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10 Six weeks of sprint interval training improves body composition in 11 healthy young males

12 Exercise plays a key role in improving body composition and bone health.
13 This study aims to look at the effects of a sprint interval training (SIT)
14 programme, on markers of skeletal health and body composition measured
15 by dual-energy x-ray absorptiometry (DXA). Twenty-eight participants
16 (24.4 ± 6.7 y; 1.77 ± 0.09 m; 79.0 ± 14.5 kg; 25.0 ± 2.8 kg•m⁻²) were
17 randomly assigned, into either the SIT or control condition. All participants
18 made a single fasted visit to the laboratory, pre-and post a 6 week SIT
19 intervention, where they underwent a whole body, proximal femur and
20 lumbar spine DXA scan. SIT caused significant reductions in sub-total (-
21 1.08 kg) and lower limb (-0.50 kg) fat mass, as well as on upper- (-1.26%)
22 and lower-limb (-1.70%) percentage fat mass compared with non-exercising
23 controls (0.26, 0.00 kg; -0.15, -0.28 %). There were no significant changes
24 in any bone measures, but trends were observed for differences in femoral
25 neck BMD (1.75%), total proximal femur BMC (5.79%) and area (4.81%)
26 following SIT compared to the control group (-1.51, 0.05, 0.36%). This
27 study showed that SIT is a potent method of reducing fat mass, as well as a
28 potential as a method for improving bone mass.

29 Keywords: SIT, DXA, training intervention, bone mineral density

30 **1. Introduction**

31 Sprint interval training (SIT) and high-intensity interval training (HIIT)
32 have emerged over the last decade as extremely potent training methods (Gillen &
33 Gibala, 2014). These two methods, classified by Weston *et. al.* (2014), utilise
34 repeated bouts of high-intensity exercise, interspersed with recovery, either

35 between 80-100% peak heart rate (HIIT) or above VO_2max (SIT). Although, these
36 two intervention styles involve repeated anaerobic exercise, both HIIT and SIT have
37 been shown to have performance and health benefits that are traditionally seen
38 following long duration endurance training. These adaptations include, but are not
39 limited to, increased endurance capacity (Burgomaster et al., 2008), increased
40 mitochondrial enzyme action (Burgomaster et al., 2005; 2006; 2007; 2008; Gibala
41 et al., 2006), improvements in insulin sensitivity (Babraj et al., 2009; Metcalfe,
42 Babraj, Fawcner, & Vollaard, 2011), skeletal muscle protein content (Burgomaster
43 et al., 2007), as well as in fat metabolism (Babraj et al., 2009; Metcalfe et al., 2011;
44 Sim, Wallman, Fairchild, & Guelfi, 2013). These positive outcomes suggest HIIT
45 and SIT interventions have a role to play in providing individuals with alternative
46 exercise modalities to improve both health and performance, with a relatively short
47 time commitment.

48 Measuring changes in body composition due to any intervention is
49 important in both clinical and athletic populations (Guppy & Wallace, 2012), and
50 has been looked at in SIT interventions, there is limited evidence examining SIT or
51 the effect of SIT on the gold standard three-compartment model of body
52 composition measured by DXA (Wang, Pierson, & Heymsfield, 1992). The
53 majority of studies show a reduction (0.72-2.57%) in percentage fat mass following
54 SIT interventions (Boer et al., 2014; Gillen, et al., 2013; Shing, et al., 2013; Terada
55 et al., 2013; Verney, et al., 2006). The studies that utilise a three compartment model
56 report reductions in both trunk (2.5%) and lower limb (1.6%) fat mass after a 12-
57 week intervention (Terada et al., 2013), and increased lower limb lean mass (0.6
58 kg) (Gillen et al., 2013) following 6 weeks of SIT.

