

1 **TITLE: Food intake dependent and independent effects of heat stress on lactation and mammary**  
2 **gland development**

3 **RUNNING TITLE:** Heat induced changes in lactation

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11 **KEYWORDS:** hypophagia, hyperthermia, hypogalactia, mouse model, dairy, lactation

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13 **Summary Statements**

14 This study demonstrates that heat stress decreases lactation and mammary development through food  
15 intake dependent and independent mechanisms.

16

17 **ABSTRACT**

18 With a growing population, a reliable food supply is increasingly important. Heat stress reduces  
19 livestock meat and milk production. Genetic selection of high producing animals increases endogenous  
20 heat production, while climate change increases exogenous heat exposure. Both sources of heat  
21 exacerbate the risk of heat-induced depression of production. Rodents are valuable models to  
22 understand mechanisms conserved across species. Heat exposure suppresses feed intake across  
23 homeothermic species including rodents and production animal species. We assessed the response to  
24 early-mid lactation or late gestation heat exposure on milk production and mammary gland  
25 development/function, respectively. Using pair-fed controls we experimentally isolated the food  
26 intake dependent and independent effects of heat stress on mammary function and mass. Heat  
27 exposure (35°C, relative humidity 50%) decreased daily food intake. When heat exposure occurred  
28 during lactation, hypophagia accounted for approximately 50% of the heat stress induced hypogalactia.  
29 Heat exposure during middle to late gestation suppressed food intake, which was fully responsible for  
30 the lowered mammary gland weight of dams at parturition. However, the impaired mammary gland  
31 function in heat exposed dams measured by metabolic rate and lactogenesis could not be explained by  
32 depressed food consumption. In conclusion, mice recapitulate the depressed milk production and  
33 mammary gland development observed in dairy species while providing insight regarding the role of  
34 food intake. This opens the potential to apply genetic, experimental and pharmacological models  
35 unique to mice to identify the mechanism by which heat is limiting animal production.

36

37

## 38 INTRODUCTION

39 Rising global temperatures may result in global food insecurity. Heat exposure in livestock species  
40 decreases feed intake, depressing meat and milk production. Heat abatement strategies, which are largely  
41 restricted to intensive production systems limit the losses associated with heat stress. Despite  
42 implementation of heat abatement, in 2003 production losses cost \$897 million, \$369 million, \$299 million,  
43 and \$128 million for the U.S. dairy, beef, swine, and poultry industries, respectively (St-Pierre et al., 2003).  
44 By 2019, the dairy industry's annual heat induced economic loss had risen to \$1.2 billion (Key and  
45 Sneeringer, 2014). This cost is predicted to keep rising throughout the 21<sup>st</sup> century with climate change  
46 (Gunn et al., 2019). Although the economic costs are robust, the water intensive nature of most heat  
47 abatement strategies exacerbates the environmental impact of animal production. With water shortages  
48 common to many areas and environmental concerns of agriculture runoff, the development of approaches to  
49 limit production losses while restricting water use is essential.

50 The economic losses of billions of USD are due to depressed growth and milk production. Pair-  
51 feeding studies establish that across species the heat induced depression in growth is nearly entirely  
52 attributable to hypophagia (O'Brien et al., 2010; Zeferino et al., 2016; Zhao et al., 2018). Thus, by  
53 understanding the mechanism behind heat stress hypophagia we may be able to restore growth. The  
54 decrease in milk production that results from heat exposure is multi-faceted. It can be broken into short-  
55 term hypogalactia or depressed mammary development and hypophagia independent or dependent. In  
56 short-term hypogalactia 50% of the decrease in milk yield is a response to hypophagia (Rhoads et al., 2009;  
57 Wheelock et al., 2010). Heat stress during the critical stages of mammary gland development (prior to and  
58 immediately following parturition) dramatically affects mammary gland development and milk production  
59 throughout lactation (Collier et al., 1982; Tao et al., 2011). The role of decreased energy intake on this muted  
60 mammary gland development has not been isolated from possible hypophagia independent effects.

61 Rodents display a heat induced reduction in feed intake that mimics that in production animal  
62 species (Hepler et al., 2016; Huynh et al., 2005; Lu, 1989; Morera et al., 2012; Spiers et al., 2004).  
63 Moreover, rodents recapitulate the increased sensitivity to elevated external temperature during  
64 lactation and gestation observed in high producing dairy cows (Gantner et al., 2017; Simons et al., 2011;  
65 Tao et al., 2011). With the conservation of phenotype, the genetic, pharmacological, and surgical models  
66 available in rodents may open the door for research aimed at understanding the mechanism by which  
67 heat depresses food intake, milk production, and late gestation mammary development. Accordingly,

68 we report the development of mouse models to assess the food intake dependent and independent effects  
69 of heat on milk production and mammary development.

70

## 71 **MATERIALS AND METHODS**

### 72 **Animals**

73 Male and female C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME)  
74 and singly housed so that we could assess individual food intake. Mice were maintained in a 14h light,  
75 10h dark light cycle. All experimental protocols were approved by the Institutional Animal Use and  
76 Care Committee at the University of Arizona.

77

### 78 **Food intake experiment**

79 Control mice were housed at the control (CTL) environment (22°C, 50% relative humidity) and  
80 given ad libitum access to NIH-31 chow (Harlan Laboratories, Indianapolis, IN) and water. Food and  
81 water weights were measured at 0600 h and 1800 h daily to measure day and night consumption. Heat  
82 stress (HS) mice were placed in a heat chamber (Coy Lab Products, Grass Lake, MI) set at 35°C and  
83 50% humidity. These environmental settings have been shown to suppress food intake in mice  
84 previously (Hepler et al., 2016; Morera et al., 2012).

