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Original Research

Quantitation of Gait and Stance Alterations Due to Monosodium Iodoacetate–induced Knee Osteoarthritis in Yucatan Swine

Katherine T LaVallee,[1] Timothy P Maus,[2] Joseph D Stock,[3] Kenneth J Stalder,[4] Locke A Karriker,[4] Naveen S Murthy,[2] Rahul Kanwar,[5] Andreas S Beutler,[5] and Mark D Unger[5,*]

Knee osteoarthritis is one of the most common causes of chronic pain worldwide, and several animal models have been developed to investigate disease mechanisms and treatments to combat associated morbidities. Here we describe a novel method for assessment of locomotor pain behavior in Yucatan swine. We used monosodium iodoacetate (MIA) to induce osteoarthritis in the hindlimb knee, and then conducted live observation, quantitative gait analysis, and quantitative weight-bearing stance analysis. We used these methods to test the hypothesis that locomotor pain behaviors after osteoarthritis induction would be detected by multiparameter quantitation for at least 12 wk in a novel large animal model of osteoarthritis. MIA-induced knee osteoarthritis produced lameness quantifiable by all measurement techniques, with onset at 2 to 4 wk and persistence until the conclusion of the study at 12 wk. Both live observation and gait analysis of kinetic parameters identified mild and moderate osteoarthritis phenotypes corresponding to a binary dose relationship. Quantitative stance analysis demonstrated the greatest sensitivity, discriminating between mild osteoarthritis states induced by 1.2 and 4.0 mg MIA, with stability of expression for as long as 12 wk. The multiparameter quantitation used in our study allowed rejection of the null hypothesis. This large animal model of quantitative locomotor pain resulting from MIA-induced osteoarthritis may support the assessment of new analgesic strategies for human knee osteoarthritis.

Abbreviations: FP, force plate; GR, GAITRite; MIA, monosodium iodoacetate; TSP, total scaled pressure

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Knee osteoarthritis is the most common cause of chronic pain in the United States.[15] The morbidity incurred by chronic pain and loss of function due to knee osteoarthritis represents the most common cause of lower limb disability in patients older than 50 y.[8] The inadequacy of existing pharmacologic and minimally invasive therapies in addressing this disabling disease is evidenced by the ever-rising rates of knee arthroplasty.[10,23] Evolution of new treatment strategies to treat knee osteoarthritis will require a suitable large animal model of chronic locomotor pain behavior that is quantifiable and affords assessment of analgesic, orthopedic, or other novel therapies.

The disability associated with knee osteoarthritis that drives knee replacement strategies likely encompasses human discomfort, nociceptive pain, and physical dysfunction.[3,5,7,8,10,13,23] A large animal model seeking to test analgesic strategies may, at best, isolate nociceptive pain from structural dysfunction.[11,19-21] This model would ideally 1) rapidly induce a pain state that is 2) manifest as a measurable behavior, that 3) remains stable over time but can be modulated via a 4) dose–response relationship with the inducing agent in a 5) useful large animal species. We previously described a large animal model of monosodium iodoacetate (MIA)-induced osteoarthritis in the hindlimb knee of Yucatan swine.[28] This species is amenable to behavioral training, demonstrates slow and stable growth, and possesses a knee that is homologous to the human joint.[28] In addition, swine develop locomotor abnormalities in association with various degrees of clinical pain and therefore provide measurable pain-associated behaviors in both clinical and research settings.[16,25,27,29]

The tissue injury resulting from MIA-induced osteoarthritis in the swine knee has been characterized previously by using clinical-grade 3-T MRI and gross pathologic examination.[28] We found that MIA stimulates initial synovitis, manifest as joint effusion, followed by progressive cartilage erosion, bone marrow edema, and exposure of subchondral bone. Each of these changes has been observed in human osteoarthritis and is considered to be a contributor to chronic, osteoarthritis-related pain syndromes.[3,5,7,8,24] A relationship between structural injury and MIA dose was observed; however, the progression of the disease over time was the strongest predictor of tissue injury. Although these features are typical for the clinical course of human osteoarthritis, they could confound the use of MIA-induced knee osteoarthritis in swine as a model of chronic pain if the resulting pain behaviors mirrored the tendency for anatomic disease to progress in a relentless fashion, as compared with a stable and titratable behavioral phenotype.

This study sought to assess MIA-induced locomotor pain in Yucatan swine by measuring the onset, severity, and stability

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Departments of [1]Comparative Medicine, and [2]Radiology (Section of Interventional Pain Management), Mayo Clinic, Rochester, Minnesota; [3]Department of Animal Science and [4]College of Veterinary Medicine, Iowa State University, Ames, Iowa; and [5]Departments of Anesthesiology and Oncology, Translational Science Track, Mayo Graduate School, Mayo Clinic, Rochester, Minnesota
[*]Corresponding author. Email: mark.unger@iax.us