59 The three-compartment model of body composition has a distinct
60 advantage over other models as it allows for the separation of fat-free mass into
61 lean- and bone mass (Wang et al., 1992). This advantage allows for the inclusion
62 of changes in bone material to be made alongside changes in fat- and lean-mass.
63 Strain has been shown to play a critical role in bone mass development, with Frost
64 (1987) proposing the Mechanostat as a likely method of adaptation of bone material
65 to loads placed upon it. The importance of strain was further illustrated by Skerry
66 (2006), who suggests that strain stimulus, number of loading cycles and the amount
67 of rest between load cycles all play a role in skeletal adaptation to mechanical
68 loading, described by Skerry (2006) as the Customary Strain Stimulus (CSS). This
69 process of repeated load intermitted with periods of rest is the definition of both
70 HIIT and SIT (Weston et al., 2014), therefore, these methods may provide a
71 mechanism for achieving adaptations to the skeleton. There has been limited
72 investigation into the role of either HIIT or SIT on the skeleton, with only a single
73 study examining the effects of HIIT on bio-markers of bone turnover (Mezil et al.,
74 2015). This study showed that a single session of high-intensity low impact activity
75 increased osteoblast responsiveness via increased osteoprotegerin (OPG) and bone
76 alkalkine phosphatase (BAP), which are both responsible for primarily responsible
77 for regulating osteoclast activity (Datta, et al., 2008; Kwan Tat, et al., 2004). This
78 study also showed increased inflammatory cytokine response which (Mezil et al.,
79 2015) suggests are associated with increased osteoclast activity differentiation by
80 promoting production of receptor activated of NF- κ B ligand (RANKL) and OPG
81 with a net increase in RANKL (Kwan Tat et al., 2004).

82 To address the lack of detailed body composition data following SIT
83 intervention, the main aim of this study was to investigate the effects of a 6-week

84 SIT cycling programme on body composition measured using dual-energy x-ray
85 absorptiometry (DXA). The authors hypothesised that SIT will result in increased
86 lean mass in the lower limbs, whilst reducing whole body fat mass. To examine the
87 effect of a SIT programme on bone adaptation, a secondary aim of this study was
88 to examine the effect of a SIT intervention on bone mass measured in the proximal
89 femur and lumbar spine, in a sub-group of participants within the study.

90 **2. Methods**

91 ***2.1 Experimental Approach to the Problem***

92 A randomized controlled trial was conducted to examine the effects of a 6-
93 week SIT on a 3-compartment model of body composition, as well as bone mass at
94 the lumbar spine and proximal femur. Participants made two visits to the laboratory;
95 pre and post a 6-week SIT intervention. Each visit was made following a 10-hour
96 overnight fast and included three whole body dual-energy x-ray absorptiometry
97 (DXA) scans.

98 ***2.2 Participants***

99 Twenty-eight male participants (Age= 24.4 ± 6.7 y, Height= 1.77 ± 0.09 m,
100 Mass= 79.0 ± 14.5 kg, BMI= $25.0 \pm \text{kg} \cdot \text{m}^{-2}$) volunteered to take part in this study.
101 Ethical approval was sought and gained from the University Research Ethics
102 Committee, and complied with the World Medical Association Declaration of
103 Helsinki, and the ethical standards described by Harriss & Atkinson (2009).
104 Participants were randomly allocated in a blind manner by a member of the research
105 team not involved in data collection into either the SIT or a control group. There
106 were no significant differences at baseline in age (years) ($t_{(1-26)}=-0.014$, $p=0.989$),

height (m) ($t_{(1-26)}=-0.536$, $p=0.596$), mass (kg) ($t_{(1-26)}=-0.774$, $p=0.446$) or BMI ($\text{kg}\cdot\text{m}^{-2}$) ($t_{(1-26)}=-0.321$, $p=0.751$) between the groups. Activity levels out-with the study were measured using the short form of the International Physical Activity Questionnaire in a subsection of 18 participants in both the exercise ($n=9$) and control ($n=9$) groups. A 2-way analysis of variance showed no difference in activity levels outwith the study due to training ($F_{(1-29)}=0.194$, $p=0.663$) or time ($F_{(1-29)}=0.216$, $p=0.645$) with no interaction between these variables $F_{(1-29)}=0.068$, $p=0.797$).

2.3 Experimental Procedures

2.3.1 Body Composition Assessment

Total and regional body composition was estimated by DXA (Discovery A, Hologic Inc., Massachusetts, USA), with images analysed using Hologic Apex software (Apex v3.2.1, Hologic Inc., Bedford, MA, USA) for the calculation of lean and fat mass (kg) as well as percentage fat mass. The scanner was calibrated before each use by bone phantom (Hologic Inc.) for quality assurances, and no evidence of machine drift appeared during this study. Unpublished work by this laboratory showed greater repeatability when left and right limbs were combined, therefore this approach is adopted in the current study. Due to the inbuilt analysis assumptions regarding the measurement of head composition, sub-total data (total - head region) will be reported in this study. Precision of operator repeated measurements ranged from 0.26-1.69% for total mass, 1.36-2.78% for fat mass and 0.77-1.90% for lean mass.

