Time dependent changes in feeding behavior and energy balance associated with weight gain in mice fed obesogenic diets

Payam A. Fathi[1], Michelle B. Bales[1,†], Julio E. Ayala[1,2,3*]

[1]Department of Molecular Physiology & Biophysics, Vanderbilt University School of Medicine, Nashville, TN, 37232

[2]Vanderbilt Mouse Metabolic Phenotyping Center, Nashville, TN, 37232

[3]Vanderbilt Center for Addiction Research, Nashville, TN, 37223

[†]Current address: Department of Psychology, Florida State University, Tallahassee, FL, 32306

*Corresponding author:

2201 Garland Avenue

702 Light Hall

Nashville, TN, 37232

Email: julio.e.ayala@vanderbilt.edu

Phone: (615) 323-7252

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Abstract

Obesity is characterized by dysregulated homeostatic mechanisms resulting in positive energy balance, yet when this dysregulation occurs is unknown. We assessed the time course of alterations to behaviors promoting weight gain in male and female mice switched to obesogenic 60% or 45% high fat diet (HFD). Switching mice to obesogenic diets promotes transient bouts of hyperphagia during the first 2 weeks followed by persistent caloric hyperphagia. Energy expenditure increases but not sufficiently to offset increased caloric intake, resulting in a sustained net positive energy balance. Hyperphagia is associated with consumption of calorically larger meals (impaired satiation) more frequently (impaired satiety) particularly during the light-cycle. Running wheel exercise delays weight gain in 60% HFD-fed male mice by enhancing satiation and increasing energy expenditure. However, exercise effects on satiation are no longer apparent after 2 weeks, coinciding with weight gain. Thus, exposure to obesogenic diets engages homeostatic regulatory mechanisms for ~2 weeks that ultimately fail, and consequent weight gain is characterized by impaired satiation and satiety. Insights into the etiology of obesity can be obtained by investigating changes to satiation and satiety mechanisms during the initial ~2 weeks of HFD exposure.

35 **Introduction**

36 Obesity has reached epidemic proportions worldwide, and since 2013, the American Medical
37 Association has recognized obesity as a disease state (Rosen, 2014). Significant effort has been
38 given to uncovering factors that contribute to excessive weight gain and the mechanisms that
39 become dysregulated in the setting of obesity. Put simply, obesity results from calories being
40 consumed and absorbed in excess of calories expended. Several factors and mechanisms have
41 been identified that play a role in the regulation of caloric consumption and energy
42 expenditure. These discoveries have led to a general model for the regulation of body weight
43 characterized by feedback mechanisms to maintain homeostasis (Myers et al., 2021). In this
44 model, excess weight gain triggers changes in several hormonal and neural factors that
45 primarily engage the central nervous system to subsequently reduce caloric intake and increase
46 energy expenditure, thus reducing body weight. With this framework in mind, obesity has been
47 characterized as a state associated with defects in many of these feedback mechanisms.
48 However, much of the evidence that these mechanisms are dysregulated has been obtained by
49 comparing subjects (mice, rats, humans) that are lean and those that already have obesity. Little
50 is known about events leading to obesity, such as the timing for the changes in factors that
51 contribute to excess weight gain. Characterizing the changes in parameters that regulate body
52 weight when rodents are provided access to obesogenic diets can provide insights into potential
53 behaviors and mechanisms associated with excess weight gain and obesity.

54 Studies in rodents have uncovered several factors associated with weight gain when exposed to
55 obesogenic diets. Early studies examining the effects of high-fat diets on weight gain in rats

56 showed that differences in distinct feeding behaviors influence weight gain (Drewnowski et al.,
57 1984). Subsequent studies demonstrated that rats prone to greater weight gain consume more
58 calories on a per meal basis without altering circadian meal numbers (Farley et al., 2003). A
59 sustained increase in per meal caloric content is observed in rats exposed to a long time course
60 of high-fat diet, and this is associated with an in increase in meal consumption rates without
61 changing daytime meal numbers (Furnes et al., 2009). Sex is also a key contributor to propensity
62 for weight gain, with male rodents broadly increasing overall caloric intake and relative body
63 weight more so than female rodents (Yang et al., 2014). Timing of exposure to obesogenic diets
64 also influences weight gain. Mice fed obesogenic diets only during the light phase gained more
65 weight compared to mice fed the same diet only during the dark phase (Arble et al., 2009).
66 Similarly, access to high-fat diet rapidly disrupts the circadian variability in food intake, and
67 mice no longer consume the majority of meals during the dark cycle (Espinosa-Carrasco et al.,
68 2018). Taken together, these studies suggest that factors such as meal patterns, sex, and timing
69 of food intake can influence weight gain associated with obesogenic diets.

70 While these studies provide insight into factors contributing to weight gain, specifically when
71 these factors become relevant during the progression towards obesity is not known. This
72 requires comprehensive metabolic phenotyping of longitudinal changes in male and female
73 mice switched from standard to obesogenic diets. Multiplexed indirect calorimetry systems
74 generate high-resolution, real-time datasets of multiple components that contribute to the
75 regulation of body weight. Using this technology, the present studies investigated the time scale
76 and magnitude of changes to these components during the progression towards obesity. Our
77 findings demonstrate that upon exposure to high-fat diets, behaviors and metabolic phenotypes

78 that are engaged to prevent weight gain rapidly become dysregulated. Furthermore, the
79 magnitude of these changes is influenced by the type of obesogenic diet (i.e., 60% vs. 45% high-
80 fat diet) and sex and are more apparent during specific times of the 24h cycle. These findings
81 provide a blueprint for investigating specific molecular mechanisms whose dysregulation
82 contributes to the progression towards obesity.

83

84 **Experimental Procedures**

85 ***Animals***

86 Male and female mice on a C57Bl6/J background at 10-12 weeks of age generated from in-house
87 breeding colonies were utilized in these experiments. Experimental procedures were approved
88 and conducted within the guidelines set by the Institutional Animal Care and Use Committee at
89 Vanderbilt University.

90 ***Diet Switch and Running Wheel Access Experiments***

91 Diet switch experiments: male or female mice (n=8 per group) were removed from our animal
92 colony and single-housed in Promethion Metabolic chambers (Sable Systems International) with
93 ad-lib access to chow (2.86 kCal/gram; PicoLab Rodent Diet 5L0D). Following a 5-7 day data
94 acquisition period on chow diet, mice were switched to either 60% HFD (5.24 kCal/gram;
95 Research Diets Inc, D12492) for 4 weeks or 45% HFD 4.73 kCal/gram; Research Diets Inc.
96 D12451) for 3 weeks.

97 Wheel running experiments: male mice (n=16) were removed from our animal colony and single
98 housed in Promethion Metabolic chambers with access to chow as noted previously. All mice
99 had access to locked running wheels during the chow period. Mice where then switched to a
100 60% HFD and were randomly selected to either remain with running wheels locked (n=8) or
101 have the running wheels unlocked (n=8) for the duration of the 4-week study period. Mice
102 comprising both groups were evenly distributed throughout the Promethion cabinets to
103 minimize any potential impact of cage positioning within the cabinet on data acquisition.

104 ***Metabolic Measurements***

105 Food intake, water intake, locomotor activity, respiratory exchange ratio, and energy
106 expenditure were continuously measured throughout the study period across five-minute
107 intervals. Energy balance was determined by taking the difference between energy expenditure
108 and intake across 12h light-dark cycles. Total energy expenditure over a given 12-hour
109 light:dark cycle was calculated by determining the average energy expenditure over the 12h
110 time interval and multiplying by 12. Energy intake was calculated by multiplying the mass of
111 food consumed over the corresponding 12h-intervals with the caloric density of diet consumed.
112 We would like to note that technical failure prevented us from capturing locomotor activity
113 beyond the first week in the female 60% HFD group.

114 ***Meal Parameters***

115 Meals parameters were chosen based on previous studies in rodents.(Zorrilla et al., 2005) A
116 meal was defined as any removal of food from the food hopper spanning a duration of at least
117 30 seconds and weighing a minimum of 10 milligrams to account for inherent sensor limitations

118 and a maximum of 500 milligrams to account for instances of spillage. The threshold for the
119 intermeal interval was set at 5 minutes (i.e., a meal was defined as terminated when there was a
120 period of at least five minutes of no hopper activity).

121 ***Meal Type/Pattern Analysis***

122 Meal types were defined by assembling all meal sizes consumed by male mice during the chow
123 feeding period in Promethion chambers across all experiments. Quartile distributions were used
124 to determine cut-offs for different meal types assigned from smallest (Type I) to largest (Type
125 IV) as follows: Type I: 10 mg – <60 mg; Type II: 60 mg – <160 mg; Type III: 160 mg – 280 mg; and
126 Type IV: >280 mg. Meal types were compared by determining the weekly proportion of a given
127 meal type within each animal across each week of diet access. Parts of whole analysis used
128 compiled averages of meal types across sex and diet groups. Histograms were generated using
129 bin sizes of 20 mg (mass analysis) or .2 kCal (calorie analysis) and curve-fit using non-linear
130 regression.

131 ***Statistical Analysis***

132 Raw data were processed using the Sable Systems Macro Interpreter (v23.6), assembled and
133 analyzed using Microsoft Excel. Graphs were generated and data were statistically analyzed
134 using GraphPad Prism Software (v10). Figures were assembled using Adobe Illustrator (v28.0).

135 Data were statistically analyzed using Repeated Measures One-Way ANOVAs and multiple
136 comparisons were assessed using the Tukey Test or Two-Way ANOVAs with the Sidak multiple

137 comparisons tests as necessary. Significance thresholds were set to a p-value of 0.05. Data are
138 reported as means with the standard error of the mean unless stated otherwise.

139

140 **Results**

141 ***Food-intake and metabolic phenotyping of mice switched to a 60% high-fat diet***

142 We first investigated the effect of switching mice from a chow diet to a 60% high-fat diet (60%
143 HFD) in male and female C57Bl/6J mice. Mice were switched from chow to 60% HFD while in
144 metabolic chambers and remained on the 60% HFD for 28 days. There is no appreciable increase
145 in the daily (**Figure 1A**) or weekly (**Figure 1B**) mass of food consumed throughout the 60% HFD
146 feeding period, although ~60% of male mice increased their mass intake during the first week
147 while the remaining ~40% actually reduced their mass intake (**Figure 1B**). Interestingly, when
148 analyzing the mass of food consumed by light:dark cycle, there was an increase in the daily and
149 average weekly mass of food consumed during the light cycle but not during the dark cycle in
150 male mice (**Supplemental Figure 1A-C**). Contrasting male mice, female mice decrease the mass
151 of food consumed throughout the 4-week exposure to 60% HFD and significantly so at weeks 2
152 and 3 (**Figure 1G, 1H**). This decrease in the mass of food consumed is primarily due to an effect
153 during the dark cycle (**Supplemental Figure 1D-F**).

154 When accounting for the differences in caloric density between the chow diet and the 60% HFD,
155 both sexes display a rapid and sustained increase in the total daily (**Figure 1C**, **1I**) and weekly
156 (**Figure 1D**, **1J**) calories consumed. This is accounted for by increased caloric consumption

157 during both the light and dark cycles (**Supplemental Figure 1G-L**). Analysis of daily caloric
158 intake reveals that after a rapid initial increase in caloric intake immediately following the
159 switch to the 60% HFD, there is a transient decrease during the first week of 60% HFD exposure
160 in both male and female mice (**Figure 1C, 1I**). This is followed by a second phase characterized
161 by a rapid increase in caloric intake and another attempt at lowering daily caloric intake over
162 the next 7 days. After these two cycles of elevated and then decreased caloric intake, there is a
163 sustained elevation in daily caloric intake for the remainder of the 60% HFD exposure period
164 (**Figure 1C, 1I**).

165 Switching to a 60% HFD increases daily and weekly average energy expenditure in both male
166 and female mice (**Figure 1E, 1F, 1K, 1L**) during both the light and dark cycles (**Supplemental**
167 **Figure 1M-R**). Interestingly, daily energy expenditure followed a similar pattern as daily caloric
168 intake, characterized by a rapid increase in daily energy expenditure upon switching to the 60%
169 HFD followed by a transient decrease, and then a sustained increase after the first two weeks of
170 the diet switch (**Figure 1E, 1K**). Utilizing measures of energy intake and energy expenditure we
171 calculated total energy balance across the diet exposure period. Male mice show a sustained
172 positive energy balance starting on the second week 60% HFD exposure (**Figure 1M, 1O,**
173 **Supplemental Figure 2A**), and this is associated with a significant increase in body weight
174 beginning on the second week of 60% HFD exposure (**Figure 1P**). Female mice also display a net
175 positive energy balance and weight gain upon switching to the 60% HFD, although the
176 magnitude of this effect is not as robust as in male mice (**Figure 1N, 1Q, 1R, Supplemental**
177 **Figure 2B**).

178 We assessed other behavioral and metabolic parameters in response to a switch to the 60% HFD
179 (**Supplemental Figure 2**). The respiratory exchange ratio significantly decreases upon exposure
180 to the 60% HFD in both males and females, reflecting the increased consumption of a diet
181 primarily composed of fat (**Supplemental Figure 2C-F**). This is observed during both the light
182 and dark cycles but is more robust during the dark cycle (**Supplemental Figure 1S-X**) Switching
183 to the 60% HFD also rapidly decreases water intake and locomotor activity in both males and
184 females (**Supplemental Figure 2G-N**) primarily due to changes during the dark cycle
185 (**Supplemental Figure 1Y-AJ**).

186 ***Meal pattern analysis of mice switched to a 60% high-fat diet***

187 We next assessed effects of switching male and female mice to a 60% HFD on meal patterns.
188 Meal numbers rapidly increase in both sexes and are significantly sustained throughout the 60%
189 HFD feeding period (**Figure 2A-C**). This is due to increases in meal numbers during both the
190 light and dark cycles, with a more robust effect in male mice (**Supplemental Figure 3A-F**). The
191 increase in the number of meals results in decreased inter-meal interval and meal duration,
192 particularly in male mice (**Figure 2D-I**). The decrease in inter-meal interval is most robust
193 during the light cycle (**Supplemental Figure 3G-L**), whereas the decrease in meal duration is
194 observed during both the light and dark cycles (**Supplemental Figure 3M-R**). Upon switching
195 to a 60% HFD, average meal sizes by mass decrease in both male and female mice (**Figure 2J-L**),
196 and this is mostly accounted for by a decrease in meal sizes during the dark cycle
197 (**Supplemental Figure 3S-X**). However, when accounting for the caloric density of each diet, the
198 average meal size significantly increases in male mice after the second week of 60% HFD

199 exposure but does not significantly increase in female mice (**Figure 2M-O**). The increase in the
200 caloric content per meal observed in male mice is primarily due to an increase in meal size
201 during the light cycle (**Supplemental Figure 3Y-AA**). As with total caloric intake, there are
202 transient increases and attempts to restore meal size during the first two weeks of 60% HFD
203 exposure. The rate of meal consumption by either mass or caloric content increases upon
204 switching to a 60% HFD in both males and females, with males displaying a more robust effect
205 (**Figure 2P-U**). Following from other meal pattern results, these increases are primarily
206 accounted for by effects observed during the light cycle (**Supplemental Figure 3AE-AP**).

207 ***Food-intake and metabolic phenotyping of mice switched to a 45% high-fat diet***

208 We next investigated the effect of switching mice to a 45% high fat diet (45% HFD) on
209 mechanisms and behaviors regulating body weight. When switched from chow to a 45% HFD
210 there is a consistent trend towards a decrease in total food intake by mass in both male and
211 female mice; however these differences are only significant at two weeks post diet switch
212 (**Figure 3B, 3H**). The decrease in mass consumed is primarily due to an effect during the dark
213 cycle (**Supplemental Figure 4A-F**). Accounting for the caloric value of each diet, there is a trend
214 towards an increase in caloric intake, although this is not significant in either sex (**Figure 3C,**
215 **3D, 3I, 3J**). This trend is evident during both light and dark cycles (**Supplemental Figure 4G-L**).
216 Similar to results obtained with the 60% HFD, albeit with a much lower magnitude and
217 duration, there is a transient hyperphagia followed by restoration to normal caloric
218 consumption that then gradually settles into a tendency for elevated caloric intake (**Figure 3C,**
219 **3I**).