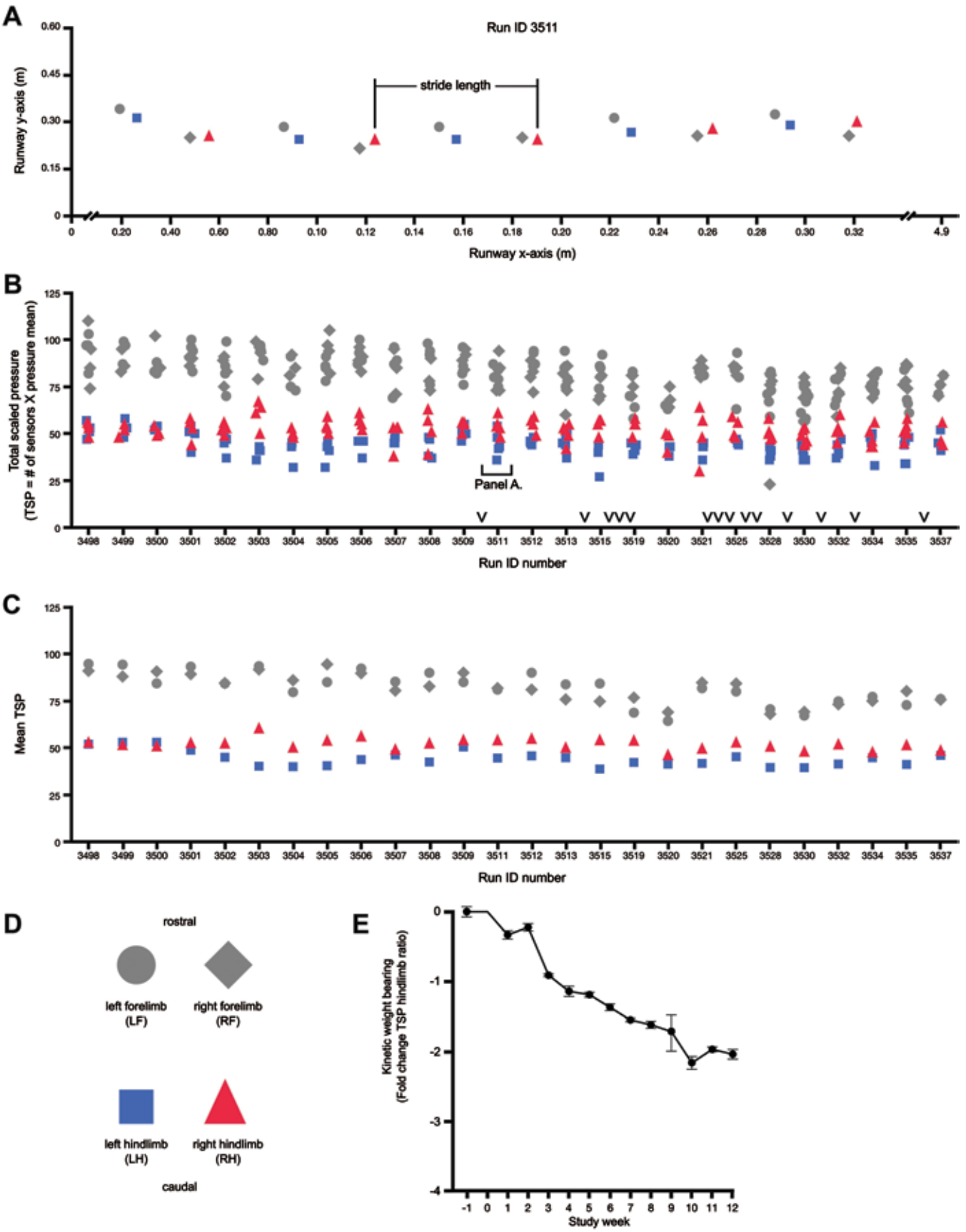

Figure 1. Detailed quantification of swine gait by GAITRite (GR) system and data processing. (A) Footfalls contributing to a successful GR run for one pig. As the animal trots across the runway (here from left to right), sensors embedded in the runway transmit pressure data to a dedicated laptop computer. GR software logs the time (s), location (m), number of sensors, and pressure received by each sensor. This information

of pain behaviors after the induction of knee osteoarthritis. We hypothesized that locomotor pain behaviors after osteoarthritis induction would be detected by multiparameter quantitation for at least 12 wk in a novel large animal model of chronic osteoarthritis. Multiple modes of assessment were used, thereby allowing testing of functionally related parameters to define phenotype stability. Live, subjective lameness evaluation was performed by blinded experimenters by using a previously validated, large animal scoring system.[17] A commercial pressure runway system, GAITRite (GR), was used to record kinetic and spatiotemporal gait parameters,[9] and an electronic force plate (FP) system[22] was used to quantify static weight bearing. Five dose groups of intraarticular MIA (0, 1.2, 4, 12, and 40 mg per knee) were tested to determine whether a dose–response relationship allowed the creation of discrete states of locomotor behavioral abnormality.

Materials and Methods

Animals. The study was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*[14] and the Mayo Clinic IACUC. Female (n = 12) and castrated male (n = 15) Yucatan swine (age, 8 to 15 mo) were included in this study. Swine were purchased from an institutionally approved vendor (Exemplar Genetics, Sioux Center, IA) from a herd that was free of major pathogens. On arrival to the facility, all animals were vaccinated against porcine circovirus type 2, *Mycoplasma hyopneumoniae*, *Erysipelothrix rhusiopathiae*, and porcine parvovirus. Study animals were group-housed indoors in an AAALAC-accredited facility with controlled temperature (66 ± 5 °F [18.9 ± 2.8 °C]), humidity (30% to 60%), and ventilation (10 to 15 air changes hourly). The animals were provided free access to filtered municipal water by using automatic watering devices and were fed a commercial swine diet (Pine Island Mayo Gestation, Purina Animal Nutrition , Shoreview, MN) twice daily, along with a variety of food treats. Swine were monitored daily by veterinary staff and were weighed at least once weekly by using a standalone scale. Behavioral analysis extended for as long as 12 wk in the current study. In addition to institutionally mandated endpoints, animals were to be euthanized in cases of uncontrolled bleeding, antibiotic-resistant infection, cyanosis, inability to drink or eat, or inability to stand or ambulate that did not improve clinically after 48 h. Swine that were observed to be in overt pain, as evidenced by vocalization during ambulation or knee manipulation, were evaluated by a clinical veterinarian to determine appropriate analgesic treatment. Analgesic trials were initiated for 48 to 72 h to test whether symptoms were transitory, during which time behavior data were not collected. When pain was determined to be unmanageable by analgesic therapy, the animal was to be euthanized. To reduce the number of animals used for related studies, swine were simultaneously enrolled in a separate protocol to study knee imaging and gross pathology, where additional animal-specific information including weight has been reported.[28] Experimental data contained herein have not previously been reported.

