Review

The effect of relaxin on the musculoskeletal system

F. Dehghan1, B. S. Haerian2, S. Muniandy3, A. Yusof4, J. L. Dragoo5, N. Salleh1
1Department of Physiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, 2Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, 3Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, 4Department of Physiology, Sports Center, University of Malaya, Kuala Lumpur, Malaysia, 5Department of Orthopaedic Surgery, Stanford University, Redwood City, California, USA

Corresponding authors: Jason L. Dragoo, Department of Orthopaedic Surgery, Stanford University, Redwood City, CA 94063-6342, USA. Tel: +1 650 723 5643, Fax: +1 650 721 3422, E-mail: jdragoo@stanford.edu; Naguib Salleh, Department of Physiology, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia. Tel: +60 3 7967 7532, Fax: +60 3 7967 4775, E-mail: naguib.salleh@yahoo.com.my

Accepted for publication 2 October 2013

Relaxin is a hormone structurally related to insulin and insulin-like growth factor, which exerts its regulatory effect on the musculoskeletal and other systems through binding to its receptor in various tissues, mediated by different signaling pathways. Relaxin alters the properties of cartilage and tendon by activating collagenase.

Relaxin, the mammalian 6-kDa heterodimeric polypeptide hormone, is a member of the insulin-like superfamily (Hisaw, 1926) and consists of seven peptides of high structural but low sequence similarity. Relaxin plays an essential role in the biological processes such as metabolism, growth, pregnancy, and parturition in different species including humans and rodents. Relaxin circulates in these species during pregnancy emanating from the corpus luteum (Conrad & Baker, 2013) and placenta (Goh et al., 2013); however, temporal pattern of change and serum concentrations of this hormone are different. In rodents, circulating relaxin peak concentrations at the end of pregnancy reach 100 ng/mL, two times greater than in human (Sherwood, 1994). While relaxin plays important role in collagen catabolism of the pubic symphysis during gestation in lower mammals such as mice and rats (Samuel et al., 1998), the role of this hormone on pubic symphysis of human is however unknown (Hashem et al., 2006; Wang et al., 2009). Several studies have highlighted the therapeutic potential of relaxin for ectopic pregnancy, male infertility, and heart failure, cardiovascular and musculoskeletal diseases. Currently, there are seven known relaxin family peptides (RXFP) that are structurally related to insulin which include relaxin (RLN1, RLN2, RLN3, and insulin-like peptide (INSL)3, INSL4, INSL5, and INSL6 (Bathgate et al., 2013). RLN1 and RLN2 are strong regulators of collagen expression and metabolism in fibroblasts, and are differentially expressed in the corpus luteum, decidua, and endometrium, as well as prostate tissue, while RLN3 is specific to the brain (Sherwood, 2005). Relaxin1 and 2 reconcile the hemodynamic changes occurring during pregnancy such as cardiac output, renal blood flow, and arterial compliance (Conrad, 2011), as well as weakening the pelvic ligaments for parturition in species such as guinea pigs and mice (Sherwood et al., 1993). RLN3 is a highly conserved neuropeptide in vertebrates, and is involved in a wide range of neuroactivities such as response to stress and cognition, as well as in neurological disease (Smith et al., 2011).

Relaxin binds to RXFP receptors and exerts its action through a ligand-receptor system in multiple pathways. The relaxin receptor is involved in signal transduction between extracellular/intracellular domains. Relaxin1–4 hormones are ligands for the RXFP1, RXFP2, RXFP3, and RXFP4, respectively (Fig. 1). This family peptides act on four G-protein-coupled receptors (GPCRs; formerly LGR7, LGR8, GPCR135, and GPCR142) (Kong et al., 2010). RXFP1 and RXFP2 are composed of large extracellular domains which encompass of leucine-rich repeats. On the other hand, RXFP3 and RXFP4 proteins are more similar to small peptide ligands (Summers et al., 2013).
Recently, it has been shown that there is a difference in the ligand binding mode between RXFP1 and RXFP2 (Scott et al., 2012). RXFP1 and RXFP2 exist in uterus, cervix, vagina, brain, and heart of a number of animal species. However, production of these proteins differs among tissues of various species. For example, RXFP1 is expressed in rats and mice myometrium (Vodstrcil et al., 2010), whereas in human, this receptor is mainly localized to the endometrium (Campitiello et al., 2011). Moreover, RXFP1 is expressed in the rats and mice heart localized to the atria where it mediates positive inotropic and chronotropic responses (Piedras-Renteria et al., 1997), while there is currently no report of this receptor binding or function in the human heart.

