Effects of a Novel Zinc-Magnesium Formulation on Hormones and Strength
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L.R. BRILLA AND VICTOR CONTE. Effects of a Novel Zinc-Magnesium Formulation on Hormones and Strength. JEP\(_{\text{online}}\), 3(4): 26-36, 2000. Muscle attributes and selected blood hormones of football players were assessed in response to a nightly supplementation regimen during spring football, over an 8-week period, with pre-post measures. A double-blind randomized study was conducted with ZMA (30 mg zinc monomethionine aspartate, 450 mg magnesium aspartate, and 10.5 mg of vitamin B-6) and placebo (P), \(n=12\) and \(n=15\), respectively. Plasma zinc and magnesium levels were ZMA (0.80 to 1.04 \(\mu\)g/ml; 19.43 to 20.63 mcg/ml) and P (0.84 to 0.80 \(\mu\)g/ml; 19.68 to 18.04 \(\mu\)g/ml), respectively (\(P<0.001\)). Free testosterone increased with ZMA (132.1 to 176.3 pg/mL), compared to P (141.0 to 126.6 pg/mL) (\(P<0.001\)); IGF-I increased in the ZMA group (424.2 to 439.3 ng/mL) and decreased in P (437.3 to 343.3 ng/mL) (\(P<0.001\)). Muscle strength via torque measurements and functional power were assessed with a Biodex dynamometer. Differences were noted between the groups (\(P<0.001\)): ZMA (189.9 to 211 Nm at 180º/s and 316.5 to 373.7 Nm at 300º/s) and P (204.2 to 209.1 Nm at 180º/s and 369.5 to 404.3 Nm at 300º/s). The results demonstrate the efficacy of a Zn-Mg preparation (ZMA) on muscle attributes and selected hormones in strength-trained, competitive athletes.

Key Words: vitamin B\(_6\), anabolic hormones, testosterone, IGF-I, muscle

INTRODUCTION
Zinc (Zn) and magnesium (Mg) may enhance levels of Insulin-like Growth Factor-I (IGF-I)(1); and zinc, in particular, may contribute to elevating serum testosterone (2). Both IGF-I and testosterone are anabolic factors that enhance muscle function and physical performance. Testosterone's role in physical performance enhancement has been studied for a number of years. The IGF-I response to intense muscular activity has not been well defined, relatively. Training may lead to a short-term catabolic state hormonally expressed by reductions in IGF-I. Baseline serum concentrations of testosterone, GH, and IGF-I were unaffected by 16-wk resistive training program which elicited an approximate 40% increase in muscular strength in men, 60±4 yr. It was intimated that training-induced increases in IGF-I could occur in muscle without altering serum IGF-I concentration (3).

A condition named somatopause due to decreased IGF-I and GH has been identified with aging. To countermeasure somatopause, 33 moderately obese women (67.1±5.2 yr), self-injected IGF-I. Weight loss with muscle strength increases were greater in IGF-I group due to training (12-wk: walk 3 days,
strength trained 2 days) (4). IGF-I may mediate the action of GH on skeletal muscle as a paracrine agent. In male rats, larger mean muscle weight and fiber cross-sectional area occurred when functional overload was combined with GH/IGF-I administration, and myonuclear number increased concomitantly with fiber volume. Increases in myonuclear numbers in rats may be a prerequisite for prolonged and substantial skeletal muscle fiber hypertrophy (5). IGF-I plus exercise resulted in an increase in the size of each predominant fiber type (I, IIa).

In contrast, the nutrients, Zn and Mg, may not be at optimal status in physically active individuals to facilitate function of these anabolic factors. Zn losses may be exacerbated through exercise (6), both long duration and high intensity, sweating (7), and inadequate intake (8). Additionally, exogenous testosterone administration results in significant reductions of Zn (9). Also, Mg has a putative effect on muscle strength in clinical applications and previously untrained individuals (10). Mg may be reduced due to intense and/or long-term exercise (10). These diminutions in Zn and Mg may lead to a situation of latent fatigue with decreased endurance (7,10,11). A special aspect of the zinc-magnesium supplement used in this study was the inclusion of vitamin B₆ to enhance the absorption of Zn and Mg (12,13), in addition to the known properties of vitamin B₆ in protein metabolism.