85

### 86 **Lactation experiment**

87 Eight-month-old multiparous females were used for these studies to ensure that mammary gland  
88 development was not limiting milk production. Within one day post-parturition, litters were culled to 6  
89 pups. Dams with a litter of only 5 pups remained on the study, while those with smaller litters were  
90 culled. Pair-fed (PF) mice were housed in the control environment, but fed to match food consumed by  
91 HS mice. To match diurnal food consumption patterns, ~25% of daily feed allotment was provided to  
92 PF mice at 1000 h and ~75% at 1800 h. Food intake, water intake, body mass and litter mass were  
93 recorded daily at 1000 h from 4 to 11 days postpartum (dpp). Treatments (CTL, HS or PF) began at 5  
94 dpp. The weigh-suckle-weigh method was employed to assess milk production (Hernandez et al., 2012).  
95 Briefly, pups were separated from dams for 4 h (1000 h to 1400 h). At the end of this 4 h separation, the  
96 litter was weighed and transferred back into the original cages with the dam. After 1h, the litter was  
97 again weighed. The litter mass gain during the 1 h of suckling acted as a proxy of dam milk production.

98

## 99 **Mammary gland experiment**

100 We used 4 months old virgin females to assess the effect of heat stress on mammary gland  
101 development. To time breeding, 2~4 females were group housed for two weeks, exposed to bedding  
102 from a male cage for 3 days, then individually exposed to a male for 24 h. Females were weighed on 6,  
103 8, 10, and 12 days post coitum (dpc) to assess pregnancy status. The females that gained substantial  
104 body weight were considered pregnant (Heyne et al., 2015). Food, water and mice were weighed daily  
105 at 1000 h from 13 dpc to parturition. Treatments (CTL, HS and PF) were initiated on 14 dpc. On the  
106 day of delivery, we assessed litter size, litter mass, and pup survival rate. Pups were sacrificed by  
107 decapitation. Dams were sacrificed by decapitation under isoflurane anesthesia. Pair 2, 3, 4, and 5  
108 mammary glands were dissected and weighed as previously described (Plante et al., 2011).

109

## 110 **Measuring lactogenesis of mammary glands**

111 *Ex vivo* mammary gland lactogenesis was measured as previously reported with minor  
112 modifications (Mellenberger et al., 1973). Pair 4 and 5 mammary glands were sliced at an average  
113 thickness of 0.2 mm with a Thomas tissue slicer (Thomas Scientific, Swedesboro, NJ). Two slices of  
114 tissue from each gland were weighed and put into individual wells of a 24 well plate with 1 mL of  
115 Krebs-Ringer bicarbonate buffer without glucose. Slices were incubated at 37 °C with 5% CO<sub>2</sub> for 0.5  
116 h to allow for release of endogenous lactose within the tissue. Subsequently, tissue slices were  
117 transferred into another well containing 0.5 ml of Krebs-Ringer bicarbonate buffer supplemented with  
118 10 mM glucose and 5 µg/ml of insulin (Sigma-Aldrich, St. Louis, MO) for a 3h incubation at 37 °C  
119 with 5% CO<sub>2</sub>. We collected media to assess lactose using a commercial assay kit (BioVision, Milpitas,  
120 CA). Lactose was corrected for tissue mass.

121

## 122 **Metabolic rates of mammary glands**

123 We used a resazurin based assay that measures reducing equivalent production to assess mammary  
124 gland metabolic function (Beckett et al., 2018; Renquist et al., 2013). 1-3mg mammary tissue biopsies  
125 were isolated from mammary glands and weighed. Two biopsies from each gland were placed into 0.3  
126 ml of pre-incubation medium (Dulbecco's Modified Eagle's Medium without glucose and phenol red  
127 (Bio5, University of Arizona, Tucson, AZ) supplemented with 1mg/ml BSA (Sigma-Aldrich), 0.1%

128 DMSO and 1% Penicillin-Streptomycin solution (Thermo, Waltham, MA)) in a 96-well plate for 0.5h  
129 at 37 °C with 5% CO<sub>2</sub>. After a 30-minute pre-incubation, tissue biopsies were transferred into the assay  
130 medium for a 4h incubation (pre-incubation medium supplemented with 4 mg/ml glucose and 4.3%  
131 Alamar Blue solution (Bio-rad, Hercules, CA)). Fluorescence (excitation 530nm, emission 590nm) was  
132 measured at 0 and 4h of incubation in the assay medium. Change in fluorescence from 0 to 4h/mg tissue  
133 was calculated to understand tissue metabolic function.

134

## 135 **Statistics**

136 We used the mixed procedure in SAS with main effect of treatment to analyze dependent variables  
137 that we had measured at a single timepoint, multiple comparisons were accounted for by Tukey's  
138 adjustment (SAS Institute, Cary, NC). When appropriate, we performed repeated measures analyses  
139 with the dependent variables being treatment, day and their interaction. A Bonferroni correction was  
140 used to allow for multiple comparisons. Means were considered different when corrected P-value was  
141 less than 0.05. Means ± SEM were plotted using GraphPad PRISM (GraphPad Software, San Diego,  
142 CA).

143

## 144 **RESULTS**

### 145 **Heat stress decreases food and water intake in adult male mice**

146 We measured food and water intake of adult male mice while housed at the control environment  
147 for 4 days (CTL; 4d; 22 °C, relative humidity 50 %), during 5 days exposure to heat (HS; 5d; 35 °C,  
148 relative humidity 50 %), and again at CTL during a recovery period (CTL2; 3d). Heat exposure  
149 decreased food intake by 68.4% on the first day ( $P<0.0001$ ; Fig. 1A) and heat continued to maintain  
150 food intake below that seen at CTL throughout the 5 days of heat exposure ( $P<0.05$ ; Fig. 1A). Heat  
151 stress decreased average daily food intake by 46.5% ( $P<0.05$ ; Fig. 1B). HS decreased dark cycle food  
152 intake throughout the 5 days ( $P<0.05$ ), but only affected light cycle food intake on the first day of heat  
153 exposure (Figs 1C and 1D).

154 Heat exposure less robustly affected water intake, which decreased 24.7% on the first day of HS  
155 ( $P<0.05$ ; Fig. 1E) and was not affected thereafter. Interestingly, heat did affect the diurnal pattern of  
156 water intake, decreasing water intake during the dark period and increasing water intake during the light  
157 period ( $P<0.05$ ; Figs 1G and 1H).

