Weight-bearing exercise and markers of bone turnover in female athletes

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Creighton, Dana L., Amy L. Morgan, Debra Boardley, and P. Gunnar Brolinson. Weight-bearing exercise and markers of bone turnover in female athletes. J Appl Physiol 90: 565–570, 2001.—Weight-bearing activity provides an osteogenic stimulus, while effects of swimming on bone are unclear. We evaluated bone mineral density (BMD) and markers of bone turnover in female athletes (n = 41, age 20.7 yr) comparing three impact groups, high impact (High, basketball and volleyball, n = 14), medium impact (Med, soccer and track, n = 13), and nonimpact (Non, swimming, n = 7), with sedentary age-matched controls (Con, n = 7). BMD was assessed by dual-energy X-ray absorptiometry at the lumbar spine, femoral neck (FN), Ward’s triangle, and trochanter (TR); bone resorption estimated from urinary cross-linked N-telopeptides (NTx); and bone formation determined from serum osteocalcin. Adjusted BMD (g/cm; covariates: body mass index, weight, and calcium and calorie intake) was greater at the FN and TR in the High group (1.27 ± 0.03 and 1.05 ± 0.03) than in the Non (1.05 ± 0.04 and 0.86 ± 0.04) and Con (1.03 ± 0.05 and 0.85 ± 0.05) groups and greater at the TR in the Med group (1.01 ± 0.03) than in the Non (0.86 ± 0.04) and Con (0.85 ± 0.05) groups. Total body BMD was higher in the High group (4.9 ± 0.12) than in the Med (4.5 ± 0.12), Non (4.2 ± 0.14), and Con (4.1 ± 0.17) groups and greater in the Med group than in the Non and Con groups. Bone formation was lower in the Non group (19.8 ± 2.6) than in the High (30.6 ± 3.0) and Med (32.9 ± 1.9, P ≤ 0.05) groups. No differences in a marker of bone resorption (NTx) were noted. This indicates that women who participate in impact sports such as volleyball and basketball had higher BMDs and bone formation values than female swimmers.

osteooporosis; physical activity; mechanical loading

OSTEOPOROSIS IS A DISEASE that is characterized by low bone mass and microarchitectural deterioration of bone tissue. This can lead to enhanced bone fragility and a consequent increase in fracture risk, particularly at the vertebrae and proximal femur (5, 8). Osteoporosis affects millions of Americans, and the risk increases progressively with age. The mechanisms that lead to osteoporosis are not fully understood, but two major contributing factors are the level of peak bone mass developed during childhood and adolescence and the rate at which bone is lost during the aging process. In recent years, pharmaceutical interventions have been developed to treat osteoporosis after manifestation of the disease. Clearly, reducing the prevalence through early intervention may be a more economical and health conscious approach. One avenue to prevent osteoporosis in the elderly may involve elucidating the factors that contribute to increasing the amount of peak bone mass attained in the young.

Bone mineral density (BMD) is a result of the dynamic process of bone formation and bone resorption, also called bone remodeling. Bone resorption causes a breakdown of bone tissue, and bone formation rebuilds and strengthens lost bone. Bone mass accumulates throughout childhood and into young adulthood. During this time, increases in bone resorption and/or decreases in bone formation can lead to less-than-optimal peak bone mass. Attaining higher peak values may lend protection as age-related bone loss occurs (24). Therefore, identifying contributors and disruptors to the process of skeletal development may aid in the prevention and treatment of osteoporosis.

It is commonly accepted that weight-bearing activity provides an osteogenic stimulus to the bones. For example, athletes involved in sports that increase the mechanical stress placed on the bones (i.e., weight-bearing activities) have an increased BMD compared with the general population (2, 3, 6, 9, 12, 25). However, swimmers, who train in a non-weight-bearing environment, have been shown to gain less skeletal benefits than do athletes who participate in weight-bearing activities (7, 9, 12, 15, 22).