Evidence also suggests that the functional domains of RXFP1, the cell type in which it is expressed, and the ligand used to activate the receptor all have important roles in the musculoskeletal system (Fig. 2). Relaxin alters cartilage and tendon stiffness by activating collagenase (Hashem et al., 2006; Pearson et al., 2011). Relaxin is also involved in bone remodeling process and in healing of injured ligaments and skeletal muscles (Li

### Table: Roles of Relaxin in Different Organs

| Organ     | Proliferation/Remodeling | Increases in ACL tears | Increases response | Activate collagenase | Osteoclastogenesis | Decrease stiffness | Increase length | Increase laxity | Antithrombus | Regulation | Expansion | Healing | PTGS |
|-----------|--------------------------|------------------------|-------------------|---------------------|--------------------|-------------------|-----------------|----------------|-------------|------------|-----------|---------|------|
| 1         | ✓                        | ✓                      | ✓                 | ✓                   | ✓                  | ✓                 | ✓               | ✓              | ✓           | ✓          | ✓        | ✓      |
| 2         | ✓                        | ✓                      | ✓                 | ✓                   | ✓                  | ✓                 | ✓               | ✓              | ✓           | ✓          | ✓        | ✓      |
| 3         | ✓                        | ✓                      | ✓                 | ✓                   | ✓                  | ✓                 | ✓               | ✓              | ✓           | ✓          | ✓        | ✓      |
| 4         | ✓                        | ✓                      | ✓                 | ✓                   | ✓                  | ✓                 | ✓               | ✓              | ✓           | ✓          | ✓        | ✓      |
| 5         | ✓                        | ✓                      | ✓                 | ✓                   | ✓                  | ✓                 | ✓               | ✓              | ✓           | ✓          | ✓        | ✓      |
| 6         | ✓                        | ✓                      | ✓                 | ✓                   | ✓                  | ✓                 | ✓               | ✓              | ✓           | ✓          | ✓        | ✓      |

**Musculoskeletal system**

Fig. 1. Interaction of RLN1, RLN2, and RLN3 proteins with their receptors RXFP1, RXFP2, and RXFP3, respectively, as well as with insulin-like growth factor (INSL3) and rearranged L-myc fusion (RFL) in the network (http://www.genecards.org/).

Fig. 2. A summary of relaxin role in the locomotor system.
et al., 2005; Dragoo et al., 2009). The soft tissue healing cascade is composed of three phases, inflammation, regeneration, and fibrosis, and relaxin is a regulator of both inflammation and fibrosis (Mu et al., 2010). Relaxin also acts as an antifibrotic agent, and favors muscle regeneration and against muscle fibrosis to promote regrowth of myofibers in skeletal muscle healing. In this review, our aim is to summarize and critically investigate the available data, strictly related to relaxin and its regulatory effect on the musculoskeletal system.

Relaxin function in the musculoskeletal system

The musculoskeletal system is composed of bone, synovium, ligament, muscle, tendon, articular cartilage, and the related connective tissues that support the body’s ability to move (Farley et al., 2012). Relaxin plays an integral role in the remodeling of multiple tissues of the musculoskeletal system.

Bone

Relaxin along with hormones such as estrogen and growth factors such as transforming growth factor-beta (TGF-β) helps orchestrate the bone remodeling process. These factors regulate a cytokine system containing three fundamental molecules: the receptor activator of nuclear factor κB ligand (RANKL), RANK, and osteoprotegerin (OPG). In the RANKL/RANK/OPG system, RANKL on the preosteoblastic/stromal cells binds to its receptor (RANK) on the osteoclastic precursor cells and induces expression of a variety of genes to provide the crucial signal to drive osteoclast recruitment and development (Faccioli et al., 2009). OPG regulates the system through blocking the effects of RANKL and interfering with RANK signaling. Relaxin facilitates differentiation of peripheral blood mononuclear cells into mature osteoclasts during osteoclastogenesis by stimulating osteoblastic/stromal cell production, while estrogen inhibits this process through increasing OPG production (Faccioli et al., 2009). Therefore, relaxin is one of the osteoclast-activating factors that increase bone resorption. It is also overexpressed in tumors that promote growth, differentiation, and invasiveness, which lead to osteolytic metastases (Clezardin & Teti, 2007). Together, these data indicate a possible role of relaxin in osteoclastogenesis (Faccioli et al., 2009; Ferlin et al., 2010). Relaxin 2 (RLX2) regulates bone metabolism and proliferation in human osteoblasts. Stimulation of osteoblasts with RLX2 activates adenylyl cyclase (AC) and increases cAMP production by G-proteins and thereby increases cell proliferation (Ferlin et al., 2009). Previous studies have identified an inactivating mutation in the RXFP2 gene (T222P), which caused idiopathic osteoporosis in young men through functional osteoblast impairment and reduced bone density (Ferlin et al., 2009). A similar result was also observed in knockout mouse model (Ferlin et al., 2008, 2011). There is also some evidence to suggest that higher levels of estrogen and relaxin in pregnant women correlated with an increased prevalence of congenital dysplasia of the hip in neonates (Uden & Lindhagen, 1988; Saugstad, 1991; Steinetz et al., 2008). In view that relaxin affects both osteoclast and osteoblast, therefore this hormone is involved in bone remodeling process, and stimulation of osteoblast by RLX2 suggests that this hormone is potentially useful in the treatment of bone condition such as osteoporosis.

