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Squat kinematics of osteoarthritic knees after intra-articular viscosupplementation: an analysis of secondary outcomes from a double-blinded randomized controlled trial

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Abstract

Background Viscosupplementation for knee osteoarthritis (OA) aims to minimize pain and improve joint function. However, its effects on knee biomechanics during squat activities have not been investigated. This study aimed to assess the effects of viscosupplementation on squat biomechanics of older adults with late-stage knee osteoarthritis utilizing three-dimensional (3D) motion capture technology.

Methods This study is a multiple-blinded, randomized, single-center, placebo-controlled trial with a 1:1 allocation ratio. Forty-two older individuals (72.6 ± 6.5 years) with advanced knee OA were randomly allocated into two groups to receive viscosupplementation or placebo (saline injection). Kinematic data were collected by a 3D motion analysis system 1 week before and 1, 6, and 12 weeks after the intervention. Dependent variables included maximal vertical displacement of center of mass (CoM), CoM position in the mediolateral axis, knee range of motion between initial and lowest CoM vertical position, and knee angles at lowest vertical CoM position in sagittal, coronal and axial planes (primary outcomes). Data were compared between groups using mixed linear models, with a significance level of 0.05. A repeated measures ANOVA was conducted within each group to assess changes over time if significant differences between groups were observed.

Results The viscosupplementation group showed a statistically significant difference in maximum knee internal rotation at lowest vertical CoM position (4.1° 95%CI [0.6 to 7.5]– $p = 0.02$) during squat at 12 weeks. None of the other variables showed statistically significant results ($p > 0.05$). There was no difference in knee internal rotation angle at 1, 6, or 12 weeks compared to baseline in the viscosupplementation group ($p = 0.307$).

Conclusion This study suggests that a single shot of intra-articular viscosupplementation may help preserve knee biomechanics during squatting in patients with late-stage knee OA in the medium term. Future studies should explore the relationship between biomechanical improvements and clinical symptoms.

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Keywords Knee, Osteoarthritis, Kinematics, Motion analysis/Kinesiology, Viscosupplementation

Introduction

Knee osteoarthritis (OA) is a prevalent degenerative condition with a rising incidence in the aging population, as established by prior research [1]. Notably, data from the United States demonstrate a prevalence of 19% of symptomatic knee OA in individuals aged 45 and older [2]. Individuals with knee OA report pain, loss of functionality, and decreased quality of life for several years before a definitive treatment [3, 4]. According to the World Health Organization, osteoarthritis is responsible for the second largest financial and social burden globally and causes functional limitations for at least 526 million people [5]. As a result, there has been a global initiative to explore cost-effective, non-surgical treatment options. However, studies evaluating these treatments' outcomes predominantly rely on self-reported measures, which may lack the precision needed to comprehensively assess joint functionality [6]. Therefore, the use of tools that provide reproducible and objective outcomes, such as three-dimensional (3D) motion analysis, is crucial for evaluating the effects of conservative treatments.

Squatting is one of the most frequent motor tasks in daily activities and it is a validated measure to assess lower limb functionality [7, 8]. It resembles functional daily activities such as sitting down and getting up from a chair. Pain and difficulty with squatting are frequently reported symptoms as knee OA progresses [9]. Previous studies reported kinematic impairments during squat in individuals with knee OA, such as less tibial internal rotation, greater hip adduction angles, and medialization of the femur relative to the tibia among others, which represents loss of functionality [10–12]. The screw-home mechanism and adequate tibial internal rotation have gained particular attention due to their influence on knee function. Loss of tibial internal rotation is commonly reported as knee OA progresses and is recognized as a biomechanical marker of disease progression [13–15]. Furthermore, targeted exercises designed to improve tibial rotation have demonstrated early evidence of enhancing clinical symptoms and biomechanical parameters during gait and squatting [16].

Intra-articular viscosupplementation of the knee with hyaluronic acid has emerged as a therapeutic choice to avoid systemic side effects. Some positive effects expected with viscosupplementation are pain reduction and functional improvement compared to oral anti-inflammatories or steroid injections, with lower adverse effects [17]. Several clinical trials and meta-analyses have evaluated the effect of viscosupplementation on knee OA, with controversial results [18]. While recent studies have reported objective outcomes of viscosupplementation - including muscle strength, joint ROM, and synovial biomarkers [19, 20] - there remains a lack of investigations examining dynamic motor behavior during real-world activities such as squatting [21]. To address this gap, some investigations have explored functional outcomes in the context of gait analysis [22]. However, there is a notable scarcity of studies investigating the effects of viscosupplementation on activities such as squats or high-flexion movements.

