F	10–12 wk	5-day rhRLX (4 μg/h) sc	No effect in mesenteric arteries	77
Wistar Hannover	Obesity	F	10–12 wk	5-day rhRLX (4 μg/h) sc	No effect in mesenteric arteries	78
Wistar Hannover/SHR	Essential hypertension	F	21–24 wk	5-day rhRLX (4 μg/h) sc	Mesenteric artery: ↑ flow mediated vasodilation	79
WKY/SHR	Essential hypertension	F	14–16 wk	14-day rhRLX (4 μg/h) sc	↔ MAP ; Brain parenchymal arteriole: ↑ distensibility, outward remodeling	15
Sprague-Dawley	Myocardial infarction	M	250–300 g	14-day rhRLX (500 $mg \cdot kg^{-1} \cdot day^{-1}$) sc	↓ cardiac fibrosis and apoptosis and reduces vulnerability to tachycardia	80

Relaxin has differential effects in the various models of disease. WKY, Wistar-Kyoto rat; SHR, spontaneously hypertensive rat; F, female; M, male; RLX, relaxin; rhRLX, recombinant human relaxin; sc, subcutaneous; iv, intravenous; MAP, mean arterial pressure; ANG II, angiotensin II; CO, cardiac output; AC_g, global arterial compliance; SVR, systemic vascular resistance; L-NAME, nitro-L-arginine methyl ester.

restriction. Preeclampsia can quickly escalate into HELLP (hemolysis, elevated liver transaminases, low platelets) syndrome or eclampsia. These serious complications are life threatening for both mother and baby. There are limited treatment options for preeclampsia, and the only cure is delivery of the placenta (49). Unfortunately, when preeclampsia is severe, this often results in extremely premature babies at risk of developing further complications. Although the placenta is central to disease development, it is maternal systemic and renal vasoconstriction that leads to hypertension and impaired renal function. Relaxin treatment could potentially counteract these negative characteristics by targeting the endothelial dysfunction to help stabilize systemic vascular function (21, 75). This rationale was used as the basis for initiating two exploratory clinical trials in which women with preeclampsia were treated intravenously for 72 h with recombinant human relaxin (15 $\mu g \cdot kg^{-1} \cdot day^{-1}$; clinicaltrials.gov NCT00333307 and NCT01566630). The objectives were to assess the safety of relaxin and its effects on blood pressure and other symptoms of preeclampsia. Both trials were closed due to the difficulty in recruiting patients.

The challenge in enrolling patients into the trials reflects the need for further rigorous preclinical testing of relaxin in preeclampsia. The main limitation to achieving this goal is the lack of an appropriate animal model of preeclampsia that encompasses all elements of the disorder (46). Models of preeclampsia exist, but there are limitations to each of them, aside from the large differences in placentation between animals and human. The reduced uterine perfusion pressure (RUPP) model is a promising model if the main purpose of the study is to investigate drug effects on the vasculature and renal function, i.e., downstream of placentation. This model presents with characteristics that are observed in women with preeclampsia (hypertension, systemic and renal vasoconstriction, oxidative stress, and intrauterine growth restriction). The model has been successfully implemented in a number of species (27), but it is most consistent with human preeclampsia in rats and primates (36). The primate model most closely resembles the human disease, but as noted above, primate studies have their own set of limitations; therefore, studies in rats are preferred. However, there is a major consideration in the RUPP model. RUPP is generally applied in midpregnancy after the placenta is established. Unfortunately, this prevents the study of abnormal placentation, which leads to placental ischemia in early pregnancy in preeclamptic women. Nevertheless, being able to treat the symptoms of the disorder in mid- to late-pregnant women is a significant clinical goal. A recent study indicated that treatment with relaxin in the rat RUPP model improves a number of these symptoms (62). Specifically, relaxin treatment reduced blood pressure and uterine artery resistance, increased nitric oxide bioavailability in the kidney, and improved circulating levels of preeclamptic biomarkers (sFlt-1, TNF-α, and PPET-1). However, it is yet to be determined whether or not relaxin improves maternal systemic vasculature function.

