Effect of Litter Size Manipulation on the Reproductive Performance of BALB/C Mice

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Abstract

Many of previous studies on lactating animals with natural litters did not support the negative link between litter size and pup mass; however, artificial manipulation of litters could be an option to support that. In this study, we manipulated the litters of lactating BALB/C mice with having two groups. Enlarged litter SL (LS=8; N=5) and reduced litters SS (LS=2; N=6) were used in this experiment and also 7 virgin female mice were assigned for control group. Mother's body mass and food intake were daily measured throughout the experiment. Litter size, litter mass, pup mass, mortality rate of pups in the studied groups were also daily measured throughout lactation. The lactating mice in both groups significantly ate more food intake compared to non –reproducing mice. Mice with enlarged litters had significantly greater food intake compared to the mice with reduced litters. Litter masses at weaning of SL mice were significantly higher compared to the SS mice. However, pup mass at weaning was significantly lighter in SL mice compared to the pup mass in the SS mice. Our present study concludes that the litter size of manipulated litters was negatively associated with pup mass at weaning.

Keywords: *Litter size, reproductive efforts, manipulation of litters, lactation*

Introduction

Litter size (LS) is defined as the total number of little pups in a litter. It considers the main vital trait of animal's life history that contributed to the fitness [1] . Species are different in their litters and the litters can also be varied within species [2, 3, 4, 5] . Many of eco-physiological studies have interested in addressing the question about why there are inter and intra variations in LS [6, 7, 8, 9]. In these studies, it was focused on the energetic cost of raising different litter sizes within one species [10, 11, 12, 13, 14, 15, 16] or comparing different species raising the same litter size [17, 18]. Previous studies indicated that are several strategies that animals can follow when rearing larger litters. Firstly, animals can increase their food intake to support the pups. Reducing the available energy for each pup can be another option. In addition, inducing the energy derived from fat store. Animals could also increase the digestive efficiency towards the growth of pups. Finally, lactating animals could kill some of their pups to ensure having an optimize reproductive success. It has been shown that the mother with large litters ate more food compared to animals raising small litters [19, 20, 17, 8, 21, 10, 22, 23, 24, 25]. However, it is not always the case that mother's food intake is positively correlated with number of litters [26, 27]. Generally speaking, pups reared in large litters are lighter than pups reared in small litters [28, 29, 30, 31, 22, 23, 24, 25]. and this was explained that mothers with large litters cannot provide their pups with enough energy. Mothers with large litters could also increase their milk production due to the continuous sucking of litters to the mammy glands [32, 33]. In some cases, mothers faced with large litters could rely on their fat stores to produce more milk during lactation [34]. Another strategy by lactating animals is that the lactating animals may kill some of the raised pups to ensure supporting the rest of the pups [25]. Nevertheless, many of previous studies on lactating animals with natural litters did not support the negative link between litter size and pup mass compared to the negative link between the above traits observed

in the studies with manipulation of litters [35].Therefore, experiments with manipulation litters could expand our understanding on the nature of limits on energetics of reproduction.

Materials and Methods

1. Animals Housing and Mating:

The study included (10) male and (27) female BALB/C mice. Mice were housed in a plastic cage (48"×15"×13") provided with sawdust, cardboard tube, and paper wool for bedding and also with water and food at *ad libitum*. The mice were kept under ambient temperature (21±2°C) with daily 12hrs:12hrs dark and light, respectively.

Twenty female mice were in pairs housed with one male in each cage and 7 female individually housed as a control. The mated mice were provided with standard diet and water as *ad libitun*. The body mass of pairs female were daily monitored for 11 days. If the pregnancy occurred, the male was removed and the pregnant female was then individually housed in a new cage. Daily body mass and food intake of pregnant female were measured until the day of parturition using a balance (± 0.01g, Sartorius). At day of delivery, the pups were counted and weighted and then left with their mother without interruption for 3 days of lactation.

