Abstract

Intermittent exercise might be an efficient means of exercise for improving bone strength and quality. The aim of our study was to examine the effect of intermittent running on bone turnover markers using altered exercise-to-rest intervals. Twelve males completed one control (no exercise), and three, 45-minute intermittent protocols (5 s, 20 s, and 80 s intervals) matched for distance and speed. Fasted venous blood samples were collected at baseline, 1 h, 2 h and 24 h post-exercise. Carboxyterminal crosslinked telopeptide (CTX-I) and procollagen type 1 amino terminal propeptide (P1NP) were used as markers of bone resorption and formation. After adjustment for baseline, CTX-I concentration at 1 h was higher (very likely to most likely small) for 5 s (30.2%; ±90% confidence limits: 10%), 20 s (2.9.0%; ±10%) and 80 s (32.0%; ±10%) compared to control. The very likely small effect remained for 5 s at 2 h (30.2%; ±15%). The effect for 20 s and 80 s was possibly trivial and possibly small/possibly trivial (~14.5%; ±~15%). Differences in P1NP concentrations were likely to very likely trivial (~7.4%; ±~7.6%). CTX-I concentration is affected acutely by intermittent running with short-interval (5 s) intermittent loading resulting in a prolonged attenuation in circadian rhythm of CTX-I up to 2 h that was not demonstrated as clearly by longer intervals despite matched internal and external training load.

Key words: CTX-I, Bone metabolism, Non-motorised treadmill, Mechanical loading, Intermittent exercise.

Introduction

Exercise stresses bone tissue via mechanical stimuli from gravitational impact forces and muscular activity causing bone deformation (strain) [1]. Consequently, physical activity and exercise have been shown to augment bone density and strength in both the growing [2], and mature skeleton [3] where bone remodelling prevails. Animal studies have been instrumental in separating the mechanisms by which bone responds to mechanical load. Exercise which delivers unaccustomed impacts of high-strain magnitude, and strain rate above a customary strain stimulus are thought to be most osteogenic [4]. Moreover, owing to the desensitisation of bone tissue following multiple loading cycles, osteogenesis is enhanced if the mechanical loads are applied intermittently with periods of rest [5] allowing the mechanosensory network to re-sensitise. As such, intermittent exercise characterised by periods of high-intensity loading interspersed with periods of rest or lower intensity loading might provide an ideal training environment to stimulate bone remodelling [6]. This concept has been well established using well-controlled animal models, in both the growing [5] and mature skeleton, [7] with as little as 10 s [8] and up to 8 h [5] recovery enhancing osteogenesis.

Soccer training, which is characterised by varied mechanical strains to bone via multiple accelerations, decelerations, and rapid changes-of-direction, has been shown to increase bone density in pre-menopausal females [9] and elderly males [10]. However, these training studies failed to; 1) appropriately control the external training load (e.g. distance, external work, and number of loading cycles), 2) accurately prescribe and evaluate biomechanical internal load [11], and 3) used a percentage of maximal heart rate to dose the internal physiological load, which is prone to large inter- and intra-individual variation [12]. Furthermore, the uncontrolled exercise-to-rest ratios in small-sided soccer games (SSGs) make it difficult to establish the independent effects of the rest duration, exercise duration, exercise intensity, and frequency of in-series changes of exercise intensity on the bone's response to mechanical loading.

Manipulating these aforementioned elements separately can alter both the physiological [13] and biomechanical responses to intermittent exercise [14]. Shorter exercise and rest intervals might increase the amount of in-series changes in speed leading to more varied mechanical loading which could alter the osteogenic environment [2]. Indeed, Ravnholt et al. [15] demonstrated an increase in bone remodelling

after 7 weeks using a 5-10-15 s high-intensity intermittent running protocol in sedentary individuals. However, Nybo et al. [16] did not replicate these responses when using a 20 min interval running (2 x 1 min) protocol compared to continuous running. The intensity and duration of the intermittent exercise sessions were similar between studies. It is not clear if the shorter exercise-to-rest intervals could explain the differences between findings. Whilst short (<10 s) rest intervals may not be sufficient to re-sensitise bone [8] it is feasible that the dynamic actions and rate of frequency of loading via more accelerations and decelerations might contribute to greater loading on the bone. To date, the effect of different exercise-to-rest durations on bone remodelling has not been investigated.