158 **Heat exposure depresses body weight and food and water intake in lactating female mice**

159 Heat exposure and pair-feeding decreased dam mass similarly, both decreased dam mass over the  
160 first 2 days of treatment that was maintained during the remainder of the study ( $P < 0.05$ ; Figs 2A and  
161 2B). Although the decrease in body mass was maximal after 2 days of treatment, heat exposure  
162 suppressed food intake at all treatment days resulting in a cumulative food intake that was 41.6% lower  
163 than that of CTL dams ( $P < 0.05$ ; Figs 2C and 2D). Heat exposure similarly decreased water intake  
164 throughout the study, resulting in a cumulative 42.2% decrease in water consumption ( $P < 0.05$ ; Figs  
165 2E and 2F). Although pair-feeding resulted a similar depression of body weight and food intake, water  
166 intake was only mildly depressed by pair-feeding (11.1% cumulative decrease relative to control).

167 **Heat exposure during lactation depresses milk production through food intake dependent and**  
168 **independent mechanisms.**

169 We evaluated the effect of heat exposure on lactation performance by assessing litter mass and  
170 performing daily weigh suckle weigh measurements throughout the treatment duration (Fig. 3A-D).  
171 Within two days, heat exposure significantly decreased cumulative litter mass gain, which remained  
172 depressed throughout the treatment period ( $P < 0.05$ ; Fig. 3B). After 6 days, litters from heat exposed  
173 dams had gained 35% less than those from control dams. Pair-feeding over those 6 days resulted in a  
174 20% decrease in litter mass gain, with cumulative litter mass gain significantly differing from controls  
175 from days 4-6 of treatment. Accordingly, nearly 60% of the decrease in litter mass gain was explained  
176 by decreased food intake, while approximately 40% of the heat induced decrease in litter mass gain was  
177 independent of food intake. By using a weigh-suckle-weigh method, we were able to more directly  
178 assess milk production. A single day of heat exposure significantly decreased weigh-suckle-weigh  
179 litter mass change ( $P < 0.05$ ; Fig. 3C). This heat-induced depression in litter mass weight gain during  
180 a suckling bout was maintained throughout the 6 days of heat exposure. As we previously observed  
181 with litter mass gain, pair-feeding resulted in daily weigh-suckle-weigh measures that were intermediate  
182 to those of control and heat-exposed mice. By expressing the change in mass that resulted from  
183 suckling as a cumulative measure across 6 days, we observed that heat stress decreased cumulative  
184 weigh-suckle-weigh mass change by 37.5% (Fig. 3D), very similar to the 35% decrease in total litter  
185 mass change (Fig. 3B). Pair-feeding resulted in a 17.5% decrease in cumulative weigh suckle weigh  
186 mass change (Fig. 3D), again very similar to the 20% decrease in total litter mass gain (Fig. 3B). Thus,  
187 53.3% of the heat induced decrease in weigh suckle weigh mass change was independent the depression

188 in food intake.

189 **Heat exposure during late gestation limits body weight gain and food intake without altering**  
190 **water consumption.**

191 Neither heat stress nor pair-feeding during late gestation affected dam mass (Fig. 4A). Surprisingly,  
192 pair-feeding more robustly decreased dam mass gain during late gestation (34.9%) than did heat stress  
193 (18.1%; Fig. 4B).

194 Heat exposure decreased daily food intake (Fig. 4C) on 4 out of 5 days and cumulative food intake  
195 throughout the entire study ( $P < 0.05$ ; Fig. 4D). In fact, heat exposure decreased cumulative food  
196 intake by nearly 40%. Surprisingly, heat exposure only decreased water intake on the first day of  
197 treatment ( $P < 0.05$ ; Fig. 4E). Since heat did not affect water intake after the first day of exposure  
198 there was no effect of heat on cumulative water intake in the end (Fig. 4F). Pair-feeding late gestation  
199 dams did not alter daily or cumulative water intake (Figs. 4E and 4F).

200

201 **Heat exposure during late gestation affects litter viability and mean pup mass without altering**  
202 **gestation length, litter size, or litter mass**

203 Gestation length, litter size and litter mass at birth were not affected by either heat stress or pair-  
204 feeding (Figs 5A, 5B and 5D). However, dam heat exposure decreased pup survival (60%) and mean  
205 pup mass (16 %;  $P < 0.05$ ; Figs. 5C and 5E). These effects on survival and pup mass are independent of  
206 food intake, as pair-feeding did not affect either variable.

207

208 **Heat exposure during late gestation depresses mammary gland mass and function.**

209 Heat stress and pair-feeding similarly depressed mammary gland mass at parturition ( $P < 0.05$ ; Fig.  
210 6A). Heat exposure during late gestation depressed *ex vivo* reducing equivalent production per mg  
211 mammary tissue, while there was no effect of pair-feeding ( $P < 0.05$ ; Fig. 6B). Heat exposure during  
212 the last 5-6 days of gestation decreased *ex vivo* lactose production independent of the decrease in food  
213 intake ( $P < 0.05$ ; Fig. 6C and 6D). In fact, pair-feeding did not affect either the lactose production/mg  
214 tissue or lactose production/gland. This data proposes that heat exposure decreases mammary gland  
215 mass dependent on decreased food intake, while affecting mammary function through a food intake  
216 independent mechanism.

217



## 218 **DISCUSSION**

219 The hypophagic response to heat exposure is conserved across homeothermic animals, decreasing  
220 growth and product synthesis (milk, eggs) (Barrett et al., 2019; O'Brien et al., 2010; Rhoads et al., 2009;  
221 Zhao et al., 2018). We aimed to understand the hypophagia dependent and independent effects of heat  
222 exposure during peak lactation and late gestation on milk production and mammary gland development,  
223 respectively.

