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Geometric Morphometric Evaluation of the Effect of Collagen on the Musculi Lumbales Profundus in Experimentally Induced Diabetic Rats

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In the study, the effect of collagen on the musculi lumbales profundi of 16 diabetic male rats was evaluated. The specimens were photographed from the top, with ten landmarks marked on each image. Statistical analyses, including principal component analysis (PCA) and discriminant function analysis (DFA), were conducted using MorphoJ software. The analysis yielded 15 principal components (PCs), the first three of which explained 72.663% of the total shape variance. The LM4 landmark was observed to be located more laterally in the lollipop and line plots obtained from the discriminant function analysis in diabetic rats compared to diabetic+collagen rats. The LM5, LM6 and LM7 landmarks were positioned more cranially in the diabetic rats than in the diabetic+collagen group. Conversely, LM8 was situated more caudally in the diabetic group than in the diabetic+collagen group. The other landmarks were found to be in close proximity to each other. In the PCA plot, distinct separation between the groups was evident based on collagen administration. The PCA indicated that the collagen administration affected the morphological changes in the muscle. According to the cross-validation scores, eight diabetic and eight diabetic+collagen subjects were correctly classified into their respective groups. In conclusion, evaluation of all the data revealed that, despite the detrimental effect of diabetes on muscle mass, collagen supplementation increased muscle mass.

Key Words: *Anatomy, collagen, geometric morphometry, musculi lumbales profundi, rat*

Deneysel Diyabet Oluşturulmuş Ratlarda Kolajenin Musculi Lumbales Profundi Üzerine Etkisinin Geometrik Morfometrik Değerlendirmesi

Çalışmada, kolajenin; 16 diyabetik erkek sıçanın musculi lumbales profundi (derin bel kasları) üzerindeki etkisi değerlendirilmiştir. Örnekler üstten fotoğraflanmış ve her görüntü üzerinde on adet işaretleme noktası (landmark) belirlenmiştir. Temel bileşenler analizi (PCA) ve ayırıcı (diskriminant) fonksiyon analizi (DFA) dahil olmak üzere istatistiksel analizler, MorphoJ yazılımı kullanılarak gerçekleştirilmiştir. Analiz sonucunda 15 temel bileşen (PC) elde edilmiş; bunlardan ilk üçü toplam şekil varyansının %72.663'ünü açıklamıştır. Diyabetik sıçanlarda, ayırıcı fonksiyon analizinden elde edilen lolipop ve çizgi grafiklerinde LM4 işaretleme noktasının, diyabetik+kolajen grubuna kıyasla daha lateralde yer aldığı gözlemlenmiştir. LM5, LM6 ve LM7 işaretleme noktaları, diyabetik sıçanlarda diyabetik+kolajen grubuna göre daha kraniyalde konumlanmıştır. Aksine, LM8 noktası diyabetik grupta diyabetik+kolajen grubuna göre daha kaudalde yer almıştır. Diğer işaretleme noktalarının ise birbirine yakın olduğu tespit edilmiştir. PCA grafiğinde, kolajen uygulamasına bağlı olarak gruplar arasında belirgin bir ayrım olduğu görülmüştür. PCA, kolajen uygulamasının kastaki morfolojik değişiklikleri etkilediğini göstermiştir. Çapraz doğrulama (cross-validation) skorlarına göre, 8 diyabetik ve 8 diyabetik+kolajen deneği kendi gruplarına doğru şekilde sınıflandırılmıştır. Sonuç olarak, tüm verilerin değerlendirilmesi; diyabetin kas kütlesi üzerindeki zararlı etkisine rağmen, kolajen takviyesinin kas kütlesini artırdığını ortaya koymuştur.

Anahtar Kelimeler: *Anatomi, geometrik morfometri, kolajen, musculi lumbales profundi, rat*

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Introduction

Laboratory animals are used as models in many contemporary experimental and biomedical research projects, including metabolic and immunological studies, tumour and cancer research, and anatomical, physiological and biochemical examinations, as well as transplantation procedures. The frequent use of laboratory mammals as animal models in veterinary and human research highlights the importance of keeping up to date with anatomical variations. Comprehensive knowledge of these variations is also crucial for experimental research and surgical procedures (1-3).

