EFFECTS OF AEROBIC TRAINING AND GENDER ON HDL-C AND LDL-C SUBFRACTIONS IN YUCATAN MINIATURE SWINE

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ABSTRACT

Effects of Aerobic Training and Gender on HDL-C and LDL-C Subfractions in Yucatan Miniature Swine. WILLIAM B. KIST, TOM R. THOMAS, KRISTEN E. HORNER and M. HAROLD LAUGHLIN JEPonline 1999, 2(2):7-15. The purpose of this study was to determine if either aerobic training or gender influence HDL-C and LDL-C subfractions in miniature swine. Thirty-five Yucatan miniature swine were randomly assigned to either a sedentary group (n = 19, 8 males, 11 females) or a training group (n = 16, 7 males, 9 females). Swine progressed in training to one hour a day, 5/days week, for 16 weeks. Swine were fed Purina mini pig chow throughout the study. At the conclusion of training, deltoid citrate synthase (CS), heart weight to body weight (HW/BW), plasma triglyceride (TG), total cholesterol (TC), HDL-C, HDL₂-C, HDL₃-C, LDL-C, LDL₁-C, LDL₂-C, and LDL₃-C were assessed. ANOVA (training x gender) with post hoc Tukey tests indicated that trained swine had significantly elevated CS and HW/BW values (p < 0.05). Training had no significant effect on either HDL-C or LDL-C subfractions. In contrast, there were significant gender differences with females exhibiting greater post-exercise values on TC (69.9 > 49.0 mg dl⁻¹), LDL-C (27.4 > 17.5), LDL₁-C (2.3 > 0.7), LDL₂-C (11.6 > 6.3), LDL₃-C (13.5 > 10.4), and HDL-C (36.2 > 31.4). Results suggest that 16 weeks of aerobic training was not effective in altering the lipoprotein subfractions of miniature swine. Female miniature swine demonstrated higher lipoprotein values than males, regardless of training status.

KEY WORDS: Pigs, Endurance, Exercise, Lipoprotein profile, Cholesterol

INTRODUCTION

Although the role of plasma triglycerides (TG) cardiovascular disease is not clearly defined, the relationship between plasma total cholesterol (TC) and cardiovascular disease is more completely characterized (1, 2). Specifically, atherosclerosis is thought to be related to decreased high density lipoprotein cholesterol (HDL-C), decreased HDL₂-C, and elevated low density lipoprotein cholesterol (LDL-C) subfractions (3, 4).
HDL$_2$-C is considered to be important in reverse cholesterol transport, with higher levels thought to be protective against the development of atherosclerosis (5). Conversely, LDL-C, the major carrier of cholesterol, is positively correlated with atherosclerosis (6). When blood concentrations of the small dense LDL subfraction, LDL$_3$-C, is elevated it has been demonstrated that there is a three fold greater risk for atherosclerosis (3, 6). Humans with low HDL$_2$-C and elevated LDL$_3$-C are thought to be at risk for atherosclerosis. Humans who exercise train aerobically generally demonstrate an increase in HDL$_2$-C and a decrease in LDL-C and experience associated increased longevity (7, 8, 9).

Swine generally demonstrate human-like lipoprotein profiles, aerobic capacities and cardiac responses to training, and are therefore considered a good animal model for the study of atherosclerosis (10, 11, 12). Despite the high degree of similarity in cardiovascular function between humans and swine, research of how exercise training influences lipoprotein parameters in swine have yielded inconsistent findings. Neither Pedersoli (TC, TG) (13) nor Van Oort et al. (TC, HDL-C, LDL-C, TG) (14) were able to demonstrate significant lipoprotein differences among swine with training. Likewise, Link et al. (15) exercise trained swine and did not see significant training effects on TC or TG. In contrast, Forsythe et al. (16) did observe significant percentage changes in TC and HDL-C with exercise training, while Stucchi et al. (17) demonstrated absolute increases in HDL$_2$-C. Differences in exercise training intensity, duration, and/or frequency could account for discrepancies among these studies.