224

### 225 **Relationship between hypophagia and hypogalactia under heat stress conditions**

226 In lactating mice, the energetic demands of lactation are robust. In fact, 44% of gross energy  
227 intake is used for milk production (Johnson et al., 2001). To meet the energetic demand for lactation,  
228 lactating dam food intake is nearly 3 times higher than in male mice of similar mass and 2 time higher  
229 than in the late gestation dam. Similarly robust effects of lactation on food intake have been reported  
230 in the C57Bl6/J mouse strain used here (Makarova et al., 2010). With this increased metabolic demand  
231 our lactating dams recapitulate the increased metabolic demand in the lactating cow and the resulting  
232 increased sensitivity to exogenous heat (Collier et al., 2012). As evidence, lactation rises body  
233 temperature by 1.1°C in mice maintained at 21°C causing chronic hyperthermia (Gamo et al., 2013).

234 Reduced food intake decreases the milk production across species. Consuming 32% less energy  
235 (less than 1500 kcal/d) for a week caused 15% depression in milk production in women (Strode et al.,  
236 1986). In rats, 50% food intake restriction leads to a 66% decrease in milk yield (Warman and  
237 Rasmussen, 1983). Given that heat depresses food intake, we aimed to understand the role of heat  
238 induced hypophagia in the depression of milk production. By using litter mass gain and weigh suckle  
239 weigh measures as proxies for milk production, we showed that approximately 50% of heat induced  
240 hypogalactia was independent on the heat induced depression in food intake. Our findings recapitulate  
241 findings in the dairy cow which have established a nearly identical relative role for hypophagia (50%)  
242 in heat induced hypogalactia (Rhoads et al., 2009; Wheelock et al., 2010).

### 243 **Heat exposure induced food intake depression impairs mammary gland weight but not mammary** 244 **function and fetal growth**

245 From late gestation through early lactation there is robust mammary gland expansion through  
246 proliferation (Howard and Gusterson, 2000; Knight and Peaker, 1982; Lu and Anderson, 1973;  
247 Sorensen et al., 2002). Late gestation heat stress limits mammary gland development, depressing milk

248 production throughout lactation (Collier et al., 1982; Hooper et al., 2019; Tao et al., 2011). This  
249 depression in milk production that lasts the entire lactation is economically disastrous.

250 Heat exposure during late gestation decreased dam weight gain (Fig. 4A) as previously observed  
251 in the dairy cow (Collier et al., 1982; Tao et al., 2011). We found gestational heat exposure caused a 20%  
252 reduction in mammary gland weight at parturition. Indeed, mammary cells proliferation, but not  
253 apoptosis, is vulnerable to thermal stress during pregnancy (Tao et al., 2011). Food intake and body  
254 weight gain during pregnancy are both associated with mammary gland DNA content (Kumaresan and  
255 Turner, 1968). We found that pair-feeding recapitulated the impaired mammary growth that we  
256 observed in heat-exposed animals (Fig. 6A). Mammary gland function measured as mammary gland  
257 mitochondrial activity and lactose production were impaired by heat exposure (Fig. 6B-6C). Pair-  
258 feeding did not recapitulate the heat-induced loss in function. Similarly, in the cow, heat stress  
259 decreases mRNA expression of genes involved in production of key milk proteins (casein and  
260 lactalbumin) and transport of amino acids and glucose (Gao et al., 2019) when compared to that  
261 observed in pair-fed cows. In cows, heat exposure also appears to limit the ability of the mammary  
262 gland to mobilize fatty acids from triglycerides and catabolize those fatty acid through  $\beta$ -oxidation  
263 (Adin et al., 2009; Gao et al., 2019). Accordingly, depressed mammary function appears to be a direct  
264 response to heat and may be a result of limited metabolite flux.

265 Late gestation heat stress has been reported to either shorten or not affect gestation length in human  
266 and farm animals (Collier et al., 1982; Porter et al., 1999; Tao et al., 2012; Williams et al., 2013). We  
267 found no effect of gestational heat stress on gestation length in mice. However, we did observe that late  
268 gestation heat stress depressed neo-natal mass and survival. In fact, the degree of decrease we  
269 observed (16%) is in the range (6% ~ 30%) of that observed in ruminant species (Laporta et al., 2017;  
270 Tao and Dahl, 2013). Gestational heat stress caused malfunction of placenta and decreases placental  
271 blood flow, in turn resulting in depressed fetal growth (Alexander et al., 1987; Reynolds et al., 1985).  
272 In mice, the stress hormone corticosterone reduced blood vessel density in the placenta, which led to  
273 fetal growth restriction (Vaughan et al., 2012). Since we observed normal fetal growth in pair-fed dams,  
274 nutrient accessibility to fetus rather than nutrient intake by the dam likely contributed to the fetal  
275 restriction. Depressed fetal development may negatively impact subsequent performance of the  
276 offspring. In fact, *in utero* heat stress has been shown to depress milk production and mammary gland  
277 structure in offspring's first lactation (Fabris et al., 2019; Monteiro et al., 2016; Skibieli et al., 2018).

278 Our studies establish that the mouse recapitulates the heat stress phenotypes observed in production  
279 animals. These include depressed food intake, decreased lactation that is both dependent and  
280 independent of feed intake, and depressed mammary gland development. Using this mouse model,  
281 we observed the novel finding that heat-induced depression of mammary gland mass was completely  
282 attributable to depressed feed intake, while the decreased mammary function was entirely independent  
283 of hypophagia. Our late gestation heat exposure caused restricted fetal growth and reduced liveborn  
284 rate, which is similar to that observed in farm animals (Laporta et al., 2017; Monteiro et al., 2016; Tao  
285 and Dahl, 2013). These in utero effects were independent of changes in food consumption. Together,  
286 our data validate these mouse models as valuable tools for studying the physiological responses to heat  
287 stress. Thereby opening the door for mechanistic studies using genetic and pharmacologic models to  
288 identify the mechanism by which heat exposure causes these physiological changes.