Both of these minerals have been reported by the USDA to be low in typical diets: 68% of diets have less than two-thirds of the RDA for Zn and 39% contain less than two-thirds of the RDA for Mg. Some dietary surveys of athletes have demonstrated that these nutrients may meet the RDAs (2,14). It may be necessary for athletes to supplement these nutrients in order to get dietary adequacy through meeting the RDA, or beyond, for physical performance effects. The purpose of this study was to assess the effect of a novel Zn, Mg, and vitamin B-6 formulation (ZMA) on anabolic hormones and muscle function in varsity football players during their spring football practice season.

METHODS
After approval of the project by the Western Washington University (WWU) Human Subjects Committee, the study commenced with the recruitment of subjects from the WWU football team, NCAA, Division II. Varsity football players were solicited for a randomized, double blind supplementation study. Fifty-seven players were involved in the initial testing which included anthropometric data, a 3-day diet analysis with Nutritionist IV software to determine dietary intake of nutrients of interest, a venipuncture blood draw, and muscle isokinetic torque and power assessments. All investigators were appropriately trained in the various aspects of the testing protocols. Anthropometric data collection was supervised by an individual trained in kinanthropometric troika methodology. A Certified Nutrition Specialist conducted the nutrition analysis. The blood draws were completed by trained phlebotomists. The isokinetic data was collected by trained and experienced testers, one with 15 years experience. Twenty-seven players completed the supplementation regimen and testing so their data were included in the analysis. Activity consisted of supervised spring football practice.

All tests were performed pre-post the spring practice season, for a total supplementation period of seven weeks. The first week was familiarization with the practice routine and the assessments were made at the first and eighth weeks. No intervening samples were taken because of the variability of such elements as zinc and magnesium for tissue saturation or steady state to be reached, approximately 3-5 weeks depending on baseline status. All subjects were tested between 0700 and 1030, with the isokinetic testing held between 1030 and 1330. Since the study was randomized, double-blinded, the tests were not controlled by group although it was attempted to test each subject at the same time of day, pre-post. Subjects reported to the lab, in the vicinity of the weight room, weekly to pick up their supplements. Subjects had been randomly assigned to one of two groups: control who took a placebo and treatment
who took the supplement, ZMA (SNAC System, Inc., Burlingame, CA), the equivalent of 30 mg zinc monomethionine aspartate, 450 mg magnesium aspartate, and 10.5 mg vitamin B-6. All subjects took three capsules nightly between dinner and bedtime. Failure to comply with the supplementation regimen resulted in subjects being dropped from the study. The players were asked to not take any other nutrient supplements during the course of the study. This request was monitored by-weekly questioning when they picked up their supplement/placebo. A 10-hour fasting blood sample was obtained early-morning via venipuncture before any physical activity was undertaken. Blood samples were prepared for analysis of plasma zinc and magnesium, and serum insulin-like growth factor-1 (IGF-I), total testosterone, free testosterone, and percent testosterone.

