

Effects of Circadian Variation on Ankle Screening Tests

Title: The influence of circadian variation on aetiological markers of ankle injury

Abstract

Context: Clinical and functional assessments are performed regularly in sporting environments to screen for performance deficits and injury risk. Circadian rhythms have been demonstrated to affect human performance, however the influence of time of day on a battery of multiple ankle injury risk factors has yet to be established within athletic populations. **Objectives:** To investigate the influence of circadian variation on a battery of tests, used to screen for ankle aetiological risk factors. **Design:** Randomised crossover design. **Setting:** University laboratory. **Participants:** Thirty-three semi-professional soccer players (age 24.9 ± 4.4 years; height 1.77 ± 0.17 m; body mass 75.47 ± 7.98 kg) completed three randomized experimental trials (07:00 h, 12:00 h, 19:00 h) **Main Outcome Measures:** Trials involved the completion of a standardized test battery comprising Biodex Stability System (BSS), Star Excursion Balance Test (SEBT), isokinetic inversion: eversion ratio, joint position sense, and a drop landing inversion cutting manoeuvre. **Results:** Repeated measures analysis of variance revealed significantly ($P < 0.05$) lower values for all BSS indicia; Overall Stability Index (1.10 ± 0.31 a.u), Anterior-Posterior (0.76 ± 0.21 a.u) and Medio-Lateral (0.68 ± 0.23) at 12:00 h when compared to 07:00 h. (1.30 ± 0.45 a.u; 0.96 ± 0.26 a.u; 0.82 ± 0.40 a.u) respectively. However, no significant ($P \geq 0.05$) main effects for time of day were reported for any other test. **Conclusions:** Circadian influence on ankle aetiological risk factors was task dependent, with measures of proprioception, strength and SEBT displaying no circadian variation, indicating no association between time of day and markers of injury risk. However, the BSS displayed improved performance at midday, indicating postural stability tasks requiring unanticipated movements to display a time of day effect and potential increased injury risk. Consequently, time of testing for this task should be standardized to ensure correct interpretations of assessments and/or interventions.

Introduction

The epidemiology of ankle sprains in soccer has previously been well described, accounting for approximately 13% of all soccer injuries sustained, with average rehabilitation periods of 16 ± 27 days^{1,2}. Literature proposes several modifiable risk factors for initial ankle sprains, including; proprioception^{3,4}, postural stability⁵ and isokinetic strength⁶. Consequently, strategies such as screening of risk factors are commonplace in the assessment of ankle joint function, with multiple tests used to replicate the multifactorial nature of ankle injury occurrence⁷. The accurate interpretation of movement screening is fundamental to the subsequent design of prehabilitation and injury management strategies.

Circadian rhythms is a term used to describe variations in many human physiological variables⁸, and factors influencing athletic performance, relative to time of day^{9,10}. It has been postulated that fine motor control movements are often enhanced in the morning¹¹, whilst activities requiring gross muscular movements including strength and power exercises typically exhibit peak performance in the early evening⁹ when core temperature is high and melatonin levels low¹². Physiologically, optimal physical performance closely follows increases in core body temperature (CBT) and readying of norepinephrine signalling cascades¹³. If physical performance does not align with endogenous biological peaks, it can initiate both physical and mental fatigue, which could potentially increase the risk of injury incidence¹⁴. These theories are further supported by an increase in match-play American Football (NFL) injuries, which have been shown to correspond with match start times, which are nearer to endogenous biological troughs in alertness, thus accentuating fatigue and injury risk¹⁴.

Physical performance variations with time of day have been observed in measures of dynamic and static postural control ^{15 - 17} and measures of isokinetic knee strength ¹⁸, tasks which are commonly used during screening of athletes. However, most studies ^{15 - 18} investigating circadian variation and aetiological risk factors have involved non-sporting populations, whilst often only including a single outcome measure. Given the multifactorial nature of sports injury aetiology ⁷, the influence of circadian variation should be applied to a battery of aetiological tests, which better represent the epidemiology and aetiology of particular sports.