Synovium

Relaxin in combination with estrogens may also have therapeutic value in the treatment of rheumatoid arthritis (RA) (Santora et al., 2005; Ho et al., 2011). RA is a chronic and systemic inflammatory disorder that may affect many tissues and organs, but also causes bone destruction through synovial hypertrophy. However, the incidence and severity of this disease during pregnancy is lower than normal. During pregnancy, relaxin and estrogen levels in the serum are elevated leading to decrease in inflammation in RA patients (D’elia et al., 2003; Ho et al., 2011). Relaxin exerts its anti-inflammatory effect through down-regulation of neutrophil function (Bani et al., 1998) and stimulates leukocyte adhesion and migration in human mononuclear cells (Figueiredo et al., 2006). A combined treatment using relaxin and estrogen appears to reduce circulating tumor necrosis factor-α level in rat adjuvant-induced arthritis model of RA and increased the anti-inflammatory cytokine IL-10 in human cells. (Santora et al., 2005; Figueiredo et al., 2006). In view of this, relaxin has a potential beneficial effect in the treatment of synovial diseases.

Ligament

Relaxin hormone alters ligament mechanics due to its collagenolytic effect mediated by discharge of matrix metalloproteinases (MMPs) (Qin et al., 1997), collagenase (Wiqvist et al., 1984; Granstrom et al., 1992), and plasminogen activator (Koay et al., 1983). Relaxin treatment in pregnant cattle increased pelvic width and height (Perezgrovas & Anderson, 1982; Musah et al., 1986), but not in other joints such as wrist and knee (Weinberg, 1956; Marnach et al., 2003). Increase in serum relaxin concentration may also correlate with joint laxity (Lubahn et al., 2006; Dragoo et al., 2011a, b), but this effect during pregnancy is controversial (Forst et al., 1997). Some studies have reported higher relaxin levels in pregnant women with pelvic joint instability or hip joint laxity as compared with controls (Saugstad, 1991; Steinetz et al., 2008), while other studies did not (Ohtera et al., 2002). Two studies on the relationship between serum relaxin levels and joint laxity reported no significant association between this hormone level and knee and generalized joint laxity (Arnold et al., 2002; Wolf et al.,
two principle signaling pathways: AC and nitric oxide

Musculoskeletal system

Muscle

Relaxin helps regulate normal skeletal muscle through two principle signaling pathways: AC and nitric oxide (NO). Relaxin activates the AC signaling pathway in skeletal muscles through the following signal chain: relaxin receptor tyrosine kinase → Gi protein (βγ-dimer) → phosphatidylinositol 3-kinase (PI3K) → protein kinase Cζ (PKCζ) → heterotrimeric Gs protein → AC → protein kinase A (Kuznetsova et al., 1999; Shpakov et al., 2004, 2006a, b, 2007a, b; Pertseva et al., 2006; Plesneva et al., 2008). Relaxin also activates the NO pathway in skeletal muscle via relaxin-mediated activation of receptor tyrosine kinase → Gi protein → PI3K → protein kinase D1 → protein kinase B → NO (Plesneva et al., 2008). NO regulates various biological processes, and is produced by NO synthase (Stamler & Meissner, 2001). There are data that indicate relaxin stimulates NO synthase signaling in the skeletal muscles of type 2 diabetic rats, leading to NO dysfunction (Kuznetsova et al., 2010).

Relaxin may be implicated in the skeletal muscle healing process by regulating inflammation, tissue remodeling, and fibrosis (Formigli et al., 2005; Sherwood, 2005). The degree of fibrotic response varies with the level of inflammation and injury. Relaxin may improve spontaneous regeneration of injured skeletal muscle as illustrated in an injured muscle mouse model (Fukushima et al., 2001; Sato et al., 2003). During this process, skeletal muscle cells regenerate and repair to reduce the size of a damaged or necrotic area and replace it with new living tissue. Degeneration/inflammation is a retrogressive change in cells and tissues characterized by abnormal structural changes and decreased functions (Li et al., 2005; Merchav et al., 2005; Negishi et al., 2005; Mu et al., 2010). Relaxin has been reported to regulate several steps during inflammation which include inhibition of platelet aggregation (Bani et al., 2007), inhibits activation and recruitment of neutrophils to the site of inflammation (Emanuela et al., 2004), and promotes migration of mononuclear leucocytes through RXFP1-dependent mechanism (Figueiredo et al., 2006).

