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**The repeated bout effect influences lower-extremity biomechanics during a 30-min
downhill run**

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Abstract

The repeated bout effect in eccentric-biased exercises is a well-known phenomenon, wherein a second bout of exercise results in attenuated strength loss and soreness compared to the first bout. We sought to determine if the repeated bout effect influences changes in lower-extremity biomechanics over the course of a 30-min downhill run. Eleven male participants completed two bouts of 30-min downhill running (DR1 and DR2) at 2.8 m.s⁻¹ and -11.3° on an instrumented treadmill. Three-dimensional kinematics and ground reaction forces were recorded and used to quantify changes in spatiotemporal parameters, external work, leg stiffness, and lower extremity joint-quasi-stiffness throughout the 30-min run. Maximum voluntary isometric contraction (MVIC) and perceived quadriceps pain were assessed before-after, and throughout the run, respectively. DR2 resulted in attenuated loss of MVIC ($P=0.004$), and perceived quadriceps pain ($P<0.001$) compared to DR1. In general, participants ran with an increased duty factor towards the end of each running bout; however, increases in duty factor during DR2 (+5.4%) were less than during DR1 (+8.8%, $P<0.035$). Significant reductions in leg stiffness (-11.7%, $P=0.002$) and joint quasi-stiffness (up to -25.4%, all $P<0.001$) were observed during DR1 but not during DR2. Furthermore, DR2 was associated with less energy absorption and energy generation than DR1 ($P<0.004$). To summarize, the repeated bout effect significantly influenced lower-extremity biomechanics over the course of a downhill run. Although the mechanism(s) underlying these observations remain(s) speculative, strength loss and/or perceived muscle pain are likely to play a key role.

Keywords: Biomechanics, 3D analysis, Exercise

Word count: 4226

Highlights

- A 30-min downhill running bout increased contact time and reduced flight time transitioning to an increased duty factor.
- Lower-extremity stiffness also decreased and mechanical energy absorption increased over the course of the first 30-min downhill running bout.
- When the same bout of 30-min downhill running was performed three weeks later, the observed changes to lower extremity biomechanics were significantly attenuated.
- The findings from this study demonstrated, for this first time, a repeated bout effect for lower extremity biomechanics associated with downhill running.

1. Introduction

Running is characterized by repetitive stretch-shortening cycles, with the magnitude of the eccentric muscular work being dependent on the speed and grade of running (Khassestarash et al., 2020). Eccentric loading is associated with muscle damage that has both acute (Chen, Chen, et al., 2007) and delayed effects (Chen et al., 2009) on running biomechanics. The total eccentric work over the course of a run is a function of the magnitude of eccentric work per step and the number of steps per run. Among these, the magnitude of eccentric work appears to contribute more to the amount of muscle damage (Chapman et al., 2006), which would be expected to have a larger influence on biomechanics.

Downhill running has been utilized as a model to induce muscle damage as well as alterations in neuromuscular function (e.g. Giandolini et al., 2016; Martin et al., 2004), energetics (Baumann et al., 2014; Chen et al., 2009), and running biomechanics (Chen, Nosaka, et al., 2007; Lima et al., 2020). The eccentric work, and corresponding muscle damage and strength loss, associated with downhill running has been well quantified (e.g. Buczek & Cavanagh, 1990; Byrnes et al., 1985; Chen, Chen, et al., 2007; Giandolini et al., 2016), with prolonged downhill running associated with dramatic strength reductions. For instance, the amount of strength loss induced by a 6.5-km intense downhill run (-14.7% to -22.5% knee extensor maximum voluntary contraction torque loss) (Giandolini et al., 2016) is comparable to a 30-km mountain race (-23.5% knee extensor maximum voluntary contraction torque loss) (Millet et al., 2003). High amounts of lower extremity muscle damage and associated pain are likely to compromise the muscle's ability to resist tension (MacIntyre et al., 1996) and joint excursion upon landing during running, resulting in a more compliant leg spring. Lower-extremity compliance (or stiffness) in biomechanics has traditionally been measured one-of-two ways: i) at the joint level, as the slope of the joint moment/joint angle

curve (Farley & Morgenroth, 1999), or ii) at the system level, as the peak ground reaction force divided by the change in “leg length” (McMahon & Cheng, 1990). The former is frequently referred to as joint quasi-stiffness because it is dependent on active muscular contraction (Latash & Zatsiorsky, 1993). Given the positive correlation between joint quasi-stiffness and leg stiffness (Farley & Morgenroth, 1999), reduced joint quasi-stiffness is typically associated with reduced leg stiffness and increased contact time (McMahon & Cheng, 1990). At a constant speed of running, an increase in contact time, along with a reduction in flight time, would result in an increased duty factor. Increased duty factor has been suggested to reduce peak eccentric forces in lower extremity muscles (Bonnaerens et al., 2019); hence, this transition could be protective in nature.

An important consideration for eccentric-biased exercise is a phenomenon known as the repeated bout effect, which has been widely documented [reviewed in Hyldahl et al. (2017)]. In downhill running, an initial bout of exercise produces a protective effect such that a secondary bout of downhill running, up to a few weeks afterwards, causes less muscle damage (Byrnes et al., 1985; Chen, Chen, et al., 2007; Khassetarash et al., 2021) and strength loss (Rowlands et al., 2001) than the initial bout. If muscle damage and strength loss over the course of a prolonged downhill run causes a transition towards an increased duty factor, it is plausible to assume that the repeated bout effect will attenuate this transition. To the best of our knowledge, the influence of the repeated bout effect on running biomechanics during 30-min of downhill running has never been investigated.

Thus, the purpose of this study was to investigate alterations in running biomechanics and the influence of the repeated bout effect on running biomechanics over the course of a 30-min downhill running bout. We hypothesized that: H1) participants would transition to a gait pattern characterized by an increased duty factor during the first downhill run (DR1); and H2) the repeated

bout effect would reduce the extent of alterations in running biomechanics during the second downhill run (DR2).