Previous research ^{15 - 18} appears to be influenced by reduced participant ability and training status. It has been suggested that athletes display improved levels of postural stability when compared to the general population ¹⁹, whilst also possessing enhanced athletic abilities at times of the day where they regularly train or perform ²⁰. Additionally, there appears to be a dearth of literature pertaining to the circadian variation in a battery of other clinical and functional tests related to aetiological risk factors in trained populations. If aetiological risk factors are shown to be affected by circadian variation, athletes may need to be screened at the time of day where deficits in performance are most pronounced. Previous research ²⁰ has demonstrated that athletic ability at a particular time of day can be improved by regularly training at that specific time. Consequently, sports medical staff could advocate (p)rehabilitation programs at times of day whereby aetiological risk factor performance is diminished, in an attempt to potentially improve athletic function, reduce injury incidence and long term health issues and provide more players for match-day selection, which has been correlated to increased team success ²¹. Consequently, the aim of the current study was to investigate the effect of time-of-day on clinical tests associated with ankle aetiological risk factors in soccer players.

Methodology

Design

Participants were required to attend the laboratory on four occasions to complete a familiarization trial, followed by three experimental trials (07:00 h, 12:00 h and 19:00 h) in a randomized order. The testing times were selected to represent different phases of the circadian rhythm for clinical testing, soccer training and match play in this population. A minimum of 48 h recovery interspersed each trial. The familiarization and experimental trials consisted of the completion of all six tasks assessing postural stability, proprioception, strength and functional performance. All tests were completed in a standardized order, interspersed by a minimum of 5 minutes to avoid the accumulation of fatigue. Intra-class correlation coefficients (ICC) scores were recorded for each variable. ICC scores were analysed using the participant's final familiarisation trial conducted at 19:00 h and the corresponding experimental trial at the same time of day.

Participants attended the ambient temperature controlled laboratory in a 3-hour post-absorptive state, following 24 hours abstinence from alcohol, caffeine and vigorous exercise. Prior to the completion of each testing sessions, participants undertook a ten-minute standardized warm up protocol. Participants were also required to wear the same lightweight athletic clothing and footwear for each session.

Participants

Thirty-three male semi-professional soccer players (age = 24.9 ± 4.4 years, height 1.77 ± 0.17 m, body mass = 75.47 ± 7.98 kg) who competed in the fifth tier of English Soccer completed the study within a 2-week period immediately after the conclusion of the competitive soccer season. Inclusion criteria required players to be normotensive, to have no injury history in the

lower limb for the previous six months, and to exhibit no neurologic or balance disorder or chronic ankle instability as determined by the Cumberland Ankle Instability Tool (CAIT) ³. In addition to weekly matches, the participants were required to have completed typical training volumes equating to $> 8 \text{ h}\cdot\text{wk}^{-1}$ during the preceding soccer season. All participants were moderate or intermediate Chronotypes ²², who reported at least 6-8 hours sleep per night and were not involved in any shift work. All participants provided written informed consent and the study was approved by the University Ethics Committee.

Procedures

Biodex Stability System (BSS)

The Biodex Stability System (BSS) (Biodex Medical Systems, Shirley, NT, USA) measures postural stability via an oscillating platform, providing quick, communicable and objective results ²³. Participants stood on their dominant barefoot whilst centring a visual stimulus on an electronic screen directly in front of them, upon which the subject's foot co-ordinates (vertical and horizontal) were recorded and inputted. The BSS has a manually pre-set degree of surface instability ranges between level 8 a completely firm surface, to a very unstable surface, stability level 1. For the current study, the BSS was set to an unstable level 1 setting. During the familiarization session, participants were provided five practice trials ²³ in order to negate any learning effect. Participants were required to maintain their balance by attempting to maintain a visual cursor on a screen in the centre of a specific marker for 10 seconds. During the experimental trials, one additional practice trial was provided to remind the participant of the procedure, followed by three consecutive experimental trials. The average score across the three experimental trials was used in subsequent analysis ²⁴. Participants performed each test for 10 seconds, with a period of 20 seconds rest provided between each test. The BSS was utilized to objectively record and evaluate outcome measures, overall stability index (OSI),

which is a function of the variance of platform displacement in both the anterior-posterior (AP) and medial-lateral (ML) planes of movement. The intra-class correlation (ICC) values for BSS variables ranged from AP (0.80), ML (0.84) to OSI (0.89), demonstrating good to excellent levels of reliability.