BREAKING RESEARCH: POTENTIAL OF RELAXINAS A THERAPEUTIC FOR LIVER DISEASE

Over a decade ago it was discovered that relaxin receptors (RXFP1) are expressed in cirrhotic livers but not healthy liver in rats (9). This brought to light the hypothesis that relaxin may be therapeutically useful in liver injury. In subsequent studies, chronic relaxin treatment significantly reduced fibrosis in mouse models of established hepatic fibrosis (10). Recent preclinical studies in rat models of cirrhosis also demonstrated a therapeutic potential for relaxin (67). These studies targeted hepatorenal syndrome (a common complication of cirrhosis) and focused on the ability of relaxin to enhance renal flow and vasodilation. The rat studies translated well to humans, with comparable findings in a phase II clinical trial (67). Patients with alcohol-related cirrhosis were treated intravenously with recombinant human relaxin (80 $\mu g \cdot kg^{-1} \cdot day^{-1}$ for 60 min, followed by 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ for 60 min). Relaxin infusion increased total renal arterial blood flow by 65% from baseline in patients and was not associated with any detrimental effects on systemic blood pressure or hepatic perfusion. Importantly, this exciting new evidence highlights that the therapeutic potential of the vasodilatory actions of relaxin is not limited to cardiovascular disease.

Perspectives and Significance

Relaxin is a naturally occurring hormone and has actions in the majority of organ systems throughout the body. Exogenous relaxin treatment also shows a good safety profile in several clinical trials in men and women. Despite the apparent failure of the recent acute heart failure clinical trial, relaxin has significant beneficial effects in the vascular system because of its consistent and reproducible vasoprotective actions in animals and humans. It is important to remember that the cardioprotective properties of relaxin appear to be driven largely by the vascular effects of relaxin. Ultimately, we believe that the lack of robust animal models of acute in vivo vascular dysfunction is hindering the discovery of the full potential of relaxin as a therapeutic. Moreover, the potential for relaxin to reduce or abolish the effects of ischemia-reperfusion injury in conditions such as kidney transplants is worthy of investigation as does its potential as a therapeutic in preeclampsia and liver disease. Clearly, more attention needs to focus on the development of appropriate animal models that are more representative of the human disease under consideration.

GRANTS

M. Jelinic and S. A. Marshall received an Australian Postgraduate Award. C. H. Leo received the JN Peter's Research Fellowship and an Early Career Grant (Faculty of Science, The University of Melbourne). The research was funded by an Australian Research Council Linkage Grant, the National Health and Medical Research Council, and Investigator-Initiated Trials from Novartis Pharmaceuticals Australia.

DISCLOSURES

We disclose that some of this research (described in this review) was partially funded by Novartis Pharmaceuticals, who also provided the relaxin as a condition of the Australian Research Council Linkage Grant. L. J. Parry was also a paid consultant for Novartis Pharmaceuticals and is a coinventor on a patent for relaxin use in the cervix, kidney, and brain. D. Stewart and E. Unemori are former employees of Corthera, a Novartis Pharmaceuticals company, and inventors on relaxin use patents.

AUTHOR CONTRIBUTIONS

M.J., L.J.P., and C.H.L. drafted manuscript; M.J., S.A.M., D.S., E.U., L.J.P., and C.H.L. edited and revised manuscript; M.J., S.A.M., D.S., E.U., L.J.P., and C.H.L. approved final version of manuscript.

