

**Mice With A Targeted Disruption of the Dopamine-3 Receptor Gene Have  
Increased Adiposity**

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## **Abstract**

Monoamine signaling has been implicated in the control of energy balance. Specifically, dopamine (DA) has been focused on as a potential target for understanding ingestive behavior. While the DA circuitry that is involved in ingestive behavior has been elucidated, little is known concerning the involvement of the DA receptors. We sought, in the present study, to characterize body weight regulation in a mouse with a targeted disruption of the dopamine 3 receptor (D3-R). In the first set of experiments male and female wild-type and mutant mice were group-housed and given access to two different diets varying in fat content. Body weight, food intake, carcass analysis and plasma leptin and insulin were analyzed in these animals. From these studies we identified an increased body weight and body fat in mutant males given access to high fat diet. The female mutants did not demonstrate increased body weight when given access to either diet, but did have increased body fat on both diets. In a second set of experiments, we evaluated wild-type and mutant male and female mice housed individually for 30 days. Findings from the first set of experiments were replicated, however short-term analysis of food intake revealed no differences in food intake. Finally, the third set of experiments evaluated the effects of food restriction and metabolism in both genders of mutant and wild-type mice. Findings from these experiments indicate no measurable metabolic differences exist between wild-type and mutant mice. Overall, these findings suggest that mutant male and female mice have increased body weight attributable to increased body fat without alterations in food intake and energy expenditure. This effect is exacerbated in animals given access to high-fat diet.

Key words: Food intake, mesolimbic dopaminergic system, reward, metabolism

## **Introduction**

By its nature, ingestive behavior must be sensitive to a number of external and internal variables. Therefore, the CNS circuitry that controls ingestive behavior must be complex to integrate these numerous signals and regulate caloric intake to maintain adequate supplies of metabolic fuels and other nutrients. A variety of lines of evidence indicate that the CNS dopamine (DA) system, among other monoamines, is part of these complex integrative processes to control ingestive behavior (12); (16); (42). It has also been demonstrated that DA systems may mediate the reinforcing properties associated with food and liquid intake. To date the majority of work concerning the DA system has focused on depletion studies(), microinjection() and microdialysis analysis of the lateral hypothalamus (LH) and its projection sites following food ingestion. DA projections, which project in part to the LH appear to inhibit food (ref) and liquid ingestion (ref). In contrast, DA within the NAcc is elevated during feeding behavior (19); (17). In addition DA is also elevated when animals have access to highly palatable diets (29). Taken together, these data suggest a distinct role for DA in the regulation of ingestive behavior.

DA exerts its effects via 5 identified receptors, divided into two classes. The D1 class of receptors includes the D1-R and D5-R. These receptors have been demonstrated to activate cAMP formation via adenylyl cyclase. The D2 class, which includes the D2-R, D3-R and D4-R, inhibits cAMP formation (46); (22). The D2-R antagonist, sulpiride, when injected into the LH induces feeding and DA release in NAcc (ref). Other studies focusing on the D1-R, have demonstrated an involvement of the receptor on ingestive

behavior (41); (49); (48). However, DA-R agonists and antagonists delivered into the NAcc, have been demonstrated to dose-dependently attenuate lever pressing for food reward, while increasing consumption of ad lib chow (25). These data establish a role for the DA-R subtypes in ingestive behavior. However, these data do not provide a distinct role for the DA-R subtypes in reducing or increasing ingestive behavior and/or energy balance.