2. Manipulation of Litter Size:

On day 3 of lactation, mother's body mass, litter size, litter mass, and pup mass were measured. On this day, the manipulation of litters was made by adding or removing pups from the lactating female to others which were born on the same day (± 1). Lactating mice were randomly allocated for mice with 2 pups (called reduced litters) or mice with 8 pups (called enlarged litters). On the day of manipulation, lactating mice and control ones were randomly assigned for 3 groups. Group 1 refers to the control (CS; N=7), group 2 refers to the lactating mice with enlarged litter (SL; N=5), and group 3 refers to the lactating mice with reduced litters (SS; N=6). Daily maternal body mass and maternal food intake were measured in lactation period until the day of weaning (day 18 of lactation) as well as in control mice at the same period. Reproductive efforts such as litter size, litter mass, and pup mass were daily measured until the day of lactation. The death of any pup throughout lactation period was also recorded.

3. Statistical analysis:

Prior to perform the statistical analysis, data were checked for normality using shipro test and the transformation was made for non normal-distributed data. Statistical changes in daily measured variables such as maternal body mass, food intake, litter mass, pup mass, and liter size throughout the experiment were tested using repeated measures of General Linear Model (GLM) followed by tukey post-hoc test to establish the changes among days. Differences in the maternal body mass, asymptotic food intake, litter mass, pup mass and litter size between groups were assessed using GLM. Pearson correlation was used to assess the potential relationship between measured variables. The statistical analysis was done using Minitab software 17 [55].

Results and Discussion

1-Maternal body mass:

At the baseline, the body mass was not significantly different in mothers that assigned for enlarged or reduced litters (see table 1). During pregnancy, body mass of pregnant mothers increased significantly throughout the last ten days (RM GLM, day of pregnancy, $F_{9, 100}$= 40.72, P<0.001; see figure1). During lactation, the body mass of mothers were significantly changed throughout the days of lactation with no changes being detected between manipulated mice (RM GLM, day of lactation, $F_{15,150}$= 36.39, P<0.001; group, $F_{1,150}$= 0.54 , P=0.481; see figure3 and table 1). The body mass in lactating mice with enlarged litters was similar to the body mass found in mice with reduced litters. These results were in line with the previous findings. [25]. found that the body mass of lactating mice with manipulated larger litters were not significantly changed compared to the values of mice with manipulated smaller litters. The absence of the changes in mother's body mass during lactation found in the current study could indicate that the lactating mice have not lost their weight, maintained a stable energy balance by not taking out any energy stores in their tissues to support reproduction, and supported their pups by only relying on the energy derived from eaten food [25, 53, 54].

Table 1: Measured physiological traits of mothers raising enlarged and reduced litters. Means that do not share a letter are significantly different

Traits	SL Mice	SS Mice	T value	p value
LS at birth	6.67±1.3	7.33±0.99	-0.42	0.686
LS at day 3	8a±0	2b±0	4.49	<0.001
LS at day 18	7.6a±0.54	2b±0	1.98	<0.001
FI baseline	3.78±0.39	3.77±0.35	-5.8	0.36
Asymp FI 8-13	20.63a±0.64	17.262b±0.39	4.49	0.004
BM at baseline	25±0.95	25.14±0.52	-2.08	0.35
BM at day 3	35.05±0.50	35.405±0.26	-0.64	0.547
BM at day 18	33.96±0.52	34.318±0.23	-0.64	0.55
LM at day 3	22.81a±0.32	6.283b±0.15	46.49	<0.001
LM at day 18	69.43a±1.4	26.717b±0.04	30.45	<0.001
PM at day 3	2.8505a±0.04	3.142b±0.07	-3.5	0.01
PM at day 18	9.152a±0.14	13.358b±0.13	-22.42	<0.001

SL= standard enlarged litters; SS=standard reduced litters; LS= litter size; FI= food intake; Asymp=Asymptotic; BM=body mass; LM= litter mass; PM= pup mass

