

Elastic Modulus of Bovine and Human Dentin at the Enamel-Dentin and Dentin-Pulp Junctions: An *In Vitro* Experimental Study *

**Módulo de elasticidad de las dentinas bovina y humana en las uniones amelo-dental y dentino-pulpar.
Estudio experimental in vitro**

**Módulo de elasticidade da dentina bovina e humana nas junções amelodentinária e dentina-polpa: um
estudo experimental in vitro**

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ABSTRACT

Background: The use of bovine dentin in dental research has gained relevance due to its easy availability, lower structural and compositional variability, and morphological similarity to human dentin. **Purpose:** To compare the elastic modulus of human and bovine dentin at the dentinoenamel junction and the dentin-pulp junction. **Methods:** An *in vitro* experimental study was conducted on 30 recently extracted human molars (20–30 years old) and 30 bovine incisors (2–3 years old). The teeth were preserved in 2% chloramine T to maintain moisture. Thirty dentin bars were obtained: 15 from the dentinoenamel junction and 15 from the dentin-pulp junction. Each bar measured 2 mm wide by 1.5 mm thick. The specimens were observed under a stereomicroscope and measured with a digital caliper. The data were entered into the three-point bending test program (Instron® universal testing machine) to calculate the elastic modulus. The test was performed using two instruments designed for the setup: a sample holder and a load application pin ($p = 0.05$). **Results:** In human dentin, the elastic modulus was 8.89 GPa at the dentinoenamel junction and 6.7064 GPa at the dentin-pulp junction. In bovine dentin, it was 9.0078 GPa at the dentinoenamel junction and 6.0388 GPa at the dentin-pulp junction. **Conclusions:** The elastic modulus values in human and bovine dentin, both at the dentinoenamel junction and the dentin-pulp junction, do not suggest significant differences.

Keywords: biophysics; bovine dentin; dental biology; dentin biomechanics; dentinoenamel junction; dentin-pulp junction; dentistry; elastic modulus; experimental research; human dentin

RESUMEN

Antecedentes: El uso de dentina bovina en investigación odontológica ha adquirido relevancia debido a su fácil obtención, su menor variabilidad estructural y composicional, y su similitud morfológica con la dentina humana. **Objetivo:** Comparar el módulo elástico de la dentina humana y bovina en la unión amelo-dental y en la unión dentino-pulpar. **Métodos:** Se realizó un estudio experimental *in vitro* en el que se analizaron 30 molares humanos (20–30 años) y 30 incisivos bovinos (2–3 años), recién extraídos. Los dientes se conservaron en cloramina T al 2% para mantener la humedad. Se obtuvieron 30 barras de dentina: 15 de la unión amelo-dental y 15 de la unión dentino-pulpar. Cada barra midió 2 mm de ancho por 1,5 mm de espesor. Los especímenes se observaron con estereomicroscopio y se midieron con calibrador digital. Los datos se ingresaron al programa del ensayo de flexión en tres puntos (máquina universal de pruebas Instron®) para calcular el módulo elástico. La prueba se realizó con dos instrumentos diseñados para el montaje: un sujetador de la muestra y un pin de aplicación de carga ($p = 0.05$). **Resultados:** En dentina humana, el módulo elástico fue 8,89 GPa en la unión amelo-dental y 6,7064 GPa en la unión dentino-pulpar. En dentina bovina, fue 9,0078 GPa en la unión amelo-dental y 6,0388 GPa en la unión dentino-pulpar. **Conclusiones:** Los valores del módulo elástico en dentina humana y bovina, tanto en la unión amelo-dental como en la unión dentino-pulpar, no sugieren diferencias relevantes.