289

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293

#### 294 **Competing interests**

295 The authors declare no competing or financial interests.

296

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423

424 **FIGURE LEGEND**

425 Fig. 1. Food and water intake in 6 adult male mice (12-14 wks old) that were singly housed under  
426 control condition (CTL; 22°C, 50% humidity) from days 1-4 of the study, put in heat stress (HS; 35°C,  
427 50% humidity) conditions from days 5-9, and returned to control condition (CTL2) from days 10-12.  
428 (A-B) 24h food intake, (C-D) light cycle and dark cycle food intake. (E-F) 24h water intake, (G-H) light  
429 cycle and dark cycle water intake. \* indicates significant difference from mean of CTL (days 1-4;  $P <$   
430 0.05). -\* indicates significant differences between indicated treatments ( $P < 0.05$ ).

431  
432 Fig. 2. The response to heat stress (HS,  $n=9$ ; 35°C, 50% humidity) and pair-feeding (PF,  $n=10$ ; fed  
433 equivalent to HS mice) on from days 5-11 of lactation in multiparous dams on dam mass (A-B), food  
434 intake (C-D), and water intake (E-F). # control (CTL,  $n=10$ ; 22°C, 50% humidity)  $>$  HS and PF groups,  
435 † indicates CTL and PF  $>$  HS, ^ indicates CTL  $>$  PF  $>$  HS ( $P < 0.05$ ).

436  
437 Fig. 3. Litter mass (A-B) and lactation response (C-D) to heat stress (HS,  $n=9$ ; 35°C, 50% humidity)  
438 or pair-feeding (PF,  $n=10$ ; fed equivalent to HS mice) from days 5-11 postpartum. \* indicates CTL  
439 (control,  $n=10$ ; 22°C, 50% humidity)  $>$  HS, † indicates CTL and PF  $>$  HS, ^ indicates CTL  $>$  PF  $>$  HS,  
440 # CTL  $>$  PF and HS.

441  
442 Fig. 4. The effect of heat stress (HS,  $n=10$ ; 35°C, 50% humidity) and pair-feeding (PF,  $n=10$ ; fed  
443 equivalent to HS mice) from 14 days post coitum to parturition on dam mass (A-B), food intake (C-D),  
444 and water intake (E-F). \* indicates control (CTL,  $n=11$ ; 22°C, 50% humidity)  $>$  HS, † indicates CTL  
445 and PF  $>$  HS, # indicates CTL  $>$  PF and HS, ^ indicates CTL and HS  $>$  PF, & indicates CTL and HS  $>$   
446 PF, ‡ indicates CTL  $>$  PF.

447  
448 Fig. 5. The effect of heat stress (HS; 35°C, 50% humidity) and pair-feeding (PF; fed equivalent to  
449 HS mice) of virgin female mice from 14 days post coitum to parturition on A) gestation length, B)  
450 pups/litter, C) pup survival rate, D) litter mass, and E) mean pup mass. Numbers under the treatment  
451 denote number of biological replicates. \* indicates significant differences between treatments ( $P <$   
452 0.05).