Osteoarthritis induction. Intraarticular injection of MIA into one hindlimb knee was used to induce osteoarthritis. Five dose groups were used, as described previously.[28] Briefly, the 5 MIA dose groups were chosen by scaling the doses previously reported in small animal studies according to the difference in total body surface area from rodents to swine.[12,19] Doses comprising 1.2 (n = 9), 4 (n = 12), 12 (n = 2), or 40 (n = 2) mg MIA dissolved in either 2 or 3 mL PBS were injected into one hindlimb knee per animal (n = 25) under ultrasound guidance, as previously described.[15] In MIA-injected animals, the contralateral hindlimb knee received either PBS (n = 10) or no injection (n = 15). Control animals (n = 2) received PBS injected into bilateral hindlimb knees. All procedures were performed under general anesthesia and strict aseptic technique. Anesthesia was induced by using intramuscular tiletamine–zolazepam (5 mg/kg), xylazine (2 mg/kg), and glycopyrrolate (0.01 mg/kg). Swine were then intubated, and anesthesia was maintained by isoflurane (1.5% to 2.5%, titrated to effect) in 50% oxygen. Total duration of anesthesia for osteoarthritis induction was 45 to 60 min per animal. Animals were monitored continuously, and vital signs were documented at least every 15 min for heart rate, respiratory rate, body temperature, ventilation, oxygenation, and depth of anesthesia by eliciting reflex responses. The skin over both knee joints was surgically clipped and scrubbed with 1% chlorhexidine. Under ultrasound guidance, a 23-gauge needle was inserted from lateral to medial into the intraarticular, synovial space between the patella and trochlea. A 100-µL PBS test injection confirmed dispersion of injectate in the synovial space under real-time ultrasound observation. Intraarticular injections were performed in a blinded fashion by a musculoskeletal radiologist during week 0 of the study. The side that received MIA was assigned randomly, and personnel involved in subsequent behavior experiments were blind to the side injected and type of injectate.

Lameness scoring through live observation. As previously described in ponies[6] and swine,[17] a large animal-specific, categorical, 5-point scoring system was used to define and evaluate lameness. Animals were observed during spontaneous

is used to compute the spatiotemporal and kinetic gait parameters described in Methods. The embedded sensors are located every 1.27 cm in 2 dimensions within the runway. Footfalls are plotted according to a Cartesian coordinate system. The x-axis is plotted along the length of the runway, parallel to the direction of animal locomotion, and the y-axis is plotted along the runway width, perpendicular to the direction of animal locomotion. Derivation of one spatiotemporal parameter, stride length, is depicted as the distance between 2 consecutive footfalls of the same foot. The software computes the time for this stride by subtracting the time of a subsequent footfall from the former. Stride velocity was computed as stride length divided by stride time. The particular run represented (A) was considered successful because it met the minimum required number of sequential footfalls (i.e., 12) after filtering out asynchronous footfalls. The x-axis is truncated to illustrate the selection for synchronous footfalls, generally occurring over the center of the runway, which corresponded to a trotting gait. (B) Total scaled pressure (TSP) per footfall grouped by run ID number for the complete set of successful GR runs during a representative week for one pig. The footfalls for run ID number 3511 correspond to those depicted in panel A. Run ID number was assigned by the software for each new run, beginning when the animal first pressurizes an embedded sensor. Missing run ID numbers (locations marked 'V') represent unsuccessful runs not included in subsequent analysis due to asynchronous pattern, failure to meet the minimum of 12 consecutive footfalls, or superfluous runway activation by the animal. (C) Mean TSP per limb grouped by run ID number for all successful GR runs during the representative week depicted in panel B. A total of 13,220 successful GR runs comprising 116,008 hindlimb footfalls were included in this study. (D) Graphical key corresponding to quadruped limbs. Limbs are named according to laterality and relative position along the rostrocaudal (head to tail) axis. Forelimbs are shown in gray, with the left forelimb (LF) as a circle, and the right forelimb (RF) as a diamond. Hindlimbs are shown in color, with the left hindlimb (LH) as a blue square, and the right hindlimb (RH) as a red triangle. (E) Fold change in weekly TSP hindlimb kinetic weight-bearing ratios for one pig in the highest dose group (40 mg MIA). Data points depict the means for all successful runs obtained in a given week. Error bars, ± 1 SD from the mean.

Figure 2. Force plate analysis of static weight-bearing asymmetry. The force plate apparatus and enclosure are shown from the (A) rear and (B) side views. Animals were encouraged to enter the enclosure and stand on the force plate during the scheduled morning mealtime. Swine chow was provided in the centrally positioned holster (arrow). Each limb was positioned on 1 of 4 force plate platforms corresponding to the left forelimb, right forelimb, left hindlimb, and right hindlimb while weight-bearing data was captured. Each animal was allowed as long as 15 min per session.

exploration on level, dry ground without obstacles for 5 min, and lameness was scored once daily 5 times each week by a veterinary technician trained in the scoring system. Individual lameness scores were defined as: 0, pig moves freely and uses all 4 limbs and feet evenly; 1, animal shows weight-shifting activities away from affected limb on standing but shows little or no lameness or limping when walking; 2, pig overtly shifts weight away from affected limb when standing and shows limping or adaptive behavior (e.g., head bob, quickened step) on affected limb when walking; 3, animal is reluctant to stand or walk and shows obvious limping and adaptive behaviors when walking; and 4, pig is nonweight-bearing on the affected limb when either standing or walking. A lameness score of 1 was considered to be consistent with mild lameness, scores of 2 and 3 as moderate lameness, and a score of 4 as severe lameness. Baseline lameness scores were collected over 3 wk prior to osteoarthritis induction.

Gait analysis. Quadrupedal gait was quantified during ambulation on level, dry ground without obstacles. Gait data were captured by using the GAITRite Electronic Walkway and software (CIR Systems, Sparta, NJ),[9] The GR system consisted of a rubber runway with embedded sensors connected to a computer. The runway measured 4.9 m × 0.6 m × 0.3 cm. For 3 sessions prior to collection of baseline experimental data, pigs were acclimated and subsequently trained to trot from one end of the runway to the other, by using positive reinforcement in the form of food rewards. Baseline gait parameters were collected over 3 wk prior to osteoarthritis induction. The GR software recorded and calculated gait parameters per footfall in sessions as long as 15 min. After each session, raw GR data were processed to remove unsuccessful runs. The video acquired during each run was used to manually assign footfall data to corresponding limbs within the software. Footfalls were then considered with regard to their sequence and speed, and only those runs that demonstrated a trotting gait rhythm[22] and contained at least 12 consecutive footfalls were considered successful and were used for subsequent analysis. Runs that did not demonstrate the trotting form or contained fewer than 12 consecutive footfalls were removed from subsequent analysis, thereby retaining only those runs with a consistent speed and rhythm. The direction of a run across the GR runway, for example, from left to right, was not a factor in determining whether a given run was considered successful. The GR software was used to compile a database of spatiotemporal gait parameters for each footfall, including stride length, stride time, stance time, and swing time, and the kinetic parameters of number of sensors and mean pressure. Stride velocity was calculated as stride length divided by stride time. Total scaled pressure (TSP), indicative of kinetic weight bearing, was calculated by multiplying the number of sensors and mean pressure per footfall. Each sensor measured the force transmitted to the GR runway over an area of 1.613 cm^2. Thus, the computed total pressure was scaled to the minimal area of detection per sensor. Kinetic weight-bearing values reported in Results for each week represent the mean fold change in TSP hindlimb ratio (MIA-injected hindlimb:contralateral control hindlimb) per dose group after mean values were calculated for each successful run per animal. A graphic depiction of data capture and handling is shown in Figure 1.