This study aimed to analyze changes in squat biomechanics in patients with knee OA after viscosupplementation. In this study, it is hypothesized that viscosupplementation will lead to significant improvements in squat biomechanics.

Materials and methods

Trial design

This trial is designed as a multiple-blinded (patient, data collectors, outcome assessors, and data analyst) randomized, single-center, placebo-controlled trial with two parallel groups. Allocation was 1:1. There were no changes to the methods after trial commencement.

The trial protocol is in accordance with Helsinki Declaration, National Health Council Resolution, 466/12, and was approved by the ethics committee of the National Institute of Trauma and Orthopedics (INTO), number: 2.308.876 and Federal University of São Paulo (UNIFESP), number: 3.408.775. The trial was previously registered at the Brazilian Clinical Trials Registry (No. RBR-3n52h4).

The study adhered to the CONSORT guidelines for reporting randomized controlled trials [23].

Patients and study setting

This study took place at a military tertiary hospital, accessible to militaries and their relatives, and in a private motion analysis laboratory, accessible to the general public. Recruiting and intervention were conducted in the Hospital and all data collection was in the motion analysis laboratory. The eligible population consisted of patients on the Hospital waiting list for total knee arthroplasty. The first contact for recruiting and screening was given by two orthopedic surgeons by phone, following the order from the oldest to the most recent in the queue. After initial screening that involved identity and age confirmation, patients were scheduled for an in-person

evaluation in the Hospital facility. All patients underwent weight-bearing anteroposterior and lateral knee x-rays.

The inclusion criteria were: patients over 60 years old, with Kellgren-Lawrence grades III and IV knee OA, and who were able to walk without any assistance during the tests [24]. The exclusion criteria were: neurologic functional impairment, secondary OA (i.e., trauma, septic arthritis, crystal-related disease, rheumatoid), ipsilateral or contralateral symptomatic joint disease, general health conditions that affect mobility, and history of previous knee joint injections in the last 6 months. Those who did not agree with the research terms or refused to perform the kinematic evaluation were excluded from the study. No restrictions were made based on gender, body mass index, and level of pain. No benefits were offered for participation in the study.

Although viscosupplementation is commonly studied in mild to moderate OA, we targeted patients with advanced disease (Kellgren-Lawrence grade III/IV) to ensure a homogeneous clinical profile. All participants were already approved for total knee arthroplasty, minimizing variability in treatment plans and allowing for a clearer assessment of the intervention's biomechanical effects.

Sample size

This is an analysis of the secondary outcomes of a randomized controlled trial [22]. The original study was powered to address research questions related to gait. Subjects performed the squats at the same time as the gait testing of the original study.

The post-hoc power analysis revealed that, with the given sample size of 42 participants, there was an 80% chance of detecting a treatment difference at a two-sided 0.05 significance level for the following biomechanical measurements: 9.8 cm for maximal vertical displacement of center of mass (CoM), 1.4 cm for mediolateral lowest vertical CoM position, 21.8° for knee flexion angle, 25.6° for sagittal plane knee range of motion (RoM), 10.0° for knee angle in the coronal plane, 4.8° knee coronal RoM, 10.4° for knee angle in the axial plane and 5.1° for knee axial RoM.

The selected biomechanical variables serve as indicators of squat performance. These values are not meant to represent minimal clinically important differences or thresholds of clinical significance, as such parameters have not been established. The power calculations were based on standard deviations listed for each variable in Table 1, at 12 weeks. Assumptions made during these calculations included the normal distribution of data.

These findings should be interpreted with an understanding of the limitations inherent in post-hoc analyses, particularly in terms of statistical power and the exploratory nature of the analysis. The power to detect smaller differences in these secondary outcomes may be limited due to the original study's sample size, which was calculated with primary outcomes in mind. Thus, while significant findings in these areas are robust, non-significant findings should be interpreted cautiously.

Randomization

Sequence generation

For allocation of the participants, a computer-assisted simple randomization was performed using "http://randomization.com/" with a 1:1 allocation. The allocation arrangement was placed in sequentially sealed and opaque envelopes. The individual responsible for this task was one of the authors who was not involved with the intervention procedure.

Allocation concealment mechanism

Sealed and opaque envelopes were used to ensure allocation concealment. The envelopes contained the random allocation sequence, which consisted of a unique number for each subject and the assigned intervention. Each of these envelopes was opened just prior to the injections, after completion of all pre-injection procedures, as described in the Intervention section above.