Bone turnover markers (BTMs) which reflect the un-coupling between resorption and formation have been successfully used to quantify bone's immediate and recovery cellular response to various exercise modes [17-19]. Acute, continuous load-bearing [17] and non-load-bearing [20] exercise has been shown to increase bone turnover, in favour of bone resorption. However, SSGs lasting ~15 and 60 min demonstrated no statistical change from baseline in resorption markers (C-terminal telopeptide of Type I collagen) with an early increase in formation markers (procollogen type 1 amino terminal propeptide) [21]. Additionally, short duration high-intensity intermittent cycling resulted in an increase in bone alkaline phosphatase (B-ALP), a marker of bone formation [22], and an increase in CTX-I [23] suggesting brief (< 20 min) intermittent exercise is capable of stimulating bone remodelling. However, it is unlikely that B-ALP would change acutely with exercise. Thus the response likely reflects diurnal variation rather than bone formation. The inclusion of a non-exercising control condition which is absent in the aforementioned the literature [21-23] would aid in the interpretation of BTM responses. . Furthermore, previous studies [22,23] did not manipulate the individual elements of intermittent exercise (e.g. different exercise-to-rest intervals) whilst controlling both the internal (physiological) and external training load imposed by the exercise.

Therefore, the aim of our study was to establish the magnitude of the effect of both internal and external load-matched intermittent exercise protocols of varying exercise-to-rest durations, on traditional BTMs. We anticipated that protocols of shorter exercise-to-rest intervals might have a greater magnitude of effect

on bone turnover in the hours proceeding exercise compared to less intermittent exercise, and a non-exercising control condition.

Materials and Methods

Participants

Twenty-two healthy males were initially recruited; however ten participants withdrew from the study at different stages, with seven participants failing to complete the full 45 min of at least one exercise protocol due to fatigue leading to premature termination of exercise. Therefore, twelve healthy male participants (mean ± SD age 23 ± 4 y, stature 179.6 ± 4.4 cm, body mass 79.7 ± 7.0 kg, $\dot{V}O_{2max}$ 53 ± 7 $mL \cdot kg^{-1} \cdot min^{-1}$) successfully completed all testing sessions and were included in the final analyses.

The inclusion criteria for participants were: non-smokers, between the age of 18-30 years, had not recently (last 12 months) suffered from a broken bone or fracture, were not regularly ingesting non-steroidal anti-inflammatory medication, or other medications that may affect bone metabolism, and participated in at least three sessions of impact exercise per week including both continuous and intermittent forms of exercise. The study was approved by a Departmental Ethics Committee and conformed to the Declaration of Helsinki.

Equipment

All trials were conducted on a Woodway Force 3.0 (Woodway Ltd) non-motorised treadmill (NMT). The NMT was chosen because: 1) it allows continuous measurement of horizontal and vertical ground reaction forces (GRFs; hGRF and vGRF, respectively) to quantify the mechanical loads imposed by the

exercise, and to calculate the number of loading cycles, 2) the combination of hGRF and distance covered allows for the quantification of external work performed by the exerciser, and 3) the self-paced nature of the treadmill allows the exerciser to accelerate and decelerate at a natural rate reflecting real-world intermittent locomotion that could not be replicated as easily on a motorised treadmill (MT).