Palabras clave: biofísica; biología dental; biomecánica dentinal; dentina bovina; dentina humana; investigación experimental; módulo de elasticidad; odontología; unión amelo-dental; unión dentino-pulpar

RESUMO

Antecedentes: O uso de dentina bovina em pesquisas odontológicas tem ganhado relevância devido à facilidade de obtenção, menor variabilidade estrutural e composicional e semelhança morfológica com a dentina humana. **Objetivo:** Comparar o módulo de elasticidade da dentina humana e bovina na junção dentina-esmalte e na junção dentina-polpa. **Métodos:** Foi realizado um estudo experimental *in vitro* no qual 30 molares humanos recém-extraídos (20-30 anos de idade) e 30 incisivos bovinos (2-3 anos de idade) foram analisados. Os dentes foram preservados em cloramina T a 2% para manter a umidade. Trinta barras de dentina foram obtidas: 15 da junção dentina-esmalte e 15 da junção dentina-polpa. Cada barra tem 2 mm de largura por 1,5 mm de espessura. Os espécimes foram observados com um estereomicroscópio e medidos com um paquímetro digital. Os dados foram inseridos no software de teste de flexão em três pontos (máquina de ensaio universal Instron®) para calcular o módulo de elasticidade. O teste foi realizado utilizando dois instrumentos projetados para montagem: um porta-amostras e um pino de aplicação de carga ($p = 0.05$). **Resultados:** Na dentina humana, o módulo de elasticidade foi de 8,89 GPa na junção dentina-esmalte e de 6,7064 GPa na junção dentina-polpa. Na dentina bovina, foi de 9,0078 GPa na junção dentina-esmalte e de 6,0388 GPa na junção dentina-polpa. **Conclusões:** Os valores do módulo de elasticidade na dentina humana e bovina, tanto na junção dentina-esmalte quanto na junção dentina-polpa, não sugerem diferenças significativas.

Palavras-chave: biofísica; biologia dental; biomecânica da dentina; dentina bovina; dentina humana; junção dentina-esmalte; junção dentina-polpa; módulo de elasticidade; odontologia; pesquisa experimental

INTRODUCTION

Understanding the mechanical properties of dentin is essential to explain how the masticatory system distributes forces through the tooth and to predict how stress and strain change with restorative procedures, aging, or different pathologies (1-3). Dentin is the tissue that accounts for most of the tooth volume and lies between enamel and the pulp. Its thickness varies by tooth type, ranging from 1–1.5 mm in mandibular incisors to approximately 3 mm in canines and molars. Throughout life, dentin thickness increases due to the continuous deposition of secondary dentin. It is typically protected by enamel in the crown and by cementum in the root. Its composition is approximately 70% hydroxyapatite crystals, 20% collagenous organic matrix, and 10% water (4-5).

Dentin is the main structural support of the tooth and the substrate to which restorations adhere. Its integrity and elastic modulus directly influence the durability of the restoration–dentin interface. A higher elastic modulus indicates a stiffer material with less deformation under load (5). When restorative materials have an elastic modulus similar to that of dentin, restorations can be biomechanically more stable and exhibit lower stress concentration (6-7).

The use of bovine dentin in dental research has gained relevance due to its easy availability, lower structural and compositional variability, and morphological similarity to human dentin (8-9). Several authors have indicated that bovine dentin can serve as an appropriate experimental model, particularly in studies where access to human teeth is limited (9). Nevertheless, important structural differences exist depending on the anatomical region evaluated, because both human and bovine dentin show significant variations between the enamel–dentin junction and the dentin–pulp junction.

The enamel–dentin junction (EDJ) is the undulating structural boundary between enamel and dentin. Mantle dentin, the first dentin formed, interdigitates with enamel and extends approximately 150 μm toward the pulp. Interglobular dentin is observed next. Beneath these layers lies circumpulpal dentin, with a thickness of 6–8 mm. Mantle dentin exhibits thicker, more organized collagen fibers (0.2–0.4 μm), whereas circumpulpal dentin contains thinner fibers (50–200 nm). These fibers run parallel to the tubules in the crown and perpendicular to them in the root (10). Differences in orientation and mineralization account for mechanical variations across dentin thickness.