The musculi lumbales profundi are a group of deep muscles located in the lumbar region of the spine. They play a crucial role in spinal stability, fine locomotor control, postural regulation and segmental movement coordination (4).

Diabetes mellitus (DM) is a complex metabolic disease resulting from impaired insulin release (5, 6). As DM continues to affect people, studies are being conducted in various animal models to investigate its pathogenic mechanisms and potential treatments (1).

Collagen is a protein that provides structural support for connective tissues, such as skin, bones, tendons and ligaments (7). It is composed of three polypeptide chains that form a triple helix, which contributes to the structure of the extracellular matrix. Collagen peptides (CPs) are produced by the action of proteases on gelatin (thermally denatured collagen) (8). Following intestinal digestion of orally administered CP, di- and tripeptides, particularly prolyl-hydroxyproline (Pro-Hyp), as well as amino acids, accumulate in human peripheral blood and persist in the body (9). Collagen constitutes over 90% of the skin's mass and ensures its mechanical integrity. As people age, collagen synthesis decreases by 1–1.5% annually (10).

The concept of morphometrics emerged in the scientific literature in the 1960s, with studies statistically analysing intra- and inter-group morphological variations (11). Traditional morphometrics quantify shape using linear measurements, such as length and width, as well as numerical ratios and angular assessments (12). Over time, the limitations of these methods led to the development of geometric morphometrics (13, 14).

Geometric morphometrics is the analysis of geometric information based on Cartesian coordinates (15, 16). In such studies, photographs are taken of the shapes to be measured (17). Geometric morphometric analyses are founded on reference points known as 'landmarks', which are used to quantitatively describe shape and reveal structural differences (18). The standardisation of these landmarks is of considerable importance. This is achieved using a method called Generalised Procrustes Analysis (GPA) which eliminates non-shape variation, such as differences in position, orientation and scale (19). Consequently, the coordinates are realigned, standardising the shape variables across different populations or samples. Following Procrustes analysis, principal components analysis (PCA) is performed on the resulting coordinate data. This procedure determines the mean shape of the individuals and the extent of their morphological differentiation. Geometric morphometrics involves plotting homologous anatomical points, or landmarks, on the shape under investigation. Subsequent analysis of the resulting shape reveals intra-group and inter-group variation (20).

This study aimed to compare the effects of collagen on the musculi lumbales profundi in male Wistar albino rats with experimental diabetes, using geometric morphometrics. The increasing use of collagen today has raised questions about its benefits and risks. The information obtained from this study, which compares the effects of collagen on the muscular system in diabetes, is expected to provide valuable preliminary data for this and similar scientific studies. This research will also inform future anatomical and morphometric studies.

Materials and Methods

Research and Publication Ethics: This study was approved by the Van Yuzuncu Yıl University Animal Experiments Local Ethics Committee (dated 07.11.2025) (Approval no: E-27552122-604.01-783711). Local ethical guidelines have been followed.

A total of 16 adult male Wistar albino rats, aged 8–12 weeks, were used in the study. The animals were housed in an environment with a room temperature of 23 ± 1°C and a 12 h light–dark cycle. The animals had ad libitum access to drinking water and standard rat feed. Following a two-week acclimatisation period to allow the animals to adapt to their new surroundings, the experimental groups were formed and the application phase of the study commenced.

The experimental diabetes model was induced via a single intraperitoneal injection of 55 mg/kg of streptozotocin (STZ), which was dissolved in a 0.1 M citrate buffer solution with a pH of 4.5. Seventy-two h after the administration of STZ, blood glucose levels were measured using a glucometer on blood samples obtained from the tail veins of the rats. Rats with blood glucose levels exceeding 200 mg/dL were considered diabetic.

Experimental Design: The groups were established as follows:

Group 1: Diabetic group; no treatment was administered.

Group 2: Collagen-treated diabetic group; 600 mg/kg of collagen peptide was administered via gastric gavage for 28 days.

