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# Relaxin affects the dentofacial sutural tissues

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**Abstract:** This is a report of an exploratory study of how the hormone relaxin might modulate the remodeling of connective tissue within the craniofacial sutures and periodontal tissues. Relaxin is a hormone that was discovered to be produced by the pregnant female. It is responsible for the relaxing of the pubic symphysis; the birth canal is widened for parturition. It has also been shown to have effects on other areas of the body, including ligaments and regions containing collagen and fibroblastic activity. Twenty-one Swiss retired-breeder mice were used to: 1) immunohistochemically demonstrate the presence of relaxin within the sutures; 2) demonstrate its effects on the integrity of the suture-like tissues; and 3) assay its effects on protease activity. Relaxin in concentrations of 250 and 500 ng/ml was used in the treated samples and allowed to incubate in complete tissue culture for 24 h. The results indicate the presence of relaxin within the cranial suture. Histological observations revealed definite changes in the collagen fibril arrangement in the PDL – from being dense and highly organized with a perpendicular direction between tooth and bone to randomly organized and loose, lacking any direction between tooth and bone. An elevation in the protease activity was evident in the relaxin-treated samples. This naturally occurring hormone might be used as an adjunct to orthodontic therapy as it appears to have the capacity to alter the physical properties of the connective tissue within sutures, gingival tissue, and the PDL. Potential indications for use include instances of sutural and soft tissue adaptation of orthopedic expansion in non-growing patients by a reduction in the tension of the stretched soft tissue envelope following orthognathic surgery (particularly the expanded palatal mucosa), periodontal ligament remodeling during or after tooth movement promoting stability, rapid gingival tissue remodeling during space closure in extraction sites, and by a decrease in the amount of scar tissue formation following frenectomies.

**Key words:** connective tissue; relaxin; remodeling; sutures

## Introduction

Orthodontic treatment is the rearrangement of skeletal or dental tissues. The clinician's ability to do so is facilitated by an extensive network of sutures and soft tissues within the orofacial complex: facial and cranial sutures, periodontal ligament, and the gingiva. Unfortunately, as much as they allow movement of dental or skeletal units, they are also thought to be responsible for the relapse of corrected relationships. Movement and relapse both require remodeling of these soft tissue systems. At the microscopic level, they are highly organized. Mechanical forces evoke a remodeling response, and remodeling involves either synthesis or degradation of collagen, or both (1–3). Arguably, if an agent or stimulus could break down the structural organization or interfere with collagen metabolism, then the orthopedic or orthodontic corrections might be easier and more stable.

One such phenomenon is physiologically present in the body: the relaxin-induced widening of the pubic symphysis during childbirth. This hormone is also present in the male, but its role remains obscure. In the pregnant female, it interferes with the collagen types I and III gene expression, reduces total collagen, and increases the collagenase activity. Currently, there are no reports of its effect on craniofacial sutures, but it was suggested as the responsible element in the degradation of the temporomandibular joint (TMJ) disc (4). Actually, the scope of relaxin's effect outside the reproductive system is largely unknown (5, 6). The potential is exciting for an application of this hormone in dentofacial orthopedics or orthodontics. Accordingly, this study was designed to 1) demonstrate the presence and effects of relaxin in the connective tissue and 2) show relaxin's effects on the proteinase activity.

## Materials and methods

Experiments in this study employed 18 Swiss retired-breeder mice. In addition, two female pregnant mice and one adult male mouse were included in the investigation for a total of 21 mice. One milligram of lyophilized mouse anti-rat relaxin monoclonal antibody (MCA1) was kindly provided by Dr. O.D. Sherwood, Department of Molecular and Investigative Physiology, University of Illinois at Urbana-Champaign. It was reconstituted in 1 ml of phosphate-buffered saline (PBS) for a final concentration of 1 mg/ml. Purified porcine relaxin hormone was also a gift from Dr.

O.D. Sherwood. Purification procedures for porcine relaxin have been described by Sherwood (6).

Reagents used in the experimental protocol were as follows: HistoMouse-SP kit (Zymed Laboratories Inc., San Francisco, CA) was used for immunohistological staining. For protein concentration determination, Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA) was used. Protease activity was determined with EnzChek™ Protease Assay Kit (Molecular Probes, Eugene, OR). Finally, a minimum essential medium (MEM; BRL), containing 15% fetal calf serum (FCS) (HyClone) was used to culture mandible and calvaria samples.

### Immunohistochemistry

Two female pregnant mice and one male adult mouse were used to demonstrate if the hormone relaxin was indeed present in the cranial sutures. Freshly isolated calvaria containing frontal sutures were embedded in OCT medium (TissueTek, Fort Washington, PA) and frozen in a  $-70^{\circ}\text{C}$  freezer. Ten-micron thick frozen sections of frontal sutures were mounted on precoated slides (Fischer Scientific, Pittsburgh, PA) and air dried. The periodontium was not used because the samples were too hard to cut into sections. MCA1 was used as the primary antibody.

