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Framework for modulating ambulatory load in the context of *in vivo* mechanosensitivity of articular cartilage

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SUMMARY

Objective: Different stress modalities have been used to provoke a load induced mechanoresponse in blood markers of articular cartilages. The challenge in *in vivo* experiments is to alter specific loading characteristics. Here, we aimed to develop a load modification framework that changes ambulatory load magnitude without changing load frequency or joint kinematics.

Design: Spatiotemporal parameters, sagittal joint kinematics and vertical ground reaction force (vGRF) of 24 healthy participants were recorded while walking with reduced (80%), normal (100%) and increased (120%) bodyweight (BW) on three separate test days in a block randomized cross-over design. The reduced and increased load conditions were compared to the normal load condition using paired sample t-tests for spatiotemporal parameters and statistical parametric mapping for vGRF and joint kinematics.

Results: Load modification resulted in measured vGRF differences of −19.5%BW (reduced) and +16.8%BW (increased). Spatiotemporal parameters with reduced and increased load did not differ from normal load except of a shorter stance time under reduced load (−21 ms). Joint kinematics for both conditions did not differ from normal load except of decreased ankle dorsiflexion (maximum −5.9°) and increased knee flexion (maximum +6.5°) for the reduced load condition during pre-swing when the support limb is already unloaded.

Conclusion: Overall, we did not observe relevant differences in spatiotemporal parameters or joint kinematics between loading conditions. Mean absolute joint angle deviations below 4.1° demonstrate that the proposed load modification framework changes ambulatory load magnitude without changing load frequency or joint kinematics.

1. Introduction

Pathogenic changes in the articular cartilage structure happen long before symptoms of osteoarthritis (OA) are recognized [1]. Although the molecular mechanisms that trigger the pathological changes in the initiation of OA are largely unknown, the ability of chondrocytes to respond to load is believed to play a critical role in maintaining healthy tissue [2,3] and the initiation of OA [4,5]. In this context, biomarkers of OA are investigated in urine, synovial fluid and blood [6]. Yet, to date there is limited information on the *in vivo* response of OA biomarkers to loading in general and especially to load magnitude [7].

Previous studies have used physiologic stress to provoke a load induced mechanoresponse assessed as a change in concentration of articular biomarkers at a systemic level [7,8]. Stress modalities range from prolonged bedrest [9], walking [10–12] to running [13–18] short distance to multistage marathons [15,19–21], and only few included other modalities such as deep knee bends [18], resistance training [22], drop jumps [17,23], cycling [24], or orthoses that increase external knee flexion moments while running [25,26].

While some studies have compared the effects of different physical loads on cartilage biomarkers, several load characteristics (i.e., load magnitude, frequency, different loaded regions of articular cartilage due

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to different range of joint motion, number of loading cycles and/or duration) of the employed physical loading modalities were modified simultaneously precluding associations of the response to any specific load characteristic [10,11,17,18,25,26]. For instance, Firner et al. [25, 26] applied an active orthosis to achieve a 30–40% increase in knee flexion moments but also reported changes in knee flexion angles by more than 10° throughout most of stance. Moreover, Denning et al. [29] reported a greater load induced increase in biomarkers when walking for 30 min with 140% bodyweight (BW) than with normal bodyweight and no difference in load induced increase in biomarkers between walking with 60%BW and normal bodyweight. However, unloading was achieved with a hyperbaric pressure chamber inducing reduced blood circulation in the lower extremity, and actual vertical ground reaction force (vGRF), spatiotemporal parameters or joint kinematics were not reported. Hence, previously reported differences in load induced changes in biomarkers cannot be clearly attributed to circulation, load magnitude, load frequency, number of loading cycles or loaded region of cartilage.

Clearly, an experimental framework is needed to systematically investigate the dose-response relationship between ambulatory load magnitude and load induced changes in cartilage biomarkers and to attribute these changes to altered load but not joint motion. A dose-response relationship between load-induced changes in biomarker concentration in response to a 30-min treadmill walking stress test at three different loading conditions (80%, 100% and 120%BW) has been reported [30]. However, Herger et al. [30] did not report detailed information on gait biomechanics with these three experimental conditions. Although considerable research has been performed on walking biomechanics with modulated bodyweight [31–34], to date reduced bodyweight and increased bodyweight have only been investigated within a single experimental setup in a small number of participants (n = 10) by McGowan et al. [35]. Moreover, a rigorous description of associated changes in individual load characteristics including load magnitude, load frequency, loading pattern or range of joint motion is lacking. In the gait context, these load characteristics can be described by peak vGRF loading rate, cadence, step length, maximum lower extremity joint angles and joint kinematic patterns, and differences between conditions can be evaluated based on statistical and pre-defined relevance criteria [36,37].