Intervention

The procedures were performed at the ambulatory surgery center of the Hospital. A systematic process was created and trained by the physicians and nurses to assure allocation concealment, providing the same experience to every subject. Videos demonstrating this systematic process can be found in the supplementary material of Metsavaht et al. [22].

Table 1 Demographic characteristics of the participants

	Placebo Group	Intervention Group
Sex (M/F)	5/16	5/16
Age (mean ± SD)	73.2 ± 7.8 years	71.8 ± 5.4 years
Body mass (mean ± SD)	81.8 ± 18.0 kg	79.0 ± 10.7 kg
Height (mean ± SD)	159 ± 0.08 cm	159 ± 0.09 cm
Body Mass Index (mean ± SD)	32.5 ± 7.1 kg/m^2	31.2 ± 4.6 kg/m^2
Kellgren-Lawrence Classification (KL)(n)	13 (KL III) / 8 (KL IV)	13 (KL III) / 8 (KL IV)

SD: Standard deviation. M: Male. F: Female

All the injections were performed by the same orthopedic surgeon and assistants. The surgeon was fellowship-trained in knee surgery, board-certified, and had more than 15 years in practice.

The procedure started with the patient in supine position with knees extended at the surgical table. The knee was scrubbed with antiseptic solution following the surgical preparation technique, and was prepped with a knee arthroscopy surgical field, blinding the patient's view to the injection procedure. Skin anesthesia with 2 ml of lidocaine 2% in a 3 ml syringe with a 0.3 mm/13 mm needle was performed at the superolateral aspect of patellofemoral joint of all patients. After that, a sealed envelope containing the randomization order was opened and the name of the patient and knee laterality were written. The paper was sealed and returned to the same envelope. All the envelopes were kept in a secured folder by an assistant who was not involved in the other procedures.

According to the assignment to the viscosupplementation group or placebo group (placebo) either 4 ml of the active 80 mg high-density hyaluronic acid associated with 160 mg sorbitol (Synolis VA®, Genebra, Switzerland) or 4 ml of sodium chloride at 0.9% for the placebo group were injected. The hyaluronic acid injection had a molecular weight of 2 MDa in its sterilized form, was non-crosslinked, derived from biofermentation (non-animal origin), and was phosphate-buffered to quantity sufficient. All material used was always kept out of sight of the patient. All infiltrations were performed with a 0.8 mm/40 mm needle following the superolateral knee approach [25] technique and were guided under direct ultrasound visualization (Toshiba Nemio 17, 2018) to ensure the intraarticular placement of the needle at the time of injection of viscosupplementation or saline solution. After the infiltration, all supplies were disposed of before the surgical field was removed to prevent loss of allocation concealment.

Post-treatment protocol

All patients received written aftercare instructions, orientation about possible side effects, and a 24/7 exclusive emergency phone number for 2 blinded orthopedic surgeons, who did not participate in the intervention, data collection, or data analysis. They were instructed and trained to provide general orientations and decide on any necessary follow-up appointment. All patients were oriented to maintain their non-pharmacological treatment routines such as braces, insoles, and physical therapy. Analgesics and non-steroidal anti-inflammatories were allowed as rescue drugs for pain, with clear instructions to avoid taking any medications at least 48 h before every analysis. Medications containing corticosteroids (creams, capsules, or injections) were not allowed. Unblinding by attending surgeons and patients was permissible if fever, inability to move the knee, disproportional pain, swelling, or redness localized on the knee were reported or any situation considered necessary for guaranteeing safety of patients.

Data collection

Kinematic data of squat was collected at a private motion analysis laboratory. The data collection team did not have access to the allocation of the participants in the groups. Motion capture was performed with an optoelectronic system with an 8-camera high-speed motion analysis system (VICON, Oxford Metrics, UK), with a sample frequency of 100 Hz. Reflective markers were placed bilaterally on specific anatomical landmarks, including the anterior and posterior superior iliac spines, iliac crest, medial and lateral femoral epicondyles, tibial tuberosity, fibular head, medial and lateral malleoli, as well as on the foot at the first, second, and fifth metatarsal heads and the calcaneus (heel). Rigid shells with four reflexive markers attached to the leg and shank were used to describe 3D knee motion [26] with mathematical models already used in prior studies [27].

Evaluation of the dynamic bilateral squat began with the individual in an upright position, knees, and hips fully extended. Initially, subjects performed 3 squat repetitions to familiarize themselves with the task. A total of 6 repetitions were recorded and analyzed. Patients were instructed to squat as depth as possible six times without removing the heel from the ground and to return to the initial upright position after this. They were asked to keep the arms crossed around the trunk, feet parallel at shoulder with, and pointing forward. There was no command about squat speed or feedback regarding squatting form [20].