In regeneration phase, immature granulation tissue containing active fibroblasts produces abundant type III collagen, which fills the defect left by an open wound (Volk et al., 2011). Granulation tissue moves, as a wave, from the border of the injury toward the center. As granulation tissue matures, fibroblasts produce less collagen and become more spindly in appearance, which then begin to produce a much stronger type I collagen (Syed et al., 2011). Some of the fibroblasts mature into myofibroblasts containing similar actin to the smooth muscle, which enables them to contract and reduce the size of the wound (Sarrazay et al., 2011). Fibrosis is the last phase of healing where a non-functional scar tissue is formed caused by excessive accumulation of connective tissue following damage. Fibrosis often delays and impairs the recovery of damaged tissue (Diegelmann & Evans, 2004). Relaxin has been shown to inhibit fibrosis formation through several mechanisms that include neutralization of the effect of TGFβ1 and activation of the collagenolytic system, which increases collagenase
synthesis (Garibay-Tupas et al., 2004; Guttridge, 2004; Mendias et al., 2004, 2012; Mu et al., 2010; Vinall et al., 2011). Through these mechanisms, relaxin reduces the formation of fibrous scar tissue (Fig. 3). Relaxin administration to the injured skeletal muscle promotes activation of satellite cells, induces angiogenesis and revascularization, as well as represses the extended inflammatory reaction (Mu et al., 2010). Recently, relaxin administration to diabetic wound in mice has been shown to up-regulate the mRNA expression of vascular endothelial growth factor, epithelial NO, and stromal-cell-derived factor 1-α, stimulates angiogenesis and vasculogenesis, enhances MMP-11 expression, and increases wound-breaking strength (Bitto et al., 2013). In view that relaxin plays important role in the healing process, it can potentially be used as a therapeutic agent to treat damaged skeletal muscle (Negishi et al., 2005).

**Tendon**

Relaxin has been reported to effect tendon metabolism by controlling the length of tendon growth (Maclennan et al., 1986; Wood et al., 2003) and reduce tendon stiffness by increasing tendon laxity through activation of collagenase (Pearson et al., 2011). An in vivo study investigating the growth of rat tails and human patellar tendons showed that relaxin levels correlate with tendon length (Wood et al., 2003; Pearson et al., 2011). Rat tail tendons treated with relaxin exhibited alterations in collagen through interfering with fibril association and collagen sliding (Wood et al., 2003). Another study in women with normal menstrual cycle, who did not take any contraception pills, demonstrated a significant link between serum relaxin levels and patellar tendon stiffness (Pearson et al., 2011). Besides the reported effects of relaxin on the tendon, potential benefits of relaxin on tendon repair and remodeling are largely unknown.

**Cartilage**

Relaxin appears to decrease knee articular cartilage stiffness (Bonaventure et al., 1988; Hellio Le Graverand et al., 1998) through induction of collagenase-1, MMP-1, and MMP-3, which reduces collagen content and expression in fibrocartilaginous cells. Modulation of MMPs to loss of collagen by hormones may contribute selectively to degeneration of specific joints fibrocartilaginous explants facilitated by proteinases (Naqvi et al., 2005; Hashem et al., 2006). The degradation of extracellular matrix in fibrocartilage is synergized by β-estradiol. Relaxin exerts its effect through binding to RXFP1 and RXFP2 receptors (Hellio Le Graverand et al., 1998; Wang et al., 2009). The ratio of RXFP2 in knee meniscus of pregnant rabbits was shown to be more than RXFP1, which may indicate differential role of these receptors in the remodeling of fibrocartilage (Hellio Le Graverand et al., 1998; Wang et al., 2009). Comparison of collagen content in articular cartilage of nonpregnant and pregnant rabbits showed that the total RNA levels and chondrocyte metabolism decreased during pregnancy. Depending on the level of skeletal maturity, pregnancy can exert both general and specific effects on the RNA levels in articular cartilage of the rabbit knee (Hellio Le Graverand et al., 1998). Thus, relaxin may play a role in women’s susceptibility to musculoskeletal disease (Naqvi et al., 2005). Taken together, these findings suggested that in female, increased relaxin level may result in undesirable effects on the articular cartilage.