In contrast, gender effects upon some swine lipoprotein parameters were found to be consistent in two studies (13, 15). Both Link et al. (15) and Pedersoli (13) noted that female miniature swine demonstrated higher lipoprotein values (TG, TC) than males. Neither study investigated the effects of gender on HDL-C and LDL-C subfractions. It is noteworthy that Forsythe et al. (16) used castrated male swine while Stucchi et al. (17) used female swine in their exercise training studies. It is plausible that swine with low levels of testosterone demonstrate a better response to training. This effect has not been investigated. Understanding gender effects may aid in interpreting training effects in the swine model of atherosclerosis. The combined effects of gender and training on HDL-C and LDL-C subfractions in swine have not been thoroughly investigated and need to be characterized (10). Therefore the purpose of this study was to determine if either aerobic training or gender influence HDL-C and LDL-C subfractions in miniature swine.

**METHODS**

**Animals and training protocol.**

Yucatan miniature swine (n = 35) (Charles Rivers Laboratories) were used to test the responses of HDL-C and LDL-C subfractions to training. The swine were approximately one year of age (12-14 months) when the study began and were randomly divided into sedentary (S) (n = 19, 8 males, 11 females) and training (T) (n = 16, 7 males, 9 females) groups. Thus, a total of 15 male and 20 female pigs were utilized in this investigation. This study was approved and conducted in conformance with the University of Missouri’s animal care committee’s guidelines on animal research.

The aerobic endurance training period lasted 16 weeks with swine training five consecutive days a week on a treadmill. The training protocol began at approximately 30 minutes (week 1) progressing to 60 minutes at 5 m/hr (week 16). A 10% grade was used throughout the training program. At the conclusion of the training program swine exceeded 20 miles/week, total mileage. S swine were not trained. The training program has been previously detailed (18). All
swine received a typical pig diet (Purina, mini pig chow, PMI Feeds Inc., St Louis, MO 63144) throughout the study. The diet consisted of 16% protein, 2.5% fat, 14% fiber, 8% ash, 3% minerals and the balance nitrogen free extract (3.89 kilocalories/g). Food intake was adjusted to prevent a weight loss or gain. Although individual food consumption was not recorded, the trained animals probably received a greater quantity of pig chow to offset increased energy expenditure. Water was given ad libitum.

Markers of training and termination of animals.
At the conclusion of the training period, a final exercise stress test was performed (18). “Stage 3” heart rate (HR) and “Stage 4” endurance duration were recorded. Animals were subsequently sacrificed via removal of the heart after administration of intravenous ketamine (1ml/2.85 kg), rompun (1ml/44 kg), and thiopental (1ml/2.5 kg). Citrate synthase (CS) from the deltoid muscle (µm/min/g tissue) and wet heart weight (HW) to live body weight (BW) ratio (HW/BW) were obtained to document physiological and anatomical evidence of training effects (19, 20).

HDL-C and LDL-C subfractions.
At the conclusion of training, jugular blood samples for lipoprotein assay were obtained via venipuncture and collected in vacutainers containing EDTA. Blood samples were obtained following a 12 hour fast. Samples from females were drawn without regard for estrous cycle variations. Plasma for lipoprotein measurement was separated by centrifugation (Beckman TJ-6R centrifuge, Palo Alto, CA) at 4 °C for 15 minutes at 3750 rev/m. All plasma was stored at -70 °C until analyzed.

TG was assayed spectrophotometrically (Beckman model DU-20, Fullerton, CA 92634) using a Sigma Diagnostic kit (Triglyceride GPO-Trinder #339, St Louis, MO 63145). HDL fractions were assayed for cholesterol content using a Sigma Diagnostic kit (Cholesterol, #352). HDL-C was determined following a heparin manganese precipitation process to remove very low density lipoprotein cholesterol (VLDL-C) and LDL-C from the plasma (21). This was followed by precipitation of HDL2-C using dextran sulfate to determine HDL3-C (21). HDL2-C was deduced as HDL-C minus HDL3-C (HDL2-C = HDL-C - HDL3-C). LDL subfractionation was performed by separating plasma LDL into three levels using density gradient ultracentrifugation (160,000g for 21 hours) with the LDL subfractions individually analyzed for cholesterol content (21).

