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Intermittent applied mechanical loading induces subchondral bone thickening that may be intensified locally by contiguous articular cartilage lesions

B. Poulet † [a], R. de Souza ‡ [a], A.V. Kent §, L. Saxon §, O. Barker §, A. Wilson §, Y.-M. Chang §, M. Cake ‖ [b], A.A. Pitsillides § * [b]

† *University College London, Royal Free Campus, London, UK*
‡ *Universidade Federal do Mato Grosso, Mato Grosso, Brazil*
§ *Royal Veterinary College, London, UK*
‖ *Murdoch University, Perth, Australia*

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SUMMARY

Objectives: Changes in subchondral bone (SCB) and cross-talk with articular cartilage (AC) have been linked to osteoarthritis (OA). Using micro-computed tomography (micro-CT) this study: (1) examines changes in SCB architecture in a non-invasive loading mouse model in which focal AC lesions are induced selectively in the lateral femur, and (2) determines any modifications in the contralateral knee, linked to changes in gait, which might complicate use of this limb as an internal control.
Methods: Right knee joints of CBA mice were loaded: once with 2weeks of habitual use ($n = 7$), for 2weeks ($n = 8$) or for 5weeks ($n = 5$). Both left (contralateral) and right (loaded) knees were micro-CT scanned and the SCB and trabecular bone analysed. Gait analysis was also performed.
Results: These analyses showed a significant increase in SCB thickness in the lateral compartments in joints loaded for 5weeks, which was most marked in the lateral femur; the contralateral non-loaded knee also showed transient SCB thickening (loaded once and repetitively). Epiphyseal trabecular bone BV/TV and trabecular thickness were also increased in the lateral compartments after 5 weeks of loading, and in all joint compartments in the contralateral knee. Gait analysis showed that applied loading only affected gait in the contralateral himd-limb in all groups of mice from the second week after the first loading episode.
Conclusions: These data indicate a spatial link between SCB thickening and AC lesions following mechanical trauma, and the clear limitations associated with the use of contralateral joints as controls in such OA models, and perhaps in OA diagnosis.

Introduction

Osteoarthritis (OA) is a heterogeneous group of conditions typified by pain, joint space narrowing, osteophytosis, subchondral bone (SCB) sclerosis and loss of articular cartilage (AC) integrity. Despite anatomical intimacy between AC and SCB changes, their relationship in OA remains incompletely defined[1,2]. Unravelling this relationship is complicated by the fact that mechanical loading is both a major risk factor for AC trauma and a strong driver of local bone mechanoadaptation. Their association is further obscured because AC also adapts in response to applied loads and by the fact that AC damage and increased SCB turnover both occur in OA[3].

Many studies in spontaneous and surgical OA models, including by Radin and Rose[4], provide support for the notion that SCB thickening precedes loss in AC integrity in OA[5,6]. The reliability of this specific hierarchy remains, however, questionable. Thus, it has been proposed that SCB stiffening is not needed for initial AC fibrillation, but rather contributes only to accelerate later OA[7–9]. It

* Address correspondence and reprint requests to: A.A. Pitsillides, Comparative Biomedical Sciences, The Royal Veterinary College, Royal College Street, University of London, NW1 0TU, UK. Tel: 44-207-468-5245.
E-mail address: apitsillides@rvc.ac.uk (A.A. Pitsillides).
[a] Drs. Poulet and de Souza contributed equally to this work.
[b] Profs Cake and Pitsillides contributed equally to the design of this work.

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is still also unclear to what extent SCB and AC interact in normal joints, what sequence leads to SCB thickening in OA and how SCB thickening is related to AC lesions. Herein, we exploit a controllable joint loading model to examine these links between SCB thickening and AC damage[10].

Surgically-induced mechanical joint destabilisation and spontaneous OA mouse models are often used to represent human OA[11–13]. We have described a non-invasive murine model in which transient joint loading produces either focal AC trauma, which is restricted to the lateral femur after a single episode, or lesions that became more severe with time after multiple loading episodes[10]. This study describes SCB and epiphyseal trabecular bone changes that occur following identical application of either single and multiple loading regimens, with the aim of establishing their link to load-induced AC lesions and spontaneous progression.

This study describes bone changes, adjacent to the joint, in response to controlled loading of mouse joints in order to determine new links between SCB and load-induced AC damage. Accordingly, micro-computer tomography (μCT) will be used to measure SCB thickness and epiphyseal trabecular parameters in both loaded and contralateral knees after application of specific experimental loading regimes; known to produce local AC trauma and which also, upon additional loading, prime spontaneous AC deterioration. Finally, longitudinal monitoring of load-related modifications in gait will evaluate whether these might explain any changes in bone architecture.

Methods

Animals

Eight-week-old male CBA mice were housed at 21 ± 2°C with 12-hour light/dark cycles (n = 8/group) and fed standard RM1 maintenance diet *ad libitum* (Rat and Mouse No.1; Special Diet Services, Witham UK). All procedures complied with the Animals (Scientific Procedures) Act 1986 and local ethical approval.