129 2.3.2 *Bone Mass Assessment*

130 Areal Bone Mineral Density (BMD, $\text{g}\cdot\text{cm}^{-2}$), Bone Mineral Content (BMC,
131 g) and bone mineral area (cm^2) was measured in the lumbar spine (L1-L4) and the
132 left proximal femur by DXA and analysis was conducted using the preinstalled
133 machine software. Total lumbar spine, proximal femur, femoral neck and Ward's
134 triangle were analysed separately, with all scanning and analysis conducted by the
135 same operator, with precision of repeated measurements ranging from 1.51-3.15%
136 for BMD, 3.46-8.66% for BMC and 1.87-8.08% for bone mineral area.
137

138 2.3.3 *Sprint Interval Training Programme*

139 The SIT group undertook 18 supervised SIT sessions over a 6-week period,
140 with 1-2 days recovery between sessions. SIT consisted of 4-6 repeated 30-s
141 maximal cycling efforts against a resistance equivalent to 7.5% body mass on a
142 cycle ergometer (Monark, 874E, Varberg, Sweden). Participants completed four
143 sprints per session in weeks 1 and 2, five sprints in weeks 3 and 4 and six sprints in
144 weeks 5 and 6, based on protocols of similar (Burgomaster et al., 2007) and 2-weeks
145 (Babraj et al., 2009; Burgomaster et al., 2005; 2006; 2008; Gibala et al., 2006)
146 duration. Exercise sessions commenced with a 5-minute warm up at a nominal
147 resistance (approximately 60 W) prior to the first sprint with each of the sprints
148 separated by a 4-minute recovery period. Participants were encouraged to remain
149 on the ergometer during recovery periods and to maintain a low pedal cadence. The
150 total time commitment ranged from 19 to 28 minutes, with the sprint exercise
151 totaling 2-3 minutes per session.

152 2.3.4 Statistical Analysis

153 Data were analysed using a factorial analysis of covariance (ANCOVA),
154 with the baseline value used as a covariate to adjust post-test values relative to the
155 baseline values. This approach is deemed to show the greatest statistical efficiency
156 (Vickers, 2001). Alpha level was set at 0.05 and data is presented as mean $\pm$
157 standard error (SEM).

158 3. Results

159 3.1 Body Composition

160 Total body mass was unchanged as a result of SIT (SIT; 80 ± 4 -v- 81 ± 5 ,
161 CON; 76 ± 6 -v- 77 ± 6 kg; $p < 0.05$). Dual energy x-ray absorptiometry revealed
162 reduced sub-total and lower limb fat mass (Table 1, Figure 1; $p < 0.05$), but no
163 significant reduction in fat mass of the upper limbs (Table 1) following SIT
164 intervention. There was also reduced percent fat in both the upper and lower limbs
165 (Table 2, $p < 0.05$) but not in sub-total percentage fat. There were no significant
166 changes in either lean or total mass due to SIT (Table 1).

167 Table 1 Changes in Body Composition

168 ***TABLE 1 SOMEWHERE HERE***

169

170 ***FIGURE 1 SOMEWHERE HERE***

171 **Figure 1** Effect of 6-weeks of sprint interval training (SIT) or no training (Control)
172 on change in fat mass (kg) of the upper limbs, trunk, lower limbs and sub-total
173 regions measured by DXA. Circles/squares show individual change and horizontal

bars show mean group change from baseline. */# denotes different from control condition (* p<0.05; # p<0.1).

3.2 Bone Mass

SIT led to near significant increases in BMD at the femoral neck (1.75% PRE: 1.03 ± 0.21 -v- 1.05 ± 0.19 g•cm⁻², p=0.059), total hip area (4.81%; 44.16 ± 6.47 -v- 46.17 ± 6.25 cm⁻², p=0.083), and total hip BMC (5.79%; 51.84 ± 15.13 -v- 54.35 ± 14.25 , p=0.059, Figure 2), compared with non-exercising controls (-1.52, 0.36 & 0.05%). There were no other changes in either bone density, content or area as can be seen in Table 2.