2. Materials and methods

2.1. Participants

A previous study that used downhill running to induce neuromuscular fatigue observed a large effect size of Cohen's $d = 1.09$ for maximum voluntary contraction force loss (Giandolini et al., 2016). For this study, a minimum sample size of $N = 10$ was computed using GPower (GPower, Version 3.1.9.2) for effect size Cohen's $d = 1.09$ and statistical significance $\alpha = 0.05$ to reach a statistical power of $1 - \beta = 0.8$. Eleven physically active males (height: 1.75 ± 0.1 m, body mass: 75.1 ± 10.5 kg, age: 26.4 ± 3.2 years) volunteered for this study. The participants were familiar with treadmill running, but they had not performed any prolonged downhill running bouts and had not sustained any musculoskeletal injuries within the 6-months prior to data collection. All participants provided written informed consent and the study procedure was approved by the Conjoint Research Ethics Board at the University of Calgary (REB 18-3093).

2.2. Protocol

Participants visited the laboratory on three occasions. During the first visit (familiarization), knee extensor maximum voluntary isometric contraction (MVIC) was assessed as a global fatigue criterion. MVIC was assessed on the knee extensors of the right leg. The participant sat on an isometric chair with 80° hip flexion and 90° knee flexion. The ankle was fixed to the chair just

slightly above the malleolus using a non-compliant strap attached to a calibrated force transducer. The participant performed two MVICs for 5-seconds each and the average of the two trials were reported for each assessment. Then, a series (less than 5) of intermittent 1-min downhill running bouts were performed at target speed and grade (i.e., -11.3° and $2.8 \text{ m}\cdot\text{s}^{-1}$) on an instrumented treadmill (Bertec Corps., Columbus, OH) by the participants until they felt comfortable. The second session began with a MVIC assessment. Retroreflective markers were then adhered to the left leg and pelvis for motion analysis (Vicon Motion analysis Ltd, Oxford, UK). Tracking markers were attached to the left and right anterior superior iliac spine, sacrum, mid-thigh, mid-calf, and foot. A cluster of four tracking markers was adhered to the mid-thigh and mid-calf, and single tracking markers were adhered to the fifth metatarsal, distal foot, and the heel. Calibration markers were attached to the left and right greater trochanters, the medial and the lateral knee joint (i.e., at the knee joint line between the femur and the tibia), and the medial and the lateral malleoli. A static trial was captured with the participants standing still in the neutral position. The calibration markers were then removed. The participants then warmed up with 5-min of level running at $2.8 \text{ m}\cdot\text{s}^{-1}$. Afterwards, the participants performed a 30-min downhill running bout at a grade of -11.3° and the speed of $2.8 \text{ m}\cdot\text{s}^{-1}$. The speed and duration were chosen according to Martin et al., (2004) who observed a $\sim 15\%$ reduction in knee extensor MVIC after the downhill running bout which was indicative of substantial neuromuscular fatigue. Force (2000 Hz) and three-dimensional motion data (200 Hz) were recorded synchronously for 20 s at the end of minute 1, 5, 10, 15, 20, 25, and 29, hereafter referred to as T1, T5, T10, T15, T20, T25, and T29, respectively. At the same time points, the participants were asked to rate their perceived overall exertion (RPE) and perceived quadriceps pain according to Borg's CR10 Scale[®] (Borg, 1998). Immediately after ($152 \pm 13 \text{ s}$) the termination of downhill run, the MVIC assessment was performed. Session three mimicked that of session two but was performed exactly three weeks later at the same time of the day (\pm one hour). This three-

week window was chosen in accordance with previous downhill running research investigating muscle soreness and the repeated bout effect (Byrnes et al., 1985). We have previously shown that the current protocol elicited indirect markers of muscle damage such as delayed onset muscle soreness and serum creatine kinase rise that persisted up to 96 hours after DR1, along with persistent reduction in MVIC up to 72 hours after DR1 (Khassetarash et al., 2021). This protocol also elicited a repeated bout effect on indirect markers of muscle damage, where creatine kinase rise and MVIC loss were attenuated following the second downhill running bout (Khassetarash et al., 2021).

2.3. Data analysis

The ground reaction force was rotated 11.3° (i.e., the grade of the treadmill) to coincide with the vertical and horizontal directions of the global reference frame. The vertical component of the ground reaction force was used to identify foot-strike and toe-off using a 50 N force threshold. Contact time, flight time, step frequency, and duty factor were then calculated. Contact time was defined as the time from left foot touchdown to left foot toe-off and flight time was defined as the time from left foot toe-off to right foot touchdown. Duty factor defined as the fraction of stride when each foot is on the ground (Alexander & Jayes, 1980) then calculated as:

$$duty\ factor = \frac{contact\ time}{2(contact\ time + flight\ time)} * 100$$

Center of mass (COM) velocity and position were calculated by integrating, and double integrating the ground reaction force after subtracting body weight and dividing by body mass, respectively.

The time-history of COM power was calculated as:

$$P_{\text{COM}} = F_z \cdot V_{z\text{COM}} + F_y \cdot V_{y\text{COM}}$$

where F_z and F_y are the vertical and horizontal components of ground reaction force. $V_{z\text{COM}}$ and $V_{y\text{COM}}$ are the velocity of the center of mass in the vertical and horizontal directions, respectively. The $F_x \cdot V_{x\text{COM}}$ component was eliminated due to its small magnitude relative to other components. Finally, energy absorption was defined as the time integral of negative power, and energy generation was defined as the time integral of positive power. Leg stiffness was calculated similar to McMahon et al. (McMahon & Cheng, 1990) with some subtle differences to account for downhill running (see Online Supplementary Materials).

A flexion-extension, adduction-abduction, and internal-external sequence of Cardan rotations were used to calculate joint kinematics. The internal joint moments were calculated from inverse dynamics considering the pelvis, thigh, leg, and foot as rigid bodies. The segment masses, center of mass locations, and moments of inertia were calculated using the equations of Vaughan et al. (Vaughan et al., 1992). Details of the joint kinematics and inverse dynamics analyses can be found in our previously published work (Khassetarash et al., 2020). Ankle and knee joint quasi-stiffness were then calculated via linear regression on the linear portion of the joint angle-joint moment curves (Figure S1) (Farley & Morgenroth, 1999).