The aim of this study was to determine the suitability of an experimental framework for modulating ambulatory load in the context of *in vivo* mechanosensitivity of articular cartilage by assessing differences in spatiotemporal, kinematic and kinetic gait parameters between conditions. We hypothesized that treadmill walking with 20% less bodyweight achieved using a dynamic unloading system and 20% additional bodyweight achieved by wearing a weight vest will result in correspondingly altered vGRF without changes in spatiotemporal parameters or joint kinematics.

2. Methods

Data presented in this study were collected as part of a larger study on the dose-response relationship of ambulatory load and load induced changes in cartilage biomarkers [30]. The study was approved by the regional ethics board and conducted in accordance with the Declaration of Helsinki, and participants provided written informed consent prior to participation.

2.1. Participants

Twenty-four healthy young subjects participated in this study (12 female, mean ± standard deviation (SD), age: 25.7 ± 1.4 years; body height: 1.67 ± 0.09 m; body mass: 62.7 ± 8.4 kg; body mass index (BMI): 22.3 ± 1.6 kg/m^2; 12 male, age: 25.0 ± 2.2 years; body height: 1.81 ± 0.08 m; body mass: 79.1 ± 11.6 kg; BMI: 24.0 ± 2.7 kg/m^2). Inclusion criteria were: age between 18 and 30 years; physically active (>2 times/week); and BMI <30 kg/m^2. Exclusion criteria were: previous lower extremity injury and neuromuscular conditions that could have affected their gait.

2.2. Experimental framework

Participants walked for 30 min with one of three different ambulatory loads on an instrumented treadmill on three separate test days. The order of load was block randomized and the loads were: normal bodyweight (100%BW = normal load); reduced bodyweight (80%BW = reduced load); increased bodyweight (120%BW = increased load). Walking speed was self-selected before the experiment and kept consistent for all three loading conditions (1.3 ± 0.1 m/s).

The reduced load condition was achieved by reducing the participants' bodyweight dynamically using a dynamic unloading system (airwalk®, h/p cosmos sports & medicinal GmbH, Nussdorf-Traunstein, Germany). This system lowers the bodyweight of a participant dynamically through a harness connected to a pneumatic pulley system set to the intended 20% of the participants' bodyweight (Fig. 1, left).

The increased load condition was achieved using a weight vest (CAPITAL SPORTS Monstervest 20 kg, Chal-Tec GmbH, Berlin, Germany). Weights (1 kg increments) corresponding to 20% of the participants body mass were placed symmetrically in front and back pockets of the vest (Fig. 1, right).

2.3. Gait analysis

Bilateral sagittal plane joint kinematics of the hip, knee and ankle were measured using an inertial sensor gait analysis system (RehaGait®, Hasomed, GmbH, Magdeburg Germany; sampling rate 400 Hz, dimensions, 0.06 × 0.015 × 0.035 m triaxial accelerometer, ±16 g; gyroscope, ±2000°/s; magnetometer, ±1.3 Gs) with seven sensors placed on the sacrum, and bilaterally on the lateral thigh, lateral shank, and lateral foot using Velcro straps. Good repeatability and reliability of measured sagittal joint kinematics in walking have been reported by studies comparing the RehaGait® with an instrumented treadmill [38] and an optoelectronic (Vicon) system [37]. The test re-test root mean square error of kinematic waveforms was 2.7°, 3.1° and 3.0° for ankle, knee and hip, respectively [37]. Ankle, knee and hip kinematic trajectories time normalized to one gait cycle were computed by the manufacturer's software and exported. Connection to inertial sensors was interrupted during data transfer leading to two respective three lost datasets per condition. All available data (subject * joint * load condition) were included in the statistical models resulting in varying numbers of included subjects between joint and load condition (min N = 21, max N = 22).

vGRF during walking was measured as surrogate for load magnitude using the pressure plate built into the instrumented treadmill (mercury® 3p, h/p/cosmos sports & medical GmbH, Nussdorf-Traunstein, Germany, with built-in Zebris FDM-THM-S pressure plate, zebris Medical GmbH, Isny, Germany; sampling rate, 120 Hz; range, 1–120 N/cm^2; precision, 1–120 N/cm^2±5%). The pressure mat (length, 1.084 m; width, 0.474 m) comprises 7168 force sensors embedded in the treadmill beneath the treadmill belt. Stance time was defined as the time from initial heel-strike (vGRF exceeding a threshold of 1 N/cm^2) to toe-off (the last frame before vGRF dropped below 1 N/cm^2) and step time as the time from initial heel-strike to the contralateral heel-strike.