In vivo loading

Animals were isoflurane-anaesthetised and the right knee loaded as described previously[10]. Briefly, axial compressive loads were applied by a servo-hydraulic materials testing machine (Model HC10, Dartec, UK) via custom-made cups which hold knee and ankle joints flexed and the tibia vertically. The loading pattern consisted of a trapezoidal wave, with peak 9 N loads for 0.05 s, rise and fall times 0.025 s each and baseline hold time of 9.9 s at 2 N. Forty cycles were applied in each loading episode.

Three groups were used, with right knees loaded: (1) once and examined 2weeks later ("Once", n = 8); (2) 3 times per week for 2weeks and examined 2days thereafter ("2wks", n = 8); (3) 3 times each week for 5weeks ("5wks", n = 5). To control for any inherent potential left/right differences and to provide baseline data, right and left knees of untreated mice were examined at 10 (n = 4) and 13weeks (n = 4) of age.

Gait analysis

Gait was recorded using a DigiGait™ Imaging system (Mouse Specifics Inc., Boston, MA) the day before and after the first loading episode, at the end of the first and second week of loading (Day 5 and Day 12), and 1 day after each of the three daily carprofen treatments, as described previously[14,15]. Gait was monitored whilst mice travelled on a transparent treadmill under which a video camera is positioned to image footfall from the ventral view. DigiGait software automatically analyses the images to define each paw area, generates a set of periodic waveforms and identifies the segments when the paw is in treadmill contact as the stance phase and portions when not in contact as the swing phase. Other postural and kinematic measurements are calculated, including stride time and length, and paw area. Treadmill speed was set at 17 cm/s for this experiment. Gait from both right (loaded) and left (non-loaded) hind limbs was used to reveal changes induced by applied loading of the right limb. Pain relief was achieved by administration of three consecutive daily intraperitoneal injections of carprofen (5 mg/kg) at the end of the loading regimen[16].

Micro-computed tomography (micro-CT)

After cervical dislocation, knees were dissected, fixed in neutral buffer formalin, stored in 70% alcohol and scanned with an isotropic 5 μm voxel size (40 kV, 250 μA respectively, Aluminium 0.5 mm filter; 0.6° rotation angle, no frame averaging; μCT, Skyscan 1176, Belgium). Each dataset was rotated in Dataviewer (Skysan, Belgium) to ensure similar orientation and alignment for analysis (Fig. 1). Hand-drawn regions of interests (ROI) of the trabecular bone for each femur/tibia lateral/medial compartments was first achieved in the posterior compartments, demarcated from a position where the growth plate was no longer visible in the femoral condyles or, for the tibia, when the ossified menisci appeared, and/or the condyle was flattened (~300 slices per joint used for analysis). SCB ROIs was subsequently selected for each compartment. Analysis of SCB thickness and trabecular bone was achieved using 3D algorithms in CTAn (Skyscan, Belgium) to provide bone volume fraction (BV/TV) and trabecular thickness (Tb.Th).

Statistical analysis

All data are shown as means and 95% confidence intervals (CIs) (lower limit, upper limit). A Shapiro–Wilk normality test (GraphPad Prism) was performed on all the datasets and were all passed with P-values < 0.05. Statistical significance of microCT data was tested using 1-way analysis of variance (ANOVA) with Bonferroni's *post-hoc* test (GraphPad Prism). The significance of the change between the lateral femur and lateral tibia after 5 weeks of loading was assessed using 2-way ANOVA (GraphPad Prism). For gait analyses, repeated-measures models (fixed effect of time points and random effect of mice) were used to assess difference between loading regimens over time and also before and after carprofen treatment between measurements on each of the different days at which gait data were collected (in R 3.1.0); P-value <0.05 was considered significant.

Results

Applied loading of lateral joint compartment promotes local SCB thickening

We first compared SCB thickness of both 10 and 13-week-old untreated CBA mice and found no difference between left and right limbs in any joint compartment (Table I). This confirms left-right joint symmetry in untreated mice and their validity as baselines for comparison. Analyses of right knees in treated groups showed that 5 weeks of applied loading significantly increased SCB thickness in femoral and tibial lateral joint compartments, compared to the right limb of 13-week-old non-loaded control mice (Fig. 2). In contrast, increases in SCB were not apparent after only 2 weeks of loading (compared to 10-week-old controls) nor in most compartments loaded only once. Our analyses also revealed a more pronounced SCB thickening in the lateral femur (30.5% increase) compared with the corresponding tibial aspect (15.1% increase;