REFERENCES

1. **Abduljalil K, Furness P, Johnson TN, Rostami-Hodjegan A, Soltani H.** Anatomical, physiological and metabolic changes with gestational age during normal pregnancy: a database for parameters required in physiologically based pharmacokinetic modelling. *Clin Pharmacokinet* 51: 365–396, 2012. doi:10.2165/11597440-000000000-00000.
2. **Alexiou K, Wilbring M, Matschke K, Dschietzig T.** Relaxin protects rat lungs from ischemia-reperfusion injury via inducible NO synthase: role of ERK-1/2, PI3K, and forkhead transcription factor FKHRL1. *PLoS One* 8: e75592–e75592, 2013. doi:10.1371/journal.pone.0075592.
3. **Anderson J, Anderson-Bialis D.** In vitro fertilization success rates by patient occupation, income and educational level. *Fertil Steril* 106: e39, 2016. doi:10.1016/j.fertnstert.2016.07.124.
4. **Anumba DO, El Gelany S, Elliott SL, Li TC.** Serum relaxin levels are reduced in pregnant women with a history of recurrent miscarriage, and correlate with maternal uterine artery Doppler indices in first trimester. *Eur J Obstet Gynecol Reprod Biol* 147: 41–45, 2009. doi:10.1016/j.ejogrb.2009.07.008.
5. **Bani D.** Relaxin: a pleiotropic hormone. *Gen Pharmacol* 28: 13–22, 1997. doi:10.1016/S0306-3623(96)00171-1.
6. **Bani D, Masini E, Bello MG, Bigazzi M, Sacchi TB.** Relaxin protects against myocardial injury caused by ischemia and reperfusion in rat heart. *Am J Pathol* 152: 1367–1376, 1998.
7. **Bell RJ, Permezel M, MacLennan A, Hughes C, Healy D, Brennecke S.** A randomized, double-blind, placebo-controlled trial of the safety of vaginal recombinant human relaxin for cervical ripening. *Obstet Gynecol* 82: 328–333, 1993.
8. **Bell RM, Bøtker HE, Carr RD, Davidson SM, Downey JM, Dutka DP, Heusch G, Ibanez B, Macallister R, Stoppe C, Ovize M, Redington A, Walker JM, Yellon DM.** 9th Hatter Biannual Meeting: position document on ischaemia/reperfusion injury, conditioning and the ten commandments of cardioprotection. *Basic Res Cardiol* 111: 41, 2016. doi:10.1007/s00395-016-0558-1.
9. **Bennett RG, Dalton SR, Mahan KJ, Gentry-Nielsen MJ, Hamel FG, Tuma DJ.** Relaxin receptors in hepatic stellate cells and cirrhotic liver. *Biochem Pharmacol* 73: 1033–1040, 2007. doi:10.1016/j.bcp.2006.12.007.
10. **Bennett RG, Heimann DG, Singh S, Simpson RL, Tuma DJ.** Relaxin decreases the severity of established hepatic fibrosis in mice. *Liver Int* 34: 416–426, 2014. doi:10.1111/liv.12247.
11. **Bergeron LH, Willcox JM, Alibhai FJ, Connell BJ, Saleh TM, Wilson BC, Summerlee AJ.** Relaxin peptide hormones are protective during the early stages of ischemic stroke in male rats. *Endocrinology* 156: 638–646, 2015. doi:10.1210/en.2014-1676.
12. **Boccalini G, Sassoli C, Formigli L, Bani D, Nistri S.** Relaxin protects cardiac muscle cells from hypoxia/reoxygenation injury: involvement of the Notch-1 pathway. *FASEB J* 29: 239–249, 2015. doi:10.1096/fj.14-254854.
13. **Boudjenah R, Molina-Gomes D, Torre A, Boitrelle F, Taieb S, Dos Santos E, Wainer R, de Mazancourt P, Selva J, Vialard F.** Associations between individual and combined polymorphisms of the TNF and VEGF genes and the embryo implantation rate in patients undergoing in vitro fertilization (IVF) programs. *PLoS One* 9: e108287, 2014. doi:10.1371/journal.pone.0108287.
14. **Brennand JE, Calder AA, Leitch CR, Greer IA, Chou MM, MacKenzie IZ.** Recombinant human relaxin as a cervical ripening agent. *Br J Obstet Gynaecol* 104: 775–780, 1997. doi:10.1111/j.1471-0528.1997.tb12019.x.
15. **Chan SL, Sweet JG, Cipolla MJ.** Treatment for cerebral small vessel disease: effect of relaxin on the function and structure of cerebral parenchymal arterioles during hypertension. *FASEB J* 27: 3917–3927, 2013. doi:10.1096/fj.13-230797.
16. **Chapman AB, Abraham WT, Zamudio S, Coffin C, Merouani A, Young D, Johnson A, Osorio F, Goldberg C, Moore LG, Dahms T, Schrier RW.** Temporal relationships between hormonal and hemodynamic changes in early human pregnancy. *Kidney Int* 54: 2056–2063, 1998. doi:10.1046/j.1523-1755.1998.00217.x.
17. **Collino M, Rogazzo M, Pini A, Benetti E, Rosa AC, Chiazza F, Fantozzi R, Bani D, Masini E.** Acute treatment with relaxin protects the kidney against ischaemia/reperfusion injury. *J Cell Mol Med* 17: 1494–1505, 2013. doi:10.1111/jcmm.12120.
19. **Conrad KP.** Maternal vasodilation in pregnancy: the emerging role of relaxin. *Am J Physiol Regul Integr Comp Physiol* 301: R267–R275, 2011. doi:10.1152/ajpregu.00156.2011.