2.4. Statistical analysis

Results were reported as mean \pm standard deviation or median and interquartile range when the variables were normally or non-normally distributed, respectively (Figures 2-4). When the values were not different between DR1 and DR2 at baseline [first data collection at the end of minute one (T1)], data were normalized to values at T1 to ensure the bout effect was isolated when assessing

the repeated bout effect. This was the case for all variables except energy absorption and energy generation; therefore, statistical analyses were performed on the absolute values for energy absorption and energy generation. For all other variables, statistical analyses were performed on normalized values. A longitudinal analysis (bout \times time) was performed using generalized estimating equations (Liang & Zeger, 1986). If significant main effects or interactions were observed, pairwise comparisons were performed and adjusted for multiple comparisons using Bonferroni's correction. Pearson or Spearman correlation analyses, depending on data normality, were used to examine the relationship between changes in biomechanical as well as MVIC variables from T1 to T29 during DR1. The procedures described by Benjamini and Hochberg (Benjamini & Hochberg, 1995) were used to correct for the P -values for multiple testing with a false discovery rate of 5% and significance defined as adjusted P -values < 0.05 . All statistical analyses were performed using IBMTM SPSSTM Statistics (version 26.0.0; IBM Corp., Somers, New York, NY) with the criterion α -level set to 0.05.

3. Results

3.1. RPE, perceived quadriceps pain, and MVIC

The changes in RPE and Pain were reported with regards to baseline (T1). RPE and perceived quadriceps pain progressively increased with time during both downhill running sessions (Figure 1A and 2B), while changes at the end of the run were larger during DR1 compared to DR2 (all $P < 0.001$). Both RPE and perceived quadriceps pain demonstrated bout \times time interactions [χ^2 (6) = 40.335, $P < 0.001$ and χ^2 (6) = 121.918, $P < 0.001$, respectively], where RPE and perceived quadriceps pain were smaller during DR2 compared to DR1 after T20 (all $P < 0.008$). A bout \times

time interaction was observed for MVIC [$\chi^2(6) = 11.212, P = 0.001$]. MVIC was reduced by 15.7% (from 701 ± 114 N at PRE to 590 ± 112 N at POST) after DR1 and by 7.7% (from 676 ± 118 N to 625 ± 121 N) after DR2 (both $P < 0.01$), while the MVIC reduction was smaller after DR2 than after DR1 ($P = 0.004$).

3.2. Spatiotemporal parameters

The changes in spatiotemporal parameters were reported with regards to baseline (T1). Main effects of time [$\chi^2(6) = 53.738, P < 0.001$] and bout [$\chi^2(6) = 4.192, P = 0.041$] were observed for contact time (Figure 1C). Contact time progressively increased from 0.7% at T5 to 8.5% at T29 (all $P < 0.017$). The difference in contact time between the two bouts became significant at T15 and T25 (all $P < 0.033$). The bout \times time interaction [$\chi^2(6) = 18.509, P = 0.005$] was significant for flight time (Figure 1D). During DR1, flight time gradually decreased from -2.7% at T15 to -32.1% at T29 (all $P < 0.003$), while during DR2, flight time decreased up to -19.6% at T29 ($P < 0.001$). The difference in flight time reduction between the two bouts became significant at T25 and T29 (all $P < 0.05$). Step frequency did not change between the beginning and the end of either running bout (Figure 1E). A bout \times time interaction [$\chi^2(6) = 17.319, P = 0.008$] was observed for duty factor (Figure 1F). During DR1, duty factor increased progressively to reach +8.8% at T29 (all $P < 0.035$) while it increased by only 5.4% at the same time point during DR2 (all $P < 0.006$). The increases in duty factor during DR1 were higher than DR2 at T15, T25, and T29 (all $P < 0.043$).

3.3. ENERGY ABSORPTION AND ENERGY GENERATION

Energy absorption and energy generation showed main effects of time [$\chi^2(6) = 31.157, P < 0.001$ and $\chi^2(6) = 57.701, P < 0.001$, respectively] and bout [$\chi^2(2) = 6.877, P = 0.009, \chi^2(6) = 11.494, P = 0.001$, respectively] (Figure 2A and B). During both downhill running bouts, energy absorption increased up to 4.8 % and 8.2% at T10 and T15, respectively compared to T1 (all $P < 0.014$). Energy absorption was larger during DR1 compared to DR2 at all time points ($P = 0.002$). During both DR bouts, energy generation progressively decreased from -2.2% at T5 to -5.2% at T29 compared to T1 ($P < 0.05$). Energy generation was higher during DR1 compared to DR2 at all time points (all $P < 0.034$), except at T5 and T20, and T29 (all $P > 0.54$).

3.4. Stiffness

The changes in stiffness were reported with regards to baseline (T1). A significant bout \times time interaction was observed for leg stiffness as well as ankle and knee quasi-stiffness [$\chi^2(6) = 15.736, P = 0.015, \chi^2(6) = 39.093, P < 0.001$, and $\chi^2(6) = 15.299, P = 0.018$, respectively] (Figure 3). During DR1, leg stiffness gradually decreased to -11.7% at T29 (all $P < 0.003$), while this variable did not change during DR2 (all $P > 0.144$). The change in leg stiffness was larger during DR1 compared to DR2 at T29 only ($P = 0.027$). Ankle quasi-stiffness was reduced progressively from -14.2% at T5 to -25.5% at T29 (all $P < 0.001$) during DR1 but it did not change during DR2 (all $P > 0.105$). Additionally, knee quasi-stiffness progressively reduced from -9.3% at T15 to -14.8% at T29 (all $P < 0.023$) during DR1 while it did not change during DR2 (all $P > 0.12$). The observed change in knee quasi-stiffness was greater during DR1 than DR2 at T20 onwards (all $P < 0.049$).

3.5. Correlation analysis

Table 1 summarizes the correlation matrix between the changes in biomechanical as well as MVIC variables from T1 to T29 during DR1. Changes in knee quasi-stiffness was the only variable that had a significant correlation with changes in MVIC force [$r = 0.86$ (confidence interval range: 0.48 to 0.96), $P = 0.003$]. Furthermore, changes in contact time were correlated with changes in flight time [$r = 0.8$ (confidence interval range: 0.38 to 0.95), $P = 0.004$] and changes in leg stiffness [$r = -0.713$ (confidence interval range: -0.92, -0.20), $P = 0.018$]. Changes in flight time were also correlated with changes in energy absorption [$r = 0.675$ (confidence interval range: 0.13, 0.91), $P = 0.032$]. Finally, changes in step frequency were correlated with changes in energy generation [$r = 0.778$ (confidence interval range: 0.33, 0.94), $P = 0.006$].