In 1994 it was noted that more recent examining bone density was needed that focused on athletes in a variety of sports as well as the effect of mechanical loading forces on different skeletal sites (20). Furthermore, in 1997, The American College of Sports Medicine identified in a position stand on the female athlete triad (i.e., disordered eating, amenorrhea, and osteoporosis) the need for more research examining the causes, prevalence, treatment, and consequences of these disorders (21). While several studies have been

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performed in women to examine the effects of different activities on BMD using dual-energy X-ray absorptiometry (DEXA), most of these studies have not quantified the athletes' training in terms of impact as determined by the ground reaction forces associated with each sport, nor have they used biomarkers to study bone remodeling.

By the time they reach college age, women who are apparently healthy and eumenorrheic have accumulated a large percentage of their adult mass, especially at the proximal femur and vertebrae (17, 18). There are several measures of BMD that may be evaluated. For example, DEXA provides a static, rather than a dynamic, picture of bone. The examination of biomarkers in serum and urine can be utilized to provide an understanding of the dynamic course of bone remodeling. This may also allow us to determine the osteogenic stimulus of various conditions of skeletal loading, i.e., loading associated with different sports. The purpose of this study was to determine how regular training in three different types of competitive sports affects BMD, bone formation, and bone resorption in young women.

METHODS

Subjects. Fifty healthy women aged 18–26 yr were recruited from the University of Toledo. The athletic subjects all competed as National Collegiate Athletic Association (NCAA) Division I athletes and were recruited from their respective sport; the sedentary subjects were recruited from the general student population. Of the 50 volunteers, 41 completed the study. Of the nine subjects who did not complete the study, six were excluded as described in the exclusion criteria below and three did not complete the necessary blood tests or DEXA scans. Written informed consent was obtained before testing. This investigation was approved by the institutional review boards of the University of Toledo and The Toledo Hospital in accordance with the policy statements concerning research with human subjects.

Competitive athletes were divided into three groups according to the degree of impact as determined by ground reaction forces associated with their sport. Basketball (n = 8) and volleyball (n = 6) comprised the high-impact (High) group (n = 14). The ground reaction forces of netball, a game very similar to basketball, have been reported at 3.9–4.6 times body weight (26), while the ground reaction forces of volleyball have been reported at 3–6 times body weight (27). Soccer (n = 9) and middle- to short-distance track (n = 4; training 10–30 miles/wk) comprised the medium-impact (Med) group (n = 13). The ground reaction forces associated with track approximate two to three times body weight (4). Soccer was combined with track because it involves running on a softer surface and less vertical jumping than the high-impact sports. Swimmers comprised the nonimpact (Non) group (n = 7), inasmuch as training in the water simulates a non-weight-bearing environment. Individuals exercising <1 h/wk for ≥1 yr comprised the control (Con) group (n = 7).

Activity assessment. All the athletic subjects were NCAA Division I athletes who trained year-round for their respective sport. The athletes were assigned to an impact group on the basis of the nature of the conditioning regimen and the competitive nature of each sport. Each in-season training period for High, Med, and Non groups was 3–6 mo, and all programs involved resistance training for ~2 h/wk. The High group trained for their sports utilizing primarily running, jumping, and cutting on hard surfaces (10–13 h/wk). The Med group trained for their sports mainly by running and jumping, usually on grass (soccer, 6 h/wk) or a track (14 h/wk). The predominant method of training for the Non group was swimming. All training programs were regulated by the NCAA, which allows 20 h/wk of in-season and 8 h/wk of pre- and postseason organized practice. All subjects trained ≥8 mo/yr, including pre-, post-, and in-season conditioning, and all athletes reported conditioning in a similar manner to their in-season sport on their own during the off-season. Furthermore, all athletes had participated in their sport for ≥4 yr and participated in only one sport at the collegiate level. Subjects in the Con group participated in <1 h of physical activity per week for ≥1 yr.