Statistical analyses.
Statistical analysis was performed (Sigma Stat, Jandel Scientific, San Rafael, CA) using a two way ANOVA (training x gender). Post hoc Tukey tests were performed when significant F values (p < 0.05) were noted. Values are reported as means ± standard deviations (SD).

RESULTS
Anatomical and physiological effects of training.
CS, HW/BW, stage 3 HR, and stage 4 endurance time means are reported in Table 1. CS means were significantly elevated in the T animals. Likewise, HW/BW ratios were significantly elevated in the T animals. Similarly, significant training effects were demonstrated by lowered stage 3 HR and prolonged stage 4 endurance times.

HDL and LDL subfractions, effects of training and gender.
TG and LDL-C subfraction means and standard deviations are reported in Table 2. There was no significant training effect for any parameter. There was, however, a significant interaction on TG for both males and females. The interaction was inconsistent, as S males had lower TG values than T males, while S females had higher TG than T females. TG and LDL-C pooled means (training status and gender) are reported
Exercise Training and Blood Cholesterol

Table 1. Markers of training.

<table>
<thead>
<tr>
<th>Group</th>
<th>CS (μM·min⁻¹·g⁻¹)</th>
<th>HW/BW</th>
<th>Stage 3 HR (bpm)</th>
<th>Endurance time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>15.57 ± 3.37</td>
<td>4.51 ± 0.58</td>
<td>271.8 ± 15</td>
<td>20.77 ± 3.45</td>
</tr>
<tr>
<td>Trained</td>
<td>20.03 ± 3.59*</td>
<td>5.57 ± 0.86*</td>
<td>251.0 ± 33*</td>
<td>30.15 ± 3.66*</td>
</tr>
</tbody>
</table>

CS = citrate synthase (deltoid), HW/BW = heart weight/body weight ratio (g/kg).
Stage 3 HR = Submaximal heart rate in stage 3 of stress protocol, Endurance time = exercise time, stage 4 of stress protocol. Values are means ± SD.
* Indicates significant difference, trained vs. sedentary (p < 0.05).

in Table 3. There was a significant main effect of gender on TG, TC, LDL-C, LDL₁-C, LDL₂-C, and LDL₃-C with females consistently demonstrating greater values.

Table 2. LDL-C subfractions.

<table>
<thead>
<tr>
<th>Lipid (mg/dL)</th>
<th>Sedentary males</th>
<th>Trained males</th>
<th>Sedentary females</th>
<th>Trained females</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>17.3 ± 8.5</td>
<td>28.0 ± 8.4</td>
<td>33.0 ± 16.5</td>
<td>26.4 ± 8.7</td>
</tr>
<tr>
<td>TC</td>
<td>47.0 ± 13.9</td>
<td>51.7 ± 8.2</td>
<td>67.0 ± 9.9</td>
<td>73.5 ± 9.2</td>
</tr>
<tr>
<td>LDL-C</td>
<td>16.0 ± 3.3</td>
<td>19.2 ± 3.5</td>
<td>26.4 ± 6.6</td>
<td>28.5 ± 7.7</td>
</tr>
<tr>
<td>LDL₁-C</td>
<td>0.7 ± 0.4</td>
<td>0.7 ± 0.4</td>
<td>2.3 ± 2.7</td>
<td>2.3 ± 1.9</td>
</tr>
<tr>
<td>LDL₂-C</td>
<td>6.1 ± 1.8</td>
<td>6.6 ± 1.3</td>
<td>10.4 ± 4.3</td>
<td>12.8 ± 4.8</td>
</tr>
<tr>
<td>LDL₃-C</td>
<td>9.3 ± 1.8</td>
<td>11.7 ± 2.5</td>
<td>13.7 ± 3.0</td>
<td>13.3 ± 3.4</td>
</tr>
</tbody>
</table>

TG = triglycerides, TC = total cholesterol, LDL-C = low density lipoprotein cholesterol.
Values are means ± SD. Values with different superscripts are statistically different – a vs. trained males, b vs. trained females, c vs. sedentary females. (p < 0.05).

Table 3. LDL-C subfractions pooled by group and gender.

