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Retrospective Comparative Study

INFLUENCE OF EARLY *VS* LATE RETURN TO SPORT ON OUTCOMES IN ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION: A RETROSPECTIVE COMPARATIVE STUDY

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ABSTRACT

The optimal timing for return to sport (RTS) following anterior cruciate ligament (ACL) reconstruction remains controversial. Accelerated rehabilitation protocols may increase the risk of re-injury, whereas delayed RTS could enhance recovery outcomes. To investigate the effects of rehabilitation timelines on functional outcomes and reinjury rates post-ACL reconstruction, comparing standard RTS protocols with delayed RTS approaches. In this retrospective cohort study, 54 highly active athletes aged 15-35 years who underwent primary ACL reconstruction using a hamstring autograft were analyzed. Participants were divided into two groups based on rehabilitation duration: the Standard RTS group (returning to sport within 5-7 months, n=28) following MOON guidelines, and the Delayed RTS group (returning after≥9 months, n=26) adhering to Delaware-Oslo guidelines. Outcomes measured included the Lysholm Knee Score for functional assessment, Tegner Activity Scale (TAS) difference for activity level changes, ACL Return to Sport Index (ACL-RSI) for psychological readiness, and incidence of ACL re-rupture and contralateral ACL injuries over a 30-month follow-up. The Delayed RTS group demonstrated significantly better functional outcomes (Lysholm score: 94.1±4.7 *vs* 79.6±6.9, p <0.001), smaller reductions in activity levels (TAS difference: -0.33±0.8 *vs* -1.9 ±0.8, p <0.001), and higher psychological readiness (ACL-RSI: 85.6±13.2 *vs* 51.9±16.4, p <0.001) compared to the Standard RTS group. The Standard RTS group had a seven-fold increased risk of ACL re-rupture (Relative Risk (RR)=7.0, Odds Ratio (OR)=6.5) and doubled the risk of contralateral ACL injury (RR=2.0, OR=2.17). The combined risk of an ACL injury was significantly higher in the Standard RTS group (RR=3.53, OR=5.27), with an absolute risk increase of 29%. Delaying

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RTS beyond nine months post-ACL reconstruction significantly improves functional outcomes psychological readiness, and reduces the risk of reinjury compared to standard rehabilitation timelines. These findings support the adoption of extended, criterion-based rehabilitation protocols to optimize patient recovery and enhance long-term knee health.

KEYWORDS: *anterior cruciate ligament reconstruction, return to sport, rehabilitation timeline, re-injury risk, functional outcomes, MOON guidelines, Delaware-Oslo guidelines*

INTRODUCTION

Anterior cruciate ligament (ACL) reconstruction is an extremely delicate surgery that significantly affects an athlete's life. From the time of injury to return to sports activities, many crucial steps must be carried out perfectly to ensure optimal recovery, both in knee function and return to sport at pre-injury levels.

The incidence of these injuries has been increasing, with an estimated 120,000 to 200,000 ACL injuries occurring annually in the United States (1, 2). ACL injuries are most prevalent in high school and college-aged individuals, with a peak incidence occurring between 15 and 25 years of age (3, 4).

The impact of ACL injuries on athletes is serious, often resulting in significant time lost from sports, surgical intervention, and long-term health consequences. These injuries are associated with an increased risk of early-onset osteoarthritis, regardless of treatment approach (5-7). Furthermore, ACL injuries can have substantial psychological impacts, affecting an athlete's confidence and potentially altering their future athletic pursuits (8, 9). Gender and sport type significantly influence ACL injury risk. Females consistently demonstrate a higher incidence of ACL injuries compared to males in sex-comparable sports, with risk ratios ranging from 1.5 to 4.6 (10, 11). High-risk sports include soccer, basketball, and football, with soccer showing particularly high rates for females (12, 13).

The optimal timing for return to sport following ACL reconstruction remains debatable. While traditional protocols often suggest a 6–9-month recovery period, recent evidence indicates that only about two-thirds of athletes return to their pre-injury level of competition within 12 months post-surgery (14, 15). Some researchers argue for a more conservative approach, suggesting that delaying return to sport beyond 12 months may reduce the risk of re-injury (16).

The high incidence and significant impact of ACL injuries underscore the importance of prevention programs and optimized rehabilitation protocols. Future research should focus on refining injury prevention strategies, improving surgical techniques, and developing evidence-based guidelines for safe return to sport to mitigate the long-term consequences of these injuries on athletes' health and careers.

Objectives

This study aimed to investigate the effects of two different rehabilitation timelines on the success rates of ACL reconstruction, focusing on the timing of return to sport as a critical factor. Specifically, the study aimed to compare functional outcomes, the frequency of reinjury, and overall knee stability between patients who adhered to a standard RTS protocol and those who delayed their return to sport. Through this comparison, the study seeks to provide clear evidence to guide clinicians in recommending the most effective rehabilitation strategy for minimizing the risk of reinjury and ensuring long-term knee health post-ACL reconstruction.

MATERIALS AND METHODS

Study design

This retrospective cohort study was conducted to compare the clinical outcomes of two distinct rehabilitation protocols following anterior cruciate ligament (ACL) reconstruction. The study focused on the timing of return to sport (RTS) and its impact on functional recovery and reinjury rates. Data were collected from patients who underwent primary ACL reconstruction between 2017 and 2020.

Participants

A total of 127 patients who met the inclusion criteria were initially enrolled in the study. Inclusion criteria were as follows: age between 15 and 35 years at the time of injury, highly active or competitive athletes with a pre-injury Tegner Activity Score (TAS) of 5 or higher, and those who had undergone ACL reconstruction using a hamstring autograft

with a double-bundle technique (gracilis-semitendinosus). Exclusion criteria included patients with previous ACL injuries, those who did not complete the follow-up, and those with a TAS lower than 5. After applying these criteria, 54 patients remained for the final analysis.

Intervention

Participants were divided into two groups based on their rehabilitation timeline:

- **Standard RTS Group:** patients who returned to sport within 5-7 months post-surgery, following the MOON guidelines.
- **Late RTS Group:** patients who delayed their return to sport until at least 9 months post-surgery, following the Delaware-Oslo guidelines.

Outcome measures

To assess the outcomes of the two rehabilitation protocols, the following validated tools were employed:

- **Lysholm Knee Scoring Scale:** used to evaluate knee function, with scores ranging from 0 to 100, where higher scores indicate better knee stability and function.
- **Tegner Activity Scale (TAS):** employed to measure the level of physical activity before and after the injury. The difference between pre-injury and post-rehabilitation TAS scores was calculated to determine the impact on activity levels.
- **ACL Return to Sport Index (ACL-RSI):** a psychological measure assessing readiness to return to sport, with scores ranging from 0 to 100, where higher scores represent greater psychological readiness.

Data collection

Patients were interviewed retrospectively regarding the duration of their rehabilitation, any relapses of ACL injury, and the occurrence of new contralateral ACL injuries within a 30-month follow-up period. Data on Lysholm, TAS, and ACL-RSI scores were collected and analyzed for both groups.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) to compare the outcomes between the two groups. Continuous variables such as Lysholm, TAS, and ACL-RSI scores were analyzed using t-tests, while categorical variables, such as the incidence of relapses and new injuries, were compared using chi-square tests. Statistical significance was set at $p < 0.05$. Odds ratios (OR), relative risks (RR), and absolute risks (AR) were calculated to quantify the risk associated with the standard and late RTS protocols.

RESULTS

Patient demographics

Out of an initial cohort of 127 patients, 54 were eligible for inclusion after excluding those lost to follow-up, had Tegner Activity Scale (TAS) scores lower than 5, or did not provide consent. The patients, aged between 15 and 35 at the time of injury, had undergone primary ACL reconstruction using the same surgical technique. The study population consisted of 38 men and 16 women, with an average age of 26.7 ± 4.7 years. Participants were assigned to two groups based on the duration of their rehabilitation:

- **Standard RTS Group**: patients in this group returned to sport after an average rehabilitation period of 5.6 ± 0.7 months.
- **Delayed RTS Group**: these patients delayed their return to sport, with an average rehabilitation duration of 9.9±1.2 months.

No gender- or sport-specific distinctions were made among the participants, all of whom were competitive or highly active athletes based on their pre-injury TAS scores.

The Lysholm Knee Scoring Scale was employed to evaluate functional outcomes post-ACL reconstruction, providing insights into knee stability, pain, swelling, and overall activity performance. Scores range from 0 to 100, where values above 84 indicate excellent function, while those between 65 and 83 denote good function. This metric was instrumental in comparing the effectiveness of the standard versus delayed rehabilitation protocols.

Lysholm score

In the standard RTS group, the mean Lysholm score was 79.6±6.9 (range: 64 to 95; 95% CI: 75.0-84.0) points. The delayed RTS group demonstrated significantly superior functional recovery (p <0.001), with a mean Lysholm score of 94.1±4.7 (range: 84 to 100; 95% CI: 91.0-99.0) (Fig. 1).

The independent t-test showed a highly significant difference between the two groups (p <0.001), with a mean difference of 14.5±1.7 points in favor of the delayed RTS group.

Statistical analyses confirm that delayed rehabilitation yields significantly better functional outcomes than standard rehabilitation in terms of knee stability, pain management, and overall knee function.

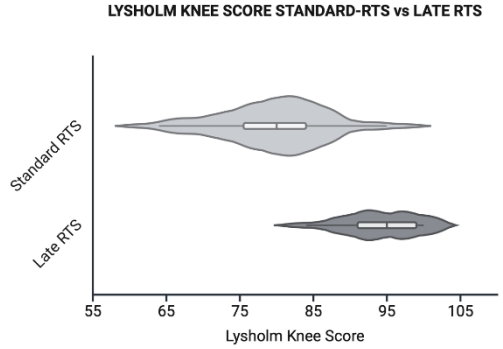

Fig 1. *Lysholm Knee Score for standard vs late RTS*

Tegner activity scale

The standard RTS group showed a mean TAS difference of -1.9 ± 0.8 (range: -3.0 to 0) (Fig. 2). The delayed RTS group performed significantly better, with a mean TAS difference of -0.3 ± 0.8 (range: -2.0 to 1.0). A Mann-Whitney U test confirmed a significant difference between the groups (p <0.001), with a median difference of 2.0 in favor of the delayed RTS group. The Shapiro-Wilk test showed an abnormal distribution of values.

ACL-RSI

The standard RTS group had a mean ACL-RSI score of 51.9 ± 16.4 (range: 18.3 to 84.6), indicating moderate psychological readiness to return to sport. The lower scores reflect ongoing concerns among patients about knee stability and fear of re-injury, which is common in shorter rehabilitation protocols. In contrast, the delayed RTS group demonstrated significantly better psychological readiness, with a mean ACL-RSI score of 85.6 ± 13.2 (range: 30.8 to 97.5) points (Fig. 3). A Mann-Whitney U test revealed a significant difference in ACL-RSI scores between the two groups (p <0.001), with a median difference of 38.3 points favoring the delayed RTS group. The

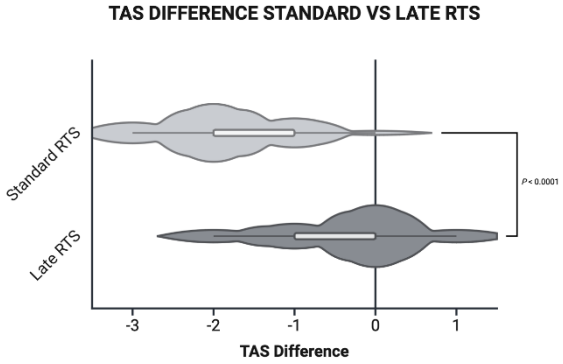

Fig. 2. *TAS Difference Standard vs Late RTS.*

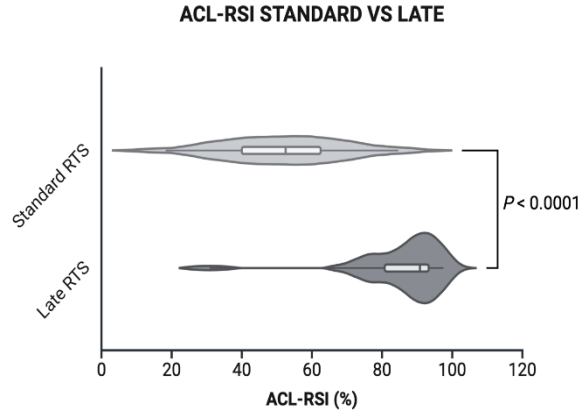

Fig. 3. *ACL-RSI standard vs late RTS.*

Shapiro-Wilk test showed non-normality in the delayed RTS group, but Levene's test indicated no significant difference in variance between groups (p=0.060).

Risk of re-injury

For the event of re-injury, the data showed a pronounced difference in risk between the two groups. The Odds Ratio (OR) was found to be 6.5, meaning that the patients in the Standard RTS group were 6.5 times more likely to experience a relapse compared to those in the Late RTS group. This substantial difference indicates that the likelihood of ACL re-injury is dramatically increased with

an accelerated rehabilitation program. The Relative Risk (RR), calculated to be 7, further emphasizes this point by showing that patients in the Standard RTS group were seven times more likely to suffer a relapse than those in the Late RTS group.

Additionally, the Absolute Risk (AR) for relapse was 0.22, signifying a 22% higher chance of experiencing a relapse if a faster rehabilitation program was followed (Fig. 4).