4. Discussion

The purpose of this study was to quantify: i) the effects of a 30-min downhill running bout on biomechanics and ii) the influence of repeated bouts of downhill running on alterations to running biomechanics. A 30-min bout of downhill running resulted in substantial increases in perceived exertion, perceived muscle pain, strength loss, and alterations in running biomechanics, in particular a transition towards a gait pattern characterized by an increased duty factor. During the second downhill running bout, performed three weeks later, perceived exertion, perceived pain, strength loss, and alterations to running biomechanics were attenuated.

4.1. Biomechanical alterations during DR1

We hypothesized that running biomechanics over the course of the 30-min downhill running bout would gradually transition to a more grounded running pattern. Indeed, 30-min of downhill running was accompanied by progressive reductions in flight time and increases in duty factor and contact time confirming our hypothesis. These alterations in spatiotemporal parameters were

accompanied by large (up to ~25%) reductions in stiffness measures indicating that the leg spring and lower extremity joints became more compliant as the downhill run progressed. Contact time is negatively correlated with leg stiffness (Moore et al., 2019), which can be mechanically explained by a mass-spring model (Ferris et al., 1999); it takes a longer time for a more compliant spring to recoil, thereby elongating contact time. Leg stiffness is modulated by alterations in joint quasi-stiffness (Farley & Morgenroth, 1999), which is related to active muscle tension (Latash & Zatsiorsky, 1993). Hence, when the tension-resistance capacity of the neuromuscular unit is reduced during the 30-min downhill running bout, the joint quasi-stiffness and eventually spatiotemporal parameters would be affected.

A gradual loss of strength in the knee extensor muscles due to repeated eccentric contraction may have played a key role in the observed biomechanical alterations. This is supported by the significant correlation observed between the loss of MVIC force and the reduction in knee quasi-stiffness. It should be noted that runners lost substantial quadriceps strength (>15% MVIC loss) after the downhill run in this study which could be the result of peripheral (such as muscle damage) and/or central factors (Khassetarash et al., 2021; Martin et al., 2004). Previous studies have shown that prolonged unaccustomed downhill running produces substantial muscle damage evident in muscle biopsies (Féasson et al., 2002), or measured indirectly by serum creatine kinase activity (Byrnes et al., 1985; Féasson et al., 2002; Lima et al., 2020; Westerlind et al., 1994). Eccentric exercise may also induce central fatigue through knee extensors/plantar flexors group III and IV afferent feedback, which play a role in force production capacity (Martin et al., 2005). The activation of those sensitive fibers also explains the high perception of pain reported by the participants (Figure 1B). Higher perceived pain may have contributed to a reduction in central

command during running and eventually to the reduction in knee quasi-stiffness leading to alterations to running pattern.

The large biomechanical alterations observed in this study appear to be specific to downhill running. For example, leg stiffness and contact time did not change during a 1-hour submaximal exhaustive level run (Hunter & Smith, 2007) or during a 10 kilometer level run at near-maximum effort (Sanno et al., 2018). In contrast, a small (~1.5%) increase in contact time has been reported during a 10 kilometer exhaustive run on a level treadmill (Cigoja et al., 2021), which is minimal compared to 8.5% increase observed in the current study. This discrepancy is likely associated with the larger amount of eccentric contraction in prolonged downhill running compared to level running (Buczek & Cavanagh, 1990; Khassetarash et al., 2020).

4.2. Repeated Bout Effect: running biomechanics alterations during DR2 vs DR1

We hypothesized that the observed alterations in running biomechanics associated with 30-min of downhill running would be attenuated during DR2, eliciting a repeated bout effect in running biomechanics. The present results confirmed our hypothesis, in that a repeated bout effect was evident in spatiotemporal parameters, leg stiffness, along with the ankle and knee quasi-stiffness. Although the running pattern still transitioned towards a more grounded running during DR2, the alterations in running pattern was significantly less than that during DR1. For instance, comparing the slope of duty factor over time between the two downhill running bouts shows that extending the running time further than 30 min during DR2 would likely result in similar alterations as DR1 (i.e., a significant ~9% increase in duty factor). From an energetics point of view, the energy absorption (i.e., negative work) and energy generation (i.e., positive work) were significantly

lower during DR2 compared to DR1 (Figure 3). This means that a lower amount of energy was expended to accomplish the same running task which can further explain the reduced exertion and reduced pain during DR2 compared to DR1. Ultimately, it can be suggested that the repeated bout effect does not eliminate but delays the transition to grounded running during a 30-min downhill running bout.

We observed changes in joint quasi-stiffness during DR1 but not DR2. This can be explained by the repeated bout effect paradigm and in the context of better peripheral and central adaptations: i) in terms of peripheral adaptation, the first eccentric-biased exercise confers a protective effect which increases the muscles' ability to mechanically withstand tension (Hyldahl et al., 2017; Lau et al., 2015), ii) in terms of central adaptation the first eccentric-biased exercise confers a protective effect, which reduces the recruitment of faster motor units, increases the recruitment of additional slower motor units or increases motor unit synchronization, thereby redistributing the workload across the muscle fibres (Hyldahl et al., 2017). In the current study, one may speculate that less muscle damage during the second bout (Khassetarash et al., 2021) led to lower sensation of pain, which in turn led to improved central command (Farina et al., 2004; Graven-Nielsen et al., 2002) by lowering pain-related afferent feedback and consequently, better resistance against tension.

In summary, the repeated bout effect attenuated alterations to running biomechanics that may have been protective in nature. The biomechanics of running were characterized by reduced energy absorption and energy generation during DR2, which is highly important from a performance perspective, as it would increase an individual's capacity to run longer. Thus, downhill training is essential to improve the performance in trail, mountain running, and hilly urban running.

4.3. Limitations

Treadmill speed during downhill running was set the same for all participants. While some studies prescribed different speeds based on participant's maximum oxygen uptake (Chen, Chen, et al., 2007; Hamill et al., 1991), the intensity of downhill running cannot solely be determined by aerobic capacity since downhill running is more mechanically demanding. Furthermore, the speed determined during a level running incremental test is not an accurate measure for downhill running and the runners cannot reach their absolute maximal oxygen uptake during downhill running incremental tests (Lemire et al., 2020). Therefore, prescribing the right downhill running intensity to produce a specific amount of muscle damage remains an open question (Bontemps et al., 2020). The rate of perceived exertion and perceived quadriceps pain only reached a moderate to strong level, which may suggest that the downhill running protocol was not challenging enough. However, we observed that the downhill running bout induced a substantial reduction in MVIC along with the dramatic alterations observed in running biomechanics. Finally, although we calculated mechanical energy absorption and energy generation throughout the downhill run, we did not measure the energy cost of running.