Spatiotemporal and vGRF trajectories normalized to stance phase were computed by the manufacturer's software and exported. The software additionally divides the foot into three zones (heel, midfoot, toes) of

Fig. 1. Left: participant walking in the reduced load condition with a 20% dynamical reduction in bodyweight. Right: participant walking in the increased load condition with increase in bodyweight corresponding to 20% body mass.

equal length, and vGRF trajectories for each of these zones were also computed. Loading rate was defined as the maximum slope of vGRF during the first 10% of stance and expressed as percent relative to bodyweight.

Both systems were calibrated immediately before the treadmill walking exercise. Sixty seconds (approx. 110 steps) of kinematic and vGRF data were recorded simultaneously during minute 4 of the 30-min walking exercise while participants remained uninformed of the time of recording. Mean and SD of spatiotemporal parameters were exported for each condition and subject. Kinematic and force trajectories of all steps were imported into MATLAB (Version 2017b, MathWorks Inc., Natick, MA, USA) and time normalized and averaged across all steps for each condition and subject.

2.4. Statistics

Statistical analysis was performed using MATLAB and SPSS Version 25 (IBM Corporation, Amonk, NY, USA). Differences in spatiotemporal and discrete kinematic and kinetic parameters between 80%BW and 100%BW, and 120%BW and 100%BW conditions, respectively, were analyzed using paired t-tests. The significance level was adjusted for multiple comparisons and set to alpha = 0.025. We also examined differences between entire times series (kinematics and vGRF) using statistical parametric mapping (SPM) conducted in MATLAB using the open-source software package spm1D 0.4 (www.spm1d.org) [39]. Between-condition statistical analyses were conducted as described by Pataky [39]. Briefly, paired t-tests were performed to compare the 100%BW condition with the 80%BW and 120%BW condition. The null hypothesis was rejected if the experimentally computed t-value for trajectory 1D data exceeded the critical value that smooth, 1D multivariate Gaussian data would reach in an infinite number of experiments involving smooth 1D data. The significance level for all statistical tests of the SPM analysis was adjusted for multiple comparisons and set a priori to 0.025. Additionally, mean differences in the joint angles and vGRF waveforms were calculated by first subtracting the mean trajectory of the normal load condition from the mean trajectories of the reduced load and increased load condition, respectively for each subject and then averaging across subjects. Results are represented as mean ± SD.

3. Results

3.1. Spatiotemporal parameters

Walking speed, step time, step length and cadence did not differ significantly between the reduced and normal load conditions or between the increased and normal load condition. For the reduced load condition stance time was significantly lower than for the normal load condition (−21 ms; Table 1).

3.2. Joint kinematics

Kinematic parameters did not differ between the left and right side. Therefore, results are presented for the left side only. Most discrete kinematic parameters for the reduced load condition did not differ significantly from parameters for the normal load condition. Although maximum ankle dorsiflexion, hip flexion at initial contact and maximum hip flexion during stance for the reduced load condition were significantly lower than for the control condition (Table 1, all $p < 0.025$), these differences were small (mean differences, −1.7°, −3.4°, and −3.6°, respectively). Discrete kinematic parameters for the increased load condition did not differ significantly from parameters for the normal load condition except for greater maximum knee flexion during the stance phase ($p = 0.013$, +1.2°). All differences in discrete kinematic parameters between the reduced and normal load conditions and between the increased and normal load conditions were on average well below 3.6°.