Surgical Procedure: The rats used in this study were anaesthetised with a mixture of ketamine (Ketasol 10% injectable, 50 mg/kg intraperitoneally) and xylazine hydrochloride (Alfazyne 2% injectable, 10 mg/kg intraperitoneally).

Following the 28-day treatment period, the animals that had reached the desired level of maturity were anaesthetised with ketamine and xylazine. A midline incision was made in the thoracic wall and the rats were euthanised by exsanguination, in accordance with ethical guidelines.

The rats' abdominal regions were dissected to expose the superior position of the musculi lumbales profundi. Photographs were taken of the musculi lumbales profundi of each rat from a fixed distance and at an identical angle. These images were saved as JPEG files, each coded according to the animal's identification number. These data were subsequently compiled into a TPS file. For the landmark analysis, landmarks were plotted in two stages using the TPS software suite. First, the photographs were prepared for the second stage (tpsDig2) using tpsUtil (version 1.78) (21). The tpsDig2 program was then used to digitise 10 homologous landmarks on the apex, base, lateral and medial surfaces of the musculi lumbales profundi in both groups (Figure 1). The muscle tissues of the animals were recorded for analysis using the geometric morphometric method.

Geometric Morphometric Analysis: In this study, geometric morphometric analysis was performed using MorphoJ software to enable comparisons between groups (22). The numerical x and y coordinate data calculated for the marked landmarks were imported into MorphoJ. These data were then superimposed using the Procrustes analysis test (15). The diabetic groups were then defined within MorphoJ based on the collagen factor and Principal Component Analysis (PCA) was conducted to identify shape deformations (23). Furthermore, discriminant function analysis (DFA) was performed to determine shape differences between the groups (19).

The effect of collagen on the lumbalis profundus muscles was evaluated using geometric morphometrics in 16 Wistar albino rats, which were divided into two groups: one with diabetes and one with diabetes and collagen.

Statistical Analysis*:* A power analysis was performed to determine the sample size for this study, which aimed to conduct a geometric morphometric evaluation of the effects of collagen on the musculi lumbales profundus in rats with experimentally induced diabetes. The sample size for this study (n1=8 ve n2=8) was determined using G*Power software (version 3.1.9.6, Germany). A post-hoc power analysis was conducted within a t-test experimental design with a Type I error rate of 0.05 and an effect size of 1.4, yielding a post-hoc power of 85%. The power analysis indicated that the number of rats used in the study was statistically sufficient. The statistical significance level was set at (α) 5%, and all calculations were performed using MorphoJ (version 1.08.02).

Results

The results of the principal component analysis (PCA) performed on the superior images of the lumbales profundus muscle are presented in Table 1. A total of 15 principal components (PCs) were calculated. The percentage variance values for PCs 1, 2 and 3 were found to be 41.182%, 19.420% and 12.061% respectively. The variance values of the first three PCs were significantly higher than those of the remaining PCs, accounting for 72.663% of the total shape variation. The remaining 12 PCs in the analysis explained the remaining 27.337% of the variation. The respective eigenvalues were found to be 0.00228379, 0.00107693, and 0.00066884 for PCs 1, 2, and 3 (Table 1).

The lollipop graph is a common visualisation technique used in geometric morphometric analysis. It depicts the direction and magnitude of deviation from the mean shape for PC1(Figure 2), which accounts for 41.182% of total shape variation. Examining the displacements reveals that points P3, P6 and P9 exhibited the most significant shifts (Figure 3-A). In contrast, the graph showing the same shape variation in a linear manner uses outline shapes formed by connecting the landmarks (Figure 3-B).