**Perspective**

Relaxin plays a vital role in biological processes including metabolism, growth, and reproduction. Among the four relaxin types, RLN1 and RLN2 regulate the musculoskeletal system via multiple pathways through a ligand-receptor system, depending on cell type and ligand (Table 1). Our investigation of relaxin’s role in the musculoskeletal system showed some limitations in the literature. For example, most of the reports did not delineate the relaxin isoform or its specific receptor. Additionally, despite relaxin’s accepted role in the regulation of AC and NO pathways, few studies have focused on the regulatory effect of these pathways in the
| Organ       | Author (year)                        | Sample                                      | Model     | Treatment                                    | Relaxin | Role of relaxin                                                                 |
|------------|--------------------------------------|---------------------------------------------|-----------|----------------------------------------------|---------|--------------------------------------------------------------------------------|
| Skeleton   | Bonaventure et al. (1988)            | Chondrocyte cell                           | Rb/vitro | Porcine relaxin                              | NI      | Modulation of type I, II, III collagen expression                              |
|            | Naqvi et al. (2005)                  | Joint fibrocartilaginous cells             | Rb/vitro | Human relaxin, β-estradiol                   | NI      | No increase collagenase1 and MMP3 expression                                   |
|            | Hashem et al. (2006)                 | Knee meniscus fibrocartilage and articular cartilage | Rb/vitro | Human relaxin, β-estradiol, progesterone     | NI      | No significant change of GAGs and collagen metabolism                           |
|            | Wang et al. (2009)                   | Joint fibrocartilaginous cells             | M/vitro  | NI                                            | 1,2     | Expression of RXFP2 > RXFP1                                                    |
| Cartilage  | Santora et al. (2005)                | Arthritis paw                              | R/vivo   | Porcine relaxin and 17-β-estradiol          | NI      | Combination hormone therapy reduced arthritis inflammation, Relaxin            |
|            | Kristiansson et al. (2005)           | Normal osteoblast cell                     | H/vitro. | Agonists INSL3, relaxin, forskolin          | 1,2     | Bone resorption by mediators                                                   |
|            | Ferlin et al. (2008)                 | Bone densitometry, cryptorchidism, osteoblast cell | H,M/vivo | 2                                              |         | Links RXFP2 gene mutations with human osteoporosis                             |
|            | Facciolli et al. (2009)              | Osteoclast cell                            | H/vitro  | Relaxin                                       | 1       | Facilitation of the differentiation of osteoclasts                             |
|            | Ferlin et al. (2009)                 | Osteoclast cell                            | H/vitro  | Relaxin                                       | 1       | Relaxin is a potent stimulator of osteoclastanogenesis, RXFP2 system is involved in bone metabolism |
|            | Ferlin et al. (2011)                 | Femoral heads murine osteoblast cell       | H/vitro  | Relaxin 2                                    | 2       | RANKL-OPG system                                                               |
|            | Ho et al. (2011)                     | Joint tissues, murine osteoblast cells     | R/vivo and vitro | 17-β-estradiol, porcine relaxin      | NI      | Modulation of RANKL-OPG system                                                 |
| Joint      | Weinberg (1956)                      | Four nonpregnant and 11 pregnant Pelvic joints | H/vivo   | Relaxin as releasin                          | NI      | No change in pelvic measurement                                                |
|            | Crelin and Brightman (1957)          | Pelvic joint                               | R/vivo   | Relaxin, estrogen                            | NI      | No difference in pelvic joint flexibility                                       |
|            | Perezgrova and Anderson (1982)       | Patients with late pregnancy Pelvic joint  | H/vivo   | NI                                            | NI      | Expansion of the pelvic area (p)                                               |
|            | MacLennan et al. (1986)              | CDH patients                               | H/vivo   | NI                                            | NI      | Increased sensitivity of the receptors of the fibroblasts                      |
|            | Musah et al. (1986)                  | 153 pregnant women                         | H/vivo   | NI                                            | NI      | Congenital hip dysplasia rate, consistent with estrogen and relaxin levels     |
|            | Udén and Lindhagen (1988)            | 472 pregnant women                         | H/vivo   | NI                                            | NI      | Not associated with pregnancy pelvic pain                                       |
|            | Saugstad (1991)                      | 19 women                                   | H/vivo   | Oral contraceptive                          | NI      | Higher relaxin with posterior pelvic and lumbar pain                           |
|            | Petersen et al. (1994)               | 21 women                                   | H/vivo   | NI                                            | 2       | No correlation with serum relaxin and joint laxity                             |
|            | Wreje et al. (1995)                  | 90 newborn children                       | H/vivo   | NI                                            | NI      | NI lower relaxin in newborns with pelvic presentation hip instability          |
|            | Schauberger et al. (1996)            | 12 boys newborn boys                      | H/vivo   | NI                                            | 2       | Reduction of relaxin concentration with increasing sonographic hip             |
|            | Forst et al. (1997)                  | 200 pregnant women                        | H/vivo   | NI                                            | NI      | Relaxin correlated with pelvic pain in early pregnancy                        |
|            | Vogel et al. (1998)                  | Knee joint of nonpregnant and pregnant Athlete eumenorrheic women and men | H/vivo | NI                                            | NI      | Relaxin preventing the development of joint contracture                       |
|            | Kristiansson et al. (1999)           | Athlete eumenorrheic women and men         | H/vivo   | NI                                            | NI      | No effect on knee laxity                                                       |
|            | Ohtera et al. (2002)                 | Pregnant women                             | H/vivo   | NI                                            | NI      | No correlation of wrist joint laxity and relaxin level                         |
|            | Arnold et al. (2002)                 | Pregnant women                             | H/vivo   | NI                                            | NI      | Higher relaxin and fall significantly faster in women with PFD                 |
|            | Marnach et al. (2003)                | Pregnant women                             | H/vivo   | NI                                            | NI      | Contribution with pelvic joint laxity but no responses to pain and disability  |
|            | Harvey et al. (2008)                 | 212 women pelvic joints                   | H/vivo   | NI                                            | 2       | No link between serum relaxin and generalized joint laxity                     |
|            | Vollestad et al. (2012)              | 289 healthy human                         | H/vivo   | NI                                            | NI      | Higher relaxin and fall significantly faster in women with PFD                 |
|            | Wolf et al. (2013)                   | 289 healthy human                         | H/vivo   | NI                                            |        | No link between serum relaxin and generalized joint laxity                     |