The specimen-preparation method used for plasma zinc and magnesium analysis was a 50/50 nitric/perchloric acid digestion. The instrumentation used in the analysis was an inductively coupled plasma atomic emission spectrometer (ICP/AES) (Applied Research Laboratories, Dearborn, MI; model 34000 simultaneous ICP). The detection limits of the ICP-AES for Zn and Mg are 0.009 and 0.014 parts per million (ppm), respectively. The ICP-AES inter-assay precision was determined from 20 assays on human plasma pools. The standard deviation and coefficient of variation (%CV) were 0.05 ppm and 5.9% for Zn and 1.0 ppm and 4.4% for Mg. Following organic extraction, a competitive radioimmunoassay (RIA) which uses the 1125 isotope as the competing antigen was the method used in the analysis of total and free testosterone. The instrumentation used was a dialysis beta counter. The precision for the quality control samples ranged from a high of 335 ng/dL with a standard deviation (SD) of 27 and %CV of 8.0% to low sample of 13.8 ng/dL with SD 1.26 and %CV of 9.2%. IGF-I analysis was done through a combination of equilibrium dialysis, extraction, chromatography, and radioimmunoassay (RIA) with use of a gamma counter. The sample reproducibility for this methodology ranges from high pool of 688±22.6 ng/mL and %CV of 3.3%, to a low pool ng/mL SD of 10.1 and %CV of 8.3%. These quality control values meet the acceptable criterion of coefficient of variation of less than 15.0%. Torque and power measurements were preformed with the lower extremity on a BIODEX® isokinetic dynamometer. The set-up was adjusted for each subject, and the same subject positions were recorded to use pre and post. Three trials were given at two separate settings: 180 °/s and 300 °/s. Torque and power data were recorded from the best trial.

Means and standard deviations were calculated. A MANOVA was used to assess the mineral and hormone data sets. ANCOVA was used to test for muscle attributes of torque and power. P was set at <0.05. For significant interactions, multiple pairwise comparisons with a Bonferroni adjustment were used.

RESULTS
Data sets were completed on 27 subjects with resultant group sample sizes of ZMA: 12, P: 15. The attrition may be accounted for through inability to comply with supplementation regimen, injuries, and aversion to testing such as phlebotomy. Any injuries were documented with the athletic training staff. Other factors were self-report. Body weights were 99.1 kg and 99.0 kg, pre-post in the ZMA group, and 95.9 kg and 95.6 kg, pre-post in placebo subjects. Diet records (3-day) showed that mean values of selected nutrients exceeded the RDA for Zn (17.0±7.4 mg), Mg (539±272 mg), and vitamin B-6 (3.6±1.6 mg). There was a significant treatment by group interaction effect (P<0.001) for plasma values of zinc, magnesium, and the serum anabolic hormone profile, except percent testosterone. Subject characteristics plus mineral and hormone data are presented in Table 1. Graphical display of the specific variables is provided in Figure 1. Statistical comparisons of the significant interactions of the mineral and hormone data are presented in Table 2. Overall, control values dropped and ZMA supplemented values increased for within groups comparisons, pre-post changes (P<0.0125, Bonferroni adjustment). For the between groups analysis, no pre test comparisons were significant. These findings demonstrate
that the groups were comparable at the commencement of the study for the plasma measures. However, the post analysis showed significant differences in all comparisons, except for a trend towards significance for IGF-I (P=0.0195) with the Bonferroni adjustment. These findings indicate that ZMA reverses the drops in these nutrients and anabolic hormones seen with an intensive 8-week training program such as spring football practice.

Table 1. Subject characteristics and measures for selected minerals and anabolic hormones (Mean±SD).

<table>
<thead>
<tr>
<th>Variables</th>
<th>ZMA</th>
<th>Placebo</th>
<th>Treatment x Group</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT (cm)</td>
<td>182.96±4.77</td>
<td>180.21±6.57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT (kg)</td>
<td>99.09±16.01</td>
<td>95.66±11.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZN (µg/mL)</td>
<td>0.80±0.10</td>
<td>0.80±0.07</td>
<td>0.08±0.09</td>
<td>33.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MG (µg/mL)</td>
<td>19.43±1.20</td>
<td>19.68±1.62</td>
<td>18.04±1.13</td>
<td>23.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TOTT (ng/mL)</td>
<td>567.92±131.96</td>
<td>588.80±180.35</td>
<td>526.80±128.86</td>
<td>24.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FRET (pg/mL)</td>
<td>132.10±36.16</td>
<td>141.02±37.91</td>
<td>126.53±29.44</td>
<td>26.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PCT (%)</td>
<td>2.32±0.33</td>
<td>2.42±0.29</td>
<td>1.70</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>IGF (ng/mL)</td>
<td>424.17±111.44</td>
<td>437.27±124.04</td>
<td>341.93±97.98</td>
<td>17.91</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a HT= Height; WT= weight; ZN= zinc; MG= magnesium; TOTT= total testosterone; FRET= free testosterone; PCT= percent testosterone; IGF= insulin-like growth factor; b = treatment by group interaction.