FIGURE 2 SOMEWHERE HERE

Figure 2. Percentage change from baseline in Total Proximal Femur (Total) bone mineral density (BMD), bone mineral content (BMC), and Area as well as Femoral Neck (FN) and Wards triangle (Wards) BMD following 6 weeks SIT. Circles/squares show individual change and horizontal bars show mean group change from baseline. * denotes different from control group (p<0.1).

Table 2. Changes in Bone Mineral Content, Density and Area measured by DXA following 6 weeks SIT.

TABLE 2 SOMEWHERE HERE

4. Discussion

4.3 Body Composition

This study aimed to examine the effects of 6-weeks SIT on a 3-compartment model of body composition. This study showed reductions in sub-total (1.08 kg),

196 and lower limb (0.5 kg) fat mass, with these reductions being of similar magnitude
197 to those previously shown (Gillen et al., 2013; Smith et al., 2009). There was also
198 a non-significant increase in sub-total lean mass (0.41 kg) of similar magnitude to
199 that reported by Gillen *et. al.* (2013) (0.4 kg). These changes in body composition
200 occurred without subsequent reductions in sub-total mass, reinforcing the use of
201 more complex measures of body composition assessment rather than simply using
202 mass alone when assessing the efficacy of training and/or dietary interventions on
203 body composition and mass.

204 The potential mechanisms behind reduced fat mass in response to SIT
205 include changes in fat oxidation, both during and post exercise, as well as alterations
206 in appetite and energy intake (Deighton & Stensel, 2014; Sim et al., 2013). These
207 changes, particularly in appetite, have also shown that supramaximal interval
208 training may be more effective at inducing changes in body composition via
209 appetite regulation than MICT (Sim, et al., 2015). These alterations in appetite
210 regulation appear alongside reductions in energy intake (Sim et al., 2013) and
211 increased energy expenditure (Hazell, et al., 2012). This increased energy
212 expenditure could be due to increased glycogen resynthesis, dissipation of blood
213 lactate (Boutcher, 2011) or increased catecholamine induced metabolism and
214 lipolysis (Bracken, Linnane, & Brooks, 2009), as well the thermic effect of exercise
215 or increased muscle protein turnover (Hazell et al., 2012).

216

217 **4.2 Bone Mass**

218 The major mechanisms behind skeletal adaptation to mechanical load are
219 through changes in lean mass and increased strain placed upon the skeleton.
220 However, in this study there were no observed changes in lean mass, with only

221 reductions seen in fat mass, suggesting that increased strain alone is driving the
222 changes seen in this study. The literature has previously suggested that weight-
223 bearing exercise is key to this change, with non-weight bearing activities such as
224 rowing and swimming often having a detrimental effect on bone (Gómez-Bruton,
225 et al., 2013; Olmedillas, et al., 2012). However, this study has shown near
226 significant increases in femoral neck BMD (3.25% -v- controls), total hip area of
227 bone material (4.45%), and total hip BMC (5.74%) in a non-weight bearing activity.
228 The changes seen in this study are of greater magnitude to those seen in studies of
229 24 (Maddalozzo & Snow, 2000) and 52 weeks (Vainionpää, et al., 2004) and of
230 similar magnitude to results reported after 48 weeks of ground reaction force
231 inducing exercise by Kohrt *et. al.* (1997). These near clinical changes in BMD after
232 only 6-weeks are potentially important as researchers suggest that a minimum of
233 24-weeks are required to induce skeletal changes (Kelley, Kelley, & Kohrt, 2013a;
234 2013b). The rapid changes observed in this study show that SIT has the potential
235 for increasing BMD over a much shorter time frame than traditional modalities for
236 improving BMD due to the supramaximal nature of this exercise type.

237 Although the observed changes fall short of reaching statistical significance,
238 the site specific nature points to the likelihood of the Mechanostat as the mechanism
239 behind any adaptation. Bone adaptation occurs when it is loaded, when it becomes
240 deformed, or under strain (ratio of change in length divided by original length)
241 (Skerry, 2006). Due to the high intensity nature of SIT the CSS is potentially being
242 exceeded, therefore causing adaptations to the site being loaded. As cycling is a
243 predominantly lower limb activity, with limited whole body involvement, changes
244 would only be expected at the proximal femur, as seen in this study, in addition to
245 the lower limbs. Although SIT also induces metabolic adaptations that could

246 influence bone metabolism and therefore density, including changes in insulin
247 action (Babraj et al., 2009), it is unlikely that these changes are the major influence
248 on the skeleton. This is shown by the lack of change in the lumbar spine (Table 3),
249 which if bone were being altered via systemic rather than local factors would show
250 some adaptation.