Experimental Design

We used a randomised, repeated measures crossover design to compare the effects of intermittent exercise on BTMs. Participants attended the exercise physiology laboratory over a five-week period. In week one, participants completed three preliminary testing sessions, consisting of three familiarisation sessions and an assessment of the participant's $\dot{V}O_{2max}$ and velocity at $\dot{V}O_{2max}$ ($v\dot{V}O_{2max}$). All testing was performed on the NMT. In the following weeks, participants completed three 45 min exercise protocols, of varying exercise-to-rest intervals, and one non-exercise (control) condition in which participants remained seated in the laboratory.

Participants were required to adhere to the following pre-exercise guidelines [24]: 1) 12 h fasted state prior to each visit, 2) avoid any exercise at least 48 h prior to the testing session, and limit physical activity the morning of the testing session, 3) remain in a euhydrated state by drinking to thirst, and 4) avoid alcohol and any psychoactive substances at least 24 h prior to the exercise testing session. Participants attended the laboratory in the morning between 7:00 and 8:00, at the same time of day for all conditions to control for diurnal variation of BTMs.

Exercise protocols

A warm-up was performed on the NMT prior to all exercise trials which consisted of five-minute interval running at 55% $v\dot{V}O_{2max}$ varying between a speed of 4 km·h^{-1} and the target speed every 15 s. The three

exercise trials consisted of a highly intermittent (5 s), a moderately intermittent (20 s), and a low intermittent (80 s) protocol. For the 5 s protocol the exercise-to-rest interval changed every five seconds. Participants were required to run between 95% and 55% $v\dot{V}O_{2max}$ (mean of 75% $v\dot{V}O_{2max}$), interspersed with recovery walking at 4 km·h^{-1} every five seconds. Participants were required to reach the target speed in two seconds. Visual and auditory feedback was provided to ensure the target speed was achieved.

For the 5 s trial there were a total of 48 changes of speed in each 4 min exercise period, with nine, 4 min bouts interspersed with one min of passive recovery between each bout to enable capillary blood and gas sampling. The total exercise time (not including the warm-up) was 45 min to reflect a similar duration to previous studies utilising SSGs [9]. The 20 s protocol was the same as the 5 s protocol; however, participants varied between speeds every 20 s. The protocol had exactly one quarter fewer changes in speed compared to the 5 s protocol. The 80 s protocol was considered the least intermittent. Participants ran for 80 s at 75% $v\dot{V}O_{2max}$ interspersed with 80 s of recovery walking. The protocol had one quarter fewer changes in speed compared to the 20 s protocol and 1/16th fewer changes in speed than the 5 s protocol. All protocols were controlled for mean speed, duration, total rest and distance with a similar volume of external work done.

Internal (physiological) training load

Respiratory data were measured using an online gas analyser (Oxycon Pro, Jaegger, Hoechberg, Germany), collected continuously over five phases during the 45 min exercise session: 0-4, 5-9, 24-29, 31-35 and 40-45 min. Heart rate (HR) was monitored continuously throughout each trial by a Polar chest strap, with HR sent wirelessly to the Oxycon Pro during the same collection periods. Oxygen consumption and HR data were obtained directly from the Oxycon Pro with a 60 s mean calculated for each minute. The 60 s means were pooled together to obtain a global mean for each 4 min exercise phase.

External (mechanical) training load

Treadmill speed and distance were monitored by two optical photomicrosensors. Four force transducers under the treadmill belt sampling at 200 Hz were used to record the vGRF. The raw data were exported from the NMT Pacer Performance software into a custom designed MATLAB programme (MathWorks, Inc., 83 Natick. MA, USA). Data were digitally filtered using a bi-directional low-pass Butterworth filter, with the frequency cut-off optimised via residual analysis. A step detection algorithm was used to detect all loading cycles in the exercise phase. The peak vGRF (taken as the maximum peak of all vGRF curves) and mean vGRF (the sum of all the loading peaks divided by the number of peaks) were used to indirectly quantify the strain magnitude on the skeletal system between exercise conditions. Total number of peaks detected represented the total number of steps (loading cycles) in each exercise condition. The mean vertical impact loading rate (VILR) served to quantify the rate or intensity of the mechanical load. The VILR was calculated using the first derivative method and applied to all loading cycles.