4.4. Conclusion

Running biomechanics during a 30-min downhill run transitioned to a gait characterised by an increased duty factor and a corresponding decrease in leg stiffness and joint quasi-stiffness. A major finding of the present study was that the repeated bout effect attenuated this transition during the second bout, during which ankle and knee quasi-stiffness remained constant. Furthermore, the repeated bout effect resulted in lower energy absorption and energy generation during the second

bout compared to the first bout. Although the reduction in energy absorption may have played a role in minimizing muscle damage and pain during the second bout, the mechanism(s) of the observed repeated bout effect on running biomechanics remain to be fully elucidated. It can be concluded that strength loss (as surrogate measures of muscle damage) and perceived pain play a key role in alterations in running biomechanics during downhill running.

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Conflict of interest

The authors declare no conflicts of interest.

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Table 1. Correlation matrix between changes in biomechanical variables as well as changes in MVIC at the beginning (minute 1: T1) and at the end (minute 29: T29) of the first downhill run (DR1). The superscripts P or S indicate Pearson's or Spearman's correlation coefficient, respectively. The *P*-values were adjusted for false discovery rate using Benjamini-Hochberg method (Benjamini & Hochberg, 1995). Statistically significant *P*-values are accompanied with an asterisk.

	Variable	Δ Duty factor	Δ Contact time	Δ Flight time	Δ Step frequency	Δ Leg stiffness	Δ Ankle quasi-stiffness	Δ Knee quasi-stiffness	Δ Energy absorption	Δ Energy generation
Δ MVIC	Correlation coefficient	-0.20 ^P	-0.11 ^S	-0.10 ^P	0.14 ^P	0.05 ^S	-0.03 ^P	0.86 ^P	0.12 ^P	-0.05 ^P
	CI: [LL, UL]	[-0.71, 0.45]	[-0.67, 0.52]	[-0.66, 0.53]	[-0.50, 0.68]	[-0.57, 0.63]	[-0.61, 0.58]	[-0.48, 0.96]	[-0.52, 0.67]	[-0.63, 0.57]
	Adjusted <i>P</i> -value	0.942	0.942	0.942	0.942	0.942	0.942	0.003*	0.942	0.942
Δ Duty factor	Correlation coefficient	-	0.797 ^S	-0.356 ^P	0.232 ^P	-0.452 ^S	-0.059 ^P	-0.322 ^P	-0.162 ^P	-0.248 ^P
	CI: [LL, UL]	-	[0.38, 0.95]	[-0.78, 0.31]	[-0.42, 0.73]	[-0.82, 0.21]	[-0.63, 0.56]	[-0.77, 0.34]	[-0.69, 0.49]	[-0.74, 0.41]
	Adjusted <i>P</i> -value	-	0.004*	0.341	0.546	0.210	0.856	0.384	0.704	0.525
Δ Contact time	Correlation coefficient	-	-	-0.608 ^S	-0.315 ^S	-0.713 ^S	-0.406 ^S	-0.483 ^S	0.035 ^S	-0.112 ^S
	CI: [LL, UL]	-	-	[-0.88, -0.013]	[-0.77, 0.351]	[-0.92, -0.20]	[-0.81, 0.26]	[-0.84, 0.16]	[-0.58, 0.62]	[-0.67, 0.52]

	Adjusted <i>P</i> -value	-	-	0.054	0.383	0.018 [*]	0.239	0.149	0.914	0.851
Δ flight time	Correlation coefficient CI: [LL, UL]	-	-	-	-0.184 ^P [-0.47, 0.71]	0.563 ^P [-0.06, 0.87]	0.005 ^P [-0.60, 0.60]	0.215 ^P [-0.44, 0.72]	0.675 ^P [0.126, 0.91]	0.272 ^P [-0.39, 0.75]
	Adjusted <i>P</i> -value	-	-	-	0.680	0.086	0.987	0.628	0.032 [*]	0.523
Δ step frequency	Correlation coefficient CI: [LL, UL]	-	-	-	-	0.368 ^P [-0.30, 0.79]	0.582 ^P [-0.03, 0.88]	0.304 ^P [-0.36, 0.76]	-0.382 ^P [0.80, 0.28]	0.778 ^P [0.33, 0.94]
	Adjusted <i>P</i> -value	-	-	-	-	0.299	0.071	0.337	0.293	0.006 [*]
Δ Leg stiffness	Correlation coefficient CI: [LL, UL]	-	-	-	-	-	0.512 ^S [-0.13, 0.85]	0.322 ^S [-0.34, 0.77]	-0.544 ^S [-0.86, 0.08]	-0.253 ^S [-0.74, 0.41]
	Adjusted <i>P</i> -value	-	-	-	-	-	0.134	0.409	0.134	0.427
Δ Ankle quasi- stiffness	Correlation coefficient CI: [LL, UL]	-	-	-	-	-	-	0.072 ^P [-0.55, 0.64]	-0.308 ^P [-0.77, 0.36]	-0.578 ^P [-0.87, 0.03]
	Adjusted <i>P</i> -value	-	-	-	-	-	-	0.823	0.497	0.099
Δ Knee quasi- stiffness	Correlation coefficient CI: [LL, UL]	-	-	-	-	-	-	-	0.006 ^P [-0.60, 0.60]	0.026 ^P [-0.58, 0.61]
	Adjusted <i>P</i> -value	-	-	-	-	-	-	-	0.986	0.986
Δ Energy absorption	Correlation coefficient CI: [LL, UL]	-	-	-	-	-	-	-	-	0.034 ^P [-0.58, 0.62]
	Adjusted <i>P</i> -value	-	-	-	-	-	-	-	-	0.823

CI = confidence interval, LL = lower limit, UL = upper limit

**The repeated bout effect influences lower-extremity biomechanics during a 30-min
downhill running**

Online Supplementary Materials

ACCEPTED MANUSCRIPT

S1. Leg stiffness during downhill running

McMahon and Cheng Model

The McMahon and Cheng (1990) mass-spring model has been used to calculate leg stiffness during level running (Ferris et al., 1999; Rabita et al., 2013). The assumption in this model is that the leg is vertical at midstance and the maximum vertical ground reaction force (VGRF) occurs at midstance. While this assumption is correct during level running, it is not true during downhill running (DR). In the current study, we adjusted the model and calculated leg displacement assuming that the leg is vertical when the horizontal GRF crosses zero. Equivalently, the leg is normal to treadmill surface when the force parallel to treadmill crosses zero.