220 When switched to a 45% HFD, both male and female mice rapidly increase energy expenditure
221 (**Figure 3E, 3F, 3K, 3L**), and this is evident during both the light and dark cycles (**Supplemental**
222 **Figure 4M-R**). Net energy balance in response to the 45% HFD switch shows a transient net
223 positive energy balance during the first week that is not significantly maintained in either sex
224 (**Figure 3M, 3N, 3O, 3Q**, **Supplemental Figure 5**). In contrast to observations made when mice
225 are switched to a 60% HFD, body weights significantly increase during each week of 45% HFD
226 diet exposure (**Figure 3P, 3R**) even though energy balance is not significantly different
227 compared to the chow period. The magnitude of weight gain in the 45% HFD fed mice is not as
228 robust as mice fed a 60% HFD suggesting that slight increases in overall energy balance are
229 sufficient to drive significant increases in body weight.

230 Similar to the observations in mice switched to a 60% HFD, switching mice to a 45% HFD
231 results in decreased respiratory exchange ratio and water intake in both males and females, but
232 there is only a significant decrease in activity in males (**Supplemental Figure 5C-N**). These
233 decreases are observed almost exclusively during the dark cycle (**Supplemental Figure 4S-AJ**).

234 ***Meal pattern analysis of mice switched to a 45% high-fat diet***

235 Meal pattern analysis of mice switched to a 45% HFD shows a rapid, yet transient increase in
236 daily meal numbers during the first week of 45% HFD exposure followed by a slow, non-
237 significant increase during subsequent weeks (**Figure 4A-C**). This pattern is observed during
238 both light and dark cycles in male mice but primarily during the dark cycle in female mice
239 (**Supplemental Figure 6A-F**). There is no significant effect of switching to a 45% HFD on inter-
240 meal intervals in either sex (**Figure 4D-F**) and during either light or dark cycle (**Supplemental**

241 **Figure 6G-L**). Similar to observations in mice switched to a 60% HFD, average meal duration is
242 significantly decreased in mice of both sexes when switched to a 45% HFD (**Figure 4G-I**), and
243 this is observed during both the light and dark cycle (**Supplemental Figure 6M-R**). Also similar
244 to switching mice to a 60% HFD, meal sizes by mass decreases in both sexes during exposure to
245 45% HFD (**Figure 4J-L**) primarily due to an effect during the dark cycle (**Supplemental Figure**
246 **6S-X**). Accounting for the caloric density results in slight increases in the average caloric intake
247 per meal (**Figure 4M-O; Supplemental Figure 6Y-AD**). Meal rates by mass and caloric content
248 significantly increase in both male and female when switched to a 45% HFD, and these
249 increases are apparent during both the light and dark cycle (**Figure 4P-U, Supplemental Figure**
250 **6AE-AP**).

251 ***Meal-typing analysis of mice switched to obesogenic diets***

252 Histograms capturing the percent frequency of meal sizes by mass and caloric content were
253 generated to more accurately describe changes that occur in feeding behaviors when mice are
254 switched to obesogenic diets. When fed a chow diet, meals of very low mass and caloric content
255 comprise the largest set of meals consumed (~15% of meals by mass and ~25% of meals by
256 calorie). The remaining meal sizes are almost equally distributed (**Figure 5A-D**). When switched
257 to a 60% HFD, male mice decrease the intake of meals of very low mass but also meals of larger
258 masses (**Figure 5A**). When accounting for the caloric density, there is a rightward shift towards a
259 larger proportion of meals with higher caloric content (**Figure 5B**). In female mice, switching to
260 60% HFD promotes a leftward shift indicating a decrease in the proportion of meals of large
261 mass and an increase the proportion of meals that are small in mass (**Figure 5C**). When

262 accounting for caloric density, there is a pronounced increase in histogram spread, indicating an
263 increase in the proportion of meals with larger caloric content (**Figure 5D**). When switched to a
264 45% HFD meal mass histograms show a consistent left-ward shift in both male and female mice,
265 and a corresponding flattening of histogram spread when examining the calories consumed per
266 meal (**Figure 5E-H**).

267 To further characterize the shifts in feeding behavior related to the mass of food consumed
268 when mice were switched to obesogenic diets, meal types were subcategorized based on the
269 quartile distribution of meal sizes by mass in chow-fed mice. These cut-offs yielded four meal
270 types: Type I (<60 mg), Type II (>60 mg – <160 mg), Type III (>160 mg < 280 mg) and Type IV
271 (>280 mg) (**Figure 5I**). Using these definitions of meal types we observe that when switched to
272 60% HFD, male mice increase the proportion of type II meals consumed while decreasing the
273 proportion of very small type I meals and large and very large type III and type IV meals,
274 respectively (**Figure 5J, 5N**). These changes are observed across both light and dark cycles
275 (**Supplemental Figure 7A-7D**). When switched to 60% HFD, female mice also increase the
276 proportion of type II meals consumed and decrease the proportion of very large type IV meals
277 consumed, but, unlike male mice, they also increase the proportion of very small type I meals
278 consumed (**Figure 5K, 5O**). These shifts are primarily observed during the dark cycle
279 (**Supplemental Figure 7E-7H**). Similar shifts than those observed in male and female mice fed
280 60% HFD are observed in mice switched to 45% HFD although the magnitude of the shift is not
281 significant (**Figure 5L, 5M, 5P, 5Q** and **Supplemental Figure 7I-7P**).

282 ***Metabolic phenotyping and meal patterns in mice switched to a 60% high-fat diet with***

283 ***concurrent running wheel access***

284 Switching male mice from a chow diet to a 60% HFD increases both caloric intake and energy

285 expenditure, yet the degree to which energy expenditure increases is not sufficient to offset the

286 increase in caloric intake (**Figure 1**). We investigated whether providing mice with access to a

287 running wheel concomitant with switching to a 60% HFD increases energy expenditure to

288 sufficiently offset the caloric content of the 60% HFD. Male mice were acclimated to cages with

289 locked running wheels during chow feeding. Mice were then switched to a 60% HFD, and

290 running wheel access was provided to a subset of mice at the time of the switch (Wheels

291 Unlocked) while the remaining mice had wheels locked through the study period (Wheels

292 Locked). Overall food intake was similar across both groups with similar patterns of transient

293 changes during the first ~2 weeks culminating in a sustained increase in daily caloric intake

294 (**Figure 6A-D**). Energy expenditure is increased to a greater degree in the Wheels Unlocked

295 group (**Figure 6E, 6F**) due exclusively to increased energy expenditure during the dark cycle

296 (**Supplemental Figure 8T**). This results in a lesser deviation towards positive energy balance in

297 mice with access to running wheels, although the net energy balance is not significantly

298 different between mice with and without access to running wheels (**Figure 6G, 6H,**

299 **Supplemental Figure 8B**). While the difference in energy balance is not statistically significant,

300 mice with running wheel access are protected against significant increases in body weight

301 during the first two weeks of 60% HFD exposure and overall gain significantly less weight

302 (**Figure 6I, Supplemental Figure 8C**). However, mice with access to running wheels do begin to

303 gain weight after the second week, and this is associated with increased caloric consumption
304 during the dark cycle (**Supplemental Figure 9A, 9B**).

305 Even though there were no differences in overall food intake, meal pattern analysis shows that
306 mice with running wheel access display a greater increase in meal number compared to mice
307 with wheels locked during the first week (**Figure 6J, 6K**). These increases are mainly driven by
308 effects during the dark cycle (**Supplemental Figure 9C**). Inter-meal interval (**Figure 6L, 6M**) and
309 meal duration (**Figure 6N, 6O**) decrease equally between both groups. Interestingly, access to
310 running wheel significantly lowers meal size by mass and calories during the first week of 60%
311 HFD exposure, and these differences are observed during both the light and dark cycles (**Figure**
312 **6P-S; Supplemental Figure 9F, 9G**). This difference in meal sizes is no longer evident from the
313 second week onwards, which is coincident with the greater deviations towards positive energy
314 balance and weight gain in mice with access to running wheels. Meal rates are not significantly
315 different between mice with and without access to running wheels (**Figure 6T-W, Supplemental**
316 **Figure 9H, 9I**). Histogram analysis of meal size by grams shows similar meal patterns across
317 both groups; however, the proportion of meals with a lower caloric content is higher in the mice
318 with running wheels blocked (**Figure 6X-AA**). Furthermore, both groups increase their
319 consumption of type II meals and decrease their consumption of very large type IV meals, but
320 mice with access to running wheels also increase the consumption of very small type I meals
321 and have a larger decrease in the proportion of very large type IV meals consumed during the
322 first week of 60% HFD exposure (**Supplemental Figure 9J-L**).

323 The respiratory exchange ratio is significantly lower in mice with running wheel access during
324 the first two weeks following diet switch (**Supplemental Figure 8D-G**). Locomotor activity (off
325 wheel) decreases in both groups, although mice with running wheel access have significantly
326 higher locomotor activity during the last two weeks of diet exposure, particularly during the
327 dark cycle (**Supplemental Figure 8L-O**). As expected, running wheel access results in
328 progressively increased wheel running activity over the first two weeks of the diet switch and
329 subsequently levels out by week four. Notably, daytime wheel running transiently increases
330 during the first two weeks (**Supplemental Figure 8P-R**).

331

332 **Discussion**

333 This study utilized metabolic phenotyping of male and female mice fed obesogenic diets to
334 observe time-dependent changes in parameters that influence body weight during the
335 progression towards obesity. Similar studies have investigated processes occurring during the
336 early phases of exposure to a high-fat diet. However, most of those studies are short-term (i.e.,
337 high-fat diet exposure for 3-7 days) and focus on effects on gross food intake as the primary
338 endpoint (Clyburn et al., 2023; Enriquez et al., 2022; Kless et al., 2017; Oraha et al., 2022). We
339 hypothesized that when placed on high-fat diets, mice would engage feedback mechanisms and
340 consequent behaviors to account for the increased caloric content of the diet in order to
341 maintain a normal body weight trajectory. Our results reveal the time course over which these
342 feedback processes are engaged and then ultimately fail, thus resulting in weight gain. These
343 important findings can be leveraged towards future studies focusing on mechanisms

344 characterizing the transition from attempts to maintain weight homeostasis to the failure of
345 these attempts. Furthermore, we provide detailed analyses of other parameters, such as meal
346 patterns, that we hypothesize are significant contributors to the propensity to gain weight.

347 When examining behavioral and metabolic differences in mice switched from chow to a 60%
348 HFD, a general pattern arises in which there is significant hyperphagia upon exposure to the
349 HFD that is rapidly followed by attempts to reduce food intake to pre-HFD exposure levels
350 during the first ~7 days, as previously shown.(Hwang et al., 2010) We extend these observations
351 to show that transient hyperphagia followed by attempts to restore normal food intake occurs
352 during the first ~14 days of HFD exposure. Following this ~2-week period, mice enter a period
353 of sustained hyperphagia. These results suggest that homeostatic mechanisms are engaged
354 during the first ~2 weeks of 60% HFD exposure, but they ultimately fail or are overtaken by
355 other contributors of feeding behavior such as hedonic mechanisms. Identifying not only the
356 mechanisms engaged during the first 2 weeks of exposure to a 60% HFD but also which of these
357 mechanisms fail or are overcome following this period will provide important information
358 about molecular targets that are essential for body weight control.

359 It is important to note that hyperphagia associated with exposure to a 60% HFD is in terms of
360 caloric intake but not mass consumed. Indeed, following a transient increase in the mass of food
361 consumed during the first 24h of 60% HFD exposure in male mice, as previously shown
362 (Clyburn et al., 2023; Enriquez et al., 2022), the average daily mass of food consumed gradually
363 decreases slightly. In female mice the average daily mass of food consumed decreases upon
364 switching to a 60% HFD, and this remains consistent for most of the study period. Similar

365 effects were observed in male and female mice switched to a 45% HFD, albeit to a lesser
366 magnitude. These observations suggest that mice can sense changes in diet caloric content and
367 adjust their intake accordingly. However, this adjustment is not sufficient to fully compensate
368 for the caloric density of the diet. Studies suggest that both humans and mice can perceive the
369 caloric density of ingested foods (Flynn et al., 2022; Hu et al., 2018; Su et al., 2017). Similar to
370 what we have shown, previous studies demonstrate that mice do not appreciably increase their
371 total food intake by mass when placed on obesogenic diets, and they actually decrease the total
372 mass of food consumed as the percentage of dietary fat exceeds 60% (Hu et al., 2018).
373 Identifying mass-sensing (e.g., mechanosensing) vs. calorie-sensing molecular mechanisms and
374 how these mechanisms are coupled or uncoupled could provide valuable insight into
375 approaches for restraining excess caloric intake in the presence of obesogenic diets.

376 Studies in rodents suggest that meal size by caloric content is a strong predictor of weight gain
377 (Furnes et al., 2009; Melhorn et al., 2010). Our findings show that meal size by calorie increases
378 immediately upon exposure to 60% HFD, with initial attempts to lower meal size during the
379 first ~2 weeks of exposure that eventually fail. This was most strongly evident in male mice
380 switched to a 60% HFD with a lesser effect in female mice and mice of both sexes switched to a
381 45% HFD. Interestingly, average meal size by mass actually tended to decrease, thus
382 contributing to the overall decrease in average daily mass consumed (see above). However,
383 analysis of meal type by mass shows that while chow-fed mice primarily eat very small meals
384 (what we refer to as Type I, <60 mg meals) with remaining meals being somewhat evenly
385 distributed by size, switching to a 60% HFD compresses this distributions by increasing the
386 relative consumption of what we refer to as Type II (<160 mg) meals at the expense of both very

387 small Type I meals and large/very large (Type III, <280 mg and IV, >280 mg) meals. When
388 accounting for the caloric content of the diet, this effect of exposure to a 60% HFD results in a
389 shift towards consumption of larger meals by calorie. This increase in meal size suggests that
390 switching to a HFD impairs mechanisms that regulate satiation. Switching mice to a HFD also
391 increased the number of meals consumed per day and, consequently, reduced the time between
392 meals. This suggests an impairment in mechanisms regulating satiety. Given the relationship
393 between meal patterns, particularly meal size, and propensity for weight gain (Furnes et al.,
394 2009; Melhorn et al., 2010), these findings further suggest that emphasis should be given to
395 investigating mechanisms regulating satiation and satiety and how these mechanisms become
396 dysregulated following ~2 weeks exposure to HFD.

397 Analysis of feeding behavior by light:dark cycle shows that, when switched to a 60% HFD, male
398 mice increase food intake primarily during the light cycle due to an increase in meal numbers
399 without compensatory changes in average meal size. The relative importance of meal timing has
400 been noted in multiple human and rodent studies. A study in rats showed that increases in
401 caloric intake were driven by inactive phase (light cycle) food consumption and removing food
402 access during this period blunts weight gain even though total caloric consumption remains
403 similar between groups (Olsen et al., 2017). Similarly mice fed a HFD during the inactive phase
404 (i.e., light cycle) gained more weight than those fed the same diet during the active phase (i.e.,
405 dark cycle) (Arble et al., 2009). Furthermore, human studies show that caloric consumption later
406 in the day is associated with greater weight gain (Bo et al., 2014; Jakubowicz et al., 2013;
407 Ruddick-Collins et al., 2022; Zhao et al., 2023). These results suggest that the increased caloric
408 consumption during the light phase in mice switched to HFD may contribute to weight gain in

409 male mice. Contrasting the findings in male mice, female mice increase meal numbers during
410 both cycles but reduce the mass of food consumed during the dark cycle, suggesting that female
411 mice are better able to recognize the caloric density of food and adjust nighttime feeding
412 accordingly (Goldstein et al., 2021; Su et al., 2017). Interestingly, meal duration during the light
413 cycle decreases in both male and female mice. This coupled with the fact that in male mice light
414 cycle meal numbers increased and average meal size did not change would indicate that male
415 mice eat larger meals (by calorie) in a shorter period. This is further reflected through a
416 significant increase in light cycle meal rates. Ultimately, the propensity of male mice to consume
417 more frequent shorter meals with higher caloric content per meal during the light cycle may be
418 a significant contributor to their greater weight gain. Cycle-dependent effects on food intake
419 and meal patterns were similar in mice switched to 45% HFD, although effects were less robust.
420 These smaller effects relative to those observed in mice fed 60% HFD likely contribute to the
421 delay in weight gain onset in mice fed a 45% HFD. In sum, changes to overall caloric intake
422 when fed HFD are driven by consumption of more frequent and shorter meals with higher
423 caloric content particularly during time spans during which significant food intake typically
424 does not occur in chow-fed mice (i.e., light cycle). Importantly, these changes follow a pattern
425 suggesting attempts to maintain homeostasis during the first two weeks of obesogenic diet
426 exposure that eventually fail.