**Musculoskeletal system**
musculoskeletal organs. Although relaxin may affect many ligaments and tendons of the musculoskeletal system, previous studies have mostly concentrated on the anterior cruciate and wrist ligaments. Future studies are warranted to gain a better understanding of relaxin’s role in the musculoskeletal system.

**Key words:** relaxin, motor organs, skeletal muscle, tendon, ligament.

**Acknowledgements**

This work is supported by PPP grant (PV104/2012A), University of Malaya, Kuala Lumpur, Malaysia.

**References**

Albert H, Godskesen M, Westergaard JG, Chard T, Gunn L. Circulating levels of relaxin are normal in pregnant women with pelvic pain. Eur J Obstet Gyn R B 1997: 74 (1): 19–22.

Arnold C, Van Bell C, Rogers V, Cooney T. The relationship between serum relaxin and knee joint laxity in female athletes. Orthopedics 2002: 25 (6): 669–673.

Bani D, Masini E, Bello MG, Bigazzi M, Sacchi TB. Relaxin protects against myocardial injury caused by ischemia and reperfusion in rat heart.

[Research Support, Non-U.S. Gov’t], Am J Pathol 1998: 152 (5): 1367–1376.

Bani D, Nistri S, Cinci L, Giannini L, Princivalle M, Elliott L, Bigazzi M, Masini E. A novel, simple bioactivity assay for relaxin based on inhibition of...
beta-estradiol modulate targeted matrix degradation in specific synovial joint fibrocartilages: progesterone prevents matrix loss [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. Arthritis Res Ther 2006: 8 (4): R98. doi: 10.1186/ar1978.

Hellio Le Graverand MP, Reno C, Hart DA. Influence of pregnancy on gene expression in rabbit articular cartilage [Research Support, Non-U.S. Gov't]. Osteoarthritis Cartilage 1998: 6 (5): 341–350.

Hissaw FL. Experimental relaxation of the pubic ligament of guinea pig. Proc Soc Exp Biol Med 1926: 23: 661–663.

Ho TY, Santora K, Chen JC, Frankshun Hisaw FL. The use of relaxin improves diabetes mellitus. Dokl Biochem Mol Biol 1999: 341–350.

Kristiansson P, Holding C, Hughes S. Effects of relaxin, pregnancy and estrogens on bone remodeling markers, receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG), in rat adjuvant-induced arthritis [Research Support, Non-U.S. Gov't]. Bone 2011: 48 (6): 1346–1353.

Koay ES, Too CK, Greenwood FC, Bryant-Greenwood GD. Relaxin stimulates collagenase and plasminogen activator secretion by dispersed human amnion and chorion cells in vitro [Comparative Study]. JCEM 1983: 56 (6): 1332–1334.

Kong RCK, Shilling PJ, Lobb DK, Gooley PR, Bathgate RAD. Membrane receptors: structure and function of the relaxin family peptide receptors. Mol Cell Endocrinol 2010: 320 (1–2): 1–15.

Kristiansson P, Holding C, Hughes S, Haynes D. Does human relaxin-2 affect peripheral blood mononuclear cells to increase inflammatory mediators in pathologic bone loss? Ann Ny Acad Sci 2005: 1041 (1): 317–319.

Kristiansson P, Svardsudd K, Von Schoultz B. Reproductive hormones and aminoterminal propeptide of type III procollagen in serum as early markers of pelvic pain during late pregnancy. Am J Obstet Gynecol 1999: 180 (1 Pt 1): 128–134.