251 The frequency of stimulation has been suggested as important when
252 examining improvements in bone mass induced by loading, as shown by low
253 frequency stimulation inducing the proliferation of osteoblasts in formation and
254 remodelling (Scott, et al., 2008). Therefore, with frequency playing a crucial role,
255 more frequent, shorter sprints may play a similar role in causing bone adaptations,
256 and are therefore an interesting avenue for further research.

257 ***4.3 Limitations and Future Directions***

258 Both SIT and SIT have limitations that need to be explored, particularly the
259 achievability of this type of exercise in clinical populations. The SIT protocol used
260 in this study is based upon a Wingate test, with the resistance set at 7.5% of body
261 mass, which although achievable in non-clinical populations, is this the case in
262 clinical populations. Changes in body composition are often required in disease
263 states such as type-2 diabetes mellitus, and specifically required in obesity. As
264 obese individuals will, and T2DM patients often do, have a large amount of
265 unfunctional, fat mass (Strasser, 2012), 7.5% of body mass may make this
266 resistance unrealistic in clinical populations. Further work is needed to establish the
267 most effective protocol that balances between health benefits, and the ability of the
268 patient to undertake the intervention. The exclusion of unfunctional fat mass from
269 the calculation of resistance may provide a more realistic intensity of exercise for
270 clinical populations. In addition future studies, should measure energy intake and

271 expenditure, as well as markers of appetitete regulation and other factors that
272 influence fat loss. This would allow determination of what mechanisms are driving
273 fat mass loss and the lack of lean mass gains.

274 When examining bone mass, it has been suggested that the frequency of the
275 stimulation is important regarding the improvement of bone tissue. This has been
276 shown with low frequency stimulation of osteoblasts playing a role in the
277 proliferation of these key cells in formation and remodelling (Scott et al., 2008).
278 Therefore, with frequency playing a crucial role, would more frequent, shorter
279 sprints play a similar role in causing bone adaptations, following SIT. The
280 effectiveness of this method in clinical populations is dependent on researchers
281 identifying the optimum level of intervention.

282

283 ***4.4 Conclusion***

284 In conclusion, although these findings did not reach statistical significance,
285 this study illustrates the potential that HIIT may have at maintaining or improving
286 skeletal health. Further studies are needed exploring how HIIT can induce changes
287 in bone material. The current trend in the literature is to reduce the time
288 commitment of this form of exercise in order to establish the lowest volume of
289 training needed (Metcalf, et al., 2016; Ruffino et al., 2017), therefore trying to
290 strike a balance between the positive outcomes associated with HIIT and the
291 feasibility of this intervention in the populations that need it most. However, this
292 approach may not be beneficial when considering bone health, but further
293 investigation is needed to establish the effectiveness of this intervention at
294 improving bone mass.

295

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460

Table 1. Changes in Body Composition

		SIT (n=19)					Control (n=9)			
		Pre	Post	Δ	d		Pre	Post	d	Δ
ST	Fat	17.43 (1.28)	16.36 (1.20)	-1.08	0.60	*	17.05 (2.62)	17.31 (2.81)	0.14	0.26
	Lean	58.19 (1.93)	59.34 (2.01)	1.15	0.64		53.76 (4.61)	54.67 (4.79)	0.51	0.92
	Mass	75.62 (2.78)	75.70 (2.83)	0.08	0.03		70.80 (6.11)	71.98 (6.50)	0.50	1.18
	%Fat	22.75 (1.21)	21.33 (1.10)	-1.42	0.71		23.93 (2.90)	23.84 (2.97)	0.04	-0.09
UL	Fat	2.13 (0.13)	2.01 (0.12)	-0.12	0.56	#	2.09 (0.28)	2.10 (0.30)	0.05	0.01
	Lean	7.40 (0.28)	7.55 (0.32)	0.15	0.27		6.56 (0.70)	6.60 (0.71)	0.08	0.04
	Mass	9.53 (0.32)	9.57 (0.37)	0.03	0.05		8.65 (0.76)	8.70 (0.78)	0.08	0.05
	%Fat	22.36 (1.19)	21.10 (1.05)	-1.26	0.66	*	25.15 (3.90)	25.00 (4.02)	0.08	-0.15
LL	Fat	6.77 (0.47)	6.28 (0.42)	-0.50	0.85	*	7.33 (1.09)	7.33 (1.17)	0.00	0.00
	Lean	20.67 (0.78)	21.08 (0.74)	0.41	0.42		19.16 (1.81)	19.27 (1.80)	0.11	0.11
	Mass	27.45 (1.08)	27.36 (1.02)	-0.09	0.08		26.49 (2.35)	26.60 (2.39)	0.10	0.11
	%Fat	24.43 (1.18)	22.73 (1.05)	-1.70	0.86	*	27.61 (3.52)	27.33 (3.65)	0.14	-0.28