Star Excursion Balance Test (SEBT)

The SEBT is an inexpensive, quick and simple functional test used to assess postural stability of the lower limb, requiring participants to demonstrate strength, range of motion and coordination. The SEBT was performed using the modified three directions (anterior, posteromedial and posterolateral) ²⁵. Prior to testing, the participant lay in a supine position and their dominant leg was measured from the anterior superior iliac spine to the distal tip of the medial malleolus. Participants were then instructed to perform the SEBT test as per recommended guidelines ²⁵. Participants performed six practice trials during the familiarization visit. For experimental trials, the same six practice trials were provided, with the seventh trial recognized as the test result with 30 s rest intervening each set. The distance was recorded from the centre of the grid to the marked point of maximum reach for each direction. Leg length was then used to normalize each reach distance by dividing the distance reach by leg length and multiplying by 100 ²⁵. Good to excellent levels of reliability were observed for measures of Star Excursion Balance Test Anterior Direction (SEBT_{AD}), Posterolateral Direction (SEBT_{PLD}), Posteromedial (SEBT_{PMD}) and composite score total (SEBT_T), with ICC scores ranging from 0.85 – 0.93.

Ankle Joint Position Sense (JPS)

Joint position sense (JPS) is the most commonly used test to assess proprioception in most body regions. JPS was assessed using an isokinetic dynamometer (IKD) (Biodex Medical

System 2, Shirley, New York). All participants were positioned in accordance with manufacturer guidelines. To reduce all feedback other than internal proprioception, participants were blindfolded whilst the barefoot of the dominant limb was aligned with axis of the dynamometer and attached to the footplate by a small wrap⁴. The talocrural joint was then placed into 15° of plantarflexion; with the lower leg secured using hooks and loops. Two target positions were tested, 15° of INV (θ_{15}) and maximal active INV minus 5 degrees (θ_M). All participants were provided three familiarization trials. Participants were initially passively moved into the desired target position for a period of 5 seconds, before being passively returned to the starting position at a speed of 5 °/s. Once returned to anatomical neutral, the participant was instructed to actively move their dominant foot to their perceived testing position at which point they pressed a stop button. The participant's foot was then passively returned to the anatomical neutral position, at which point the procedure was then repeated a further two times, with 30 s rest between each set⁴. Absolute error scores were recorded as the difference between the actual position the participant is asked to achieve and the position they move their ankle to when asked to replicate the original position. ICC scores of 0.87 and 0.89 were recorded for θ_{15} and θ_M respectively.

Isokinetic Dynamometer (IKD) Inversion (IN) and Eversion (EV) Ratio (IE)

Participants were positioned according to manufacturer guidelines on the IKD (Biodex Medical System 2, Shirley, New York), which was calibrated prior to testing commencement. The knee of the dominant leg was positioned in 80 - 110° flexion, with the lower foot parallel to the ground. The barefoot of the dominant leg was placed into the IKD footplate, with the ankle positioned at 10° of plantar flexion and the subtalar joint placed in a neutral position. Two straps were then placed across the dorsum of the foot, securing it against the footplate. Additionally, a thigh stabilizer pad and strap secured the distal portion of the thigh, with a belt

placed around the abdomen, preventing excessive torso movement. Participants familiarized themselves with the dynamometric system by completing a submaximal force of muscles contractions ⁶. Four submaximal trials (50% effort) were followed by five maximal effort concentric/eccentric reciprocal contractions at a velocity of 60°/s for inversion and eversion. During concentric conditions, participants were instructed to push the lever arm throughout the whole range of motion in both inversion and eversion movements ⁶. During eccentric conditions, participants were instructed to actively resist the lever arm. Isokinetic muscle movements were performed throughout the subject's active range of motion ⁶. A two-minute rest was provided between tests for inversion and eversion joint motions to prevent accumulation of fatigue. The values of peak torque (N.m) were obtained from the peak repetition at 60°/s, using only the isokinetic (constant angular velocity) phase of the movement. The ankle evertor/invertor muscles torque ratio (I:E) was then calculated. Good to excellent (ICC = 0.85) levels of reliability were reported for the IE task.