427 Male mice, particularly of the C57Bl/6 background, are prone to weight gain on a HFD
428 compared to female mice. We observe that male mice display more robust caloric hyperphagia
429 and increases in meal size relative to female mice when switched to a HFD. Regarding meal
430 size, both sexes increase the relative intake of Type II meals, but this increase is comparatively

431 greater in male mice. This suggests that while exposure to 60% HFD impairs satiation in both
432 sexes, this effect is greater in male mice. A similar observation is made when examining the
433 impact of 45% HFD in that male mice show no significant change to the proportion of meal
434 types whereas females increase the proportion of very small Type I meals while decreasing the
435 proportion of larger Type III and IV meals. This suggests that in response to 45% HFD, satiation
436 is somewhat preserved in male mice but is actually enhanced in female mice. Overall, this
437 further suggests that the propensity towards weight gain in response to HFD feeding is
438 associated with impairments in both satiety and satiation that depend on the dietary fat content
439 and differ according to sex. These sex differences can be leveraged to investigate potential
440 mechanisms regulating meal patterns. For example, previous studies suggest that sex
441 differences in feeding behavior may due to steroid hormone signaling (Alonso-Caraballo and
442 Ferrario, 2019; Lenert et al., 2021; Petersen, 1976). Additionally, differences in energy
443 expenditure may also contribute to sex-dependent differences on weight gain (see below).

444 Our observed changes to satiety and satiation fall in line with the idea that obesogenic diet
445 exposure rapidly impairs: 1- the latency or ability to appropriately convey hunger signals
446 (satiety) and 2- the fidelity of- or ability to process signals that would appropriately account for
447 both mass and caloric density to regulate meal size (satiation). Obesogenic diets have been
448 shown to impact satiety by impairing vagal afferent responsiveness to distension as well as
449 change gastric emptying (Kentish et al., 2015; Loper et al., 2021). Previous studies also show
450 decreased responsiveness to cholecystokinin (CCK)-induced satiation in male mice fed a 45%
451 HFD (Huang et al., 2020). Importantly, HFD impairs vagal afferent circadian rhythms (Kentish
452 et al., 2018, 2016), and this may contribute in part to our observed increases in daytime food

453 intake. Interventions used to increase intestinal glucagon-like peptide-1 (GLP-1) levels have
454 been associated with decreased inactive phase food-intake in mice in a vagal GLP-1 receptor-
455 mediated manner, presumably through modulating meal termination (Borgmann et al., 2021;
456 Iwasaki et al., 2018). Thus, future studies should address whether defects in these mechanisms
457 occur in a time scale corresponding to the changes in feeding behavior we demonstrate in the
458 present studies.

459 Concomitant with an increase in caloric consumption, energy expenditure also increases in both
460 male and female mice when switched to a 60% HFD. This increase in energy expenditure
461 further adds to the notion that mice can sense the difference in the caloric content of HFD
462 relative to chow and attempt to compensate for this, much like the reduction in mass of food
463 consumed. Similarly to the latter, however, the increase in energy expenditure is not sufficient
464 to compensate for the increased caloric intake. The net result of this is that following the first ~2
465 weeks of exposure to 60% HFD, mice display a relative and consistent positive daily energy
466 balance of ~5 kCal/day for males and ~3 kCal/day for females. Interestingly, energy expenditure
467 also increases when mice are switched to a 45% HFD, and the magnitude of this increase is
468 similar to what is observed in mice switched to a 60% HFD. This, combined with a lesser degree
469 of caloric hyperphagia (see above) results in smaller deviations towards positive energy
470 balance, thus contributing to the reduced weight gain associated with 45% HFD relative to 60%
471 HFD exposure. Male and female mice experience quantitatively similar increases in energy
472 expenditure. Oraha et al. and Huang et al. (Huang et al., 2020; Oraha et al., 2022) suggest that
473 protection from weight gain in female relative to male mice is heavily driven by relative
474 increases in energy expenditure and the ability of female mice to better utilize fat. Our data

475 support this by showing lower relative respiratory exchange ratio in female compared to male
476 mice switched to a 60% HFD. When switched to a 45% HFD both males and females lower
477 respective respiratory exchange ratio to similar levels. This contrasts findings from Huang et al.
478 study (Huang et al., 2020) that used the same 45% HFD but suggest that female mice rely more
479 on fat oxidation. These discrepancies may stem from methodological differences since in our
480 studies, mice were switched to the 45% HFD while obtaining metabolic chamber measurements
481 whereas the studies of Huang et al. performed metabolic chamber measurements after mice had
482 been on the 45% HFD for 5 weeks. Nevertheless, we find that when exposed to a 45% HFD, the
483 propensity towards weight gain in 45% HFD male mice compared to female mice is associated
484 with changes in specific aspects of feeding behaviors in conjunction with alterations in energy
485 balance. This is in agreement with recent studies showing that female mice balance energy
486 expenditure and intake more efficiently than do male mice (Morris et al., 2020).

487 Energy expenditure increased in response to HFD exposure even as locomotor activity
488 decreased, as previously observed (Bjursell et al., 2008; Kless et al., 2017). Given the dramatic
489 sustained decrease in locomotor activity concurrent with switching to a HFD, we hypothesized
490 that this constrains energy expenditure and likely contributes to the observation that energy
491 expenditure does not increase sufficiently to account for increased caloric intake. A previous
492 study showed that decreased locomotor activity in mice fed a 40% HFD accounted for over 60%
493 of the bodyweight gain in these mice (Bjursell et al., 2008). We examined whether wheel
494 running access would increase energy expenditure sufficiently to modify weight gain
495 trajectories. Wheel running access provides a relative protection against weight gain in male
496 mice fed a 60% HFD (Cho et al., 2023), as we also show here. Our results show that the shortfall

497 between energy expenditure and caloric intake is partially mitigated in male mice given access
498 to a running wheel concomitant with exposure to a 60% HFD, and this is associated with a
499 greater increase in energy expenditure. However, this only leads to a delay in weight gain for
500 the first ~2 weeks of exposure to the HFD. This resistance to weight gain during early time
501 points of HFD exposure in the wheel running group was associated with specific changes in
502 feeding behaviors. While total energy intake was not different between the groups, wheel
503 running mice significantly increased meal numbers during the dark cycle with a corresponding
504 decrease in meal sizes across both the light and dark cycle. This suggests that wheel running
505 enhances satiation at the expense of satiety. In further support of this, mice with running wheel
506 access had an increased proportion of Type I very small meals. Following the first 2 weeks,
507 caloric intake was actually increased in mice with access to a running wheel during the dark
508 cycle relative to mice with no wheel access. This partially offset the significant increase in
509 energy expenditure observed in mice given access to running wheels and likely contributed to
510 the initiation of weight gain in this group. Meal patterns became indistinguishable between
511 mice with and without access to running wheels, and this corresponded to the period during
512 which mice with access to running wheels were no longer protected from weight gain. This
513 further emphasizes the potential important role of meal size as a contributor to weight gain in
514 the setting of consuming obesogenic diets. Untangling the mechanisms mediating the satiation
515 may prove useful in identifying molecular targets that can be leveraged to modulate weight
516 gain by controlling feeding behaviors.

517 Access to a running wheel is associated with subsequent decreases in off wheel locomotor
518 activity in mice fed a chow diet, suggesting the presence of a counterregulatory mechanism that

519 serves to stabilize the exercise driven increase in energy expenditure (Lark et al., 2018).
520 Similarly, the increase in energy expenditure resulting from wheel running plateaus after one
521 week even though on-wheel activity increases in mice fed a chow diet and decreases over time
522 in mice fed a HFD (O'Neal et al., 2017; Vellers et al., 2017). We expand on this by showing that
523 when switched to a 60% HFD, male mice rapidly increase energy expenditure resulting from an
524 increase in wheel running activity exclusively during the dark cycle that peaks by week 2. While
525 off-wheel locomotor activity decreased in mice with or without access to a running wheel, mice
526 with access to a running wheel displayed greater off-wheel locomotor activity, particularly
527 during the dark cycle, beginning around week 2 of the HFD exposure. Interestingly, this
528 coincided with a slight decrease in wheel running activity. The compensation of off-wheel
529 locomotor activity acts to preserve the increase in energy expenditure.

530 In sum, our study provides insights into how time-dependent changes in feeding behaviors
531 following obesogenic diet exposure can modify weight gain. Specifically, we found that
532 following exposure to HFD, mice engage mechanisms in an attempt to maintain homeostasis
533 and prevent weight gain during the first two weeks of diet exposure, but these mechanisms
534 eventually give way to the cumulative compounding effect of small increases in positive energy
535 balance and, thus, weight gain. Importantly, this positive energy balance is associated with
536 increases in the average calories consumed per meal. Wheel running activity increases energy
537 expenditure and prevents an increase in meal size, but this effect on meal size is temporary and
538 is only sufficient to delay but not prevent weight gain on obesogenic diets. These findings
539 provide valuable insight for future studies focusing on mechanisms that regulate satiation as
540 important regulators of weight gain and in particular highlight the value of investigating how

541 these mechanisms are affected during the transition from initial attempts to maintain neutral
542 energy balance to the eventual development of consistent positive energy balance.

543

544 ***Author Contributions***

545 Conceptualization: JEA, MBB, PF; Methodology: JEA, MBB, PF; Analysis: JEA, PF; Writing: JEA,
546 PF; Funding: JEA. All authors reviewed and have approved the final version of this manuscript.

547

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554

555 **References**

556 Alonso-Caraballo Y, Ferrario CR. 2019. Effects of the estrous cycle and ovarian hormones on
557 cue-triggered motivation and intrinsic excitability of medium spiny neurons in the Nucleus
558 Accumbens core of female rats. *Horm Behav* **116**:104583. doi:10.1016/j.yhbeh.2019.104583

559 Arble DM, Bass J, Laposky AD, Vitaterna MH, Turek FW. 2009. Circadian timing of food intake
560 contributes to weight gain. *Obesity (Silver Spring)* **17**:2100–2. doi:10.1038/oby.2009.264

561 Bjursell M, Gerdin A-K, Lelliott CJ, Egecioglu E, Elmgren A, Törnell J, Oscarsson J, Bohlooly-Y
562 M. 2008. Acutely reduced locomotor activity is a major contributor to Western diet-induced
563 obesity in mice. *Am J Physiol Endocrinol Metab* **294**:E251-60. doi:10.1152/ajpendo.00401.2007

564 Bo S, Musso G, Beccuti G, Fadda M, Fedele D, Gambino R, Gentile L, Durazzo M, Ghigo E,
565 Cassader M. 2014. Consuming more of daily caloric intake at dinner predisposes to obesity.
566 A 6-year population-based prospective cohort study. *PLoS One* **9**:e108467.
567 doi:10.1371/journal.pone.0108467

568 Borgmann D, Ciglieri E, Biglari N, Brandt C, Cremer AL, Backes H, Tittgemeyer M, Wunderlich
569 FT, Brüning JC, Fenselau H. 2021. Gut-brain communication by distinct sensory neurons
570 differently controls feeding and glucose metabolism. *Cell Metab* **33**:1466-1482.e7.
571 doi:10.1016/j.cmet.2021.05.002

572 Cho C, Ji M, Cho E, Yi S, Kim JG, Lee S. 2023. Chronic voluntary wheel running exercise
573 ameliorates metabolic dysfunction via PGC-1α expression independently of FNDC5/irisin

574 pathway in high fat diet-induced obese mice. *J Physiol Sci* **73**:6. doi:10.1186/s12576-023-
575 00864-6

576 Clyburn C, Carson KE, Smith CR, Travagli RA, Browning KN. 2023. Brainstem astrocytes
577 control homeostatic regulation of caloric intake. *J Physiol* **601**:801–829. doi:10.1113/JP283566

578 Drewnowski A, Cohen AE, Faust IM, Grinker JA. 1984. Meal-taking behavior is related to
579 predisposition to dietary obesity in the rat. *Physiol Behav* **32**:61–67. doi:10.1016/0031-
580 9384(84)90071-4

581 Enriquez JR, McCauley HA, Zhang KX, Sanchez JG, Kalin GT, Lang RA, Wells JM. 2022. A
582 dietary change to a high-fat diet initiates a rapid adaptation of the intestine. *Cell Rep*
583 **41**:111641. doi:10.1016/j.celrep.2022.111641

584 Espinosa-Carrasco J, Burokas A, Fructuoso M, Erb I, Martín-García E, Gutiérrez-Martos M,
585 Notredame C, Maldonado R, Dierssen M. 2018. Time-course and dynamics of obesity-
586 related behavioral changes induced by energy-dense foods in mice. *Addiction Biology*
587 **23**:531–543. doi:10.1111/adb.12595

588 Farley C, Cook JA, Spar BD, Austin TM, Kowalski TJ. 2003. Meal pattern analysis of diet-
589 induced obesity in susceptible and resistant rats. *Obes Res* **11**:845–851.
590 doi:10.1038/OBY.2003.116

591 Flynn AN, Hall KD, Courville AB, Rogers PJ, Brunstrom JM. 2022. Time to revisit the passive
592 overconsumption hypothesis? Humans show sensitivity to calories in energy-rich meals.
593 *Am J Clin Nutr* **116**:581–588. doi:10.1093/ajcn/nqac112

594 Furnes MW, Zhao C-M, Chen D. 2009. Development of obesity is associated with increased
595 calories per meal rather than per day. A study of high-fat diet-induced obesity in young
596 rats. *Obes Surg* **19**:1430–8. doi:10.1007/s11695-009-9863-1

597 Goldstein N, McKnight AD, Carty JRE, Arnold M, Betley JN, Alhadeff AL. 2021. Hypothalamic
598 detection of macronutrients via multiple gut-brain pathways. *Cell Metab* **33**:676-687.e5.
599 doi:10.1016/J.CMET.2020.12.018

600 Hu S, Wang L, Yang D, Li L, Togo J, Wu Y, Liu Q, Li B, Li M, Wang G, Zhang X, Niu C, Li J, Xu
601 Y, Couper E, Whittington-Davies A, Mazidi M, Luo L, Wang S, Douglas A, Speakman JR.
602 2018. Dietary Fat, but Not Protein or Carbohydrate, Regulates Energy Intake and Causes
603 Adiposity in Mice. *Cell Metab* **28**:415-431.e4. doi:10.1016/j.cmet.2018.06.010

604 Huang K-P, Ronveaux CC, Knotts TA, Rutkowsky JR, Ramsey JJ, Raybould HE. 2020. Sex
605 differences in response to short-term high fat diet in mice. *Physiol Behav* **221**:112894.
606 doi:10.1016/j.physbeh.2020.112894

607 Hwang L-L, Wang C-H, Li T-L, Chang S-D, Lin L-C, Chen C-P, Chen C-T, Liang K-C, Ho I-K,
608 Yang W-S, Chiou L-C. 2010. Sex Differences in High-fat Diet-induced Obesity, Metabolic
609 Alterations and Learning, and Synaptic Plasticity Deficits in Mice. *Obesity* **18**:463–469.
610 doi:10.1038/oby.2009.273

611 Iwasaki Y, Sendo M, Dezaki K, Hira T, Sato T, Nakata M, Goswami C, Aoki R, Arai T, Kumari P,
612 Hayakawa M, Masuda C, Okada T, Hara H, Drucker DJ, Yamada Y, Tokuda M, Yada T.

