1	Revision No.1
2	Bone mineral density in male weight-classified athletes is higher than that in male
3	endurance-athletes and non-athletes
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20 Abstract

Background: Weight-bearing physical activity and intense mechanical stimuli affect the 21bone through the endocrine system; hence, bone-loading sports affect bone mineral 22density. We hypothesized that weight-classified athletes, such as those practicing 23wrestling and judo, have relatively high bone mineral density because these activities 24have a higher impact on the entire body during daily training compared to low- or 25non-impact activities. We aimed to investigate the bone mineral density of 26weight-classified athletes (participating in wrestling and judo) to compare the 2728parameters with those of endurance-athletes and non-athletes.

Methods: Thirty-three college athletes (aged 18-22 years) were divided into three
groups, wrestlers, judoka, and endurance-athletes, according to their sports history.
Eight non-athletes participated as controls. Bone mineral density was determined by
whole-body dual-energy X-ray absorptiometry.

Results: Mean whole-body bone mineral density of wrestlers and judoka was higher than that of endurance-athletes and non-athletes (P<0.01). The bone mineral density of athletes competing in wrestling and judo was higher than that of non-athletes when adjusted for body mass.

37 Conclusions: The present study demonstrated that weight-classified athletes have

39	despite rapid weight loss before competitions.
40	
41	Keywords: Weight-cycling, Dual-energy X-ray absorptiometry, Athletes
42	
43	Abbreviations:
44	BMD, bone mineral density
45	DXA, dual-energy x-ray absorptiometry
46	SD, standard deviation
47	ANOVA, one-way analysis of variance
48	ANCOVA, analysis of covariance

significantly higher bone mineral density compared to endurance- and non-athletes,

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50 INTRODUCTION

51	It is important to maintain or enhance bone health and therefore minimize bone
52	injury in weight-classified athletes who participate in direct contact sports such as
53	wrestling, judo, and boxing. Energy, vitamin D, and calcium intake all play a role in
54	bone health $[1-3]$. Thus, there are concerns that insufficient food intake in athletes could
55	result in low energy availability and nutrient deficiency, which could lead to low bone
56	mineral density (BMD) [4]. Energy availability is defined as energy intake (kcal) minus
57	exercise energy expenditure (kcal) divided by kilograms of fat-free mass [5].
58	Weight-classified athletes are likely to experience low energy availability because they
59	usually lose weight rapidly before a competition, maintaining high-volume training
60	activity with severe energy restriction [6]. Since these athletes lose weight several times
61	a year for tournaments, it is expected that their BMD would be negatively affected. This
62	means these athletes have to experience some episodes of low energy availability over a
63	year, otherwise the weight loss would be short-term. Furthermore, there is a risk of
64	vitamin D insufficiency or deficiency, which may also impact bone health, and is of
65	particular concern since most of the weight-classified sports are practiced indoors [7].
66	It is widely known that weight-bearing physical activity benefits the bone.

67 Tenforde et al. [8] showed that the BMD z-score of athletes in non-weight-bearing

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68	sports, such swimming and cycling, were low compared to that of other athletes. Recent
69	research has confirmed these findings, where a high prevalence of low BMD (z-score <
70	-1.0) was found in competitive cyclists [9]. Hence, it is possible that repeated bone
71	impact may be associated with BMD. For example, cross-country running and other
72	high energy-expenditure sports involve repetitive, lower-impact forces that may not
73	result in high bone strength compared to that in team sports such as soccer [10]. It has
74	already been shown that jumping, which creates higher bone impact than running,
75	results in stiffer, more fracture-resistant bones [11]. Consequently, sports that involve
76	high bone impact may result in higher BMD in athletes if high impact activity does
77	indeed increase BMD. In wrestling and judo, athletes experience high impact on the
78	whole body during daily training because combat sports involve direct contact with
79	competitors. Therefore, it is possible that the BMD of weight-classified athletes, such as
80	wrestlers and judoka, are significantly higher than that of athletes in other sports despite
81	weight-cycling and indoor training. A recent study reported that low BMD in male
82	athletes in different types of sport activity was associated with bone stress injuries [12].
83	However, there have been a few studies that have focused on weight-classified athletes
84	subjected to severe energy restriction and weight loss during a competitive season

85	several times in a year. Their BMD should be monitored because their bodies are
86	directly exposed to punches or tackles.
87	In this study, we aimed to investigate the BMD of weight-classified athletes
88	and to compare it with that of endurance-athletes and non-athletes. We hypothesized
89	that the BMD of weight-classified athletes would be higher than that of
90	endurance-athletes and non-athletes.