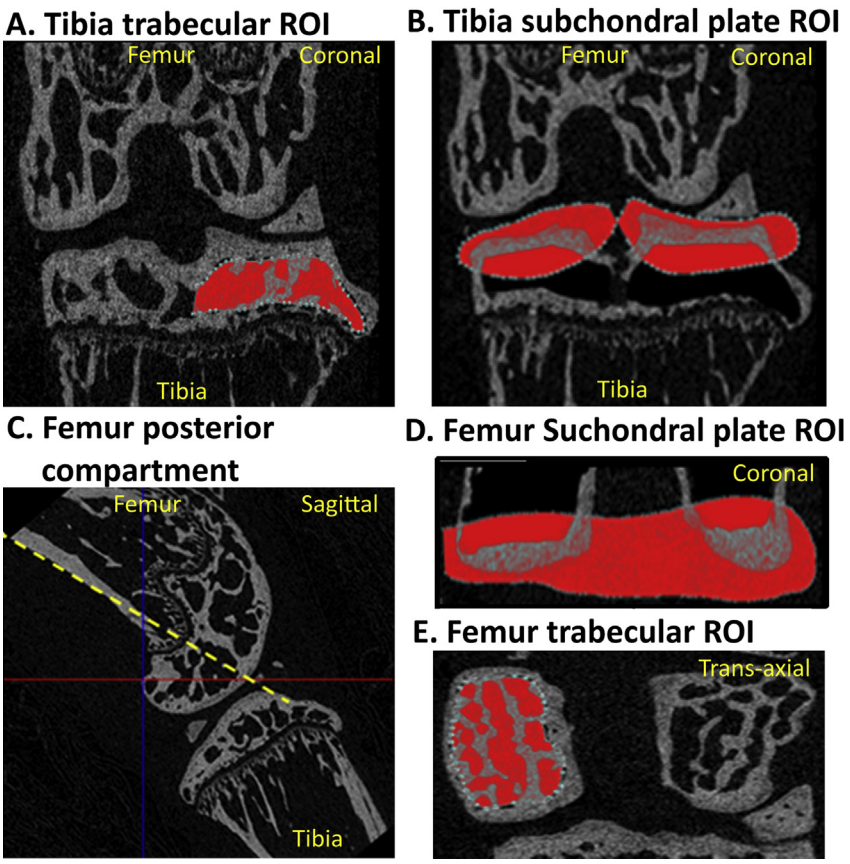

Fig. 1. Selecting ROI in tibia and femur. Red shapes represent the hand-drawn ROI. A and B: coronal knee joint view of ROI for trabecular (A) and SCB (B) regions. C: posterior compartment of the femur analysed. D: coronal view of femur for SCB plate ROI selection and E: trans-axial view for trabecular ROI. All pictures were taken from CTAn analysis software (Skysan, Belgium), except for C which used Dataviewer (Skysan, Belgium).

$P = 0.02$). This is consistent with the proposed transferral of applied loads between the lateral aspects of the tibia and femur and also with induction of AC lesions in only the lateral femur in our model[17]. These data indicate that prolongation of a recurring applied loading episode produces SCB thickening and that this is more marked where load-induced AC lesions are generated.

Non-loaded contralateral joints show transitory SCB thickening

Many studies have used contralateral limb comparison in OA joint destabilisation models. To examine whether such comparison is always valid and justified in our model, we measured SCB in the contralateral joint in loaded and age-matched control groups. This showed that SCB thickening was also evident in non-loaded contralateral limbs in the lateral femur after a single loading episode and in almost all compartments after 2weeks of repetitive loading. This contralateral limb SCB thickening was, however, only transitory as they were not observed when mice were subjected to the longer 5week-long period of applied loading (compared to age-matched controls; Fig. 2). These data question the validity of making only left-right comparisons when assessing acute SCB changes in this joint loading model and that these contralateral increases in SCB thickness are more rapid and widespread but, notably, much less persistent than in limbs subjected to direct applied loading.

Applied loading promotes widespread increases in epiphyseal trabecular bone mass in loaded and contralateral joints

In addition to modifying SCB thickness, we found that 5weeks of repetitive loading produced increases in BV/TV in the lateral compartment of right femur and tibia as well as increases in

Table I
SCB thickness in control non-loaded CBA mouse knee joints at 10 and 13weeks of age. Data shown as mean (lower-upper limit 95% CI). No significant differences were seen between left and right limbs

SCB thickness (mm)	Femur		Tibia	
	Lateral	Medial	Lateral	Medial
10wks_Left	0.110 (0.104–0.117)	0.098 (0.092–0.104)	0.104 (0.099–0.115)	0.120 (0.113–0.126)
10wks_Right	0.115 (0.107–0.124)	0.097 (0.086–0.109)	0.105 (0.099–0.111)	0.122 (0.110–0.135)
P-value (10wks)	*0.215556*	*0.911552*	*0.884548*	*0.496189*
13wks_Left	0.125 (0.114–0.135)	0.128 (0.108–0.149)	0.119 (0.115–0.124)	0.128 (0.119–0.137)
13wks_Right	0.121 (0.100–0.141)	0.130 (0.120–0.139)	0.118 (0.110–0.126)	0.127 (0.112–0.142)
P-value (13wks)	*0.54866*	*0.672606*	*0.640792*	*0.716166*