Trajectory analyses revealed that at the end of stance ankle plantarflexion (50–60% gait cycle) and knee flexion angles (50–56% gait cycle) for the reduced load condition were significantly greater than for the normal load condition with a maximum average difference of 5.9° for the ankle and 6.5° for the knee in the stance phase (Fig. 2). Hip flexion angles in the first 6% of the gait cycle were significantly smaller for the reduced than the normal load condition with a maximum average difference of 4.2°. Moreover, hip and knee flexion in the swing phase were significantly smaller for the reduced load than the normal load condition. Mean absolute joint angle deviation during the entire stance phase between the reduced and the normal load condition in ankle, knee and hip joint were 1.7 ± 1.4°, 4.1 ± 2.8° and 2.8 ± 2.1°, respectively (Fig. 2). In contrast, ankle, knee and hip angle trajectories during stance for the increased load

Table 1
Mean (standard deviation) spatiotemporal and discrete kinematic and kinetic parameters for the reduced (80%BW), normal (100%BW), and increased (120%BW) load condition for all participants (N = 24).

Parameter	Reduced load	Normal load	Increased load	95% CI reduced-normal	95% CI increased-normal	P-Value reduced-normal	P-Value increased-normal
Spatiotemporal parameters							
Walking speed (m/s)	1.3 (0.1)	1.3 (0.1)	1.3 (0.1)	[−0.0; 0.0]	[−0.0; 0.0]	0.142	0.757
Step time (s)	0.538 (0.026)	0.543 (0.030)	0.541 (0.032)	[−0.011; 0.000]	[−0.008; 0.003]	0.063	0.393
Step length (m)	0.694 (0.065)	0.700 (0.059)	0.698 (0.063)	[−0.014; 0.001]	[−0.009; 0.005]	0.100	0.551
Stance time (s)	0.672 (0.045)	0.693 (0.049)	0.699 (0.051)	[−0.027; −0.014]	[0.000; 0.014]	**<0.001**	0.045
Cadence (steps/min)	112.0 (5.4)	110.8 (6.0)	111.3 (6.2)	[0.2; 2.3]	[−0.6; 1.7]	0.026	0.341
Ankle							
Initial contact (°)	1.5 (3.6)	2.8 (2.7)	2.9 (3.2)	[−2.7; −0.0]	[−1.2; 1.3]	0.047	0.906
Minimum plantarflexion weight acceptance (°)	6.1 (1.8)	6.8 (1.9)	7.6 (2.0)	−1.5; −0.0]	[−0.1; 1.4]	0.049	0.074
Maximum dorsiflexion (°)	8.4 (3.4)	10.1 (3.9)	10.2 (4.0)	[−2.8; −0.6]	[−1.1; 1.5]	**0.004**	0.782
Minimum Plantarflexion at toe-off (°)	24.7 (6.6)	22.8 (6.6)	23.0 (5.1)	[−1.7; 5.4]	−-2.0; 3.0]	0.283	0.665
Knee joint							
Initial contact (°)	4.8 (3.7)	3.9 (1.8)	4.6 (2.9)	[−0.9; 2.6]	[−0.2; 1.5]	0.330	0.148
Maximum flexion stance (°)	18.3 (5.1)	17.7 (5.5)	18.9 (4.9)	[−1.8; 3.0]	[0.3; 2.4]	0.617	**0.013**
Minimum knee flexion at toe-off (°)	10.1 (5.4)	7.4 (4.3)	6.6 (3.2)	[0.0; 5.4]	[−1.2; 0.7]	0.047	0.571
Hip joint							
Initial contact (°)	20.9 (4.8)	24.3 (4.4)	24.9 (3.7)	[−5.7; −1.1]	[−0.8; 2.5]	**0.006**	0.308
Maximum flexion stance (°)	21.9 (4.5)	25.5 (4.3)	25.8 (3.7)	[−5.7; −1.4]	[−1.1; 2.2]	**0.003**	0.475
Maximum extension (°)	8.7 (3.3)	9.6 (3.3)	8.4 (3.8)	[−2.9; 1.0]	[−2.4; 0.3]	0.329	0.108
Ground reaction force							
First peak (N/kg)	86.2 (6.2)	101.9 (8.3)	118.2 (10.7)	[−19.0; −12.4]	[14.2; 18.4]	**<0.001**	**<0.001**
Second peak (N/kg)	52.6 (7.3)	63.1 (6.7)	73.1 (8.2)	[−12.5; −8.6]	[8.2; 11.8]	**<0.001**	**<0.001**
Loading rate (%BW/s)	1296.6 (344.7)	1472.4 (335.5)	1678.3 (354.3)	[−264.5; −87.0]	[151.1; 260.6]	**<0.001**	**<0.001**

BW—bodyweight; CI—confidence interval.

condition did not differ significantly from trajectories for the normal load condition (Fig. 2). Mean joint angle differences between the increased and the normal load condition in ankle, knee and hip joint were 1.5 ± 1.5°, 1.9 ± 0.8° and 2.3 ± 1.7°, respectively.