Biochemical analyses

The use of C-terminal telopeptide of Type I collagen (CTX-I), which represents type I collagen degradation, and procollogen type 1 amino terminal propeptide (P1NP), which reflects changes in type I collagen synthesis, are currently recommended for markers of bone resorption and bone formation, respectively [24]. Venepuncture was performed and blood samples were collected 30 min prior to exercise (pre), 1 h, 2 h and 24 h post exercise similar to previous studies [25,26]. Plasma was used for the measurement of CTX-I and P1NP on the IDS-iSYS multi-discipline automated analyser. The reportable range of the IDS-iSYS CTX-I assay kit is 0.033-6.000 $ng \cdot mL^{-1}$. The expected mean (95% CI) for fasted healthy males is reported as 0.294 $ng \cdot mL^{-1}$ (0.115–0.748). Intra-assay precision for CTX-I ranged from 3.2% to 3.5%, with the inter-assay precision of the assay ranging from 4.4% to 5.3%. For P1NP the

detectable reference range is 27.7-127.6 ng·mL^{-1}, the intra-assay precision 3.4% to 5.3% and the inter-assay precision range 3.9% to 5.5%.

A sample of blood was obtained from one Ethylenediaminetetraacetic acid (EDTA) tube and measured in duplicate for measurement of haematocrit (Hct) and haemoglobin (Hb). Haemoglobin was measured on an automated haemoglobin analyser (Hemocue Ltd, Sheffield, UK). The method of microcentrifugation was used for the measurement of Hct. Bone markers were adjusted for plasma volume (PV) shifts at 1 h, 2 h and 24 h. Capillary blood samples (finger-tip) were obtained at baseline (pre), 4 min, 24 min, and immediately post exercise (45 min) for measurements of blood lactate [La], quantified via a Radiometer (ABL 800, Radiometer, UK). Blood [La] was adjusted for PV shifts at 45 min. For transparency, outcomes for the unadjusted BTM and blood [La] data are presented as supplementary material.

Statistical analysis

All variables except HR were first log transformed to reduce non-uniformity of error [27] and subsequently back-transformed (post-analysis) to express effects as percentage change [27]. All raw data were deemed to be normally distributed following visual assessment of Q–Q plots and histograms. BTMs were the main outcome variables, and were analysed using general linear models (GLM) via Proc Mixed in the SAS studio (University edition, version 9). The analysis of covariance (ANCOVA) approach was adopted, whereby the change scores (change from Baseline at 1 h, 2 h and 24 h) were the dependent variables, condition was entered as the independent variable (fixed effect), and baseline measures (i.e. pre) were set as covariate to adjust for any confounding effects of regression to the mean [28].

Measures of external load were analysed using GLM via Proc Mixed procedure with Condition set as a fixed effect. For HR, $\dot{V}O_2$ and blood [La], within-athlete modelling for clustered repeated measures of time series data was performed using linear mixed models (LMM) via Proc Mixed procedure. Initially,

conditions were split and assessed separately, with time re-scaled and specified as a fixed effect (covariate) to estimate the linearized rate of change in internal load per 10 minutes of each protocol. Models were also fitted with a random intercept for participant and a random slope for time, using an unstructured covariance matrix. This approach was adopted to assess the fidelity of the protocols by allowing participants to differ in their absolute internal physiological load, and in its rate of change per 10 minutes of each protocol. We subsequently quantified these individual differences as standard deviations (SD). Finally, the baseline ('pre') blood [La] measure was added as a covariate for blood [La] only.