Data processing

The coordinates of each marker were filtered by a second-order low-pass Butterworth filter applied in the direct and reserve directions to avoid phase distortions, with a cutoff frequency of 6 Hz. 3D knee angles were calculated according to Grood and Suntay [28]. The orthostatic posture of each individual was initially used for purposes of calibration, anthropometric, and inertial parameters. A functional calibration trial was used to calculate knee and hip joint centers [29, 30].

Data was collected one week before (baseline) and 1, 6, 12 weeks (W1, W6, W12) after intervention, with variables observed being the maximal vertical displacement of center of mass (CoM Vert), position of center of mass (CoM) in the mediolateral axis at lowest vertical CoM position (CoM Lat), knee range of motion (RoM) between initial and lowest CoM vertical position in the sagittal, coronal, and axial planes and the knee angle in

the sagittal, coronal and axial plane at lowest vertical CoM position.

The CoM was estimated based on the geometric center of the pelvis, calculated as the arithmetic mean of the coordinates from four reflective markers placed at the following anatomical landmarks: right and left anterior superior iliac spines and right and left posterior superior iliac spines [31].

Data analysis

All biomechanical data were processed using custom-written routines in Matlab 2015 (The Mathworks, USA). Results were analyzed by comparing the kinematics differences between the injected knees of both groups in W1, W6, and W12.

A Student's t-test was conducted to compare the demographic characteristics between groups at baseline. All statistical procedures were performed according to the principles of intention to treat, and missing data was imputed as the last observation carried forward. Initially, descriptive analyses and histogram inspections were conducted to determine the probability distribution of data. The between-group comparisons to obtain the mean effects were conducted employing interaction terms (group versus time interactions) within linear mixed models. The differences were considered statistically significant when $p \leq 0.05$. The software SPSS (Version 19, IBM Corp.) was used for analyses. The statistician responsible for data analysis received a codified spreadsheet with de-identified data and remained blinded to group allocation.

When differences between groups were observed, a repeated measures ANOVA was conducted to evaluate changes within the group across four time points (baseline, 1 week, 6 weeks, and 12 weeks) to assess whether there was an improvement in the biomechanical variable over time. Mauchly's Test of Sphericity was performed to assess the sphericity assumption. Corrections (Greenhouse-Geisser) were applied when the assumption was violated. Statistical significance was set at $p < 0.05$.

Results

Sample

Recruiting occurred from February 2018 to June 2018. Of 156 patients assessed for eligibility, 42 met inclusion criteria and were enrolled in the randomization protocol for viscosupplementation or saline injection (Fig. 1). Of the initial 42 patients, four did not complete the entire follow up period (two in each group) and were included in the study with their data imputed as last observation carried forward. In the viscosupplementation group, two patients did not perform the 6-week and 12-week evaluations. One due to a stroke and another reported difficulty going to the collection site on the scheduled days. In the placebo group, one patient did not return for evaluations at 6 weeks and 12 weeks, also reporting difficulty in attending the collection site, and another did not return for analysis at 12 weeks due to Chikungunya fever. Local pain lasting less than 24 h was the only adverse event reported ($n = 1$). No patients or research team members reported unblinding during the study.

Demographics

Mean participant age was 72.6 years (standard deviation [SD] 6.5, minimum-maximum 64–91), mean body mass index (BMI) was 31.8 kg/m^2 (SD 5.9, minimum-maximum 23.7–50.1), and 76% of the patients were female. No significant differences in demographic characteristics were observed between groups ($p > 0.05$), as detailed in Table 2.

Baseline measures

All measures were similar between groups ($p > 0.05$), except the maximum knee angle on coronal plane (Table 1).

Primary outcomes

On the axial plane, viscosupplementation group showed a statistically significant improvement in knee internal rotation angle (tibial rotation) at lowest vertical center of mass (CoM) position (4.1° 95%CI [0.6 to 7.5]– $p = 0.02$) during squat at 12 weeks (Table 3). At one and six weeks, tibial internal rotation angle at lowest CoM were not statistically different between groups.

On the coronal and sagittal plane, knee angles at lowest vertical CoM position were not statistically different between groups at 1, 6, and 12 weeks.

The range of motion between initial and lowest CoM vertical position on the axial, coronal, and sagittal plane was not statistically different at 1, 6, and 12 weeks.

