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Impact of supplemented undenatured type II collagen on pain and mobility in healthy Labrador Retrievers during an exercise regimen

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ABSTRACT

The aim of this experiment was to evaluate the effect of undenatured type II collagen supplementation on inflammation and pain using gait analysis and industry-accepted pain and mobility questionnaires during an exercise regimen in healthy dogs. Forty healthy Labrador Retrievers (20 male/20 female; range: 5 to 12 yr) were sorted into two groups: undenatured type II collagen group receiving 40 mg UC-II product (10 mg total collagen and ≥3% undenatured type II collagen) and placebo group receiving 40 mg maltodextrin daily by capsule. After 2 wk loading, all dogs began an 11 wk endurance exercise regimen consisting of two weekly runs, starting at 5 km and increasingly incrementally to 8 km, with one final 16 km run. Gait analysis was performed at baseline; before, 24 and 48 h after the first 5 km run; and before, 24 and 48 h after the final 16 km run. Gait analysis was calculated to obtain a Four Rivers Kennel (FRK) Inflammation Index score. Dogs were scored according to the Liverpool Osteoarthritis in Dogs (LOAD) and Canine Brief Pain Inventory (CBPI) assessments at baseline, before and after the first 5 km run, and before and after the final 16 km run. On the LOAD questionnaire, undenatured type II collagen group had improved "how active is the dog" ($P = 0.03$) and less "stiffness after a lie down" ($P = 0.041$) compared with placebo at pre 5 km. Undenatured type II collagen appeared to mitigate the development of pain after exercise compared with placebo, as related to the CPBI assessment. Undenatured type II collagen dogs had lower "pain at worst" pre 5 km ($P = 0.021$), "pain at least" post 5 km ($P = 0.015$), "pain at average" post 5 km ($P = 0.046$), and "pain as it is now" post 16 km ($P = 0.006$) compared with placebo dogs. Undenatured type II collagen was more effective than placebo at mitigating inflammation on gait analysis per the FRK Inflammation Index. Undenatured type II collagen dogs had a 6.42 lower FRK Inflammation Index score at 24 h post 5 km ($P = 0.032$) and 6.3 lower score at 24 h post 16 km ($P = 0.029$), indicating the mitigation of inflammation on gait analysis. When considering the change between timepoints, undenatured type II collagen had a lower increase in FRK Inflammation scores compared with placebo for baseline to pre 5 km ($P < 0.001$), pre 16 km to 24 h post 16 km ($P = 0.028$), and pre 16 km to 48 h post 16 km ($P = 0.027$). Undenatured type II collagen supplemented Labrador Retrievers improved pain assessment variables and improved FRK Inflammation Index on gait analysis.

Key words: canine nutrition, collagen supplementation, FRK index, inflammation

INTRODUCTION

Joint support and pain management in veterinary medicine are areas that are important to provide diverse options for canine patients to avoid potential side effects, drug interactions, and choices for pets with different tolerances. Supplements, nutraceuticals, and prescription medications all have their roles in the reduction of symptoms of arthritis and joint pain. Undenatured type II collagen is a glycoprotein derived from chicken sternum cartilage that has recently been shown to prevent the increase of pro-inflammatory and cartilage degeneration biomarkers in Labrador Retrievers after exercise (Varney et al., 2021). Undenatured type II collagen (UC-II supplementation) works by stabilizing cartilage through an increased composition of amino acids glycine and proline (Walrand et al., 2008), as well as preventing immune reactivity and destruction of cartilage via glycosylation (Bagchi et al., 2002). Previous studies have reported greater therapeutic effectiveness of undenatured type II collagen compared with other supplements such as glucosamine-hydrochloride and chondroitin-sulfate (Gupta et al., 2011). Undenatured type II collagen also had similar efficacy compared with the non-steroidal anti-inflammatory drug (NSAID) robenacoxib (Stabile et al., 2019), however, NSAIDs are documented to cause possible side effects such as gastrointestinal irritation and organ dysfunction and can exacerbate those issues if already present (KuKanich et al., 2012) (Monteiro-Steagall et al., 2013). Undenatured type II collagen is generally considered non-toxic (Marone et al., 2010) with no negative impact to the liver or kidneys in canines (Deparle et al., 2005), making it a viable option compared with NSAIDs. The goal of this experiment was to evaluate the impact of undenatured type II collagen supplementation on inflammation and pain using objective gait analysis and subjective pain and mobility assessments in exercised Labrador Retrievers.