20. **Conrad KP.** Unveiling the vasodilatory actions and mechanisms of relaxin. *Hypertension* 56: 2–9, 2010. doi:10.1161/HYPERTENSIONAHA.109.133926.
21. **Conrad KP, Shroff SG.** Effects of relaxin on arterial dilation, remodeling, and mechanical properties. *Curr Hypertens Rep* 13: 409–420, 2011. doi:10.1007/s11906-011-0231-x.
22. **Dallenbach-Hellweg G, Dawson AB, Hisaw FL.** The effect of relaxin on the endometrium of monkeys histological and histochemical studies. *Am J Anat* 119: 61–77, 1966. doi:10.1002/aja.1001190105.
23. **Debrah DO, Conrad KP, Jeyabalan A, Danielson LA, Shroff SG.** Relaxin increases cardiac output and reduces systemic arterial load in hypertensive rats. *Hypertension* 46: 745–750, 2005. doi:10.1161/01.HYP.0000184230.52059.33.
24. **Dschietzig T, Bartsch C, Baumann G, Stangl K.** Relaxin-a pleiotropic hormone and its emerging role for experimental and clinical therapeutics. *Pharmacol Ther* 112: 38–56, 2006. doi:10.1016/j.pharmthera.2006.03.004.
25. **Du XJ, Hewitson TD, Nguyen MN, Samuel CS.** Therapeutic effects of serelaxin in acute heart failure. *Circ J* 78: 542–552, 2014. doi:10.1253/circj.CJ-14-0014.
26. **Fuquay R, Renner B, Kulik L, McCullough JW, Amura C, Strassheim D, Pelanda R, Torres R, Thurman JM.** Renal ischemia-reperfusion injury amplifies the humoral immune response. *J Am Soc Nephrol* 24: 1063–1072, 2013. doi:10.1681/ASN.2012060560.
27. **Fushima T, Sekimoto A, Minato T, Ito T, Oe Y, Kisu K, Sato E, Funamoto K, Hayase T, Kimura Y, Ito S, Sato H, Takahashi N.** Reduced uterine perfusion pressure (RUPP) model of preeclampsia in mice. *PLoS One* 11: e0155426, 2016. doi:10.1371/journal.pone.0155426.
28. **Goldsmith LT, Weiss G, Palejwala S, Plant TM, Wojtczuk A, Lambert WC, Ammur N, Heller D, Skurnick JH, Edwards D, Cole DM, Lieberman S.** Relaxin regulation of endometrial structure and function in the rhesus monkey. *Proc Natl Acad Sci USA* 101: 4685–4689, 2004. doi:10.1073/pnas.0400776101.
29. **Hayes ES, Curnow EC, Trounson AO, Danielson LA, Unemori EN.** Implantation and pregnancy following in vitro fertilization and the effect of recombinant human relaxin administration in Macaca fascicularis. *Biol Reprod* 71: 1591–1597, 2004. doi:10.1095/biolreprod.104.030585.
30. **Hisaw FL.** Experimental relaxation of the pubic ligament of the guinea pig. *Proc Soc Exp Biol Med* 23: 661–663, 1926. doi:10.3181/00379727-23-3107.
31. **Jelinic M, Kahlberg N, Parry LJ, Tare M.** Does serelaxin treatment alter passive mechanical wall properties in small resistance arteries? *Microcirculation* 23: 631–636, 2016. doi:10.1111/micc.12321.
32. **Jelinic M, Leo CH, Marshall SA, Senadheera SN, Parry LJ, Tare M.** Short-term (48 hours) intravenous serelaxin infusion has no effect on myogenic tone or vascular remodeling in rat mesenteric arteries. *Microcirculation* 24: e12371, 2017. doi:10.1111/micc.12371.
33. **Jelinic M, Leo CH, Post Uiterweer ED, Sandow SL, Gooi JH, Wlodek ME, Conrad KP, Parkington H, Tare M, Parry LJ.** Localization of relaxin receptors in arteries and veins, and region-specific increases in compliance and bradykinin-mediated relaxation after in vivo serelaxin treatment. *FASEB J* 28: 275–287, 2014. doi:10.1096/fj.13-233429.
34. **Leo CH, Jelinic M, Ng HH, Marshall SA, Novak J, Tare M, Conrad KP, Parry LJ.** Vascular actions of relaxin: nitric oxide and beyond. *Br J Pharmacol* 174: 1002–1014, 2017. [Correction in *Br J Pharmacol* 174: 4836–4836, 2017.] doi:10.1111/bph.13614.
35. **Leo CH, Jelinic M, Ng HH, Tare M, Parry LJ.** Serelaxin: a novel therapeutic for vascular diseases. *Trends Pharmacol Sci* 37: 498–507, 2016. doi:10.1016/j.tips.2016.04.001.
36. **Li J, LaMarca B, Reckelhoff JF.** A model of preeclampsia in rats: the reduced uterine perfusion pressure (RUPP) model. *Am J Physiol Heart Circ Physiol* 303: H1–H8, 2012. doi:10.1152/ajpheart.00117.2012.
37. **Li Y, Brookes ZL, Kaufman S.** Acute and chronic effects of relaxin on vasoactivity, myogenic reactivity and compliance of the rat mesenteric arterial and venous vasculature. *Regul Pept* 132: 41–46, 2005. doi:10.1016/j.regpep.2005.09.009.
38. **MacLennan AH, Green RC, Grant P, Nicolson R.** Ripening of the human cervix and induction of labor with intracervical purified porcine relaxin. *Obstet Gynecol* 68: 598–601, 1986.
39. **Magness R.** Maternal cardiovascular and other physiologic responses to the endocrinology of pregnancy. In: *Endocrinology of Pregnancy*, edited