Table 1. Principal component analysis (PCA) of the lumbales profundus muscle

PC	Eigenvalues	% Variance
1	0.00228379	41.182
2	0.00107693	19.420
3	0.00066884	12.061
4	0.00049415	8.911
5	0.00036016	6.495
6	0.00027213	4.907
7	0.00015328	2.764
8	0.00009170	1.654
9	0.00005892	1.062
10	0.00005102	0.920
11	0.00001755	0.316
12	0.00000844	0.152
13	0.00000629	0.113
14	0.00000226	0.041
15	0.00000009	0.002

Figure 1. Marking of the landmark on the musculi lumbales profundus

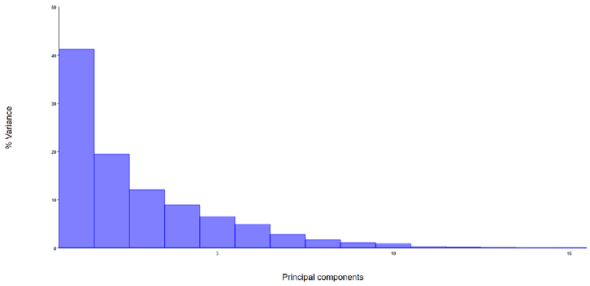

Figure 2. Principal component analysis (PCA) variation percentages on images of the lumbales profundus muscle

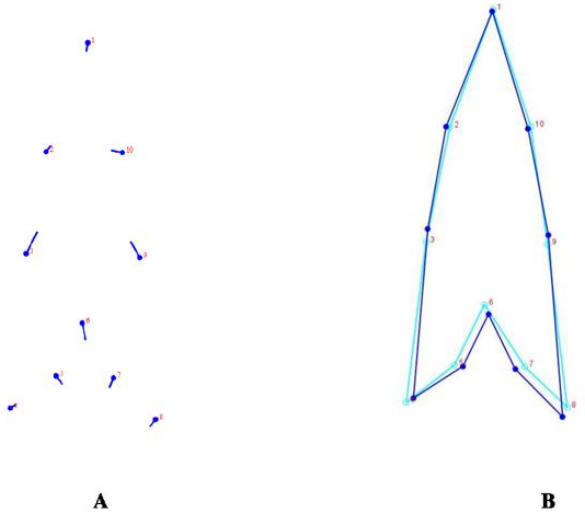

Figure 3. Lollipop (A) and wireframe (B) plots for principal component 1 (PC1), based on images of the musculi profundi lumbales. A- The points represent the mean shape and the lines indicate variation, B- The light green areas denote the mean shape and the blue areas represent variation

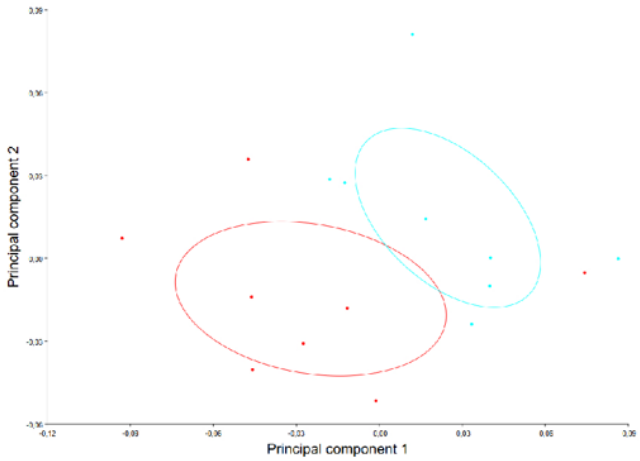

Figure 4. Scatter plot of Principal Component 1 (PC1) versus Principal Component 2 (PC2), based on images of the muscles of the lumbar region. The red dots represent the diabetic rats and the blue dots represent the rats with diabetes and collagen

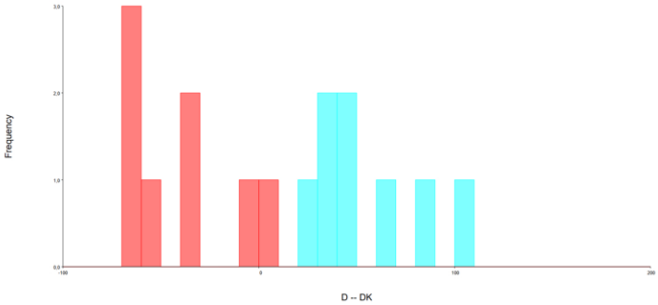

Figure 5. Discriminant function cross-validation plot of images of the musculi profundi lumbales

Since Principal Components 1 and 2 together account for 60.602% of the total variation, combining their analyses provides a clearer visualisation of the distribution of rats in the diabetes and diabetes+collagen groups. Red dots represent rats in the diabetes group and blue dots represent rats in the diabetes+collagen group (Figure 4). Regarding the 95% confidence ellipses, five rats from the diabetes group and five rats from the diabetes+collagen group were located outside the boundary. Certain values demonstrated a distinct separation with collagen treatment in diabetic rats, and no overlapping values were observed between the ellipses.