The procedure for immunological staining of the sections was performed as according to the protocol supplied with the HistoMouse-SP Kit. All sections were fixed in ice-cold acetone for 10 min. The elimination of all endogenous peroxidase activity (i.e., bloody tissues) was done with Peroxi-Block for 45 s and washed immediately with distilled water. Subsequently, sections were blocked according to the manufacturer's instructions. They were then rinsed with PBS for 2 min, three times. The anti-relaxin monoclonal antibody was added to each section and allowed to incubate for 60 min. Next, the biotinylated secondary antibody was added to each section, incubated for 10 min, and rinsed with PBS for 2 min, three times. Enzyme conjugate was added to each section, incubated for 10 min, and rinsed with PBS for 2 min, three times. Substrate–chromogen mixture (DAB) was added to each section, incubated for 5 min, and rinsed well with distilled water. Each section was then counterstained with hematoxyline for about a minute and put in PBS for 30 s and then rinsed with distilled water. Sections were dehydrated, cleared with xylene, and mounted with coverslips to preserve the stained sections. A negative control experiment was obtained following the same protocol using a mouse isotype IgG as the primary antibody.

**Organ culture**

Eighteen Swiss retired-breeder mice were sacrificed. The heads were removed from the bodies and dipped in 70% ethanol (ETOH) for 10 s to eliminate extra- and intra-oral microflora. The calvariae and mandibles were dissected out and stripped of skin and muscular tissue. The gingiva remained intact on the mandibles. Samples were placed in complete medium (a-MEM, 15% FCS medium). Relaxin hormone was added to the medium at final concentrations of 250 and 500 ng/ml. Samples from each mouse (calvaria and mandible) were divided into control and experimental groups, as listed in Table 1.

One male mouse was discarded due to suspicious tumorous growths on its body. Tissue samples were incubated for 24 h. Earlier studies have shown that the effects of relaxin are evident within the first 24 h (6–8). This was confirmed and reiterated by conversations with Dr. O.D. Sherwood. After 24 h of incubation, the samples were checked for contamination and then prepared for investigation.

**Histology**

Samples were fixed in 10% neutral formalin and decalcified with EDTA for 5 weeks and then dehydrated by increasing concentrations of ethanol (30, 50, 70, 80, 90, 90, 100, 100%) for 30 min each. The samples were then embedded in JB-4 Solution A (acrylic monomer n-butoxyethanol, Electron Microscopy Science, Fort Washington, PA). Calvarial sections consisted of the sagittal suture, while sections of the mandible were taken from distal of the incisor to distal of the molar. Glass knife-cut 7-mm thick sections were stained with epoxy tissue stain-

ing (toluidine blue and basic fuschin, EMS) and processed for histological analysis.

**Protein extraction from relaxin-treated mandible and calvarial samples**

For proteinase assays, the mandibular and calvarial samples cultured for 24 h were harvested, frozen in liquid nitrogen, and pulverized using a mortar and pestle. Next, they were homogenized using a Polytron for 1 min in 2 ml of PBS, while chilling the samples on ice. Special attention was given to preventing the samples from overheating during the homogenization. The samples were centrifuged at 10000 rpm for 10 min. The supernatant was collected and stored at -70°C until it was used. Protein concentration of each sample was performed using the Bio-Rad Protein Assay Kit.

**Measurement of protease activities: fluorescence polarization analysis**

The metalloproteinase activity was measured using Enz-Chek™ Protease Assay Kit from Molecular Probes Inc. (Eugene, OR). This is a direct fluorescence-based assay for detecting proteases. Fluorescence was measured with a PTI Alphascan fluorometer set at excitation/emission maxima of 595/615 nm.

**Results**

**Immunohistochemical analyses of calvarial sutures to determine the presence of endogenous relaxin**

First, the presence of relaxin in the cranial sutures was determined. The samples from the three mice (two pregnant females, one male) positively revealed the presence of relaxin in the sagittal sutures of all three samples. Staining was evident within the suture itself as well as along the periosteum, as seen in Fig. 1B. Negative controls using a mouse IgG isotype as the primary antibody were processed in the same way. They showed a lack of staining (Fig. 1A). There was no difference in staining between the genders. Immunohistological staining was not performed on the mandible samples as the tissue proved to be too hard for sectioning. Attempts to decalcify the tissue would have destroyed antigenicity for the relaxin antibody; thus, they were not included in this part of the analysis.