Kuznetsova L, Plesneva S, Derjabina N, Kuznetsova L, Plesneva S, Omeljanjuk E, Frankshun Hisaw FL. Characterization of the relationship between joint laxity and maternal hormones in pregnancy. Obstet Gynecol 2003: 101 (2): 331–335.

Mendias CL, Gumopio JC, Davis ME, Bromley CW, Davis CS, Brooks SV. Transforming growth factor-beta induces skeletal muscle atrophy and fibrosis through the induction of atrogin-1 and sclerasis. Muscle Nerve 2012: 45 (1): 55–59.

Mendias CL, Tatsumi R, Allen RE. Role of cyclooxygenase-1 and -2 in satellite cell proliferation, differentiation, and fusion. Muscle Nerve 2004: 30 (4): 497–500.

Merchav R, Feuermann Y, Shamay A, Ranen E, Stein U, Johnston DE, Shahar R. Expression of relaxin receptor LRG7, canine relaxin, and relaxin-like factor in the pelvic diaphragm musculature of dogs with and without perineal hernia. Vet Surg 2005: 34 (5): 476–481.

Min G, Sherwood OD. Identification of specific relaxin-binding cells in the cervix, mammary glands, nipples, small intestine, and skin of pregnant pigs [Research Support, Non-U.S. Gov't]. Biol Reprod 1996: 55 (6): 1243–1252.

Mu X, Urso ML, Murray K, Fu F, Li Y. Relaxin regulates MMP expression and promotes satellite cell mobilization during muscle healing in both young and aged mice. Am J Pathol 2010: 177 (5): 2399–2410.

Musah AI, Schwabe C, Anderson LL. Pelvic development as affected by relaxin in three genetically selected frame sizes of beef heifers. Biol Reprod 1986: 34: 363–369.

Naqvi T, Duong TT, Hashem G, Shiga M, Min G, Sherwood OD. Identification of the relaxin family peptide receptors. Mol Pharmacol 1991: 39 (4): H1791–H1797.

Negishi S, Li Y, Negishi S, Sakamoto M, Usas A, Huard J. The use of relaxin improves healing in injured muscle [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov’t Research Support, U.S. Gov’t, P.H.S.]. Ann N Y Acad Sci 2005: 1041: 395–397.

Lubahn J, Ivance D, Konieczko E, Cooney T. Immunohistochemical detection of relaxin binding to the vular oblique ligament [Research Support, Non-U.S. Gov't]. J Hand Surg [Am] 2006: 31 (1): 80–84.

MacAlman AH, Nicolson R, Green RC, Bath M. Serum relaxin and pelvic pain of pregnancy [Research Support, Non-U.S. Gov't]. Lancet 1986: 2 (8501): 243–245.

Marnach ML, Ramin KD, Ramsey PS, Song SW, Stensland JI, An KN. Disturbance of regulation of NO synthase activity by peptides of insulin MN. Disturbance of regulation of NO synthase activity by peptides of insulin MN. Biophys 2010: 432 (1): 123–125.

Li Y, Negishi S, Sakamoto M, Usas A, Huard J. The use of relaxin improves healing in injured muscle [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov’t Research Support, U.S. Gov’t, P.H.S.]. Ann N Y Acad Sci 2005: 1041: 395–397.

Lubahn J, Ivance D, Konieczko E, Cooney T. Immunohistochemical detection of relaxin binding to the vular oblique ligament [Research Support, Non-U.S. Gov’t]. J Hand Surg [Am] 2006: 31 (1): 80–84.

MacAlman AH, Nicolson R, Green RC, Bath M. Serum relaxin and pelvic pain of pregnancy [Research Support, Non-U.S. Gov't]. Lancet 1986: 2 (8501): 243–245.

Marnach ML, Ramin KD, Ramsey PS, Song SW, Stensland JI, An KN. Disturbance of regulation of NO synthase activity by peptides of insulin MN. Disturbance of regulation of NO synthase activity by peptides of insulin MN. Biophys 2010: 432 (1): 123–125.

Li Y, Negishi S, Sakamoto M, Usas A, Huard J. The use of relaxin improves healing in injured muscle [Research Support, Non-U.S. Gov’t Research Support, U.S. Gov’t, P.H.S.]. Ann N Y Acad Sci 2005: 1041: 395–397.