MATERIALS AND METHODS

All animal care and procedures for this experiment were reviewed and approved by the Institutional Care and Use Committee at Four Rivers Kennel, LLC (IACUC #FRK-22). The study was conducted over 13-wks at Four Rivers Kennel (FRK) and used a longitudinal design to compare treatments and outcomes.

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Animals and Housing

Forty healthy Labrador retrievers (20 male/20 female) were used in this experiment and averaged 8 yr of age (range: 5 to 12 yr). All dogs were housed in individual kennels overnight and allowed free access to outside airing yards for 6 to 8 h daily, weather permitting. All dogs had ad libitum access to automatic waterers inside and outside. All dogs were fed once daily in the morning as per their treatment requirements. Prophylactic heartworm prevention (Heartgard Plus (Ivermectin/Pyrantel); Merial USA) was administered monthly.

Diet and Treatments

All dogs were fed the standard kennel diet, MFA Gold N (Missouri Farmers Association, Inc.; Columbia, MO) for the duration of the experiment (Table 1). Feed amounts were determined based on previous feeding records to maintain starting body weight. Feed consumption was determined daily by weighing feed provided and feed refusals.

Each dog was sorted to one of two equalized treatment groups based on age, sex, bodyweight, and parentage. Undenatured type II collagen group received 40 mg UC-II product (10mg total collagen providing ≥3% undenatured type II collagen) (Lonza Capsules and Health Ingredients, Inc.; Morristown, NJ) daily in capsule form by mouth and placebo group received 40 mg maltodextrin daily in capsule form by mouth.

Running Exercise

After 2 wk supplement loading, all dogs began a twice weekly running regimen. The running regimen was as follows: weeks 1-2, loading; weeks 3-5, 2 × 5 km runs; weeks 6-8, 2 × 6.5 km runs; weeks 9-11, 2 × 8 km runs; week 12, tapered to 2 × 3 km runs as a rest week before the long run; week 13, 1 × 16 km run. The first 5 km run and the final 16 km run were used as the interest points for the biggest exercise insult to the dogs. All dogs ran alongside an all-terrain vehicle in the bush where they were free to run, swim, stop but met the minimum prescribed distance as determined by global positioning system (GPS) collars. All dogs wore Actical accelerometer collars (Starr Life Sciences Corp; Oa kmont, PA) to quantify activity intensity and GPS collars (Garmin Intl; Olathe, KS) to determine actual distance ran and average moving speed. Data are presented in a previous publication (Varney et al., 2021).

Table 1. As-fed nutrient composition of the basal diet, MFA Gold N Pro (Missouri Farmers Association; Columbia, MO)

Nutrient	%
Dry natter, %	93.50
Crude protein, %	28.88
Crude fat, %	16.08
Fiber, %	2.50
Ash, %	9.32
Moisture, %	6.50
Nitrogen-free extract, %	36.72
Energy content, kcal/kg	3663

Pain Assessments

Each dog was scored by two trained technicians using two veterinarian accepted (Muller et al., 2016) pain assessments, the Canine Brief Pain Inventory (CBPI) and the Liverpool Osteoarthritis in Dogs (LOAD), at baseline, pre and post first 5 km run, and pre and post final 16 km run. Canine Brief Pain Inventory (CBPI) assessments and Liverpool Osteoarthritis in Dogs (LOAD) (Walton et al., 2013) were used for the assessment and relevant questions included. The CPBI assessment measured pain severity and interference, with higher scores correlating to increased levels of pain. The LOAD assessment measures abnormalities in the activities of the dog, with higher scores associated with abnormal behavior, mobility, and exercise levels.

