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Exercise Physiology

Administration of sodium hyaluronate to adult horses prior to and immediately after exercise does not alter the range of motion in either the tarsus or metacarpophalangeal joints

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Abstract

Hyaluronic acid (HA), a glycosaminoglycan found in joint synovial fluid, is administered to horses as an anti-inflammatory with lubrication properties. This experiment examined the effects of HA administered before and shortly after an exercise test on metacarpophalangeal (MCP; fetlock) and tibiotarsal (hock) joint range of motion (ROM). Horses were injected intravenously (IV) with placebo (4 mL, saline) or HA (4 mL, 40 mg) 24 h before performing a standardized exercise test (SET) on a high-speed treadmill and again at 6-h post-SET. Passive fetlock flexion was measured at 4 and 24 h post-SET. Hock flexion and extension were measured at 24 h post-SET by videography and kinematic evaluation at the trot. Parameters of the SET were sufficient to cause peak lactate values of 6.6 ± 0.15 mM and a maximum heart rate of 203.6 ± 4.8 bpm. A minor gain ($P = 0.08$) in fetlock flexion prior to SET was observed in HA horses that were not retained at either 4 or 24 h post-SET. Hock flexion in both limbs was greater ($P < 0.05$) at 24 h post-SET, independent of treatment. Horses receiving HA exhibited reduced ($P = 0.04$) right hock extension. No differences in either right or left hock ROM were observed between control and HA-treated horses. From these results, it is concluded that IV HA injections surrounding an exercise stressor offer no substantive gains in either fetlock or hock ROM.

Lay Summary

Repetitive compressive force placed on a horse's joints during strenuous exercise can predispose the tissue to damage and lead to early retirement. Hyaluronic acid (HA) injections are frequently administered to athletic horses as anti-inflammatory and lubrication aids. Recent popular press reports point to the use of HA injections before and after the cross-country race component performed by 3-d eventers. Because the timing of HA administration may affect subsequent mobility outcomes, this experiment examined the impact of HA given before and after an exercise test on fetlock and hock range of motion (ROM). Results demonstrate no substantial benefit of HA on joint ROM following a single bout of exercise. We conclude that a treatment program that delivers multiple injections over a longer timeframe, as typically prescribed, may be advantageous for horses performing demanding exercise by comparison to the truncated protocol described herein.

Key words biomechanics, exercise, horse, hyaluronic acid, range of motion

Introduction

Horses engaged in high-intensity exercise are prone to joint and soft tissue injuries that limit their ability to compete (Crawford and Ahern, 2022). Many of these athletes experience osteoarthritic (OA) lesions due to damage to the articular cartilage layer within the joint. Coincident with OA progression is an increase in proinflammatory cytokines and a decrease in viscosity within the synovial fluid further exacerbating the diseased condition (Sellam and Berenbaum, 2010; Fasanello et al., 2021). The diminished lubrication properties of synovial fluid are directly correlated with a loss of high molecular weight hyaluronic acid (HA) and increased concentrations of lubricin (Peal et al., 2020; Agreste et al., 2021). Intraarticular (IA) injection of HA into the afflicted joint capsule alone or in combination with corticosteroids (Neuenschwander et al., 2019), or polyacrylamide gels (Clifford et al., 2021) may provide pain relief and reduced inflammation leading to improved mobility.

Safety concerns with IA injections are an issue due to possible infections within the joint, and heat and swelling after administration (Smith et al., 2019; Krause et al., 2022). Many horse owners and trainers have opted for oral or intravenous delivery of HA as an alternative to invasion of the joint capsule. Oral HA products may provide some improvements in hock range of motion (ROM; Much et al., 2020) with others demonstrating no changes in movement through the joint (Carmona et al., 2009). In a similar manner, IV injectable HA products demonstrate an improvement in lameness scores of horses suffering from experimental OA (Kawcak et al., 1997) while others report no benefits to arthritic horses with possible declines in ROM (Frisbie et al., 2016). Thus, the effectiveness of either oral or IV HA administration as a mobility aid remains ambiguous.

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Anecdotal and popular press reports describe the administration of HA products prior to and immediately after strenuous exercise provides improved mobility during the early post-activity recovery period. This suggests that timing of administration relative to activity is important for positive outcomes. The hypothesis that an injection of HA prior to and after an exercise test would improve ROM during the recovery period was tested.