613 2018. GLP-1 release and vagal afferent activation mediate the beneficial metabolic and
614 chronotherapeutic effects of D-allulose. *Nat Commun* **9**:113. doi:10.1038/s41467-017-02488-y

615 Jakubowicz D, Barnea M, Wainstein J, Froy O. 2013. High caloric intake at breakfast vs. dinner
616 differentially influences weight loss of overweight and obese women. *Obesity (Silver Spring)*
617 **21**:2504–12. doi:10.1002/oby.20460

618 Kentish SJ, Frisby CL, Kritas S, Li H, Hatzinikolas G, O'Donnell TA, Wittert GA, Page AJ. 2015.
619 TRPV1 Channels and Gastric Vagal Afferent Signalling in Lean and High Fat Diet Induced
620 Obese Mice. *PLoS One* **10**:e0135892. doi:10.1371/journal.pone.0135892

621 Kentish SJ, Hatzinikolas G, Li H, Frisby CL, Wittert GA, Page AJ. 2018. Time-Restricted Feeding
622 Prevents Ablation of Diurnal Rhythms in Gastric Vagal Afferent Mechanosensitivity
623 Observed in High-Fat Diet-Induced Obese Mice. *J Neurosci* **38**:5088–5095.
624 doi:10.1523/JNEUROSCI.0052-18.2018

625 Kentish SJ, Vincent AD, Kennaway DJ, Wittert GA, Page AJ. 2016. High-Fat Diet-Induced
626 Obesity Ablates Gastric Vagal Afferent Circadian Rhythms. *J Neurosci* **36**:3199–207.
627 doi:10.1523/JNEUROSCI.2710-15.2016

628 Kless C, Rink N, Rozman J, Klingenspor M. 2017. Proximate causes for diet-induced obesity in
629 laboratory mice: a case study. *Eur J Clin Nutr* **71**:306–317. doi:10.1038/ejcn.2016.243

630 Lark DS, Kwan JR, McClatchey PM, James MN, James FD, Lighton JRB, Lantier L, Wasserman
631 DH. 2018. Reduced Nonexercise Activity Attenuates Negative Energy Balance in Mice
632 Engaged in Voluntary Exercise. *Diabetes* **67**:831–840. doi:10.2337/db17-1293

633 Lenert ME, Chaparro MM, Burton MD. 2021. Homeostatic Regulation of Estrus Cycle of Young
634 Female Mice on Western Diet. *J Endocr Soc* **5**. doi:10.1210/jendso/bvab010

635 Loper H, Leinen M, Bassoff L, Sample J, Romero-Ortega M, Gustafson KJ, Taylor DM, Schiefer
636 MA. 2021. Both high fat and high carbohydrate diets impair vagus nerve signaling of
637 satiety. *Sci Rep* **11**:10394. doi:10.1038/s41598-021-89465-0

638 Melhorn SJ, Krause EG, Scott KA, Mooney MR, Johnson JD, Woods SC, Sakai RR. 2010. Acute
639 exposure to a high-fat diet alters meal patterns and body composition. *Physiol Behav* **99**:33–
640 9. doi:10.1016/j.physbeh.2009.10.004

641 Morris EM, Noland RD, Allen JA, McCoin CS, Xia Q, Koestler DC, Shook RP, Lighton JRB,
642 Christianson JA, Thyfault JP. 2020. Difference in Housing Temperature-Induced Energy
643 Expenditure Elicits Sex-Specific Diet-Induced Metabolic Adaptations in Mice. *Obesity*
644 *(Silver Spring)* **28**:1922–1931. doi:10.1002/oby.22925

645 Myers MG, Affinati AH, Richardson N, Schwartz MW. 2021. Central nervous system regulation
646 of organismal energy and glucose homeostasis. *Nat Metab* **3**:737–750. doi:10.1038/s42255-
647 021-00408-5

648 Olsen MK, Choi MH, Kulseng B, Zhao C-M, Chen D. 2017. Time-restricted feeding on weekdays
649 restricts weight gain: A study using rat models of high-fat diet-induced obesity. *Physiol*
650 *Behav* **173**:298–304. doi:10.1016/j.physbeh.2017.02.032

651 O'Neal TJ, Friend DM, Guo J, Hall KD, Kravitz A V. 2017. Increases in Physical Activity Result
652 in Diminishing Increments in Daily Energy Expenditure in Mice. *Curr Biol* **27**:423–430.
653 doi:10.1016/j.cub.2016.12.009

654 Oraha J, Enriquez RF, Herzog H, Lee NJ. 2022. Sex-specific changes in metabolism during the
655 transition from chow to high-fat diet feeding are abolished in response to dieting in
656 C57BL/6J mice. *Int J Obes (Lond)* **46**:1749–1758. doi:10.1038/s41366-022-01174-4

657 Petersen S. 1976. The temporal pattern of feeding over the oestrous cycle of the mouse. *Anim*
658 *Behav* **24**:939–955. doi:10.1016/S0003-3472(76)80023-1

659 Rosen H. 2014. Is Obesity A Disease or A Behavior Abnormality? Did the AMA Get It Right? *Mo*
660 *Med* **111**:104–108.

661 Ruddick-Collins LC, Morgan PJ, Fyfe CL, Filipe JAN, Horgan GW, Westerterp KR, Johnston JD,
662 Johnstone AM. 2022. Timing of daily calorie loading affects appetite and hunger responses
663 without changes in energy metabolism in healthy subjects with obesity. *Cell Metab* **34**:1472-
664 1485.e6. doi:10.1016/j.cmet.2022.08.001

665 Su Z, Alhadeff AL, Betley JN. 2017. Nutritive, Post-ingestive Signals Are the Primary Regulators
666 of AgRP Neuron Activity. *Cell Rep* **21**:2724–2736. doi:10.1016/j.celrep.2017.11.036

667 Vellers HL, Letsinger AC, Walker NR, Granados JZ, Lightfoot JT. 2017. High Fat High Sugar
668 Diet Reduces Voluntary Wheel Running in Mice Independent of Sex Hormone
669 Involvement. *Front Physiol* **8**:628. doi:10.3389/fphys.2017.00628

670 Yang Y, Smith DL, Keating KD, Allison DB, Nagy TR. 2014. Variations in body weight, food
671 intake and body composition after long-term high-fat diet feeding in C57BL/6J mice.
672 *Obesity* **22**:2147–2155. doi:10.1002/OBY.20811

673 Zhao D, Guallar E, Woolf TB, Martin L, Lehmann H, Coughlin J, Holzhauer K, Goheer AA,
674 McTigue KM, Lent MR, Hawkins M, Clark JM, Bennett WL. 2023. Association of Eating
675 and Sleeping Intervals With Weight Change Over Time: The Daily24 Cohort. *J Am Heart*
676 *Assoc* **12**:e026484. doi:10.1161/JAHA.122.026484

677 Zorrilla EP, Inoue K, Fekete EM, Tabarin A, Valdez GR, Koob GF. 2005. Measuring meals:
678 structure of prandial food and water intake of rats. *Am J Physiol Regul Integr Comp Physiol*
679 **288**:R1450-67. doi:10.1152/ajpregu.00175.2004

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685 **Figure Legends**

686 **Figure 1. Food intake and energy balance in mice fed a 60% high-fat diet**

687 Mice were placed in metabolic chambers and were fed with chow for up to 6 days. Mice were
688 then switched to 60% HFD at day 0 (dashed line) and remained on 60% HFD for 28 days. (A-F,
689 M, O, P) represent data from male mice (black) and (G-L, N, Q, R) represent data from female
690 mice (pink). (A, G) Daily food intake by mass (grams). (B, H) Average weekly food intake by
691 mass. (C, I) Daily caloric intake. (D, J) Average weekly caloric intake. (E, K) Daily energy
692 expenditure. (F, L) Average weekly energy expenditure. (M, N) Heat map showing shifts in net
693 daily energy balance. (O, Q) Average weekly energy balance. (P, R) Average weekly body
694 weight. Data are shown as mean ± SEM for *N*=8 per sex and were statistically analyzed using a
695 repeated measures one-way ANOVA with Tukey multiple comparison tests. P-value definitions:
696 not-significant (ns) >0.05, * <0.05, **<0.01, ***<0.001, **** <0.00001.

697

698 **Figure 2. Feeding behaviors in mice switched to a 60% high-fat diet**

699 Feeding behavior analysis in male mice (black) and female mice (pink) switched from chow to
700 60% HFD in metabolic chambers at day 0 (dashed line). (A) Daily meal numbers. (B) Average
701 weekly meal number in male and (C) female mice. (D) Daily intermeal intervals. (E) Average
702 weekly intermeal intervals in male and (F) female mice. G) Daily meal duration. (H) Average
703 weekly meal duration in male and (I) female mice. (J) Daily meal size by mass. (K) Average
704 weekly meal size by mass in male and (L) female mice. (M) Daily meal size by calorie. (N)

705 Average weekly meal size by calorie in male and (O) female mice. (P) Daily meal rate in grams
706 consumed per minute. (Q) Average weekly meal rate in grams consumed per minute in male
707 and (R) female mice. (S) Daily meal rate in calories consumed per minute. (T) Average weekly
708 meal rate in calories consumed per minute in male and (U) female mice. Data are shown as
709 mean ± SEM for *N*=8 per sex and were statistically analyzed using a repeated measures one-way
710 ANOVA with Tukey multiple comparison tests. P-value definitions: not-significant (ns) >0.05, *
711 <0.05, **<0.01, ***<0.001, **** <0.00001.

712

713 **Figure 3. Food intake and energy balance in male and female mice switched to a 45% high-fat**
714 **diet**

715 Mice were placed in metabolic chambers and were fed with chow for 5 days. Mice were then
716 switched to 45% HFD at day 0 (dashed line) and remained on 45% HFD for 21 days. (A-F, M, O,
717 P) represent data from male mice (black) and (G-L, N, Q, R) represent data from female mice
718 (pink). (A, G) Daily food intake by mass (grams). (B, H) Average weekly food intake by mass.
719 (C, I) Daily caloric intake. (D, J) Average weekly caloric intake. (E, K) Daily energy expenditure.
720 (F, L) Average weekly energy expenditure. (M, N) Heat map showing shifts in net daily energy
721 balance. (O, Q) Average weekly energy balance. (P, R) Average weekly body weight. Data are
722 shown as mean ± SEM for *N*=8 per sex and were statistically analyzed using a repeated
723 measures one-way ANOVA with Tukey multiple comparison tests. P-value definitions: not-
724 significant (ns) >0.05, * <0.05, **<0.01, ***<0.001, **** <0.00001.

725

726 **Figure 4. Feeding behaviors in mice switched to a 45% high-fat diet**

727 Feeding behavior analysis in male mice (black) and female mice (pink) switched from chow to
728 45% HFD in metabolic chambers at day 0 (dashed line). (A) Daily meal numbers. (B) Average
729 weekly meal number in male and (C) female mice. (D) Daily intermeal intervals. (E) Average
730 weekly intermeal intervals in male and (F) female mice. G) Daily meal duration. (H) Average
731 weekly meal duration in male and (I) female mice. (J) Daily meal size by mass. (K) Average
732 weekly meal size by mass in male and (L) female mice. (M) Daily meal size by calorie. (N)
733 Average weekly meal size by calorie in male and (O) female mice. (P) Daily meal rate in grams
734 consumed per minute. (Q) Average weekly meal rate in grams consumed per minute in male
735 and (R) female mice. (S) Daily meal rate in calories consumed per minute. (T) Average weekly
736 meal rate in calories consumed per minute in male and (U) female mice. Data are shown as
737 mean ± SEM for *N*=8 per sex and were statistically analyzed using a repeated measures one-way
738 ANOVA with Tukey multiple comparison tests. P-value definitions: not-significant (ns) >0.05, *
739 <0.05, **<0.01, ***<0.001, **** <0.00001.

740

741 **Figure 5. Meal patterns of male and female mice fed obesogenic diets**

742 Meal size analysis in male and female mice switched from chow to 60% HFD (A-D, J-K, N-O)
743 for 4 weeks or 45% HFD (E-H, L-M, P-Q) for 3 weeks. Histograms of meal size by (A) grams and
744 (B) calories in male and (C) grams and (D) calories in female mice switched to a 60% HFD.
745 Histograms of meal size by (E) grams and (F) calories in male and (G) grams and (H) calories in
746 female mice switched to a 45% HFD. Histogram bin sizes of 0.02 (range: 0-0.5) for meals by

747 grams and bin size of 0.2 (range: 0-3) for meals by calories. Non-linear regression was used to fit
748 curves on each graph. (I) Range of meal sizes in grams consumed by mice (n=16) during the
749 chow feeding period. Quartiles were used to determine cut-offs for meal types. Type 1: <60 mg,
750 Type2: <160 mg, Type 3: <280 mg, and Type 4: >280 mg. Pie-charts depicting meal types across
751 diet switch week in (J) males and (K) females switched from chow to a 60% HFD and (L) males
752 and (M) females switched from chow to a 45% HFD. Percent frequency of meals by type across
753 each week of feeding in (N) males and (O) females switched from chow to a 60% HFD and (P)
754 males and (Q) females switched from chow to a 45% HFD. Data are shown as mean ± SEM for
755 *N*=8 per sex and were statistically analyzed using a repeated measures one-way ANOVA with
756 Tukey multiple comparison tests. P-value definitions: not-significant (ns) >0.05, * <0.05, **<0.01,
757 ***<0.001, **** <0.00001.

758

759 **Figure 6. Effects of running wheel access on feeding behavior and energy expenditure in**
760 **male mice switched to a 60% HFD**

761 Male mice were placed in metabolic chambers with chow and locked running wheels for 5 days.
762 Mice were then switched to 60% HFD on day 0 (dashed line) and concomitantly remained with
763 locked wheels (wheels locked, black) or the running wheels were unlocked (wheels unlocked,
764 red) for 28 days. (A) Daily and (B) average weekly food intake in grams. (C) Daily and D)
765 average weekly caloric intake. (E) Daily and (F) average weekly energy expenditure. (G) Heat
766 map depicting net energy balance for each mouse across time after being switched from normal
767 chow to 60% HFD with locked or unlocked running wheels. (H) Average net daily energy

768 balance across each week of diet. (I) Average body weight across each week of diet. Feeding
769 behavior analysis depicting (J) daily and (K) average weekly average meal number, (L) daily
770 and (M) average weekly intermeal interval, N) daily and O) average weekly meal duration, (P)
771 daily and (Q) average weekly meal size in grams, (R) daily and (S) average weekly meal size in
772 calories, (T) daily and (U) average weekly meal rates in grams per minute, and (V) daily and
773 (W) average weekly meal rate in calories per minute. Meal size histograms by gram for (X) mice
774 with wheels locked and (Y) mice with wheels unlocked in bin sizes of 0.02 (Range 0-0.5). Meal
775 size histograms by calories for (Z) mice with wheels locked and (AA) mice with wheels
776 unlocked in bin sizes of 0.2 (Range 0-3). Non-linear regression was used to fit curves on each
777 graph. Data are shown as mean ± SEM for *N*=8 per group and were statistically analyzed using
778 a two-way ANOVA with Sidaks multiple comparison tests. P-value definitions: not-significant
779 (ns) >0.05, * <0.05, **<0.01, ***<0.001, **** <0.00001.

780

781 **Supplemental Figure 1. Metabolic phenotyping of mice switched to a 60% high-fat diet**
782 **separated by light:dark cycle.**

783 Metabolic phenotyping parameters in male and female mice switched from chow to 60% HFD
784 on day 0 (dashed lines) in metabolic chambers and divided by light (blue) and dark (beige)
785 cycles. (A) Daily food intake in grams in male and (D) female mice. Average weekly food intake
786 by mass in the light (B) and dark (C) cycle in male mice and in the light (E) and dark (F) cycle in
787 female mice. (G) Daily caloric intake in male and (J) female mice. Average weekly caloric intake
788 in the light (H) and dark (I) cycle in male mice and in the light (K) and dark (L) cycle in female

789 mice. (M) Daily energy expenditure in male and (P) female mice. Average weekly energy
790 expenditure in the light (N) and dark (O) cycles in male mice and in the light (Q) and dark (R)
791 cycle in female mice. (S) Daily respiratory exchange ratio in male and (V) female mice. Average
792 weekly respiratory exchange ratio in the light (T) and dark (U) cycle in male mice and in the
793 light (W) and dark (X) cycle in female mice. (Y) Daily water intake in male and (AB) female
794 mice. Average weekly water intake in the light (Z) and dark (AA) cycle in male mice and in the
795 light (AC) and dark (AD) cycle in female mice. (AE) Daily locomotor activity in male and (AH)
796 female mice. Average weekly locomotor activity in the light (AF) and dark (AG) cycle in male
797 mice and in the light (AI) and dark (AJ) cycle in female mice. Locomotor activity data are only
798 available for first seven days of diet switch in female mice due to instrument malfunction for
799 this feature. Data are shown as mean ± SEM for N=8 per sex and were statistically analyzed
800 using a repeated measures one-way ANOVA with Tukey multiple comparison tests. P-value
801 definitions: not-significant (ns) >0.05, * <0.05, **<0.01, ***<0.001, **** <0.00001.