Descriptive information. To obtain descriptive data, all subjects completed questionnaires pertaining to their daily intakes of calcium and total calories, eating behaviors, health history, and training history. Exclusion criteria included current smokers (1 subject), excessive alcohol users (>15 drinks/wk), users of corticosteroids, and individuals with a body mass index (BMI) >30 kg/m², history of diabetes mellitus, thyroid dysfunction, or renal disease. Subjects reporting a diagnosed eating disorder were excluded. The subjects were required to complete an eating attitudes test (EAT-26) (13). The eating attitudes test has been shown to be an effective screening instrument to identify individuals with eating disorders when used as part of a two-stage screening process in which those who score >20 are interviewed to diagnose an eating disorder. For the purposes of this investigation, any individual who scored >25 was excluded (1 subject).

Menstrual function. Subjects reported age of menarche and number of menstrual cycles in the previous 12 mo. All subjects were eumenorrheic and reported ≥10 menstrual cycles in the previous 12 mo (10). Individuals reporting use of oral contraceptives (OCs) to induce menses (2 subjects) or <10 cycles in the past year (1 subject) were excluded. Of the remaining subjects, six from the High, five from the Med, one from the Non, and three from the Con group reported using OCs. To identify whether OC users differed from nonusers, descriptive statistics and comparisons of mean bone mineral data were analyzed using a two-tailed t-test (P < 0.05). No statistical difference between the means of the OC users and nonusers was found. Therefore, the two groups were combined for all subsequent analysis.

Protocol. Within 2 wk of completing their respective in-season training, all athletes underwent a radiological evaluation of bone density (DEXA scan) and blood and urine collection for assessment of metabolic markers of bone formation and resorption. All control subjects also completed the same testing within a 2-wk period. Between 8 and 12 AM, urine samples (4 ml) were obtained at the second morning void after a 12-h fast and frozen at −20°C within 30 min of collection. At the time of the urine collection, blood samples (10 ml) were drawn from an antecubital vein, stored on ice, and centrifuged at 10°C at 2,500 rpm for 15 min. Serum pipetted from the sample was stored at −70°C within 1 h of collection.

Anthropometric and dietary variables. Each subject completed a food frequency questionnaire (Health Habits and History Questionnaire, Scan 92), which was analyzed by DietSystem Computer software (National Cancer Institute, Bethesda, MD) and calculated average daily dietary intake. Height and weight were self-reported on this questionnaire. Skinfold thickness was measured at three sites (triceps, suprailliac, and midthigh) with Lange skinfold calipers. From the skinfold measures, body density was estimated according
to Jackson et al. (16); percent fat was estimated according to Siri (23). Subject characteristics are shown in Table 1.

**Evaluation of bone density.** A dual-energy X-ray bone densitometer (model DPX-A, Lunar, Madison, WI) was used to measure BMD at the lumbar spine, femoral neck, Ward’s triangle, and trochanter. Subjects were placed in a supine position with knees bent at a 90° angle for the lumbar spine measurement. Subjects were placed in a supine position with the left hip abducted and rotated inward at a 45° angle for evaluation of hip measurement. BMD was analyzed using Lunar software (version 1.13). The coefficients of variation for these measures were 0.5 and 1.2% for the lumbar spine and femoral neck, respectively. Trained radiology technicians performed subject placement and DEXA analysis. Board-certified radiologists read and interpreted the DEXA scan results.

**Evaluation of bone metabolic markers.** Bone resorption markers were assessed by ELISA for the measurement of cross-linked N-telopeptides of type I collagen (NTx) in human urine (Osteomark, Ostex International). Bone formation markers were assessed by immunoradiometric assay for the quantitative determination of osteocalcin levels in human serum (Nichols Institute Diagnostics). The interassay variation was 6.3% for the NTx and 11.1% for the osteocalcin. Results from two fewer subjects are reported for bone resorption values from the NTx and 11.1% for the osteocalcin. When the ANOVA indicated a significant difference, Tukey’s comparison test was used for a post hoc analysis.

**RESULTS**

Descriptive statistics (Table 1) indicate that the High group was significantly taller and heavier than all other groups. When the variables of height and weight were expressed as BMI, no significant differences were found between any of the groups. The Con group was significantly older than all the other groups.