Materials and Methods

Animals and Exercise

Animal procedures were reviewed and approved by the Virginia Tech Institutional Animal Care and Use Committee (22-170). Clinically sound, adult Thoroughbred geldings (7.3 ± 0.8 yr) participated in a moderate-intensity exercise conditioning program for 6-wk. On week 7, horses were injected intravenously (IV) with a placebo (4 mL sterile saline, Control, $n = 8$, 528 +/− 17 kg) or with 40 mg sodium hyaluronate (4 mL HA, $n = 8$, 532 +/− 14 kg; LEGEND, Boehringer Ingelheim, Duluth, GA), 24-h prior to performing a standardized exercise test (SET) on a high-speed treadmill (EquiGym, Paris, KY). The SET was designed to mimic an entry-level Concours Complet International cross-country race with sequential parameters of 15 min of trot (4 m/s), 2 min walk (2 m/s), 5 min trot (4 m/s), 7 min gallop (10 m/s), and 2 min walk (2 m/s). All segments of the SET were performed at a 6% incline. Six hours post-SET, horses received a second IV injection of placebo (4 mL) or HA (4 mL, 40 mg).

Physical and Biochemical Measures

Heart rates (HRs) were collected throughout the SET (Polar Equine H10, Kempele, Fin-land) at 1,000 Hz and stored digitally. HR recovery was calculated as the time in seconds to reach 50% of maximal HR (HRmax) upon completion of the SET. Blood lactate was analyzed from plasma obtained prior to exercise, 10 min, 1 h, and 4 h post-SET using a colorimetric assay (MAK064, Sigma Aldrich, St. Louis, MO) at an absorbance reading of 570 nm.

Kinematic Measures

Hock ROM was measured at 24-h post-SET by videography of the horses moving in both directions in a straight line at the trot on a flat surface. A single handler was used for all post-SET trot activities to minimize speed variability. Digital images were captured using high-speed cameras (Quintic High-Speed LIVE, Quintic Consultancy Ltd, Birmingham, UK) equipped with an f/1.2, 8- to 48-mm zoom lens (Computar H6Z0812, CBC America, Cary, NC) and a capture rate of 150 frames/s. Quantitative biomechanical data were collected by sampling consecutive strides using Quintic Biomechanics Video Analysis Software (v.31, Quintic Consultancy Ltd., Birmingham UK). Reference points for measurement were a linear line drawn from the proximal epiphysis of the tibia to the central region (talus) of the tibiotarsal joint (hock) and the central region of the metatarsophalangeal joint (fetlock; Walker et al., 2017). Digital images were enlarged to establish the correct localization of the reference points (mid-tibiotarsal, mid-metatarsophalangeal). Maximal flexion and extension were corrected for angular irregularities and reported as a change (Δ) in angle calculated as Δ, degree = max extension/flexion degree—degree at 20% of stance phase. Five consecutive strides in each direction were assessed for angular and linear measurements (Busse et al., 2021).

Maximal passive flexion of the left and right forelimb fetlocks was measured at 4 and 24 h post-SET using a digital goniometer (Halo Medical Devices, Sydney, NSW, Australia). All angle measures were completed by a single technician with the horse standing on a flat surface (Bergh et al., 2020). Reference points used the center of the metacarpophalangeal (MCP) joint as the axis of rotation and laser alignment along the lateral aspect of the third metacarpus and the long axis of the proximal phalanx (Adair et al., 2016).

Statistical Analysis

Data were analyzed and graphed using GraphPad Prism (v.10; Boston, MA). Joint kinematics and lactate data were analyzed as a 2-way ANOVA with repeated measures for the effects of treatment, time, and their interaction. Maximum HR and HR recovery were analyzed by unpaired student T-test. Significance was established as $P < 0.05$ and a trend as $P > 0.06 < 0.10$.

Results

Horses reached a peak blood lactate of 6.6 mM at 10 min post-gallop with values returning to normal within 4 h ($P = 0.05$; Figure 1A). No differences were observed between controls and horses injected with HA. In a similar manner, no differences were noted between the groups for HRmax (202.5 ± 5.1 bpm, 204.6 ± 4.7 bpm, control and HA, respectively) or HR recovery (273 ± 32 s, 286 ± 37 s, control and HA, respectively) (Figure 1B and C).