Leg Stiffness during DR

Suppose an individual is running on an instrumented treadmill angled α° from the horizontal axis at the velocity of v . This individual experiences GRF of F during ground contact. Figure S1 depicts the decomposition of this force into its parallel and normal to treadmill components (F_p and F_n , respectively) and into its horizontal and vertical components (F_y and F_z , respectively).

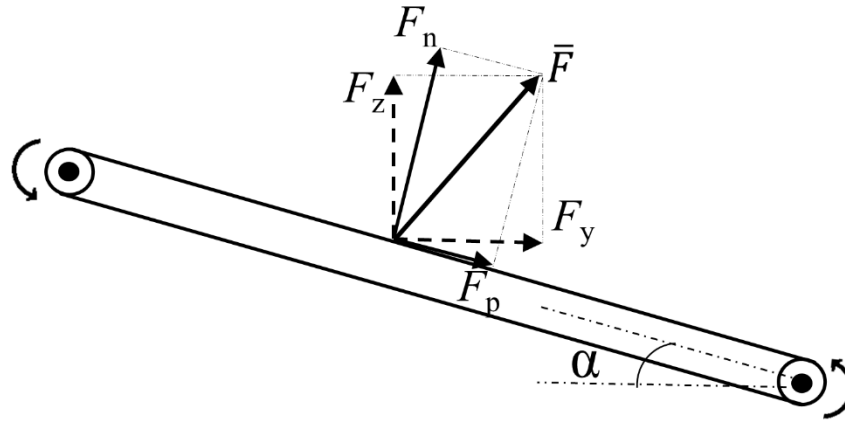
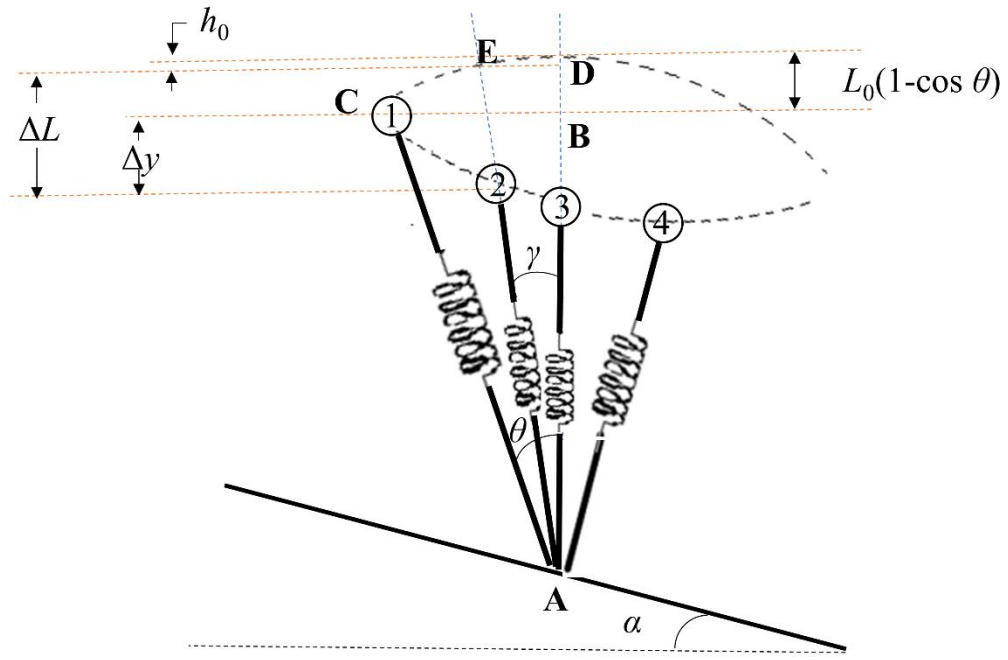


FIGURE S1. DECOMPOSITION OF THE GROUND REACTION FORCE INTO PARALLEL, NORMAL, HORIZONTAL AND VERTICAL COMPONENTS.

Now assume the configuration of the mass-spring model in different instances of ground contact (Figure S2). The lower dashed curve is the trajectory of center of mass during ground contact. The upper dashed curve is part of the circle that the center of mass would form if it were to purely rotate around the point of ground contact (point A in Figure S2) with no force on the spring. Configuration 1 is the leg at touchdown. Configuration 2 is the time when the VGRF reaches its active peak. Configuration 3 is when the leg is in vertical position. Configuration 4 is when the leg is normal to the treadmill surface. The individual is in push-off phase when the leg is normal to the treadmill surface (i.e. configuration 4), therefore, calculating leg stiffness based on this configuration would not yield information about the leg stiffness during ground contact. Ideally, calculating the leg stiffness at the time of peak VGRF (i.e. configuration 2) would be comparable to the method McMahon and Cheng (1990) applied during level running. The vertical displacement of the leg (ΔL) at configuration 2 is therefore required in order to calculate leg stiffness based on the following:



$$K_{leg} = \frac{VGRF_{max}}{\Delta L}$$

FIGURE S2. CONFIGURATION OF LEG SPRING AT DIFFERENT TIMES OF STANCE PHASE

A simple geometrical relationship can be utilized to calculate ΔL . Consider the angle θ and the angle γ representing the angles between configurations 1 and 3, and 2 and 3, respectively. Assume T_2 and T_3 are the time when the leg is in configurations 2 and 3 respectively. Δy is the center of mass displacement at configuration 2 that can be determined by double integrating the VGRF after accounting for the mass of the individual. The parameter h_0 is the difference in leg displacement between configurations 3 and 2. Then, ΔL can be calculated as following:

$$\Delta L = L_0(1 - \cos\theta) - h_0 + \Delta y$$

where the angle between configurations 3 and 1 can be calculated from trigonometry knowing that ABC is a right-angle triangle:

$$\theta = \sin^{-1}\left(\frac{T_3 * v * \cos\alpha}{L_0}\right)$$

where the numerator is the horizontal distance that the center of mass travels from configuration 1 to 3, and L_0 is the resting length of the leg spring. Subsequently, h_0 can be calculated as following:

$$h_0 = L_0(1 - \cos\gamma)$$

The angle γ is as following knowing that ADE is a right-angle triangle:

$$\gamma = \sin^{-1}\left(\frac{(T_3 - T_2) * v * \cos\alpha}{L_0}\right)$$

The numerator is the horizontal distance the center of mass travels from configuration 2 to configuration 3.