The repeated measures ANOVA indicated no significant difference in knee internal rotation angle within the viscosupplementation group across the four time points ($p = 0.307$). Mauchly's Test of Sphericity was not significant ($W = 0.981$, $p = 0.980$), indicating that the assumption of sphericity was met, and no corrections were necessary.

Secondary outcomes

Lowest vertical and lateral position of CoM were not statistically different between groups at 1, 6 or 12 weeks (Table 3).

Discussion

The research presented examined the effects of viscosupplementation on the knee kinematics of individuals with advanced osteoarthritis. The main finding of this study is that the group receiving viscosupplementation

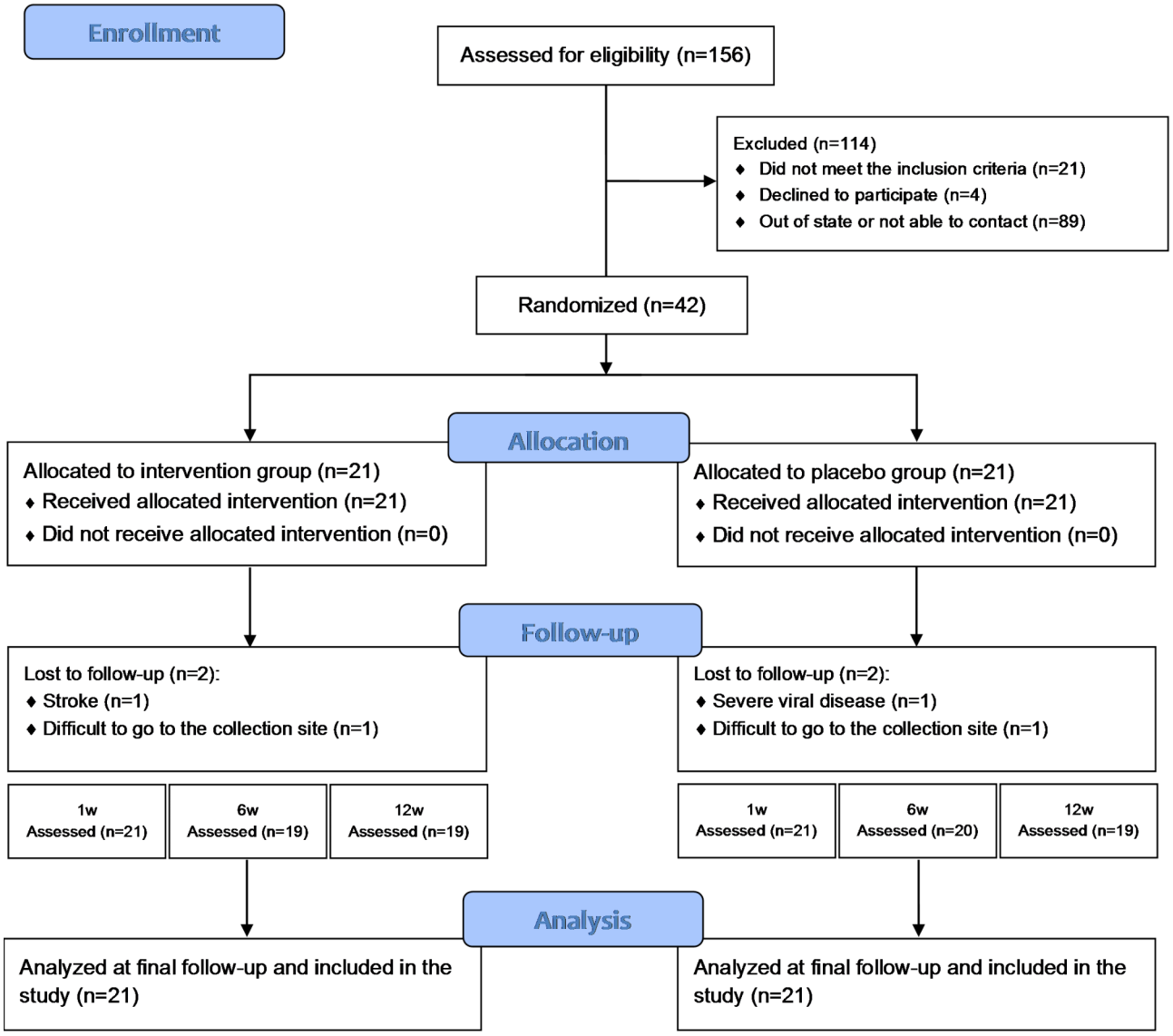

Fig. 1 Enrollment diagram for sampling and randomization. All 156 patients on the waiting list for total knee arthroplasty were assessed for eligibility and 114 were excluded. The remaining 42 were randomized and allocated into two groups of 21 patients. Two patients of each group did not complete all follow-up visits and had their data imputed as "last observation carried forward" for final statistical analysis

demonstrated higher tibial internal rotation during squatting after 12 weeks, compared to the placebo group. This suggests that a single injection of hyaluronic acid may positively influence a biomechanical parameter closely associated with functional decline in late-stage knee OA [14, 15]. However, intra-group analysis revealed no significant change from baseline, suggesting that viscosupplementation may play a role in preserving joint function and mitigating further decline, rather than directly enhancing knee internal rotation.