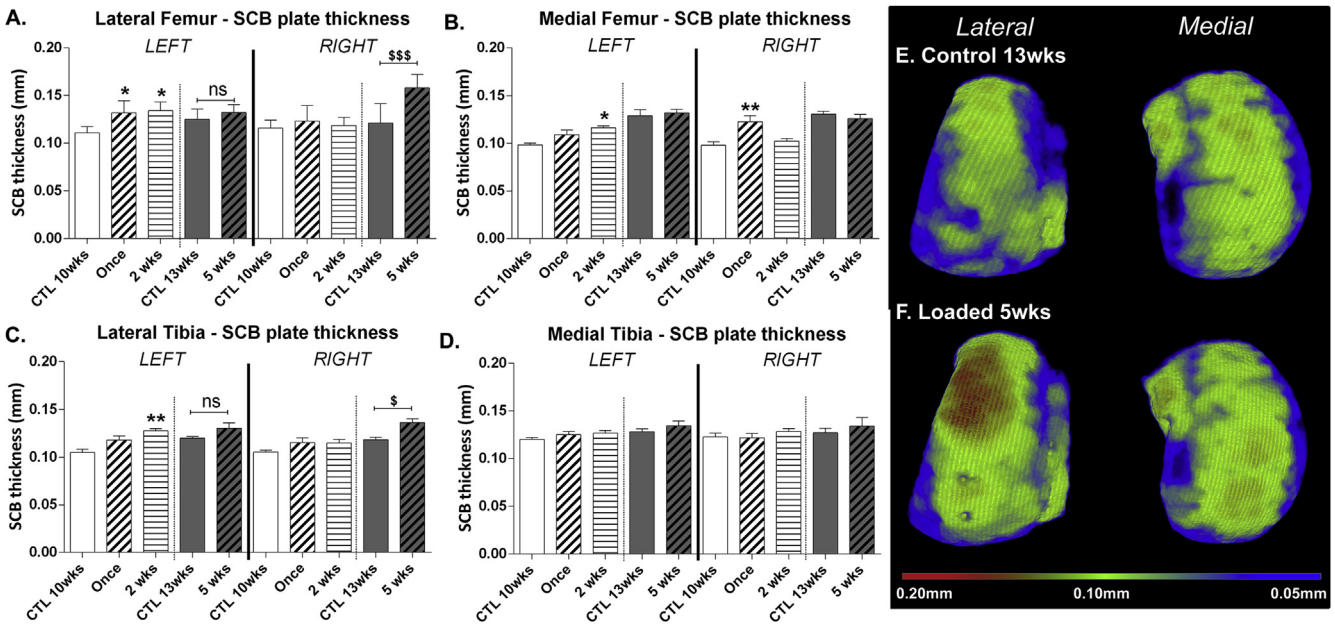

Fig. 2. Applied loading increases SCB thickness in right (loaded) and contralateral (non-loaded) knees. Data shown as mean with 95% CI for each joint compartment, *for statistical significance compared to 10wks control and $ compared to 13wks control. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. (A: Left once *$P = 0.0328$, Left 2wks *$P = 0.0119$, Right 5wks $^{\$\$\$}P = 0.0001$; B: Left 2wks *$P = 0.0301$, Right once **$P = 0.0018$; C: Left 2wks **$P = 0.0013$, Right 5wks $^{\$}P = 0.0373$). E–F. Heat map representing SCB thickness in right femur condyles in non-loaded 13week-old control (E) and joint loaded for 5weeks (F), with red showing localised load-induced thickening the lateral femur compared to age-matched controls.

trabecular thickness in almost all joint compartments compared to non-loaded control joints of 13week-old mice (Fig. 3). Similarly to SCB, changes were more pronounced in the lateral femur than lateral tibia (increases of 55.6% and 31.4% in BV/TV, 41.9% and 20.5% in trabecular thickness, respectively); loading once or repetitively for 2weeks did not induce such changes. Surprisingly, such epiphyseal changes were also apparent in all joint compartments of non-loaded, left contralateral limbs after 5weeks of loading (Fig. 3, vs equivalent joints in 13-week non-loaded mice). These data suggest that epiphyseal trabecular bone is also affected by applied loads, particularly in the lateral compartment, and that contralateral changes are more widespread, despite the unilateral application of limb loading (right). Trabecular number and SMI (Supplementary Fig. 1) showed little changes in any of the groups, with some changes in trabecular SMI in the medial femur after 2 and 5 weeks of loading.

Gait changes are restricted to non-loaded contralateral hind-limbs

To identify the likely source of these contralateral changes in bone architecture, we undertook gait analyses and found initially that no detectible load-induced change in limb use was evident 1 day after right hind-limb loading (Table II). As this loading is known to create lateral femur AC lesions, these data suggest that such trauma alone is insufficient to modify gait. Weekly monitoring of mice loaded only once or repetitively for 2weeks failed to reveal any significant gait change in right, loaded hind-limbs even 3weeks later. In contrast, significant increases in stance and stride times, stride length and paw area were observed in the contralateral left hind-limbs, and this persisted into the third week after initial load application (day 12–19, Fig. 4).

To determine whether these changes in contralateral hind-limb use were associated with pain, gait of loaded mice was monitored during 3 days of carprofen treatment during the third week. Carprofen treatment promoted a return toward baseline gait (decreasing left contralateral hind-limb stance time, stride length and paw area), in mice subjected to a single loading episode, but not in mice loaded repetitively for 2weeks (Fig. 4); right loaded hind-limbs showed no gait modifications in response to carprofen. This shows association between gait changes, SCB plate and trabecular bone thickening in contralateral non-loaded limbs and that bone changes in loaded limbs is likely unrelated to changes in habitual gait.