**Table 1. Control and experimental groups**

	Female			Male		
	0	250	500	0	250	500
Relaxin (ng/ml)	0	250	500	0	250	500
Number of mice for:						
Histology	1	1	1	1	1	1
Protease assay	2	2	2	2	2	2

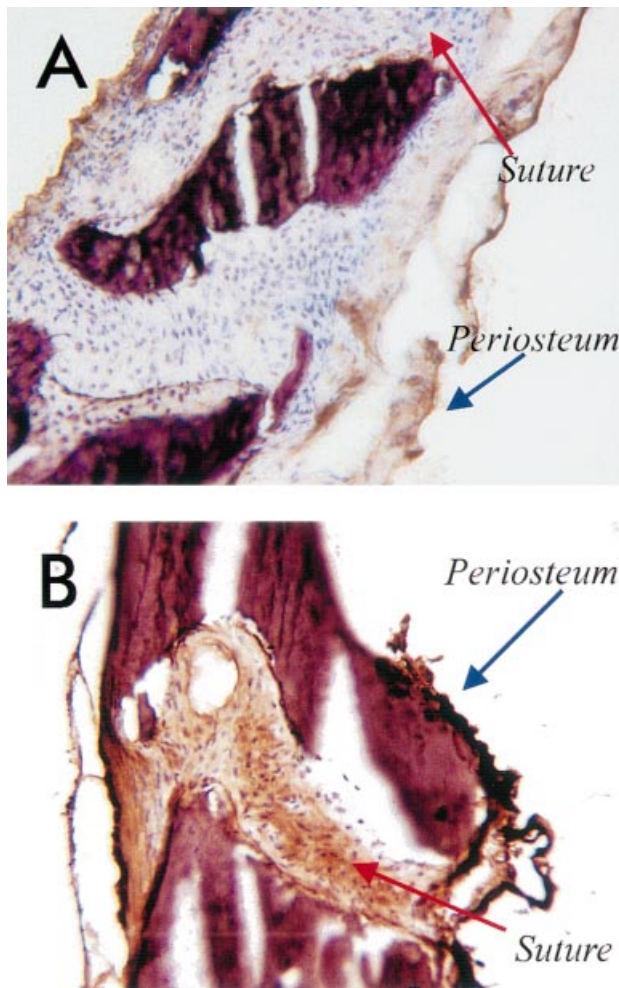


Fig. 1. Immunohistochemical staining of female cranial suture (200 $\times$ ). The lack of staining in the negative control (A) indicates the immunohistochemistry assay is reliable. The high-power view reveals the presence of relaxin hormone in cranial suture by the positive brown staining of relaxin antibody (B).

#### Histological examination of periodontium and calvarial sutures treated with relaxin

Histological staining of the periodontium was performed to allow qualitative comparison of relaxin-treated versus non-treated samples. In the mandible, control samples displayed a normal arrangement of collagen fibers in the PDL space. Fibers can be seen to run in a certain perpendicular direction from tooth to bone surface, with distinct insertion via the Sharpey's fibers. The fibers appear to be highly organized. This was evident in both female and male samples, as seen in Fig. 2A.

The PDL of the samples treated with relaxin demonstrated an irregular organization and loose arrangement from tooth to bone surface. Fibers lacked directionality and appeared short and curled. Furthermore, there

seemed to be similar dissolution of the Sharpey's fibers insertion both for females and males, as seen in Fig. 2B.

In the sagittal sutures, it was difficult to distinctly see a difference between experimental and control samples as the normal anatomical arrangement of collagen fibers within this suture is rather tortuous to begin with. This meandering of fibers follows the shape of the path made by the bony interdigitations of the suture. Thus, a change in directionality of fibers is not clearly evident. What is slightly evident is a dissolution of the fibrils from having a dense, packed arrangement to being short, curled, loose, and irregular. Male calvaria samples were lost during the sectioning process and thus could not be reported along with the female results.

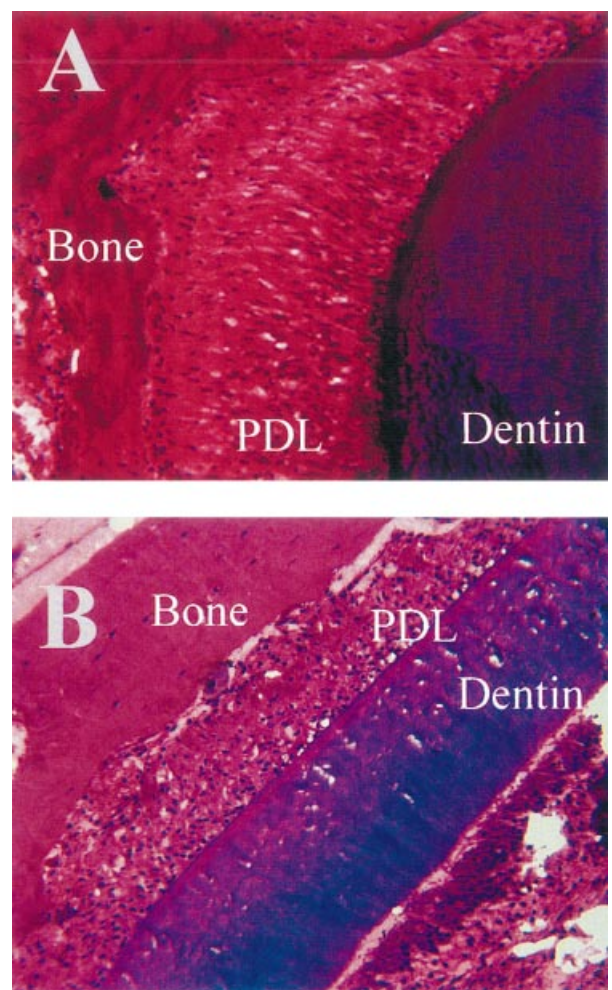


Fig. 2. Female mandible control and experimental samples (200 $\times$ ). (A) Control: note the perpendicular orientation of the PDL. Fibrils appear dense and tightly packed in nature with insertions of Sharpey's fibers. (B) Experimental sample treated with 500 ng/ml of relaxin: note the random nature and irregularity of the PDL with dissolution of Sharpey's fiber insertions.