BMD measurements were reported for lumbar spine, femoral neck, Ward’s triangle, and trochanter (Table 2). In addition, the sum of all spine and hip measurements was calculated and reported as TBMD. The High group had significantly greater BMD at the femoral neck, Ward’s triangle, and trochanter and TBMD than the Non and Con groups as well as a significantly higher lumbar spine BMD than the Con group. The Med group had significantly greater BMD at the trochanter than the Non and Con groups.

Activity is only one of a multitude of factors that may affect BMD. To determine the effects of possible confounding factors, a stepwise linear regression procedure was used. For each of the BMD sites (dependent variable), the independent variables age, height, weight, BMI, percent body fat, age of menarche, average daily calorie intake, and average daily calorie intake were entered into the equation. Different covariates appeared to influence BMD at different sites. Therefore, for the ANCOVA, all covariates found to affect BMD at any site were included in the subsequent analysis. It was established that there were no interaction effects between the covariates and the impact groups before the ANCOVA was run. Table 3 gives BMD measurements of each impact group, adjusted for weight, BMI, total calories, and calcium intake. A post hoc comparison test indicated several significant differences between groups in adjusted BMD. Specifically, the High group had significantly greater adjusted BMD at the femoral neck and trochanter than the Med and Con groups. The Med group also had greater adjusted TBMD than all the other groups (Med, Non, and Con). Finally, the Med group had a significantly greater adjusted BMD at the trochanter and TBMD than the Non and Con groups.

**Table 1. Subject characteristics by impact group**

<table>
<thead>
<tr>
<th>Impact Group</th>
<th>High (n = 14)</th>
<th>Med (n = 13)</th>
<th>Non (n = 7)</th>
<th>Con (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>19.9 ± 0.3</td>
<td>20.6 ± 0.3</td>
<td>19.4 ± 0.3</td>
<td>22.9 ± 0.6†</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178.0 ± 1.7</td>
<td>167.1 ± 1.9</td>
<td>172.4 ± 2.6</td>
<td>164.0 ± 2.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70.3 ± 2.0§</td>
<td>64.0 ± 1.5</td>
<td>66.2 ± 2.0</td>
<td>55.9 ± 2.0</td>
</tr>
<tr>
<td>BMI</td>
<td>22.2 ± 0.5</td>
<td>23.1 ± 0.6</td>
<td>22.3 ± 0.7</td>
<td>20.7 ± 0.5</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>18.1 ± 0.7</td>
<td>18.4 ± 1.1</td>
<td>19.0 ± 0.9</td>
<td>19.1 ± 1.9</td>
</tr>
<tr>
<td>Age of menarche, yr</td>
<td>13.5 ± 0.4</td>
<td>13.1 ± 0.3</td>
<td>12.4 ± 0.5</td>
<td>12.9 ± 0.4</td>
</tr>
<tr>
<td>Calcium intake, mg*</td>
<td>1,061 ± 136</td>
<td>803.5 ± 89</td>
<td>911 ± 197</td>
<td>950 ± 114</td>
</tr>
<tr>
<td>Calorie intake, kcal*</td>
<td>1,784 ± 228</td>
<td>1,629 ± 240</td>
<td>1,717 ± 240</td>
<td>1,750 ± 307</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of subjects. BMI, body mass index. *Average daily intake. †Greater than High, Med, and Non (P < 0.05); §greater than Med and Con (P < 0.05).

| Age of menarche, yr | 13.5 ± 0.4 | 13.1 ± 0.3 | 12.4 ± 0.5 | 12.9 ± 0.4 |
| Calcium intake, mg* | 1,061 ± 136 | 803.5 ± 89 | 911 ± 197 | 950 ± 114 |
| Calorie intake, kcal* | 1,784 ± 228 | 1,629 ± 240 | 1,717 ± 240 | 1,750 ± 307|