Discussion

This study describes bone changes in treated and contralateral joints in response to unilateral loading. We find load-related increases in SCB thickness and epiphyseal trabecular thickness and mass in the lateral joint compartments and more marked SCB thickening in the femur, than tibia, after 5weeks. This aligns with our previous findings that applied loads were transferred predominantly across the joint's lateral aspect, where only articular femoral lesions also occur, in this non-invasive mouse model[10]. Our data are consistent with a conclusion that repetitive loads, applied directly across articular surfaces, can produce SCB thickening in intact joints and that load-induced SCB thickening is further accentuated in regions underlying cartilage lesions.

Our studies also reveal bone changes in the knee of the contralateral limb, with SCB thickening evident at the early 2-week time point (in animals loaded once or multiply) and widespread increases in epiphyseal trabecular thickness and mass in non-loaded contralateral joints after 5weeks. Such bone changes occur in contralateral limbs, in which gait modifications were found to be exclusively clustered, suggest that altered compensatory limb use underpins both the transient SCB thickening observed at 2weeks and the later accrual of underlying epiphyseal bone.

Epiphyseal changes are a well-known OA hallmark in both humans and animal models, yet the relationships between AC lesions and SCB remain unclear. It has been speculated that changes in SCB stiffness predispose AC to fibrillation[4]. In addition, it is also possible that AC damage itself may accelerate SCB remodelling and