3.3. Vertical ground reaction force

vGRF did not differ between the left and right side. Therefore, results are presented for the left side only. Mean vGRF curves for all loading conditions showed the typical two peak pattern with force maxima in early and late stance and a minimum in midstance. Compared to the normal load condition, the vGRF was lower for the reduced load condition and higher for the increased load condition during the entire stance phase ($p < 0.001$; Fig. 3). The average difference in vGRF between the reduced load condition and the normal load condition across the entire stance phase was −19.5 ± 6.8%BW. The amount of relative load reduction increased from initial loading response to toe-off where it reached its maximum (Fig. 3). The amount of relative load increase for the increased load condition was relatively constant across the entire stance (Fig. 3). The average difference in vGRF between the increased load condition and the normal load condition across the entire stance phase was +16.8 ± 3.9%BW. The magnitude of difference in vGRF between conditions was greatest in the forefoot region, which corresponded to the region with the greatest vGRF magnitude. Maximum average vGRF difference to the normal load condition in the forefoot region was −23.4%BW and +14.4%BW for the reduced and increased load condition, respectively (Fig. 3).

Mean loading rate for the reduced and increased load conditions differed significantly from the normal load condition (reduced load condition: 12.0%; increased load condition: +14.0%; both $p < 0.001$; Table 1).

4. Discussion

Previous research on the sensitivity of load-induced changes in cartilage biomarkers to different physiological loads compared ambulatory activities with different loading modes and duration [9–28]. Walking is the most common physical and daily activity thus representing the most frequent mode of *in vivo* load. The aim of our study was to determine the suitability of an experimental framework for modulating ambulatory load in the context of *in vivo* mechanosensitivity of articular cartilage by assessing differences in spatiotemporal, kinematic and kinetic gait parameters between conditions. With this experimental framework we were able to modulate ambulatory load magnitude without relevant changes to joint kinematics. Specifically, the advantage of this experiment is that only load magnitude (vGRF) but not load frequency (cadence), nor total number of load cycles (number of steps) was modified without relevant changes in ankle, knee or hip kinematics. Hence, this experimental framework is suitable for studying the dose-response relationship between ambulatory load and load-induced changes in cartilage biomarkers.

vGRF trajectories and peaks, spatiotemporal parameters, discrete joint angles, and joint kinematic trajectories for the reduced and increased load conditions were compared to the normal load condition. These values and patterns were comparable to the literature. For instance, the first and second peak of the vGRF for the normal load condition were similar in magnitude and around 100%BW. Similar values have been reported by studies investigating treadmill walking [40] that measured slightly lower forces than in over ground walking [41]. Other studies comparing treadmill walking with over ground walking reported lower [42] or comparable [40] peak forces for treadmill walking. Because in our study, all conditions were measured on the same pressure plate of the same treadmill, the same basic principles of