Because no difference ($P = 0.68$) was observed between left and right MCP joint flexion, the combined average was analyzed over time (Figure 2). Results demonstrate that HA-injected horses tended ($P = 0.08$) to have greater MCP flexion prior to the exercise test than controls. No differences were noted between controls and HA-treated horses at either 4 h ($P = 0.54$) or 24 h ($P = 0.92$) post-SET. Importantly, average MCP flexion at 4 and 24 h post-SET in HA-treated horses remained less ($P < 0.01$) than pre-SET values indicating that any positive effects of HA did not translate to the later time points.

Flexion through the hock was examined before and after the SET by post-videography kinematic measurement. Right hock flexion was greater ($P = 0.001$) in both groups 24 h after exercise by comparison to pre-exercise values (Figure 3A). No treatment effect was noted for left hock flexion, although both groups demonstrated a gain in flexion post-exercise ($P = 0.05$, Figure 3B). Right hock extension was less ($P = 0.04$) in horses receiving HA by comparison to controls (Figure 3C) with no differences noted for the left hock ($P = 0.29$, Figure 3D). ROM for the hock was calculated as the sum of extension and flexion angles (Figure 3E and F). No difference for either left or right tarsus joint ROM was noted as a function of treatment or time.

Discussion

Lameness is one of the primary determinants of wastage in the racing industry and can be a product of excessive workload leading to tendon and ligament strains, bone fractures, and osteoarthritis (Wilsher et al., 2006; Plevin et al., 2019).

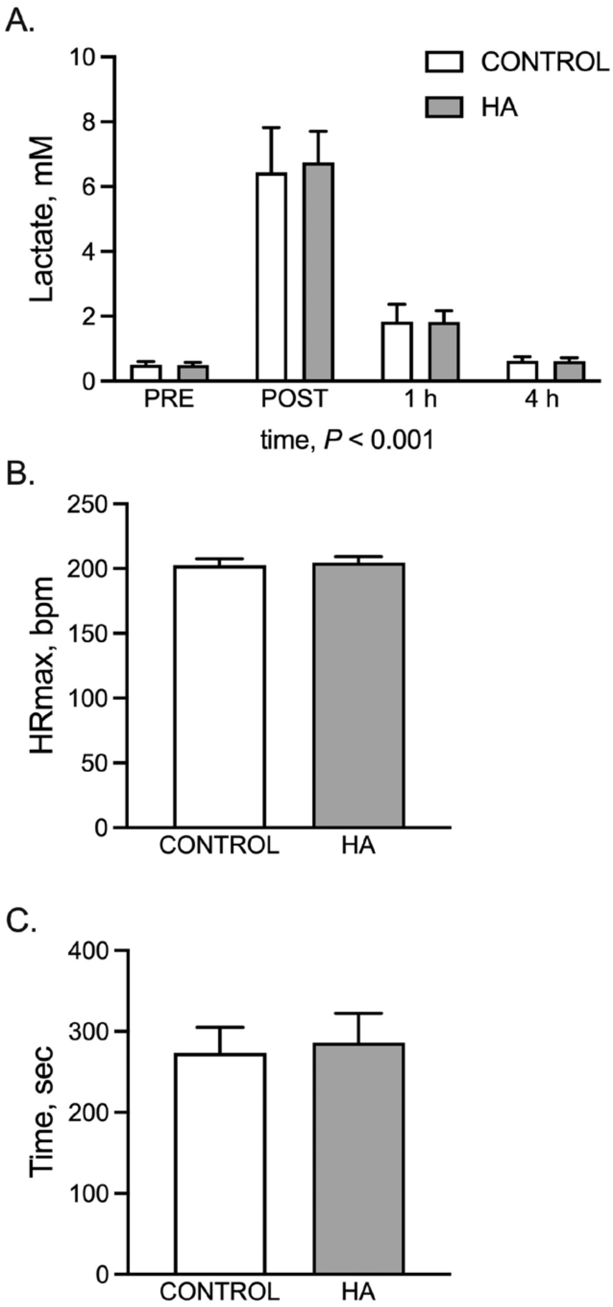

Figure 1. Physiological parameters of the standardized exercise test. Horses receiving a placebo (CONTROL, $n = 8$) or HA injection ($n = 8$) were exercised on a motorized treadmill. Blood was collected for plasma lactate concentration before and at the indicated time after exercise (**A**). Heart rate (HR) was recorded throughout the exercise test and maximal HR in beats per minute (HRmax, bpm) was extracted (**B**). The time to reach 50% of HRmax was extracted from the data (**C**) as a measure of recovery time. Means and SEMs are shown. No treatment differences were noted for any physical or biochemical parameter.