Dentin at the dentin–pulp junction (DPJ), in contrast, surrounds the dental pulp and constitutes the largest volume of dentin. Its collagen fibers are thinner and are arranged irregularly, forming a dense meshwork. Its globular mineralization contrasts with the more uniform mineralization of mantle dentin. Between this region and the odontoblasts lies predentin, a non-mineralized layer measuring 20–30 μm . Its matrix, rich in sulfur-containing components, resembles osteoid substance and is essential for continuous dentin formation (11-16).

Differences in tubular density explain variability in dentin hardness and mechanical behavior, according to Craig *et al.* (16), Fusayama and Maeda (7), and Pashley *et al.* (14). These authors report that hardness decreases toward the pulpal region. This change is attributed to increased tubule density and possible variations in mineral content. In turn, Kawamoto *et al.* (1) showed that the relative position between the pulp and the EDJ determines structural variations that contribute to the wide range of values reported for the mechanical properties of dentin tissue. Tubular microstructure, as well as its orientation and distribution, directly influence strength and elastic modulus.

Subsequent studies have reported wide ranges for dentin elastic modulus (17.7–21.1 GPa) (17-18), consistent with historical values such as those reported by Bowan and Rodríguez (1962): 19.3 GPa (wet, tensile), Craig and Peyton (1958): 18.3 GPa (wet, compressive), and dry measurements of up to 16.5 GPa. However, more recent studies have reported considerably lower values, such as 13.7 GPa in Sano *et al.* (1994) and 10.16 GPa in Jameson *et al.* (1993) (8). These discrepancies remain controversial and hinder precise interpretation of dentin biomechanics.

The aim of this study was to assess whether bovine dentin can replace human dentin by measuring and comparing the elastic modulus in two anatomical regions: the enamel–dentin junction and the dentin–pulp junction. Unlike previous studies, this research examines specific regions rather than treating dentin as a homogeneous tissue. This approach is essential because microstructure and tubular density vary substantially across regions. This differentiation enables a better understanding of biomechanical response, improves prediction of restorative behavior, and provides quantitative evidence on the validity of bovine dentin as a biomechanically reliable substitute.

MATERIALS AND METHODS

This study corresponds to an in vitro experimental investigation in which two main factors were evaluated: species (human dentin versus bovine dentin) and dentin region (EDJ versus DPJ). Each factor combination was analyzed independently to determine the effect of species and anatomical location on the elastic modulus.

The study population consisted of sound human third molars, recently extracted from patients aged 20 to 30 years for orthodontic reasons at the Dental Clinics of the Pontificia Universidad Javeriana's Dental School, after patients/caregivers signed informed consent. On the other side, sound incisors from young Holstein cattle, approximately 2 to 3.5 years old, were obtained after slaughter through the livestock trade in the municipality of Mosquera, Cundinamarca, Colombia.

The selection of ages for the human and bovine samples was based on the need to ensure morpho-structural comparability between both species. Fonseca *et al.* (13) showed that human teeth from individuals aged 46 to 80 years exhibit a marked reduction in the number of dentinal tubules and increased sclerosis, which significantly alters their mechanical properties. In contrast, young bovine teeth, approximately 2 to 3 years old, display a tubular pattern more similar to that of young human teeth aged 20 to 30 years, in both tubule density and diameter. Because this structural similarity is crucial for a valid biomechanical comparison of elastic modulus, the present study selected the ages described by these authors. This approach aimed to minimize the influence of age-related dentin changes on the observed differences.

The sample was selected by convenience, based on expert judgment and related literature. Thirty human third molars and 30 young bovine incisors were included. From these teeth, 15 dentin specimens were obtained from the human and bovine EDJ, and 15 dentin specimens were obtained from the DPJ, for a total of 60 specimens. Human third molars with fractures, caries, or restorations were excluded, as were bovine incisors from animals older than 4 years or with severe wear, caries, or fractures.