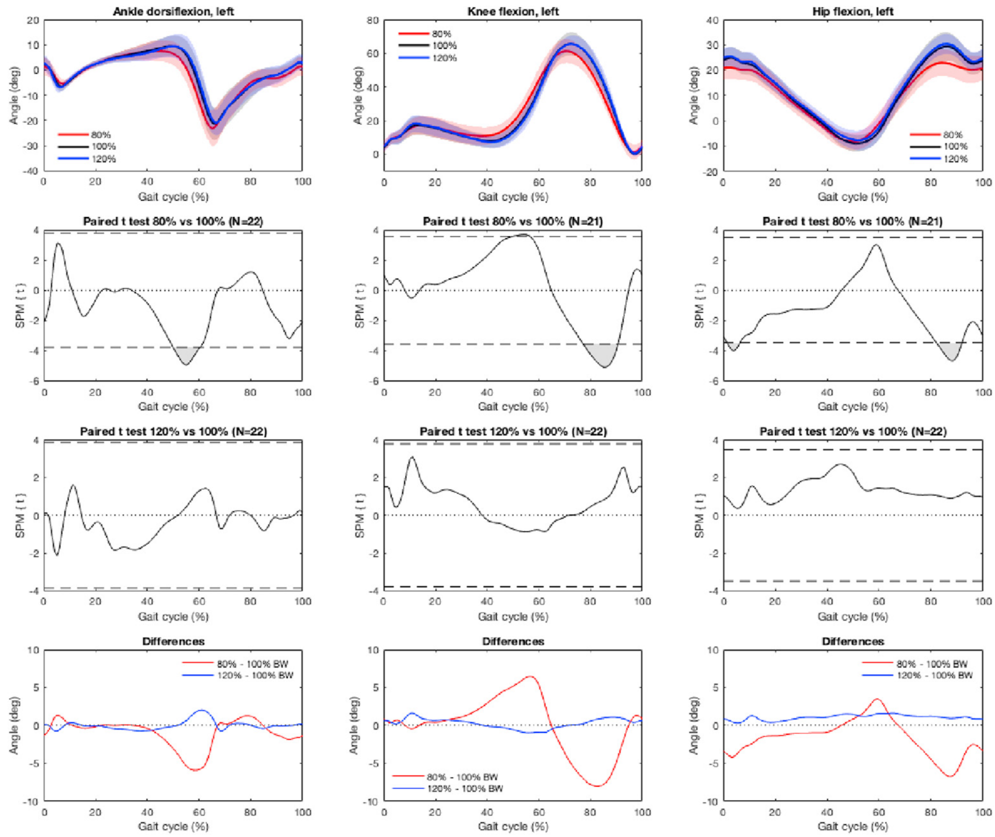

Fig. 2. Mean (1 standard deviation) joint angle for the ankle, knee and hip (top; left to right), results of SPM paired *t*-test for the 80% and 120% vs. 100%BW conditions (1st and 2nd middle), and absolute difference in joint angle for the 80% and 120% vs. 100%BW conditions grey areas indicate statistical differences between conditions ($p < 0.025$). Datasets included in joint angle calculations were expressed as N.

treadmill walking applied to all conditions and thus the measured vGRF can be used to quantify the degree of load modification.

The observed reduction in vGRF by 19.5%BW indicates that the targeted load reduction of 20%BW was achieved. Greater load reductions towards toe-off are in line with results by McGowan et al. [43] who reported a decrease in propulsive impulse from 40 to 70% of the gait cycle for the reduced load conditions. With increasing load reduction, progressive reductions in propulsive impulse came with a reduction in internal plantarflexion moment and power [44]. This observation of a reduced push-off in the reduced load condition is supported by our data showing that the load reduction occurs mainly in the forefoot region.

The reduced load condition resulted in a significantly shorter stance time and although not statistically significant shorter step time and step length compared to the normal load condition. However, differences in step time and step length were small and around the detection limits of the measuring system (0.008 s, 0.009 m, respectively). Interestingly, McGowan et al. [43] reported no reduction in stance time for load reductions of 25% and 50%BW whereas Lewek [44] and Threlkeld et al. [31] reported a tendency for a reduction in stance time with greater load reduction. The same inconsistent behavior in stance phase was reported in a systematic review by Apte et al. [45]. Since step time for the normal and reduced load condition did not differ but stance time differed, we assume that the pre-swing phase for the reduced load condition was shorter.

In our study, we measured a small but not significant increase in cadence for the reduced load condition. This increase in cadence corresponded to around 37 additional steps during the entire 30-min walking exercise compared to the normal load condition (average total number of steps: 3324) and hence was deemed negligible. Contrary to our findings, Threlkeld et al. [31] reported that cadence decreased with load reductions of 30% and 50%BW, and McGowan et al. [43] found longer stride times and hence lower cadence for load reductions of 25% and 50%BW achieved using similar pneumatic unloading systems. No definitive trend of increase or decrease in cadence was found across five studies in healthy participants [45]. Moreover, in our study step time was not affected by the 20%BW load reduction which is supported by other studies where only reductions in bodyweight of 40% or 50%BW resulted in significantly reduced [44] or increased [31] step length, respectively. Overall, the different loading conditions in our experimental framework does not appear to impose major changes to the subjects' gait cycle.

For the reduced load condition, discrete ankle dorsiflexion angles were lower in mid stance phase. Considering the temporal course of ankle angles in Fig. 2, an earlier initiation of plantar flexion was observed confirming other observations for load reductions [31,43]. For instance, Threlkeld et al. [31] reported that reducing load to 70%BW initiated plantarflexion as early as in midstance. The earlier initiation of push-off came along with an earlier knee flexion in late stance (50–60% of the gait cycle) resulting in a significant difference between the reduced and normal load conditions during terminal stance that exceed 5°.