802

803 **Supplemental Figure 2. Metabolic phenotyping of mice switched to a 60% high-fat diet**

804 Metabolic parameters in male (black) and female (pink) mice fed a chow diet and switched to
805 60% HFD at day 0 (dashed line) for 28 days. Average daily energy balance plots showing energy
806 intake (Energy In, circle), energy expenditure (Energy Out, square), and net energy balance
807 (triangle). (C) Daily and (D) average weekly respiratory exchange ratio in male and (E, F) female
808 mice. G) Daily and (H) average weekly water intake in male and (I, J) female mice. (K) Daily and
809 (L) average weekly locomotor activity in male and (M, N) female mice. Locomotor activity data

810 are only available for first seven days of diet switch in female mice due to instrument
811 malfunction for this feature. Data are shown as mean ± SEM for *N*=8 per sex and were
812 statistically analyzed using a repeated measures one-way ANOVA with Tukey multiple
813 comparison tests. P-value definitions: not-significant (ns) >0.05, * <0.05, **<0.01, ***<0.001, ****
814 <0.00001.

815

816 **Supplemental Figure 3. Feeding behaviors in mice switched to a 60% high-fat diet separated**
817 **by light cycle.**

818 Feeding behavior analysis in male and female mice switched from chow to 60% HFD on day 0
819 (dashed lines) in metabolic chambers and divided by light (blue) and dark (beige) cycles. (A)
820 Daily and (B, C) average weekly meal number in male mice in the light and dark cycle. (D)
821 Daily and (E, F) average weekly meal number in female mice in the light and dark cycle. (G)
822 Daily and (H, I) average weekly intermeal interval in male mice in the light and dark cycle. (J)
823 Daily and (K, L) average weekly intermeal interval in female mice in the light and dark cycle.
824 (M) Daily and (N, O) average weekly meal duration in male mice in the light and dark cycle. (P)
825 Daily and (Q, R) average weekly meal duration in female mice in the light and dark cycle. (S)
826 Daily and (T, U) average weekly meal size by mass in male mice in the light and dark cycle. (V)
827 Daily and (W, X) average weekly meal size by mass in female mice in the light and dark cycle.
828 (Y). Daily and (Z, AA) average weekly meal size by calorie in male mice in the light and dark
829 cycle. (AB) Daily and (AC, AD) average weekly meal size by calorie in female mice in the light
830 and dark cycle. (AE) Daily and (AF, AG) average weekly meal rate in grams per minute in male

831 mice in the light and dark cycle. (AH) Daily and (AI, AJ) average weekly meal rate in grams per
832 minute in female mice in the light and dark cycle. (AK) Daily and (AL, AM) average weekly
833 meal rate in calories per minute in male mice in the light and dark cycle. (AN) Daily and (AO,
834 AP) average weekly meal rate in calories per minute in female mice in the light and dark cycle.
835 Data are shown as mean ± SEM for *N*=8 per sex and were statistically analyzed using repeated
836 measures one-way ANOVA with Tukey multiple comparison tests. P-value definitions: not-
837 significant (ns) >0.05, * <0.05, **<0.01, ***<0.001, **** <0.00001.

838

839 **Supplemental Figure 4. Metabolic phenotyping of mice switched to a 45% high-fat diet**
840 **separated by light:dark cycle.**

841 Metabolic phenotyping parameters in male and female mice switched from chow to 45% HFD
842 on day 0 (dashed lines) in metabolic chambers and divided by light (blue) and dark (beige)
843 cycles. (A) Daily food intake in grams in male and (D) female mice. Average weekly food intake
844 by mass in the light (B) and dark (C) cycle in male mice and in the light (E) and dark (F) cycle in
845 female mice. (G) Daily caloric intake in male and (J) female mice. Average weekly caloric intake
846 in the light (H) and dark (I) cycle in male mice and in the light (K) and dark (L) cycle in female
847 mice. (M) Daily energy expenditure in male and (P) female mice. Average weekly energy
848 expenditure in the light (N) and dark (O) cycles in male mice and in the light (Q) and dark (R)
849 cycle in female mice. (S) Daily respiratory exchange ratio in male and (V) female mice. Average
850 weekly respiratory exchange ratio in the light (T) and dark (U) cycle in male mice and in the
851 light (W) and dark (X) cycle in female mice. (Y) Daily water intake in male and (AB) female

852 mice. Average weekly water intake in the light (Z) and dark (AA) cycle in male mice and in the
853 light (AC) and dark (AD) cycle in female mice. (AE) Daily locomotor activity in male and (AH)
854 female mice. Average weekly locomotor activity in the light (AF) and dark (AG) cycle in male
855 mice and in the light (AI) and dark (AJ) cycle in female mice. Data are shown as mean ± SEM for
856 *N*=8 per sex and were statistically analyzed using a repeated measures one-way ANOVA with
857 Tukey multiple comparison tests. P-value definitions: not-significant (ns) >0.05, * <0.05, **<0.01,
858 ***<0.001, **** <0.00001.

859

860 **Supplemental Figure 5. Metabolic phenotyping of mice switched to a 45% high-fat diet**

861 Metabolic parameters in male (black) and female (pink) mice fed a chow diet and switched to
862 45% HFD at day 0 (dashed line) for 21 days. Average daily energy balance plots showing energy
863 intake (Energy In, circle), energy expenditure (Energy Out, square), and net energy balance
864 (triangle). (C) Daily and (D) average weekly respiratory exchange ratio in male and (E, F) female
865 mice. G) Daily and (H) average weekly water intake in male and (I, J) female mice. (K) Daily and
866 (L) average weekly locomotor activity in male and (M, N) female mice. Data are shown as mean
867 ± SEM for *N*=8 per sex and were statistically analyzed using a repeated measures one-way
868 ANOVA with Tukey multiple comparison tests. P-value definitions: not-significant (ns) >0.05, *
869 <0.05, **<0.01, ***<0.001, **** <0.00001.

870

871 **Supplemental Figure 6. Feeding behaviors in mice switched to a 45% high-fat diet separated**
872 **by light:dark cycle**

873 Feeding behavior analysis in male and female mice switched from chow to 60% HFD on day 0
874 (dashed lines) in metabolic chambers and divided by light (blue) and dark (beige) cycles. (A)
875 Daily and (B, C) average weekly meal number in male mice in the light and dark cycle. (D)
876 Daily and (E, F) average weekly meal number in female mice in the light and dark cycle. (G)
877 Daily and (H, I) average weekly intermeal interval in male mice in the light and dark cycle. (J)
878 Daily and (K, L) average weekly intermeal interval in female mice in the light and dark cycle.
879 (M) Daily and (N, O) average weekly meal duration in male mice in the light and dark cycle. (P)
880 Daily and (Q, R) average weekly meal duration in female mice in the light and dark cycle. (S)
881 Daily and (T, U) average weekly meal size by mass in male mice in the light and dark cycle. (V)
882 Daily and (W, X) average weekly meal size by mass in female mice in the light and dark cycle.
883 (Y). Daily and (Z, AA) average weekly meal size by calorie in male mice in the light and dark
884 cycle. (AB) Daily and (AC, AD) average weekly meal size by calorie in female mice in the light
885 and dark cycle. (AE) Daily and (AF, AG) average weekly meal rate in grams per minute in male
886 mice in the light and dark cycle. (AH) Daily and (AI, AJ) average weekly meal rate in grams per
887 minute in female mice in the light and dark cycle. (AK) Daily and (AL, AM) average weekly
888 meal rate in calories per minute in male mice in the light and dark cycle. (AN) Daily and (AO,
889 AP) average weekly meal rate in calories per minute in female mice in the light and dark cycle.
890 Data are shown as mean ± SEM for N=8 per sex and were statistically analyzed using a repeated
891 measures one-way ANOVA with Tukey multiple comparison tests. P-value definitions: not-
892 significant (ns) >0.05, * <0.05, **<0.01, ***<0.001, **** <0.00001.

893

894 **Supplemental Figure 7. Shifts in meal patterns across time and light-cycle in mice fed**

895 **obesogenic diets**

896 Frequency distribution of (A) Type I (<60 mg), (B) Type II (<160 mg), (C) Type III (<280 mg), (D)

897 Type IV (>280 mg) meals in male and (E) Type I (<60 mg), (F) Type II (<160 mg), (G) Type III

898 (<280 mg), and (H) Type IV (>280 mg) meals in female mice switched to a 60% HFD and

899 separated by light:dark cycle. Frequency distribution of (I) Type I (<60 mg), (J) Type II (<160

900 mg), (K) Type III (<280 mg), (L) Type IV (>280 mg) meals in male and (M) Type I (<60 mg), (N)

901 Type II (<160 mg), (O) Type III (<280 mg), and (P) Type IV (>280 mg) meals in female mice

902 switched to a 45% HFD separated by light:dark cycle. Data are shown as mean ± SEM for *N*=8

903 per sex and were statistically analyzed using a repeated measures one-way ANOVA with Tukey

904 multiple comparison tests. P-value definitions: not-significant (ns) >0.05, * <0.05, **<0.01,

905 ***<0.001, **** <0.00001.

906

907 **Supplemental Figure 8. Metabolic phenotyping of mice switched to a 60% HFD with running**

908 **wheel access**

909 Metabolic parameters in male mice switched from chow to a 60% HFD at day 0 (dashed line) in

910 metabolic chambers with wheels locked or wheels unlocked. (A) Daily and (B) average weekly

911 energy balance. (C) Average weekly body weight. (D) Daily and (E) average respiratory

912 exchange ratio and average weekly respiratory exchange ratio during the (F) light and (G) dark

913 cycle. (H) Daily and (I) average water intake and average weekly water intake during the (J)
914 light and (K) dark cycle. (L) Daily and (M) average locomotor activity and average weekly
915 locomotor activity during the (N) light and (O) dark cycle. (P) Average daily and (Q) light cycle
916 and (R) dark cycle running wheel activity in mice with access to unlocked running wheels. (S)
917 Light and (T) dark cycle average weekly energy expenditure. Data are shown as mean ± SEM
918 for *N*=8 per group and were statistically analyzed using a two-way ANOVA with Sidaks
919 multiple comparison tests. P-value definitions: not-significant (ns) >0.05, * <0.05, **<0.01,
920 ***<0.001, **** <0.00001.

921

922 **Supplemental Figure 9. Effects of running wheel access on feeding behaviors and meal**
923 **patterns**

924 Feeding behavior and meal type analysis by light:dark cycle in male mice switched from chow
925 to a 60% HFD with access to locked or unlocked running wheels. Average weekly (A) meal
926 numbers, (B) inter meal interval, (C) meal duration, (F) meal size by mass (G) meal size by
927 calorie, (H) meal rates by grams per minute, and (I) meal rates by calories per minute. Mice with
928 locked wheels are shown in light gray (light cycle) and dark gray (dark cycle) bars with data
929 points depicted by circles. Mice with unlocked wheels are shown in orange (light cycle) and
930 blue (dark cycle) with data points depicted by squares. Frequency of meal types [Type I (<60
931 mg), Type II (<160 mg), Type III (<280 mg), Type IV (>280] across time on diet in mice with (J)
932 wheels locked and (K) wheels unlocked. (L) Frequency of meal types across time on diet in mice
933 with wheels locked vs. wheels unlocked. Data are shown as mean ± SEM for *N*=8 per group and

934 were statistically analyzed using a two-way ANOVA with Sidaks multiple comparison tests. P-

935 value definitions: not-significant (ns) >0.05, * <0.05, **<0.01, ***<0.001, **** <0.00001.