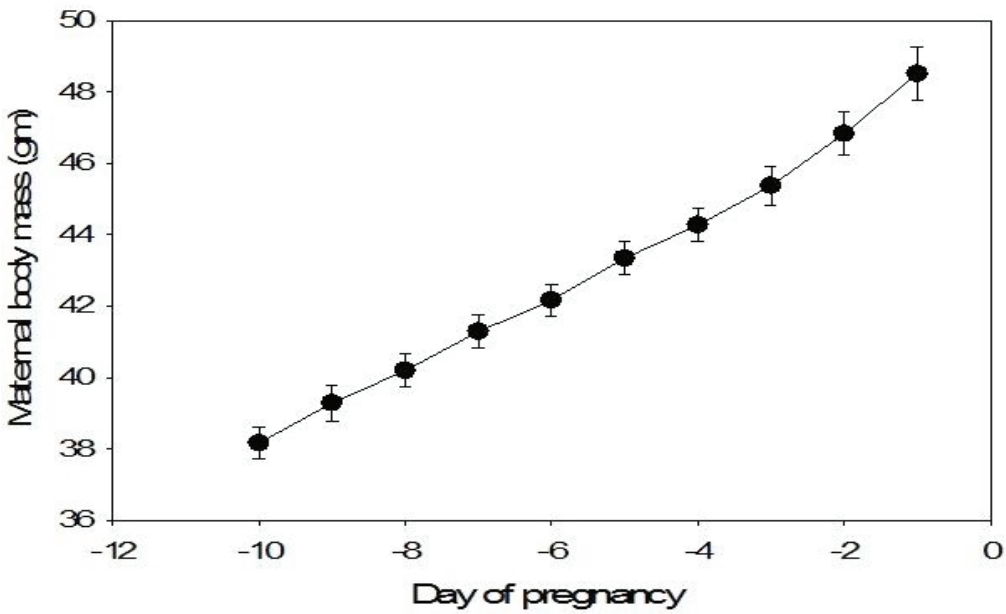

Figure 1: The maternal body mass of pregnant mice throughout pregnancy period

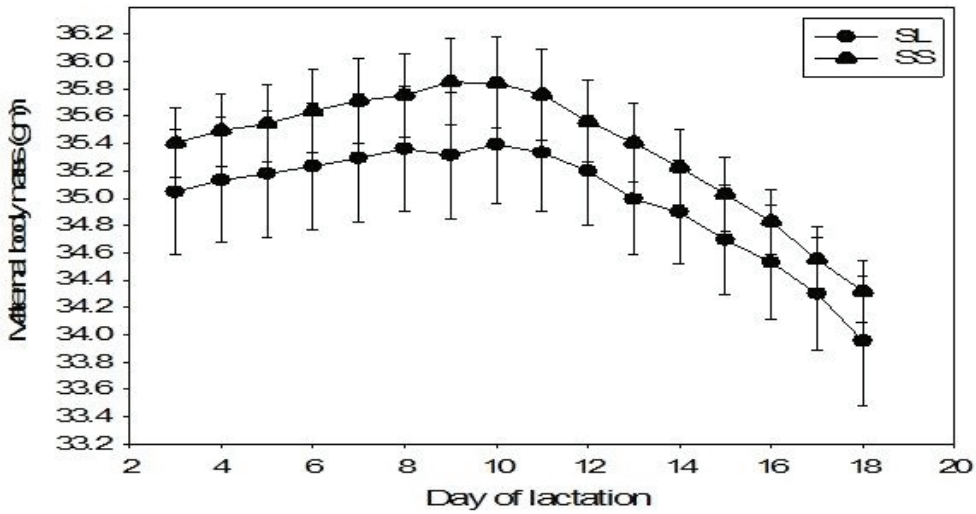

Figure 2: The maternal body mass of lactating enlarged and reduced mice fed standard diet throughout lactation period.

2- Maternal food intake:

At the baseline, the food intake was not significantly different in mothers that assigned for enlarged or reduced litters (see table 1). During pregnancy, food intake of pregnant mothers increased significantly throughout the last ten days (RM GLM, day of pregnancy, $F_{9, 100}$= 40.72, P<0.001; see figure 3). During lactation, the food intake increased significantly across the days of lactation as well as significantly different between mice with enlarged litters and mice with reduced litters (RM GLM, day of lactation, $F_{14, 139}$= 5.63 , P<0.001; group, , $F_{1, 139}$= 22.22 , P=0.00; see figure 4 and table 1). In both groups, the food intake was the highest and not significantly different among the days 8- 13 of lactation and this period considered an asymptotic food intake see table 1.