Lubahn J, Ivance D, Konieczko E, Cooney T. Immunohistochemical detection of relaxin binding to the vular oblique ligament [Research Support, Non-U.S. Gov’t]. J Hand Surg [Am] 2006: 31 (1): 80–84.
myofibroblasts and current developments. Wound Repair Regen 2011: 19: s10–s15.
Sato K, Li Y, Foster W, Fukushima K, Badlani N, Adachi N, Usas A, Fu FH, Huard J. Improvement of muscle healing through enhancement of muscle regeneration and prevention of fibrosis. Muscle Nerve 2005: 28 (3): 365–372.
Saugstad LF. Persistent pelvic pain and pelvic joint instability. Eur J Obset Gynecol Reprod Biol 1991: 41 (3): 197–201.
Schauberger CW, Rooney BL, Goldsmith L, Shenton D, Silva PD, Schaper A. Peripheral joint laxity increases in pregnancy but does not correlate with serum relaxin levels. Am J Obset Gynecol 1996: 174 (2): 667–671.
Scott DJ, Rosengren KJ, Bathgate RAD. The different ligand-binding modes of relaxin family peptide receptors RXFP1 and RXFP2. Mol Endocrinol 2012: 26 (11): 1896–1906.
Sherwood OD. Relaxin. In: Knobil E, Scott DJ, Rosengren KJ, Bathgate RAD. Shauberger CW, Rooney BL, Goldsmith SA, Pertseva MN. Role of [In Vitro Research Support, Non-U.S. Gov't]. Ann N Y Acad Sci, 1988: 59 (6): 667–668.
Smith CM, Ryan PJ, Hosken IT, Ma S, Gundlach AL. Relaxin-3 systems in the brain – the first 10 years [Research Support, Non-U.S. Gov't Review]. J Chem Neuroanat 2011: 42 (4): 262–275.
Steinmetz BG, Williams AJ, Lust G, Schwabe C, Bullesbach EE, Goldsmith GC, Vere White W, De R. Dual function and potentiation of relaxin extract in pelvic expansion. Surg Gynecol Obstet 1956: 103 (3): 303–306.
Syed F, Ahmadi E, Iqbal SA, Singh S, McGrouther DA, Bayat A. Fibroblasts from the growing margin of keloid scars produce higher levels of collagen I and III compared with intrasional and extralesional sites: clinical implications for lesional site-directed therapy. Br J Dermatol 2011: 164 (1): 83–96.
Toth AP, Cordasco FA. Anterior cruciate ligament injuries in the female athlete [Review]. J Gend Specif Med 2001: 4 (4): 25–34.
Uden A, Lindhagen T. Inguinal hernia in patients with congenital dislocation of the hip. A sign of general connective tissue disorder [Research Support, Non-U.S. Gov't]. Acta Orthop Scand 1988: 59 (6): 667–668.
Vatnall LR, Mahaffey MC, Davis RR, Luo ZP, Regina GE, Ghosh MP, Tepper GC, Vere White W, De R. Dual blockade of PKA and NF-kB inhibits H2 relaxin-mediated castrate-resistant growth of prostate cancer sublines and induces apoptosis. Horm Cancer 2011: 2 (4): 224–238.
Vlodrscil LA, Shynlova O, Verlander JW, Wlodek ME, Parry LJ. Decreased expression of the rat myometrial relaxin receptor (RXFP1) in late pregnancy is partially mediated by the presence of the conceptus. Biol Reprod 2010: 83 (5): 818–824.
Vogel I, Andersson JE, Uldbjerg N. Serum relaxin in the newborn is not a marker of neonatal hip instability. J Pediatr Orthoped 1998: 18 (4): 535–537.
Volk SW, Wang Y, Mauldin EA, Liechty KW, Adams SL. Diminished type III collagen promotes myofibroblast differentiation and increases scar deposition in cutaneous wound healing. Cells Tissues Organs 2011: 194 (1): 25–37.
Vollestad NK, Torjesen PA, Robinson HS. Association between the serum levels of relaxin and responses to the active straight leg raise test in pregnancy. Man Ther 2012: 17 (3): 225–230.
Wang W, Hayami T, Kapila S. Female hormone receptors are differentially expressed in mouse fibrocartilages [Research Support, N.I.H., Extramural]. Osteoarthritis Cartilage 2009: 17 (5): 646–654.
Weinberg A. An x-ray pelvimetric study of relaxin extract in pelvic expansion. Surg Gynecol Obstet 1956: 103 (3): 303–306.
Wiqvist I, Norstrom A, O'byrne E, Wiqvist N. Regulatory influence of relaxin on human cerebral and uterine connective tissue. Acta Endocrinol (Copenh) 1984: 106 (1): 127–132.
Wojtys EM, Huston LJ, Boynton MD, Spindler KP, Lindenfeld TN. The effect of the menstrual cycle on anterior cruciate ligament injuries in women as determined by hormone levels. Am J Sports Med 2002: 30 (2): 182–188.
Wolf JM, Williams AE, Delaronde S, Leger R, Clifton KB, King KB. Relationship of serum relaxin to generalized and trapezio-metacarpal joint laxity. J Hand Surg [Am] 2013: 38 (4): 721–728.