Figure 1. Food intake and energy balance in mice fed a 60% high-fat diet

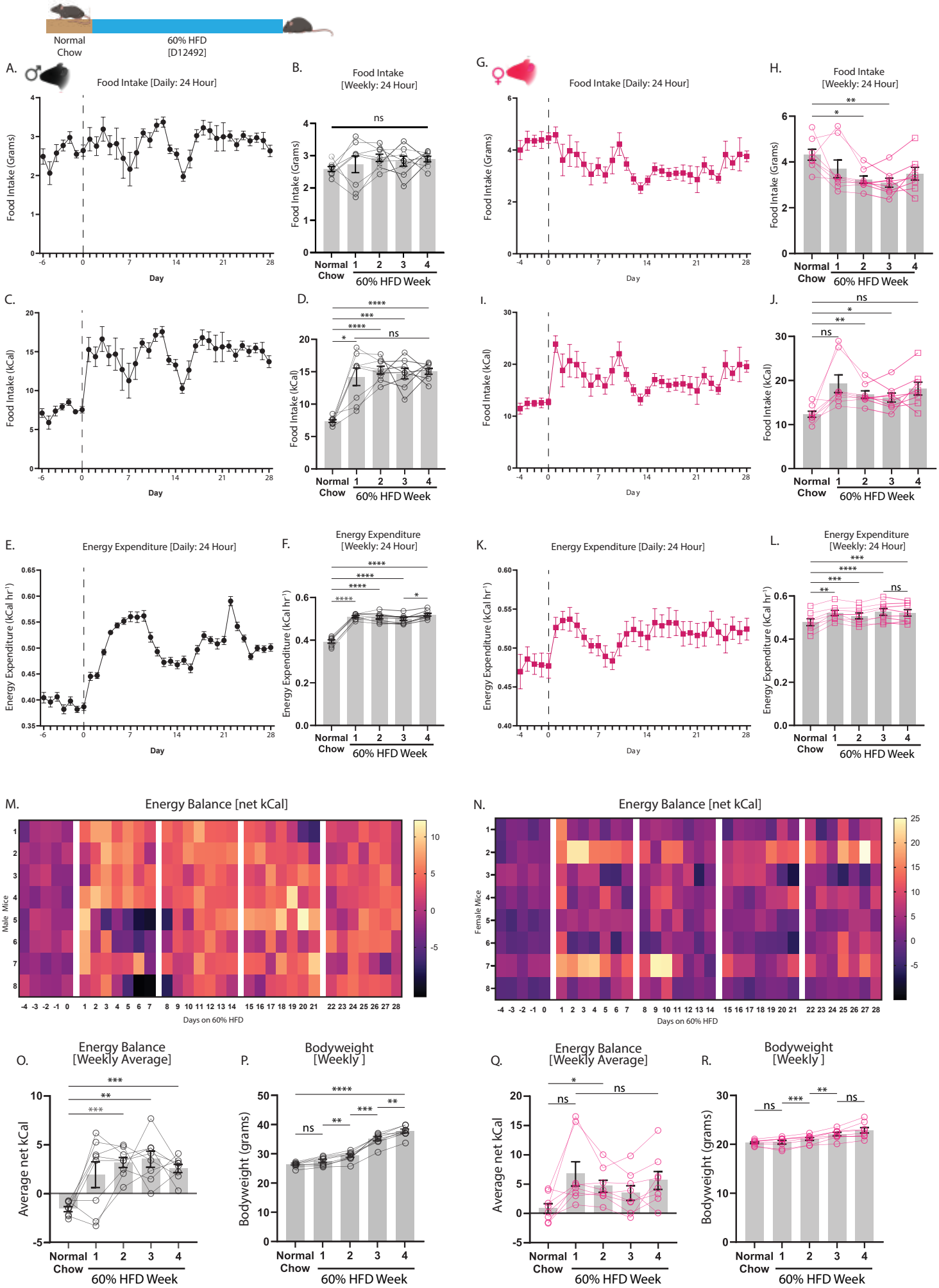


Figure 2. Feeding behaviors in mice switched to 60% high-fat diet

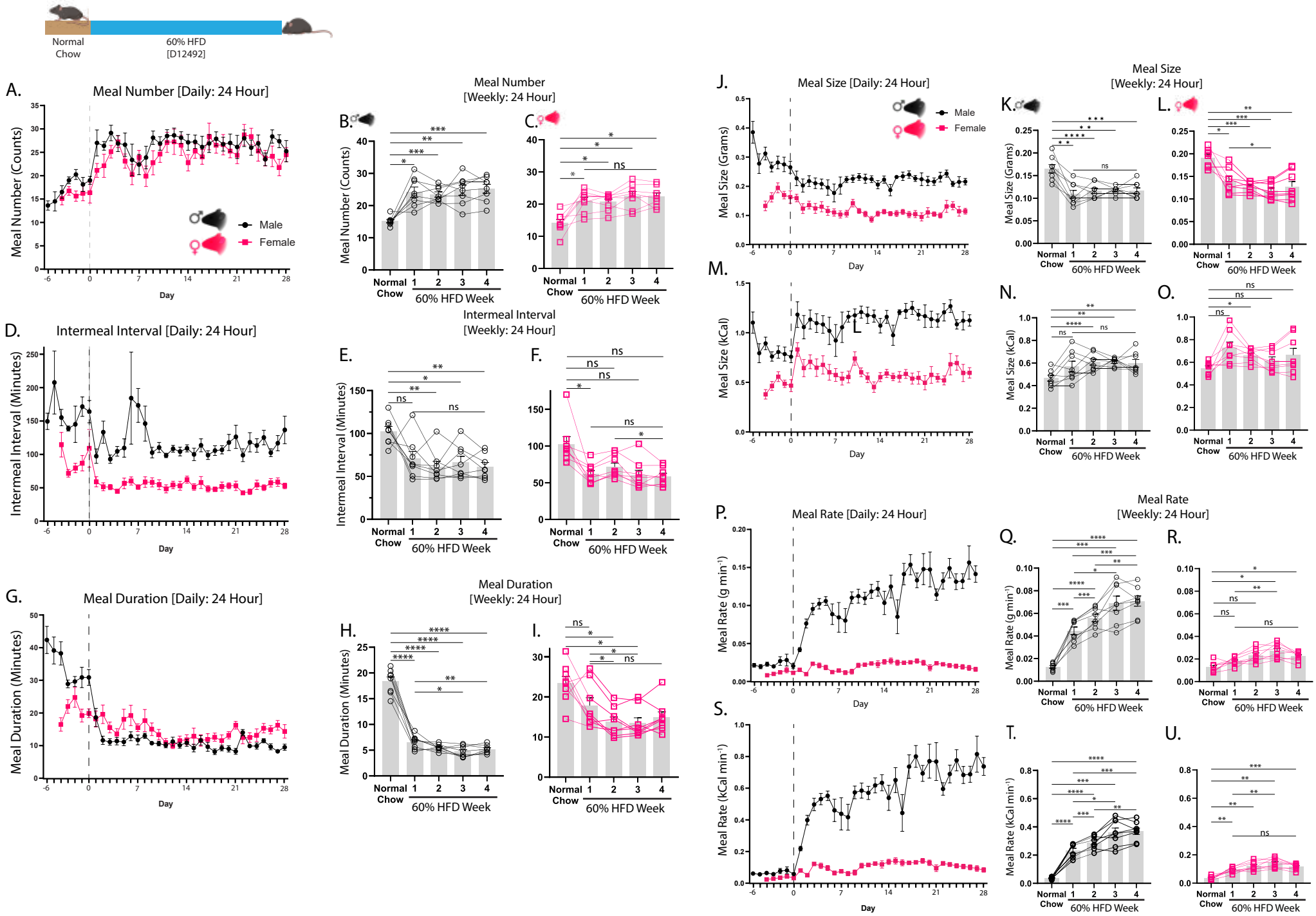


Figure 3. Food intake and energy balance in mice swithced to a 45% high-fat diet

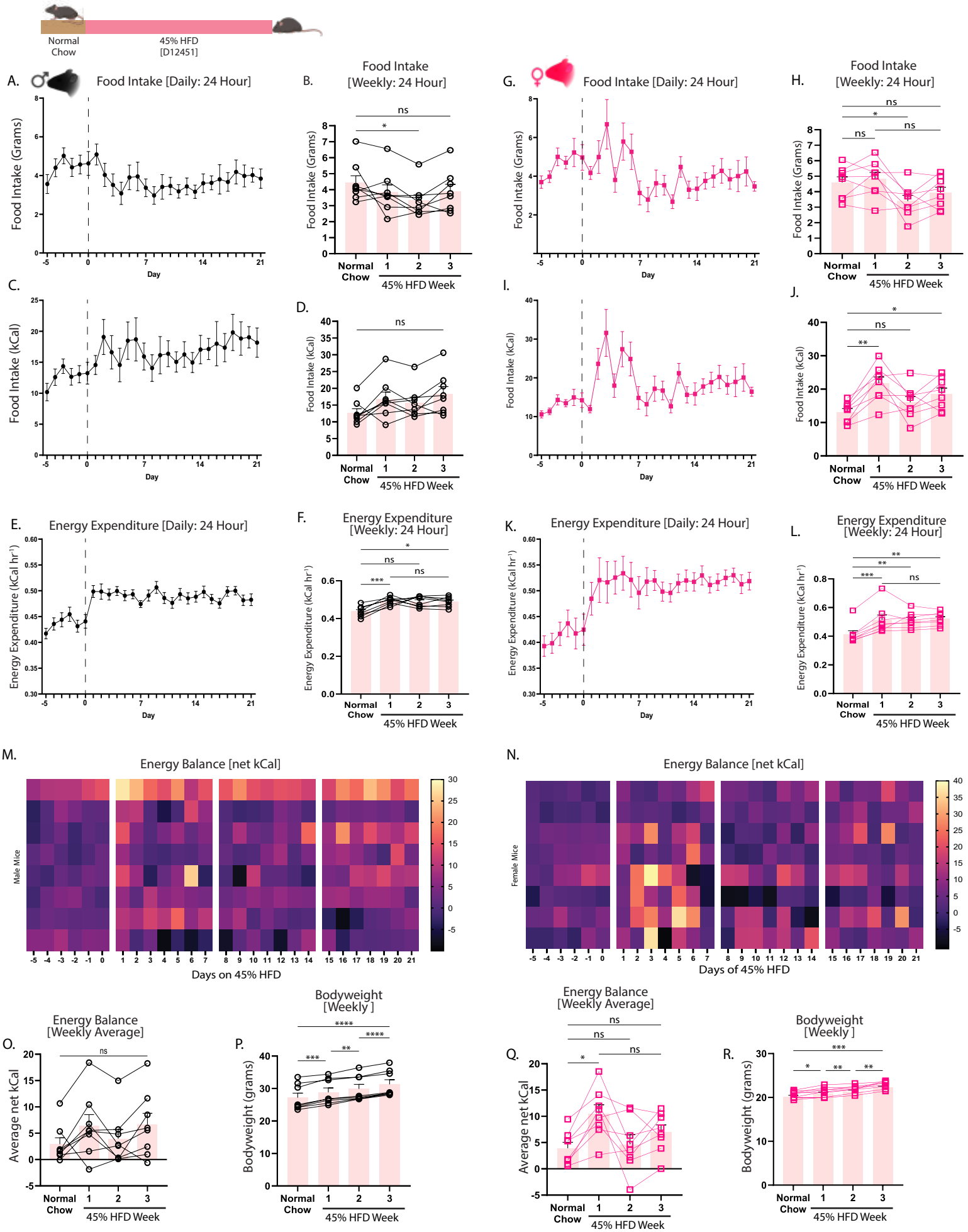
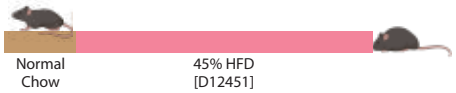


Figure 4. Daily and weekly feeding behaviors in mice fed a 45% high-fat diet



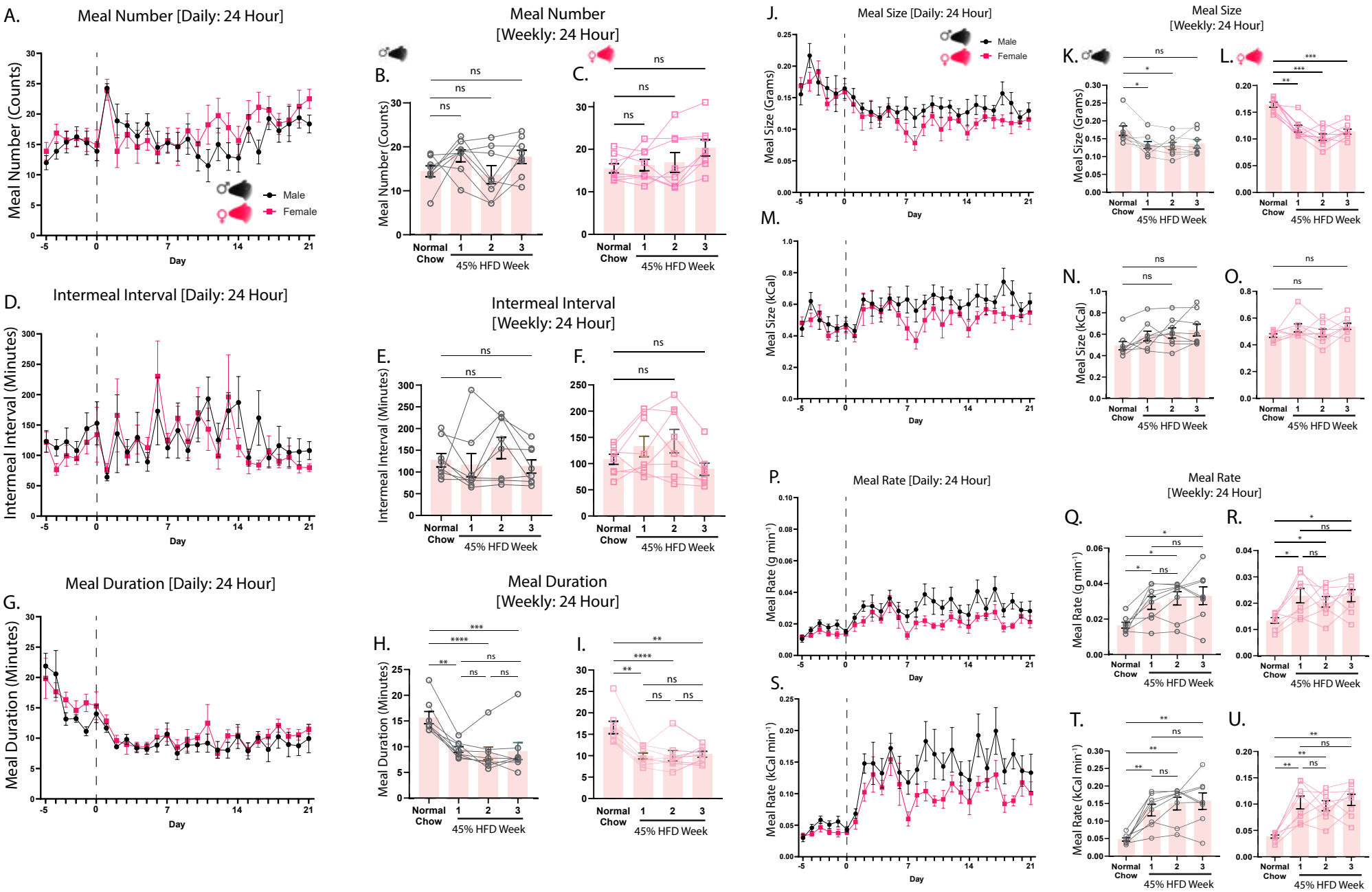


Figure 5. Meal patterns of male and female mice fed obesogenic diets

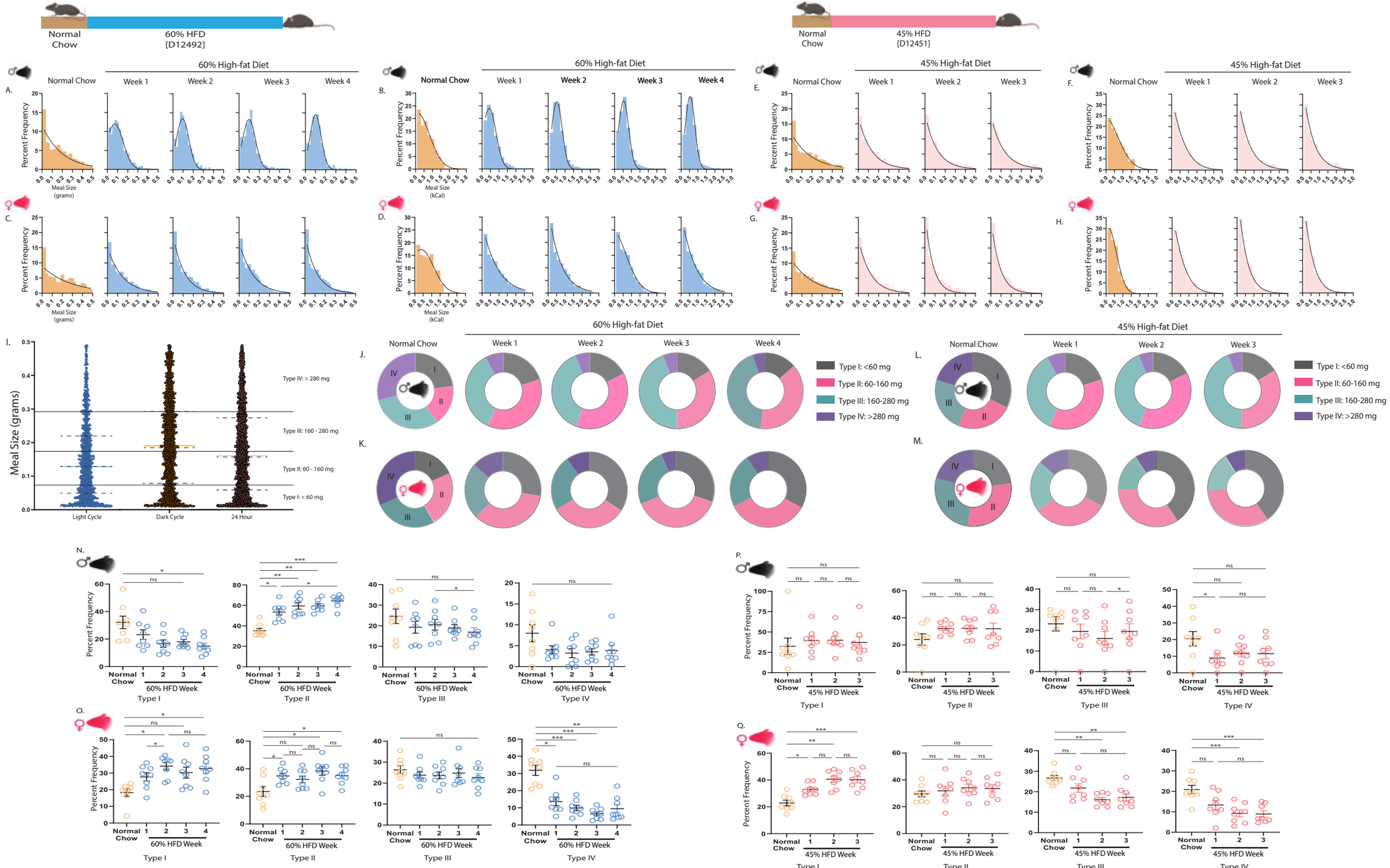
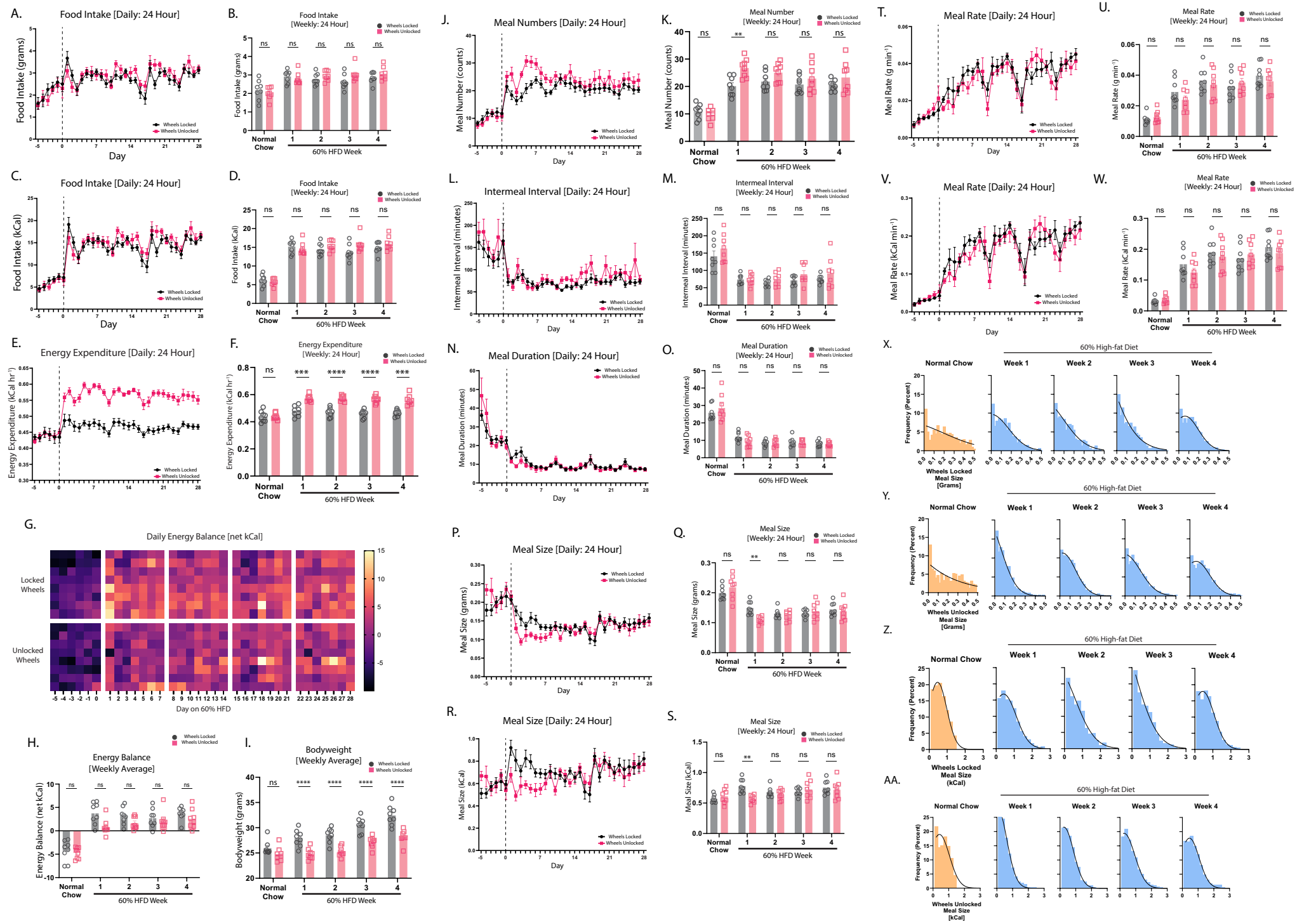
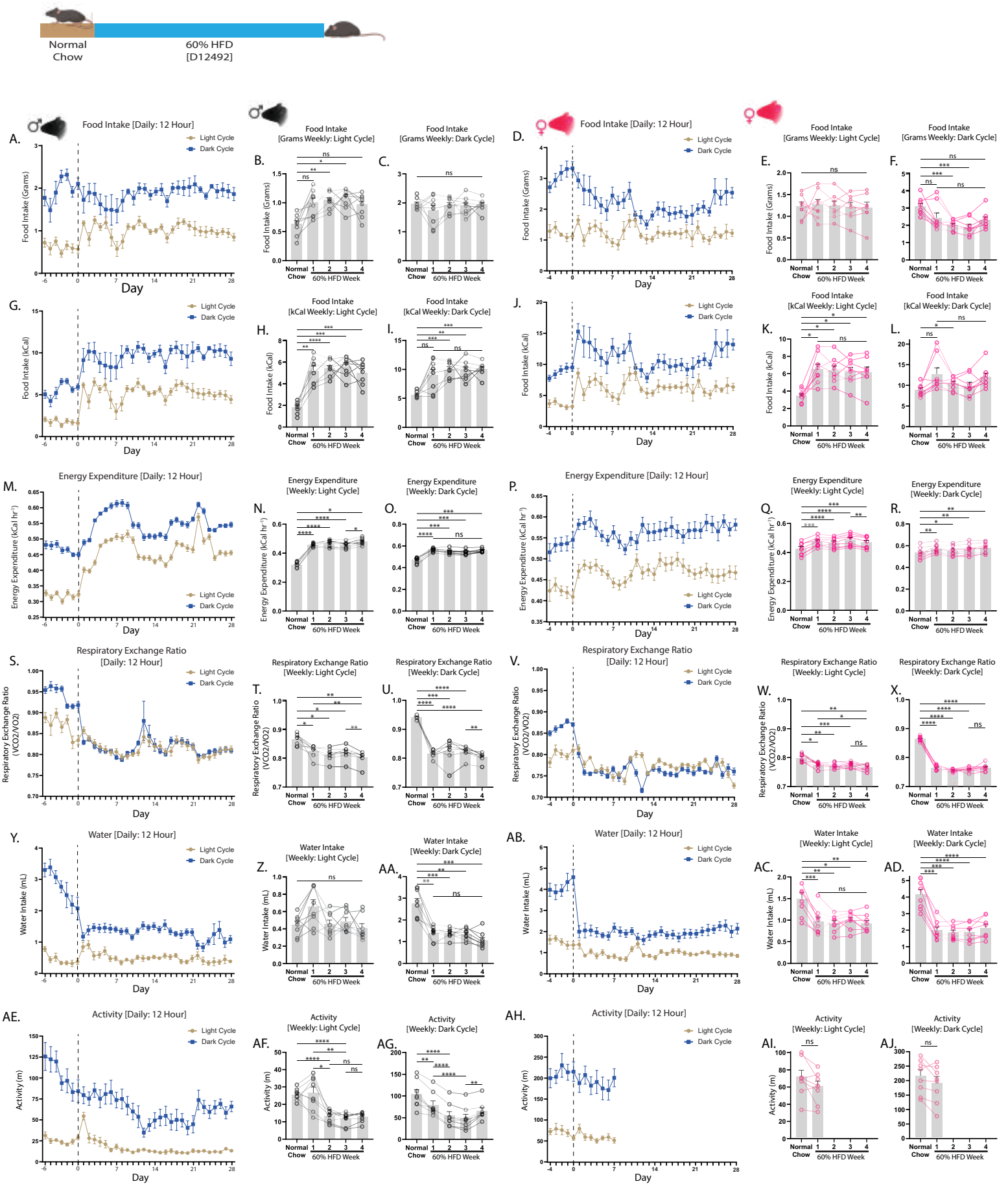