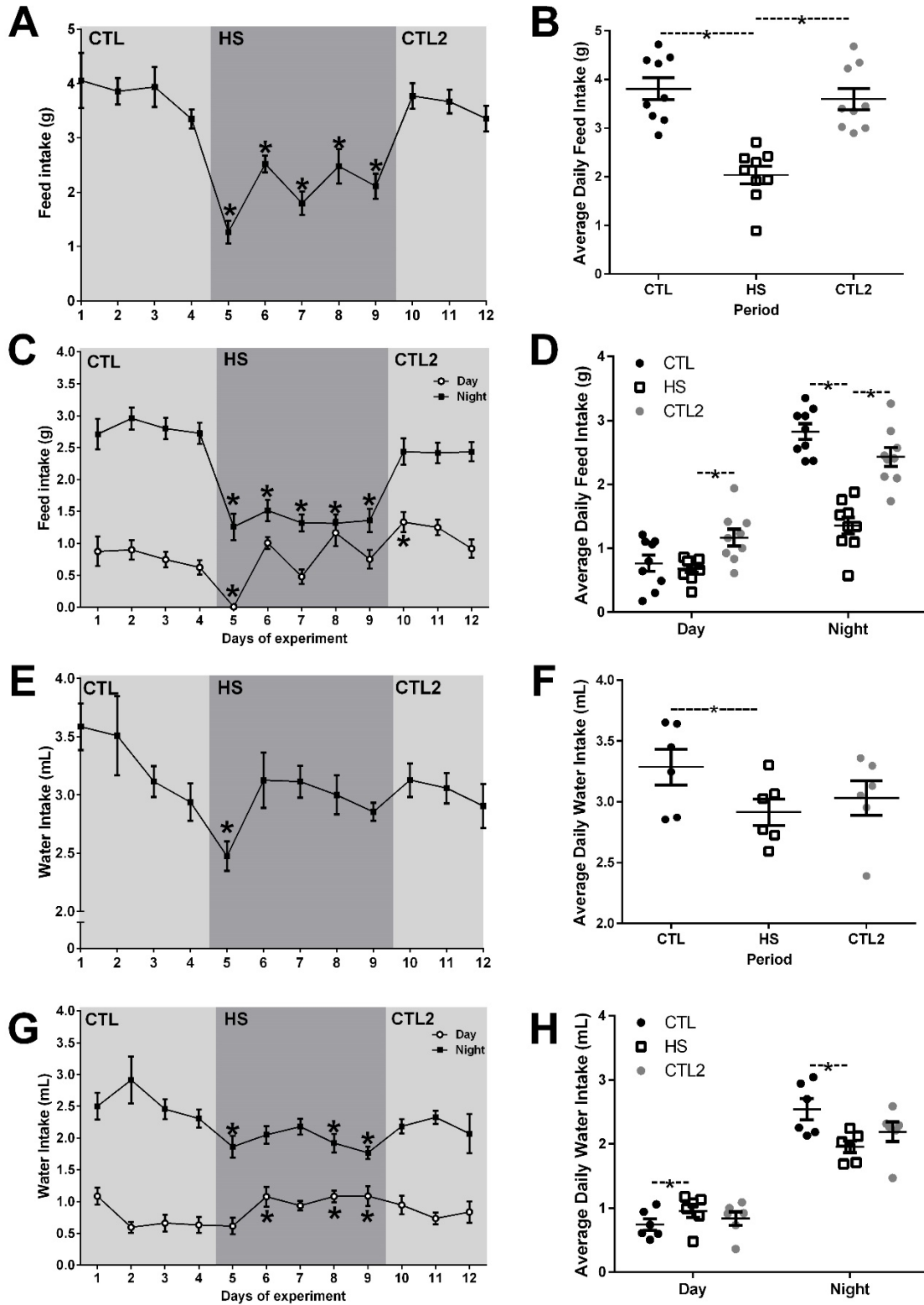
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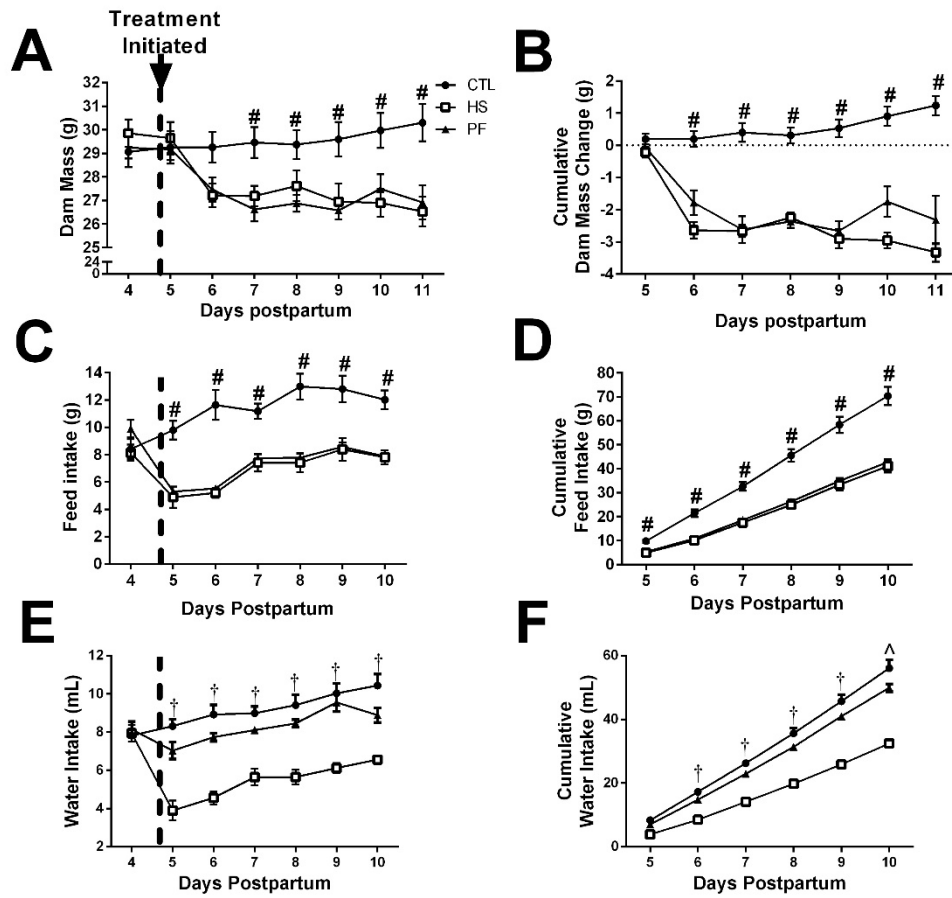
454 Fig. 6. The effect of heat stress (HS; 35°C, 50% humidity) and pair-feeding (PF; fed equivalent to  
455 HS mice) from 14 days post coitum to parturition on A) mammary gland mass, B) metabolic rate, C)  
456 lactogenesis ex vivo, D) predicted lactose production per gland. Numbers under the treatment denote  
457 number of biological replicates. \*indicates significant differences between treatments ( $P < 0.05$ ), \*\*\*  
458  $P < 0.001$ .  
459

460 FIGURES

461 Fig. 1.



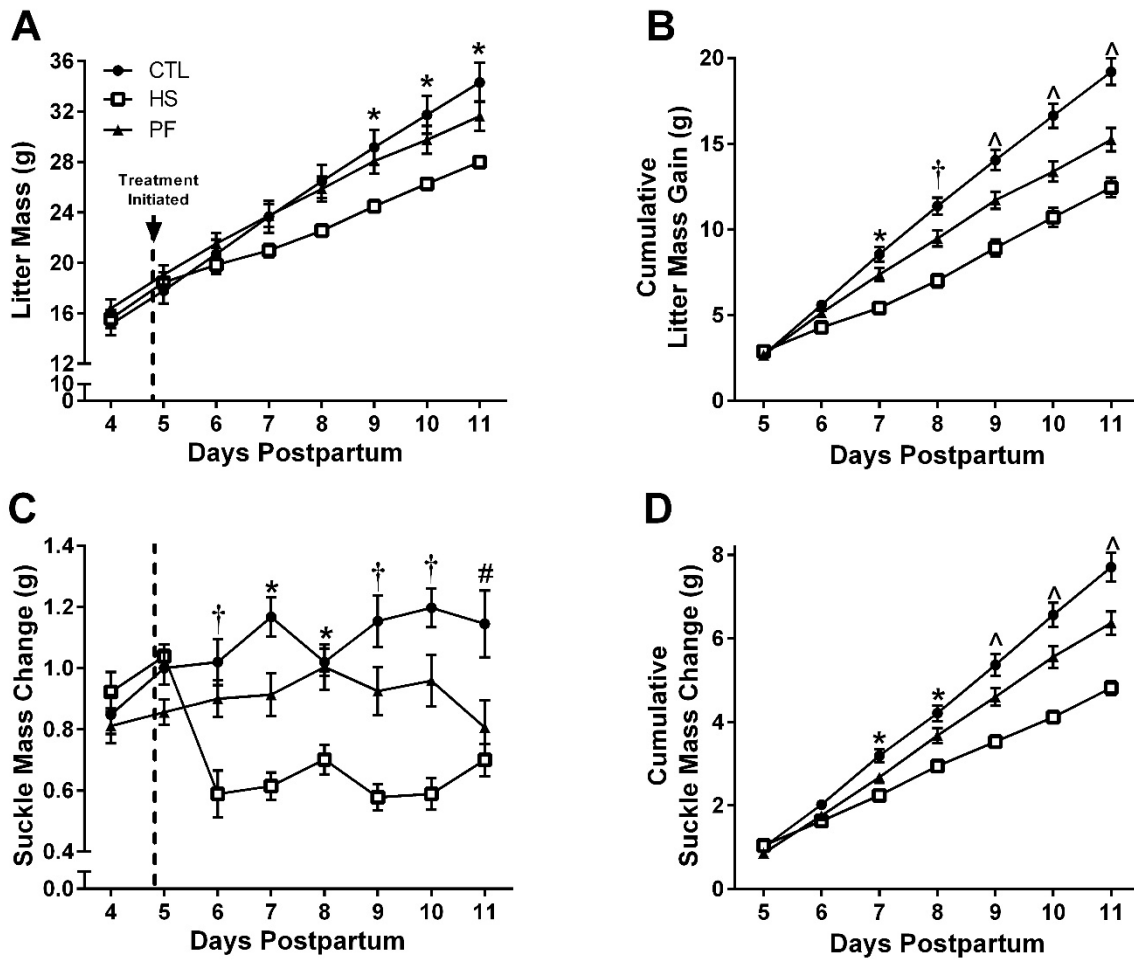
463 Fig. 2.