The 4.1° difference (95% CI: 0.6 to 7.5) in maximal internal rotation is nearly four times greater than the differences observed in the first and sixth weeks (W1: 1.0°; 95% CI: -2.6 to 4.6; W6: 1.3°; 95% CI: -2.4 to 5.1), as shown in Table 1. This indicates a meaningful change in this parameter over time, supporting the hypothesis of a delayed protective effect.

The clinical significance of this finding is that the difference in knee internal rotation between groups can be interpreted as a decreased decline in joint functionality in the viscosupplementation group. Loss of internal rotation is frequently reported on gait among this population and is a marker of disease progression [13–15]. Therefore, worsening of knee function was expected in this population due to the advanced disease. The observed difference in knee internal rotation at 12 weeks indicates that the placebo was less effective at preserving knee function over time. This could reflect a protective effect

Table 2 Mean (Standard Deviation) of angular Biomechanical outcomes at baseline and 1 week, 6 weeks and 12 weeks after the viscosupplementation and placebo injection

Outcome	Intervention							
	Baseline		1 week		6 weeks		12 weeks	
	Visco	Placebo	Visco	Placebo	Visco	Placebo	Visco	Placebo
CoM Vert (cm)	17.4 (12.1)	19.6 (9.3)	17.9 (10.3)	21.0 (9.3)	18.1 (10.8)	19.9 (8.2)	18.3 (11.1)	21.0 (9.5)
CoM Lat (cm)	0.3 (1.6)	0.1 (2.1)	0.2 (1.4)	0.4 (1.4)	0.6 (1.6)	-0.1 (1.3)	0.4 (1.6)	-0.1 (1.5)
Flex/ext (°)	73.9 (24.2)	76.1 (21.8)	72.0 (19.7)	76.6 (22.8)	71.1 (23.6)	73.2 (20.2)	71.9 (24.6)	75.9 (20.4)
RoM Flex/ext (°)	62.8 (28.8)	66.7 (22.1)	62.3 (24.3)	67.9 (21.4)	64.2 (26.3)	66.3 (19.3)	63.2 (28.9)	69.9 (20.1)
Varus/Valgus (°)	1.7 (8.1)	-3.2 (9.9)	0.9 (8.6)	-3.7 (10.9)	-0.4 (8.0)	-4.3 (11.5)	0.5 (8.0)	-3.8 (11.3)
RoM Varus/Valgus (°)	0.3 (5.5)	1.1 (7.0)	-0.9 (5.5)	2.1 (7.3)	-1.3 (5.2)	0.3 (6.1)	-1.1 (5.4)	-0.1 (5.5)
Int/Ext (°)	-7.6 (9.7)	-10.2 (12.2)	-8.7 (8.6)	-12.0 (12.8)	-7.4 (9.9)	-11.0 (12.1)	-7.1 (9.4)	-13.3 (11.8)
RoM Int/Ext (°)	3.7 (6.9)	1.1 (6.5)	3.0 (7.7)	-0.4 (6.7)	3.0 (5.2)	0.1 (6.3)	3.2 (4.6)	-0.4 (5.8)

Visco: Viscosupplementation group; ROM: maximum range of motion between initial and lowest CoM vertical position; CoM Vert: maximal vertical displacement of center of mass; CoM Lat: position of CoM in the mediolateral axis at lowest vertical CoM position. Flex/ext: flexion(+)/extension(-) of knee in sagittal plane at lowest vertical position of center of mass; Varus/Valgus: knee varus(+)/valgus(-) on coronal plane at lowest vertical position of center of mass; Int/Ext: knee rotation internal(+)/external(-) in the axial plane at lowest vertical position of center of mass

Table 3 Differences between groups for angular Biomechanical outcomes 1 week, 6 weeks, and 12 weeks after the viscosupplementation and placebo injection