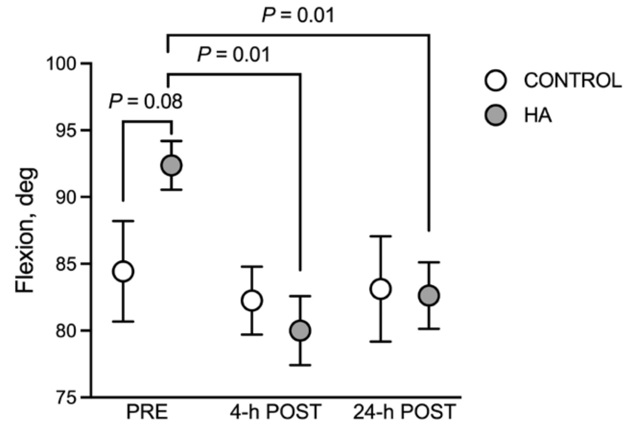

Figure 2. Fetlock flexion in adult horses following a standardized exercise test (SET). Horses received an IV injection of saline (CONTROL, $n = 8$) or HA ($n = 8$) prior to and after a SET. Flexion through the left and right metacarpophalangeal joint was measured and values were averaged. Means and SEMs are shown.

Similar challenges are experienced by eventing horses due to the strain of speed and jumping on the musculoskeletal system (Munsters et al., 2013). HA, by acting as a lubricant and anti-inflammatory, may lessen exercise-induced damage and prolong the career of the horse. To date, the effectiveness of HA alone or in combination with other supplements and aids is debatable with equal numbers of horses showing positive responses as those with no measurable improvement (Xavier et al., 2021). Our results show a modest improvement in passive fetlock flexion when HA is administered as a preventative aid 24-h prior to exercise. No differences between controls and HA-treated horses after exercise argue that the glycosaminoglycan does not benefit joint ROM during the immediate recovery period (24 h post SET). This is further supported by a reduction in right hock extension at the trot by horses administered HA. Although no structural or functional gains were noted for joint mobility, this does not preclude the absence of secondary effects of HA such as relief from inflammation or pain suppression. Hyaluronate products act as anti-inflammatories in humans and horses as indicated by a reduction in inflammatory cytokine gene expression in human neutrophils and lung extracellular vesicles (Zhang et al., 2021; Jia et al., 2023) and equine skeletal muscle tissue (Gregg et al., 2023). A reduction in systemic TNFα and IL1β levels is a common outcome following the administration of non-steroidal anti-inflammatory drugs (NSAID) while also offering analgesic properties (Macpherson et al., 2021; Mercer et al., 2023; Urayama et al., 2023). Treatment of microglia, modulators of neuropathic pain, with HA results in a reduction in *TNFα* and *ILβ* expression supporting both anti-inflammatory and analgesic activities (Austin et al., 2012; Ji et al., 2016). Thus, any perceived mobility improvements by horse owners and clinicians may be related to the suppression of pain experienced following exercise.

A limitation of the study was the use of an unconventional exercise stressor. The SET was designed to mimic a lower-level cross-country race with gallop and trot times extended to offset the absence of jumps. Adult warmbloods completing a show jumping course with 12 obstacles exhibit blood lactate values that are equivalent to running 600 m at 8 m/s (Leguillette et al., 2020). Using this as a reference point, our test was sufficiently arduous to meet the demands of jumping. Peak lactate values of 6.5 mM are less than the peak values of 16 mM experienced by elite eventing horses firmly placing our test in the lower-level class (Burger et al., 2023). A field test using an actual course and competitive horses may reveal differences in mobility for those administered the dual HA treatment.

In summary, injection of HA before and immediately after a single bout of exercise offered little substantive gains in