Static weight-bearing asymmetry. Static weight bearing was quantified for each hindlimb by using the Iowa State University FP system and software (Figure 2).[17,22] For 3 sessions prior to collection of baseline experimental data, positive reinforcement training in the form of food rewards was used to train animals to freely walk onto the FP system and stand with one foot

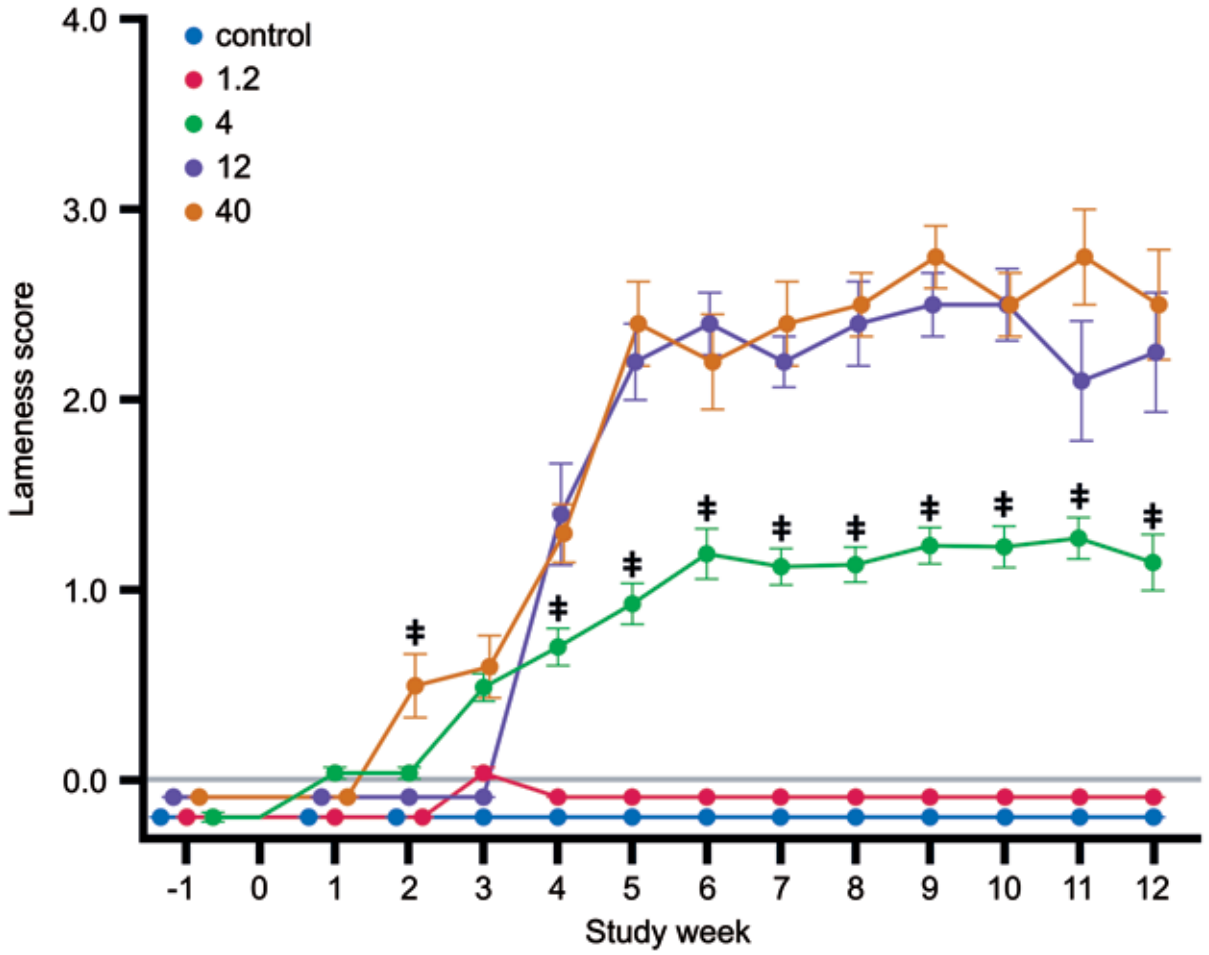

Figure 3. Severity and progression of lameness measured during live observation is correlated to monosodium iodoacetate (MIA) dose. Time series depicts lameness scores before and after osteoarthritis induction (study week 0). Individual data points represent the weekly mean lameness score per dose group. Dose groups are indicated by color as follows: blue, control (0 mg, $n = 2$); red, 1.2 mg MIA ($n = 9$); green, 4 mg MIA ($n = 12$); violet, 12 mg MIA ($n = 2$); orange, 40 mg MIA ($n = 2$). Lameness scores reflect overall animal lameness irrespective of injection laterality. Control animals did not receive a score greater than 0 at any time point. All data points displayed below the zero line (gray) reflect a score of 0. Error bar, ± 1 SEM. Tukey multiple comparison test, ‡, $P < 0.001$.

on each of the 4 force plates. The individual force plates were connected to a dedicated laptop computer. The FP software recorded the weight transmitted to each plate at a frequency of 2 measurements per second. FP sessions lasted a maximum of 15 min, and pigs continually received food rewards throughout each session. A blinded experimentalist gently corrected the animal by guiding limbs to the appropriate plate when a given limb deviated from the correct position. After each session, FP data were processed to remove measurements corresponding to limb deviation and during periods of animal mounting and dismounting from the FP system. The resulting FP data, reported by the software as vertical ground reaction force per limb, were divided by total body weight to yield static weight bearing per limb as a percentage of total body weight. We assumed that force transmission would be almost entirely along the vertical axis without significant loss to another vector, because animals were stationary during static weight-bearing data acquisition. FP analysis became available during the course of the study, limiting its use to data collection for the lower dose groups of 1.2 and 4 mg MIA. Baseline static weight-bearing parameters for these cohorts were collected over 3 wk prior to osteoarthritis induction.