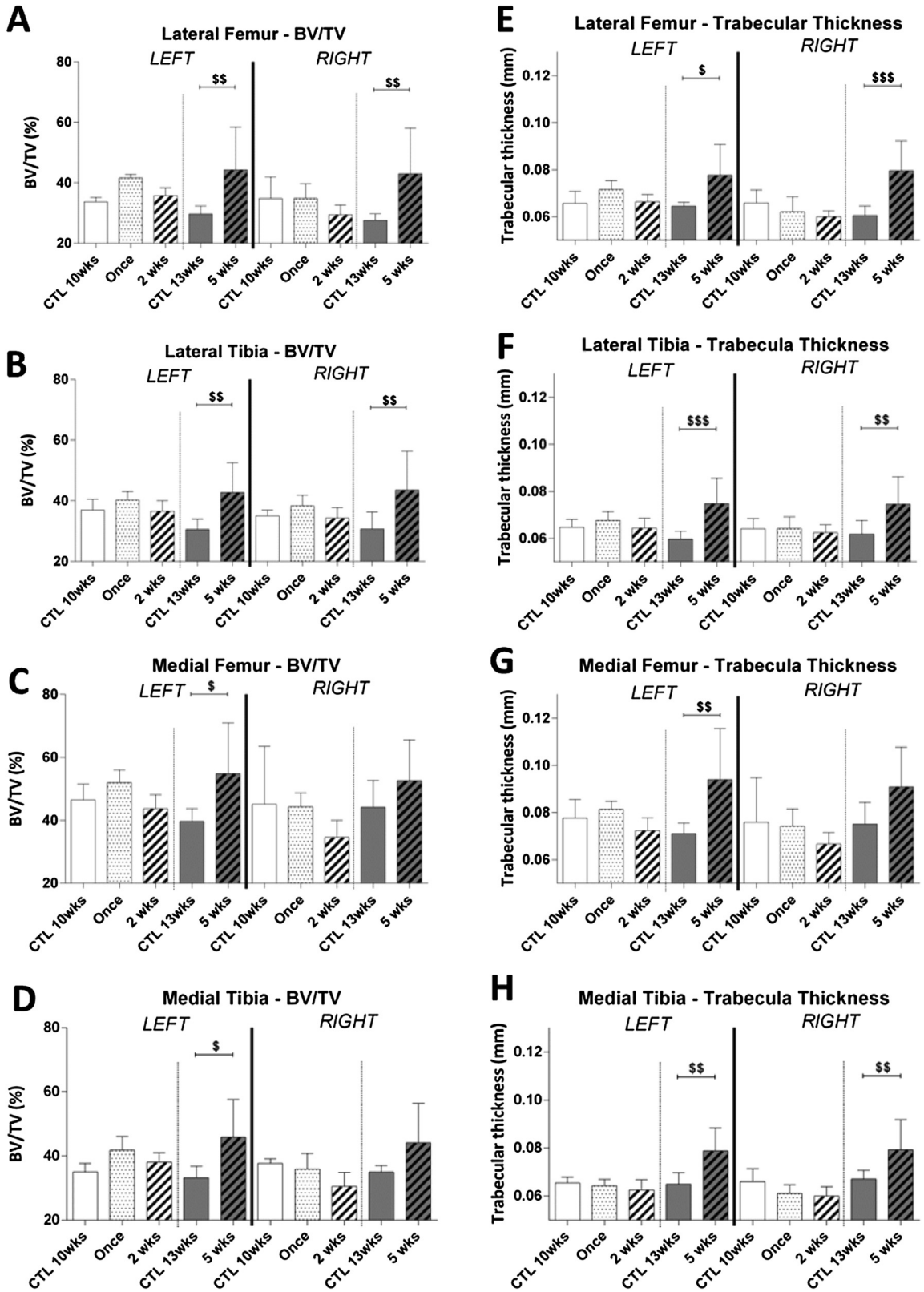

Fig. 3. Applied loading increases BV/TV and trabecular thickness in the right (loaded) and left (contralateral) knee. Data shown as mean with 95% CI for each joint compartment, *for statistical significance compared to 10wks control and $ when compared to 13wks control. $$P < 0.05$, $$ $P < 0.01$, $$$$P < 0.001$. (A: Left 5wks $^{\$\$}P = 0.0041$, Right 5wks $^{\$\$}P = 0.0022$; B: Left 5wks $^{\$\$}P = 0.0052$, Right 5wks $^{\$\$}P = 0.0028$; C: Left 5wks $^{\$}P = 0.0281$; D: Left 5wks $^{\$}P = 0.0104$; E: Left 5wks $^{\$}P = 0.0178$, Right 5wks $^{\$\$\$}P = 0.0003$; F: Left 5wks $^{\$\$\$}P = 0.0009$, Right 5wks $^{\$}P = 0.0079$; G: Left 5wks $^{\$\$}P = 0.0031$; H: Left 5wks $^{\$\$}P = 0.0019$, Right 5wks $^{\$\$}P = 0.0083$).

Table II
Gait measurements in the left and right rear limbs (LR and RR, respectively) before and after the first loading episode. All gait measurements are shown as mean (lower-upper limits 95% CI) in each group

	Repetitive loading group		Single loading group	
	Before loading	Day 1	Before loading	Day 1
		First load		First load
LR Swing time (sec)	0.050 (0.042–0.058)	0.045 (0.037–0.052)	0.047 (0.042–0.051)	0.047 (0.035–0.060)
LR Stance time (sec)	0.102 (0.083–0.121)	0.091 (0.072–0.109)	0.087 (0.076–0.098)	0.098 (0.073–0.123)
LR Stride length (cm)	2.612 (2.233–2.992)	2.328 (1.937–2.720)	2.287 (2.120–2.455)	2.5 (2.079-2.921)
LR Paw area at peak stance (cm^2)	0.369 (0.295–0.442)	0.355 (0.259–0.451)	0.385 (0.297–0.472)	0.412 (0.212–0.611)
RR Swing time (sec)	0.050 (0.046–0.054)	0.046 (0.037–0.054)	0.047 (0.041–0.053)	0.048 (0.039–0.057)
RR Stance time (sec)	0.111 (0.087–0.135)	0.095 (0.076–0.113)	0.107 (0.093–0.121)	0.103 (0.074–0.132)
RR Stride length (cm)	2.75 (2.363–3.137)	2.385 (2.038–2.734)	2.643 (2.342–2.946)	2.62 (2.083–3.157)
RR Paw area at peak stance (cm^2)	0.506 (0.387–0.625)	0.42 (0.288–0.551)	0.579 (0.501–0.658)	0.51 (0.210–0.809)

thickening by changing the joint's mechanics[18]. These diverging opinions have mostly been informed by use of models where joint mechanics are intransiently altered by permanent surgical anatomical disruption. Our use of a model that allows for transient *in vivo* joint loading, in which load-induced cartilage lesions are created in only a single compartment, provides a different basis for determining these temporo-spatial links, indicating that SCB thickening can indeed be induced by applied load beneath intact AC and that lesion-containing AC regions, which share applied loads, may act to accentuate this thickening.

Increases in SCB thickness and volume are known to occur in OA[4] and the resultant increases in stiffness thought to adversely affect AC biomechanics and to precede loss in its integrity. Epiphyseal bone changes are now known to accompany this SCB thickening in mouse models of both post-traumatic OA and destabilization of the medial meniscus[11,19,20]. This latter model has also shown less SCB thickening along with decreased levels of AC damage in ADAMTS5-deficient mice, thus strengthening their association[11]. SCB sclerosis has also been described in spontaneous OA models[21,22]. Others studies have shown, however, that early timepoints following mechanical trauma can induce decreases in epiphyseal bone parameters[23]. It is noteworthy that joint injury induced in these earlier studies was more severe than ours, with clear joint subluxation leading to severe OA development. Direct comparison between right, loaded and contra-lateral, left non-loaded knees in our analyses show corresponding decreases in SCB thickness at early timepoints (2 weeks), which are reversed by 5 weeks. This further emphasises the need to use appropriate controls. Our study is also somewhat limited by examining responses at only particular time-points. Highly dynamic bone loading responses have been emphasised previously and caution should therefore be exercised when comparing each of the various parameters measured, including gait, at any specific time-point.