Discriminant Function Analysis is one of the comparative analyses available in the MorphoJ software package. Procrustes and Mahalanobis distances were calculated between the diabetes and diabetes+collagen groups, yielding values of 0.062 and 11.678, respectively. Permutation tests (1.000 permutations) yielded a *p*-value of>0.001 for the Procrustes distance. Cross-validation produced a classification table indicating that seven out of eight diabetic rats and all eight rats in the diabetes+collagen group were correctly classified. Consequently, 87.5% of the diabetic rats and 100% of the diabetes+collagen rats were successfully assigned to their respective groups (Figure 5). The cross-validation results are presented in Figure 5.

Analysis of the lollipop and wireframe graphs derived from discriminant function analysis revealed that landmark 4 (LM4) was situated more laterally in diabetic rats than in diabetic rats given collagen. Landmarks LM5, LM6 and LM7 were found to be in a more cranial position relative to the diabetic+collagen group. Conversely, LM8 was found to be located more caudally in the diabetic group than in the diabetic+collagen group. The remaining landmarks were found to be in close proximity to one another (Figure 6-A, B).

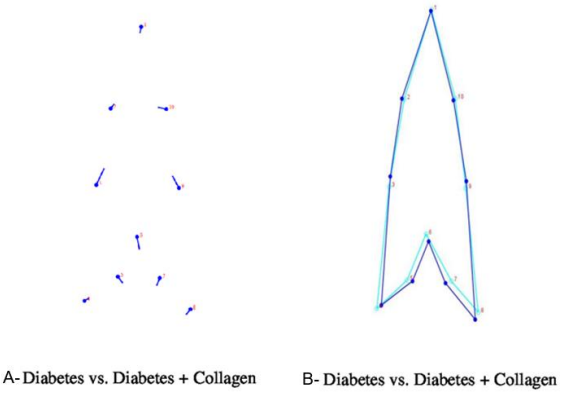

Figure 6. Lollipop and line plots based on the results of a discriminant function analysis of images of the musculi profundi lumbales. A- Dots represent diabetic rats and lines represent diabetic rats with collagen, B- Light blue areas indicate diabetic rats and blue areas indicate diabetic rats with collagen

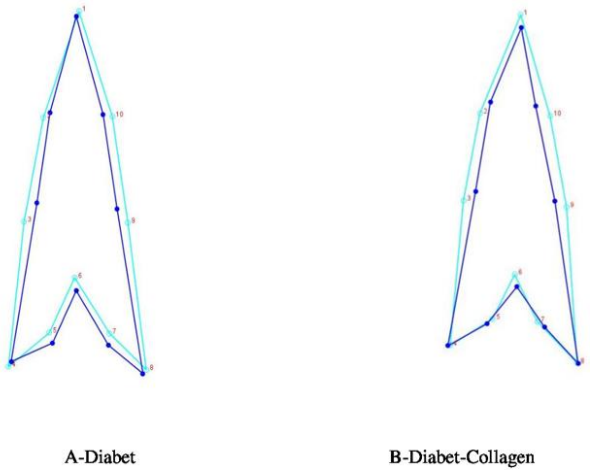

Figure 7. Line plots showing the mean and variation in the diabetes and diabetes + collagen groups, based on images of the musculi profundi lumbales. A-In the diabetes group, light blue represents the mean shape and dark blue represents the variation, B- In the diabetes + collagen group, light blue represents the mean shape and dark blue represents the variation

Comparisons of size and shape between the diabetes and diabetes+collagen groups were conducted using MorphoJ software and analysed via ANOVA. The results indicated that there were no statistically significant size differences between the two groups. However, the difference in shape between the two groups was found to be statistically significant (p<0.001). The shape means and variations within the two groups are presented in the wireframe graph below (Figure 7-A, B). In the graph, the light blue lines represent the mean shape of each group, while the dark blue lines show the variation within each group.