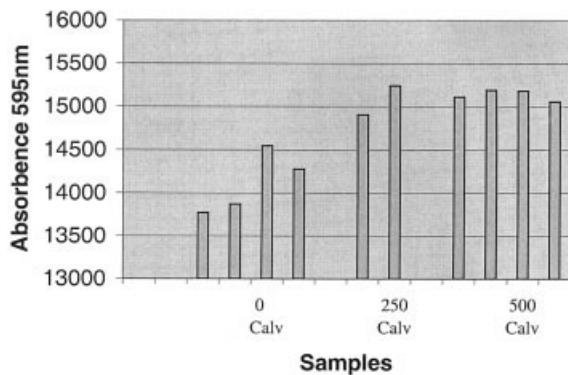


Fig. 3. Absorbance values of the male calvaria protease assay. Note the overall increase in absorbance values in the samples (0–500 ng/ml of relaxin), indicating an increase in protease activity due to relaxin treatment.

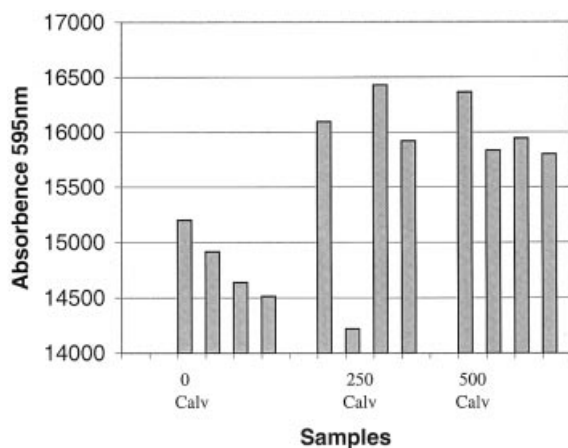


Fig. 4. Absorbance values of the female calvaria protease assay. Note the overall increase in absorbance values in the samples (0–500 ng/ml of relaxin), indicating an increase in protease activity due to relaxin treatment.

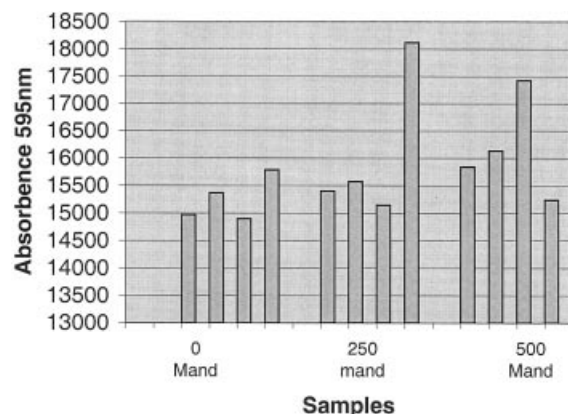


Fig. 5. Absorbance values of the male mandible protease assay. Note the overall increase in absorbance values in the samples (0–500 ng/ml of relaxin), indicating an increase in protease activity due to relaxin treatment.

**The effect of relaxin on protease activities**

Earlier studies have shown that relaxin upregulates metalloproteinase gene expression in skin fibroblasts (14). Thus, it is quite plausible that the altered histological appearance shown above in the relaxin-treated samples may be, in part, due to the breakdown of the matrix scaffolding by the increased protease activities. To test this hypothesis, the levels of protease activities were measured in both mandibular and calvarial bone samples that had been treated with 250 or 500 mg/ml of relaxin for 24 h in organ cultures (Table 1).

We initially determined how much of the sample should be subjected to each assay. To do that, varying amounts (0, 1.2, 6, 12, 24, and 48 mg, based on the protein content) of an untreated female mandibular bone homogenate were assayed for protease activities using the fluoresceine-conjugated casein substrate. The result revealed a linear relationship between the protease activity and the sample amount up to 6 mg (data not shown). Based on this result, we decided to use aliquots of sample homogenates containing 6 mg of protein in subsequent assays to evaluate the effect of relaxin on protease activities. In this experiment, we also determined the contribution of metalloproteinase activities to total protease activities by pretreating samples with EDTA. EDTA chelates metallic ions, effectively inhibiting metalloproteinase activities. The EDTA pretreatment reduced protease activities by almost 20% (data not shown), suggesting that about 20% of total protease activity accounts for the metalloproteinase activity.