by Bazer F. Totowa, NJ: Humana, 1998, p. 507–539. doi:10.1007/978-1-4612-1804-3_18.
40. **Magness R, Ford SP.** Maternal cardiovascular adaptation and uterine circulation-physiology and pathophysiology. In: *Stress and Developmental Programming of Health and Disease: Beyond Phenomenology*, edited by Zhang L, Longo LD. Hauppauge, NY: Nova Science, 2014, p. 341–374.
41. **Malassiné A, Frendo JL, Evain-Brion D.** A comparison of placental development and endocrine functions between the human and mouse model. *Hum Reprod Update* 9: 531–539, 2003. doi:10.1093/humupd/dmg043.
42. **Marshall SA, Ng L, Unemori EN, Girling JE, Parry LJ.** Relaxin deficiency results in increased expression of angiogenesis- and remodelling-related genes in the uterus of early pregnant mice but does not affect endometrial angiogenesis prior to implantation. *Reprod Biol Endocrinol* 14: 11–11, 2016. doi:10.1186/s12958-016-0148-y.
43. **Masini E, Bani D, Bello MG, Bigazzi M, Mannaioni PF, Sacchi TB.** Relaxin counteracts myocardial damage induced by ischemia-reperfusion in isolated guinea pig hearts: evidence for an involvement of nitric oxide. *Endocrinology* 138: 4713–4720, 1997. doi:10.1210/endo.138.11.5520.
44. **Masini E, Cuzzocrea S, Mazzon E, Muià C, Vannacci A, Fabrizi F, Bani D.** Protective effects of relaxin in ischemia/reperfusion-induced intestinal injury due to splanchnic artery occlusion. *Br J Pharmacol* 148: 1124–1132, 2006. doi:10.1038/sj.bjp.0706811.
45. **Massicotte G, Parent A, St-Louis J.** Blunted responses to vasoconstrictors in mesenteric vasculature but not in portal vein of spontaneously hypertensive rats treated with relaxin. *Proc Soc Exp Biol Med* 190: 254–259, 1989. doi:10.3181/00379727-190-42857.
46. **McCarthy FP, Kingdom JC, Kenny LC, Walsh SK.** Animal models of preeclampsia; uses and limitations. *Placenta* 32: 413–419, 2011. doi:10.1016/j.placenta.2011.03.010.
47. **McCullough PA.** How trialists and pharmaceutical sponsors have failed us by thinking that acute heart failure is a 48-hour illness. *Am J Cardiol* 120: 505–508, 2017. doi:10.1016/j.amjcard.2017.04.056.
48. **Metra M, Cotter G, Davison BA, Felker GM, Filippatos G, Greenberg BH, Ponikowski P, Unemori E, Voors AA, Adams KF Jr, Dorobantu MI, Grinfeld L, Jondeau G, Marmor A, Masip J, Pang PS, Werdan K, Prescott MF, Edwards C, Teichman SL, Trapani A, Bush CA, Saini R, Schumacher C, Severin T, Teerlink JR; RELAX-AHF Investigators.** Effect of serelaxin on cardiac, renal, and hepatic biomarkers in the Relaxin in Acute Heart Failure (RELAX-AHF) development program: correlation with outcomes. *J Am Coll Cardiol* 61: 196–206, 2013. doi:10.1016/j.jacc.2012.11.005.
49. **Mol BW, Roberts CT, Thangaratinam S, Magee LA, de Groot CJM, Hofmeyr GJ.** Pre-eclampsia. *Lancet* 387: 999–1011, 2016. doi:10.1016/S0140-6736(15)00070-7.
50. **Musah AI, Schwabe C, Willham RL, Anderson LL.** Induction of parturition, progesterone secretion, and delivery of placenta in beef heifers given relaxin with cloprostenol or dexamethasone. *Biol Reprod* 37: 797–803, 1987. doi:10.1095/biolreprod37.4.797.