Evaluating the Efficacy of the Model

We further evaluated the efficacy of the presented modeling on a dataset that was published previously (Khassetarash et al., 2020). Briefly, nineteen physically active individuals (10 females and 9 males; age: 26.6 ± 7.9 years, body mass: 66.2 ± 9.5 kg, height: 171.2 ± 8.8 cm) ran on an instrumented treadmill (Bertec, Bertec Corps, Columbus, OH) at three velocities (2.5 m/s, 3.33 m/s, and 4.17 m/s) at a grade of -10° for 30 seconds each. The ground reaction forces were recorded and analyzed to calculate leg stiffness. A longitudinal analysis was performed using generalized estimating equation (Liang and Zeger, 1986) to determine the speed effect. Pairwise comparisons were performed and adjusted for multiple comparison using Bonferroni's correction when main effect of speed was observed. A main effect of speed [$\chi^2(2) = 18.964, P < 0.001$] was observed for the leg stiffness (Table S1). Leg stiffness was higher at $4.17 \text{ m}\cdot\text{s}^{-1}$ compared to $3.33 \text{ m}\cdot\text{s}^{-1}$ ($P = 0.004$) and $2.5 \text{ m}\cdot\text{s}^{-1}$ ($P < 0.001$).

TABLE S1. MEAN \pm STANDARD DEVIATION OF THE LEG STIFFNESS CALCULATED AT DIFFERENT VELOCITIES.

Velocity	2.5 m.s ⁻¹	3.33 m.s ⁻¹	4.17 m.s ⁻¹
Leg stiffness [kN.m ⁻¹]	12.3 \pm 7.2	13.4 \pm 5.2	18.1 \pm 7.7

It has been previously shown that the leg becomes stiffer as running velocity increases during level running (Arampatzis et al., 1999; McMahon and Cheng, 1990). The same situation was expected during downhill running. Our results demonstrated that the modified method for leg stiffness calculation in downhill running is able to detect the difference in leg stiffness at different running velocities.