Overall, the reduced and increased load conditions elicited only few changes in discrete kinematic parameters, and these changes were well below previously reported relevance criteria [36,37]. While analysis of the kinematic patterns revealed additional differences in ankle and knee angle trajectories exceeding 5° between the normal and reduced load conditions, these differences occurred during pre-swing. This phase of the gait cycle coincides with the time when the vGRF decreases rapidly to 0%BW. Therefore, it is reasonable to assume that these kinematic differences have little relevance in the context of cartilage mechanosensitivity. The region of articular cartilage of the knee that may be affected by the slightly altered kinematics and the associated low load during this phase likely will not affect load-induced changes in cartilage biomarkers considering the total accumulated load during stance. Similarly,

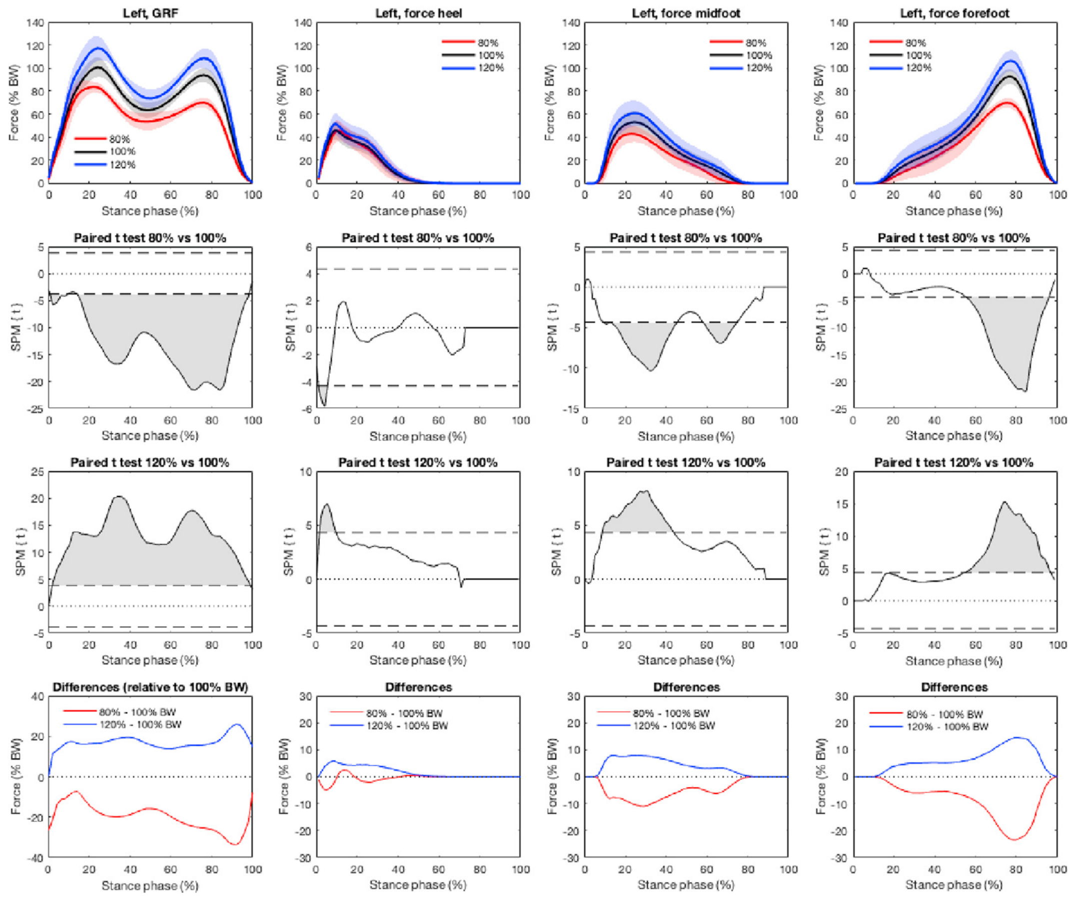

Fig. 3. Mean (1 standard deviation) overall, heel, midfoot and forefoot ground reaction during the three loading conditions (top; left to right), results of SPM repeated measures *t*-test for the 80% and 120% vs. 100%BW conditions (1st and 2nd middle), and mean difference in ground reaction force for the 80% and 120% vs. 100%BW conditions (N = 24). Grey areas indicate statistical differences between conditions.

differences in knee and hip flexion during swing for the reduced load conditions compared to normal load occurred during times with modelled joint moments of one third of maximal forces [46]. Hence, these differences should be deemed as not relevant in the context of cartilage mechanosensitivity. Recently, Apte et al. [45] affirmed in their systematic review that load reductions up to 30%BW have limited influence on kinematic and spatiotemporal parameters but higher effects on kinetic parameters.