<table>
<thead>
<tr>
<th>Lipid (mg/dL)</th>
<th>Sedentary</th>
<th>Trained</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>26.4 ± 15.6</td>
<td>27.0 ± 8.3</td>
<td>21.8 ± 9.8</td>
<td>30.0 ± 13.7</td>
</tr>
<tr>
<td>TC</td>
<td>58.6 ± 15.2</td>
<td>64.8 ± 14.0</td>
<td>49.0 ± 11.6</td>
<td>69.9 ± 9.9*</td>
</tr>
<tr>
<td>LDL-C</td>
<td>21.5 ± 7.4</td>
<td>24.4 ± 7.7</td>
<td>17.5 ± 3.7</td>
<td>27.4 ± 7.0*</td>
</tr>
<tr>
<td>LDL₁-C</td>
<td>1.6 ± 2.0</td>
<td>1.6 ± 1.7</td>
<td>0.7 ± 0.4</td>
<td>2.3 ± 2.2*</td>
</tr>
<tr>
<td>LDL₂-C</td>
<td>8.4 ± 3.9</td>
<td>10.1 ± 4.8</td>
<td>6.3 ± 1.6</td>
<td>11.6 ± 4.6*</td>
</tr>
<tr>
<td>LDL₃-C</td>
<td>11.6 ± 3.4</td>
<td>12.6 ± 3.0</td>
<td>10.4 ± 2.4</td>
<td>13.5 ± 3.1*</td>
</tr>
</tbody>
</table>

TG = triglycerides, TC = total cholesterol, LDL-C = low density lipoprotein cholesterol.
Values are means ± SD. * Indicates significant difference by gender (p < 0.05).

HDL-C subfraction means and standard deviations are illustrated in Figure 1. There was no significant training effect on any subfraction. HDL-C pooled subfraction means are illustrated in Figure 2. There was a significant gender effect on HDL-C with females exhibiting greater values.

Data of statistical power (1-β) for specific statistical mean comparisons are reported in Table 4. Power greater than or equal to 0.80 was considered adequate. Power analysis for gender demonstrated that Stage 4 endurance, TC, LDL-C, LDL₂-C, and LDL₃-C were adequate. Power analysis for training effects demonstrated that HW/BW, CS, Stage 3 HR, and Stage 4 endurance were adequate. For the interaction of gender and training, power was inadequate for all parameters.

DISCUSSION

Training effects on anatomical and physiological markers.

Trained swine in this study exhibited a significantly higher HW/BW, lower stage 3 HR, longer stage 4 endurance, and higher CS (Table 1). The HW/BW is a relative anatomical indicator of heart size that was used to reveal cardiac muscle hypertrophy in response to training (14,16,18). Hypertrophy was conclusively demonstrated as T swine had significantly heavier hearts relative to body size. Stage 3 HR values demonstrated that T animals had significantly lower HR at this submaximal exercise intensity compared to the S group. Consistent with this, the T group was able to continue significantly longer during Stage 4 of the exercise test. Both a lower submaximal HR and greater exercise duration would be considered positive markers of a training effect (14, 19). Consistent with these parameters was the significant physiological increase in muscle CS in T swine (Table 1). Increased CS reflects increased oxidative capacity of a trained muscle (19, 20). Thus, in the T swine the efficacy of the aerobic...
training was both anatomically and physiologically demonstrated.

Table 4. Statistical power data for key variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gender</th>
<th>Training</th>
<th>Gender x Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>HW/BW</td>
<td>0.050</td>
<td>0.999</td>
<td>0.096</td>
</tr>
<tr>
<td>CS</td>
<td>0.225</td>
<td>0.951</td>
<td>0.050</td>
</tr>
<tr>
<td>STAGE 3 HR</td>
<td>0.207</td>
<td>0.826</td>
<td>0.590</td>
</tr>
<tr>
<td>STAGE 4 endurance</td>
<td>0.825</td>
<td>1.000</td>
<td>0.179</td>
</tr>
<tr>
<td>TG</td>
<td>0.254</td>
<td>0.050</td>
<td>0.409</td>
</tr>
<tr>
<td>TC</td>
<td>1.000</td>
<td>0.185</td>
<td>0.050</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.998</td>
<td>0.170</td>
<td>0.050</td>
</tr>
<tr>
<td>LDL_{1}C</td>
<td>0.714</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>LDL_{2}C</td>
<td>0.974</td>
<td>0.145</td>
<td>0.050</td>
</tr>
<tr>
<td>LDL_{3}C</td>
<td>0.872</td>
<td>0.080</td>
<td>0.161</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.423</td>
<td>0.210</td>
<td>0.050</td>
</tr>
<tr>
<td>HDL_{1}C</td>
<td>0.076</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>HDL_{2}C</td>
<td>0.281</td>
<td>0.175</td>
<td>0.050</td>
</tr>
</tbody>
</table>