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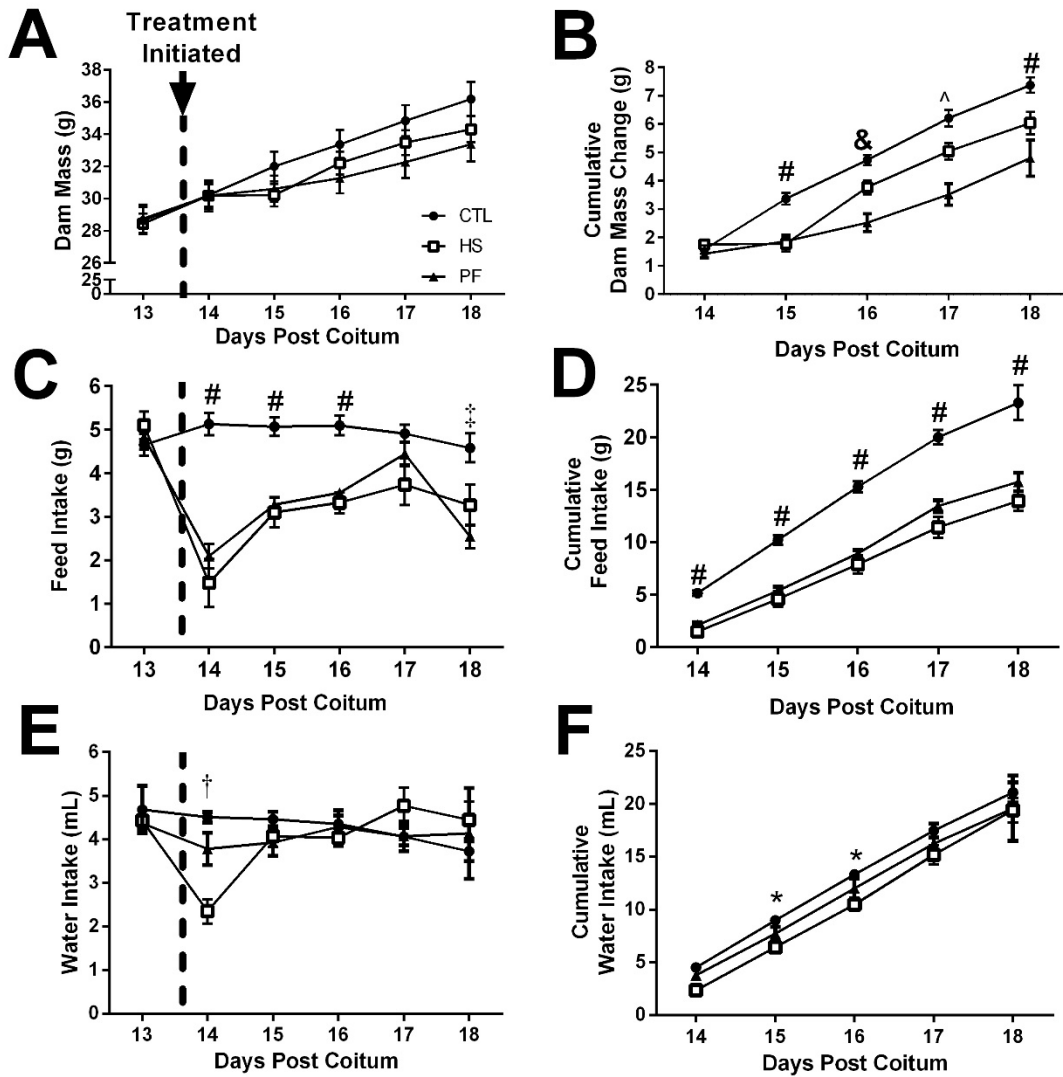
466 Fig. 3.