Drop Landing

Participants were required to step off and land on the dominant barefoot from a 35cm high platform, positioned to ensure landing was centred on the force platform (Bertec, Columbus, USA) ²⁶. Upon landing players were required to react to a light stimulus and accelerate through a set of timing gates (SmartSpeed, Fusion Sport, Australia) positioned at a 45° cutting angle from the mid-line of the force platform, at a distance of 4m. The stimulus was triggered by the players stepping through a beam as they initiated the drop. During the familiarization visit, participants were required to complete 5 trials of the previously discussed task. For right footed dominant participants, the inversion trial was recorded when the stimulus required them to respond to a 45° cut to their left, as this placed them into a position of plantarflexion and inversion. Eversion trials required subject to respond to a 45° cut to their right. Left foot

dominant participants inversion and eversion trials appear in the opposite direction. Participants were provided two practice trials, followed by an experimental trial, from which results were recorded for analysis. Inversion and eversion trials were counter-balanced, with 5 minutes passive recovery between trials. Data presented for this test were measured during the eversion trial, given the relevance to the mechanism of ankle injury; with the inversion, trial was included to ensure a reactive task ²⁶. The drop landing task was selected in the multifactorial battery of tests as the drop, land, cut and drive elements of the test are functionally demanding and ecologically valid in relation to the subject used within the current study.

Performance was quantified as the time taken to complete the task (T). Kinetic measures at a sampling frequency of 1000 Hz enabling the magnitude (\dot{F}_{xy}) and angle of the take-off vector (θ) to be determined. Resultant ground reactions forces were then normalized to individual body weights (BW). Good to excellent (ICC = 0.86 – 0.97) levels of reliability were recorded for aforementioned measures during the drop and drive task.

Statistical Analyses

Statistical analysis was conducted using PASW Statistics Editor 22.0 for windows (SPSS Inc., Chicago, IL USA). Before parametric analysis, the assumptions of normality of the residual values were assessed using a Shapiro-Wilk test, with significance set at $P \leq 0.05$. Subsequently, a repeated measures ANOVA was used to investigate the time-of-day effect for each variable. Where appropriate, post hoc pairwise comparisons with a Bonferonni correction were applied. Between session reliability was assessed using Intraclass correlation coefficients (ICC).

All data is reported as mean \pm SD unless otherwise stated. For all significant interactions, 95% confidence intervals (CI) are reported in conjunction with the traditional statistical approach.

Cohen's d effect sizes (< 0.50 = small, $0.50 - 0.80$ = small to moderate, > 0.8 = large) were calculated to further assess differences in measures.

Results

Figure 1 highlights a significant main effect for time of day and AP indicia of the BSS ($P \leq 0.01$) with significantly ($P \leq 0.01$; $d = 0.54$) lower values identified at 12:00 h (0.76 ± 0.21 a.u.) when compared to 07:00 h (0.96 ± 0.26 a.u.). The 95% CI of this difference was -0.29, to -0.05 a.u.). The ML indicia demonstrated the same pattern, with Figure 1 demonstrating significantly ($P = 0.04$; $d = 0.43$) lower values at 12:00 h (0.68 ± 0.23 a.u.) when compared to 07:00 h (0.82 ± 0.40 a.u.). The 95% CI of this difference was -0.26 to -0.01 a.u. In terms of the BSS composite score, OSI, significantly lower values ($P \leq 0.01$) were identified at 12:00 h (1.10 ± 0.31 a.u.) when compared to 07:00 h (1.30 ± 0.45 a.u.; 95% CI = 0.38 to 0.07 a.u.; $d = 0.49$) and 19:00 (1.32 ± 0.49 a.u.; 95% CI = 0.60 to 0.37 a.u.; $d = 0.54$).