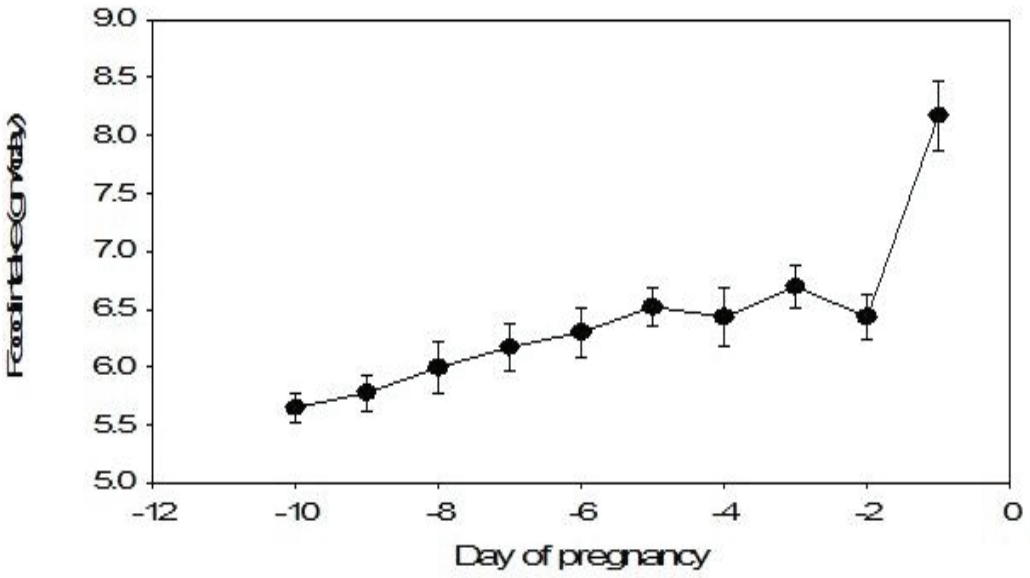

Figure 3: The food intake of pregnant mice throughout pregnancy period.

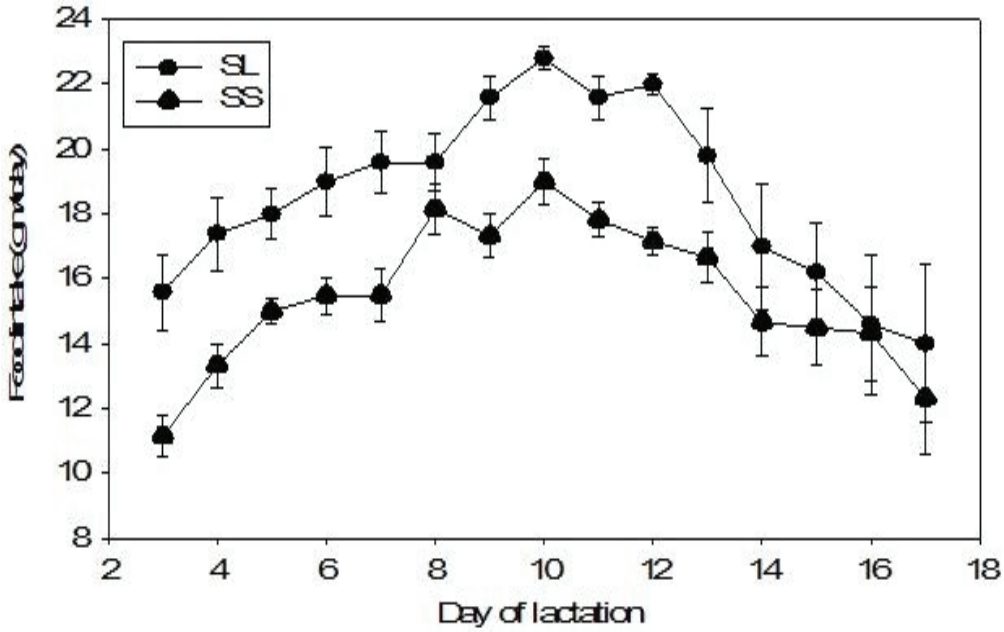

Figure 4: The food intake of lactating enlarged and reduced mice fed standard diet throughout lactation.