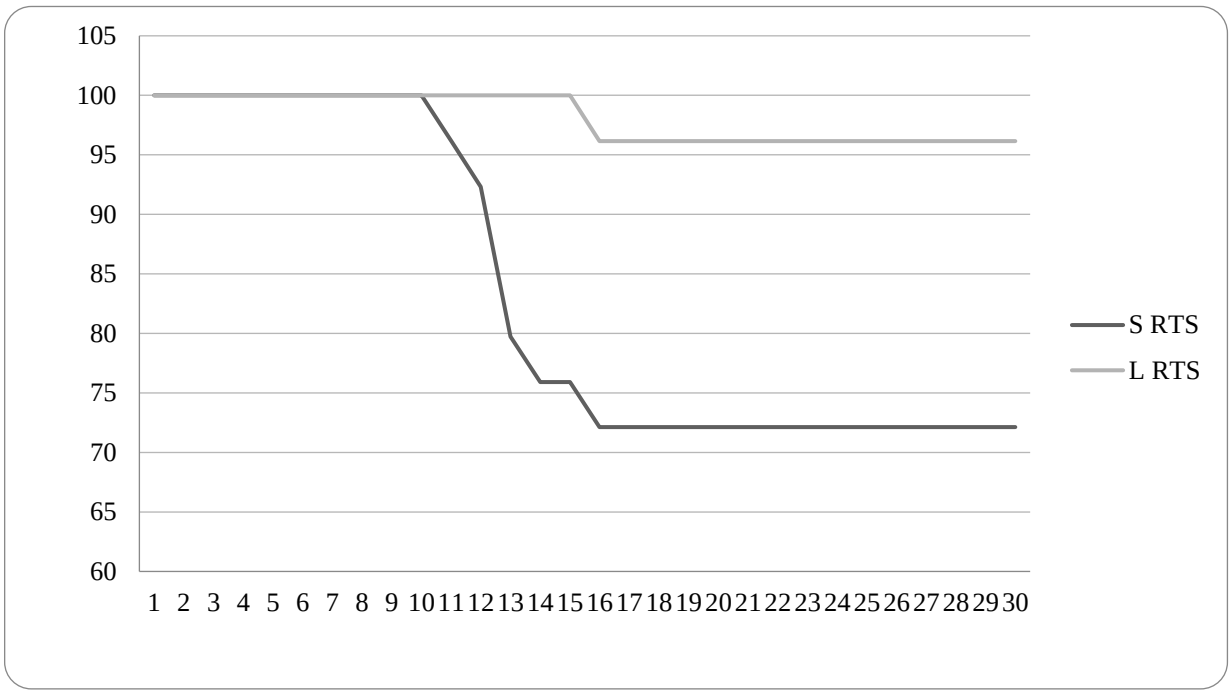

Fig. 4. *Risk of re-rupture standard vs late RTS.*

In contrast to re-injury, the risk of contralateral injuries was less pronounced but still noteworthy. The data showed an OR of 2.17, meaning that patients in the Standard RTS group were approximately twice as likely to experience a contralateral injury as those in the Late RTS group. The Relative Risk (RR) for contralateral injuries was calculated to be 2, indicating that the faster rehabilitation group had double the risk of sustaining a contralateral injury. The Absolute Risk (AR) for contralateral injuries was 0.07, reflecting a 7% increase in the absolute probability of developing a contralateral ACL injury in the Standard RTS group (Fig. 5).

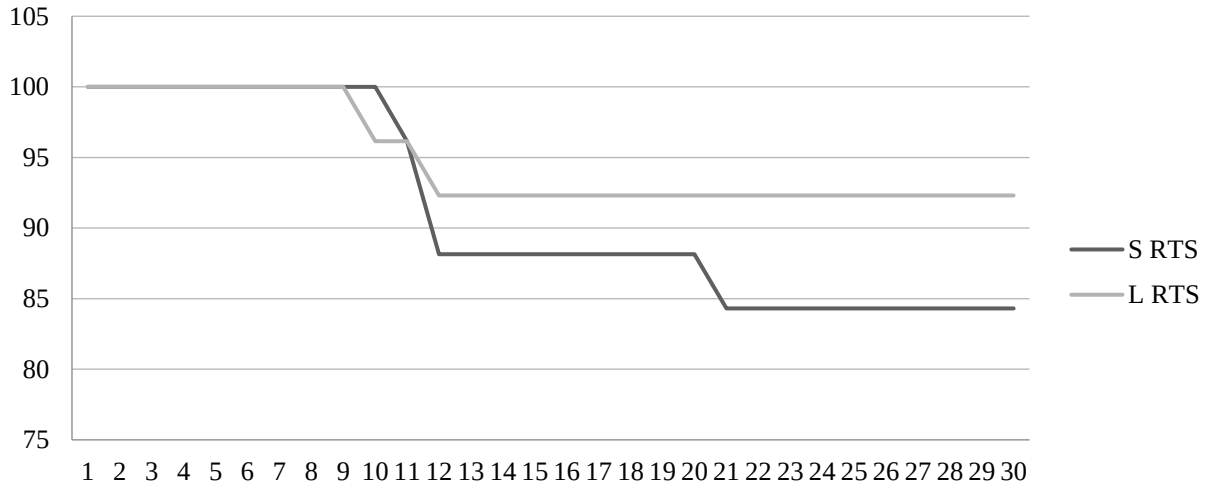

Fig. 5.*Risk of contralateral injury standard vs late RTS.*

Combined event (re-injury + contralateral ACL injury)

When considering the event of injury as both re-injury and contralateral ACL injuries, the overall risk remains elevated for the Standard RTS group. The Odds Ratio (OR) for this combined injury event was calculated to be 5.27, indicating that patients in the Standard RTS group were 5.27 times more likely to experience an injury (either relapse or contralateral) compared to those in the Late RTS group. The Relative Risk (RR) was 3.5, meaning that patients in the Standard RTS group were more than three times as likely to suffer from an injury, whether a relapse or contralateral injury. The Absolute Risk (AR) for the combined injury event was 0.29, signifying a 29% higher chance of encountering either a relapse or a contralateral injury when following a faster rehabilitation program.

Kaplan-Meier survival analysis

To better understand the long-term impact of the rehabilitation protocols, Kaplan-Meier survival curves were employed to compare the injury-free survival between the Standard RTS and Late RTS groups over a 30-month follow-up period. The Kaplan-Meier analysis showed a significant divergence between the two groups, with the Late RTS group maintaining a considerably higher probability of remaining injury-free throughout the 30 months compared to the Standard RTS group.

In the Standard RTS group, the survival curve showed a steep decline early in the follow-up period, indicating a higher frequency of injuries (both relapses and contralateral injuries) soon after the return to sport. In contrast, the Late RTS group exhibited a more gradual decline in the survival curve, with far fewer injuries observed over the same period.

DISCUSSION

The findings of this study indicate that delaying return to sport (RTS) beyond nine months after anterior cruciate ligament (ACL) reconstruction significantly enhances functional outcomes and reduces the risk of reinjury compared to a standard rehabilitation timeline of 5–7 months. These results support the growing body of evidence advocating for extended, criterion-based rehabilitation protocols to optimize patient recovery.

The results of the present study align closely with the recommendations of the Delaware-Oslo ACL cohort study, which emphasized that delaying RTS until certain functional milestones are achieved can substantially reduce the risk of re-injury.

Grindem et al. (16) found that each additional month of rehabilitation up to nine months decreased the reinjury rate by 51%, and patients who met specific strength and hopping criteria before RTS had an 84% lower risk of a second ACL injury compared to those who did not meet these criteria. This underscores the importance of not only time but also the quality of rehabilitation in ensuring safe RTS.

Similarly, the Multicenter Orthopaedic Outcomes Network (MOON) group has developed guidelines that stress the importance of individualized, criterion-based progression through rehabilitation phases (8, 17). The MOON guidelines recommend that patients achieve specific functional benchmarks—such as quadriceps strength symmetry and successful completion of hop tests—before considering RTS. Our delayed RTS group, which adhered more closely to these principles by allowing an average of 9.9 months for rehabilitation, demonstrated significantly better Lysholm Knee Scores and ACL-RSI scores, suggesting both better physical function and psychological readiness.

The superior functional outcomes observed in the delayed RTS group are consistent with the findings of Kyritsis et al. (18), who reported that patients not meeting specific discharge criteria before RTS were at a fourfold greater risk of graft rupture. This study highlights the critical role of objective functional assessments in determining RTS readiness. The delayed RTS group's longer rehabilitation period likely allowed for a more comprehensive recovery of muscle strength, proprioception, and neuromuscular control, which are essential for knee stability and function.

Psychological readiness is another crucial factor influencing RTS outcomes. Ardern et al. (8) emphasized that psychological responses significantly impact the likelihood of returning to preinjury levels of sport. The higher ACL-RSI scores in the delayed RTS group suggest that extended rehabilitation may provide additional time for patients to rebuild confidence in their knee function, reducing fear of reinjury—a common barrier to successful RTS.

The markedly lower rates of ACL re-rupture and contralateral injuries in the delayed RTS group have important clinical implications. Paterno et al. (19) demonstrated that young athletes who returned to high-risk sports had a significantly higher incidence of second ACL injuries within 24 months post-reconstruction. Our study's findings reinforce

the need for cautious progression through rehabilitation and suggest that accelerated RTS protocols may inadequately prepare patients for the demands of competitive sports.

Moreover, the Kaplan-Meier survival analysis in our study illustrates a clear divergence in injury-free survival between the two groups over 30 months, favoring the delayed RTS group. This long-term benefit supports the notion that extended rehabilitation improves immediate postoperative outcomes and contributes to sustained knee health and function.

While the study provides valuable insights, several limitations must be acknowledged. The retrospective design may introduce selection bias, and the sample size of 54 patients, although adequate for detecting significant differences, limits the generalizability of the results. Additionally, the study did not control for potential confounding variables such as the exact rehabilitation protocols followed, patient adherence, or the presence of concomitant injuries. Future studies should employ a prospective, randomized, controlled design to validate these findings and account for these variables.

The results advocate for a shift towards more conservative, individualized rehabilitation timelines, as recommended by both the MOON guidelines and the Delaware-Oslo study. Clinicians should consider incorporating objective functional tests and psychological assessments into their RTS criteria.

CONCLUSIONS

This study provides robust evidence that delaying return to sport (RTS) after ACL reconstruction leads to significantly better outcomes compared to standard, accelerated rehabilitation protocols. The delayed RTS group demonstrated superior results across all measured outcomes. Future research should focus on identifying specific biological and functional markers that indicate readiness for return to sport, as well as developing and validating standardized, evidence-based protocols for extended ACL rehabilitation. Additionally, long-term follow-up studies are needed to assess the impact of delayed RTS on career longevity and the development of post-traumatic osteoarthritis.

In conclusion, this study provides strong evidence in favor of delaying return to sport following ACL reconstruction to optimize functional outcomes and minimize re-injury risk.

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Investigative study

XENOGRAFT ACTS ON STEM CELLS

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ABSTRACT

Xenogeneic bone substitute derived from bovine cancellous bone has become a widely used biomaterial in dentistry and maxillofacial surgery for bone augmentation and regeneration procedures. It is composed of deproteinized bovine bone minerals with organic components removed to minimize immunogenicity and enhance biocompatibility. Xenogeneic bone substitute exhibits excellent osteoconductivity, allowing for the ingrowth of host bone and facilitating long-term stability and integration with surrounding tissues. For this reason, we investigated how xenogeneic bone substitutes act on dental pulp stem cells to differentiate them into osteoblasts, measuring the expression levels of bone-related genes and stem cell markers by Real-Time Polymerase Chain Reaction (real-time RT-PCR). The results indicated that RUNKS and FOSL1 strongly increased gene expression after 4 days of treatment. Although its role in bone biology is still being elucidated, FOSL1 and MMP XII appear to exert effects on osteoblasts and osteoclasts, modulating their activity and contributing to bone homeostasis and disease pathogenesis. Xenograft bone substitutes act on dental stem cells and promote osteoblast differentiation.

KEYWORDS: *graft, bone, osteoblasts, stem, expression*

INTRODUCTION

Xenogeneic bone substitute (XBS) derived from bovine cancellous bone has become a widely used biomaterial in dentistry and maxillofacial surgery for bone augmentation and regeneration procedures. It is composed of deproteinized bovine bone mineral, with the organic components removed to minimize immunogenicity and enhance biocompatibility.

It closely resembles human bone in terms of its structure and composition, providing an ideal scaffold for new bone formation (1, 2). XBS exhibits excellent osteoconductivity, allowing for the ingrowth of the host bone and facilitating long-term stability and integration with surrounding tissues. XPS is used in various dental and maxillofacial procedures, including ridge augmentation, sinus floor elevation, socket preservation, and guided bone regeneration (3, 4).

It serves as a filler material in bone defects and provides support for dental implants, thereby enhancing their stability and success rates. XBS is compatible with autogenous bone grafts and other biomaterials, allowing versatile treatment approaches tailored to individual patient needs (5, 6). XBS offers several advantages for bone augmentation procedures, including biocompatibility, osteoconductivity, and predictable clinical outcomes. Its natural origin and structure minimize the risk of adverse reactions or rejection, making it suitable for a wide range of patients. XBS eliminates the need for additional donor-site surgery and reduces patient morbidity and surgical

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complexity. Moreover, its availability in various particle sizes and formulations enables customized treatment strategies for different clinical scenarios. Despite its widespread use and clinical efficacy, XBS presents challenges, including limited resorption and remodeling capacity. XBS may elicit immune responses in some patients, necessitating careful selection and pre-operative evaluation. Among XBS, Bio-Oss (Geistlich, Germany) is one of the most commonly used, so we decided to investigate the impact of Bio-Oss on dental pulp stem cells (DPSC).

Dental pulp stem cells (DPSCs) residing within the dental pulp tissue of teeth have garnered significant attention in regenerative medicine and tissue engineering because of their multipotent differentiation potential and accessibility.

DPSCs are a heterogeneous population of cells with self-renewal capacity and multi-lineage differentiation potential. They can differentiate into various cell types, including odontoblasts, osteoblasts, adipocytes, and neural-like cells, making them promising candidates for tissue regeneration and repair. DPSCs can be isolated from the dental pulp of deciduous and permanent teeth, exfoliated deciduous teeth (SHED), and third molars through minimally invasive procedures (7, 8).

DPSC has immense therapeutic potential for various medical and dental applications. They have been investigated for their ability to regenerate dental tissues, such as dentin, pulp, and the periodontal ligament, in cases of dental caries, trauma, and pulpitis. Additionally, DPSCs have shown promise in regenerating non-dental tissues, including bone, cartilage, nerve, and cardiac tissues, making them versatile tools for tissue engineering and regenerative medicine therapies. Therefore, we investigated how Bio-Oss act on DPSCs to differentiate them into osteoblasts.

MATERIALS AND METHODS

Dental pulp stem cells (DPSCs) isolation

Dental pulp was extracted from the third molars of healthy subjects and digested for 1 h at 37°C in a solution containing 1 mg/ml collagenase type I and 1 mg/ml dispase dissolved in phosphate-buffered saline (PBS) supplemented with 100 U/ml penicillin 100 µg/ml streptomycin, and 500 µg/ml clarithromycin. The solution was then filtered using 70 µm Falcon strainers (Sigma Aldrich, St Louis, Mo, U.S.A.) to separate mesenchymal stem cells from fibroblasts. Stem cells were cultivated in α-MEM culture medium (Sigma Aldrich, St Louis, Mo, U.S.A.) supplemented with 20% Fetal Bovine Serum (FBS), 100 µM 2P-ascorbic acid, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (Sigma Aldrich, St Louis, Mo, U.S.A.). The flasks were incubated at 37°C with 5% CO2, and the medium was changed twice weekly.

DPSCs were characterized by immunofluorescence for the cytoskeletal component vimentin, positive mesenchymal stem cell markers CD90 and CD73, and the negative marker CD34, as described in Sollazzo et al. (9).

Cell treatment

DPSCs were seeded at a concentration of 1.0 × 105 cells/ml in 9 cm^2 (3 ml) wells containing DMEM supplemented with 10% serum and antibiotics, and Bio-Oss (Geistlich, Wolhusen, Switzerland) was added at a concentration of 10 mg/ml. Another set of wells containing untreated cells was used as the control. The treatment was performed at two time points: 24 h and 4 days.

Cells were maintained in a humidified atmosphere containing 5% CO2 at 37°C. At the end of the treatment period, cells were lysed and processed for total RNA extraction.