The stimulatory effect of relaxin on protease activities is clearly demonstrated in calvarial bone samples from both male and female mice (Figs. 3 and 4). A similar effect is also shown in mandibular bone samples also, but it is much less prominent (Figs. 5 and 6). There were no significant differences in the response to relaxin between male and female samples. One male calvarial bone sample treated with 250 ng/ml of relaxin was not processed due to a suspicious lesion on its body prior to sacrifice.

**Discussion**

The purpose of this study was to explore the possibilities of how the hormone known as relaxin might modulate the remodeling of the connective tissue within the craniofacial sutures and the periodontium. The objectives included

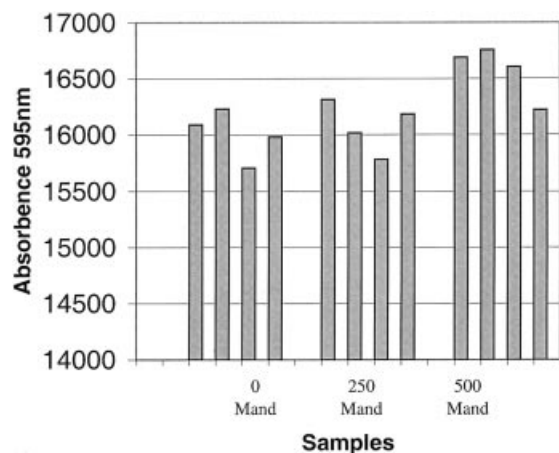


Fig. 6. Absorbance values of the female mandible protease assay. Note the overall increase in absorbance values in the samples (0–500 ng/ml of relaxin), indicating an increase in protease activity due to relaxin treatment.

showing the presence and effects of the hormone within the target tissues. The presence was positively shown using immunohistochemical staining with an antibody to relaxin, while the effects were positively seen via histological and enzyme fluorescence analysis.

It is reported that relaxin is produced in highest levels by female reproductive organs during pregnancy, especially during the third trimester, closest to term. The structures responsible for circulating relaxin in the peripheral blood stream are the corpus luteum, decidua, and placenta. In non-pregnant females, relaxin is mainly produced by the corpus luteum, 9–10 days after the surge of luteinizing hormone. Other sources of relaxin include endometrial glands, thecal cells of ovarian follicles, and mammary gland cells. There have also been reports of relaxin originating from cardiac atria sources (9). Relaxin has also been found to be produced in the male, the primary source being the prostate gland, though it has not been possible to demonstrate its release into the peripheral blood (6, 9). Additionally, relaxin gene expression has been found in a range of rat tissues using reverse transcriptase-polymerase chain reaction and immunohistochemistry. These rat tissues include the brain, uterus, prostate gland, pancreas, and kidneys. These findings support the notion of proposed paracrine actions of relaxin, which may be important in non-pregnant female and male rats, in addition to pregnant females (10).

With these reports borne in mind, this study sought to demonstrate the presence of the hormone within the sagittal suture of the cranium. The immunohistochemical analyses showed the occurrence of relaxin in the cranial

sagittal suture. The specificity was ascertained by the lack of staining in the negative control samples. The presence of relaxin within the sagittal suture of the mouse cranium is a previously unreported finding. This result corroborates the notion that relaxin may have paracrine activity, since its gene expression has been reported to originate from the brain in the rat. Paracrine hormone activity occurs when hormone molecules secreted by endocrine cells of the brain can act on target cells within the same locale, the sagittal suture, by a simple diffusion through the interstitial fluid. The mandible was not used for immunohistochemistry because the hardness of the tissue (bone and tooth) would have been impossible to section. Furthermore, attempts to decalcify the tissue would have destroyed the antigenicity of the tissue.

The histological evidence in this study exhibits positive effects of relaxin on the connective tissue within the PDL and cranial sutures. The connective tissue fibrils that were once defined and exhibiting a certain directionality became amorphous and random in nature.

These histological findings parallel other investigations in comparable tissues. Sherwood (6) refers to histological findings reported earlier on the pubic symphyses of guinea pigs. They showed that untreated samples displayed collagen fibrils that were dense and organized, while treated samples had collagen fibers that were disrupted, random, and exhibited evidence of collagen digestion. Furthermore, they reported these findings to be evident within 6–8 h following the administration of relaxin. Similar results on collagen organization of the pregnant rat cervix were described by Cheah et al. (11). Relaxin-treated samples had collagen fibrils that were disorganized and dispersed. The cervixes became soft and more extensible.

In two rodent models of induced fibrosis, relaxin was shown to inhibit collagen accumulation in a dose-dependent manner, as measured by hydroxyproline content. Histologically, control samples displayed densely packed, parallel collagen fibrils, whereas treated samples exhibited fibrils that were not parallel, loosely packed, and less abundant (13). This study is also important because it shows that the effects of relaxin may not be and, perhaps, should not be restricted to the reproductive system. Applications of its effects could lie in other areas of medicine and even orthodontics.