92 METHODS

This study was a cross-sectional trial sample of young Japanese men. In total, 41 93healthy adults aged ≥ 18 years were enrolled through a prospective open recruitment 94 95between universities in the Kanto region of Japan (age 18-24 years). In the present study, individuals participating in 4 or more sessions of high-volume training per week as 96 ascertained from weekly training records were defined as athletes [n=33], as in our 97previous study (Table 1) [13]. The number of training days for athletes was 5.9 ± 1.0 98 (range 4–7) days per week, which was obtained within 7 days of dual-energy x-ray 99 100 absorptiometry (DXA) measurement; whereas, the non-athletes were recruited and confirmed as none or one exercise per week [n=8] at screening. The athletes were 101 further divided into three groups, "wrestlers" [n=11], "judoka" [n=9], and 102"endurance-athletes" [n=13] according to each individual's sporting background. 103 Weight-classified athletes, who participated in wrestling or judo, usually conducted 104 105weight-cycling before competition at least 3 times per year and had experienced loss of >5% of their body mass before a major competition. The athletes participated in these 106 107 events within or after the season. The endurance-athletes were 10 triathletes, 2 108 endurance cyclists, and one orienteer. We confirm that none of the participants were taking any medications, and none had thyroid or heart disease. All participants provided 109

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written informed consent. The study protocol was approved by the ethics committee of

111 Japan Institute of Sports Sciences (approval no. 036 and 050).

112 Measurements

113The participants refrained from hard training and consuming alcohol or stimulant beverages for at least 24 h prior to the measurements. They were instructed to abstain 114 from consuming all food and beverages after 23:00 of the day preceding the evaluation. 115116 The participants presented to the laboratory at 06:30 and voided urine. The participants' height was measured within the nearest 0.1 cm with a stadiometer and their body mass 117118 was measured to the nearest 0.01 kg using an electronic scale (A&D Co. Ltd., Tokyo, Japan). BMD and body composition were determined using DXA (Discovery A, 119 Hologic, Waltham, MA, USA) whole-body scans. The same technician positioned the 120participants, performed all scans, and executed the analysis according to the operator's 121manual using the standard analysis protocol. Participants wore a swimsuit, were 122123barefoot, and positioned on the scanner table with arms out to the side, hands flat on the table, and feet in the plantar-flexed position for 5-10 min according to the 124manufacturer's guidelines and our previous studies [6,14]. For data analysis of the 125126whole body, the trunk, arm, and leg measurements were considered [15]. Body composition is presented as fat mass and fat-free mass including bone mineral content. 127

128 Statistical analysis

Results are presented as mean \pm standard deviation (SD). The differences 129between-groups were examined using one-way analysis of variance (ANOVA). Pearson 130correlation coefficients were used as measures of correlation between whole-body BMD 131and body mass, age, and height. To eliminate confounding effects, analysis of 132133covariance (ANCOVA) was used to analyze between-group differences whole-body 134BMD adjusting with body mass. When a significant difference was detected, a multiple comparison test was performed using Tukey's post-hoc test for the least significant 135difference between the groups. All analyses were performed using IBM SPSS 23.0 for 136Mac (IBM, New York, NY). For all the analyses, P<0.05 was used to denote statistical 137significance. 138

140 **RESULTS**

In total, 41 participants (33 athletes and 8 non-athletes) completed the DXA 141 measurements for BMD. The characteristics of the participants included in the analysis 142are summarized in Table 1. There were significant differences in body mass, BMI, 143percent of fat, fat mass, and fat-free mass but no differences in age or height between 144the groups. There was a significant, moderately positive correlation between 145146whole-body BMD and body mass (r = 0.439, P=0.004), but whole-body BMD was not significantly correlated with age (r = -0.230, P = 0.204) or height (r = -0.061, P = 1471480.703).