For the CBPI assessment, dogs were scored on a scale of 0-10 (0 at least, 10 at worst) for the following questions (Table 2): in the past 2 wk, what was the pain of the dog at worst, pain at least, pain at average, pain now, pain during general activity, enjoyment of life, ability to rise, ability to run and ability to climb up. For the LOAD assessment, dogs were scored on an ordinal continuous scale which were later converted to numerical values, with higher scores correlating to increased levels of pain. The following questions were included on the LOAD assessment (Table 3): how is the quality of life of the dog, mobility of the dog, how disabled is the dog by lameness, how active has the dog been, effect of cold or damp weather on mobility, stiffness after lying down, activity at exercise, keenness to exercise, ability to exercise, effect of exercise on lameness, and how often does the dog rest during exercise.

Gait Analysis

A commercially available pressure mat walkway connected to software (Gait4Dogs, CIR Systems, Inc; Franklin, NJ) was used to evaluate spatial, temporal, and pressure variables for gait analysis. Each dog was familiarized with the mat prior to testing, and then walked on the mat between 6 and 12 times at each time point to obtain at least 3 valid walks for data analysis. Data were reviewed to ensure a minimum of three similar walks were collected in order to determine the representative data for that dog. Walks were excluded from the data set if the dog exhibited any behaviors other than a calm forward walk down the center of the walkway; e.g., stepped off the mat, stopped, trotted, pulled on leash, turned head significantly, had inconsistent velocity, or too few gait cycles, according to the manufacturer literature (CIR Systems, Inc; 2017). Gait analysis was performed at baseline; before, 24 and 48 h after the first 5 km run; and before, 24 h and 48 h after the final 16 km run.

Calculation of FRK Inflammation Index

The calculation of Four Rivers Kennel (FRK) Inflammation Index was developed using automatically generated parameters which have a known ideal score for each limb. Gait Lameness Score (combination of weight distribution and reach) has a known ideal score of "100" for each limb. Total pressure index (weight distribution of all four limbs) has an ideal score of "25" for each limb. Step/stride ratio (ratio of the length of step and length of stride, which shows torque around the cervical or lumbar spine) has an ideal score of 50% per limb. Hind reach (length of reach of hind limbs, shows flexion and extension of the hip) has an ideal score of 50% of the step length. To calculate the FRK Inflammation Index, the distance away from the

Table 2. Canine Brief Pain Inventory (CBPI) assessment comparison of undenatured type II collagen (UC-II) (Lonza Capsules and Health Ingredients, Inc; Morristown, NJ) versus placebo supplemented Labrador Retrievers