The effects of relaxin on enzymatic activities have been investigated. Unemori et al. (13) reported a dose-dependent enhancement of matrix metalloproteinase-1 (procollagenase) expression in lung fibroblasts treated with

relaxin *in vitro*. The same authors in 1989 reported a stimulation of metalloproteinase-1 (procollagenase) expression in dermal fibroblasts in addition to a decrease in collagen synthesis and secretion following treatment with relaxin. In 1996, Qin et al. (15) described a dose-dependent increase in the expression of the metalloproteinase-1 gene, its secreted protein, and its enzyme activity. Similar findings have been reported by Mushayandebvu and Rajabi (14).

In the present study, we sought to determine if there were changes in protease activity and not changes in the amount of proteases as a result of relaxin treatment. The addition of EDTA revealed a decrease in absorbance, indicating that metalloproteinase accounts for a part of protease activities in calvarial and alveolar bones. The difference was roughly 20%, which is consistent with the portion of metalloproteinases among all proteinases in other tissues. This finding corroborates the previous reports. What is uncertain is exactly what type of differences between the treated and control samples should be expected in the tissues examined. With differences ranging from roughly 3 to 9%, is this enough to elicit a significant physiological response, or should a greater difference be expected? Histologically, there is clearly a difference. Can one assume, then, that this differing range of enzyme activity is adequate for altering the connective tissue? Answers to these questions require clinical investigations.

Because this is a novel and exploratory study, certain questions remain unanswered. For example, we measured the response of non-specific protease activity. These findings could be strengthened by the use of specific inhibitors to identify which specific enzymes are responsible for the degradation of the connective tissue within the craniofacial complex and periodontium. The mouse model proved to be adequate for the needs of this study. If mechanical manipulation of the sutures or periodontal ligament is an objective, a larger animal may prove to be more useful as the oral cavity of the mouse is rather small for such manipulation. Perhaps better demonstrative stains could be employed to show the effects of relaxin on the connective tissue of the craniofacial complex and periodontium.

Mechanical perturbation was not included in the experimental protocol as it was not within the scope of the study. Future studies might employ appliances to widen the cranial or maxillary sutures (16) and explore relaxin's ability to enhance sutural separation and stability in non-

growing samples. Furthermore, relaxin's effect on the rate of tooth movement may be investigated along with the tendency for relapse once the tooth is moved or rotated. Gingival tissue invagination during the closure of extraction sites in the presence of this hormone is also worth investigating. Scar tissue formation following frenectomies, which may prevent space closure or cause space to open between teeth, may be reduced with relaxin application. There might be a possible application for relaxin in the subluxation of partially ankylosed teeth to prevent them from becoming ankylosed again. Perhaps the effects of relaxin might aid in reducing the stretching tension of the soft tissue envelope following orthognathic surgery. This may be especially noteworthy for the relapse associated with stretching the palatal mucosa in surgically assisted expansion. This stands to reason as previous studies have found that relaxin increases the extensibility of the cervix in the rat during pregnancy while increasing the solubility of collagen (12, 17). Both tissues contain collagen types I and III (16).

Actual application of relaxin in the clinical setting might occur in a few different ways. Delivery systems might include a transdermal patch impregnated with relaxin that could be placed over the palatal suture or in the vestibule by the zygomatic buttress during expansion. It could be placed over gingival tissue during space closure. Delivery into the PDL could be via injections or the Stabi-Dent delivery system, which is promoted as being less painful.

While it is interesting to think about relaxin's ability to decrease the tension within the connective tissue associated with tooth movement and sutural expansion, it is even more intriguing to think of the notion of an adjunct to increase the tension associated with tooth movement. This might enable the clinician to selectively enhance tooth anchorage or prevent dental tipping associated with maxillary expansion.

Before utilizing relaxin as an adjunct to orthodontic therapy, the side effects on other physiological systems within the body must be fully understood. Relaxin has been reported to have an inhibitory effect on smooth muscle contraction, especially myometrial contractility (6). It is said to do this by decreasing myosin light-chain kinase phosphorylation. This smooth muscle relaxation is why relaxin is also believed to have vasodilatory effects in several organs and tissues. It has been shown to increase coronary blood flow more powerfully than acetylcholine. This relaxin-enhanced increase in coronary blood flow was paralleled with an increase in the production of nitric

oxide, which is also a powerful vasodilatory agent (6). There are studies investigating the association of nitric oxide with tooth movement, and perhaps relaxin might enhance the outcome (18, 19). Relaxin has been shown to inhibit granule exocytosis and histamine release by mast cells and to inhibit platelet activation (6). This implies that relaxin may counteract antigen-induced asthma. Indeed, histological studies have shown a decrease in bronchoconstriction, mast cell degranulation, and an accumulation of inflammatory leukocytes. This may be the explanation as to why some clinical reports discuss an improvement of asthma during pregnancy (9). It is tempting to speculate that this particular aspect of the hormone might even affect orthodontic treatment-induced root resorption.