149	The mean whole-body BMD of athletes in the weight-classified sports, i.e.,
150	wrestlers (1 366 \pm 0 060 g/cm ² · 95% CI: 1 325–1 466) and judoka (1 282 \pm 0 066 g/cm ² ·

 $151 \quad 95\%$ CI: 1.231, 1.333) was higher than those of endurance-athletes (1.175±0.081 g/cm²;

152 95% CI: 1.125, 1.224) and non-athletes (1.161±0.092 g/cm²; 95% CI: 1.084, 1.238)

153 (P<0.01, see **Table 1**). The whole-body BMD of wrestlers $(1.360\pm0.052 \text{ g/cm}^2; 95\% \text{ CI}:$

154 1.325, 1.395) and judoka (1.264±0.074 g/cm²; 95% CI: 1.207, 1.321) remained higher

than that of non-athletes $(1.164\pm0.092 \text{ g/cm}^2; 95\% \text{ CI: } 1.087, 1.241)$, when adjusted for

body mass (Figure 1). The difference between judoka and endurance-athletes

157 disappeared when adjusted for body mass (Figure 1). The distribution of whole-body

158	BMD z-score value for each participant separated by group is displayed in Figure 2.
159	The average whole-body BMD z-score values for each group were: wrestlers, 3.6±1.0;
160	judoka, 2.2±1.1; endurance-athletes, 0.5±1.3; and non-athletes, 0.3±1.5. Low
161	whole-body BMD (z-score $<$ -1.0) was observed in endurance- and non-athletes. High
162	whole-body BMD (z-score > 1.0) was found in wrestlers and judoka.
163	The segmental BMD values, which are separated by trunk, arms, and legs, are
164	shown in Table 2. The segmental BMD of wrestlers were higher than those of
165	endurance- and non-athlete groups in the trunk, arms, and legs (P<0.01). These
166	remained higher when adjusted for body mass (P<0.01). Moreover, BMD values for
167	judoka were higher than those in the endurance- and non-athlete groups in the trunk and
168	arms, but the difference in trunk BMD disappeared when compared to that of
169	non-athletes after adjusting for body weight.

171 **DISCUSSION**

Results from the present study show that the BMD of wrestlers and judoka are higher than that of endurance- and non-athletes. Wrestlers had the highest BMD when adjusted for body weight. This aligns with previous findings of weight-classified athletes participating in wrestling and judo who had very high z-scores compared to that of the general population of Asian youth [16]. This is the first study to describe a higher BMD for weight-classified athletes who regularly participate in whole-body high-impact activity and rapid weight-cycling several times a year.

179It is known that athletes who receive whole-body mechanical stimuli have higher BMD. According to the literature, the BMD of the average Japanese male is 1.10 180g/cm² [17]. Previous studies have reported the BMD reference for swimmers (1.167 181 g/cm²) and triathletes (1.153 g/cm²), who participate in sports with less bone 182mechanical stimuli than combat sports, which are lower compared to athletes 183participating in sports with more physical contact such as basketball (1.299 g/cm^2) and 184 handball (1.364 g/cm²) [18]. Another epidemiological study reported that BMD is 185higher in runners compared to cyclists [19] and this result may account for significantly 186 187 higher femoral BMD in recreational male runners [20]. Participants in endurance sports in this study comprised triathletes (1.188±0.083 g/cm²), two cyclists (1.116 and 1.069 188

 g/cm^2), and one orienteer (1.207 g/cm^2) in this study. Indeed, the type of sport may 189 involve different mechanical stimuli and BMD as indicated by the prior studies 190 described above. We speculated that the BMD was greater in wrestling and judo than in 191 these endurance sports, although our study could not directly compare different 192endurance sports. The BMD for wrestlers in the present study was similar to the 193 National College Athletic Association average for wrestlers (1.459 g/cm²) [21], and was 194 higher than for previously described sports. This is in line with a recent study showing 195196 that athletes participating in high-impact sports have significantly higher BMD than 197those in low-impact sports and a control group [22]. In a previous study describing the physiological results of bone impact, a relationship was shown between bone loading 198activity and the release of bone alkaline phosphatase and osteocalcin secreted from 199 osteoblasts [23]. Since osteocalcin is secreted only from osteoblasts and is involved in 200bone mineralization and calcium ion concentration homeostasis, the repetitive impact of 201202wrestling and judo may be a factor in osteocalcin secretion from the bone. Different sports are associated with different BMD depending on the part of the 203204body most impacted. For example, in kendo and tennis, the BMD of the dominant hand 205tended to be higher [24,25]. In the present study, wrestlers had higher BMD in their trunks, arms, and legs when compared with endurance- and non-athletes, and the same 206