Statistics. For all behavioral modalities, values represent the mean ± 1 SEM per dose group for each study week,. Data obtained prior to intervention are represented graphically throughout the study during week –1. To assess the significance of various factors on the severity of observed pain-associated behaviors, a multivariate, ANOVA-type analysis with time as repeated measures, MIA dose group as fixed effect, and combined 'MIA dose group:time' as interaction effect was performed by using the function LMER in *R*. This analysis was conducted according to previously described methods.[28] The Tukey multiple comparison test was used to determine statistically significant differences between dose groups for lameness scores, GR data, and FP data each week. FP static weight-bearing data were compared by using the Student *t* test to determine statistically significant differences within dose groups between paired values obtained from the MIA-injected hindlimb and the contralateral noninjected hindlimb. For all statistical analyses, a *P* value of less than 0.05 was considered significant.

Results

Association between MIA-induced osteoarthritis and persistent and stable lameness. All pigs demonstrated symmetric gait and stance during live observation prior to osteoarthritis induction (Figure 3). In the 40-mg dose group, lameness was first observed during week 2 and remained significantly ($P < 0.001$) different from controls for the remainder of the study. Beginning at week 4, lameness scores for the 12-mg dose group were comparable to

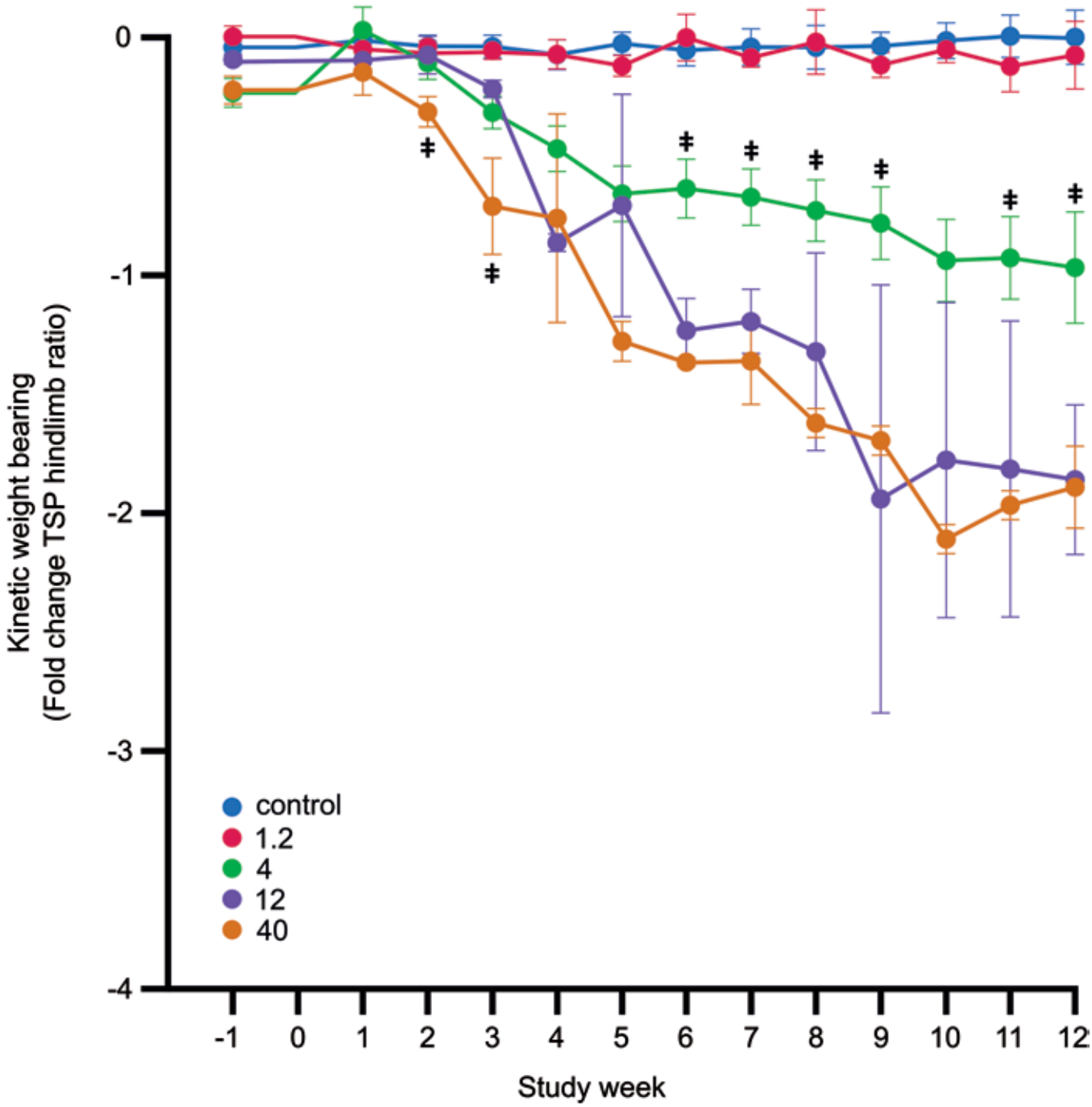

Figure 4. Dose-dependent decrease in kinetic weight bearing of monoiodoacetate (MIA)-injected hindlimbs. Time series depicts fold change in kinetic weight bearing before and after osteoarthritis induction (study week 0). Individual data points represent the weekly mean kinetic weight-bearing value per dose group. Dose groups are indicated by color as follows: blue, control (0 mg, $n = 2$ hindlimb pairs); red, 1.2 mg MIA ($n = 9$ hindlimb pairs); green, 4 mg MIA ($n = 12$ hindlimb pairs); and violet, 12 mg MIA ($n = 2$ hindlimb pairs); orange, 40 mg MIA ($n = 2$ hindlimb pairs). Kinetic weight bearing was calculated as the total scaled pressure (TSP) ratio between the MIA-injected hindlimb and contralateral control hindlimb per animal within dose groups. Errors bar, ± 1 SEM. Tukey multiple comparison test, ‡, $P < 0.001$.

the 40 mg dose group ($P \geq 0.28$ at all time points), and this relationship was sustained throughout the study until the primary endpoint (week 12). A minority of animals in the 4-mg group ($n = 4$) demonstrated transient lameness during weeks 1 and 2, which became significant compared with controls by week 3 ($P < 0.001$) and persisted for the remainder of the study. The 1.2-mg cohort remained visually unaffected and indistinguishable from control animals throughout the study ($P = 0.999$).