51. **Ng HH, Leo CH, Prakoso D, Qin C, Ritchie RH, Parry LJ.** Serelaxin treatment reverses vascular dysfunction and left ventricular hypertrophy in a mouse model of Type 1 diabetes. *Sci Rep* 7: 39604, 2017. doi:10.1038/srep39604.
52. **Nistri S, Cinci L, Perna AM, Masini E, Mastroianni R, Bani D.** Relaxin induces mast cell inhibition and reduces ventricular arrhythmias in a swine model of acute myocardial infarction. *Pharmacol Res* 57: 43–48, 2008. doi:10.1016/j.phrs.2007.11.001.
53. **O'Byrne EM, Carriere BT, Sorensen L, Segaloff A, Schwabe C, Steinetz BG.** Plasma immunoreactive relaxin levels in pregnant and nonpregnant women. *J Clin Endocrinol Metab* 47: 1106–1110, 1978. doi:10.1210/jcem-47-5-1106.
54. **Palejwala S, Tseng L, Wojtczuk A, Weiss G, Goldsmith LT.** Relaxin gene and protein expression and its regulation of procollagenase and vascular endothelial growth factor in human endometrial cells. *Biol Reprod* 66: 1743–1748, 2002. doi:10.1095/biolreprod66.6.1743.
55. **Perna AM, Masini E, Nistri S, Briganti V, Chiappini L, Stefano P, Bigazzi M, Pieroni C, Bani Sacchi T, Bani D.** Novel drug development opportunity for relaxin in acute myocardial infarction: evidences from a swine model. *FASEB J* 19: 1525–1527, 2005. doi:10.1096/fj.04-3664fje.
56. **Ponikowski P, Mitrovic V, Ruda M, Fernandez A, Voors AA, Vishnevsky A, Cotter G, Milo O, Laessing U, Zhang Y, Dahlke M, Zymlinski R, Metra M.** A randomized, double-blind, placebo-controlled, multicentre study to assess haemodynamic effects of serelaxin in patients with acute heart failure. *Eur Heart J* 35: 431–441, 2014. doi:10.1093/eurheartj/eht459.
57. **Ponticelli C.** Ischaemia-reperfusion injury: a major protagonist in kidney transplantation. *Nephrol Dial Transplant* 29: 1134–1140, 2014. doi:10.1093/ndt/gft488.
58. **Qin C, Yang YH, May L, Gao X, Stewart AG, Tu Y, Woodman OL, Ritchie RH.** Cardioprotective potential of annexin-A1 mimetics in myocardial infarction. *Pharmacol Ther* 148: 47–65, 2015. doi:10.1016/j.pharmthera.2014.11.012.
59. **Rojko JL, Evans MG, Price SA, Han B, Waine G, DeWitte M, Haynes J, Freimark B, Martin P, Raymond JT, Evering W, Rebelatto MC, Schenck E, Horvath C.** Formation, clearance, deposition, pathogenicity, and identification of biopharmaceutical-related immune complexes: review and case studies. *Toxicol Pathol* 42: 725–764, 2014. doi:10.1177/0192623314526475.
60. **Salamonsen LA, Evans J, Nguyen HP, Edgell TA.** The microenvironment of human implantation: determinant of reproductive success. *Am J Reprod Immunol* 75: 218–225, 2016. doi:10.1111/aji.12450.
61. **Salvadori M, Rosso G, Bertoni E.** Update on ischemia-reperfusion injury in kidney transplantation: Pathogenesis and treatment. *World J Transplant* 5: 52–67, 2015. doi:10.5500/wjt.v5.i2.52.
62. **Santiago-Font JA, Amaral LM, Faulkner J, Ibrahim T, Vaka VR, Cunningham MW, LaMarca B.** Serelaxin improves the pathophysiology of placental ischemia in the reduced uterine perfusion pressure rat model of preeclampsia. *Am J Physiol Regul Integr Comp Physiol* 311: R1158–R1163, 2016. doi:10.1152/ajpregu.00192.2016.