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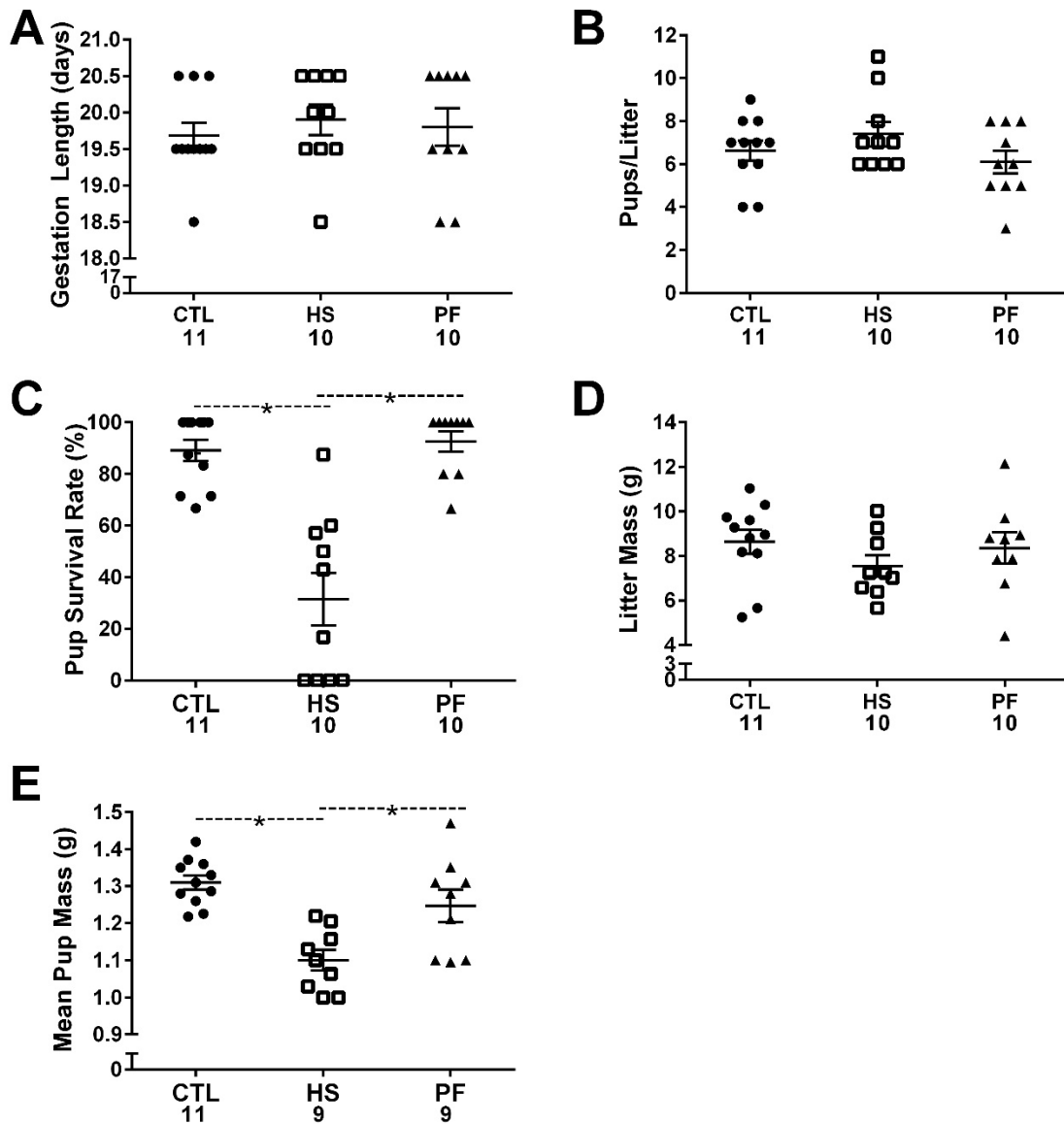
469 Fig. 4.



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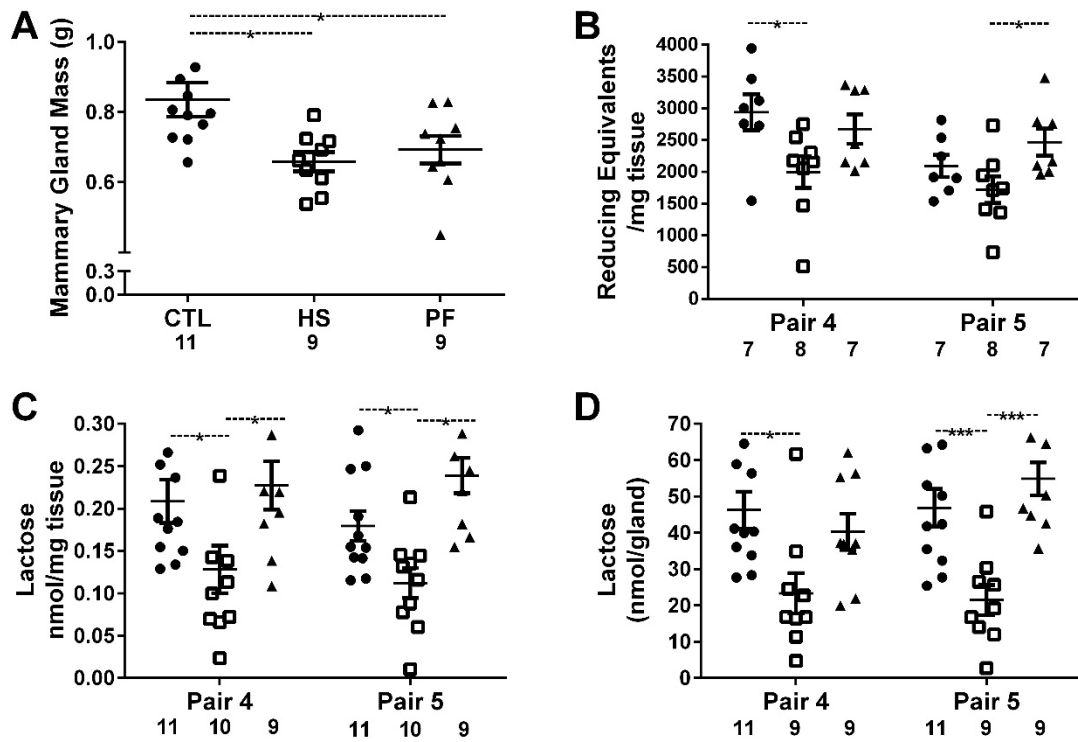
472 Fig. 5.



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475 Fig. 6.



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