Parameter	Timepoint	UC-II	Placebo	SEM	*P*-value
Pain at worst	Baseline	0.48[b]	0.58[b]	0.12	0.681
	Pre 5 km	0.45[b]	0.63[b]	0.12	0.467
	Post 5 km	0.60[ab]	1.43[ab]	0.18	0.021
	Pre 16 km	0.75[ab]	1.38[ab]	0.19	0.105
	Post 16 km	1.25[a]	2.05[a]	0.24	0.097
Pain at least	Baseline	0.15	0.35	0.07	0.155
	Pre 5 km	0.15	0.35	0.07	0.155
	Post 5 km	0.15	0.50	0.07	0.015
	Pre 16 km	0.15	0.35	0.06	0.098
	Post 16 km	0.25	0.55	0.08	0.063
Pain at average	Baseline	0.20[b]	0.45	0.09	0.176
	Pre 5 km	0.15[b]	0.45	0.09	0.101
	Post 5 km	0.23[b]	0.70	0.11	0.025
	Pre 16 km	0.33[ab]	0.58	0.09	0.177
	Post 16 km	0.73[a]	1.15	0.16	0.187
Pain as it is now	Baseline	0.18[b]	0.45[b]	0.09	0.134
	Pre 5 km	0.15[b]	0.45[b]	0.09	0.101
	Post 5 km	0.28[b]	0.60[b]	0.11	0.144
	Pre 16 km	0.30[ab]	0.58[b]	0.10	0.186
	Post 16 km	0.70[a]	1.45[a]	0.19	0.046
Pain during general activity	Baseline	0.08[b]	0.20[b]	0.06	0.287
	Pre 5 km	0.05[b]	0.23[ab]	0.06	0.152
	Post 5 km	0.10[b]	0.33[ab]	0.08	0.166
	Pre 16 km	0.25[b]	0.45[ab]	0.09	0.283
	Post 16 km	0.65[a]	0.85[a]	0.15	0.501
Enjoyment of life	Baseline	0.00[b]	0.08	0.02	0.079
	Pre 5 km	0.00[b]	0.13	0.04	0.092
	Post 5 km	0.00[b]	0.15	0.05	0.156
	Pre 16 km	0.05[ab]	0.23	0.06	0.152
	Post 16 km	0.20[a]	0.43	0.11	0.305
Ability to rise	Baseline	0.00[b]	0.08[b]	0.02	0.079
	Pre 5 km	0.00[b]	0.18[ab]	0.05	0.105
	Post 5 km	0.00[b]	0.13[b]	0.04	0.092
	Pre 16 km	0.18[a]	0.33[ab]	0.08	0.355
	Post 16 km	0.33[a]	0.63[a]	0.12	0.200
Ability to walk	Baseline	0.23	0.30[b]	0.07	0.573
	Pre 5 km	0.20	0.30[b]	0.07	0.448
	Post 5 km	0.20	0.43[ab]	0.08	0.175
	Pre 16 km	0.38	0.48[ab]	0.10	0.627
	Post 16 km	0.50	0.93[a]	0.14	0.118
Ability to run	Baseline	0.23[b]	0.30[b]	0.07	0.573
	Pre 5 km	0.23[b]	0.30[b]	0.07	0.573
	Post 5 km	0.20[b]	0.45[b]	0.09	0.159
	Pre 16 km	0.38[ab]	0.60[ab]	0.13	0.375
	Post 16 km	0.85[a]	1.28[a]	0.20	0.291
Ability to climb up	Baseline	0.03[b]	0.08	0.02	0.311
	Pre 5 km	0.00[b]	0.08	0.02	0.079
	Post 5 km	0.00[b]	0.13	0.04	0.092
	Pre 16 km	0.05[ab]	0.33	0.09	0.116
	Post 16 km	0.20[a]	0.53	0.11	0.146
Quality of life	Baseline	1.13	1.13	0.04	0.999
	Pre 5 km	1.15	1.13	0.04	0.749
	Post 5 km	1.15	1.20	0.05	0.638
	Pre 16 km	1.23	1.28	0.07	0.715
	Post 16 km	1.28	1.35	0.08	0.645

[1]Timepoints include prior to starting treatments or exercise (baseline), prior to the first 5 km run (pre 5 km), 24 h after the first 5 km run (post 5 km), prior to the final 16 km run (pre 16 km), and 24 h after the final 16 km run (post 16 km). Dogs were scored on an 11-point scale of 0 to 10, with higher values correlating to increased pain. Values are presented as least squared means with their standard error.

ideal score was calculated for each variable for each dog. These numbers were standardized, weighted, and added together to produce the FRK Inflammation Index which allows us to track overall gait between timepoints, and to compare treatments or therapies. This calculation provides advantages for identifying generalized inflammation caused by exercise, aging, or to compare overall impact of treatments by group.

Statistical Analysis

JMP 14.1.0 (SAS Institute Inc, Cary NC) was used to create a mixed model for body weights, feed intake, pain assessments, and gait analysis values to compare treatment groups by timepoint. Dog was analyzed as the random effect. If the mixed model indicated significant differences between timepoint, a post hoc Tukey's test was used for multiple comparisons. Results were considered significant at P-value <0.05, and results considered a trend at P-value <0.10. Results are presented as least square mean ± standard error.

Results

Body Weights

Overall, body weights were not different between groups, with undenatured type II collagen dogs at 28.77 ± 0.22 kg and placebo dogs at 29.12 ± 0.22 kg (P = 0.261) on daily average throughout the trial. Undenatured type II collagen males had lighter body weights on average compared with placebo males (P = 0.023), but females had no differences (P = 0.375). Body weights were not different for either treatment overall group by week.