63. **Sasser JM, Cunningham MW Jr, Baylis C.** Serelaxin reduces oxidative stress and asymmetric dimethylarginine in angiotensin II-induced hypertension. *Am J Physiol Renal Physiol* 307: F1355–F1362, 2014. doi:10.1152/ajprenal.00407.2014.
64. **Sasser JM, Molnar M, Baylis C.** Relaxin ameliorates hypertension and increases nitric oxide metabolite excretion in angiotensin II but not N(ω)-nitro-L-arginine methyl ester hypertensive rats. *Hypertension* 58: 197–204, 2011. doi:10.1161/HYPERTENSIONAHA.110.164392.
65. **Schumacher M, Van Vliet BN, Ferrari P.** Kidney transplantation in rats: an appraisal of surgical techniques and outcome. *Microsurgery* 23: 387–394, 2003. doi:10.1002/micr.10139.
66. **Sherwood OD.** Relaxin. In: *The Physiology of Reproduction*, edited by Knobil E, Neill JD. New York: Raven, 1994, p. 861–1009.
67. **Snowdon VK, Lachlan NJ, Hoy AM, Hadoke PW, Semple SI, Patel D, Mungall W, Kendall TJ, Thomson A, Lennen RJ, Jansen MA, Moran CM, Pellicoro A, Ramachandran P, Shaw I, Aucott RL, Severin T, Saini R, Pak J, Yates D, Dongre N, Duffield JS, Webb DJ, Iredale JP, Hayes PC, Fallowfield JA.** Serelaxin as a potential treatment for renal dysfunction in cirrhosis: preclinical evaluation and results of a randomized phase 2 trial. *PLoS Med* 14: e1002248, 2017. doi:10.1371/journal.pmed.1002248.
68. **Steinetz BG, Kroc RL, Greep RO.** Progress in relaxin research. In: *Relaxin '94; 2nd International Congress on the Hormone Relaxin*, edited by McLennan AH, Tregear GW, Bryant-Greenwood GD. Singapore: World Scientific, 1995, p. 3.
69. **Stewart DR, VandeVoort CA.** Relaxin secretion by human granulosa cell culture is predictive of in-vitro fertilization-embryo transfer success. *Hum Reprod* 14: 338–344, 1999. doi:10.1093/humrep/14.2.338.
70. **Summers RJ.** Recent progress in the understanding of relaxin family peptides and their receptors. *Br J Pharmacol* 174: 915–920, 2017. doi:10.1111/bph.13778.
71. **Teerlink JR.** *RELAXin in Acute Heart Failure-2-RELAX-AHF-2*. http://www.acc.org/latest-in-cardiology/clinical-trials/2017/05/07/16/09/relax-ahf-2. [June 20, 2017].
72. **Teerlink JR, Cotter G, Davison BA, Felker GM, Filippatos G, Greenberg BH, Ponikowski P, Unemori E, Voors AA, Adams KF Jr, Dorobantu MI, Grinfeld LR, Jondeau G, Marmor A, Masip J, Pang PS, Werdan K, Teichman SL, Trapani A, Bush CA, Saini R, Schumacher C, Severin TM, Metra M; RELAXin in Acute Heart Failure (RELAX-AHF) Investigators.** Serelaxin, recombinant human relaxin-2, for treatment of acute heart failure (RELAX-AHF): a randomised, placebo-controlled trial. *Lancet* 381: 29–39, 2013. doi:10.1016/S0140-6736(12)61855-8.
73. **Timmons B, Akins M, Mahendroo M.** Cervical remodeling during pregnancy and parturition. *Trends Endocrinol Metab* 21: 353–361, 2010. doi:10.1016/j.tem.2010.01.011.
74. **Unemori E.** Serelaxin in clinical development: past, present and future. *Br J Pharmacol* 174: 921–932, 2017. doi:10.1111/bph.13695.