For the increased load condition, differences in spatiotemporal parameters compared to the normal load condition were smaller than observed differences from reduced to normal load conditions. Similarly, previous studies reported that double support, stance and swing time or stride length were not altered when wearing a 15%BW backpack [32] or by a 25% increase in bodyweight [43]. While spatiotemporal gait parameters were not affected by the increased load condition, previous studies reported inconsistent results regarding joint range of motion and joint kinematics. For instance, Dames and Smith [32] observed no differences in ankle, knee and hip angle range of motion between walking with 15%BW and normal load walking. We did not reveal differences in joint kinematics between the increased and normal load conditions except the slightly increased (+1.2°) but negligible maximal knee flexion during stance. This result is in contrast to Areliano et al. [33] who found increased hip and knee flexion at initial contact for walking with increased load and Firner et al. [25,26] who reported altered knee kinematics that came along with the intended increase in external knee flexion moment for running with active orthoses. Boffey et al. [47] defined a bodyweight increase by more than 21%BW as threshold for significant changes in gait pattern. Hence, other experimental settings for altering joint load may result in changes in joint kinematics making them unsuitable for studying *in vivo* articular cartilage mechanosensitivity. These results emphasize the need for monitoring the different load characteristics in these experimental settings and demonstrate the strength of our framework.

The average increase in vGRF of 16.8%BW for most of the stance phase and the 14% increase in loading rate suggests that the increased load condition generates a systematically increased load for the entire stance phase that is slightly smaller than the targeted 20% increase. Although maximum knee flexion at midstance for the increased load condition differed significantly from the normal load condition, this difference was negligible. Hence, the increased load condition can be considered as suitable for increasing load without changing lower leg kinematics. Using this experimental framework, Herger et al. [30] have shown changes in load-induced serum levels of cartilage oligomeric matrix protein, a marker reflecting cartilage health, between loading conditions suggesting that the modulation of load achieved using this experimental framework are sufficient in magnitude to elicit different metabolic responses.

4.1. Limitations

In this study, we measured only sagittal plane joint kinematics. However, for an initial evaluation of whether joint kinematics of reduced and increased load conditions were comparable to normal load treadmill walking sagittal plane joint kinematics are sufficient because this is the plane of primary joint motion during walking. To expand this framework to populations at risk for developing knee OA such as persons after knee injury or with varus or valgus knee alignment, assessing frontal and transverse plane kinematic especially at the knee will be necessary.

Similarly, we only measured the vGRF because of the technical limitation of an instrumented treadmill with a built-in pressure plate. Employing instrumented treadmills with built-in force plates would allow measuring not only joint kinematics but also joint kinetics that may be relevant in the context of articular cartilage mechanosensitivity.

Such a setup in combination with musculoskeletal models would allow to estimate internal joint forces applied to the articular cartilage during the different load conditions. vGRF and joint kinematics were recorded during 60 s in the initial (minute 3 to minute 4) phase of the 30-min walking stress test after participants had time to habituate to the given load condition. While we also collected data at later time points during the 30-min walking stress test, data were often missing because of technical difficulties. Future studies should consider measuring continuous kinematic data to rule out kinematic changes during the test. According to Donelan and Kram [48], 1-min walking with reduced bodyweight is adequate to accustom subjects to the condition.

5. Conclusion

Similar kinematics between conditions suggest that the same cartilage regions are loaded in all loading conditions. This experimental framework may be suitable for studying the dose-response relationship of isolated changes in ambulatory load magnitude and physiological effects without changing other load characteristics or loaded cartilage regions in the context of *in vivo* mechanosensitivity of articular cartilage in health and disease. However, a framework for studying the effects of modulated load without relevant kinematic changes should be limited to load changes by a maximum of 20%BW.

Author contributions

SH and AM designed the study; SH recruited the participants and collected the data; SH, CN prepared the data for statistical analysis; CN performed the statistical analysis; SH, CN, AML, CE and AM were involved in data interpretation; SH, CN and AM prepared the manuscript; SH, CN, AML, CE and AM contributed to reviewing and revising the manuscript, and approved the final draft.

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Declaration of competing interest

The authors declare no conflict of interest.

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