HW/BW = heart weight to body weight ratio, CS = citrate synthase, STAGE 3 HR = heart rate during third stage of exercise test, STAGE 4 endurance = duration of last stage of exercise test, TG = triglycerides, TC = total cholesterol, LDL-C = low density lipoprotein cholesterol, HDL-C = high density cholesterol. Power values calculated for two way ANOVA (gender x training) with $p < 0.05$.

Training effects on HDL-C and LDL-C subfractions.
Despite the obvious aerobic training effect, trained swine did not demonstrate a significant training effect on lipoprotein subfractions (Tables 2, 3, and Figures 1, 2). These findings were consistent with those of Pedersoli (13), Van Oort et al. (14), and Link et al. (15). In contrast, Forsythe et al. (16) demonstrated a 16% increase in HDL-C, a 12% decrease in LDL-C, and a lower TC in T animals. However, these differences were based upon percentages and not absolute values. For example, there was no difference in the absolute amount of plasma HDL-C found, but the percentage of cholesterol carried in HDL was significantly greater in the T than in the S swine. Thus, the results of the present study are comparable to other swine studies in that major lipoprotein classes are resistant to change by training (13,14,15).

Figure 1. HDL-C subfractions by group and gender. HDL-C = high density cholesterol. Values are means (mg/dL) ± standard deviations. There were no significant differences by training ($p < 0.05$).

Figure 2. HDL-C subfractions pooled by group and gender. HDL-C = high density cholesterol. Values are means (mg/dL) ± SD. * significant differences by gender ($p < 0.05$).

The reason for the lack of lipoprotein response in the present study was not ascertained. Intensity of the exercise program seems an unlikely reason as evidenced by training induced changes in physiological and anatomical markers (Table 1). Stucchi et al. (17) trained Yucatan swine at 75% maximum HR using a similar protocol and had comparable CS findings (Stucchi et al. $S = 17.5, T = 22.5$, present study
S = 15.6 and T = 20.0 µm/min/g). It therefore seems likely that the swine of the present study were trained near 75% maximal HR.

In humans, training near 75% maximum HR for 30 minutes would be considered adequate for lipoprotein changes to occur. Superko (22) reviewed evidence from several studies and deduced that 15 miles per week of aerobic activity is sufficient to modify human lipoprotein profiles. The T swine in this study exceeded 20 miles/week. Thus, if swine and human lipoprotein profiles behave similarly to an equivalent stimulus, the training intensity should have been adequate in this study. The findings of the present study and other studies may suggest that in swine lipoprotein changes may lag behind other training indicators.

The length of the training period is a more plausible explanation for the lack of demonstrable lipoprotein subfraction changes in the present study. Neither Pedersoli (13) nor Van Oort et al. (14) demonstrated significant training effects, despite training periods of approximately eight months. In humans, it may take a considerable period of time for HDL and LDL to change significantly (8, 23). Therefore, if Yucatan swine HDL and LDL subfractions behave similar to human counterparts, a longer training period may be necessary to demonstrate a significant training effect in HDL and LDL subfractions. This duration hypothesis is supported by the results of Stucchi et al. (17). Only after two years of endurance training was a significant decrease of TG in LDL₁, LDL₂ and a four-fold increase in HDL₂-C demonstrated. Thus, sixteen weeks of training in the present study may be inadequate to induce a significant change despite adequate intensity.