Could there be a utility for relaxin in dentofacial orthopedics? Based upon the available literature, it is possible. According to Meikle et al. (2), the fibroblasts in naturally growing sutures synthesize type I collagen, whereas under mechanical stress, 20% of the new collagen synthesized was that of type III. Type III collagen was also found to be synthesized in greater amounts than type I following orthopedic expansion. It was noted that type III was not just localized in the suture, but also found in Sharpey's fibers (21). Additionally, Nakagawa et al. (22) have reported that cells expressing a positive signal for type I collagen are evenly distributed along the PDL. Following experimental tooth movement, there is a greater density of cells expressing type I collagen along the tension side than the pressure side. This distribution was seen as early as 12 h following tooth movement.

In a recent report, Redlich et al. (3) summarized the available data on the effect of orthodontic force on collagen, elastin, and collagenase in the gingiva, and its potential association with tooth relapse. Briefly, as the gingiva is stretched or compressed, it responds to the orthodontic force by producing more collagen and elastin while inhibiting collagenase production. These fibroblastic responses are the opposite to those seen in tissues treated with relaxin.

The biological effects of relaxin on the same collagen types have been investigated in other parts of the body. When administered to estrogen-primed rats, there was a decrease in total collagen content ( $68 \pm 6\%$ ), without altering the collagen ratios of types I and II, in the interpubic fibrocartilage. Unemori et al. (13) reported that lung fibroblasts treated with relaxin displayed a decrease

in expression of types I and III procollagen, while an increase in procollagenase was seen in the same cells. Furthermore, relaxin has been reported to promote an increase in the expression of the metalloproteinase gene, its secreted protein, and enzyme activity (15).

There appears to be a potential application for relaxin as an adjunct to orthodontic therapy. Relaxin affects the collagen types synthesized within the craniofacial sutures and periodontium. It will be necessary, however, to study specific collagen types and collagenases to get a full picture of the effects of relaxin. It would also be beneficial to examine relaxin's effects on the network of elastin fibers that make up a small, yet important, portion of the gingival proteins.

The possible clinical applications of relaxin could be in: 1) sutural adaptation following expansion in growing and non-growing patients; 2) enhancement of tooth movement and stability through reorganization of the PDL fibers; 3) decreased scar tissue formation following frenectomies; 4) enhanced movement of partially ankylosed teeth following luxation; 5) reduction in the soft tissue envelope pull following orthognathic surgery; and 6) enhanced gingival remodeling following space closure.

## Abstrakt

Die hier vorgestellte Pilotstudie untersucht die Wirkung des Hormons Relaxin auf das Bindegewebe der kraniofazialen Suturen und der parodontalen Gewebe. Relaxin ist ein Hormon, das von schwangeren Frauen produziert wird und zu einer Relaxation der Symphyse des Schambeins führt, so dass der Geburtskanal während der Entbindung geweitet wird. Außerdem wurde nachgewiesen, dass Relaxin einen Effekt auf andere Körperregionen hat, zum Beispiel auf Ligamente und Bereiche, die Kollagen und fibroblastische Aktivität aufweisen. 21 schweizer Mäuse, die keine Jungen mehr aufzogen, wurden untersucht, um 1. immunhistochemisch Relaxin in den Suturen nachzuweisen, 2. die Effekte von Relaxin auf die Integrität suturenähnlicher Gewebe zu studieren und 3. die Wirkung von Relaxin auf die Proteaseaktivität zu bestimmen. Für 24 Stunden wurden in den Untersuchungsgruppen vollständige Gewebekulturen in Relaxin-Konzentrationen von 250 ng/ml oder 500 ng/ml inkubiert. Die Ergebnisse zeigen, dass Relaxin in kranialen Suturen vorhanden ist. Histologische Untersuchungen zeigten deutliche Veränderungen in der Orientierung der Kollagenfibrillen parodontalen Gewebes: aus einem dichten, streng organisierten Arrangement der Kollagenfibrillen mit einer senkrechten Ausrichtung zwischen Zahn und Knochen wurde durch Relaxin eine lockere, unorganisierte und orientierungslose Faserausrichtung. Ferner führte Relaxin zu einer Erhöhung der Proteaseaktivität. Das natürlich vorkommende Relaxin könnte nutzbringend in der orthodontischen Therapie eingesetzt werden, das es die physikalischen Eigenschaften von Bindegewebe in Suturen, Gingivagewebe und parodontalem Ligament zu ändern vermag. Eine denkbare Indikation kann eine Beschleunigung

nigung der suturalen und der Weichteilanpassung an Gaumennahtweiterungen bei nicht wachsenden Patienten sein, indem die Spannung der chirurgisch gedehnten Weichteile (insbesondere der Gaumenschleimhaut) reduziert wird. Weiterhin kann Relaxin den Umbau des parodontalen Ligaments nach Zahnbewegungen beschleunigen und somit die Stabilität fördern, es kann zu einem schnelleren Umbau der Gingiva im Bereich von Extraktionslücken während des Lückenschlusses führen und die Bildung von Narbengewebe bei Frenektomien reduzieren.