MIA-induced osteoarthritis caused visually distinguishable changes in qualitative gait and stance behaviors in swine compared with baseline and control animals (Figure 3). Although the lowest dose tested did not result in overt lameness, the remaining dosage groups exhibited persistent gait and stance aberrations that stabilized by week 4. Severity of osteoarthritis-specific lameness during this time period was either mild (4-mg group) or moderate, with the moderate phenotype composed of animals that received either 12 or 40 mg MIA.

Quantitative gait analysis. Kinetic weight bearing measured from MIA-injected and control hindlimbs in the 40-mg dose group demonstrated a significant ($P < 0.001$) difference at 2

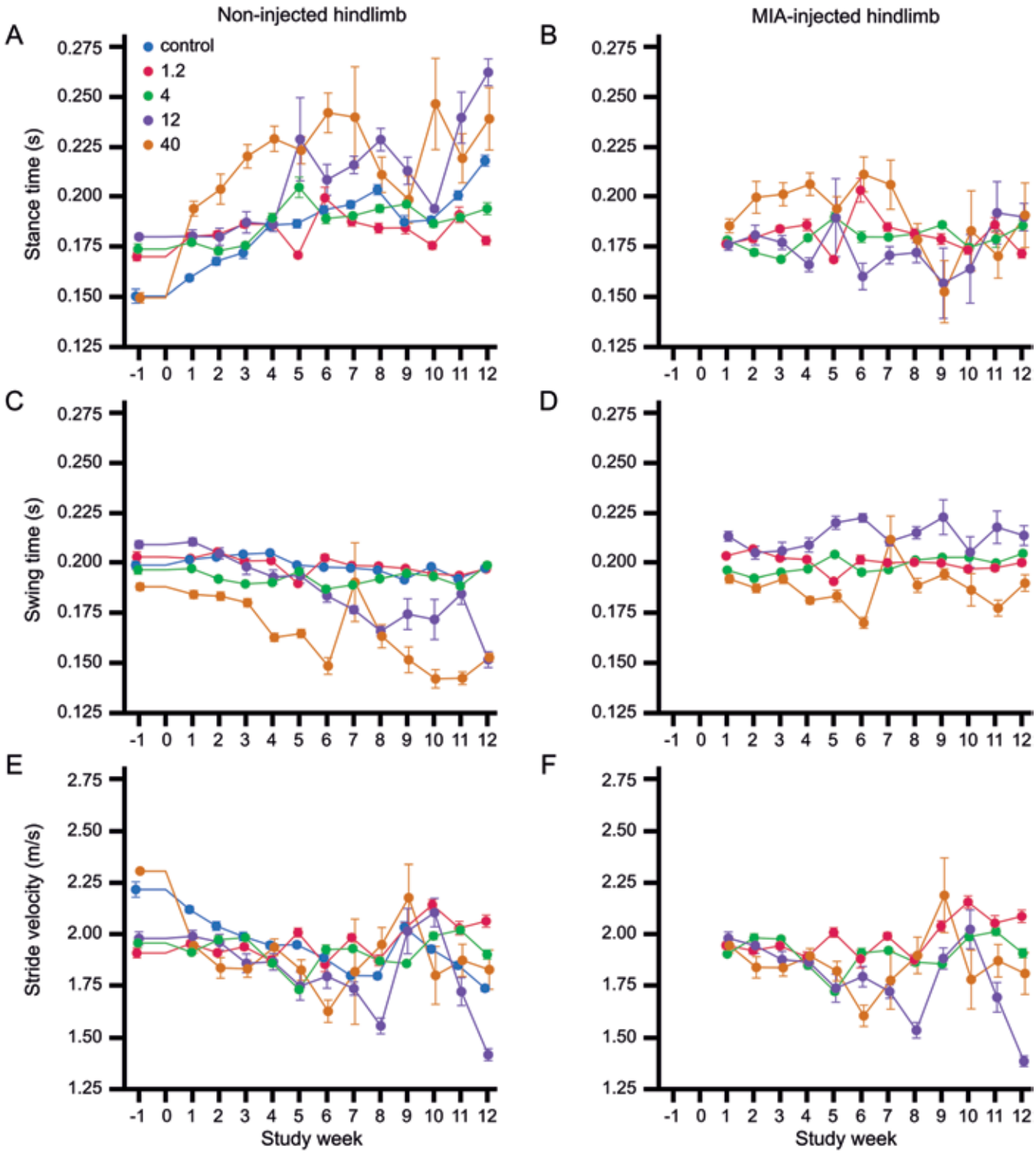

Figure 5. Spatiotemporal gait parameters are unaffected by monosodium iodoacetate (MIA)-induced osteoarthritis in swine. (A and B) Stance time (s), (C and D) swing time (s), and (E and F) stride velocity (m/s) are depicted as weekly mean values per dose group. (A, C, E) Control hindlimb contralateral to (B, D, F) MIA-injected hindlimb of each animal is depicted. Both hindlimbs of control animals (n = 2 animals, n = 4 hindlimbs) are represented in panels A, C, and E. Individual data points represent weekly mean values per dose group obtained for a single hindlimb per animal. Dose groups are indicated by color as follows: blue, control (0 mg, n = 2 animals); red, 1.2 mg MIA (n = 18 hindlimbs); green, 4 mg MIA (n = 24 hindlimbs); violet, 12 mg MIA (n = 4 hindlimbs); and orange, 40 mg MIA (n = 4 hindlimbs). Errors bar, ± 1 SEM. Study week 0 corresponds to the time of MIA injection. No trend correlating any relationship between MIA dose or time and the spatiotemporal parameters depicted was observed.