Discussion

A review of the current literature reveals that extensive research has been conducted on animal bones using geometric morphometric analyses (24-31). However, no studies investigating soft tissues and organs have been found. The present study investigated the effect of collagen administration on the musculi profundi lumbales in diabetic Wistar albino rats. Lollipop and wireframe graphs derived from discriminant function analysis revealed that Landmark 4 (LM4) was positioned more laterally in diabetic rats than in the diabetes+collagen group, while LM5, LM6 and LM7 were located more cranially in the diabetes+collagen group. Furthermore, LM8 was found to be positioned more caudally in the diabetic group than in the diabetes+collagen group. The remaining landmarks were also found to be in close proximity to one another. These findings suggest that collagen administration increases muscle mass in the muscle groups compared to the diabetic groups.

In a review of the literature, Bischof et al. (32) conducted a systematic review and meta-analysis of randomised controlled trials (RCTs) investigating the effects of long-term daily collagen peptide (CP) supplementation on strength, muscle-tendon compliance, functional recovery and body composition in healthy adults with and without concurrent exercise interventions over several weeks. Their findings suggest that long-term collagen intake can significantly increase fat-free mass (FFM) and improve tendon morphology, muscle architecture, maximum strength and reactive strength recovery following multiple sessions of muscle-lengthening exercises.

McKendry et al. (33) investigated the effect of adding high-quality protein supplements to a weight-maintenance diet versus adding lower-quality protein supplements to a diet that met the recommended daily allowance on integrated muscle protein synthesis rates. They found that, compared to dietary protein guideline conditions, supplementation with whey and pea protein increased integrated muscle protein synthesis, but no such increase was observed with the administration of an additional 50 g of collagen protein per day, as synthesis rates remained unchanged. These results demonstrate that high-quality protein supplementation is more effective than collagen supplementation.

Aussieker et al. (34) suggested that collagen protein could stimulate muscle connective protein synthesis effectively. In their study, they evaluated the ability of whey and collagen proteins to stimulate myofibrillar and muscle connective protein synthesis rates after exercise. Whey protein ingestion was found to increase myofibrillar protein synthesis rates during post-exercise recovery. However, neither collagen nor whey protein ingestion further increased muscle connective protein synthesis rates during the early stages of post-exercise recovery in male and female recreational athletes.

Sugimoto et al. (35) reviewed the existing literature on the effects of diabetes on muscle mass. The study examined the correlation between variations in glycemic control and sarcopenia, as well as the potential protective role of antidiabetic agents in patients with type 2 diabetes. The results showed that, although diabetes has a negative effect on muscle mass, a substantial increase in muscle mass and gait speed was seen in the subgroup that reduced their HbA1c levels by 1% or more. Furthermore, improvements in poor glycemic control, insulin administration and exercise habits were identified as significant correlates of increased skeletal muscle mass and gait speed.

In the literature review, the study by Stansfield et al. (36) is noteworthy because it presents a geometric morphometric analysis integrating soft tissue and bone structure. The study investigated the morphological characteristics of the soft tissue and bony pelvis in relation to predictive risk factors for the development of pelvic organ prolapse (POP) in adult women. The findings suggest that geometric morphometrics yield more comprehensive results than manual measurements.

However, studies involving superficial soft tissues, such as those of the nose and ears, have also been conducted (14, 37-39).

The present study examined the geometric morphometric characteristics of the musculi lumbales profundi in rats. There are certain limitations to our study: comparisons with other rat strains could be made, and differences between the sexes could be investigated by including female rats.

In conclusion, collagen supplementation was found to significantly increase muscle mass in the rats with induced-diabetes. This study is unique in the field as it is the first to comprehensively demonstrate the effects of widely consumed collagen supplementation on muscle mass using geometric morphometric analysis. Furthermore, the study highlights the effectiveness of geometric morphometrics in elucidating shape variations in muscle mass in the context of collagen supplementation research.

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