Before conducting the test, a pilot trial was performed to assess the behavior of the dentin specimens in the Instron® device. The performance of the digital caliper was also verified. In addition, the functionality of the specimen holder and the load-application pin designed for this study was confirmed.

Extractions in cattle slaughtered for purposes other than this research were performed while considering key anatomic features of the incisors. As a group, these teeth are inclined forward and are not firmly seated in their sockets. In addition, because there are no incisors in the maxilla, they have slight mobility to avoid injuring the mucosa of the dental pad. This mobility is also facilitated by the beveled configuration of the lingual surface. It was further considered that the root is longer than the crown, with an approximate 1.5:1 ratio, especially in worn teeth. The approximate root length was 26.5 mm from the cervical region to the apex. The mesiodistal dimension was approximately 9 mm in the coronal third, 6.5 mm in the middle third, and 4 mm in the apical third. The buccolingual thickness was about 7 mm at its widest area. Extractions were performed using surgical instruments. Each incisor was removed as follows:

- Incisor syndesmotomy was performed using a periosteotome.

- Levering movements were performed using straight and flag elevators.
- Finally, the incisors were extracted using forceps.

All freshly extracted specimens were stored in 2% chloramine T and refrigerated at 3 °C for a maximum of 6 months. Soft tissue was curetted from the 60 extracted teeth (human and bovine) to achieve debridement. The teeth were then stored separately in 2% chloramine T to maintain adequate moisture conditions. Each sample was randomly labeled with a number from 1 to 30.

The samples were placed in 12 × 12 mm silicone molds. The molds were filled with self-curing acrylic resin up to 3 mm below the cement-enamel junction. Exothermic heat was controlled by immersing the molds in cold water during polymerization. This base provided support for the samples and kept them stable during testing. Twenty-four hours before sectioning, the teeth were removed from chloramine T and decontaminated with distilled water.

Horizontal cuts were made on the incisal or occlusal surface until the EDJ was reached. Two longitudinal cuts were then performed in dentin, 2 mm apart, to a depth of 1.5 mm. Next, a horizontal cut perpendicular to the previous cuts was made to obtain an EDJ dentin bar measuring 2 mm in width (buccolingual) and 1.5 mm in thickness (occluso-gingival). The same procedure was applied to obtain DPJ bars. All cuts were performed using a microtome for organic specimens with a 320 μm -thick diamond blade, at 500 rpm, and cooled with distilled water. Human and bovine dentin bars were obtained from both the EDJ (superficial dentin) and the DPJ (deep dentin), with a thickness of 1.5 mm in the occluso-gingival direction and a width of 2 mm in the buccopalatal or buccolingual direction (Figure 1A).

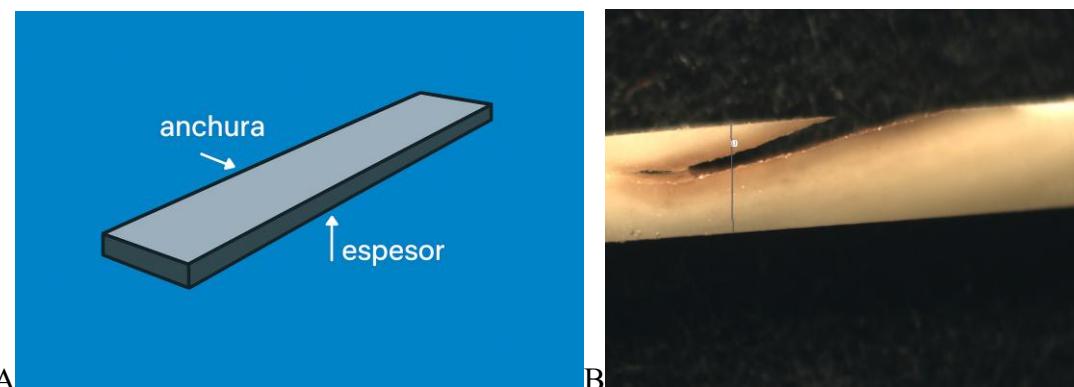


FIGURE 1

A. Specimen measurement parameters. B. Specimen crack observed under the stereomicroscope.

Once the 60 samples were obtained, they were examined under a stereomicroscope to standardize the specimens and identify irregularities in the bars, such as fractures or cracks (Figure 1B). Samples with irregularities were discarded. Subsequently, they were stored until the test day in a hygrometer at 37 °C and 60–70% relative humidity.