Feed Intake

Overall, feed intake was not different between groups, with undenatured type II collagen dogs averaging 562 ± 20 g per daily and placebo dogs consuming an average of 579 ± 20 g/day (P = 0.572) (Table 1). Male undenatured type II collagen dogs consumed an average of 605 ± 21 g/day, and male placebo dogs consumed an average of 653 ± 21 g/day (P = 0.134). Female undenatured type II collagen dogs consumed an average of 520 ± 22 g/day, and placebo females consumed an average of 505 ± 22 g/day (P = 0.999).

Pain Assessments

For the CBPI assessment, compared with placebo, undenatured type II collagen dogs had 0.83 points lower "pain at worst" compared with placebo dogs at pre 5 km (P = 0.021), 0.35

Table 3. Liverpool Osteoarthritis in Dogs (LOAD) assessment comparison of undenatured type II collagen (UC-II) (Lonza Capsules and Health Ingredients, Inc. Morristown, NJ) versus placebo supplemented Labrador Retrievers

Parameter	Timepoint	UC-II	Placebo	SEM	*P*-value
How mobile is the dog?	Baseline	1.15	1.13	0.04	0.749
	Pre 5 km	1.13	1.18	0.04	0.537
	Post 5 km	1.20	1.18	0.05	0.807
	Pre 16 km	1.28	1.35	0.07	0.597
	Post 16 km	1.30	1.43	0.08	0.414
How disabled is the dog by lameness?	Baseline	1.13	1.10	0.04	0.728
	Pre 5 km	1.10	1.10	0.03	0.999
	Post 5 km	1.15	1.10	0.04	0.505
	Pre 16 km	1.25	1.23	0.06	0.842
	Post 16 km	1.30	1.28	0.07	0.862
How active is the dog?	Baseline	1.20	1.45	0.07	0.077
	Pre 5 km	1.18	1.50	0.08	0.030
	Post 5 km	1.30	1.50	0.08	0.229
	Pre 16 km	1.40	1.58	0.09	0.328
	Post 16 km	1.43	1.70	0.10	0.151
Effect of cold or damp weather	Baseline	1.05	1.18	0.04	0.153
	Pre 5 km	1.08	1.18	0.04	0.268
	Post 5 km	1.18	1.18	0.05	0.999
	Pre 16 km	1.20	1.18	0.05	0.807
	Post 16 km	1.23	1.18	0.05	0.649
Stiffness after a lie down	Baseline	1.03	1.10	0.03	0.170
	Pre 5 km	1.00	1.10	0.02	0.041
	Post 5 km	1.05	1.10	0.03	0.402
	Pre 16 km	1.23	1.15	0.06	0.511
	Post 16 km	1.23	1.30	0.07	0.586
At exercise, how active is the dog?	Baseline	1.30	1.33	0.07	0.864
	Pre 5 km	1.30	1.40	0.07	0.500
	Post 5 km	1.48	1.45	0.09	0.889
	Pre 16 km	1.60	1.53	0.10	0.715
	Post 16 km	1.63	1.60	0.10	0.904
How keen is the dog to exercise?	Baseline	1.15[b]	1.23	0.05	0.488
	Pre 5 km	1.15[b]	1.25	0.05	0.363
	Post 5 km	1.35[ab]	1.28	0.07	0.597
	Pre 16 km	1.55[ab]	1.48	0.09	0.693
	Post 16 km	1.58[a]	1.58	0.10	0.999
How is the dog's ability to exercise?	Baseline	1.15	1.20	0.04	0.562
	Pre 5 km	1.15	1.25	0.05	0.269
	Post 5 km	1.25	1.25	0.05	0.999
	Pre 16 km	1.33	1.43	0.08	0.516
	Post 16 km	1.38	1.48	0.08	0.550
What effect does exercise have on the dog's lameness?	Baseline	1.18	1.15	0.05	0.799
	Pre 5 km	1.15	1.18	0.05	0.799
	Post 5 km	1.15	1.18	0.05	0.799
	Pre 16 km	1.25	1.33	0.07	0.579
	Post 16 km	1.35	1.50	0.08	0.358
How often does the dog rest during exercise?	Baseline	1.15	1.15	0.07	0.999
	Pre 5 km	1.18	1.18	0.07	0.999
	Post 5 km	1.30	1.23	0.07	0.573
	Pre 16 km	1.35	1.35	0.07	0.999
	Post 16 km	1.43	1.45	0.07	0.899

[1]Timepoints include prior to starting treatments or exercise (baseline), prior to the first 5 km run (pre 5 km), 24 h after the first 5 km run (post 5 km), prior to the final 16 km run (pre 16 km), and 24 h after the final 16 km run (post 16 km). Scoring was based on a 5-point scale from 1 to 5, with higher values correlating with increased abnormal behavior, mobility, and exercise. Values are presented as least squared means with their standard error.