Outcome	Difference between interventions Adjusted Mean Difference (95% CI)					
	Baseline to Follow-Up at 1 Week (95% CI), *p*		Baseline to Follow-Up at 6 Weeks (95% CI), *p*		Baseline to Follow-Up at 12 Weeks (95% CI), *p*	
	Visco vs placebo	*p*	Visco vs placebo	*p*	Visco vs placebo	*p*
CoM Vert (cm)	-1.2 [-3.8 to 1.3]	0.33	-0.1 [-3.0 to 2.7]	0.92	-0.9 [-4.0 to 2.1]	0.53
CoM Lat (cm)	-0.3 [-0.8 to 0.3]	0.37	0.6 [-0.3 to 1.4]	0.17	0.5 [-0.3 to 1.2]	0.25
Flex/ext(°)	-2.7 [-8.6 to 3.1]	0.35	-0.3 [-7.6 to 6.9]	0.92	-2.2 [-10.1 to 5.7]	0.57
RoM Flex/ext (°)	-2.4 [-8.5 to 3.6]	0.42	0.7 [-7.9 to 9.2]	0.87	-3.7 [-12.7 to 5.4]	0.42
Varus/Valgus (°)	-0.3 [-2.7 to 2.1]	0.79	-1.0 [-3.7 to 1.7]	0.46	-0.4 [-3.2 to 2.4]	0.77
RoM Varus/Valgus (°)	-2.4 [-5.1 to 0.3]	0.08	-1.1 [-3.4 to 1.2]	0.34	-0.6 [-3.1 to 1.9]	0.65
Int/Ext(°)	1.0 [-2.6 to 4.6]	0.56	1.3 [-2.4 to 5.1]	0.47	4.1 [0.6 to 7.5]	0.02*
RoM Int/Ext (°)	1.2 [-1.8 to 4.3]	0.42	1.1 [-1.2 to 3.4]	0.34	2.33 [-0.3 to 5.0]	0.09

Visco: Viscosupplementation group; ROM: maximum range of motion between initial and lowest CoM vertical position. CoM Vert: maximal vertical displacement of center of mass; CoM Lat: position of CoM in the mediolateral axis at lowest vertical CoM position. Flex/ext: flexion(+)/extension(-) of knee in sagittal plane at lowest vertical position of center of mass; Varus/Valgus: knee varus(+)/valgus(-) on coronal plane at lowest vertical position of center of mass; Int/Ext: knee rotation internal(+)/external(-) in the axial plane at lowest vertical position of center of mass.* $p < 0.05$

of viscosupplementation, potentially slowing the progression of functional decline.

This study provides high-quality preliminary evidence on the impact of viscosupplementation on knee biomechanics in advanced OA patients. It builds on previous research that analyzed gait kinematics after viscosupplementation. The study's design employed a placebo intervention, which provides a safe foundation for group comparisons. The placebo effect of interventions is well-known, and it increases with invasiveness, which means that injections and surgeries will usually cause the highest placebo effects [32, 33]. This characteristic often makes it difficult to compare invasive treatments like injections with non-invasive interventions such as physical therapy, oral medications, or others.

To the best of our knowledge, no studies so far have evaluated the effects of viscosupplementation on knee OA during squat activity, making clear comparisons with

previously reported data difficult. However, these results are in line with previous findings from a similar study conducted by this group that analyzed gait in the same population using similar methodology. Also, at 12 weeks post-viscosupplementation, Metsavaht et al. reported an increase of 4° in maximum tibial internal rotation compared to placebo group, which represents 36.3% of the total range of motion [22]. Other three studies that reported data on the effects of viscosupplementation on gait, did not analyze the axial plane, which impairs further comparisons [34–36].

Analyzing the coronal plane, there was no statistically significant difference in knee angles at the bottom position or range of motion after viscosupplementation. There has been reported increased knee adduction moment after viscosupplementation in two previous studies that analyzed gait [34, 35]. A possible reason for the divergent results could be due to differences in the tasks being analyzed. Bilateral squats normally produce smaller knee adduction moments compared to the gait [37]. Moreover, during squats, the knee angles on the coronal plane normally do not vary excessively with knee flexion and remain more stable than during the gait cycle [37, 38]. Therefore, squats may not be provocative enough in the coronal plane to induce kinematic impairments on the knee that viscosupplementation can improve. Finally, the inclusion of individuals with varus and valgus alignment could be a possible reason for the divergent results compared to the gait studies [34, 35]. These reported studies only included individuals with medial knee OA. A subgroup analysis considering static and dynamic alignment could evidence different results for the present study.