207	results were obtained after adjustment for body mass (Table 2). Judoka had higher
208	BMD in their trunks and arms when compared to endurance- and non-athletes, but there
209	was no difference in leg BMD between the groups. This may be a feature of combat
210	sports, where there is repetitive whole-body impact. However, judo athletes practice
211	"ukemi," which is a technique meant to reduce impact [26]. Additionally, there is no
212	tackling on the legs as in wrestling. Thus, it is possible that the BMD is the same in
213	judoka as in endurance- and in non-athletes due to the influence of this technique.
214	Another reason for the differences observed in BMD may due to a bidirectional
215	crosstalk from muscle to bone and from bone to muscle, which involves a complex
216	correlation between mechanical and metabolic factors [27]. Indeed, an increase in
217	muscle mass acts at the interface of the two organs and stimulates local bone growth by
218	stretching collagen fibers and periosteum [28]. In this study, wrestlers had significantly
219	larger whole and partial fat-free mass than the other groups. Thus, the BMD of wrestlers
220	with lager skeletal muscle mass may be higher than endurance-athletes who often use
221	the lower body, even if the BMD is adjusted by weight.
222	According to a previous study, BMD does not increase unless the bones endure enough
223	impact due to exercise [29]. When bone is not impacted, sclerostin is excreted, which
224	prevents osteoblasts from forming new bone. For this reason, it is important to obtain

data regarding the appropriate amount and type of physical activity. Wrestlers have a large total energy expenditure (averaging 17.9 ± 2.5 MJ) and physical activity level (2.6 ± 0.3 , where >2.40 is extremely active), as shown in a large physical activity study

[13]. Thus, it is also highly possible that the physical activity of wrestlers influences

their BMD.

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While it is theoretically possible to explain differences in BMD between judoka and wrestlers according to total energy expenditure and physical activity level, unfortunately, these parameters for judoka are unknown. Hinton et al. described that BMD can be improved by high-impact and resistance training [30]. Therefore, bones may have a mechanism that enables them to make stronger new bones through activity, especially activity that causes bone impact.

In the present study, there was no collection of dietary data and causal relationships could not be clarified. However, wrestling and judo athletes who perform rapid weight loss through energy restriction for competition several times in a year did show a higher BMD. Since bone formation is increased with weight-bearing physical activity even during weight loss [31], wrestling and judo athletes may benefit from high-impact body contact during training even when they perform weight-cycling. Moreover, since ultraviolet rays from sunlight endogenously produce vitamin D [32], it

has been pointed out that there may be a negative effect on the bones of athletes who 243participate in indoor sports and do not receive sufficient sunlight favoring bone health. 244However, results from this study indicate that athletes participating in indoor sports, 245such as a wrestling and judo, still had higher BMD than the triathlon and cycling 246athletes who participate in their sport outdoors. Therefore, we speculate that the effects 247of hormones that increase bone strength are stronger in terms of promoting BMD than 248249the negative effects of energy restriction, rapid weight loss, and indoor sports. There are several limitations of this study to consider. First, the present study 250251used cross-sectional data rather than a controlled trial design, limiting the analyses of the results. Because the participants were college athletes, it was difficult to obtain 252blood samples and collect food records, which would have been prohibitive to their 253participation in the study. Food intake during college life is an important factor for 254obtaining peak bone mass [33]. Most important is intake of various nutrients such as 255protein, minerals such as calcium, phosphorus, and vitamins D, A, K, and C [34]. 256Therefore, it will be necessary to conduct further evaluation of energy availability, food 257intake, and bone mass during this period, especially in athletes [35]. In addition, all the 258259participants were Japanese, and it has been shown that race effects BMD [16]. For future studies, it will be beneficial to compare different races with a larger sample size. 260

261 **Conclusions**

The results of the present study showed that weight-classified athletes such as 262those who participate in wrestling and judo had higher BMD when compared to 263endurance- and non-athletes despite undergoing rapid weight loss before competition. In 264addition, we found that the BMD of wrestlers was higher than that of judoka and 265endurance- and non-athletes when adjusted for body mass. This striking difference in 266267BMD between the weight-classified athletes, endurance-athletes, and non-athletes could be attributed to differences in bone-loading activity irrespective of change in weight 268status during a competitive season. This information could be used in training protocols 269to increase BMD for other male young adults in further studies. 270

272 Authors' contribution

- 273 HS conceived the study and the study design; HS, EK, YT and OT conducted
- 274 experiments; HS and EK performed data analysis; HS, EK, YT, OT, YY, and HT
- interpreted experimental results; HS prepared illustrations; HS drafted the manuscript;
- HS, EK, YT, OT, YY, and HT edited and revised the manuscript. All authors read and
- approved the final version of the manuscript.