Table 4. Gait analysis FRK Inflammation Index comparison of undenatured type II collagen (UC-II) (Lonza Capsules and Health Ingredients, Inc; Morristown, NJ) versus placebo supplemented Labrador Retrievers

Parameter	Timepoint	UC-II	Placebo	SEM	*P*-value
FRK Inflammation Index	Baseline	67.62	67.57	2.20	0.988
	Pre 5 km	64.14	67.76	2.08	0.220
	24 h post 5 km	65.20	71.62	2.10	0.032
	48 h post 5 km	64.18	65.74	2.02	0.587
	Pre 16 km	60.62	64.06	1.99	0.228
	24 h post 16 km	61.49	67.79	1.99	0.029
	48 h post 16 km	64.23	70.45	2.45	0.073
FRK Inflammation Index Change	Baseline vs pre 5 km	–6.60	0.65	1.63	0.003
	Pre 5 km vs 24 h post 5 km	0.63	0.97	1.79	0.893
	Pre 5 km vs 48 h post 5 km	–1.95	–2.58	2.09	0.832
	Pre 16 km vs 24 h post 16 km	2.04	6.94	1.50	0.028
	Pre 16 km vs 48 h post 16 km	1.55	10.44	1.55	0.027

[1]Timepoints include prior to starting treatments or exercise (baseline), prior to the first 5 km run (pre 5 km), 24 h after the first 5 km run (24 h post 5 km), 48 h after the first 5 km run (48 h post 5 km) prior to the final 16 km run (pre 16 km), 24 h after the final 16 km run (post 16 km), and 48 h after the final 16 km run (48 h post 16 km). Values are presented as least squared means with their standard error.

points lower "pain at least" at post 5 km (P = 0.015), 0.47 points lower "pain at average" at post 5 km (P = 0.025), and 0.75 points "pain as it is now" at post 16 km (P = 0.046) (Table 2).

On the LOAD assessment, compared with placebo, undenatured type II collagen group had 0.32 points lower "How active is the dog?" (P = 0.03) at pre 5 km and 0.10 points lower "stiffness after a lie down" (P = 0.041) at post 5 km compared with placebo (Table 3).

Gait Analysis Results

Undenatured collagen type II prevented significant increase of inflammation compared with placebo on the FRK Inflammation Index 24 h post 5 km (P = 0.049) and at 24 h post 16 km (P = 0.029) (Table 4). Placebo dogs had greater increases of inflammation between timepoints than undenatured type II collagen dogs, including an increase of 0.65 from baseline to pre 5 km (P < 0.001), pre 5 km to 24 h post 5 km (P = 0.03), pre 16 km to 24 h post 16 km (P = 0.048), and pre 16 km to 48 h post 16 km (P = 0.028) for the FRK Inflammation Index, indicating increased inflammation (Table 4).

DISCUSSION

The results of this study indicated that supplementation with undenatured type II collagen limits the development of pain and impaired mobility after exercise in Labrador Retrievers based on subjective pain assessments and objective gait analysis. Previously, we reported that supplementation of undenatured type II collagen limits the increase of pro-inflammatory and cartilage degeneration biomarkers in the serum of Labrador Retrievers after exercise (Varney et al., 2021). During the same experiment, all dogs were scored using the pain assessments and gait analysis with results presented herein. Pain assessments were used to evaluate each dog such as from a subjective perspective (Alves et al., 2020), to determine how undenatured type II collagen may be perceived after key events. However, caregiver bias can also be present when subjectively assessing mobility in dogs at rates of up to approximately 40% for owners and up to 45% for veterinarians (Conzemius and Evans, 2012). For an objective measure of the effectiveness of undenatured type II collagen on mobility, gait analysis and calculation of the FRK Inflammation Index were used (Assaf et al., 2019) (Nielsen et al., 2020) (Varney and Coon, 2021).