Table 2. Post hoc comparison probabilities for significant treatment by group interactions in minerals and hormones.

<table>
<thead>
<tr>
<th>Contrasts</th>
<th>Within Groups</th>
<th>Between Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZN</td>
<td>0.0004*</td>
<td>NS</td>
</tr>
<tr>
<td>MG</td>
<td>NS</td>
<td>0.00119*</td>
</tr>
<tr>
<td>TOTT</td>
<td>0.0017</td>
<td>NS</td>
</tr>
<tr>
<td>FRET</td>
<td>0.0015</td>
<td>NS</td>
</tr>
<tr>
<td>IGF-I</td>
<td>NS</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

*P<0.0125, Bonferroni adjustment; NS: not significant; b = treatment by group interaction.

Torque and power measures of the quadriceps and hamstrings were significantly different between placebo and treatment with ZMA (P<0.05). An ANCOVA was performed for the discrete measures after it was demonstrated from graphical display and initial group mean values that there was a discrepancy in baseline mean values observed which might have masked true differences in response to training and treatment on the muscle function data. The ANCOVA results (P<0.05) showed a more prominent increase in the ZMA group than the placebo subjects, except for 300 °/s torque measures for right quadriceps and hamstrings. Measurements and statistical results are presented in Table 3. Figure 2 presents percent change in isokinetic torque and power measurements that show the consistent greater improvements in the ZMA supplemented group compared to the placebo group, when baselines are relative.
Figure 1. Interaction effects of blood variables related to ZMA supplementation.
Table 3. Biodex variables for quadricep and hamstring torque and power (Mean±SD).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
<th>F</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RQ 180T</strong></td>
<td>189.85±40.01</td>
<td>211.81±22.31</td>
<td>204.21±36.23</td>
<td>209.13±37.19</td>
<td>24.61</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>RH 180T</strong></td>
<td>173.43±53.22</td>
<td>194.71±46.43</td>
<td>147.59±49.72</td>
<td>158.79±49.75</td>
<td>56.44</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>RQ 300T</strong></td>
<td>156.95±41.82</td>
<td>175.56±38.47</td>
<td>178.61±30.59</td>
<td>177.15±35.76</td>
<td>12.31</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>RH 300T</strong></td>
<td>121.51±50.73</td>
<td>123.35±30.34</td>
<td>122.59±24.91</td>
<td>125.33±13.98</td>
<td>4.51</td>
<td>0.037</td>
</tr>
<tr>
<td><strong>RQ 180P</strong></td>
<td>307.93±85.82</td>
<td>352.69±51.42</td>
<td>335.78±96.89</td>
<td>376.80±79.86</td>
<td>20.11</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>RH 180P</strong></td>
<td>206.49±102.31</td>
<td>255.49±68.95</td>
<td>240.00±94.72</td>
<td>273.66±48.14</td>
<td>31.26</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>RQ 300P</strong></td>
<td>316.51±104.86</td>
<td>373.68±98.78</td>
<td>369.50±60.71</td>
<td>404.33±87.13</td>
<td>28.10</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>RH 300P</strong></td>
<td>241.31±122.05</td>
<td>281.34±88.63</td>
<td>275.78±65.60</td>
<td>319.64±57.54</td>
<td>38.16</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

a R=right leg; Q=quadriceps; H=hamstrings; T=torque (Nm); P=power (Nm/s)

![Isokinetic Test Condition](image)

**Figure 2.** Percent change in isokinetic torque and power.
DISCUSSION
Varsity football players were solicited for a randomized, double blind supplementation study. Of 57 subjects who initially volunteered for the study, 27 successfully followed the nightly supplement regimen over the course of the study and completed the testing sessions. The attrition was due to the need for compliance not only with the supplement and placebo regimen, but also with subsequent blood sampling. There were also some injuries that occurred that prohibited some players from participating fully in practices and/or follow-up muscle function testing. The resultant groups were 15 players on the placebo and 12 with the supplement treatment. The supplement was ZMA, a novel preparation of 30 mg zinc monomethionine aspartate, 450 mg magnesium aspartate, and 10.5 mg vitamin B-6.