75. **Unemori E, Sibai B, Teichman SL.** Scientific rationale and design of a phase I safety study of relaxin in women with severe preeclampsia. *Ann NY Acad Sci* 1160: 381–384, 2009. doi:10.1111/j.1749-6632.2009.03838.x.
76. **Unemori EN, Erikson ME, Rocco SE, Sutherland KM, Parsell DA, Mak J, Grove BH.** Relaxin stimulates expression of vascular endothelial growth factor in normal human endometrial cells in vitro and is associated with menometrorrhagia in women. *Hum Reprod* 14: 800–806, 1999. doi:10.1093/humrep/14.3.800.
77. **van Drongelen J, Ploemen IH, Pertijs J, Gooi JH, Sweep FC, Lotgering FK, Spaanderman ME, Smits P.** Aging attenuates the vasodilator response to relaxin. *Am J Physiol Heart Circ Physiol* 300: H1609–H1615, 2011. doi:10.1152/ajpheart.00360.2010.
78. **van Drongelen J, van Koppen A, Pertijs J, Gooi JH, Parry LJ, Sweep FC, Lotgering FK, Smits P, Spaanderman ME.** Impaired vascular responses to relaxin in diet-induced overweight female rats. *J Appl Physiol (1985)* 112: 962–969, 2012. doi:10.1152/japplphysiol.00470.2011.
79. **van Drongelen J, van Koppen A, Pertijs J, Gooi JH, Sweep FC, Lotgering FK, Spaanderman ME, Smits P.** Impaired effect of relaxin on vasoconstrictor reactivity in spontaneous hypertensive rats. *Peptides* 49: 41–48, 2013. doi:10.1016/j.peptides.2013.08.020.
80. **Wang D, Zhu H, Yang Q, Sun Y.** Effects of relaxin on cardiac fibrosis, apoptosis, and tachyarrhythmia in rats with myocardial infarction. *Biomed Pharmacother* 84: 348–355, 2016. doi:10.1016/j.biopha.2016.09.054.
81. **Weiss G, Teichman S, Stewart DR, Nader D, Wood S, Breining P, Unemori E.** Recombinant human relaxin versus placebo for cervical ripening: a double-blind randomised trial in pregnant women scheduled for induction of labour. *BMC Preg Childbirth* 16: 260, 2016. doi:10.1186/s12884-016-1046-1.
82. **Xu Q, Chakravorty A, Bathgate RA, Dart AM, Du XJ.** Relaxin therapy reverses large artery remodeling and improves arterial compliance in senescent spontaneously hypertensive rats. *Hypertension* 55: 1260–1266, 2010. doi:10.1161/HYPERTENSIONAHA.109.149369.
83. **Yoshida T, Kumagai H, Kohsaka T, Ikegaya N.** Relaxin protects against renal ischemia-reperfusion injury. *Am J Physiol Renal Physiol* 305: F1169–F1176, 2013. doi:10.1152/ajprenal.00654.2012.