Uncertainty in all outcome measures was expressed with 90% confidence intervals (CI). A calibrated Bayes analysis with a dispersed uniform prior was used to make inference on the true magnitude and uncertainty of effects [29]. In the absence of a minimum clinically important difference for short-term (up to 24 h) changes in our main (CTX-I, P1NP) and secondary outcomes, we used standardised thresholds of 0.2, 0.6, and 1.2 multiplied by the between athlete SD (pooled from all conditions and adjusted for small sample bias) to anchor small, moderate and large effects respectively. These thresholds were 15.2%, 45.6%, 91.2% for CTX-I, and 15.9%, 47.7%, 95.4% for P1NP. Inference was then based on the probability of the distribution for the true effect being greater than these thresholds, using a custom-made spreadsheet. The likelihood of the true effect being the observed magnitude was indicated by the following scale; possibly (25 to < 75%), likely (75 to < 95%), very likely (95 to < 99.5%) and most likely (≥ 99.5%). All effects were evaluated non-clinically, whereby a difference was deemed unclear if its chance of being both substantially positive and negative was ≥ 5% (based on the threshold for a small effect)[27] Finally, SD representing individual differences in the change of internal load over time were doubled before interpreting their magnitude against the above thresholds [30].

Results

Bone turnover markers

Raw mean and SD values for plasma volume adjusted/unadjusted CTX-I and P1NP are displayed in Table 1. Back transformed mean change and 90% CI of the mean change from baseline for CTX-I and P1NP are presented in Figure 1 (A & B) with the Cohen's *d* effect size units displayed in the secondary axis.

-INSERT TABLE 1 HERE-

After adjusting for baseline (CTX-I = 1.0 ng·mL^{-1}), circulating CTX-I was very likely higher (small) for 5 s (30.2%; 90% confidence limits (CL): ±10%, effect size 0.40; 90% CL: ±0.13) and 80 s (29.0%; ±10%, 0.38; ±0.13), and most likely higher (small) for 20 s (32.0%; ±10%, 0.42 ±0.13), compared to control at 1 h post exercise. The differences between exercise conditions were very likely/most likely trivial (~0.5%; ±8.6%, ~0.01 ±0.11). The very likely higher concentration in CTX-I remained in magnitude (small) at 2 h for 5 s when compared to the control (30.2% ±15%, 0.40 ±0.18). This difference was possibly trivial for the 20 s (13.0%; ±15%, 0.18 ±0.19) and possibly higher (small)/possibly trivial for the 80 s (16.0%; ±15%, 0.21 ±0.19) protocols. The differences between 5 s, 20 s and 80 s were possibly trivial/unclear. By 24 h post exercise CTX-I had returned to baseline for all conditions, with likely trivial differences between exercise and control. The effect of exercise on P1NP (baseline adjustment = 90 ng·mL^{-1}) compared to the control for 5 s was likely trivial (~7.6%; ±~7.8%, ~0.10 ±~0.10) to very likely trivial for 20 s (~7.1%; ±~7.6%, ~~0.09 ±~0.10) and 80 s (~7.4%; ±~7.5%, ~0.09 ±~0.10) across time points.

-INSERT FIGURE 1 HERE-

External (mechanical) training load

Percentage mean difference and 90% CL between conditions for measures of external load are displayed in Table 2.

265

266 **-INSERT TABLE 2 HERE-**

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268

269 **Internal (physiological) load ($\dot{V}O_2$, HR and blood [La])**

270

271 The change in $\dot{V}O_2$ per 10 min time interval was most likely trivial for all exercise protocols, with small
272 to trivial individual differences in this effect (see Table 3). The differences between protocols in $\dot{V}O_2$
273 slopes were very likely trivial to most likely trivial. Heart rate demonstrated a most likely small increase
274 over time for 5 s and 80 s and was possibly trivial for 20 s. Individual differences in these effects were
275 small to trivial, and the between protocol differences were likely to very likely trivial. After adjusting for
276 baseline B[La], the changes per 10 min time were as follows: a possibly small increase/possibly trivial
277 change for 5s, a likely trivial change for 20 s, a most likely trivial change for 80 s (See Table 3). Standard
278 deviations representing individual differences in these effects were moderate for 5 s and 20 s, and small
279 for 80 s. Between protocol differences in the change of blood [La] per 10 min of time were possibly to
280 likely trivial (Table 3).