wk after osteoarthritis induction, similar to observed overt lameness (Figure 4). Of note, the rise in lameness within the 4-mg dose group during week 3 (Figure 3) did not coincide with decreased weight bearing recorded by the GR system (Figure 4). Decreased weight bearing in the 12-mg dose group was observed by week 3 (Figure 4), 1 wk earlier than that observed by lameness scoring alone. The decrease in weight bearing demonstrated by the 4-, 12-, and 40-mg MIA-injected

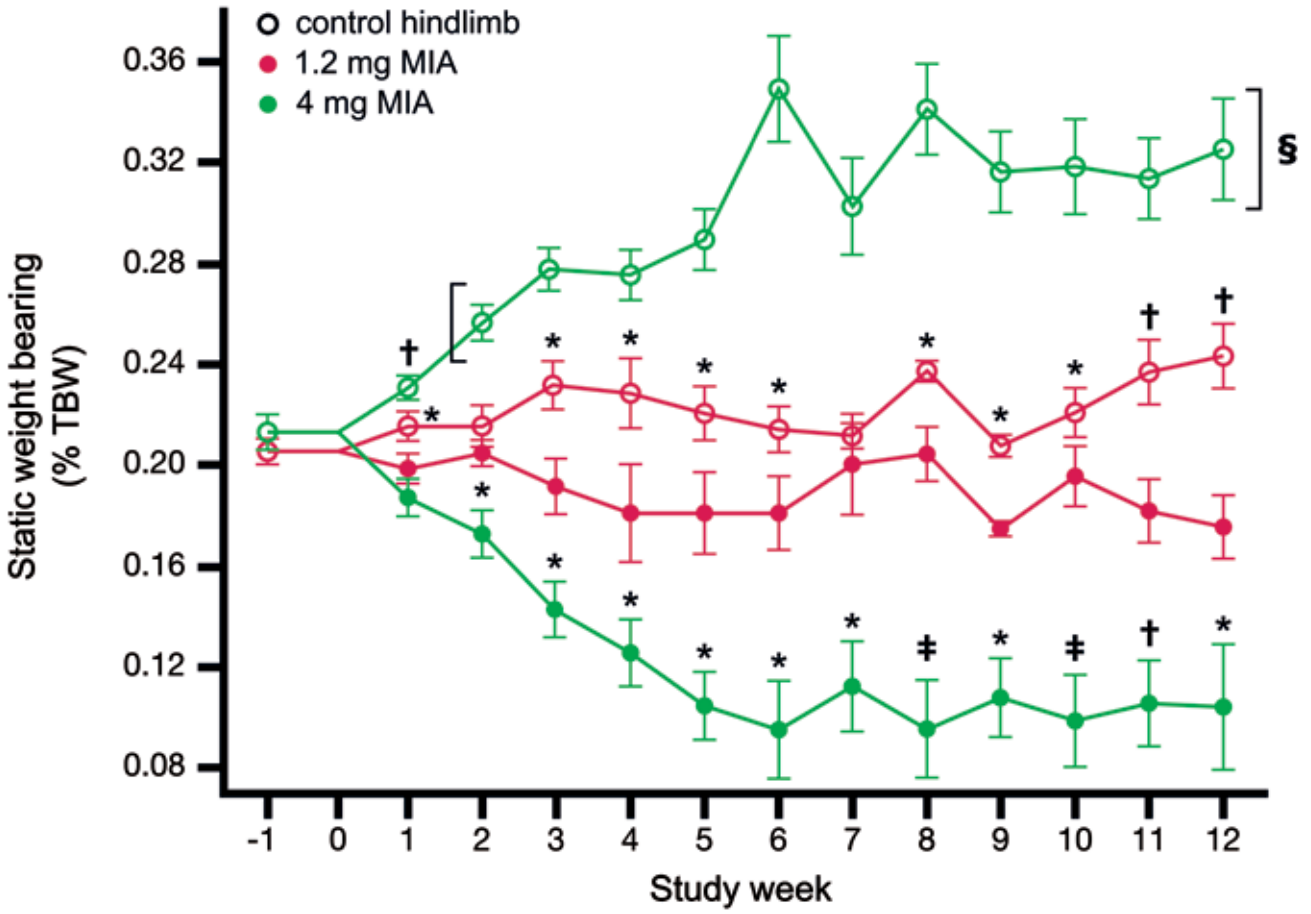

Figure 6. Quantitation of static weight bearing by using the force plate system reveals early asymmetry and hindlimb weight transfer in the lowest monoiodoacetate (MIA) dose groups. Time series depicts static weight bearing before and after osteoarthritis induction (study week 0). Individual data points represent the weekly mean static weight bearing value for each hindlimb per dose group. Open symbols indicate the control hindlimb located contralateral to the MIA-injected hindlimb per animal, indicated by closed symbols, for animals that received 1.2 mg MIA (red, $n = 9$ hindlimbs) or 4 mg MIA (green, $n = 12$ hindlimbs). Static weight bearing was calculated as the percentage of total body weight (% TBW) transmitted to each hindlimb calculated as vertical ground reaction force divided by TBW, performed independently for each limb. Data are given as mean ± 1 SE. The Student *t* test was used to determine significant differences in static weight bearing between hindlimbs within dose groups. Tukey multiple comparison test was used to determine significant differences in static weight bearing of MIA-injected hindlimbs between dose groups. *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$; §, $P < 0.0001$.

hindlimbs suggested a dose relationship by week 6 (Figure 4, $P < 0.001$), approximately 2 wk later than was observed according to lameness scores. Control and 1.2-mg dose groups were indistinguishable through gait analysis throughout the study (Figure 4).

Kinetic weight bearing measured by GR revealed that the 4-mg dose group demonstrated a stable, mild osteoarthritis phenotype between weeks 6 to 12 relative to the moderate osteoarthritis phenotype observed for the 12- and 40-mg doses over the same time period (Figure 4). All spatiotemporal gait parameters derived from GR data, including stance time, swing time, and stride velocity, did not reveal any significant differences from controls throughout the study period (Figure 5).