The non-invasive mechanical loading model used herein has shown that articular areas experiencing greatest applied loads are likely confined to the joint's lateral aspect; with very reproducible load-induced AC lesions in only the femur between studies[10]. Although we have not attempted to directly correlate spatial relationships, the restriction of SCB thickening to sites of known AC lesions and mechanical loading in the posterior femur aspect offers strong support to their likely co-localisation. Data showing most SCB thickening and trabecular architectural changes in these same regions suggests, in loaded joints at least, that they are the direct result of applied mechanical loads. The presence of AC lesions may modify load transfer to underlying subchondral tissue sufficiently to accentuate these bone responses, perhaps by stress-concentration effects. Previous finite element modelling of loaded joints has emphasised differential forces on femoral and tibial articular surfaces[17] and it remains possible that this accentuated SCB thickening is due to differences in mechanically-engendered stresses and strains, or alternatively be mediated by modified cross-talk between chondrocytes from damaged AC and SCB[24–26]. While it is possible that concomitant induction of contiguous cartilage lesions acts to increase SCB thickness, the possibility that this thickening was created by loading alone cannot be ruled out. For example, Ko *et al.*[20] show that extent of SCB thickening was not different in joints experiencing either 4.5 or 9 N loads, even though the joints loaded at 9 N exhibited significantly more cartilage injury. This suggests that the presence of AC lesions alone is not necessarily responsible for driving greater SCB thickening.

Botter *et al.*[12] highlighted a potentially serious flaw in interpreting data arising through contralateral limbs comparison. Thus, changes in gait have been described in OA, with the diseased limb exhibiting reduced peak vertical load[27,28] but similar velocity, stride length and stance time[29]. Other legs will share the extra load as the sum of loads does not change, and so load may also be preferentially distributed to contralateral limbs. Evidence for this is evident in a higher OA risk in contralateral vs ipsilateral knees, in individuals with hip OA[30]. It is therefore imperative that any trabecular or SCB changes are defined against otherwise untreated age-matched mouse limbs; hence our use of both 10 and 13-week-old untreated controls. On this basis, we found generalised epiphyseal bone changes that coincided with gait modifications clustered in only non-loaded contralateral limbs; their more widespread distribution than in loaded knees is perhaps consistent with a generalised shift in mechanical demand engendered by modified gait. From this study, it seems that subchondral and trabecular bone respond differently to mechanical loading *in vivo*. The former shows responses that are spatially concentrated to areas receiving applied loading (lateral compartment) and the latter more widespread to all knee compartments correlating with modified gait. This discrepancy could simply be due to load distribution in each of these locations, but it remains possible that these distinct bone types may exhibit different sensitivities to mechanical stimuli[31]. Alternatively, bone changes in SCB and epiphyseal BV/TV may be achieved by mechanisms controlled dissimilarly by habitual activities. These differences may be explained by their divergent structure and architecture. For example, trabecular bone is more porous and less dense than cortical bone, and its large surface area allows for a higher metabolic remodelling activity compared to cortical bone[32]. Perhaps this makes epiphyseal trabecular bone more susceptible than SCB to subtle changes in gait. In addition, bone formation is a time-dependent process and it is not expected that increases in bone mass will occur simultaneously with changes in applied load or indeed gait.

Our video-based analysis reveals that mice showed normal gait 1 day after knee joint loading. As it is known that a single episode of loading is sufficient to create focal AC lesions[10], this suggests that such trauma alone is insufficient to modify gait. Changes in contralateral limb gait were, however, apparent from the second