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283 **Conflict of interest**

284 The authors state that there are no personal conflicts of interest.

285 Compliance with Ethical Standards

286 This study was performed in compliance with current Japanese law.

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406 Figure legends

- 407 Figure 1: Whole-body bone mineral density adjusted for body mass between
- 408 **groups**
- 409 * significant difference between groups (P<0.05).
- 410 Figure 2: Distribution of whole-body bone mineral density z-score values for each
- 411 participant separated by group
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	Wrestlers	Judoka	Endurance athletes	Non-athletes	F-values	P-values
Age, years	20±1	19±1	21±2	21±4	2.194	0.11
95%CI	(20-21)	(18-19)	(19-22)	(18-24)		
Height, cm	169±4	170±8	171±5	173±4	0.826	0.49
95%CI	(166-171)	(163-176)	(167-174)	(169-176)		
Body mass, kg	73.0±7.8 ^b	79.1±10.6 ^{b,c}	62.5±5.8	68.9±6.5	8.88	<0.01
95%CI	(67.7-78.3)	(71.0-87.2)	(59.0-66.0)	(63.4-74.3)		
BMI, kg/m ²	25.6±1.7 ^{b,c}	27.4±2.7 ^{b,c}	21.4±1.6	23.0±1.9	19.191	<0.01
95%CI	(24.4-26.7)	(25.4-29.5)	(20.5-22.4)	(21.4-24.7)		
Percent of fat, %	11.2±1.9 ª	15.6±2.4 ^b	11.8±2.2	14.6±4.7	5.667	<0.01
95%CI	(9.9-12.5)	(13.8-17.4)	(10.4-13.1)	(10.6-18.5)		
Fat mass, kg	8.2±1.8 ^a	12.4±3.1 ^b	7.4±1.7	10.3±4.4	6.822	<0.01
95%CI	(7.0-9.4)	(10.0-14.8)	(6.3-8.4)	(6.6-13.9)		
Fat free mass, kg	64.8±6.7 ^b	66.7±8.1 b,c	55.1±4.8	58.6±3.6	8.666	<0.01
95%CI	(60.3-69.3)	(60.5-72.9)	(52.2-58)	(55.6-61.7)		

Table 1. Characteristics of each group

Values are mean \pm standard deviation. CI, confidence interval. Body composition was calculated using dual-energy x-ray

absorptiometry

^a significantly different in comparison to Judoka (P<0.05).

^b significantly different in comparison to Endurance athletes (P<0.05).

	Wrestlers	Judoka	Endurance athletes	Non-athletes	F-values	P-values
Whole-body	1.366±0.06 ^{a,b}	1.282±0.066 ^{a,b}	1.175 ± 0.081	1.161±0.092	17.19	<0.01
95%CI	1.325-1.406	1.231-1.333	1.125-1.224	1.084-1.238		
Trunk	1.185±0.046 a,b	1.162±0.065 ^{a,b}	0.960 ± 0.078	0.988±0.136	20.71	<0.01
95%CI	(1.154-1.216)	(1.112-1.212)	(0.912-1.007)	(0.875-1.102)		
Trunk with body weight	1.182±0.042 ^{a,b}	1.153±0.067 ^{a,b}	$0.968 {\pm} 0.074$	0.990±0.139	38.51	<0.01
95%CI	(1.154-1.210)	(1.101-1.204)	(0.923-1.013)	(0.874-1.105)		
Arms	0.982±0.042 ^{a,b}	0.955±0.037 ^{a,b}	0.802 ± 0.053	0.849 ± 0.047	6.75	<0.01
95%CI	(0.954-1.010)	(0.927-0.984)	(0.770-0.834)	(0.809-0.888)		
Arms with body weight	0.976±0.033 ^{a,b}	0.938±0.046 ^{a,b}	0.817 ± 0.049	0.851±0.046	18.84	<0.01
95%CI	(0.954-0.998)	(0.902-0.973)	(0.788-0.847)	(0.813-0.890)		
Legs	1.422±0.090 ^{a,b}	1.346±0.086	1.262 ± 0.097	1.279±0.100	31.22	<0.01
95%CI	(1.362-1.482)	(1.280-1.412)	(1.204-1.320)	(1.196-1.363)		
Legs with body weight	1.414±0.073 ^{a,b}	1.319±0.095	1.285±0.091	1.283±0.102	5.04	<0.01
95%CI	(1.365-1.463)	(1.247-1.392)	(1.230-1.340)	(1.197-1.369)		

 Table 2. Bone mineral density

Values are mean \pm standard deviation. CI, confidence interval.

^a significantly different in comparison to Endurance athletes (P<0.05).

^b significantly different in comparison to Non-athletes (P<0.05).