All values shown are mean (SE). All units are kg with the exception of % Fat. * denotes difference from control group (*p<0.05). Δ ; Change from Baseline, d ; Cohens D SIT; Sprint Interval Training, LL; Lower-limbs, ST; Sub-total, UL; Upper-limbs LL; Lower-limbs.

Table 2 Changes in Bone Mineral Content, Density and Area measured by DXA following 6 weeks SIT.

		SIT				CONTROL				
		Pre	Post	Δ	d		Pre	Post	Δ	d
Total LS	BMA	71.30 (0.92)	72.66 (0.84)	1.36	0.74		66.04 (0.77)	67.18 (0.82)	1.14	0.54
	BMC	83.99 (2.45)	86.38 (2.82)	2.39	0.45		69.40 (1.55)	70.10 (1.62)	0.50	0.44
	BMD	1.16 (0.02)	1.17 (0.03)	0.01	0.12		1.05 (0.02)	1.04 (0.02)	-0.01	0.11
Total PF	BMA	44.16 (0.72)	46.17 (0.69)	2.01	1.10	#	43.13 (0.69)	43.26 (0.67)	0.13	0.09
	BMC	51.84 (1.68)	54.35 (1.58)	2.51	0.88	#	47.71 (1.47)	47.59 (1.42)	-0.12	0.02
	BMD	1.15 (0.02)	1.16 (0.02)	0.01	0.32		1.09 (0.02)	1.09 (0.02)	0.00	0.10
FN	BMA	6.01 (0.06)	5.99 (0.05)	-0.02	0.08		5.98 (0.07)	5.73 (0.06)	-0.25	0.48
	BMC	6.28 (0.18)	6.33 (0.16)	0.05	0.02		5.80 (0.17)	5.46 (0.15)	-0.34	0.59
	BMD	1.03 (0.02)	1.05 (0.02)	0.02	0.24	#	0.96 (0.02)	0.94 (0.02)	-0.02	0.26
WT	BMA	1.17 (0.01)	1.11 (0.01)	-0.06	0.91		1.18 (0.01)	1.18 (0.01)	0.00	0.00
	BMC	1.09 (0.03)	1.06 (0.03)	-0.03	0.33		1.04 (0.04)	1.02 (0.04)	-0.02	0.36
	BMD	0.94 (0.02)	0.96 (0.03)	0.02	0.42		0.87 (0.03)	0.86 (0.03)	-0.01	0.29

Units: BMA (cm²), BMC (g), BMD (g•cm⁻²). #; denotes difference from control group (p<0.1). Δ ; Change from

Baseline, d; Cohen's d FN; Femoral Neck, SIT; Sprint Interval Training, LS: Lumbar Spine, PF; Proximal Femur,

WT: Wards Triangle

Figure 1 Effect of 6-weeks of sprint interval training (SIT) or no training (Control) on change in fat mass (kg) of the upper limbs, trunk, lower limbs and sub-total regions measured by DXA. Circles/squares show individual change and horizontal bars show mean

Figure 2 Percentage change from baseline in Total Proximal Femur (Total) bone mineral density (BMD), bone mineral content (BMC), and Area as well as Femoral Neck (FN) and Wards triangle (Wards) BMD following 6 weeks SIT. Circles/squares show individual change and horizontal bars show mean group change from baseline. * denotes different from control group ($p < 0.1$).