Insert Figure 1 near here

Table 1 demonstrates results for all performance predictors recorded at three different times of day. With the exception of all BSS indices, no other significant main effects for time of day were recorded.

Insert Table 1 near here

Discussion

The aim of the current study was to investigate the effect of circadian variation on clinical and functional tests associated with ankle aetiological risk factors in male semi-professional soccer players. The results of the current study suggest that circadian influence on measures of ankle joint performance is task dependent. Only variables measured using the biodex stability system

were affected by circadian variation. All other outcome variables including, joint position sense, drop landing, ankle joint strength and Star Excursion Balance Test were demonstrated not to be affected by circadian variation.

Biodex stability system performance in the AP and ML plane, significantly improved at 12:00 h when compared to 07:00 h. Furthermore, OSI displayed significant improvements in performance at 12:00 h when compared to both 07:00 and 19:00. Inhibited BSS performance in the early hours of the morning has implications for increased injury risk. Poor postural stability has previously been highlighted as an ankle aetiological risk factor ⁵. Additionally, postural stability is highlighted as an integral element of successful athletic performance, as athletes require balance to change direction, step, run or jump ¹⁹. Consequently, if postural stability is diminished during the early hours (07:00) of the day, it may be unadvisable to advocate the use of sport-specific training and or screening tasks which involve a high degree of balance. Alternatively, a more proactive approach would require medical practitioners to design and implement training programs to improve postural stability at the times of day where reductions in performance have been observed. It has been proposed that postural stability training programs improve neuromuscular mechanisms involved in the co-contraction of agonist and antagonistic muscles which increase active joint stability and joint stiffness, thus resulting in less joint displacement and strain placed upon joint structures ²⁷.

Reduced postural stability ability observed in the early morning hours, disagrees with previous literature ¹⁶, which reported improved balance in the afternoon (15:00 - 18:00 h) when compared to the morning (07:00 - 10:00 h). However, these results were more pronounced for static balance with minimal time of day effects reported for dynamic balance. The results of the current study and existing research ¹⁶ differ to that of previous studies ^{15, 17} which report improved postural stability in the morning when compared to other times of day. Reasons for

disparity in results could be attributed to the differences in participant's sporting experience, ability and regular participation in physical activity. The participants in the current study regularly complete soccer training in the late morning, approaching midday. Consequently, the observed improvement in postural stability performance may be a result of their body's acclimation of exercising at this time of day ²⁰.

Another measure of dynamic stability, the SEBT failed to display any significant main effects for time of day. The results of the current study contrast with previous literature ¹⁶ that identified improved SEBT performance in the afternoon when compared to the morning. However, these significant improvements were only observed in the upper quarter of the SEBT test, with performance improvements translating to only 1% percentage of change, indicating minimal clinical relevance ^{15, 16}. Furthermore, both studies ^{15, 16} used a mixed gender population. It has been hypothesised that women tend to utilize a greater quadriceps dominant pattern of knee control when compared to males ²⁸. Successful completion of the anterior reach distance requires quadriceps muscle activation ²⁹, which has been shown to peak in the morning. Subsequently, gender differences may be a potential reason for between-study differences.

Perhaps a more interesting finding is that both the SEBT and the BSS claim to measure postural stability ²⁴ subsequently if the tests are taken on face validity, one could assume that both should be influenced by circadian variation. However, the results of this study indicate potential differences between the two tasks, as only BSS indices were affected by circadian variation. Both tests share similarities, as they test underlying motor systems, functional stability limits and anticipatory postural control, whilst requiring participants to maintain as stable base of support and alter their centre of gravity (COG) accordingly ^{25, 30}. However, the SEBT entails, purposefully moving the non-weight bearing limb into pre-planned and predicted positions

away from the base of support on a stable surface, whilst attempting to limit the instability created³¹. The greater the distance achieved during these movements, the more emphasis that is placed on both balance and neuro-muscular control systems³². Conversely, the BSS requires its participants to continuously adjust their posture and fixate the required muscles, to maintain a horizontal platform, which is able to move in all directions²⁵. Muscle strength has been shown display a temporal relationship with core temperature, with increased strength observed in the evening when compared to the morning⁹. Consequently, the muscles which are required to provide strength to fixate and maintain a horizontal platform during the BSS may be affected by circadian variation, thus potentially explaining the difference in BSS and SEBT response to circadian variation.