Post blood samples and muscle function measures were obtained for comparison to the baseline testing. The results of ZMA supplementation on anabolic hormone profile in football players pre-post spring football practice indicates an amelioration of the anabolic hormones so that the ZMA group had increased concentrations of total testosterone, free testosterone, and IGF-I compared to plateaus or drops in the placebo group. Free testosterone levels have been positively correlated with IGF-I levels (15) and muscle mass (16). Previous research has demonstrated that testosterone responds to intense muscular activity through a decline over time (17) or no significant change (18). Elevated levels of testosterone may be accounted for by exercise-induced changes in plasma volume, therefore no significant differences are demonstrated when hemoconcentration is considered. The subjects in this study were well hydrated in a temperate environment, and tested at least 24 hours after the last strenuous workout of spring football practice.

The preliminary evidence from the results of the present study indicates that simple nutritional supplementation with ZMA may improve the anabolic hormone profile of athletes engaging in intense physical activity. Zinc plays an essential role in androgen metabolism and interaction with steroid receptors (19). Zinc deficiency in male rats reduced circulating luteinizing hormone and testosterone concentrations, by 34% and 68%, respectively. The livers of zinc-deficient rats exhibited a higher aromatization of testosterone to estradiol than did those of controls (19). Concentration of hepatic estrogen receptors in the liver cytosol was significantly higher in zinc deficiency. Zinc deficiency has deleterious effects similar to those of alcohol or castration on hepatic androgen metabolism and aromatization of androgens. Zinc deficiency caused a 41% reduction in the number of androgen binding sites and a 57% increase in the number of estrogen receptors. Zinc maintains the structural integrity of DNA and plays an important role in synthesis of nucleic acid and protein (2). In the present study, the reverse action of deficiency, Zn supplementation, was used to determine effects on anabolic hormones, with positive effects demonstrated on testosterone. Direct muscle function studies with manipulation of zinc status over a short time interval of 3 weeks demonstrated that zinc status positively alters the total work capacity of skeletal muscle in humans (20). The present study results contribute to those findings, although the preparation used in this study was more complex including magnesium and vitamin B-6 as well as zinc.

Exquisite sensitivity of circulating IGF-I to nutrients has been observed. Nutrition is one of the main regulators of circulating IGF-I, which is lowered by energy and/or protein deprivation (21). Enhanced nitrogen balance is demonstrated in caloric restriction with IGF-I administration. IGF is putatively strongly linked to diet, specifically carbohydrate content in caloric restriction. Although most research attention has been on the energy and macronutrient content of the diet, there have been studies that evaluated specific nutrients on IGF-I levels. When purported growth hormone enhancers, arginine and lysine, were administered together with a strength training program, there was no change in resting levels of IGF-I (22). The strongest associations may be between IGF-I and micronutrient levels. Increase in growth velocity in growth-retarded children resulted from zinc supplementation associated
with a 70% increase in plasma IGF-I concentration (23). Zinc and magnesium deficiencies lead to marked growth retardation. In a study using rats, dietary zinc and magnesium were manipulated to assess effects on IGF-I (1). When animals were deprived of magnesium, serum magnesium was reduced 76% and serum IGF-I decreased 60% from baseline. Then, diets were replete with magnesium. The serum magnesium normalized, then 2 weeks later, IGF-I reached control levels. When animals were deprived of zinc, serum zinc was reduced 80% and serum IGF-I decreased 69% from baseline. With dietary zinc repletion, serum IGF-I improved 194%. The researchers concluded that decreased IGF-I was not attributed to reduced energy intake, but seems to be a specific effect of nutritional deficiency of magnesium and/or zinc. Growth retardation in hypocaloric states may be due to magnesium or zinc deficiency mediated through reduced serum IGF-I. Serum changes of magnesium and zinc might be of importance as a mediator for regulating serum IGF-I levels. These studies on specific nutrients, specifically zinc and magnesium, were corroborated with the results of the present study. The element levels were low at the start of the study and increased, but remained within the normal laboratory ranges. Supplementation with ZMA, a novel zinc-magnesium combination, resulted in increased plasma element concentrations and concomitant stabilization of IGF-I levels compared to the placebo group, which demonstrated significant reductions in IGF-I mean values over the training period.