281

282 **-INSERT TABLE 3 HERE-**

283 **Discussion**

284

285 The principal aim of our study was to establish the magnitude of the effect of three intermittent exercise
286 protocols of different exercise-to-rest intervals but matched for internal and external training load on the
287 acute responses of CTX-I and P1NP. The novel findings of our study are; 1) all intermittent conditions at
288 1 h post-exercise attenuated the normal decline of CTX-I. However, there were very likely trivial
289 differences between exercise conditions at 1 h, 2) the very likely (small) stimulatory effect of exercise on

circulating CTX-I concentration continued up to 2 h post-exercise for the 5 s condition. However, the small effect was not as clearly demonstrated for the 20 s and 80 s protocols at 2 h (effect was possibly small/possibly trivial), and 3) differences between circulating concentrations of P1NP were likely to very likely trivial across all time points.

Exercise-induced elevations in CTX-I above baseline have been observed in previous studies investigating either endurance running, [17] plyometric jumping, [25] and short-duration high-intensity intermittent exercise [23] using both running and cycling protocols. Scott et al. [17] observed a 3% elevation in CTX-I above baseline immediately post continuous running remaining elevated up to 1 h post-exercise for the 75% $\dot{V}O_{2max}$ protocol. Furthermore, Guillemant et al. [31], and Rogers et al. [25] observed a 45-50%, and ~10% increase above baseline, respectively. The greater difference observed by the latter two studies can be explained by the provision of a standardised meal allowing CTX-I to reach its natural nadir [32] and augmenting the stimulatory effect of exercise.

.Conversely, Bowtell et al. [21] reported no statistically significant change in CTX-I during or post-exercise from baseline, with a statistically significant increase in P1NP for short (15 min) and long duration SSGs (60 min) concluding that exercise reduced bone resorption whilst increasing bone formation. However, this interpretation is limited by the absence of a control condition. We also did not observe an overall increase in CTX-I concentration above baseline. However, the circulating concentrations of CTX-I for exercise at 1 h were higher for all exercise conditions compared to the control condition suggesting intermittent running might have suppressed the diurnal decline of CTX-I, thus circulating CTX-I was elevated for intermittent exercise in our study. Recently, Kouvelioti et al. [23] reported a combined increase of 28% in CTX-I from baseline at 5 min post-exercise following high-intensity intermittent exercise (cycling + running) with a smaller increase in CTX-I following running reporting a return to baseline by 1 h. Interestingly, participants exercised for a shorter duration (~16 min) compared to our study. However, the intensity was higher, participants exercised in a fed-state, were measured later in the day, and included a 5 min post-exercise blood sample which would contribute to the differences in responses compared to our data.

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322 The reduction in CTX-1 from baseline of ~10 % at 1 h in our study is similar to reductions (10-16%)
323 observed by Scott et al. [17] for the lower-intensity (≤ 65% $\dot{V}O_{2max}$) running protocols. Thus, the
324 intermittent nature and additional rest periods in our study could be responsible for reducing overall
325 repetitive stress on the bone that occurs with high-intensity (> 75% $\dot{V}O_{2max}$) but longer duration (60 min)
326 continuous repetitive stress [5]. Comparing an intermittent and continuous running protocol matched for
327 internal and external load would be of interest to confirm this. However, due to the nature of the NMT
328 and higher cardiometabolic demands [34], it is difficult to run continuously. Furthermore, whilst running
329 on an NMT has demonstrated similar peak vGRFs to MT running, the NMT causes an attenuation of the
330 vertical impact transient, and large reductions in tibial accelerations [35] compared to MT running, which
331 could affect the osteogenic potential of the exercise and contribute to the lower magnitude of CTX-I
332 response in our study compared to previous studies [17, 23].