Inhibited BSS performance during the early morning may display implications for ankle injury risk. The perturbations and control mechanism created by the BSS are unanticipated. Although not the same, unanticipated drop landing tasks have demonstrated greater ground reaction forces, inversion angles and velocity and time from contact to peak muscle activation, thus better reflecting the mechanism of ankle sprain injury when compared to anticipated landings and further explaining the difference between BSS and SEBT circadian variation response³³. Both the SEBT and the BSS attempt to measure a similar outcome, however use different systems and methods to achieve this, potentially explaining why only the BSS task is affected by circadian variation. The practical implications of these results indicate that both the BSS and SEBT should be assessed due to varying results for the same aetiological risk factor, whilst time of day should be considered when assessing measures of postural stability using the BSS. If time of day is not standardised for this task, results could be influenced which may affect the interpretation of the outcome measures.

Circadian variation was shown not to affect measures of ankle joint strength, proprioception and functional performance during a drop landing task. Limited research has investigated the effects of strength, proprioception and drop landing performance with specific reference to the ankle joint, subsequently making it difficult to compare results to other published literature. However, it should be noted that previous measures of isokinetic peak torque have displayed circadian variation for grosser musculature such as the quadriceps at velocities greater than 3.14 rads.s^{-1} ³⁴. This could potentially be a result of speed-specific circadian variations in muscle strength, due to muscle fibre type recruitment patterns ³⁴. The current study comprised ankle inversion/eversion movements at $60^\circ/\text{s}$ (1.05 rads.s^{-1}) due to the reduced range of motion that occurs in these actions and, to ensure that an isokinetic period was ascertained. However, this speed may have limited the ability to detect circadian variation of ankle strength due to recruitment of different muscle fibre types.

Other non-significant findings observed in the current study could potentially be explained by training adaptations and the specific demographics of the subject population. Previous research ²⁰ has demonstrated that adaptations to strength training are enhanced at the time of day at which the training occurred, when compared to other times during the day, implying that training demonstrates temporal specificity. All participants in the current study were injury free, played soccer to a semi-professional standard and had training and match-play volumes of $> 8 \text{ h}\cdot\text{wk}^{-1}$. Furthermore, all participants performed their training in the late morning approaching midday and completed $> 90\%$ of match-play performance at 15:00 h and/or 19:45. Subsequently, this adaptation to a variety of performance times may explain why outcome measures of proprioception, functional performance and ankle strength display no circadian variation in a soccer-specific population. A variety of clinical and functional tests are available for medical practitioners to select from, when choosing tasks to help screen athletes for injury risk and performance deficits. Consequently, the results of this study cannot be generalised

beyond the tests, times of day and populations used within the current study. Furthermore, with previous research ² indicating increased injury incidence whilst in a fatigued state, future research should also consider whether athletes should be screened, using a battery of tests either before or after exercise induced fatigue has taken place.

Conclusions

The findings of the current study suggest that circadian variation did not influence performance of ankle aetiological risk factors quantified as proprioception, isokinetic strength and postural stability in the form of the SEBT. These findings have implications for medical practitioners and researchers as they indicate that these measures can be assessed without controlling for time of day. However, measures of postural stability involving tasks which require fixation of an unstable platform and unanticipated perturbations display a significant influence of circadian variation, with improved scores observed for all indicia at 12:00 and OSI only at 19:00. Improvements in BSS scores at these times of day may be observed in this semi-professional soccer population due to regular training and match performance at these hours. Consequently, medical practitioners should be aware of reduced postural stability performance during early morning hours and either avoid such tasks at these times or take a more proactive approach and attempt to implement (p)rehabilitation programs which aim to address deficits in postural stability performance during these hours. Furthermore, interpretations of BSS results could be influenced by circadian variation when making informed decisions on participant screening results, rehabilitation progress and/or return to play decisions, if time of day is not controlled for. Should medical and sports science practitioners choose to use the BSS as a screening tool, they should consider time-of-day when assessing athletes for movement dysfunction, athletic performance and the design and delivery of intervention programmes in healthy semi-professional soccer players.