RNA isolation, reverse transcription, and quantitative real-time RT-PCR

Total RNA was isolated from the cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The pure RNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

cDNA synthesis was performed using 500 ng of total RNA and PrimeScript RT Master Mix (Takara Bio Inc., Kusatsu, Japan). The reaction mixture was incubated at 37°C for 15 min and inactivated by heating at 70°C for 10 s.

cDNA was amplified by real-time quantitative PCR using ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA).

All PCR reactions were performed in a 20 µL volume. Each reaction contained 10 µl of 2x qPCRBIO SYGreen Mix Lo-ROX (PCR Biosystems, Ltd., London, UK), 400 nM of each primer, and cDNA.

Custom primers belonging to the "extracellular matrix, adhesion molecule" pathway, "osteoblast differentiation" and "inflammation" pathway were purchased from Sigma-Aldrich. The selected genes grouped by functional pathways are listed in Table I.

All the experiments were performed using non-template controls to prevent reagent contamination. PCR was performed using two analytical replicates.

The amplification profile was initiated by incubation for 10 min at 95 °C, followed by a two-step amplification for 15 s at 95°C and 60 s at 60°C for 40 cycles. In the final step, melt curve dissociation analysis was performed.

Table I. *Selected genes used in Real-Time PCR grouped by functional pathway.*

Pathway	**Gene**
Osteoblast differentiation	SPP1 (Osteopontin) SPARC (Osteonectin) RUNX2 (Runt-related transcription factor 2) ALP (Alkaline phosphatase) FOSL1 (FOS-like antigen 1) SP7 (Osterix) ENG (Endoglin)
Extracellular matrix, adhesion molecule	COL1A1 (Collagen type I alpha1) COL3A1 (Collagen, type III, alpha 1) COL4A1 (Collagen, type IV, alpha 1) MMP7 (Matrix Metallopeptidase 7) MMP12 (Matrix Metallopeptidase 12) MMP14 (Matrix Metallopeptidase 12)
Inflammation	IL1α (Interleukin 1 Alpha) IL1R (Interleukin 1 Receptor Type 1) IL6 (Interleukin 6) IL6R (Interleukin 6 Receptor)
Reference gene	RPL13 (Ribosomal protein L13)

Statistical analysis

The gene expression levels were normalized to the expression of the reference gene (RPL13) and expressed as fold changes relative to the expression in untreated cells. Quantification was performed using the delta-delta Ct method (10).

RESULTS

The DPCSs were phenotypically characterized using immunofluorescence. Fig. 1a shows cytoskeletal filaments stained with vimentin. The cell surfaces were positive for mesenchymal stem cell markers CD90 (Fig. 1b) and CD73 (Fig. 1c) and negative for markers of hematopoietic origin CD34 (Fig. 1d).

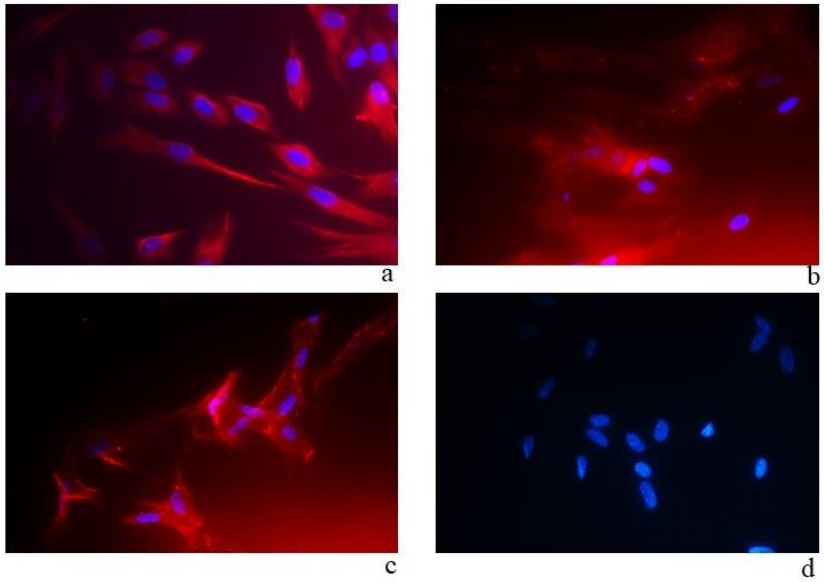

Fig. 1. *DPCSs by indirect immunofluorescence (Rhodamine). Immunofluorescence staining of vimentin (a), mesenchymal stem cell marker CD73 (b), CD90 (c), and hematopoietic markers CD34 (d). Nuclei were stained with DAPI. Original magnification x40.*

Bio-Oss treatment in DPSCs was analyzed using quantitative real-time PCR after 24 h and 4 days of treatment, and the expression levels of osteoblast-related genes, extracellular matrix, and inflammation pathways were measured.

Table II reports the significant fold changes obtained after 24 h and 4 days.

Table II. *Gene expression in DPSCs after 24h and 4 days of treatment. Numbers express the fold changes of the de-regulated genes in treated cells vs. untreated cells. In bold significant gene expression level.*

	24 h	**4 DAYS**
SPP1	nd	nd
SPARC	0.9	0.67
RUNX2	1.16	**2.38**
ALP	0.59	0.36
FOSL1	0.84	**4.31**
SP7	**5.18**	0.23
ENG	0.91	1.47
COL1A1	0.67	0.63
COL3A1	1.5	0.4
COL4A1	1.3	1.1
MMP7	1.8	0.2
MMP12	**3.8**	0.2
MMP14	**2.1**	1.8
IL1α	1.8	0.4
IL1R	1	0.4
IL6	**10.6**	**6.2**
IL6 R	0.6	1.2

Significantly upregulated genes showed ≥ 2-fold change in expression (P value ≤ 0.05), while significantly downregulated genes showed ≤ 0.5-fold change in expression (P value ≤ 0.05).

In DPSCs, after 24 h of treatment, SP7 was strongly upregulated, as were MMP12, MMP14, and IL6 (Table II). After 4 days, IL6 was still upregulated, whereas SP7, MMP12, and MMP14 decreased. RUNX2 and FOSL1 strongly increased gene expression after four days of treatment.

DISCUSSION

Dental pulp stem cells (DPSCs) have emerged as a significant focus in regenerative medicine due to their unique properties and versatile applications. Isolated from the dental pulp of deciduous and permanent teeth, DPSCs are mesenchymal stem cells capable of differentiating into various cell types.

DPSCs exhibit several key characteristics that make them valuable in regenerative medicine. DPSCs can differentiate into multiple cell lineages, including osteoblasts (bone cells), chondrocytes (cartilage cells), adipocytes (fat cells), and neurons (nerve cells). This multipotency is crucial for their application in tissue engineering and repair. DPSCs have a high proliferative rate, meaning they can rapidly multiply to generate sufficient cells for therapeutic use.

DPSCs secrete various bioactive molecules that modulate immune response, reduce inflammation, and promote tissue repair. DPSCs can be easily obtained from extracted teeth, which is a common and minimally invasive procedure, making them an accessible source of stem cells.

DPSCs have significant potential for various applications in regenerative medicine. DPSCs can be used to regenerate dental tissues, including dentin, pulp, and the periodontal ligament, offering potential treatments for tooth decay, pulpitis, and periodontal disease. DPSCs can differentiate into osteoblasts, making them suitable for bone tissue engineering and treatment of bone defects and fractures. DPSCs have the ability to differentiate into neural cells, presenting potential therapeutic options for neurodegenerative diseases and spinal cord injuries. DPSCs can contribute to the regeneration of cardiac tissues and offer promise for the treatment of myocardial infarction and other heart conditions.

DPSCs can enhance wound healing and skin regeneration, thereby providing new approaches for treating burns and chronic wounds (10, 11).

The use of DPSCs has several advantages. The collection of DPSCs from extracted teeth is a minimally invasive procedure that reduces the risk and discomfort associated with stem cell harvesting. DPSCs avoid ethical issues related to embryonic stem cells as they are derived from discarded dental tissues. DPSCs can be used in autologous therapies, where the patient's own cells are used for treatment, thus minimizing the risk of immune rejection. The ability of DPSCs to differentiate into multiple cell types renders them suitable for a wide range of regenerative applications.

For all the above-mentioned reasons, we decided to verify how Bio-Oss act on DPSCs to stimulate their differentiation into osteoblasts.

Both FOSL1 and MMP12 were activated by Bio-Oss (Table II).

FOSL1, a member of the FOS family of transcription factors, plays a significant role in regulating cellular processes, such as proliferation, differentiation, and apoptosis. Its involvement in osteogenesis, which is the process of bone formation, has garnered considerable interest in recent years.

FOSL1 belongs to the AP-1 (Activator Protein-1) family of transcription factors and is characterized by a basic leucine zipper (bZIP) domain that mediates dimerization and DNA binding. FOSL1 forms heterodimers with members of the Jun family (c-Jun, JunB, and JunD) constituting the AP-1 transcription complex. Its transcriptional activity is modulated by various signaling pathways, including the mitogen-activated protein kinase (MAPK) and Wnt/β-catenin pathways, which regulate FOSL1 expression and activity during osteogenesis. Post-translational modifications such as phosphorylation and acetylation also regulate FOSL1 function, influencing its stability, subcellular localization, and interaction with co-regulatory proteins. These regulatory mechanisms fine-tune FOSL1 activity, allowing the precise control of gene expression during osteogenesis (12).

FOSL1 plays diverse roles in osteogenesis, influencing both osteoblast differentiation and bone matrix mineralization. During the early stages of osteoblast differentiation, FOSL1 cooperates with other transcription factors such as RUNX2 and Osterix to activate the expression of osteogenic genes, including alkaline phosphatase

(ALP), osteocalcin (OCN), and collagen type I (COL1A1). FOSL1 also promotes cell cycle progression and proliferation, facilitating expansion of the osteoblast progenitor pool.

In addition to its role in osteoblast differentiation, FOSL1 regulates bone matrix mineralization by modulating the expression of genes involved in extracellular matrix (ECM) synthesis and remodeling. FOSL1 promotes the expression of matrix metalloproteinases (MMPs) and other proteases that degrade ECM components, thereby facilitating the deposition of mineralized matrices. Moreover, FOSL1 interacts with signaling pathways involved in calcium homeostasis and phosphate metabolism, thereby influencing bone mineralization processes (13, 14).

Matrix Metalloproteinase 12 (MMP12), also known as macrophage metalloelastase, is a member of the matrix metalloproteinase family involved in the degradation of extracellular matrix components. Although traditionally studied in the context of inflammation and tissue remodeling, emerging evidence suggests that MMP12 may also play a role in osteogenesis (15, 16).

MMP12 is a zinc-dependent endopeptidase characterized by a catalytic domain, prodomain, and hemopexin-like domain. It is primarily produced by macrophages and is involved in the degradation of elastin and other components of the extracellular matrix. MMP12 expression and activity are regulated at multiple levels, including transcriptional regulation by cytokines, growth factors, and inflammatory mediators as well as post-translational modifications such as proteolytic cleavage and inhibition by tissue inhibitors of metalloproteinases (TIMPs).

Although the role of MMP12 in osteogenesis is less well characterized than that of other MMPs, emerging evidence suggests that MMP12 may influence bone formation through its effects on bone cells. In vitro studies have shown that MMP12 is expressed by osteoblasts and osteoclasts and can modulate their activity. MMP12 may promote osteoblast differentiation and mineralization by facilitating turnover of the extracellular matrix and releasing bioactive factors that regulate bone cell function (17, 18).

Conversely, MMP12 may contribute to bone resorption by enhancing osteoclast activity and bone matrix degradation. MMP12 degrades collagen and other components of the bone matrix, leading to the release of growth factors and cytokines that stimulate osteoclast formation and activation. Additionally, MMP12 may indirectly influence osteogenesis by modulating the inflammatory microenvironment, which plays a critical role in bone remodeling and repair.

CONCLUSIONS

Bio-Oss is one of the most widely used xenografted bone substitutes. Is acts on DPSCs stimulates the differentiation of DPSCs into osteoblasts. DPSCs represent a versatile and promising resource in regenerative medicine. Their ability to differentiate into various cell types, coupled with their accessibility and ethical advantages, makes them an attractive option for tissue engineering and therapeutic applications. Among the investigated genes, we focused on FOSL1 and MMP12. FOSL1 is a key regulator of osteogenesis and influences osteoblast differentiation, bone matrix mineralization, and bone homeostasis. MMP12 is emerging as a potential regulator of osteogenesis and influences the bone formation and remodeling processes. Although its role in bone biology is still being elucidated, MMP12 appears to exert effects on both osteoblasts and osteoclasts, modulating their activity and contributing to bone homeostasis and disease pathogenesis. Further research is required to better understand how xenografts stimulate stem cell differentiation into osteoblasts.

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Evaluation Study

INTERACTION BETWEEN HUMAN FIBROBLASTS AND HYALURONIC ACID: OUTPUT IN THE EXTRACELLULAR MATRIX

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ABSTRACT

Hyaluronic acid is the major constituent of the extracellular matrix. It is important in cell signaling and proliferation, extracellular matrix structural organization, tissue reparation, angiogenesis, and inflammatory and immune response. Nevertheless, it was demonstrated that hyaluronic acid's biological functions and properties are strictly dependent on its molecular weight, showing opposite effects between high-molecular-weight and low molecular weight. Here, we tested the effect of hyaluronic acid at the different molecular weights (low, medium, and high) on the extracellular matrix deposition and remodeling in fibroblasts treated for 24 hours, measuring the gene expression levels of genes belonging to "Extracellular Matrix and Adhesion Molecules" pathway. The most significant effects in cell proliferation seem to occur with the administration of high and medium molecular-weight hyaluronic acid, which induces the expression of genes such as HAS1, COL4A1, and COL9A1. These results demonstrated that hyaluronic acid activates fibroblasts by stimulating the deposition of the extracellular matrix and its remodeling.

KEYWORDS: *hyaluronic acid, fibroblasts, extracellular matrix, gene expression*

INTRODUCTION

Hyaluronic acid (HA), a non-sulfated glycosaminoglycan, is a polymer of disaccharides composed of D-glucuronic acid and N-acetyl-D-glucosamine. HA is present at the extracellular matrix (ECM) level and plays a key role during wound healing phases and in any regulatory process ECM. It is important in cell signaling and proliferation, ECM structural organization, tissue reparation, angiogenesis, and inflammatory and immune response (1-4). Nevertheless, it was demonstrated that HA's biological functions and properties are strictly dependent on its molecular weight, also showing opposite effects between high-molecular-weight (HMW) (i.e., higher than 1000 KDa) and low-molecular-weight (LMW) (i.e., lower than 1000 KDa) (2-7). HA is a major constituent of ECM in the human body; it is constantly synthesized as HMW-HA and is degraded very fast by hyaluronidases (8). Moreover, it plays an important role in supporting cells during wound healing (9, 10), recognizing specific surface receptors during the healing process (11), and favoring collagen deposition and angiogenesis (9, 10). HA is known to activate fibroblasts, and it is involved during the proliferation, migration,

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and tissue maturation phases of the healing process (12). However, HA is rapidly metabolized, and its half-life is less than a day. HA is also actively degraded within 24 h by the hyaluronidase enzymes or reactive oxygen species (12).