333

334 We observed an approximate 30and 40% reduction in CTX-I from baseline at 1 and 2 h respectively for
335 the non-exercising condition (Figure 3 A) which is consistent with circadian variation in CTX-I [36] in a
336 fed- rather than fasted-state [36,37]. It is unclear why we demonstrated larger circadian variation in the
337 fasted-state during the non-exercise condition compared to previous circadian rhythm studies. One
338 explanation could be due to the different analytical techniques used by the different laboratories. We
339 quantified plasma CTX-I using the IDS iSYS which when compared with Roche Elecsys has poor
340 agreement and systematic bias[38] therefore results between the two devices are not comparable. Both
341 reference values and circadian variation data are also limited for the IDS iSYS. Furthermore, we used a
342 homogenous group of males who regularly performed vigorous intensity exercise which might also
343 account for the differences between studies.. Nevertheless, the circadian variation was supressed for all
344 exercise protocols with the 5 s protocol decreasing by ~20% from baseline at 2 h, representing a very

likely higher (small) difference in CTX-I between 5 s and the control. The 20 s and 80 s protocols decreased by ~30% representing only a possibly higher (small)/possibly trivial effect. The reduction from baseline at 2 h was similar with previous authors [17] demonstrating ~ 20-30 % reduction from baseline in CTX-I at 2 h post-exercise , and ~39-42 % reduction at 3 h post-exercise. Similar to our study, participants in the study by Scott et al. [17] exercised in a fasted state. Given BTMs were adjusted for PV changes in our study, the prolonged elevation in the 5 s protocol is likely a true response of exercise, rather than the resulting hemoconcentration artificially raising CTX-I during the 5 s protocol. This is substantiated by continued elevated CTX-I in the unadjusted data (Table 1; supplementary material).

As running speed increases the vGRF [39], and higher GRF increases bone deformation [40], it is possible that higher peak speeds might cause greater strain and ultimately greater mechanical strain on bone tissue [40] which could explain the difference between protocols at 2 h. However, while the 5 s protocol had a greater peak vGRF and VILR (Table 2) compared to 80 s, the difference between the 5 s and 20 s was trivial. Moreover, the overall mean vGRF was higher for 80 s (Table 2). Therefore, the trivial to small inconsistent differences between overall mean mechanical loading environments are unlikely to have contributed to a greater strain on bone between protocols. We also demonstrated that changes in HR, and $\dot{V}O_2$ (internal physiological load) were well-matched between protocols despite moderate individual differences in blood [La] responses. The greater heterogeneity in blood [La] responses is likely due to the difficulty in running on the NMT [34], combined with the limitations of using $v\dot{V}O_{2max}$ to dose exercise intensity [41]. The unremarkable differences between exercise protocols in both the internal physiological and external biomechanical load might explain the very likely trivial differences in BTMs between exercise protocols at 1 h. However, this does not explain the continued attenuated decline in CTX-I for 5 s compared to control at 2 h which warrants further investigation. As frequency of mechanical loading effects bone remodelling [42], the higher frequency in changes of accelerations and decelerations might have contributed to greater loading on the musculoskeletal system. Furthermore, the short (5 s) rest intervals might be too short to allow the bone to re-sensitise [8].

By 24 h post-exercise, the differences in circulating CTX-I concentrations between conditions were trivial, consistent with previous studies [17]. In contrast, several studies [18,43] have observed a prolonged elevation, likely due to the longer duration and greater mechanical stresses imparted by the exercise. However, these studies failed to standardise diurnal variation or nutritional status prior to blood sampling, and so outcomes post 24 h should be interpreted with caution. Finally, exercise had a trivial effect on circulating P1NP for all conditions (Figure 3 B). This is supported by previous research which has shown no change in P1NP immediately after 30 min of walking [44], continuous running [17], and both intermittent cycling and running [23]. Several previous authors [17,21] have shown an increase from baseline in P1NP during exercise. This increase preceded changes in CTX-I but P1NP declined in the recovery hours. Therefore, it is possible that P1NP increased during exercise in our study, but we had insufficient sampling time points to demonstrate this response. The overall increase in CTX-I combined with a trivial change in P1NP suggests an increase in bone remodelling in favour of resorption.