In the sagittal plane, the range of motion and maximum knee angle at the bottom position did not demonstrate any statistically significant differences between the two groups. Loss of active knee flexion and extension has been extensively reported in the context of OA, particularly in gait analysis and activities involving loaded knee flexion, such as squatting [12, 39]. It is worth noting that an indication of improvement following viscosupplementation would typically manifest as an increase in knee flexion during this task. However, in our study, such improvements were not observed in the sagittal plane parameters.

The lowest vertical position of CoM and the position of CoM in the mediolateral axis at lowest vertical CoM position were not statistically different among groups. As a proxy for improvement, we anticipated that higher values for CoM vertical displacement would signify enhanced functionality, as individuals would be capable of reaching lower positions during the squat. Regarding CoM lateral position, a decrease in the values was expected, indicating reduced weight shift to the contralateral knee. These anticipated changes would have been indicative of improved biomechanical function and balance during squatting. However, the observed data did not align with these expectations in our study.

This study has limitations that can affect the interpretation of the results. First, the follow-up period was 12 weeks, and any generalization to longer-term outcomes should be made with caution. Additionally, variance in maker positions due to human error must be taken into account, but this issue was minimized by a highly trained team and appropriate blinding at multiple levels. This study has included mostly patients with medial knee OA. Overall results can be influenced by different kinematics of patients with lateral knee OA. Results could be generalized with caution to patients of both sexes, medial or lateral knee OA, Kellgren-Lawrence grade 3 and 4, with more than 60 years old. By including patients regardless of mechanical limb axis alterations and patients waiting for total knee arthroplasty, we have covered cases with very advanced disease. Results could be better in patients with mild to moderate disease severity. Moreover, we opted for a pragmatic approach regarding non-pharmacological treatment routines. This strategy offers less strict control over physical therapy interventions; however, it enhances the external validity of the study and reflects how treatment is typically applied in practice. Since randomization and allocation concealment were properly implemented, any variability in physical therapy treatments would be balanced between the groups. Furthermore, individuals performed squats at different flexion angles, and although there were no differences across time points, examining knee internal rotation at a standardized angle (e.g., 90° of flexion) could provide more precise insights into the true effects of viscosupplementation. Lastly, subgroup analysis was not performed. A recent study presented different motion profiles in the same population included in this study [40]. It is possible that selected biomechanical subgroups may have a better response to viscosupplementation.

Conclusions

This study provides early evidence that patients with late-stage knee OA may prevent functional decline in knee biomechanics during squatting after a single shot of viscosupplementation in the medium term. The main outcome observed was a decline in knee internal rotation at the bottom position during a bilateral squat in the placebo group at 12 weeks, which was not observed in the viscosupplementation group. This indicates a functional decline in the placebo group and potential preservation of knee internal rotation in the viscosupplementation group. Further studies should confirm those findings, investigate the long-term effects and explore the impact on selected biomechanical subgroups.

Abbreviations

3D	Three–dimensional
ANOVA	Analysis of Variance
BMI	Body Mass Index
CI	Confidence Interval
CoM	Center of Mass
CoM Lat	Position of the center of mass in the mediolateral axis at lowest vertical CoM position
CoM Vert	Maximal vertical displacement of the center of mass
CONSORT	Consolidated Standards of Reporting Trials
Flex/Ext	Flexion/Extension
INTO	National Institute of Trauma and Orthopedics
Int/Ext	Internal/External rotation
MDa	Megadalton
OA	Osteoarthritis
PROs	Patient–Reported Outcome Measures
RoM	Range of Motion
SD	Standard Deviation
TKA	Total Knee Arthroplasty
W	Mauchly's Test of Sphericity Statistic
W1	1 week
W6	6 weeks
W12	12 weeks

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The trial protocol is in accordance with the Helsinki Declaration, National Health Council Resolution 466/12, and was approved by the ethics committee of the National Institute of Trauma and Orthopedics (INTO), number: 2.308.876, and the Federal University of São Paulo (UNIFESP), number: 3.408.775. The trial was previously registered at the Brazilian Clinical Trials Registry (No. RBR-3n52h4). Informed consent terms were obtained from all participants.

Consent for publication
Not applicable.

Competing interests
This study was financed in part by Aptissen. The company funded the hyaluronic acid injections, disposable supplies, and third-party services. Aptissen had no role in the design or realization of the study, including data analysis, interpretation, and preparation of the manuscript. Aptissen agreed to the publication of positive or negative results by the research team without sponsor review. All healthcare professionals and patients were unpaid volunteers. Each of the authors certifies that they have no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article.

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