The aim of our research was to study the effect of HA with different molecular weights on human gingival fibroblasts, assessing the role of this natural linear polysaccharide in extracellular matrix deposition and remodeling. For this purpose, we treated human fibroblasts with hyaluronic acid at three different molecular weights, high, medium, and low, for 24 hours. Then we measured the expression levels of genes involved in the "Extracellular Matrix and Adhesion Molecules" pathway by real-time PCR.

MATERIALS AND METHODS

Primary gingival fibroblasts purchased from ATCC® Cell Lines were cultured in flasks containing medium and antibiotics and incubated in a humified atmosphere. PrestoBlue™ Reagent Protocol (Invitrogen) was used to evaluate the viability of cells.

Cells were treated with the following solution: a) 10 mg/mL of high molecular weight HA; b) 10 mg/mL of medium molecular weight HA; c) 10 mg/mL of low molecular weight HA. For each treatment, three biological replicates were performed. Cell medium alone was used as a negative control. After the end of the exposure time, cells were trypsinized and processed for RNA extraction. Primers from the "Extracellular Matrix and Adhesion Molecules" pathway were purchased from Sigma Aldrich. The selected genes grouped by functional pathway are listed in Table I. Standard cDNA synthesis was performed, and cDNA was amplified by Real-Time Quantitative PCR using the ABI PRISM 7500 (Applied Biosystems). For statistical analysis, the delta/delta Ct calculation method was used. (13).

Table I. *Selected genes grouped by functional pathway.*

Pathway	Gene symbol	Gene name
Collagens & Extracellular Matrix Structural constituent	COL1A2	collagen type I alpha 2 chain
	COL2A1	collagen type II alpha 1 chain
	COL3A1	collagen type III alpha 1 chain
	COL4A1	collagen type IV alpha 1 chain
	COL5A1	collagen type V alpha 1 chain
	COL6A1	collagen type VI alpha 1 chain
	COL7A1	collagen type VII alpha 1 chain
	COL8A1	collagen type VIII alpha 1 chain
	COL9A1	collagen type IX alpha 1 chain
	COL10A1	collagen type X alpha 1 chain
	COL11A1	collagen type XI alpha 1 chain
Cell Adhesion Molecule	CCTNA1	catenin alpha 1
	CTNNB	catenin beta 1
	CTNND2	catenin delta 2
	VCAN	versican
Transmembrane Receptor	HAS1	hyaluronan synthase 1
	ILF3	interleukin enhancer binding factor 3
	ITGA1	integrin subunit alpha 1
	ITGA2	integrin subunit alpha 2
	ITGA3	integrin subunit alpha 3
	ITGA4	integrin subunit alpha 4
	ITGA5	integrin subunit alpha 5
	ITGA6	integrin subunit alpha 6
	ITGA7	integrin subunit alpha 7
	ITGA8	integrin subunit alpha 8
	ITGB1	integrin subunit beta 1
	ITGB2	integrin subunit beta 2
	ITGB4	integrin subunit beta 4
	ITGB5	integrin subunit beta 5
	LAMA1	laminin subunit alpha 1
	LAMA2	laminin subunit alpha 2
	LAMA3	laminin subunit alpha 3

	LAMB1	laminin subunit beta 1
	LAMB2	laminin subunit beta 2
	LAMB3	laminin subunit beta 3
Extracellular Matrix Protease	MMP2	matrix metallopeptidase 2
	MMP7	matrix metallopeptidase 7
	MMP8	matrix metallopeptidase 8
	MMP9	matrix metallopeptidase 9
	MMP10	matrix metallopeptidase 10
	MMP11	matrix metallopeptidase 11
	MMP12	matrix metallopeptidase 12
	MMP13	matrix metallopeptidase 13
	MMP14	matrix metallopeptidase 14
	MMP15	matrix metallopeptidase 15
	MMP16	matrix metallopeptidase 16
	MMP24	matrix metallopeptidase 24
	MMP26	matrix metallopeptidase 26
TGFβ Signaling	TGFB1	transforming growth factor beta 1
	TGFB2	transforming growth factor beta 2
	TGFB3	transforming growth factor beta 3
Extracellular Matrix Protease Inhibitor	TIMP1	TIMP metallopeptidase inhibitor 1
Housekeeping gene	RPL13	ribosomal protein L13

RESULTS

The proper concentration of hyaluronic acid to be used in treating human fibroblasts cultured in vitro was established by making serial dilutions of the stock solutions and treating the cells for 24 hours. In addition, gene expression of genes belonging to the "Extracellular Matrix and Adhesion Molecules" pathway was investigated in human fibroblasts treated with high, medium and low molecular weight hyaluronic acid solution 10 mg/ml for 24 h.

Table II shows significant gene expression levels after 24h treatment with high molecular weight hyaluronic acid (HMW-HA) compared to untreated cells. The up-regulated genes belong to "Collagens & Extracellular Matrix Structural constituent" (COL7A1, COL9A1) "Cell Adhesion Molecule" (CTNND2), "Transmembrane Receptor" (HAS1, ILF3, ITGA1, ITGA3, ITGA7, ITGA8, ITGB2, ITGB5), "Basement Membrane Constituent" (LAMA1, LAMB1, LAMB3), "Extracellular matrix protease pathway" (MMP9, MMP11, MMP24), TGFβ Signaling (TGFB3). The down-regulated genes were collagen COL6A1, metalloproteases MMP8, MMP12 and MMP26, and the transmembrane receptor TGFB2. Fig. 1 represents the gene expression profile of treated fibroblasts compared with control (untreated cells).

Table II. *Significant gene expression levels after 24h treatment with high molecular weight hyaluronic acid (HMW-HA)*

Gene	Fold change	SD (+/-)	Gene function
COL6A1	0,45	0,15	Collagens & Extracellular Matrix Structural constituent
COL7A1	2,07	0,15	Collagens & Extracellular Matrix Structural constituent
COL9A1	3,78	1,10	Collagens & Extracellular Matrix Structural constituent
CTNND2	5,43	0,35	Cell Adhesion Molecule
HAS1	3,96	0,54	Transmembrane Receptor
ILF3	3,29	0,22	Transmembrane Receptor
ITGA1	4,98	0,02	Transmembrane Receptor
ITGA3	2,42	0,28	Transmembrane Receptor
ITGA7	2,60	0,38	Transmembrane Receptor
ITGA8	2,44	0,05	Transmembrane Receptor
ITGB2	2,53	0,31	Transmembrane Receptor

ITGB5	3,41	0,25	Transmembrane Receptor
LAMA1	6,17	0,04	Basement Membrane Constituent
LAMB1	7,66	0,34	Basement Membrane Constituent
LAMB3	3,87	0,52	Basement Membrane Constituent
MMP8	0,14	0,01	Extracellular Matrix Protease
MMP9	2,63	0,12	Extracellular Matrix Protease
MMP11	2,28	0,33	Extracellular Matrix Protease
MMP12	0,44	0,04	Extracellular Matrix Protease
MMP24	8,51	1,86	Extracellular Matrix Protease
MMP26	0,35	0,03	Extracellular Matrix Protease
TGFB2	0,38	0,01	TGFβ Signaling
TGFB3	2,42	0,08	TGFβ Signaling

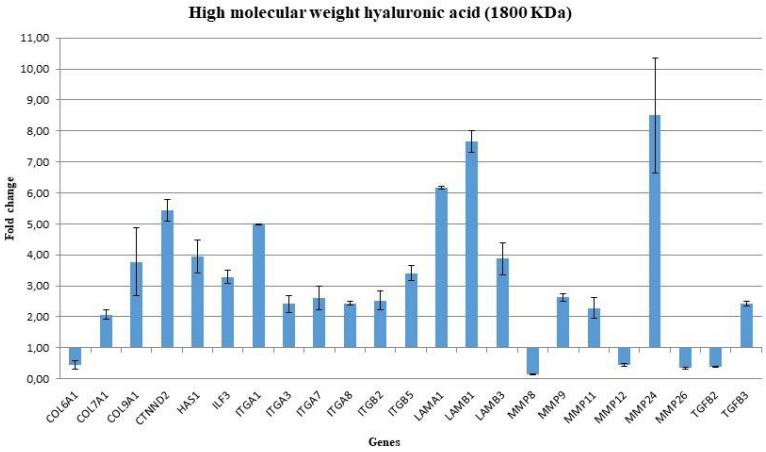

Fig. 1. *Gene expression profile of human fibroblasts treated with HMW-HA 10 mg/ml.*

Table III shows significant gene expression levels after 24h treatment with medium molecular weight hyaluronic acid (MMW-HA) compared with untreated cells. Genes differentially expressed were "Collagens & Extracellular Matrix Structural constituent" (COL4A1, COL9A1), "Cell Adhesion Molecule" (CTNND2), "Transmembrane Receptor" (HAS1), "Basement Membrane Constituent" (LAMA2) and "Extracellular Matrix Protease" (MMP8, MMP10, MMP13). All the genes were up-regulated except MMP2 and MMP15. Fig. 2 shows the expression profile of genes up-and down-regulated in treated fibroblasts with medium molecular weight hyaluronic acid.

Table III. *Significant gene expression levels are reported after 24h treatment with medium molecular weight hyaluronic acid (MMW-HA).*

Gene	Fold change	SD (+/-)	Gene function
COL4A1	2.24	0.19	Collagens & Extracellular Matrix Structural constituents
COL9A1	2.37	0.18	Collagens & Extracellular Matrix Structural constituents
CTNND2	4.91	0.47	Cell Adhesion Molecules
HAS1	4.64	0.03	Transmembrane Receptors
LAMA2	3.14	0.19	Basement Membrane Constituents
MMP2	0.34	0.01	Extracellular Matrix Proteases
MMP8	2.13	0.48	Extracellular Matrix Proteases
MMP10	2.99	0.24	Extracellular Matrix Proteases
MMP13	6.50	1.12	Extracellular Matrix Proteases
MMP15	0,18	0,00	Extracellular Matrix Proteases

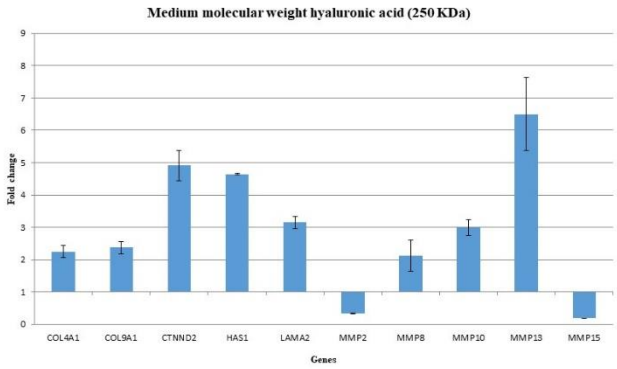

Fig. 2. *Gene expression profile of human fibroblasts treated with MMW-HA 10 mg/ml.*

Table IV reported the significant gene expression levels after 24h treatment with low molecular weight hyaluronic acid (LMW-HA) compared with untreated cells. The treatment induces the up-regulation of catenin delta 2 (CTNND2), laminin subunit beta 1 (LAMB1), and matrix metallopeptidase MMP13 and MMP26. The down-regulated genes were the transmembrane receptors ITGA7 and ITGB4 and the metallopeptidases MMP15 and MMP26. Fig. 3 shows the expression profile of genes up-and down-regulated in treated fibroblasts with low molecular weight hyaluronic acid.

Table IV. *Significant gene expression levels after 24h treatment with low molecular weight hyaluronic acid (LMW-HA).*

Gene	Fold change	SD (+/-)	Gene function
CTNND2	5.82	0.33	Cell Adhesion Molecule
ITGA7	0.44	0.10	Transmembrane Receptor
ITGB4	0.34	0.02	Transmembrane Receptor
LAMB1	7.71	0.09	Basement Membrane Constituent
MMP13	8.04	0.76	Extracellular Matrix Protease
MMP15	0.10	0.03	Extracellular Matrix Protease
MMP24	10.12	1.54	Extracellular Matrix Protease
MMP26	0.39	0.06	Extracellular Matrix Protease

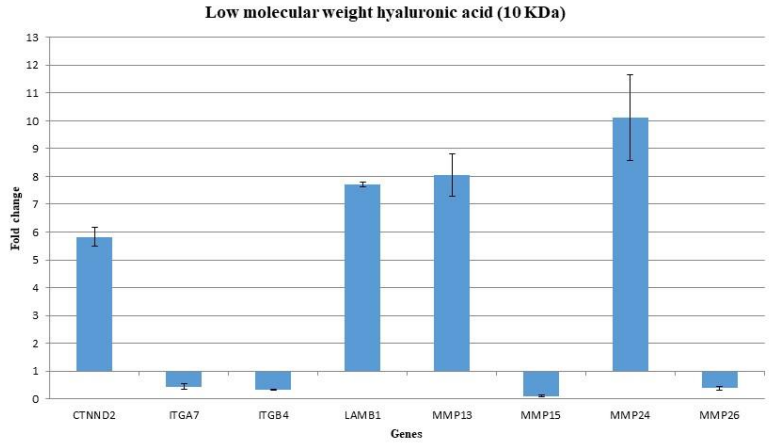

Fig. 3. *Gene expression profile of human fibroblasts treated with LMW-HA 10 mg/ml.*

DISCUSSION

The principal constituent of ECM in the human body is HA, synthesized as HMW-HA, and is degraded very fast by hyaluronidases (8). It plays an important role in supporting cells during wound healing (9, 10), recognizing specific surface receptors during the healing process (11), and favoring collagen deposition and angiogenesis. Here we tested the effect of HA at the different molecular weights (low, medium, and high) on the ECM deposition and remodeling in fibroblasts treated for 24 hours, measuring the gene expression levels of genes belonging to the "Extracellular Matrix and Adhesion Molecules" pathway. High molecular weight hyaluronic acid (HMW-HA) promotes fibrocytes' differentiation, leading to the deposition of extracellular matrix by fibroblasts (14).

In this study, fibroblasts treated with HMW-HA showed a high number of downregulated metallopeptidases, normally involved in extracellular matrix degradation. The administration of high and medium molecular weight hyaluronic acid stimulates the expression in treated fibroblasts of a series of genes involved in synthesizing high molecular weight hyaluronic acid involved in the deposition of extracellular matrix. These genes are the HAS1 enzyme responsible for synthesizing HMW-HA chains, and COL4A and COL9A1 are directly involved in the fibrillar rearrangement component of the extracellular matrix. In the same way, metalloproteinases are also differentially expressed, suggesting a modulation in the remodeling of the matrix.