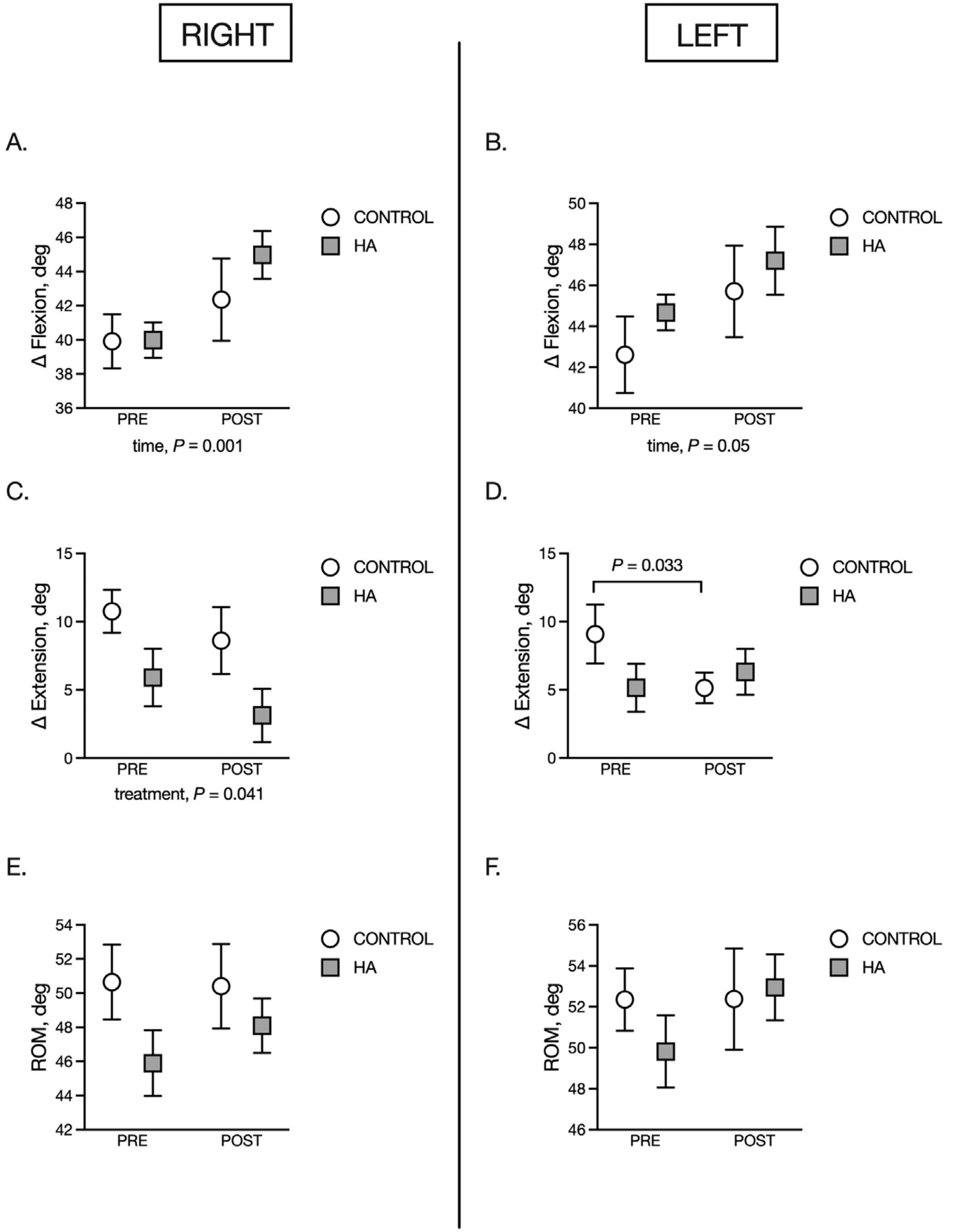

Figure 3. Hindlimb hock flexion, extension, and range of motion in adult horses following an exercise test. Horses received an IV injection of saline (CONTROL, *n* = 8) or HA (*n* = 8) prior to and after a standardized exercise test. Horses were trotted in hand across a flat surface and evaluated by high-speed videography. Change in flexion (**A**, **B**) and extension (**C**, **D**) was calculated as maximal angle minus angle at stance. Range of motion (ROM) was calculated as the sum of flexion and extension for right (**E**) and left (**F**) hock. Means and SEMs are shown.

joint ROM. The benefits of HA as a systemic anti-inflammatory were not measured and may be the underlying cause for anecdotal improvements observed by equine professionals.

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Conflict of interest statement

The authors report no conflicts of interest.

Author contributions

Julia Wells Riley (Data curation, Formal analysis, Methodology, Writing—review & editing), Madison Barshick (Investigation, Writing—review & editing), Lara Chance (Data curation, Methodology), and Sally Johnson (Conceptualization, Formal analysis, Funding acquisition, Project administration, Writing—original draft, Writing—review & editing)

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