The LOAD and CBPI assessments were useful in determining pain and mobility both between undenatured type II collagen and placebo supplementation, and between normal and exercised timepoints. Both assessments have been evaluated and accepted by veterinarians (Brown et al., 2009) (Brown et al., 2013a) (Brown et al., 2013b). The LOAD assessment was used in a previous study of dogs supplemented with undenatured type II collagen (Stabile et al., 2019). Stabile et al. (2019) found that osteoarthritic dogs supplemented with undenatured type II collagen had improved mobility similar to a prescription NSAID. Results from the present study agreed with this finding, especially during the first insult at the post 5 km time points. Placebo dogs also tended to have increased pain assessment scores after loading and before the start of the exercise regimen compared with undenatured type II collagen dogs, based on the assessment questions 'how active is the dog?' and 'stiffness after a lie down'. The CBPI assessment was used in previous studies of dogs receiving various treatments for pain or arthritis (Lascelles et al. 2015) (Daems et al., 2019), making it a valid choice for the evaluation of undenatured type II collagen. The CBPI results indicated better scores for undenatured type II collagen dogs versus placebo dogs at post 5 km for "pain at worst", "pain at least", "pain at average", and for post 16 km run "pain at it is now". These results indicate a strong protective feature of undenatured type II collagen during exercise in canines. None of the placebo dogs had significantly better scores compared with undenatured type II collagen dogs for any measurements at any timepoint.

Gait analysis using the FRK Inflammation Index worked well in this experiment both for highlighting inflammation from before to after the endurance runs and for highlighting treatment differences. After 2 wks of supplement loading,

we saw a decrease in the FRK Inflammation Index for undenatured type II collagen dogs but a slight increase for placebo dogs. Undenatured type II collagen dogs had an improved FRK Inflammation Index score compared with placebo dogs at three of the four post exercise timepoints. Undenatured type II collagen dogs also had a smaller increase in FRK Inflammation Index compared with placebo for baseline versus loading and for most pre to post exercise timepoints. These results indicate a positive effect for dogs supplemented with undenatured type II collagen, even without the insult of exercise. With strenuous exercise, it is apparent that undenatured type II collagen was effective in mitigating pain and inflammation compared with placebo dogs.

Improvements in both pain assessments and gait analysis for undenatured type II collagen supplemented dogs agreed with the previously reported results of improved activity during runs and improved biomarker activity (Varney et al., 2021). Reductions in cartilage oligomeric matrix protein (COMP) and pro-inflammatory cytokine interleukin-6 (IL-6) appear to be associated with improvement in the pain assessments and gait analysis. These results are similar to a study by Orhan et al. (2021) in which undenatured type II collagen supplemented rats received joint injections of monoiodoacetate to induce inflammation. Undenatured type II collagen supplemented rats had improved biomarker activity and gait testing, similar to the present study, as well as improved knee diameter and joint space compared with control rats. Dosage and mode of delivery appear effective under the present study. Undenatured type II collagen works through oral tolerance, by surviving the digestion process and interacting with lymph tissue in the gut (Bagchi et al., 2002). If provided by injection or immunization, undenatured type II collagen tends to have the opposite effect and can actuate arthritis (Corthay et al., 1998). Undenatured type II collagen reduces the immune system's responsiveness to antigens, limiting the production of pro-inflammatory cytokines and preventing T-cells from attacking the body's type II joint collagen (Gupta et al., 2011).

In conclusion, undenatured type II collagen supplementation was effective in reducing pain and improving mobility gait in exercised Labrador Retrievers. Modes of effectiveness were different depending on the condition of the dogs and the distance ran. By limiting the production of pro-inflammatory and cartilage degeneration biomarkers, undenatured type II collagen supplemented Labrador Retrievers had improved pain assessment variables and improved FRK Inflammation Index on gait analysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare no real or perceived conflicts of interest.

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