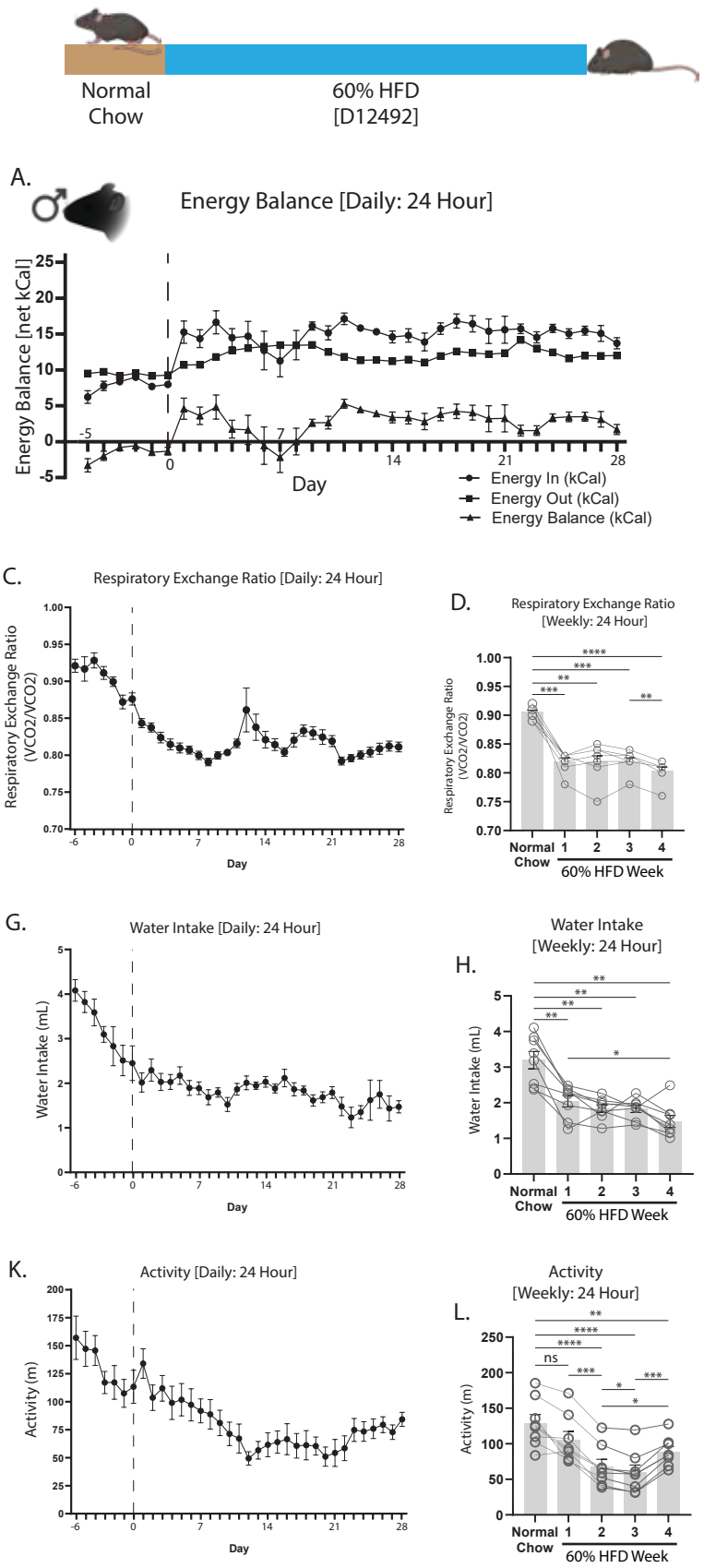
Figure 6. Effects of running wheel access on feeding behavior and energy expenditure in male mice switched to a 60% high-fat diet



Supplemental Figure 1. Metabolic phenotyping of mice switched to a 60% high-fat diet [light:dark cycle]

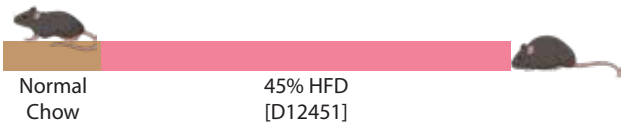


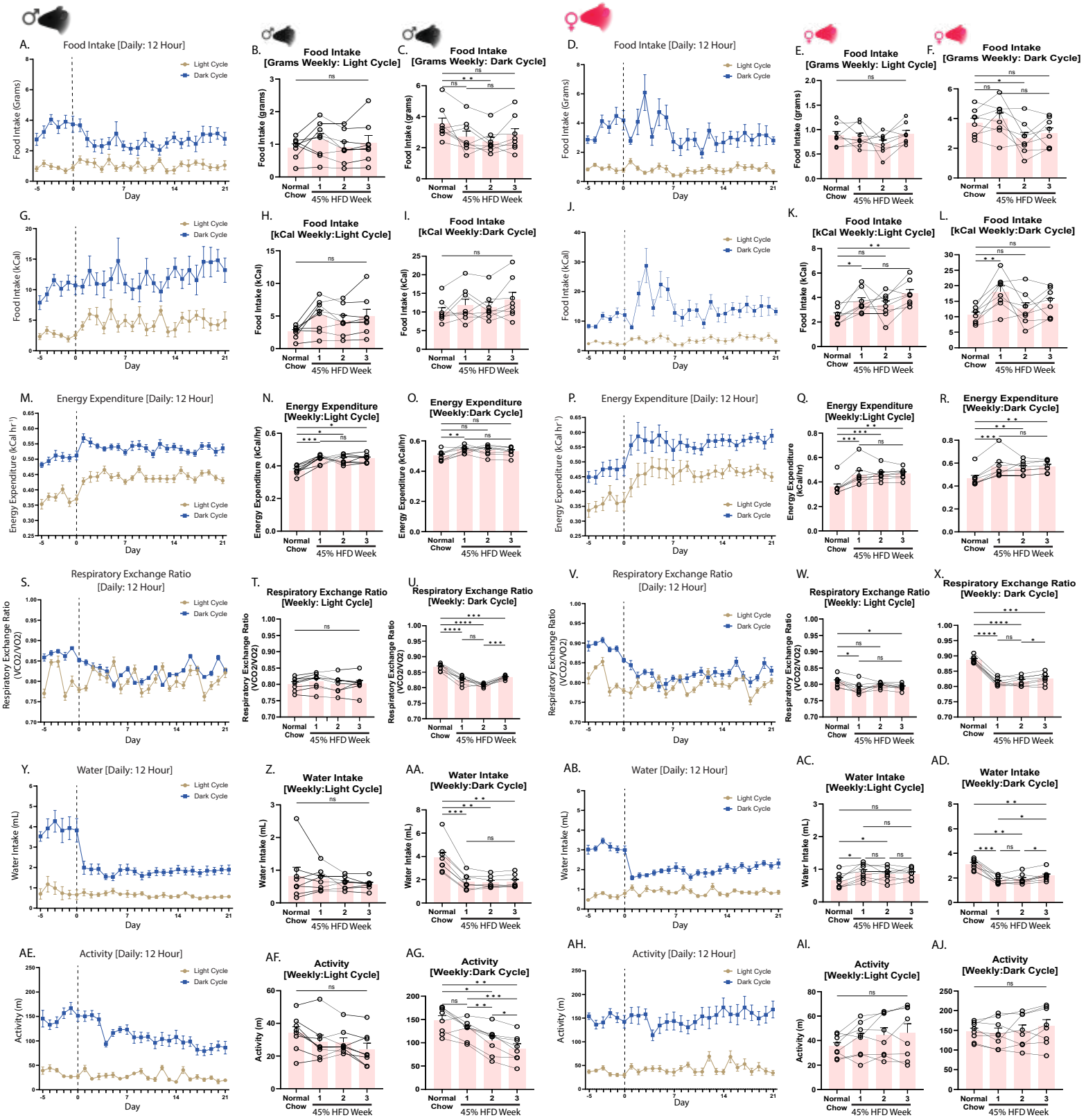
Supplemental Figure 2. Metabolic phenotyping of mice switched to a 60% high-fat diet



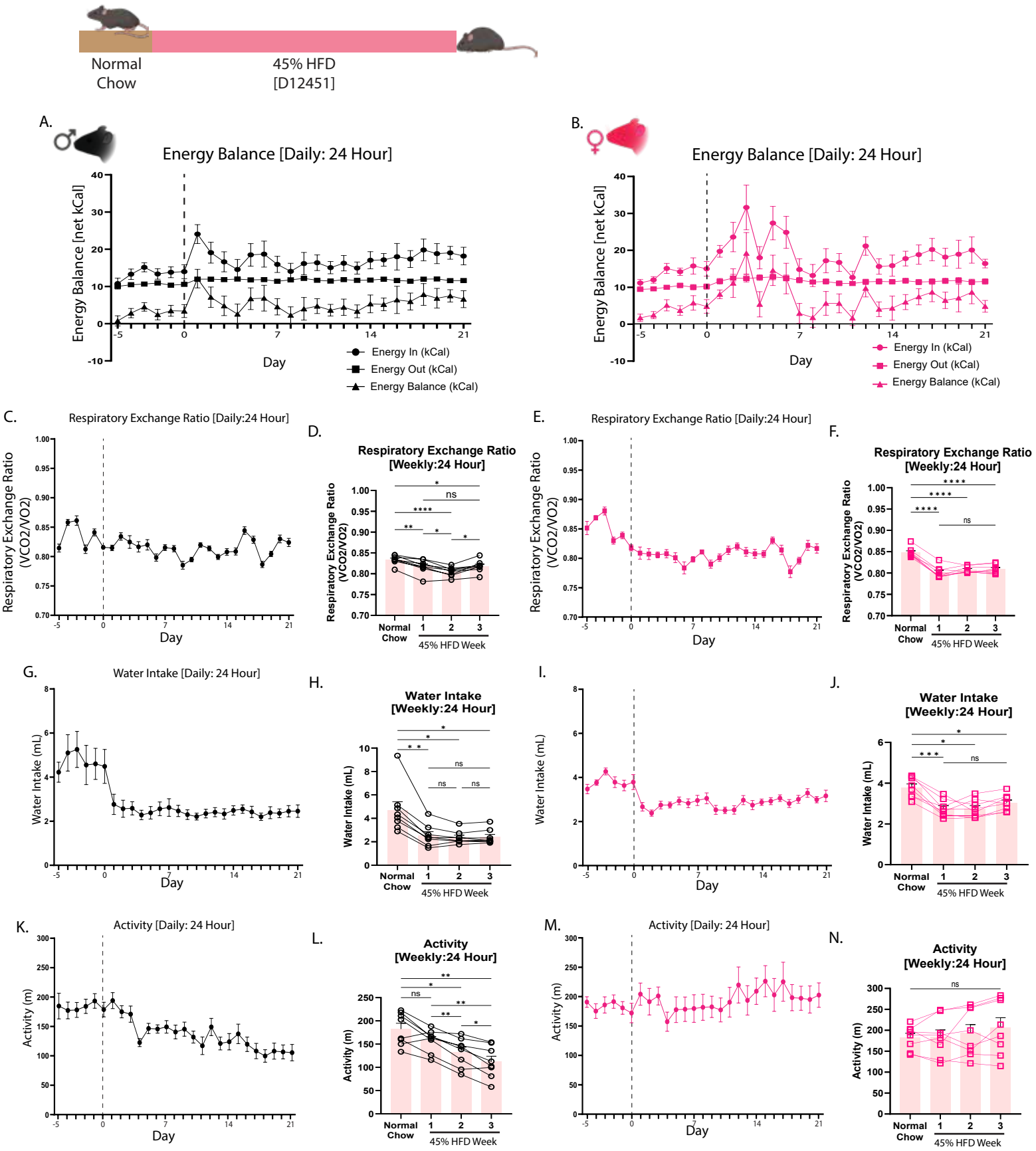
Supplemental Figure 3. Feeding behavior of mice switched to a 60% high-fat diet [light:dark cycle]