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S2. Joint quasi-stiffness

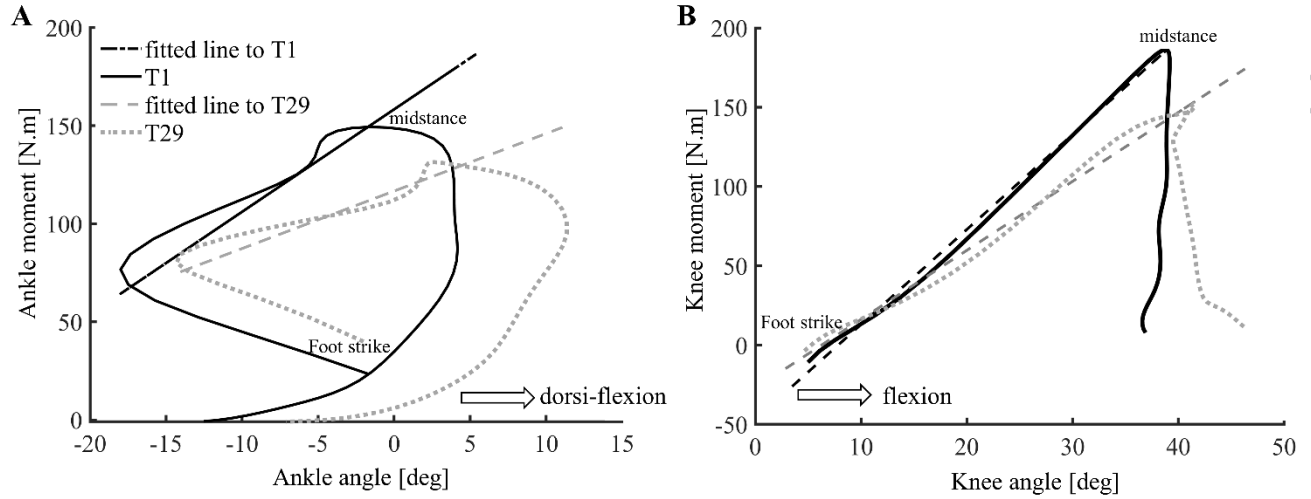
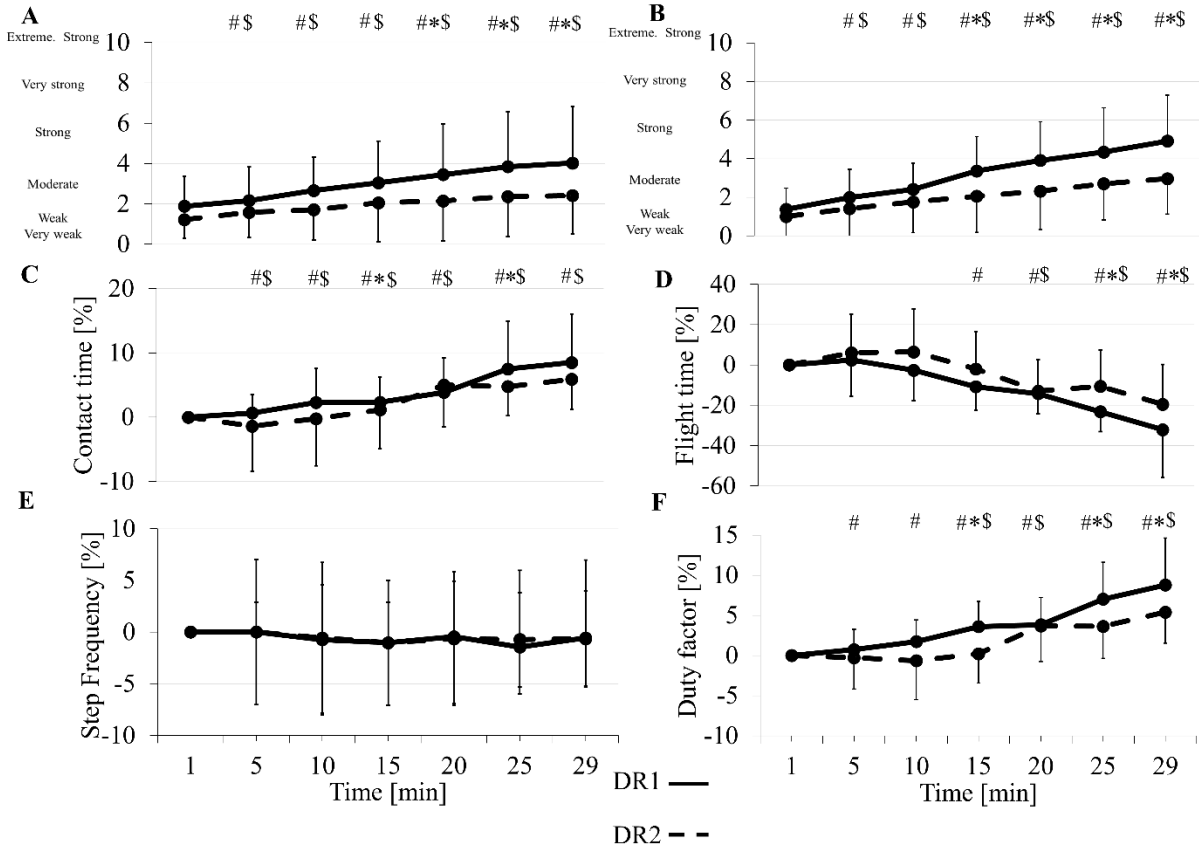
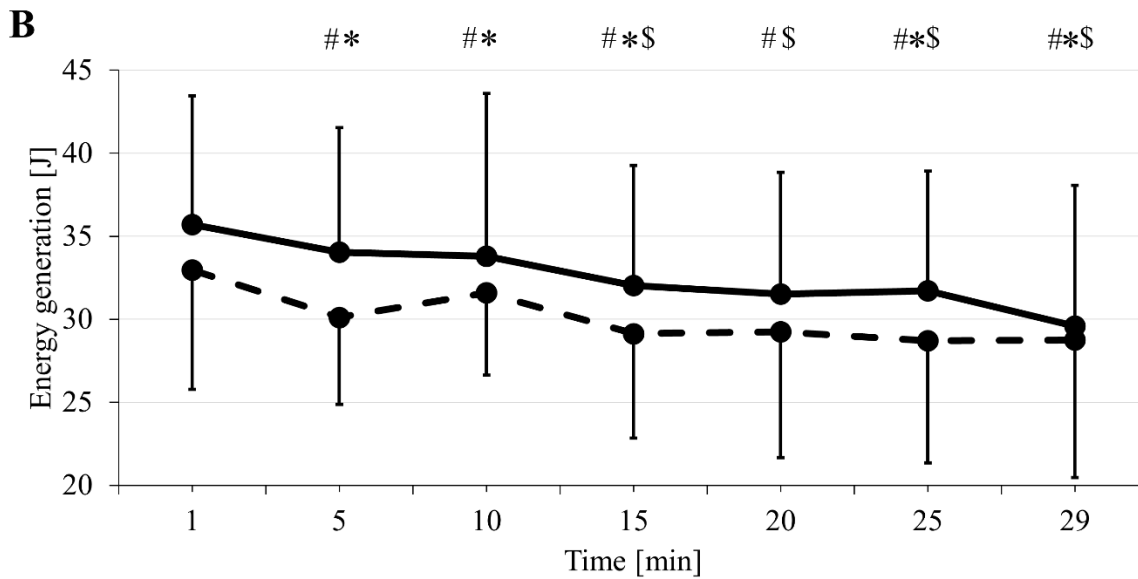
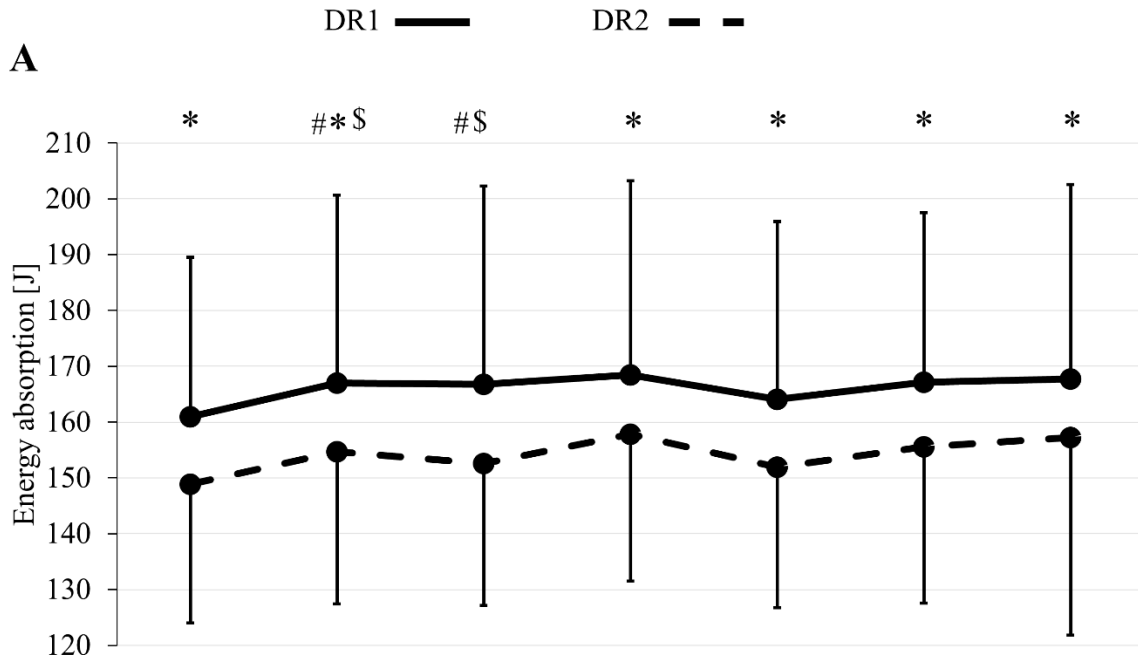


FIGURE S3. A) ANKLE AND B) KNEE MOMENT-ANGLE PLOTS FOR A REPRESENTATIVE PARTICIPANT AT THE BEGINNING (T1) AND END (T29) OF THE FIRST DOWNHILL RUN (I.E., DR1). THE SLOPE OF THE FITTED LINES INDICATED THE ANKLE AND KNEE QUASI-STIFFNESS MEASUREMENTS.



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