The frequency of training does not appear to contribute to the negative training findings. Forsythe et al. (16) trained swine 7 days/week, Van Oort et al. (14), 4 days/week, Link et al. (15), 5 days/week, and Pedersoli (13), 5 days/week, and all showed an absolute lack of lipoprotein changes with training. Consistent with this, the present study used 5 days/week of training and no significant lipoprotein changes were noted.

The diet used in the present study could contribute to the lack of demonstrable lipoprotein changes. In the present study TC, HDL-C, LDL-C, and TG plasma values were normolipidemic (Tables 2, 3, and Figures 1, 2) and were comparable, although generally lower, to Pedersoli (13) (pre-atherogenic diet trial) and Van Oort et al. (14) (control diet, S and T) values. It is reasonable to assume that the normal values of the present study were directly related to the amount of fat (2.5%) ingested. Thus, without extreme training protocols, subtle lipoprotein effects may not have been demonstrated due to the initial low normal values. It is plausible to suggest that if the swine of the present study had atherogenic lipoprotein values, a lipoprotein training effect may have been demonstrated. A training study using an atherogenic diet (high fat/cholesterol) is in progress.

Finally, the statistical power of the present study may have been inadequate to detect subtle training differences (Table 4) in lipoprotein parameters. The crux of the problem for some parameters appears to be large inter-animal variability. For example, in the present study, the range of HDL₂-C values of the S animals was 0.1 to 6.9 mg/dl. The range in the T animals was 0.1 to 9.5. With such large variances (Figures 1 and 2) within the groups, detection of differences between the groups is difficult.

Related to this, in the negative lipoprotein training studies of Pedersoli (13), Van Oort et al. (14), Forsythe et al. (16), and Link et al. (15), all employed a between subjects design. Only
Stucchi et al. (17) demonstrated significant training differences (HDL$_2$-C) using a between subjects design. It is noteworthy that Stucchi et al. (17) used only nine (n = 9) animals (5 control and 4 training). Considering the large HDL$_2$-C inter-animal variance documented in the present study, and the almost nonexistent levels of HDL$_2$-C in some Yucatan swine, it is plausible that the results from Stucchi et al. (17) may have been statistically insignificant with a larger population of animals.

**Gender effects on HDL and LDL subfractions.**

There was a significant effect of gender on TC, LDL$_1$-C, LDL$_2$-C, LDL$_3$-C (Table 3) and HDL-C (Figure 2). The gender effects demonstrated that females had greater values than males in both S and T animals. This gender effect was consistent with Pedersoli (13) (TC, TG) and Link et al. (15) (TC, TG). Thus, it appears that female swine generally have elevated lipoprotein profiles compared to their male counterparts. This contrasts with human premenopausal females who typically have healthier lipoprotein profiles than males. In human females, HDL-C subfractions are higher and LDL-C subfractions are lower, as demonstrated by Ziogas et al. (24). Thus, miniature swine simply may not parallel human HDL-C and LDL-C gender subfraction patterns.

The only parameter which demonstrated a significant interaction between gender and training was TG (Table 2). This finding, however, was inconsistent. S female swine had greater TG values than T females, as would be expected based upon human studies (24). Interestingly, Merservey et al. (25) noted that the food intake of T female Yucatan swine was slightly less than S females. A lower food intake could impact TG values. In contrast, S males had lower TG levels than T males. Pedersoli (13) also found that S male miniature swine had lower TG values than T males. Pedersoli attributed this finding to the fact that the S males ate less food than T males and therefore had lower TG values. A lower TG value in S swine conflicts with the typical human TG response where S individuals have higher TG values than T individuals (24). Similar TG responses in the present study could be attributed to food intake, however, food intake was not recorded and this suspicion cannot be confirmed. Thus, overall, HDL-C and LDL-C subfractions responses to training does not appear to be gender specific.

**CONCLUSION**

In conclusion, these results indicate that a 16 week aerobic training program was not effective in altering HDL-C and LDL-C subfractions in either male or female miniature swine fed a normal diet. The results also suggest that human and swine lipoprotein profiles may respond differently to training. However, the limited duration of the training program in this study may have contributed to the lack of change in blood lipoproteins. Additionally, our results indicate that female miniature swine generally have higher HDL-C and LDL-C subfractions than males, regardless of training status.

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