Subsequently, the exact dimensions of the bars were verified. Although they were obtained using a microtome, their size could have varied. Therefore, the actual measurements were entered into the software used to calculate the elastic modulus from the Instron® three-point bending test (Figure 2). This verification was performed with a digital caliper.

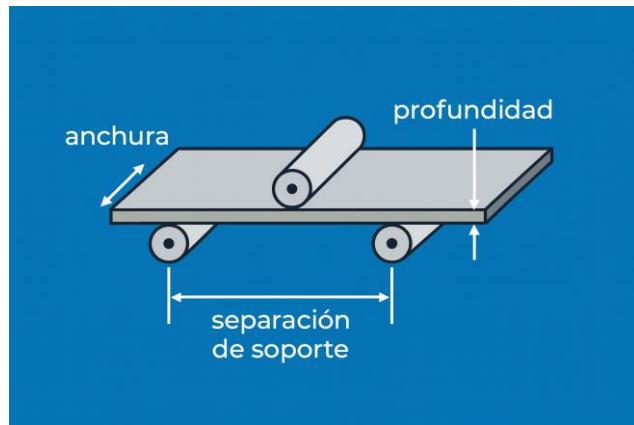


FIGURE 2
Three-point bending test using the Instron system

To perform the test, two custom instruments made of tempered stainless steel were designed: one to hold the specimen (Figures 3 and 5A) and another corresponding to the load-application pin (Figures 4 and 5B).