Low molecular weight hyaluronic acid (LMW-HA) is involved in tissue inflammation mechanisms (15, 16). In fibroblasts treated with this molecule, it would seem that cells respond to the treatment by opposing the inflammatory action of the molecule by down-regulating numerous metalloproteinases, thus trying to stop the degeneration processes of the extracellular matrix. Therefore, the most significant effects in tissue repair and cell proliferation seem to occur with the administration of medium molecular weight hyaluronic acid, which induces the expression of genes such as HAS1, COL4A1, and COL9A1 to synthesise HMW-HA involved in cell proliferation processes. These results demonstrated that HA is involved in the activation of fibroblasts by stimulating the deposition of the extracellular matrix and its remodelling.

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Case Series

EARLY ARTHROSCOPIC ANTERIOR TALOFIBULAR REPAIR: A CASE SERIES WITH PERSONAL TECHNIQUE

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ABSTRACT

Ankle sprains often disrupt the anterior talofibular ligament either partially or completely. Arthroscopy has been described for ligamentous repair yet has been implied in limited cases of acute injury. Beginning in 2015, we have treated 71 patients for anterior talofibular ligament injury. After the talofibular ligament injury diagnosis, an early surgical repair was performed for complete ruptures between days 1 and 3 following injury, which was feasible in 59 cases (83.1%) and 12 partial ruptures between days 9 and 30 following ankle sprain. One patient sustained a re-rupture during postoperative sports activity, and one patient reported longstanding ankle stiffness and pain, for which a second look determined the cause of pain to be a previously untreated osteophyte, whereas ligament integrity was confirmed. Literature suggests that arthroscopy is superior in identifying chondral lesions while permitting timely treatment. Arthroscopic repair of acute talofibular ligament ruptures is reliable, reduces the risk of chronic ankle symptoms following sprains, offers patients a higher quality of life, allows return to sports in less than 90 days, and reduces the risk of future re-injury.

KEYWORDS: *talofibular ligament, arthroscopy, ligament repair, ankle sprain*

INTRODUCTION

Ankle sprains are extremely common and often lead to anterior talofibular ligament disruptions, leading to residual chronic symptoms (1, 2). Sequelae of ankle sprains may manifest as recurrent pain, impingement, and cartilaginous damage or instability due to joint hyperlaxity (3-5).

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Currently, repair methods include open surgery used for complete ligament tears and thermal shrinkage for chronic ligament laxity. Arthroscopy has also been described in chronic symptomatic talofibular ruptures using the arthro-Brostrom (6), among other techniques.

Following this research, we introduce an arthroscopic approach that can be used in acute, partial, and complete anterior talofibular ligament repair tears.

PATIENTS AND METHODS

Beginning in 2015, we treated 71 patients for anterior talofibular ligament injury. The right ankle was affected in 53 cases, and the left ankle in 18. Males constituted 66.2% of our patients; the mean age was 26 (range minimum of 17 and maximum of 47). Patients were assessed for ligament status through clinical and magnetic resonance examinations, and the surgical indication was given whenever tears were graded III or in cases of athletic patients with injuries graded I or II.

We performed early surgical repair for complete ruptures between days 1 and 3 following injury, feasible in 59 cases (83.1%), and 12 partial ruptures between days 9 and 30 following ankle sprain. Postoperative management consisted of cast immobilization for 35 days, followed by a rehabilitation program of at least 60 days. Office work was permitted between days 20-40 from cast removal. Sports activities were allowed between 7 and 90 days from cast removal. One patient sustained a new injury during postoperative sports activity, which led to re-rupture. No further complications arose.

Technical note

A non-traumatic traction device was positioned after patient positioning and surgical field preparation. Anteromedial and anterolateral portals were established, and a third portal was placed just medial to the first anterolateral portal, minding the peroneal nerve passage. We proceeded with synovectomy of the anterior compartment when necessary.

Shaver is used to prepare the talus ligament footprint, and the torn end of the talofibular ligament is hooked with a polydioxanone 2-0 thread that is advanced into the joint for a “poor man shuttle” and is retrieved from the lateral portal (Fig. 1).

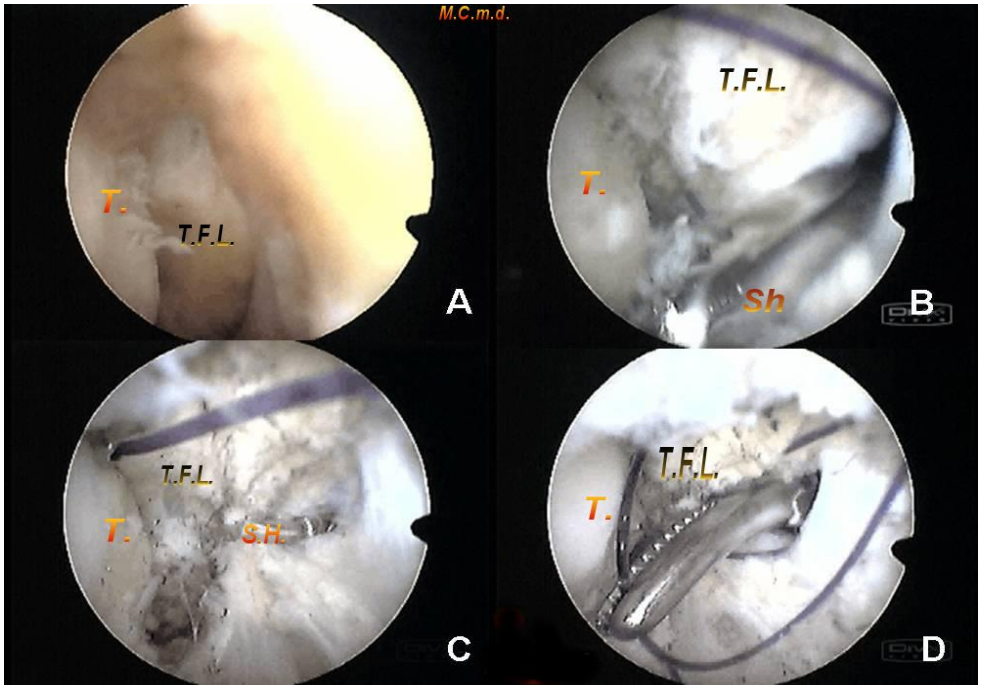

Fig. 1. *Talofibular ligament identification (**A**); shaver is used for talar bed preparation at the ligament insertion site (**B**); a suture hook is passed through the ligament (**C**); the ligament is passed through the lateral portal using a shuttle thread (**D**).*

A Minilok anchor (Depuy-Mitek, Raynham, MA, USA) is inserted on the debrided talar footprint, and the anchor strand is transported outside the portal. Using the PDS shuttle, the fibular end of the ligament is engaged, and a sliding knot is performed to secure the proximal talofibular ligament into its new distal insertion site (Fig. 2). Ligament stability is checked, and a drain and posterior leg slap is positioned for 24 hours and upon removal, the leg is cast.

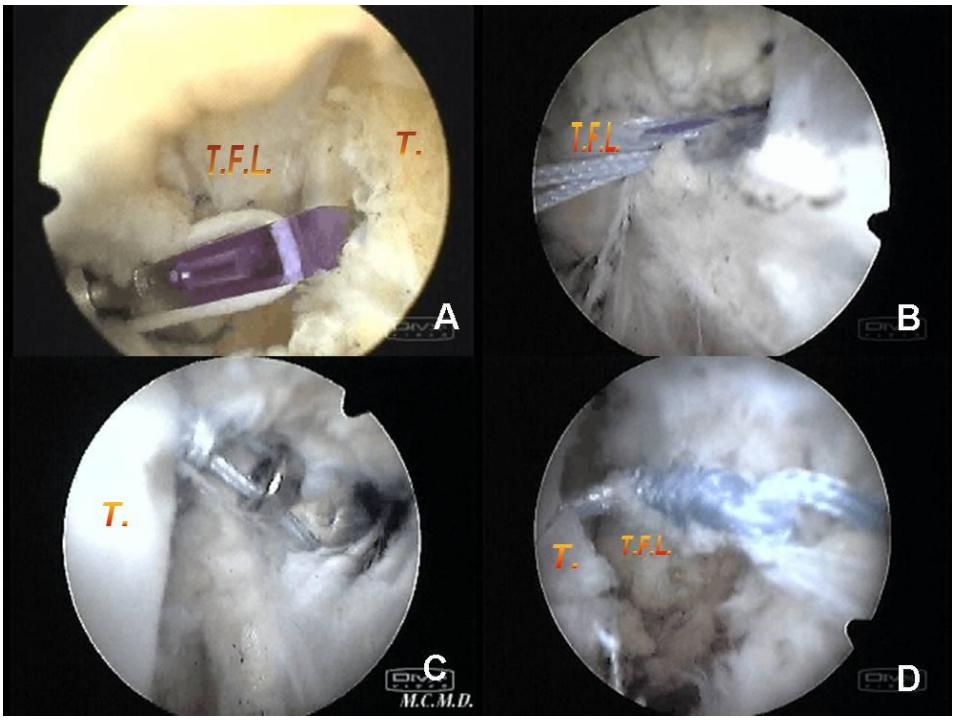

Fig 2. *A suture anchor is positioned in the talus (**A**); the anchor strand is passed through the shuttle thread (**B**); a sliding knot is performed to complete the repair (**C**, **D**).*

DISCUSSION

Literature is controversial regarding surgical ligamentous ankle repair (7-8). Studies suggest that conservative and surgical treatments are equivalent (6). However, the role of the anterior talofibular ligament and capsular integrity have been widely known to contribute to chronic ankle disorders following neglected injuries (9-12).

Despite open surgery being more frequently used for ankle ligamentous repair (13-16), arthroscopy is often used for chronic ankle instability treatment (17). The literature also suggests that arthroscopy is superior in identifying chondral lesions while permitting timely treatment (8, 9). Therefore, we recommend utilizing the described arthroscopic method to treat acute talofibular ruptures, especially when presenting talar avulsion.

As for complications, one patient sustained a new injury during postoperative sports activity, which led to re-rupture, while another patient presented with ongoing pain and underwent a second-look arthroscopy, which assessed the ligament repair integrity and evidenced a previously untreated osteophyte that was addressed.

This paper is limited by the patient number, and no statistical analysis could be performed due to the study nature. Yet, we believe that arthroscopy is feasible and reduces the risk of chronic ankle symptoms following sprains, offering patients a higher quality of life and reducing the risk of future injury. Further studies focusing on controls and long-term patient outcomes are necessary to determine the efficacy of this technique in acute talofibular ligament repair.

CONCLUSIONS

Arthroscopic repair of acute talofibular ligament ruptures is reliable, reduces the risk of chronic ankle symptoms following sprains, offers patients a higher quality of life, allows return to sports in less than 90 days, and reduces the risk of future re-injury.

Author contributions

MC ideated the surgical technique and performed the surgeries, AAM and FP wrote the article and revised it; MC and AAM performed different roles and equally contributed to the realization of the scientific work. MAM revised and submitted the article, and GP and VS coordinated the scientific work.

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Review

DELTOID LIGAMENT REPAIR IN ANKLE FRACTURES: ANATOMY, BIOMECHANICS, INJURY MECHANISMS, AND TREATMENT APPROACHES

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ABSTRACT

The deltoid ligament (DL) is a complex structure that provides medial stability to the ankle joint. Injuries to the DL, particularly in association with ankle fractures such as Weber B and C fractures, are often challenging to manage. The presence of a DL sprain generally worsens the prognosis, requiring thorough clinical and radiographic evaluation to determine whether surgical intervention is necessary to restore stability. A combination of clinical and radiographic methods is often employed to evaluate DL injuries in the context of ankle fractures. Plain radiographs are primarily used to exclude fractures or other bony abnormalities, while weight-bearing radiographs help assess any deformities, particularly in chronic cases. The surgical management of bimalleolar equivalent ankle fractures typically begins with open reduction and internal fixation (ORIF) of the fibula, performed through a lateral or posterolateral approach. However, in cases where the DL or posterior tibial tendon becomes entrapped between the talus and the medial malleolus, it can prevent proper closure of the medial clear space or obstruct fibular reduction. The common aspect of surgical techniques for DL repair is the use of suture anchors to reattach ligament fibers to their anatomic origin on the medial malleolus or medial tibia. However, variations exist regarding the location of the incision, whether both the superficial and deep fibers of the deltoid are repaired, and how avulsions (particularly from the talus or from calcaneus) are managed. The purpose of this narrative review is to provide a comprehensive overview of the ligament's anatomy, mechanics, common injury patterns, and treatment options, focusing on the surgical repair of the ligament in ankle fractures.

KEYWORDS: *deltoid ligament, ankle joint, fracture, Weber B and C fractures, ligament repair*

INTRODUCTION

The deltoid ligament (DL) is a complex structure that provides medial stability to the ankle joint. Injuries to the DL, particularly in association with ankle fractures such as Weber B and C fractures, are often challenging to manage. (1).

Understanding the anatomical structure and biomechanical role of the DL is essential for the accurate diagnosis and appropriate treatment of these injuries (2). The purpose of this narrative review is to provide a comprehensive

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overview of the ligament's anatomy, mechanics, common injury patterns, and treatment options, with a focus on the surgical repair of the ligament in ankle fractures.

Anatomy of the DL

The DL is a strong, broad ligament with a complex fascicular arrangement. It spans from the medial malleolus to the calcaneus, navicular, and talus bones, creating a triangular shape on the medial side of the ankle (1,3).

DL complex consists of two layers: a superficial and a deep layer. The superficial layer is composed of four distinct components, including the talonavicular (TN), talocalcaneal (TC), and the anterior and posterior tibiotalar (TT) ligaments. Functionally, this layer restricts the talus from moving into the valgus position and resists the eversion of the hindfoot (4). The deep layer primarily consists of the deep TT ligament, which is the principal restraint of the talus against external rotation. Together, the superficial and deep fibers of the DL provide medial stability, resisting external forces that could lead to ankle dislocation or misalignment (4).

Biomechanics and the role of the DL

The DL stabilizes the ankle joint during weight-bearing and prevents excessive eversion and valgus stress. With all lateral structures removed, the intact DL allows only 2 mm of separation between the talus and medial malleolus. When the deep DL is released, the talus can be separated from the medial malleolus by 3.7 mm (4, 5).

The superficial layers of the DL primarily limit talar abduction or negative talar tilt. The TC ligament specifically restricts talar pronation. In contrast, the deep layers of the DL rupture during external rotation, with the superficial portion remaining unaffected. The DL is the primary restraint against pronation of the talus, with the superficial and deep components equally effective.

Injury mechanisms and associated fractures

DL injuries are often linked to ankle fractures, particularly in Weber B and Weber C fractures, where the fibula is fractured at or above the syndesmosis (1, 6).