FP analysis. Static weight-bearing values obtained for the 1.2- and 4-mg dose groups demonstrated a significant difference between MIA-injected hindlimbs and contralateral control hindlimbs as early as week 1 after osteoarthritis induction (Figure 6). For the lowest dose group (1.2 mg), static weight-bearing asymmetry between hindlimbs was significant ($P < 0.05$) throughout the study except during weeks 2 and 7 (Figure 6). The final 2 wk of the study revealed increased and significant ($P < 0.01$) asymmetry. As demonstrated by lameness scores (Figure 3) and kinetic weight-bearing measurements (Figure 4), the 4-mg dose group demonstrated statistically significant ($P < 0.0001$) weight-bearing asymmetry between hindlimbs throughout the study (Figure 6). A dose relationship ($P < 0.05$) for static weight-bearing asymmetry was observed between the 1.2-and 4-mg dose groups for all time points except week 1. Qualitatively, the static weight-bearing time series demonstrated an inverse relationship between hindlimbs of the same dosage group, with a tendency toward unloading of weight from the MIA-injected hindlimb to the contralateral control hindlimb (Figure 6).

General outcomes. Over the course of the 12-wk study, no animal required preemptive euthanasia, none lost the ability to ambulate independently, and none demonstrated clinically significant weight loss. One animal in the 40-mg dose group required analgesic intervention. Multivariate, ANOVA-type analysis[28] showed that time, as an independent factor, was not correlated with the observed changes in locomotor pain behavior for overt lameness, kinetic weight bearing, or static weight bearing, whereas MIA dose was found to be a significant factor (Table 1). Control animals receiving sham injection in one knee, contralateral to a noninjected hindlimb, showed no significant difference in kinetic or static weight bearing over the course of the study.

Table 1. Summary of results from multivariate, ANOVA-type analysis

	Behavioral assessment parameter		
Factor	Lameness score	Kinetic weight bearing	Static weight bearing
MIA dose group	<0.0001	< 0.01	<0.0001
Study week	0.070	0.22	0.76
MIA dose group×study week	0.061	0.12	0.094

Data presented are *P* values generated by using the LMER function in *R*, and values less than 0.05 were considered significant.

Discussion

The induction of osteoarthritis in the swine knee joint by using intraarticular MIA resulted in detectable locomotor pain behaviors compared with controls, allowing for rejection of the null hypothesis. The onset of behavioral change occurred by 2 to 4 wk after osteoarthritis induction, with phenotypes remaining stable thereafter for at least 12 wk. Thus, as an experimental outcome, locomotor pain behaviors demonstrated greater stability than grading of structural MRI sequences, which revealed relentless progression of osteoarthritis over the same time period.[28] In contrast, time, as an independent factor, did not correlate with observed locomotor pain behaviors. Although the induced lameness was evident through live categorical observation and quantitative kinetic weight bearing, static weight bearing provided the greatest sensitivity to locomotor pain behavior at the lowest MIA doses. These findings therefore support the use of this large animal model of chronic locomotor pain for testing of new treatment strategies for osteoarthritis-related syndromes.

Live observation on an ordinal scale was found to be sensitive in the detection of gait abnormality, discriminating treated pigs from controls by 3 to 4 wk after MIA administration and showing relative stability throughout serial assessment after 6 wk. Quantitative gait analysis showed increased sensitivity in the detection of progressive behavioral changes during the 6- to 12-wk period, with less stability of measurement and more interindividual variability. Neither live observation nor gait analysis could discriminate locomotor pain behaviors between the lowest MIA dose group (1.2 mg) and control animals or between the 2 highest doses (12 and 40 mg MIA). In contrast, static weight bearing identified subtle differences between hindlimb weight-bearing in the 2 lowest dose groups (1.2 and 4.0 mg MIA). Taken together, analysis of locomotor pain behavior by lameness assessment, kinetic weight bearing, and static weight bearing suggested a dose–response relationship, wherein mild, moderate, and severe locomotor pain behaviors could be detected, approximating the range of MIA doses tested.

In addition to improved sensitivity of weight-bearing change, the FP system provided ease of use. Gait analysis required as much as 20 min of effort by 2 experimentalists to acquire 15 min of raw kinetic gait data from one animal. For 15 min of data capture from one animal, the FP system required less than 5 min of preparation. However, the combination of these kinetic measurement tools to detect osteoarthritis-associated locomotor pain was critical in characterizing the behavioral phenotype of this model, and both assays should be used in concert for future investigations in treatment effect.

Limitations in the present study were due to the resource-intensive nature of large animal experimentation. The study limitations included small group sizes, observation limited to 12 wk, differences in dose group sizes, and availability of the FP system for testing the lowest 2 MIA dose groups (1.2 and 4 mg) only. These factors diminished the statistical power of the model and, as a result, limited the ability to resolve differences between dose groups throughout study weeks. Therefore, if significant interactions existed between time, dose group, and the key variables of lameness, kinetic weight bearing, and static weight bearing, those interactions were likely missed. For these reasons, reference to study week throughout this report is used to direct the reader to descriptive observations within specific study periods and not to a trend over time.

The use of chemically induced models of osteoarthritis has been criticized for the rapid onset of disease compared with the slowly progressive nature of human osteoarthritis.[26] However, rapid onset combined with stability of the resulting disease phenotype allows for reproducible assessment of treatment effects in relieving locomotor pain between cohorts. The present study reflects the phenotype described in rodents, where osteoarthritis therapeutic testing is common.[4,12,18] Recent investigations into the homology between MIA-induced osteoarthritic rodent cartilage and human osteoarthritic cartilage found little transcriptional similarity between the 2 disease states.[2] The phylogenetic similarity between humans and swine underlies their distinct advantage over rodents,[1,30] and MIA-induced cartilage damage at a molecular level in swine may be more similar to osteoarthritic cartilage damage in humans, although no studies have tested this theory.

Demonstration of nocifensive reversal by existing analgesics, to benchmark future strategies, was not included in the present study, which focused on determining optimal MIA doses for causing clinically detectable locomotor pain while documenting the natural history of the disease model. The present study results will support the design of well-powered treatment studies, including benchmarking with reference drugs by focusing on only 1 or 2 dose levels of MIA.

In summary, we have described a novel large animal model of MIA-induced chronic knee osteoarthritis with measurable locomotor pain. Kinetic measurement tools, including quantitative gait analysis, static weight bearing, and subjective lameness evaluation, were used in concert to develop this behavioral phenotype. Studies combining the locomotor pain quantitation tools used here along with subjective comprehensive pain assessment (such as use of a modified visual-analog scale) could further describe chronic osteoarthritis pain in this model.

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