## Abstracto

Este es un reporte de un estudio exploratorio de como la hormona relaxin puede modular la remodelación de los tejidos conectivos entre las suturas craneofaciales y los tejidos periodontales. Se descubrió que la relaxin es una hormona producida por la mujer embarazada. Esta es responsable por el relajamiento de la sínfisis púbica; el canal de parto se ensancha para el alumbramiento. En adición, se ha demostrado que la relaxin tiene efectos en otras áreas del cuerpo incluyendo ligamentos y regiones que contienen colágeno y actividad fibroblástica. Veinte ratones del tipo 'Swiss retired-breeder' fueron utilizados para: 1) demostrar inmunohistoquímicamente la presencia de relaxin entre las suturas, 2) sus efectos en la integridad de los tejidos con apariencia de sutura y 3) evaluar sus efectos en la actividad de 'proteasa'. Se utilizó relaxin en concentraciones de 250 ng/ml y 500 ng/ml en las muestras tratadas y se incubaron en cultivos de tejidos completos por veinticuatro horas. Los resultados indican la presencia de relaxin en la sutura craneal. Observaciones histológicas revelaron cambios definitivos en el arreglo fibril del colágeno en el LPD: desde un denso y sumamente organizado con una dirección perpendicular entre diente y hueso, a uno organizado al azar y suelto, con una falta de dirección entre el diente y el hueso. Un incremento en la actividad de la 'proteasa' fue evidente en las muestras tratadas con relaxin. Esta hormona producida naturalmente puede ser utilizada como un tratamiento complementario a la terapia ortodóntica ya que esta aparenta tener la capacidad de alterar las propiedades físicas de los tejidos conectivos en las suturas, tejido gingival, y en el PDL. Indicaciones potenciales de uso incluyen instancias de adaptación del tejido blando y sutural de expansión ortodóntica en pacientes sin crecimiento por una reducción en la tensión de la envoltura del tejido blando estirado luego de cirugía ortognática (particularmente en la mucosa expandida palatalmente), remodelación del ligamento periodontal durante o luego de movimiento dental promoviendo estabilidad, remodelación rápida del tejido gingival durante el cierre de espacio en los lugares de extracción, y por una disminución en la cantidad de la formación de tejido cicatrizado luego de las frenotomías.

## 抄録

本研究はリラキシンが頭蓋顔面の縫合部における結合組織および歯根膜組織のリモデリングをどのように修飾するのかについての報告である。リラキシンは妊娠している動物で産生されることで知られているホルモンである。このホルモンは、恥骨結合を緩めて、分娩のとき産道を広げる働きをもつ。このほかに、靭帯を含む身体の他の部位およびコラーゲンや線維芽細胞の活性に影響を及ぼすことも示されている。本研究では、20匹のマウス(Swiss retired-breeder)を用いて、縫合部内にリラキシンが存在することを免疫組織学的に証明し、それが縫合部の組織に及ぼす影響、およびプロテアーゼ活性に及ぼす影響を分析した。250 ng/ml および 500 ng/ml の濃度のリラキシンを処理サンプルに用い、24時間の完全組織培養が行われた。その結果、頭蓋縫合部にリラキシンが存在することが示された。組織学的な観察は、PDL におけるコラーゲン線維の配列における明確な変化が明らかとなった。つまり、歯と骨との間の垂直方向に密に高度に組織された状態から、歯と骨との間で方向性が欠如し、ランダムに組織され、疎な状態へと変化した。プロテアーゼ活性の上昇はリラキシンで処理されたサンプルで明白であった。このホルモンは、縫合部、歯肉組織、歯根膜線維中の結合組織の物理特性を変えることができるので矯正歯科治療に応用できるかもしれない。可能な応用法には次のようなものが考えられる。外科的矯正治療後に伸展された軟組織(特に伸展された口蓋粘膜)に生じた張力を減じることにより成長期を過ぎた患者におかる顎骨拡大後の縫合部および軟組織を適応させること、歯の移動中や移動後における歯根膜線維のリモデリングにより安定性を向上させること、抜歯部位の空隙閉鎖中に歯肉組織を急速にリモデリングすること、小帯切除術後に形成される癒痕組織量を減少させることである。

## Structured Abstract

**Authors** – Nicozisis JL, Nah HD, Tuncay OC.

**Objectives** – To demonstrate the presence and effects of relaxin in the sutural tissues and to show the relaxin effects on the proteinase activity.

**Design** – The mouse organ culture model was employed.

**Setting and Samples** – The presence of relaxin in the cranial sutures was demonstrated immunohistochemically. Histological observation of the periodontal ligament was accomplished by hematoxyline staining. The non-specific protease activity was measured with fluorescence polarization analysis.

**Experimental Variable** – Mouse cultures were treated with 250 and 500 ng/ml of relaxin.

**Outcome Measure** – Microscopy for changes in fibril orientation and morphology. Enzyme activities were measured by fluorescence polarization assays.

**Results** – Relaxin was present in the calvarial sutures. It abolished the organization of PDL fibers and increased the protease activity.

**Conclusion** – The hormone has the potential to be used as an adjunct to orthodontic or surgical therapy to promote manipulation of sutural tissues or affecting stability.

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