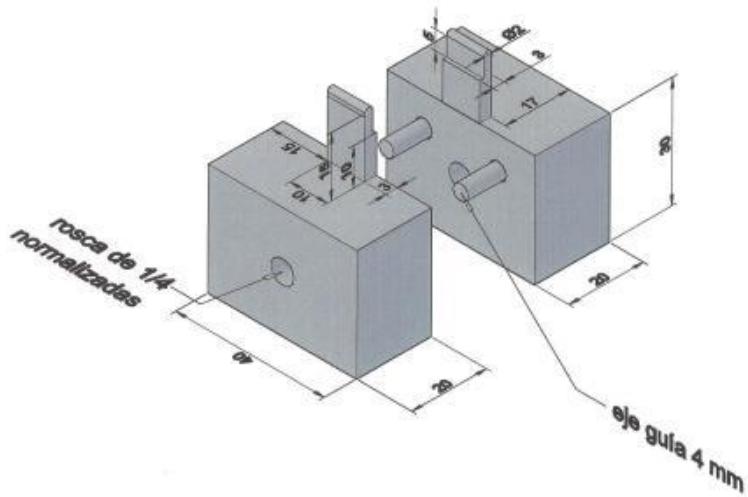


FIGURE 3
Specimen holding device

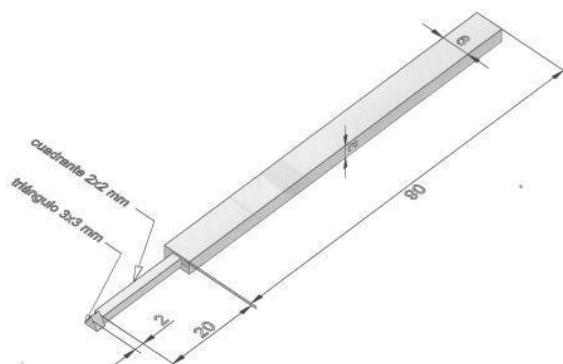


FIGURE 4
Load-application pin

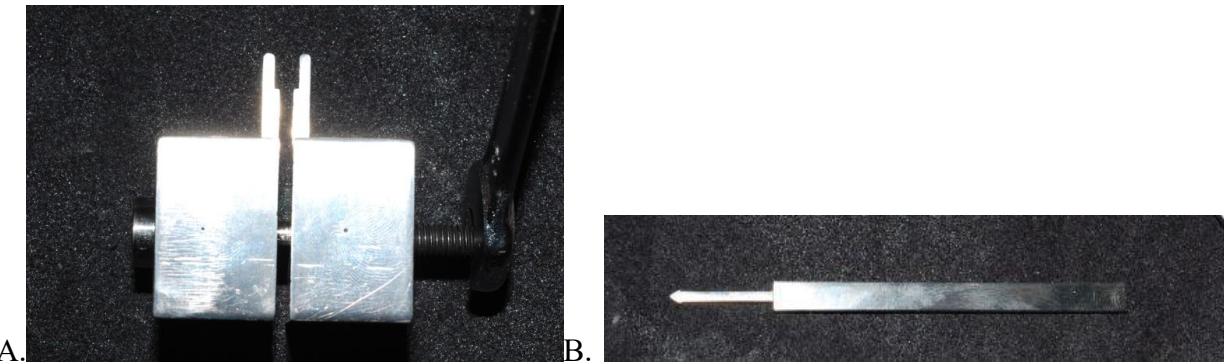


FIGURE 5
A. Specimen holding device. B. Load-application pin

The specimen holding device (Figure 5A) was designed to allow expansion of the two support semicircles from 4 mm. This enabled adaptation to the specimen with the shortest mesiodistal length of the dentin bar.

Among all specimens, the dentin bar with the shortest mesiodistal length was selected. Using this specimen as a reference, the holding device was expanded until the ridge of the two supporting semicircles was entirely positioned within the dentin area of the bar. The ridges of both semicircles were then marked with a moistened red pencil. Next, the smallest bar was placed on the supports and pressed to transfer the location of the two support points onto the bar. The distance between these marks was measured with a digital caliper. This measurement was used to define the exact opening of the holder. The smallest specimen was used to ensure that all remaining bars would be supported by dentin within the calculated opening.

After confirming the holder expansion, the elastic modulus was measured using with an Instron®. A custom load-application pin designed for this study (Figure 5B) was attached to the device using a clamp. The pin applied force to the specimen at a constant rate of 0.5 mm/min. A load cell recorded the applied force at each time point. These data were used to obtain the dentin mechanical behavior curves (Figure 6).



FIGURE 6
Actual three-point bending test

Results were analyzed using descriptive statistics, including absolute and relative frequencies, measures of central tendency (mode, median, and arithmetic mean), and measures of dispersion (range, variance, and standard deviation). In addition, the Shapiro-Wilk test was applied to assess data normality. To examine associations between variables, the t-test was used ($p = 0.05$).

RESULTS

A total of 60 measurements were performed: 30 in bovine specimens and 30 in human specimens. The measurements were then grouped by dentin region into two groups of 15 samples each: DPJ and EDJ. The following codes were used to refer to each group: bovine DPJ (updpbov), bovine EDJ (uaddbov), human DPJ (updpdhum), and human EPD (uaddhum). Table 1 presents the mean values, standard deviations, and the minimum and maximum values for each evaluated tissue. In both tissues, the data show a homogeneous distribution, with mean and median values that are close, suggesting stability in the mechanical behavior of the samples. In addition, the maximum and minimum values show similar ranges across species, indicating the absence of extreme outliers that could bias the interpretation of the results.

TABLE 1
Descriptive measures of elastic modulus (GPa)

Code	n	Average	SD	Minimum	Maximum
Updpbov	15	6.0388	1.202624	3.556	7.913
Uaddbov	15	9.0078	1.242208	7.058	11.254
Updpdhum	15	6.7064	1.810700	3.109	8.877
Uaddhum	15	8.8900	1.487575	6.868	11.083

Figure 7 allows a comparative visualization of elastic modulus behavior between human and bovine dentin. It shows that, in both the EDJ and the DPJ, the mean values for both species are close. This indicates similar stiffness patterns at the two analyzed depths. This similarity is consistent with the descriptive statistics, which do not show differences between groups. Overall, the data suggest that bovine dentin exhibits mechanical behavior comparable to human dentin under the evaluated experimental conditions.