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Tables**Table 1: Effect of time-of-day on performance outcome scores. P values for main effects for time of day are presented**

Outcome Measures	07:00 h	12:00 h	19:00 h
SEBT _{AD} (cm) (P = 0.28)	78.68 ± 6.45	79.98 ± 6.49	79.94 ± 6.88
SEBT _{PLD} (cm) (P = 0.24)	94.44 ± 8.13	94.95 ± 8.80	95.31 ± 8.53
SEBT _{PMD} (cm) (P = 0.22)	97.11 ± 7.41	96.91 ± 8.32	98.31 ± 7.23
Θ ₁₅ (°) (P = 0.20)	2.27 ± 1.22	2.37 ± 0.75	2.02 ± 0.77
Θ _M (°) (P = 0.11)	1.82 ± 0.84	2.05 ± 0.84	2.27 ± 1.14
IE (P = 0.29)	1.18 ± 0.25	1.12 ± 0.18	1.11 ± 0.27
T (s) (P = 0.19)	1.51 ± 0.13	1.54 ± 0.14	1.48 ± 0.12
Ĥ _{xy} (BW) (P = 0.44)	2.23 ± 0.62	2.26 ± 0.68	2.30 ± 0.71

Figures

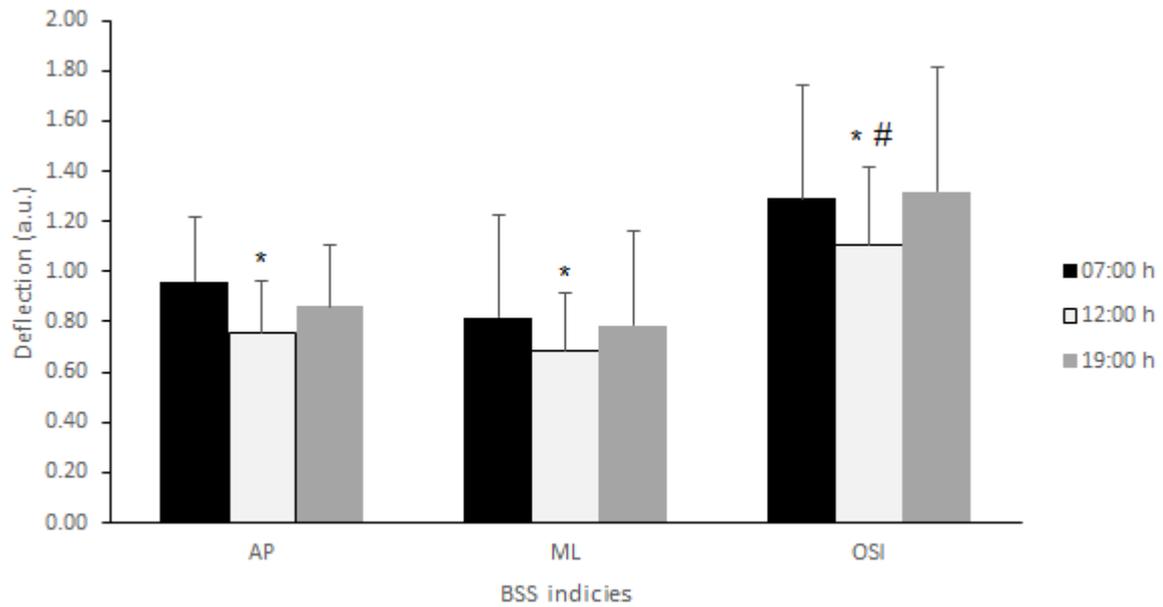


Figure 1: Effect of time-of-day on BSS indicia (* denotes a significant difference with 07:00 h; # denotes a significant difference with 19:00 h)