Supplemental Figure 4. Metabolic Phenotyping of of mice switched to a 45% high-fat diet [light:dark cycle]

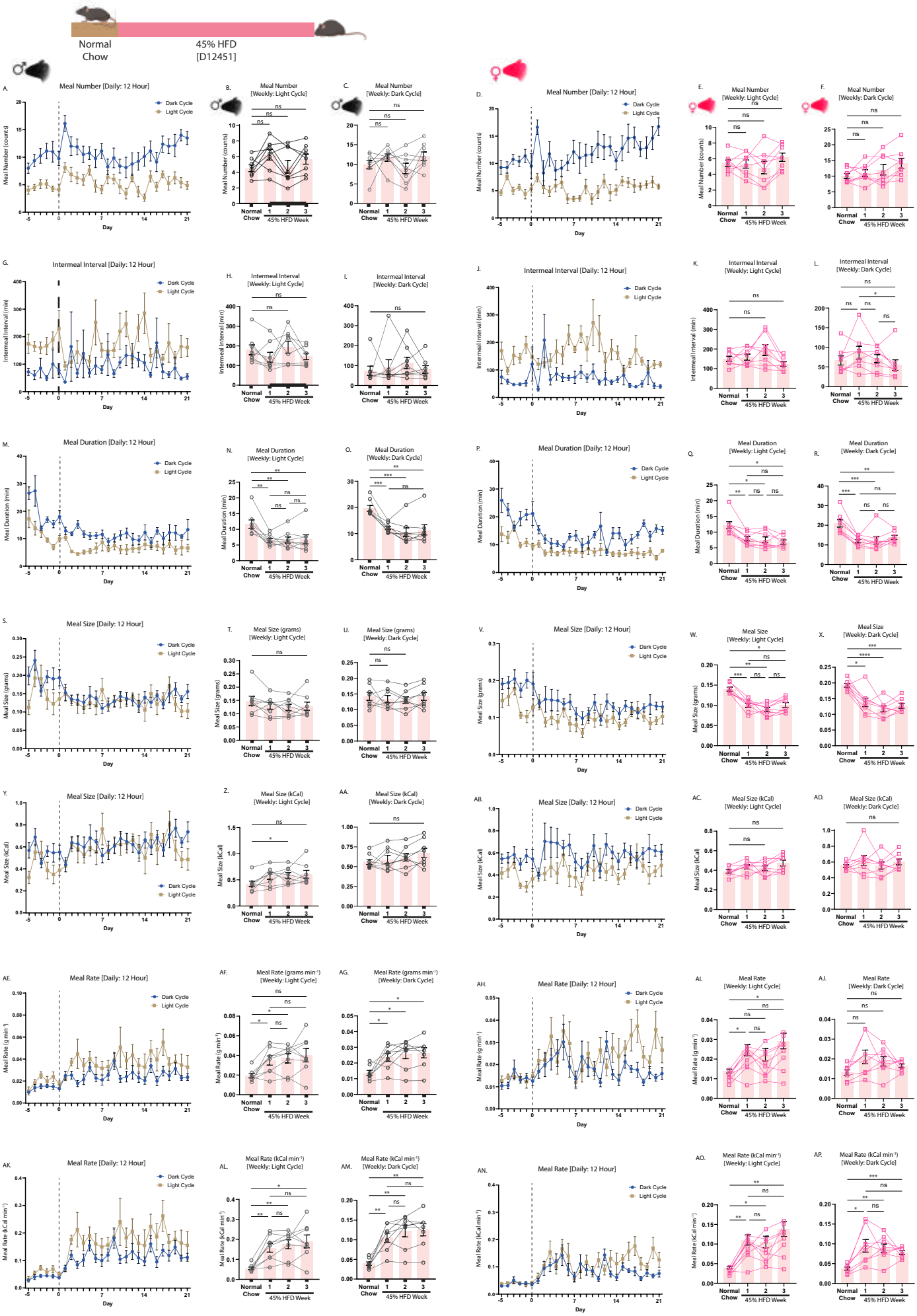




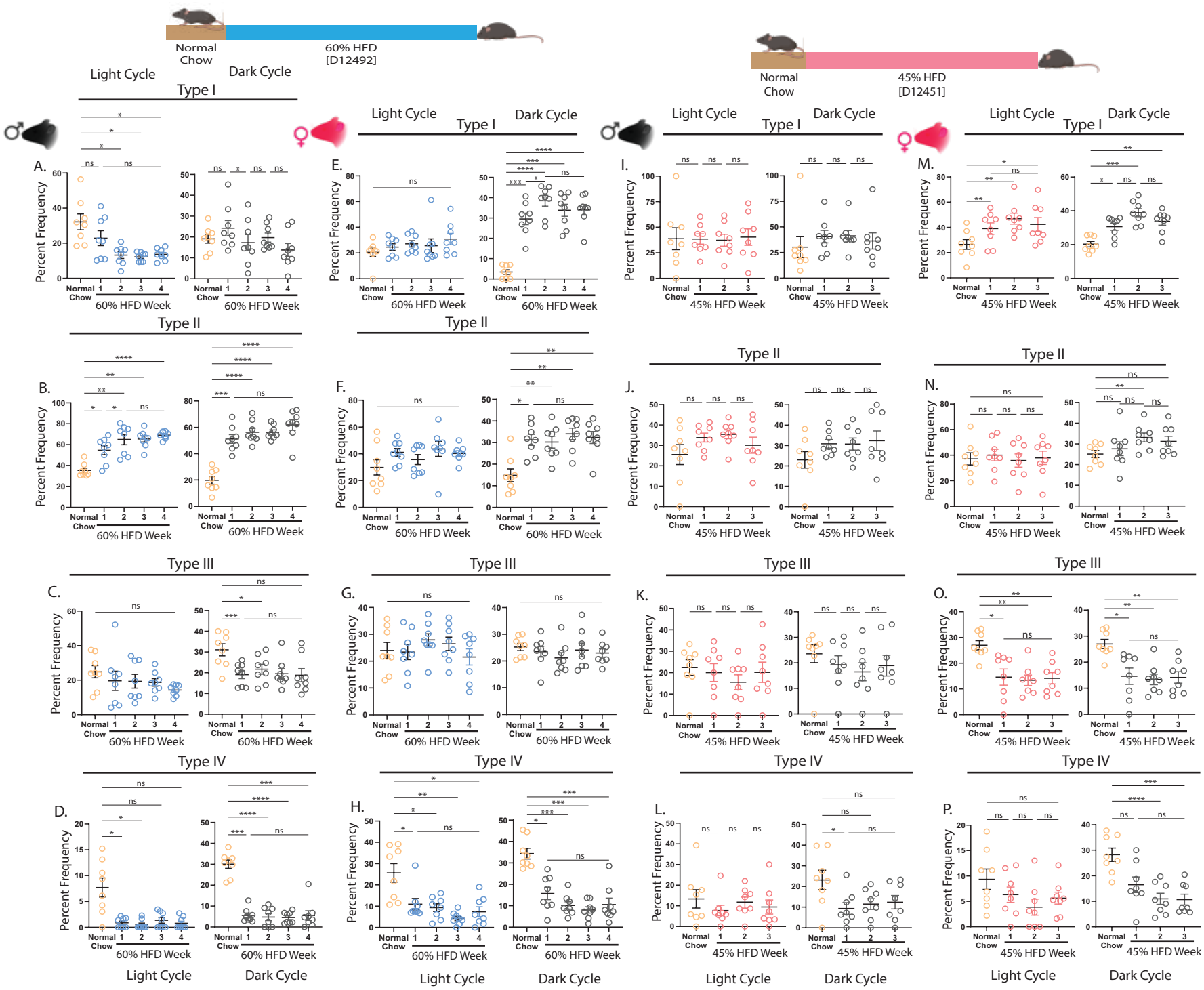
Supplemental Figure 5. Metabolic phenotyping of mice switched to a 45% high-fat diet



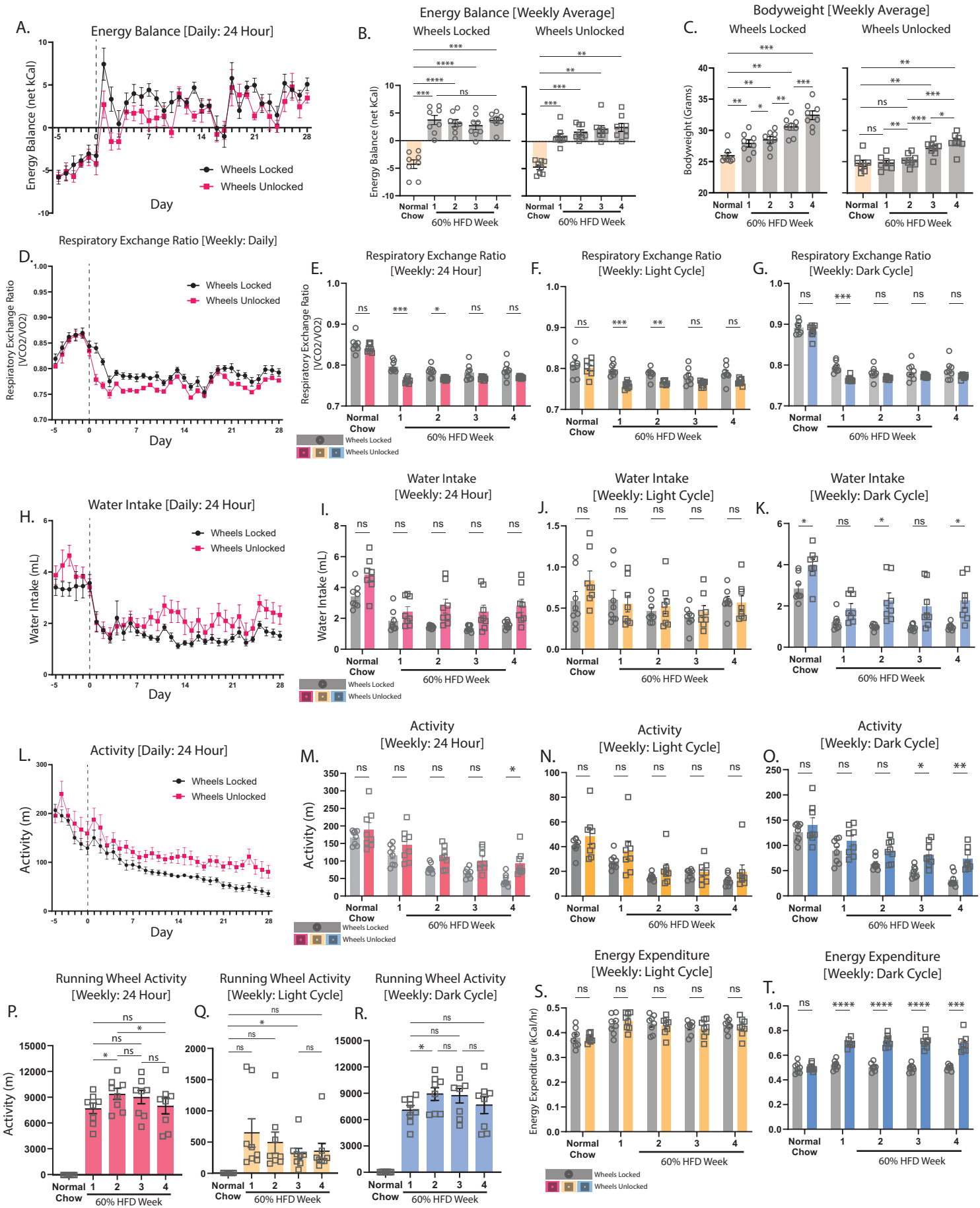
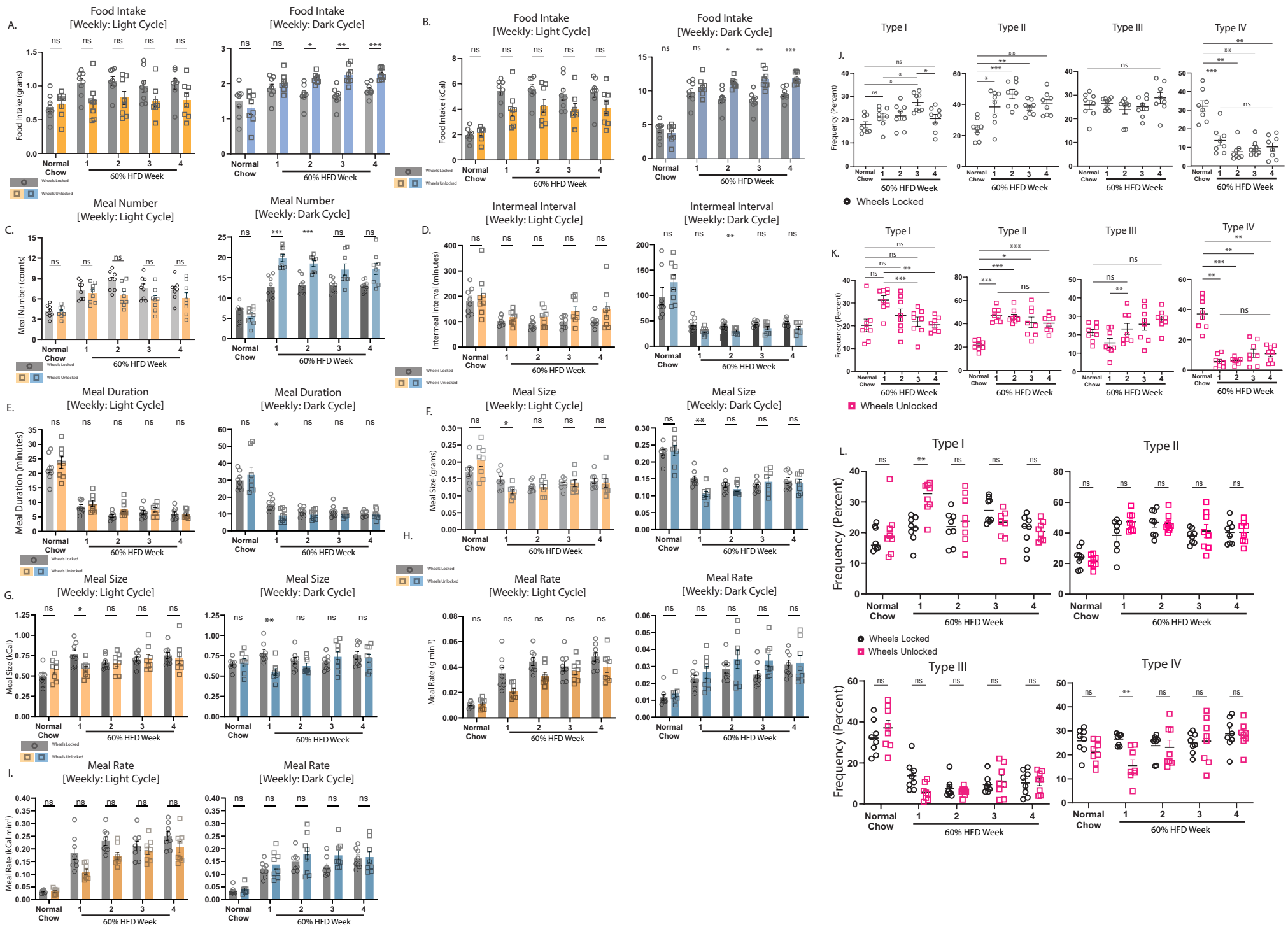
Supplemental Figure 6. Feeding behavior of mice switched to a 45% high-fat diet [light:dark cycle]



Supplemental Figure 7. Shifts in meal patterns across time and light-cycle in mice fed obesogenic diets



Supplemental Figure 8. Metabolic phenotyping of mice switched to a 60% HFD with running wheel access



Supplemental Figure 9. Effects of running wheel access on feeding behavior and meal patterns