**Table 2. BMD by impact group**

<table>
<thead>
<tr>
<th>Impact Group</th>
<th>High (n = 14)</th>
<th>Med (n = 13)</th>
<th>Non (n = 7)</th>
<th>Con (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar spine</td>
<td>1.38 ± 0.01†</td>
<td>1.30 ± 0.01</td>
<td>1.26 ± 0.001</td>
<td>1.20 ± 0.003</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>1.26 ± 0.03†</td>
<td>1.14 ± 0.01</td>
<td>1.05 ± 0.004</td>
<td>1.04 ± 0.003</td>
</tr>
<tr>
<td>Ward’s triangle</td>
<td>1.23 ± 0.01†</td>
<td>1.10 ± 0.002</td>
<td>1.04 ± 0.001</td>
<td>1.01 ± 0.001</td>
</tr>
<tr>
<td>Trochanter</td>
<td>1.04 ± 0.04†</td>
<td>1.02 ± 0.04†</td>
<td>0.86 ± 0.04</td>
<td>0.86 ± 0.02</td>
</tr>
<tr>
<td>TBMD</td>
<td>4.9 ± 0.14†</td>
<td>4.5 ± 0.12</td>
<td>4.2 ± 0.11</td>
<td>4.1 ± 0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in g/cm; n, number of subjects. BMD, bone mineral density; TBMD, total BMD (sum of spine and hip measurements). *Greater than Con (P < 0.05); †greater than Non and Con (P < 0.05).
and Con (P, possible that osteocalcin and NTx are affected by diure-
in the body and, thus, are not site specific. It is also
bone formation and resorption is that these indexes
unique addition to the use of BMD in examining female
female athletes.
level of training by using ground reaction forces, sim-
subjects by level of impact was utilized to quantify the
cess of bone remodeling. In the present study, grouping
status of bone to aid in the understanding of the pro-
ence of different levels of impact on the metabolic
High and Med groups.

DISCUSSION
The main finding of the present study was that, of
the athletes tested, those involved in the highest-im-
 pact sports displayed the greatest markers of bone for-
amation as well as the highest BMDs at weight-
 bearing sites. This study reported site-specific BMDs
(adjusted and nonadjusted) at weight-bearing sites of
young competitive female athletes engaged in sports
producing different amounts of skeletal loading. In
addition, rate of bone formation and resorption and the
uncoupling index were used to determine the metabolic
status of bone. We found that the BMD of individuals
involved in nonimpact sports, i.e., swimming, was sig-
ificantly less at the hip (trochanter and femoral neck)
than in women who participated in high-impact sports
but not different from sedentary individuals. Further-
more, examination of metabolic markers of bone for-
amation and resorption demonstrated that swimmers
had reduced bone formation values compared with the
High and Med groups.
Numerous other researchers have found greater
bone densities in individuals training in sports involv-
ing high strain rates and high-impact activity than in
nonactive controls (1–3, 7, 12, 14, 19, 22, 28). There-
fore, the focus of this study was to evaluate the influ-
ence of different levels of impact on the metabolic
status of bone to aid in the understanding of the pro-
cess of bone remodeling. In the present study, grouping
subjects by level of impact was utilized to quantify the
level of training by using ground reaction forces, sim-
ilar to the method Dook et al. (7) utilized with mature
female athletes.
The utilization of markers of bone turnover is a
unique addition to the use of BMD in examining female
athletes. One of the limitations of using markers of
bone formation and resorption is that these indexes
represent an average of turnover from all skeletal sites
in the body and, thus, are not site specific. It is also
possible that osteocalcin and NTx are affected by diur-

Table 3. BMD adjusted for weight, BMI, and average
daily calorie and calcium intakes by impact group

<table>
<thead>
<tr>
<th>Impact Group</th>
<th>High (n = 14)</th>
<th>Med (n = 11)</th>
<th>Non (n = 7)</th>
<th>Con (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar spine</td>
<td>1.35 ± 0.04</td>
<td>1.31 ± 0.03</td>
<td>1.26 ± 0.04</td>
<td>1.23 ± 0.05</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>1.27 ± 0.03*</td>
<td>1.12 ± 0.03</td>
<td>1.05 ± 0.04</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>Ward's triangle</td>
<td>1.22 ± 0.05</td>
<td>1.10 ± 0.04</td>
<td>1.05 ± 0.05</td>
<td>1.02 ± 0.06</td>
</tr>
<tr>
<td>Trochanter</td>
<td>1.05 ± 0.03*</td>
<td>1.01 ± 0.03*</td>
<td>0.86 ± 0.04</td>
<td>0.85 ± 0.05</td>
</tr>
<tr>
<td>TBMD</td>
<td>4.9 ± 0.12†</td>
<td>4.5 ± 0.12*</td>
<td>4.2 ± 0.14</td>
<td>4.1 ± 0.17</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in g/cm; n, number of subjects. *Greater than Non and Con (P < 0.05); †greater than Med, Non, and Con (P < 0.05).