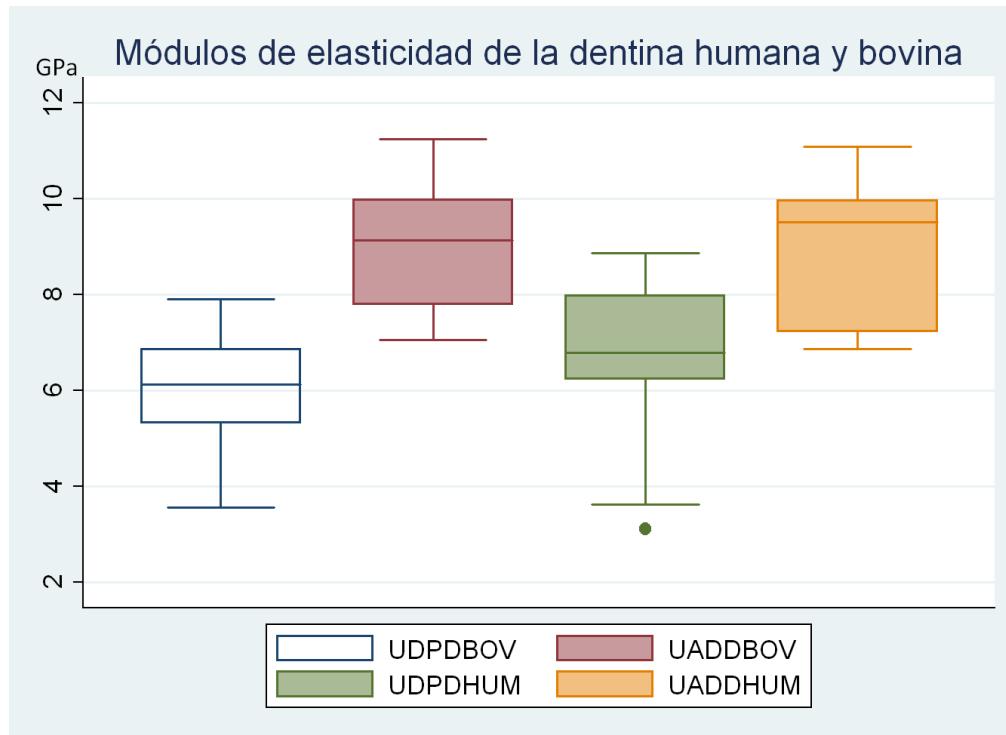


FIGURE 7
Elastic modulus of human and bovine dentin at the EDJ and the DPJ

The analysis through the Shapiro-Wilk test indicated that all groups followed a normal distribution. Based on this, the t-test was used to examine the association between variables ($p = 0.05$). The findings suggest that there are no differences in elastic modulus between bovine and human dentin, either at the EDJ or the DPJ.

DISCUSSION

Overall, recent findings comparing human and bovine dentin agree that, although both share a similar tubular architecture, relevant differences exist in parameters such as microhardness, mineralization, and elastic modulus. These differences may influence mechanical response under functional and restorative loads (17-28). Contemporary studies have emphasized the need to characterize these tissues more precisely to improve the predictability of experimental models and biomechanical simulations, particularly when bovine dentin is used as a substitute for ethical and availability reasons (27,28). However, important gaps remain regarding how these structural differences affect stress transmission, force distribution, and dentin behavior in the context of aging and clinical intervention. In this context, the present study provides original evidence by comparing the mechanical properties of both tissues within a standardized methodological framework. It thus offers information that may improve understanding of dentin performance under masticatory conditions and contribute to the development of more reliable models for dental research.