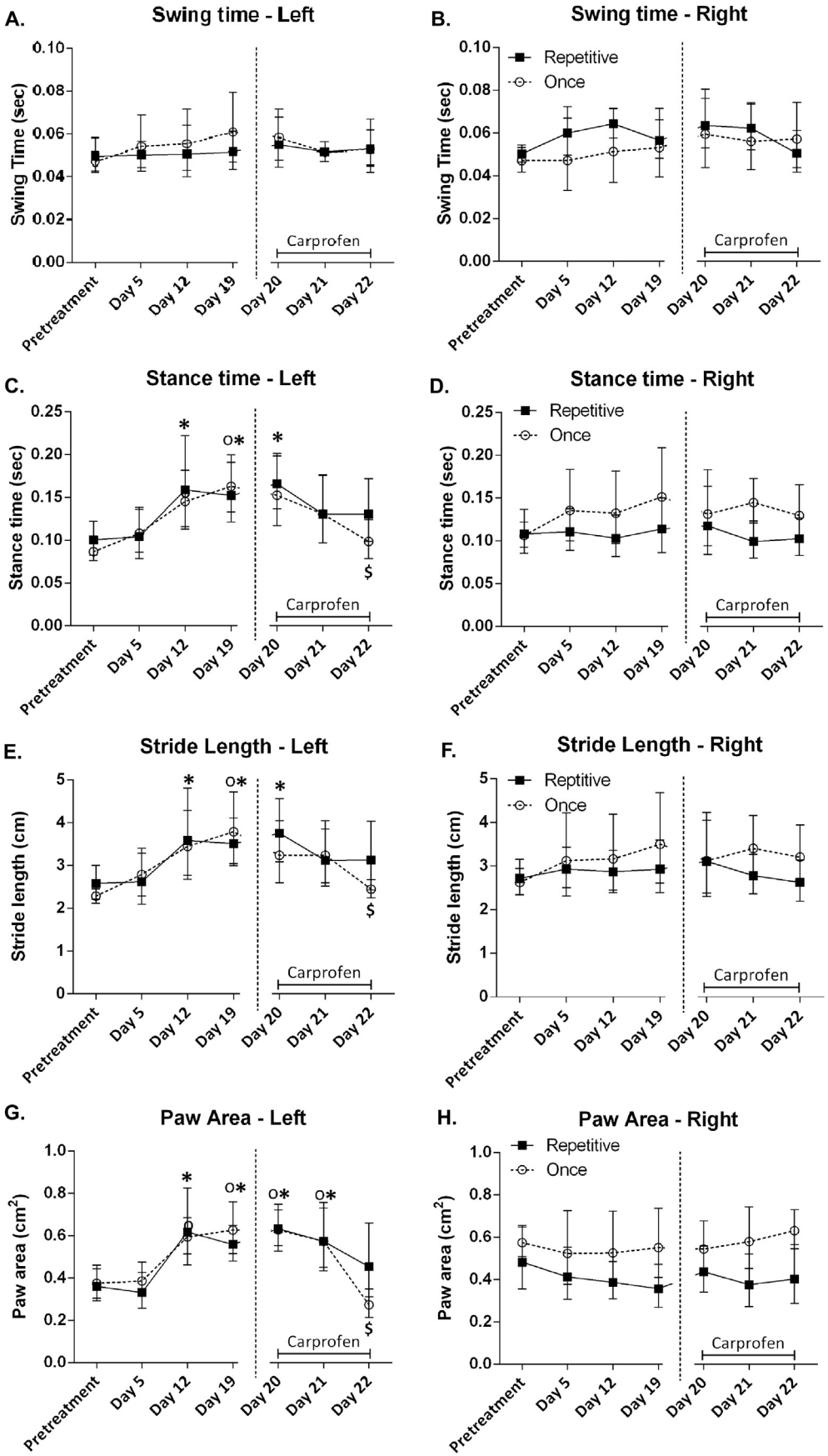

Fig. 4. Applied loading (right knee) increases only contralateral left limb stance time, stride length and paw area. Data shown as mean (with upper and lower limit 95% CI) for swing and stance times, stride length and paw area for non-loaded (left) and loaded (right) hind-limbs. Statistical significance ($P < 0.0001$; except for Day 19 Repetitive Stance time $P = 0.001$ and Stride length $P = 0.002$) compared to pretreatment, *for animals loaded repetitively (black squares) and ^{o} loaded once (open circles). $^{\$}$represents statistical significance of carprofen treatment (vs. day19).

week in animals both loaded once or multiply, and these were reversible upon pain relief only in the former group. Lack of reversibility in mice loaded on multiple occasions suggests that morphological non pain-related changes underpin increases in stance/stride times, stride length and paw area and that longitudinal changes in these specific gait parameters may allow non-invasive monitoring structural joint deficits related to OA. It is important to note that all gait changes appeared compensatory and were restricted to the contra-lateral non-loaded hindlimb. This has not, to our knowledge, previously been described in mice and is somewhat surprising We did not find any morphological/histological changes in the contra-lateral joint except for those described herein in the bone compartment, and no obvious signs of inflammation in neither loaded nor contralateral joints. Increased stance time and paw area could allow the time required in order to distribute weight from injured to contralateral limb to decrease weight impact on the loaded limb. In contrast, other animal models describe most changes taking place in the osteoarthritic limb or no significant gait changes between ipsilateral and contralateral limbs[33] and those with a more prominent inflammatory component[33,34] lead to pain-related abnormal gait of the arthritic limb. Our study suggests that gait changes in animals loaded once are due to pain, since they are rescued by carprofen treatment. Those induced by repetitive loading are more likely due to some non-painful, 'structural' compromise as they remain unaffected by pain relief. The former is consistent with the known meniscal and ligamentous remodelling and osteophyte formation in response to 2weeks of loading in this model[10]. It does remain possible, however, that the dose of carprofen used in these animals is not sufficient to alleviate load-induced pain or alternatively they switch to a neuropathic pain status. Long-term pain relief may be incorporated in future studies in order to limit the effects of gait on the contralateral limb. Any such studies would need first to explore whether such anti-inflammatory pain relief interacted in any way with load-induced bone changes-. In fact, it is well established that selective COX-2 inhibitors blunt load-induced new bone formation[35]. In addition, these treatments may also modify the progression of OA-like lesions in the cartilage in this model; this could be explored in these future studies.

In conclusion, our data support the hypotheses that concomitant AC damage aggravates focal SCB thickening induced by applied loading and, that changes in gait may produce generalised, non-focal, increases in trabecular bone mass in contralateral joints. In addition, this study appears to emphasise the importance of using appropriate age-matched non-loaded controls for studying knee joint bone changes in mouse models of OA.

Contributions

Conception and design: Pitsillides, Cake, Poulet, de Souza.
Collection and assembly of data: Poulet, de Souza, Kent, Saxon, Barker.
Analysis and interpretation of data: all co-authors.
Statistical Analysis: Poulet, Kent, Chang.
Drafting of article: Poulet, de Souza, Pitsillides, Cake, Kent, Saxon, Barker.
Critical revision: Poulet, de Souza, Pitsillides, Cake, Wilson, Chang.
Final Approval: all co-authors.
Obtaining Funding: Pitsillides.

Competing interests

The authors have no conflict of interest to declare.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2015.01.012.

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