3- Reproductive performance

The liter size (LS) at birth (prior manipulation) was not significantly different in mice that assigned for enlarged litters compared to the mice with reduced litters (see table 1). After manipulation, litter size was significantly greater in mice with enlarged litters compared to those in mice with reduced litters throughout lactation but the litter size did not significantly changed cross the days of lactation (RM GLM, group, $F_{1, 135}$= 17160.21 , P<0.001; day of lactation, $F_{15, 135}$= 1.00 , P=0.5; see figure 5 and table 1. The litter and pup masses were significantly different between mice with enlarged and reduced litters. Litter mass (RM GLM, group, F1, 135= 178.26, P<0.001; day of lactation, F15, 135= 7.62, P<0.001) and pup mass, (RM GLM, group, F1, 135= 31.92 , P<0.001; day of lactation, F15, 135= 12.47 , P<0.001). see figure 6 and 7.

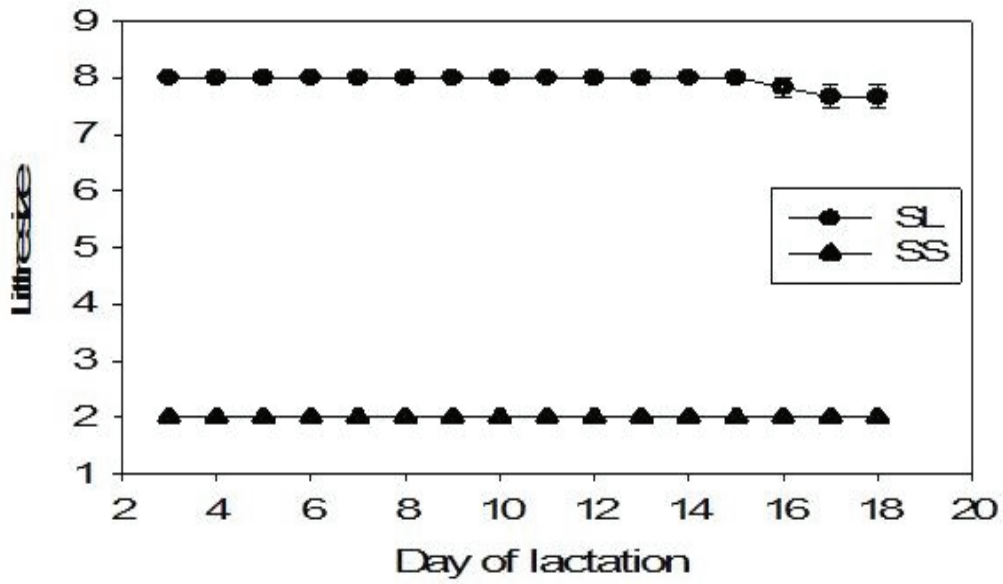

Figure 5: The litter sizes of lactating enlarged and reduced mice throughout lactation period.

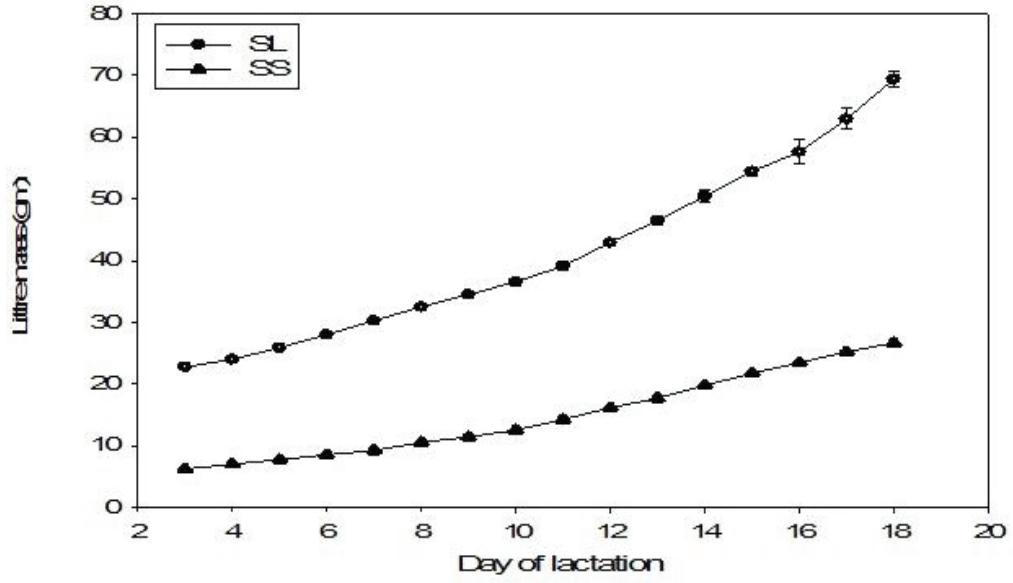

Figure 6: The litter masses of lactating enlarged and reduced mice throughout lactation