The Weber classification is a system that categorizes fibular fractures based on their relationship to the syndesmosis (7, 8). Weber B fractures occur at the level of the syndesmosis, while Weber C fractures happen above it and are typically more severe. In both types, there is a risk of DL injury, which can lead to significant instability. In Weber B fractures, the DL may be sprained or torn if there is a widening of the medial clear space, signaling ligamentous injury. In Weber C fractures, DL disruption is more often associated with severe syndesmotic injury and potential ankle dislocation. Although DL sprains are less common than lateral and syndesmotic sprains, they can lead to considerable ankle instability.

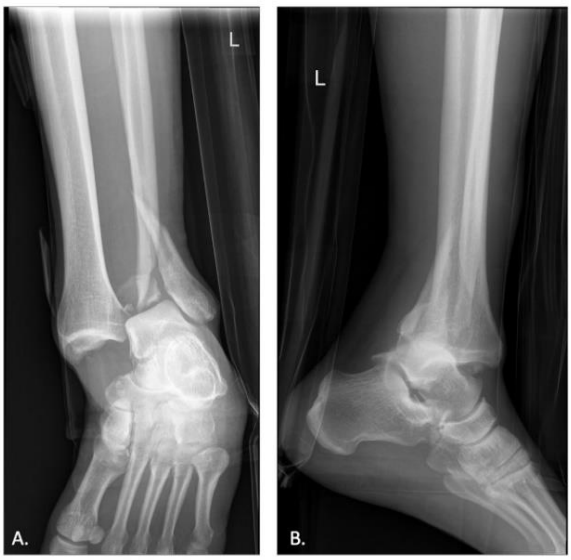

Fig. 1. *An example of X-ray antero-posterior (**A**) and lateral (**B**) view of ankle fracture dislocation with disruption of the syndesmotic distal tibio-peroneal ligaments and DL rupture.*

The presence of a DL sprain generally worsens the prognosis, requiring thorough clinical and radiographic evaluation to determine whether surgical intervention is necessary to restore stability (Fig. 1).

Clinical presentation of incompetence of the DL

Acute injuries to the DL must be suspected after an eversion and/or pronation injury (1, 4, 5). Typically, the foot is firm on the ground when an eversion force causes valgus stress to the ankle or an internal rotation force causes pronation stress to the hindfoot.

Acute injuries to the DL can also occur in association with lateral ankle fractures (6, 9). Chronic injuries typically cause medial ankle instability. This must be suspected if the patient feels the ankle "give way," especially medially, when walking on even ground, downhill, or downstairs, or if the patient experiences pain at the anteromedial or lateral aspect of the ankle, especially on dorsiflexion of the foot (10). Accurate diagnosis of DL injury is essential for determining the appropriate treatment.

Clinical findings

Acute DL injuries often present with tenderness and hematoma along the ligament. In chronic cases, a key finding is pain in the medial gutter, typically elicited by palpation of the anterior border of the medial malleolus. When the patient is weight-bearing, excessive valgus of the hindfoot and pronation of the affected foot indicate laxity on the medial side of the ankle (4). This valgus deformity and pronation usually disappear when the patient activates the posterior tibial muscle. Similarly, the valgus of the hindfoot and foot pronation resolve when the patient rises onto their tiptoes. Significantly, because there is no flattening of the medial longitudinal arch, the hindfoot valgus and forefoot abduction are not corrected by the single heel rise test, allowing clinicians to quickly rule out posterior tibial dysfunction (2-5).

A reliable clinical test involves the patient seated on an examination table with their feet hanging freely. The examiner grasps the heel of the affected ankle with one hand and the tibia with the other, applying first a varus and then a valgus tilt to the heel, comparing the results with the contralateral side. An anterior drawer test is also performed, and the findings are again compared with the unaffected ankle (10).

Imaging

To evaluate DL injuries in the context of ankle fractures, a combination of clinical and radiographic methods is often employed.

Plain radiographs are primarily used to exclude fractures or other bony abnormalities, while weight-bearing radiographs help assess any deformities, particularly in chronic cases. In cases of severe medial ligament incompetence, valgus deformity of the hindfoot may be evident (11). Additionally, stress radiographs provide indirect evidence of DL lesions in acute fractures, with a widened medial clear space, defined as greater than 4 mm and at least 1 mm more than the superior tibiotalar clear space, strongly suggesting deltoid disruption, particularly when accompanied by a fibular fracture (6, 9, 11). This often warrants surgical intervention. However, a normal medial clear space in static imaging does not necessarily exclude deltoid injury, as some cases may show widening only under stress, such as during an external rotation stress test (12).

Studies have shown that a medial clear space of 5 mm or more under external rotation and dorsiflexion is a reliable predictor of deep DL injury (11-13). In some cases, gravity stress radiographs, where the patient lies laterally and gravity induces external rotation stress, are also employed. These stress tests are typically more sensitive than simple weight-bearing radiographs for evaluating deltoid and syndesmotic integrity (11, 12).

Magnetic resonance imaging (MRI) can detect acute DL injuries, including partial tears or edema. However, it is not routinely recommended for deciding between surgical and non-surgical treatment in acute settings (13). This is due to variability in medial clear space widening even with similar MRI findings and the higher inter-rater reliability of stress test results over MRI in such cases. In rare instances, the "medial malleolus fleck sign," which indicates a small bone avulsion, may be present in bimalleolar equivalent fractures (13).

SURGICAL MANAGEMENT

Surgical sequence and indications for DL repair

The surgical management of bimalleolar equivalent ankle fractures typically begins with open reduction and internal fixation (ORIF) of the fibula, performed through a lateral or posterolateral approach (14). However, in cases where the DL or posterior tibial tendon becomes entrapped between the talus and the medial malleolus, it can prevent proper closure of the medial clear space or obstruct fibular reduction. In such instances, clearing the medial gutter via a separate medial incision is recommended (15).

Following fibular fixation, syndesmotic integrity should be assessed using the Cotton test or hook test, which involves applying lateral distraction to the fibula and evaluating for dynamic widening of the syndesmosis on a mortise view. If widening is observed, syndesmotic reduction and trans-syndesmotic fixation are required (16).

After addressing the fibula and syndesmosis, the need for DL repair remains controversial. Some surgeons advocate for routine deltoid repair in all patients with bimalleolar equivalent fractures, arguing that if the DL is incompetent enough to destabilize the fracture, it should be repaired to restore the medial tether and improve tibiotalar mechanics. Others only repair the DL if medial exposure is necessary to clear soft tissue from the medial gutter or if the patient is an athlete or shows signs of complete deltoid rupture during arthroscopy (17).

Intraoperative stress radiography is another method used to evaluate the stability of the medial ankle following ORIF. This typically involves applying an external rotation or eversion stress test to assess for persistent medial-sided instability. If the medial clear space widens by more than 4 mm and 1 mm more than the superior tibiotalar clear space, this is considered a positive result, indicating medial instability (10, 11). Additionally, talar tilt during eversion stress,

greater than 7 degrees, suggests a complete rupture of both the deep and superficial DL, warranting ligament repair (6, 9).

While the exact threshold for talar tilt or medial clear space widening necessitating deltoid repair is debated, studies suggest that talar tilt occurs in around half of patients even after proper fibular and syndesmotic fixation. DL repair is indicated in patients with positive intraoperative stress radiographs, as it helps to address persistent instability (6).

However, further research is needed to establish standardized thresholds for when DL repair should be performed during surgery.

Techniques for DL repair

DL repair techniques in ankle fractures have been extensively described by multiple authors, each proposing variations in the surgical approach (18, 19). Despite these differences, direct comparisons of these methods are lacking in the literature.

The common aspect of surgical techniques is the use of suture anchors to reattach the DL fibers to their anatomic origin on the medial malleolus or medial tibia (10, 18, 19). However, variations exist regarding the location of the incision, whether both the superficial and deep fibers of the deltoid are repaired, and how avulsions (particularly from the talus or calcaneus) are managed.

One of the first steps in the deltoid repair involves making a 5-cm curvilinear incision over the medial malleolus, after which the skin flaps are mobilized to provide adequate visualization. Often, horizontal clefts in the ligament or joint capsule are visible (18). If osteochondral lesions are present in the talus or tibia, they are treated either with traditional drilling or, more commonly, with a microfracture technique using an awl, which avoids excessive heating of the bone (20).

In cases where the DL avulses from the medial malleolus (the most frequent scenario), the malleolus is prepped for repair by drilling appropriately sized holes for suture anchor placement. Typically, one or two anchors are inserted, allowing for fixation with braided nonabsorbable sutures. The ligament is repaired using a "vest-over-pants" imbrication technique, which secures the superficial and deep fibers to the malleolus, alongside repairing any capsular disruption. The ankle is then stress-tested to confirm stability under external rotation and eversion forces (17, 18).

For less common cases of distal avulsion from the talus, the repair requires a more distal exposure, taking care to avoid injury to neurovascular structures. Two anchors are inserted on the medial aspect of the talus at the insertion sites of the deep anterior and posterior tibiotalar ligaments, and the deltoid fibers are then sutured back in place. Depending on the avulsion site, superficial deltoid repairs may also require an anchor placed into the fibula (21).

From a clinical standpoint, the decision to repair the DL remains somewhat controversial (22, 23). Some surgeons advocate routine repair in all bimalleolar equivalent fractures, reasoning that a damaged DL should be restored to optimize tibiotalar kinematics and stability (19). Others prefer to reserve deltoid repair for high-level athletes or when medial exposure is already required to clear soft tissue (6, 9, 23).

Recent studies emphasize that DL repair can optimize outcomes, especially when combined with syndesmotic fixation. A meta-analysis by Guo et al. (24) demonstrated that deltoid repair could reduce complications and improve long-term clinical outcomes associated with ankle instability. Conversely, research by Sun et al. (25) suggested that there is no indication of routine exposure and repair of the injured DL, advocating a more selective approach based on intraoperative findings and patient activity level. More work is required to establish standardized guidelines for when to repair the deltoid. Still, current techniques, especially those using suture anchors and stress radiographs, have proven effective in restoring ankle function (Fig. 2).

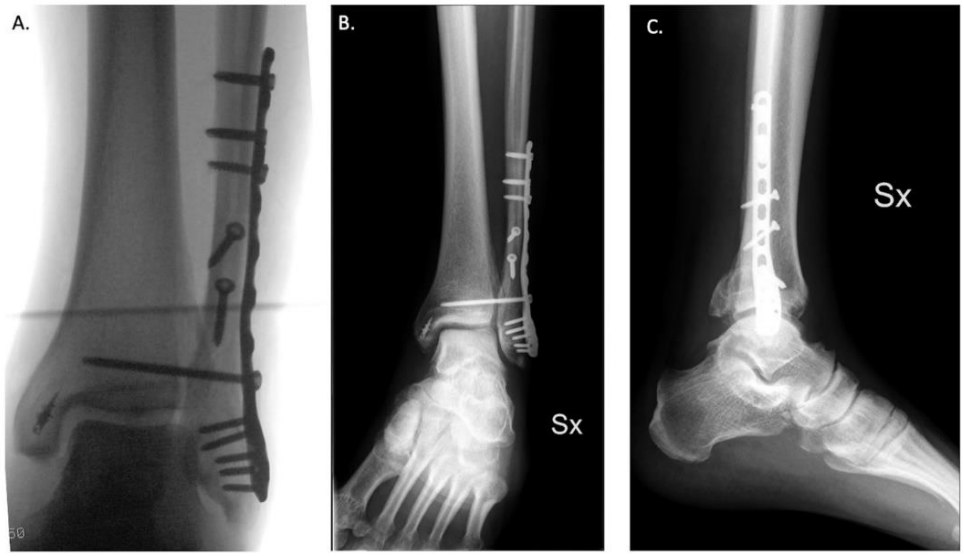

Fig 2. *Intra-operative fluoroscopic anteroposterior view (**A**) of the left ankle after open reduction internal fixation with plate and screws, trans-syndesmotic fixation, and DL repair with 5 mm anchor. The 1-month X-ray anteroposterior (**B**) and lateral (**C**) views show excellent mortise anatomy reduction and restoration.*

Outcomes of surgical repair

Early retrospective studies from the 1980s suggested that ORIF of bimalleolar-equivalent ankle fractures without repairing the DL yields acceptable long-term results (22). The theory behind this approach is that by restoring the anatomical alignment of the ankle mortise through fibular and syndesmotic ORIF, the DL would scar in and heal without the need for direct repair. For example, Zeegers and van der Werken (22) studied 28 patients with lateral malleolar fractures and associated DL ruptures who underwent lateral malleolar ORIF without DL repair. After an average follow-up of 18 months, none of the patients showed medial instability on clinical exams or stress testing, although seven showed early signs of osteoarthritis. Notably, 5 of these patients had anatomically restored mortises at the time of surgery, leading the authors to conclude that direct deltoid repair may not be necessary if the mortise is restored correctly.

Later studies with improved designs, such as comparative studies, reinforced these findings. Maynou et al. (26) compared 34 patients with bimalleolar-equivalent fractures, 18 of whom underwent deltoid repair and 17 did not. The study found no significant differences in subjective or objective outcomes, including medial instability, between the two groups, with only one patient in the non-repair group developing posttraumatic osteoarthritis. The authors concluded that repair of the DL is necessary in case of medial incongruency greater than 3 mm after the internal fixation of the fibula.

In 1995, Strömsöe et al. (27) conducted the first randomized controlled trial, comparing 50 patients with Weber B and C fractures. Half of the patients underwent deltoid repair, while the other half did not. After a mean follow-up of 17 months, there were no significant differences in functional outcomes between the groups, though deltoid repair resulted in longer surgical times. The study concluded that deltoid repair was unnecessary if the talus was adequately reduced to the medial malleolus and fibular anatomy restored. However, the study lacked a power analysis and did not assess medial instability, limiting the strength of its conclusions.

Despite the positive outcomes in most studies without deltoid repair, there have been reports of suboptimal results in some patient subgroups, including persistent medial instability, medial gutter pain, and early-onset posttraumatic arthritis (22-27). These poor outcomes have been attributed to the failure of the DL to heal anatomically. Consequently, some surgeons decide on deltoid repair in select cases, particularly in high-demand athletes or when intraoperative stress tests reveal medial instability (25-27).

Some authors strongly support the DL repair. Hsu et al. (28) reported excellent outcomes in 14 National Football League (NFL) players who underwent DL repair and ORIF for bimalleolar equivalent fractures. Eighty-six percent returned to play, and no medial pain or instability was observed at the final follow-up.

Woo et al. (29) retrospectively studied 78 patients with bimalleolar equivalent fractures over a 15-year period. The study found that those who underwent deltoid repair had significantly smaller medial clear space on final radiographs compared to those who did not have deltoid repair (3.2 mm vs. 3.7 mm). A subgroup analysis of patients who also had syndesmotic injuries revealed that the deltoid repair group had superior clinical outcomes, including better American Orthopedic Foot and Ankle (AOFAS) scores, lower pain levels, and less medial-sided pain. This suggests that deltoid

repair may be particularly beneficial in cases with combined syndesmotic injuries, as the two repairs may reinforce each other (Fig. 3).