Over the past fifty years, a wide dispersion has been reported in dentin elastic modulus values, which has hindered the establishment of a representative range and the exclusion of invalid measurements. Kinney *et al.* identified more than 31 distinct measurements between 1950 and 2000, with values ranging from 8 to 31 GPa (19). Florian *et al.* indicated that the 17.7–21.1 GPa range is consistent with previous reports, such as those by Bowan and Rodríguez (19.3 GPa) and Craig and Payton (18.3 GPa) (20).

However, more recent studies have reported lower values, such as 13.7 GPa in Sano *et al.* (1994) and 10.16 GPa in Jameson *et al.* (1993) (9). These figures are close to those found in the present study (8.89 GPa). Nakamichi *et al.* (12) noted that historical discrepancies may be due to inadequate storage conditions. Specifically, teeth stored for prolonged periods may exhibit tubule opening due to odontoblastic degeneration, which increases experimental error.

Authors such as Fusayama and Maeda (7), and Pashley and Tay (14), Craig and Peyton (16), have shown that dentin stiffness varies according to tooth location and tubular density. In this study, dentin from the enamel–dentin junction exhibited a higher elastic modulus (8.89 GPa) than dentin from the dentin–pulp junction (6.70 GPa). This pattern aligns with the literature because tubule density increases toward the pulp, which reduces tissue stiffness. Kawamoto *et al.* (1) also linked these variations to the structural gradient between the EDJ and the DPJ.

Another study correlated increased tubular density with decreased dentin hardness. This relationship was linked to variations in mineralization and in the proportion between peritubular and intertubular dentin (22). Although the present study did not assess hardness, the obtained values are consistent with this expected behavior. In deeper regions, where tubular density is higher, a lower elastic modulus was observed. Likewise, although the present study did not measure dentin hardness—a property closely related to tubular density and elastic modulus (23)—this assessment should be incorporated into future studies. Joint measurement of hardness and elastic modulus would allow a better understanding of microstructural influences on mechanical behavior and help reduce the variability reported in the literature. Tanaka *et al.* (28) noted that ethical and logistical difficulties in obtaining human teeth have encouraged the use of animal teeth. In turn, Sánchez *et al.* (4) described relevant histological similarities between human and bovine dentin, including a type I collagen-based composition, an “S”-shaped tubular trajectory, and organization into primary, secondary, and tertiary dentin. However, they also identified differences, such as the absence of interglobular dentin and greater tubular irregularity in bovine teeth. These authors reported comparable tubule diameter and density values between species, consistent with

the findings of this study. Fonseca *et al.*, (13) in turn, showed that age influences mechanical properties. Specifically, young bovine teeth (2–3 years) are more similar to young human teeth (20–30 years), whereas human teeth from individuals aged 46 to 80 years show lower tubule density. This criterion was considered when selecting the samples for the present study.

Recent studies agree that, although human and bovine dentin share architectural similarities, significant differences have also been reported in microhardness, mineralization, and elastic modulus (27,28). This underscores the need to characterize these tissues precisely to strengthen the validity of experimental models, particularly when bovine dentin is used as a substitute. The present study contributes to this line of research by comparing equivalent regions using a homogeneous and standardized protocol. The few differences observed between human and bovine dentin suggest comparable mechanical behavior under bending. These findings support the potential use of bovine dentin in experimental assays.

CONCLUSIONS

The elastic modulus, when comparing human and bovine dentin at the EDJ and the DPJ levels, as well as between the EDJ and the DPJ where similar. Therefore, bovine dentin may be used as a substitute for human dentin in future studies on restorative material behavior and analyses of the restoration–dentin interface.

RECOMMENDATIONS

Based on the results of this study and the ethical and legal implications of working with human teeth, it is recommended to evaluate other bovine dental tissues, such as enamel and radicular dentin. This would allow a more reliable determination of whether bovine teeth can replace human teeth as a research model.

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