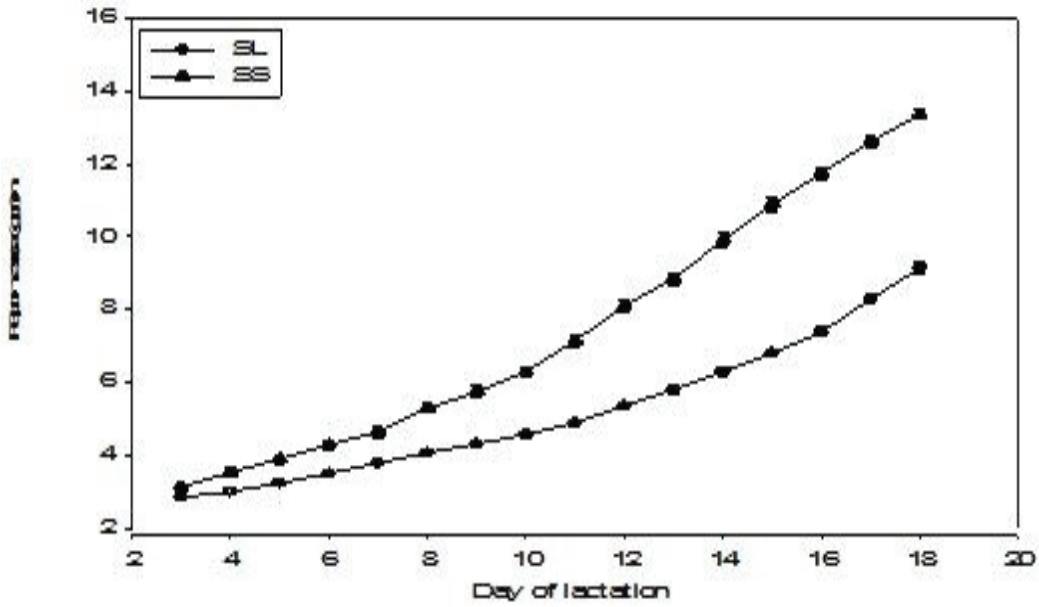

Figure 7: The pup masses of lactating enlarged and reduced mice throughout lactation

During lactation, lactating mice with both enlarged and reduced litters significantly increased the daily food intake over the first 13 days of lactation and reached a maximum of asymptotic food intake (days 8-13) compared to non-reproducing mice. These findings were consistent with many of previous studies conducted on food intake during lactation [12, 36, 37, 38, 39 , 40,41]. Lactating mice with enlarged litters ate significantly more food compared to the lactating mice with reduced litters [42]. This is explained by the fact the mice raised more pups responded by eating more food. Similar to this finding was also found in lactating MF1 mice reared large pups compared to those reared small pups [22, 23, 43, 25]. This means that the lactating mice allocated the energy derived from food intake to support their pups. However, weaned pups raised in large litters were significantly

lighter than in pups raised in small litters. These results were consistent with previous studies conducted on rodents [44, 45, 46, 31, 14,25]. These studies suggested that milk production of small mammals is physiologically limited [47, 12, 16, 48]. Liter size in mothers can be adjusted before or at birth and this adjustment can be different between mothers depending on the mother's resources. One possible way in which the lactating mothers can control their litters after giving birth is by infanticide [49, 50]. In this regard, mothers can kill some of their litters to relive the stress of extra burden [51]. In our current study, mothers with enlarged litters had about 4% mortality rate of their pups compared to zero % in mothers with reduced litters. This result was consistent with previous studied conducted on MF1 mice [25] and also in lactating Syria hamster (Mesocricetusauratus) [52].

Conclusions

Manipulation of larger litters significantly induced mothers to eat more food and weaned lighter pups compared the weaned pup reared in manipulated smaller litters.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Physiology and Pharmacology and all experiments were carried out in accordance with approved guidelines.

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