Both zinc and magnesium supplementation have been shown to significantly decrease the levels of the catabolic “stress” hormone, cortisol. In a double blind, randomized study of 23 triathletes, serum cortisol was lower in the magnesium-supplemented group before and after competition compared to controls (24). The authors concluded that the magnesium supplementation resulted in a reduced stress response without affecting competitive potential. In addition to increasing the football players anabolic hormone levels, the ZMA may have had an anti-catabolic effect as well. It would be beneficial to include cortisol measures in future studies.

Related to the improved hormone profile were enhanced posttest values of muscle measures with ZMA. There were relatively greater values with ZMA than placebo in lower extremity isokinetic torque and functional power (180 °/s and 300 °/s, except for torque at 300 °/s) compared to baseline measures as demonstrated in Figure 2.

There is extensive evidence that the anabolic hormones supported by the nutriture of the ZMA supplementation are involved in muscle anabolism and related force production changes (2, 10, 20, 21, 23, 24). Virtually every tissue type is capable of autocrine production of the IGFs. Elevated IGF-I may contribute to hypertrophy response, possibly via mobilization of satellite cells to provide increases in muscle DNA, maintaining some critical DNA-to-protein ratio (25). Increased IGF-I production coincides with increases in muscle DNA and precedes measurable increases in muscle protein. IGF-I may be acting to directly stimulate processes such as protein synthesis and satellite cell proliferation, which result in skeletal muscle hypertrophy. Purported ability of IGF-I to stimulate both anabolic and myogenic effects in vitro suggests it as a component of cellular-level signaling system in skeletal muscle. After acute exercise, IGF-I receptor mRNA was elevated. The main function of IGF-I is to regulate cellular growth and metabolism; IGF-I stimulates DNA synthesis, cell proliferation, and protein synthesis. The anabolic effects of testosterone are mediated primarily through protein synthesis and retarding muscle catabolism, as has been clearly defined over the years (26). Related to the ZMA supplementation-induced enhanced blood profile of zinc, magnesium, and anabolic hormones were significant increases in isokinetic torque and power measurements. The ZMA group increases were significantly different than the placebo group. On a relative scale, the 10%-range increases in quadriceps torque and 12.7% to 15.2% increases in quadriceps power for ZMA supplementation were comparatively greater compared to the –0.8% to 2.4% change in quadriceps
torque and 8.6% to 10.8% change in quadriceps power for the placebo group. There was a baseline difference in muscle torque and power as a result randomization, which resulted in higher values for the placebo group versus the treatment group at the outset. Further statistical analysis was applied so that the significant differences between groups were noted when analyzed with an ANCOVA. Both groups had overall increases in the training and supplementation period, but the ZMA supplementation resulted in greater increases compared to the placebo.

The results of the study are intriguing, since ZMA supplementation was associated with improved anabolic hormone profile and muscle function in already strength-trained varsity collegiate football players. Further research on applications of the novel ZMA compound and related contributing mechanisms would elucidate the effects demonstrated in this preliminary study.

REFERENCES


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**Disclosure of Commercial Interests**

Victor Conte has an equity interest in SNAC System, Inc., patent pending for ZMA.

L.R. Brilla and Western Washington University do not endorse any commercial products and disclaim any interactions other than educational and research interests.

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