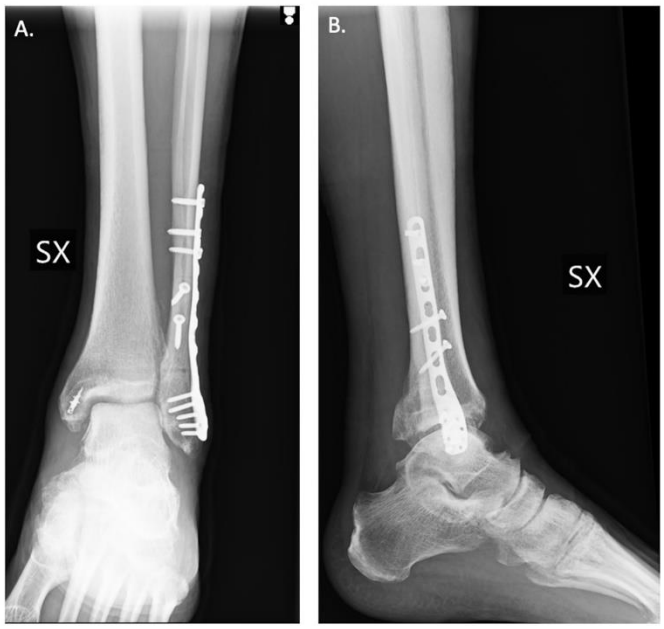

Fig. 3. One-*year follow-up X-ray assessment with anteroposterior (A) and lateral (B) view of the left ankle after open reduction internal fixation and DL repair with 5 mm anchor. Imaging demonstrated no development of ankle osteoarthritis and excellent mortise restoration.*

Early studies (22, 26, 27) suggested that DL repair might not be necessary for all bimalleolar equivalent ankle fractures, whereas recent research (23, 25, 28, 29) highlights specific groups, such as those with syndesmotic injuries or high physical demands, that may benefit from primary deltoid repair.

DISCUSSION

Proper reduction of the fibula and syndesmosis could allow the DL to heal without direct intervention. Patients with confirmed syndesmotic injury or high functional demands may benefit from DL repair when combined with syndesmotic fixation, which enhances medial stability and functional outcomes. Improved radiographic results, such as reduced medial clear space and decreased instances of posttraumatic arthritis, suggest that combining these repairs strengthens ankle stability and reduces complications like talar tilt and arthritis.

The decision to repair the DL remains controversial and is not universally accepted for all bimalleolar equivalent fractures. Some surgeons continue to reserve deltoid repair for cases where medial-sided instability persists following fibular and syndesmotic fixation. Others use it routinely in patients with combined deltoid and syndesmotic injuries, as their combined repair may enhance recovery and stability.

The lack of large-scale, randomized, controlled trials comparing outcomes with and without DL repair highlights the need for further investigation. Future research should focus on establishing clear clinical guidelines and decision-making criteria for when DL repair should be performed. Such studies could clarify which subgroups of patients, such as athletes, those with high physical demands, or individuals with syndesmotic injury, are most likely to benefit from this additional intervention.

Ultimately, the goal is to optimize treatment protocols that lead to the best possible functional, clinical, and radiographic outcomes, reducing long-term complications like arthritis and instability while facilitating rapid return to normal activity.

CONCLUSIONS

In conclusion, the DL plays a critical role in ankle stability, especially in the context of ankle fractures. While early research downplayed the necessity of its repair, evolving evidence suggests that DL repair, particularly when combined with syndesmotic fixation, may be beneficial for specific patient groups. As understanding of this topic continues to grow, surgeons must consider the individual patient's needs, activity level, and the presence of additional injuries when deciding whether DL repair is indicated. Further research will help refine these indications and improve long-term outcomes for patients with ankle fractures.

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Review

THE EFFECTIVENESS OF CORE STRENGTHENING EXERCISES IN THE REHABILITATION AND PREVENTION OF SPORTS INJURIES IN FOOTBALL PLAYERS

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ABSTRACT

This article highlights the global importance of core training in sports physiotherapy, focusing on improving performance and minimizing injuries. The core, encompassing regions from the scapula to the gluteals, plays a crucial role in athletic performance and injury prevention. The concept of the core has been at the center of attention in many media and scientific journals from the end of the last decade to the present. Since the core is the area that connects the upper and lower limbs, control of core strength, balance, and movement can optimize the entire kinetic chain, which includes isolated athletic gestures of both the upper and lower limbs. Several studies have shown that excellent core stability is associated with better physical performance across all sports. A strong and stable core enhances mobility, speed, and the performance of the lower limbs in athletes. The aim of this article is to highlight the literature examining whether postural variations and core stability are functionally related to performance in sports that require body balance and proper posture, identify gaps and deficiencies in the literature, and suggest further reviews in this area. The literature for this article is based on scientific sources such as MEDLINE, Scopus, Web of Science, PubMed, and the Cochrane Library. It is supplemented by Google Scholar, Springer Link, and Elsevier. A total of 27 publications were reviewed. This article underscores the importance of a comprehensive approach to core training in sports physiotherapy for improving athletic performance and reducing injuries. The results and recommendations presented contribute to advancing knowledge in sports physiotherapy and provide a valuable resource for professionals working with athletes at all levels.

KEYWORDS: *core training, physiotherapy, prevention, rehabilitation, stability, posture*

INTRODUCTION

Football is one of the most popular sports in the world. It is considered a sport with a very high-intensity level and a significant risk of injuries among players. Physical fitness is one of the most crucial elements influencing football performance due to the high physical demands during play.

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As a high-impact sport with injuries occurring in both contact and non-contact situations, football has the highest risk of injury. It has been demonstrated that the risk of injury for professional players is 1,000 times higher compared to other individuals (1).

The frequency of direction changes, frequent accelerations, and sudden decelerations during the game increase the possibility of muscular injuries for football players. Power and speed play a crucial role in the most decisive moments of the sport, which is why sprints and direction changes are more common during goal-scoring situations.

It has been shown that during a high-level football match, a player performs a sprint every 90 seconds, each lasting an average of 2-4 seconds (2). Some studies have found that sprints in matches are short and explosive. Although evidence is limited, integrating basic stabilization exercises into injury prevention programs, especially for the lower extremities, has demonstrated a reduction in the injury rate (3-6).

Men are at higher risk for muscular and joint injuries, so the importance of core strength and muscle strengthening for injury prevention has increased in recent years. Today, core strengthening is crucial in many preventive programs, such as "FIFA 11+."

Even though there is extensive knowledge about the biomechanics of the trunk region, defining the advantages and disadvantages of many analytically researched exercises, the focus has shifted toward a more global view of the human body. In this view, specific movement training tends to replace mere muscle conditioning, aiming for balance in a system where individual components interact harmoniously to achieve a desired function.

Thus, there has been a gradual and parallel shift from the concept of "abdominal muscles" to that of the "core region." The core concept, particularly "Core stability," has been at the center of attention in many media and scientific journals from the end of the last decade to the present. The muscles of this region are responsible for maintaining the stability of the spine and pelvis and assist in generating and transferring force from the trunk to the limbs and vice versa during daily and sports activities. The ability to maintain functional stability and good neuromuscular control of the lumbopelvic region plays a crucial role in preventing and recovering from musculoskeletal pathologies, postural control, and enhancing sports performance.

Some authors have proposed a more functional perspective, describing the core as kinetic chains that facilitate the transfer of force and energy between the lower and upper extremities (7, 8).

Core muscle strengthening protocols are widely practiced today by people of all ages and athletes at various levels. These protocols are composed of different types of exercises. The literature contains many studies demonstrating the effectiveness of "Core training" compared to traditional exercises, but it is not yet fully clear which method is the best to apply. Many muscles acting on the knee joint and the surrounding areas originate from the lumbopelvic region; thus, a loss of control and strength in the core muscles can increase the rotational forces acting on the knee, particularly during the landing phase after a jump, which may lead to ACL injury.

The aim of this article is to demonstrate the existence of therapeutic modalities for the re-education and training of the core and "core stability" based on different rehabilitative specifics, as well as various preventive modalities for muscular and joint injuries. To achieve this goal, it is essential first to identify the aspects that characterize a specific core training regimen.

Core concept

The core concept has been a focal point in media and scientific literature since the end of the last decade (9). However, a precise and universally accepted definition remains elusive, with variations in interpretation based on different authors and contexts.

Historically, the core has been described as a "cylindrical box" encompassing the abdominal muscles in the front, the gluteal and paraspinal muscles in the back, the diaphragm at the top, and the pelvic floor/coxofemoral joint as the base.

Some authors have expanded this definition to describe the core as the "lumbopelvic complex," which includes the lumbar vertebral column, pelvis, coxofemoral joint, and all associated muscles that produce or limit movements of these segments (10).

As suggested by other researchers, the definition of core endurance is context-dependent, varying between biomechanical laboratories, rehabilitation clinics, and sports centers (11-13). This definition must be grounded in engineering and biomechanics principles and the core structures' morphological and functional

characteristics. In light of these considerations, some researchers propose defining the core as the ability of osteoarticular and muscular structures, coordinated by the motor control system, to maintain or return to a desired trunk position or trajectory when subjected to internal or external forces. In training or sports medicine contexts, core endurance can be viewed as a physical quality that can be modified through training or rehabilitation, though it remains context-specific.

In sports, the core is often defined as "the group of all anatomical components between the sternum and the knees, focusing on the abdominal region, lumbar spine, and coxofemoral joint." This perspective is supported by other researchers who suggest that "core muscles" include "all muscles between the shoulders and pelvis that facilitate the transfer of forces from the vertebral column to the extremities" (11, 12). Consequently, the core is considered a muscular corset that functions as a unit to stabilize the entire body, particularly the vertebral column, both during limb movements and at rest. It acts as a bridge connecting the upper and lower body, where forces are transmitted and generated.

Researchers have highlighted the core's role in improving balance, strength, and proprioception during trunk examination, daily activities, and sports (14). In alternative medicine, the core is often viewed as "energy," serving as the source of all limb movements and the origin of all energy, referred to as the "inferior dan tian.".

The core comprises passive and active elements: passive structures include the thoracolumbar column and pelvis, while active structures involve the trunk muscles. These muscles are crucial for maintaining the stability of the vertebral column and pelvis, as well as generating and transferring forces between the trunk and limbs during sports activities.

Since the core connects the upper and lower limbs, controlling strength, balance, and movement in this region can optimize the entire kinetic chain, including upper and lower-limb athletic gestures. Some criteria for enhancing the sports specificity of core stability training programs involve performing exercises on the feet and incorporating three planes of motion. While some studies have analyzed core stability programs (15, 16), there is a noted lack of research specifically evaluating sport-specific core stability programs that apply these criteria, highlighting a limitation in existing studies. It is also important to train core muscles independently, despite their interdependence, through fascial connections, which form a dynamic system that enables efficient movement and force transmission throughout the body.

Core anatomy includes all structures between the scapula and gluteals. Core structures can be categorized into stabilizers, such as the internal and external oblique muscles, which control movement angles eccentrically, and mobilizers, such as the *rectus abdominis* and *iliocostalis*, which accelerate movement concentrically.

The muscles constituting the core are responsible for maintaining posture in various positions and facilitating safe and effective movement through different planes and directions. The core, represented by the coxo-lumbo-pelvic complex, is the center of the kinetic chains from which all upper and lower limb movements originate.

"CORE TRAINING" protocols

In addition to terminological and conceptual changes, "Core training" programs focus on improving strength and neuromuscular control of the core region (17). Core muscle strengthening protocols are used by athletes across various sports and consist of different types of exercises. The definition of a "Core training program" encompasses various types of exercises as follows (18):

- balance training;
- plyometric exercises;
- sport-specific movement exercises;
- proprioceptive exercises;
- joint stabilization exercises.

In the clinical sector, proprioceptive exercises play a crucial role, using unstable surfaces such as Fitballs, Bosu balls, Freeman tables, etc. Some authors (17) believe it is essential to perform training with various loading thresholds, as outlined below:

1. **"Motor control stability"**: stability at low loading thresholds where the central nervous system (CNS) integrates global and local muscles;
2. **"Core strength training"**: high loading thresholds for global stabilizer muscles leading to hypertrophy and functional adaptation;

3. **"Systematic strength training"**: traditional high loading for global mobilizing musculature.

It is essential that the primary objective focuses on local muscles and utilizes a low training threshold to avoid injuries. In the early stages of training, it is crucial for athletes to understand their neuromuscular patterns by training muscles with low loads and then progressing to positions and movements with functional applicability (19, 20).

Therefore, the choice of exercises plays a fundamental role, including the duration of muscle activation and the required patterns, which vary depending on the load and the objective pursued ("Core stability" or "Core strength"). The author (17) reviewed several scientific studies, and the results are presented as follows:

- in the case of "Core strength," activation should exceed 60% of maximal voluntary contraction (MVC) to achieve strength gains, while for "Core stability," activation should be below 25% MVC to achieve benefits in resistance and neuromuscular control;
- "Core stability training" should vary from isolated deep local muscle activations using weights on irregular surfaces; considering the different functional roles of muscles, it is recommended to vary exercises to stimulate the core in a three-plane level and develop stability under global conditions;
- "Core strength" development programs should include flexibility exercises for the abdominal muscles, lower back, hip flexors, and extensors, performed on unstable surfaces accompanied by static and dynamic contractions;
- since a low level of trunk muscle activation (1-3% MVC) is required to stabilize the spine, "Core endurance" plays a primary role compared to "Core strength." Essential exercises include:
    1. curl up;
    2. bird dog;
    3. side bridge;
    4. prone bridge;
    5. weighted squat to strengthen anterior, posterior, and lateral musculature without exceeding the load threshold that poses multiple injury risks (21).

Before using any protocol, a detailed assessment of each individual should be conducted, including their physical condition, goals achieved thus far, and future ones (pain reduction and performance enhancement).

In the field of sports, the predominant use of core training is in the prevention of injuries: it has been established that muscular and joint injuries at the level of the shoulders, knees, and variable movements are often related to deficits in the trunk and abdominal stabilizer muscles (22).

Identifying and correcting any weaknesses in this area by examining their connection to injuries is crucial. High-intensity training leads to changes in muscle structure (hypertrophy) and neural adaptation to external stimuli. These neural adaptations facilitate force generation, increase tissue mobilization, and accelerate the alleviating mechanisms of the nervous system.

Methodology

The training program aims to correct local body weaknesses by improving segmental and global control. This control is achieved by working through the appropriate training threshold. The protocols used have the following objectives:

- increase in joint range of motion (ROM);
- increase in joint stability;
- improvement of muscular performance;
- optimization of movement function.

However, many sports rely on high-threshold training that exclusively conditions global muscles and alters the functionality of local stabilizers, favoring "Core Strength" rather than "Core Stability." Therefore, the ideal approach would be to work with both low-threshold and high-threshold loads, as both are beneficial for enhancing both components. Low-threshold load training primarily focuses on postural control, muscular adaptation, and motor efficiency. High-threshold load training is performed through overload exercises that place more stress on the muscles and induce structural changes (18, 23).