The clinical significance of acute small transient changes in CTX-I in response to intermittent exercise is unclear. Nevertheless, type I collagen makes up nearly 90% of the organic matrix of bone [45], and an important step in bone formation and resorption is the synthesis and degradation of type 1 collagen. Multiple loading cycles above the strain stimulus might evoke micro-damage to the bone tissue stimulating a targeted remodelling response (activation – resorption – formation cycle) [46]. Therefore, the higherCTX-I concentration and attenuated circadian rhythm of CTX-I due to the intermittent exercise l might reflect an initial increase in bone resorption (degradation of type 1 collagen) stimulated to repair fatigue-induced micro-cracks caused by the exercise [47]. Providing nutritional and health status of the exerciser is optimal, the old damaged bone will be replaced by newer stronger tissue later in the remodelling cycle.

Of note is that BTMs are only a surrogate measure of the bone's response to exercise and they do not reflect site-specific changes in bone [45]. Furthermore, the limited number (up to 24 h) of sampling points in our study make it difficult to draw conclusions about longer term (days, months, years) adaptations to bone, and we may have missed the peak in CTX-I due to the absence of an immediate post-exercise

sample. Moreover, in order to induce shifts in bone tissue in a healthy adult skeleton, the intensity must be high [17,20]. The intensity combined with the difficulty of running on the NMT resulted in a large drop-out rate due to early onset of fatigue, thus reducing the power of the study. Therefore, further work is required to establish the osteogenic potential of intermittent exercise using different exercise modalities (e.g. resistance exercise), different populations, and longer exercise-to-rest intervals using longitudinal randomised controlled trials.

Our study confirms previous findings that exercise has an acute, albeit small and transient effect, on bone remodelling in favour of resorption, in the hours following exercise. A novel finding of our study is that the very intermittent protocol (shorter more frequent exercise-to-rest intervals) caused a prolonged attenuation in the diurnal decline of CTX-I compared to the non-exercising control, lasting up to 2 h post exercise. This attenuated effect on the diurnal response was not as clearly demonstrated by the 20 s and 80 s protocols at 2 h despite similar physiological and mechanical loads imposed by the different intermittent protocols.

Acknowledgements

The authors would like to thank all the participants who volunteered for the study. We would also like to thank [redacted] for her technical assistance during data collection.

Conflict of interest statement

The authors declare that all authors have no conflict of interest.

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Table and Figure descriptions

Table 1. Raw mean ± SD for the PV adjusted and unadjusted CTX-I and P1NP at baseline ('Pre'), 1 h, 2 h and 24 h post exercise (*n*=12).

Table 2. The difference in magnitude of change between the exercise protocols on measures of external load including; number of steps, total distance, mean speed, mean vGRF, peak vGRF, work done and loading rate. Data shown are raw mean and SD, with back-transformed mean differences (90% CL) (*n*=12).

Table 3. The mean slope and interactions between protocols expressed as a % mean difference (90% CL) for oxygen consumption ($\dot{V}O_2$), heart rate (HR) and blood lactate [La] per unit of time with the qualitative inference of the effect for all protocols (5 s, 20 s and 80 s). The individual differences in the slopes, and qualitative inference for the magnitude of the individual differences is also displayed (*n*=12).

Figure 1. The back-transformed percentage change (90 % CI) from baseline for PV-adjusted CTX-I (panel A) and P1NP (panel B) at 1 h, 2 h and 24 h post exercise. The right Y axis displays the Cohen's *d* effect size units. > = greater than, C = control, * = possibly, *** = Very likely, **** = Most likely, the subscripted S and T = small and trivial respectively.