ANOVAs were also run on the metabolic bone mark-
ers (Table 4). Tukey’s post hoc test determined that
mean bone formation values were significantly less in
the Non group than in the High and Med groups. The
uncoupling index was significantly greater in the Non
group than in the Med and Con groups.

Table 4. Bone formation, bone resorption,
and uncoupling index

<table>
<thead>
<tr>
<th>Impact Group</th>
<th>High (n = 13)</th>
<th>Med (n = 12)</th>
<th>Non (n = 7)</th>
<th>Con (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone formation,* ng/ml</td>
<td>30.6 ± 3.0</td>
<td>32.9 ± 1.9</td>
<td>19.8 ± 2.6†</td>
<td>30.0 ± 2.6</td>
</tr>
<tr>
<td>Bone resorption, nm BCE/mM</td>
<td>72.9 ± 11.4</td>
<td>62.5 ± 7.6</td>
<td>80.0 ± 9.2</td>
<td>60.0 ± 10.3</td>
</tr>
<tr>
<td>CR</td>
<td>72.9 ± 11.4</td>
<td>62.5 ± 7.6</td>
<td>80.0 ± 9.2</td>
<td>60.0 ± 10.3</td>
</tr>
<tr>
<td>Uncoupling index</td>
<td>0.08 ± 0.45</td>
<td>-0.53 ± 0.30</td>
<td>1.37 ± 0.26‡</td>
<td>-0.34 ± 0.24</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of subjects. BCE, bone collagen equivalents; CR, creatinine. *n = 14 and 13 for High and Med, respectively. †Less than High and Med (P < 0.05); ‡greater than Med and Con (P < 0.05).
increased BMD, elevations in bone formation would be necessary to overcome the increased bone resorption; however, this was not found in the investigation of Matsumoto et al.

Part of the reason for the discrepancy between the findings in these studies may be the use of different methods for determination of metabolic markers. Measuring NTx or cross-links of NTx for bone resorption is specific to type I bone only. The markers of bone resorption utilized in the study of Matsumoto et al. (19) measured not only bone resorption but also turnover from cartilage and other connective tissue. Because there is considerable strain imposed on connective tissue in athletes in sports such as judo, turnover from cartilage and connective tissue may have led to significant increases in bone resorption in their subjects. However, the discrepancy between these studies, which utilized similar protocols and studied similar populations, warrants further analysis.

Another possible confound to our findings is self-selection. In other words, it is possible that individuals with high BMD innately choose to participate in high-impact sports, while individuals with less density put themselves at less risk by participating in a non-weight-bearing activity. While this is a limitation in any study that tests competitive athletes, it is likely a minor contributor to these findings. Swimmers train in a non-weight-bearing environment for the majority of their exercise; thus it is likely that their training history, and not self-selection, contributes to their lower BMD and higher uncoupling index.

These findings indicate that repetitive stress applied to weight-bearing sites over extended periods of time acts as a strengthening agent for bones. However, the threshold of activity and stress required to stimulate bone to the degree shown in this investigation may not be feasible to increase BMD in the noncompetitive athlete. Furthermore, these findings demonstrate that weight-bearing exercise may be more beneficial than swimming to promote bone health.

In conclusion, the results of this study indicate that athletes involved in sports producing the greatest weight-bearing strain have higher BMD values than athletes involved in non-weight-bearing activities and sedentary individuals. In addition, the low levels of impact associated with non-weight-bearing activities appear to negatively influence bone formation and thus bone density. These findings support the theory that high strain rates are more effective at inducing new bone formation and enhancing BMD at weight-bearing sites. More research with similar populations is necessary to confirm and expand on these findings.

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