From the literature review, it is still unclear which methods and exercises are most effective for improving performance within a specific discipline. Despite the widespread use of "Core training" in every sport for recovery and prevention, its characteristics need to be better understood.

Athletic gestures in many disciplines are often performed under asymmetric and unstable conditions (one-legged balance, flight phase), involving global movements across three planes (17). Considering the role of the core, to strengthen its musculature and to have a "Core training" program specific to a sport, it is important to:

- perform exercises on unstable surfaces;
- perform exercises while standing, not sitting;
- use body weight instead of machines for muscle development;
- perform unilateral rather than bilateral movements (asymmetric loading);
- execute global rotational movements with a medicine ball.

A comprehensive "Core Training" program should improve strength, power, agility, coordination, body balance, functionality, speed, aerobic and anaerobic mechanisms, flexibility, and both static and dynamic stability of the spine (24). This article uses programs designed to prevent injuries in football players through core strengthening and core stability exercises. Here are three injury prevention programs for football players, focusing on fitness and injury prevention. Incorporating these programs into a regular training schedule can help improve strength, flexibility, stability, and overall conditioning, thereby reducing the risk of injuries.

Program 1: Comprehensive Strength and Conditioning Program (Table I);
Goal: Enhance overall strength, flexibility, and stability to prevent injuries;
Frequency: 3 times per week.

Table I. *Comprehensive strength and conditioning program.*

Day 1: Lower Body Strength and Stability	Day 2: Upper Body and Core Strength	Day 3: Plyometrics and Agility
1- Warm-Up: -Dynamic stretches (leg swings, hip circles)–5 min -Light jogging – 5 min	**1- Warm-Up:** -Arm circles, shoulder shrugs- 5 min -Light jogging – 5 min	**1- Warm-Up:** Dynamic stretches (high knees, butt kicks) – 5 minutes Light jogging – 5 minutes
2- Strength Exercises: -Squats: 3 sets of 10 reps -Deadlifts: 3 sets of 8 reps -Lunges: 3 sets of 12 reps(each leg) -Calf Raises: 3 sets of 15 reps	**2- Strength Exercises:** -Push-Ups: 3 sets of 15 reps -Dumbbell Rows: 3 sets of 10 reps (each arm) -Overhead Press: 3 sets of 10 reps -Plank: 3 sets of 1 minute	**3- Plyometric Exercises:** -Box Jumps: 3 sets of 10 reps -Lateral Bounds: 3 sets of 15 reps -Tuck Jumps: 3 sets of 10 reps
3- Stability and Balance: -Single Leg Romanian Deadlift: 3 sets of 10 reps (each leg) -Bosu Ball Balance: 3 sets of 30 seconds (each leg)	**3- Core Exercises:** -Russian Twists: 3 sets of 20 reps -Leg Raises: 3 sets of 15 reps -Bicycle Crunches: 3 sets of 20 reps	**3- Agility Drills**: -Cone Drills: 3 sets of 5 minutes -Ladder Drills: 3 sets of 5 minutes -Shuttle Runs: 3 sets of 5 minutes
4- Cool-Down: -Static stretching focusing on lower body muscles – 10 minutes	**4- Cool-Down:** Static stretching focusing on upper body and core – 10 minutes	**4-Cool-Down**: Static stretching focusing on full body – 10 minutes

Program 2: Functional Movement and Flexibility Program (Table II);
Goal: Improve functional movement patterns and flexibility to prevent injuries;
Frequency: 3 times per week.

Table II. *Functional movement and flexibility program.*

Day 1: Functional Strength	Day 2: Flexibility and Mobility	Day 3: Stability and Coordination
1- Warm-Up -Foam rolling – 5 min -Dynamic stretches – 5 min	**1- Warm-Up:** -Light jogging – 5 min -Dynamic stretches – 5 min	**1- Warm-Up:** -Foam rolling – 5 min -Dynamic stretches – 5 min
2- Functional Exercises: -Turkish Get-Ups: 3 x 5 reps (each side) -Kettlebell Swings: 3 x 15 reps -Farmer's Walk: 3 sets of 1 min -Medicine Ball Slams: 3 x 10 reps	**2- Flexibility and Mobility Exercises:** -Yoga Flow (Sun Salutations): 10 min -Hip Flexor Stretch: 3 x 30 sec (each side) -Hamstring Stretch: 3 x 30 sec (each side) -Shoulder Stretch: 3 x 30 sec (each side)	**2- Stability and Coordination Exercises:** -Single-Leg Balance: 3 x 1 min (each leg) - Bosu Ball Squats: 3 x 15 reps - Coordination Drills (hand-eye coordination with a ball): 10 min - Resistance Band Walks: 3 x 20 steps
3- Cool-Down: -Static stretching focusing on full body – 10 min	**3- Cool-Down:** -Deep breathing and relaxation – 10 minutes	**3. Cool-Down:** Static stretching focusing on lower body – 10 minutes

Program 3: Aerobic and Anaerobic Conditioning Program (Table III);
Goal: Enhance aerobic and anaerobic fitness to improve overall conditioning and prevent injuries;
Frequency: 3 times per week.

Table III. *Aerobic and anaerobic conditioning program.*

Day 1: Aerobic Conditioning	Day 2: Anaerobic Conditioning	Day 3: Mixed Conditioning
1- Warm-Up -Light jogging – 5 min -Dynamic stretches – 5 min	**1- Warm-Up:** -High knees, butt kicks – 5 minutes -Dynamic stretches – 5 minutes	**1- Warm-Up:** -Light jogging – 5 min -Dynamic stretches – 5 min
2- Aerobic Exercises -Interval Running (3 minu fast, 2 min slow): 6 sets -Continuous Running: 30 min at moderate pace	**2- Anaerobic Exercises** Sprints: 10 x 50 meters with 1-min rest Hill Sprints: 6 sets of 30 meters Shuttle Runs: 5 sets of 20 meters	**2- Mixed Conditioning Exercises:** -Fartlek Training (varying speeds): 30 min -HIIT Circuit (1 min work, 1 min rest): -Jumping Jacks -Burpees, Squat Jumps -Mountain Climbers
3- Cool-Down: -Static stretching focusing on lower body – 10 min	**3- Cool-Down:** - Static stretching focusing on lower body – 10 min	**3. Cool-Down:** Static stretching focusing on full body – 10 min

Here are three different core strength programs for soccer players, each focusing on core stability and strength (Table IV).
General Tips:
- ensure proper warm-up and cool-down sessions to prevent injuries;
- focus on maintaining proper form throughout each exercise;
- gradually increase intensity and difficulty as the player progresses;
- incorporate these programs into a comprehensive training routine that includes strength, flexibility, and agility exercises.

Table IV. *Core strength programs.*

Program 1: Stability and Endurance Focus Frequency: 3 times per week	Program 2: Power and Strength Focus Frequency: 3 times per week	Program 3: Functional Movement Focus Frequency: 3 times per week
Plank Variations -Standard Plank: 3 sets of 1 min -Side Plank: 3 sets of 45 seconds on each side -Plank with Leg Lift: 3 sets of 30 seconds each leg	**Hanging Leg Raises** 3 sets of 10-15 reps	**Turkish Get-Ups** -3 sets of 5 reps on each side
Dead Bug -3 sets of 15 reps on each side	**Medicine Ball Slams** -3 sets of 15 reps	**Stability Ball Pike** -3 sets of 10 reps
Russian Twists -3 sets of 20 reps on each side	**Weighted Russian Twists** -3 sets of 20 reps on each side	**TRX Body Saw** -3 sets of 15 reps
Bird Dog -3 sets of 15 reps each side	**Ab Wheel Rollouts** -3 sets of 10 reps	**Single-Leg Romanian Deadlift** -3 sets of 12 reps on each side
Bicycle Crunches -3 sets of 20 reps each side	**Cable Woodchoppers** -3 sets of 12 reps on each side	**Lateral Band Walks** 3 sets of 20 steps in each direction

RESULTS

One of the main reasons for core training is the development of the capacity to resist movement and create it. The importance of including exercises in the core training protocol that limit certain movements (in this case, rotation) is evident. Physiologically, training "core strength" and "core stability" leads to greater force and power generation in the shoulder, arm, and leg muscles, reducing the risk of injury and increasing speed, agility, power, and endurance (18, 25, 26).

Core stability plays a crucial role in athletic function and performance. Strengthening these muscles impacts the central nervous system, as these muscles help maintain trunk alignment and provide a stable pelvis foundation, thereby preventing instability. This stability enhances overall movement efficiency, dynamic control, and injury prevention during sports activities (27).

Core stability is closely related to preventing and rehabilitating lower limb injuries, as the core serves as the primary point where the lower limbs generate or resist forces produced during movement. A systematic review and meta-analysis evaluated the effects of injury prevention programs incorporating core stability exercises on knee and ACL injuries (27).

DISCUSSION

The aim of the article is to study the existence of therapeutic modalities in enhancing "core stability" by referring to rehabilitative objectives and highlighting the characteristics of the core region.

Many researchers question the significance of the connection between core training and performance, especially regarding injury prevention. Despite its importance in sports, scientific literature does not provide conclusive evidence about the actual impact of core training methods.

Various studies have the predominant view of abdominal muscles in preventing back pain. Lederman highlights the lack of evidence supporting the predictive role of the transversus abdominis muscle and its delayed activation in cases of lower back pain. He argues that common core stability exercises fail to restore the activation time of the abdominal muscles and that most exercises do not significantly improve core strength and endurance (26). "Core stability" is crucial in treating pubalgia, involving the synergistic training of the abdominal, adductor, and lumbar muscles to create a balanced muscular synergy among these groups.

In athletics, there is a lack of scientific evidence regarding the relationship between "core training" and performance. It is clear that all sports disciplines require good stabilization skills and neuromuscular control, considering the three-dimensional movements that demand adequate levels of strength in the trunk and pelvic regions. However, individual disciplines vary in terms of balance and symmetry, requiring a strong link between "core stability" and "core strength" (18).

Despite the lack of strong scientific evidence supporting core training's effectiveness, it remains widely used for prevention, sports performance, and rehabilitation, keeping the debate on its utility ongoing.

CONCLUSIONS

In conclusion, this article highlights the importance of a global approach to core training in sports physical therapy for improving athletic performance and reducing injuries. The core plays a crucial role in providing stability, force transmission, and preventing sports injuries.

Through a comprehensive study of core anatomy, function, various sports injuries, and clinical assessment techniques, this article provides insights for sports physical therapists. Implementing injury prevention programs for football players through core strengthening and core stability programs offers a clear, evidence-based framework for designing various effective programs. By following this approach, athletes can improve their core functions, stability, and performance while reducing the risk of injury.

Several studies have shown that a single exercise is insufficient to strengthen the entire core region; instead, a combination of exercises is needed to optimally strengthen the musculature (25).

Based on scientific research in the field of core stability rehabilitation, there is evidence that low-load exercises of this type can reduce injury rates and influence pain recovery. The results and recommendations presented in this article contribute to the enhancement of knowledge in sports physical therapy and provide a resource for professionals working with athletes of all levels.

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Letter to the Editor

COMMENTARY ON "OSTEOLYSIS IN TOTAL HIP ARTHROPLASTY IN RELATION TO METAL ION RELEASE: COMPARISON BETWEEN MONOLITHIC PROSTHESES AND DIFFERENT MODULARITIES". WHAT IS THE EVIDENCE ON THE REAL PROS AND CONS?

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Dear Editor,

The progress in the field of total hip arthroplasty (THA) has been immense over the past 20 years or so, and outcomes have definitely improved. However, there are still significant debated aspects that need further evidence and research. A great potential for better results has been brought by the introduction of modular THA implants. Their advantages and disadvantages have been hypothesized and studied, but uncertainty still remains. Particularly, further evidence is needed to establish internationally accepted indications and guidelines, able to resolve doubts, and provide surgeons with the appropriate knowledge to balance the pros and cons of the available surgical alternatives and conservative strategies in revision surgery (1-3).

A major issue of total hip arthroplasty implants is the presence of active corrosion processes at the metallic surfaces and the release of particles due to wear. These processes are thought to be more frequent in modular implants (4-6). The active corrosion process of metallic surfaces and the release of particles due to wear are a source of soluble metal ions (predominantly Cobalt and Chromium (CoCr) (7). The particles are degraded by macrophages and eliminated. However, the precise mechanisms underlying these processes are still unknown. Certainly, there is a strong inflammatory response against the particles and ions around the implants, which subsequently causes loosening and implant failure (1-4).

The paper we are commenting on sought to investigate the presence of any association between serum and urine concentrations of metal ions released in THA and periprosthetic osteolysis for modular neck and monolithic implants, with clinical, radiographic, and tribological insights (1). A significant number of patients were included in the study groups (monoblock, modular with metal head, and modular with ceramic head) and a mid-term follow-up was reached (4 years on average). The presented data included radiological evaluation (to detect any area of osteolysis around the prosthesis of both the femur and the acetabulum) and serum and urinary tests (to assess the values of Chromium and Cobalt released) (1).

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The evidence provided supports clear biomechanical advantages of modular implants but with the side effects of higher metal ion release and a greater prevalence of osteolysis. This should be well considered by surgeons when deciding what type of procedure to perform. Surgeons should appropriately balance which risks to assign to patients based on the specific clinical scenario, patient characteristics and mobility, functional goals, and specific implant indications. With our commentary, we would like to highlight the strengths and weaknesses of the published article and use the evidence provided to further discuss the matter, integrating the most recent available evidence-based knowledge with the final aim of delineating strong recommendations for surgeons when deciding on the type of THA implant to use.

Most of the previously published studies have correlated the elevation of ion levels to the type of prostheses (8-14). Osteolysis phenomena are rarely taken into account, and few clinical follow-ups have been reported. There is a particular lack of follow-ups longer than 3 years. On the other hand, the commented study has compared modular and monoblock implants clinically, radiographically, and tribologically, attempting to investigate the presence of any association between serum and urine concentrations of metal ions released in THA and periprosthetic osteolysis for modular neck and monolithic implants (1).

The combination of these data is rarely found in the available literature, and the presented results provide a significant boost to evidence-based knowledge on the topic. In fact, the paper provides both quantitative and qualitative data regarding the release of the most common periprosthetic metal ions in THAs (Cr and Co) and the presence of periprosthetic osteolysis. The evidence provided supports clear biomechanical advantages of modular implants but with the side effects of higher metal ion release and a greater prevalence of osteolysis. This should be well considered by surgeons when deciding what type of procedure to perform.

The commented study also reported a higher incidence of osteolysis in the modular group, almost the only group presenting grade 3 osteolysis. Serum and urinary chromium and cobalt values were also higher in the modular groups, with the highest levels in the metal head implants. Statistical linear correlation test results suggested positive correlations between increasing metal concentrations and incidences of osteolysis. However, no cases of pseudo-tumor were detected (1).

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