Since the D3-R was cloned in 1990 considerable effort has been devoted to understanding the function of the receptor (44). Expression of the D3-R has been localized to limbic regions within the brain, including the NAcc, islands of Calleja, ventral pallidum, and olfactory tubercle (43); (34). These regions have been implicated in motivated behaviors such as drug self-administration, sexual behavior, and food intake (35); (40); (3). Thus, the D3-R expressed in these limbic regions may be a potential mediator of the reinforcement associated with ingestive behavior. Consistent with this hypothesis, D3-R agonists injected into the NAcc have been demonstrated to reduce sucrose consumption (14). One hypothesis suggests increased DA release or tone in the NAcc contributes to elevated adiposity and the inability to regulate energy balance (19). If the D3-R exerts an inhibitory tone on DA circuitry involved in ingestive behavior then the D3-R knockout mouse may be more susceptible to obesity. Consequently, we compared mutant and wild-type mice in their food intake and metabolic response to different diet regimens.

## **Methods**

### **Experiment 1: Body weight and carcass analysis**

The mutant mice were previously backcrossed to a C57BL/6 background for 3 generations see ref. (53). Mice were housed in plastic cages the day of weaning with free access to standard rodent chow (prior to experimentation) and water. The colony room was maintained at 22 ° C with a 12:12-h light/dark cycle. All procedures used in the present study were in compliance with National Institutes of Health guidelines, and the protocols were approved by the University of Cincinnati Animal Care Committee.

#### *Body weight analysis*

D3-R mutant and wild-type mice were housed individually at weaning age. The mice had access to standard rodent chow for 2 months post weaning. This study used D3-R mutant male and female and wild-type male and female mice. The first group (wildtype male, n= 6; knockout male, n= 8; wildtype female, n= 8; knockout female, n= 6) received standard rodent chow, Teklad, 3.41 kcal/gm, 0.51 kcal/gm from fat, a low fat diet, 3.6 kcal/gm, 0.27 kcal/gm from fat, (wildtype male, n= 6; knockout male, n= 5; wildtype female, n= 8; knockout female, n= 7) and a high-fat diet, 4.4 kcal/gm, 1.71 kcal/gm from fat (wildtype male, n= 6; knockout male, n= 10; wildtype female, n= 8; knockout female, n= 8). The diets were purchased from Dyets, Inc., Bethlehem, Pennsylvania. The mice had access to each of the diets for a period of 30 days. Body weight was measured every 4 days.

### *Carcass analysis*

Mice were sacrificed following the 1-month exposure to each of the two diets. Mice were weighed and fat was extracted using an ether extraction process (4). The data is presented as percent body fat upon sacrifice.

## **Experiment 2: Food intake, carcass analysis and plasma insulin and leptin**

### *Food intake analysis*

This study used D3-R mutant and wild-type mice. Animals of each genotype and sex were divided into two groups based upon which experimental diet they would receive. The diets were, standard rodent chow (wild-type male, n= 10; wild-type female, n= 12; knockout male, n=13; knockout female, n= 10) and high-fat diet (wild-type male, n= 9; wild-type female, n= 10; knockout male, n=14; knockout female, n= 7). Animals had access to the diets for 3 months. Food intake was measured every 4 or 7 days along with body weight.

### *Carcass analysis*

Mice were sacrificed following the 3-month exposure to each of the two diets. Mice were weighed and fat was extracted using an ether extraction process (4). The data is presented as percent body fat upon sacrifice.

#### *Plasma leptin and insulin*

Mice from the previous experiments were used for these assays. Plasma was extracted from whole blood by centrifugation. Radioimmunoassay for plasma leptin used a rat leptin radioimmunoassay (RIA) kit (Linco Research, St. Charles, Missouri), and immunoreactive insulin was assayed as previously described (54). Plasma leptin is reported in ng/ml and plasma insulin is reported in *pM*.

### **Experiment 3: Metabolic analysis**

#### *Energy expenditure*

Male (n=8) and female (n=8) wild-type and male (n=8) and female (n=8) mutant mice were placed individually in cages attached to the indirect calorimeter (Columbus Instruments, Columbus, Ohio) (30); (31). Mice were housed in the device for a period of 24 hours. Measurements were taken and recorded on a computer every 15 minutes. The data was then converted to energy expenditure (kilocalorie per hour per kilogram).

#### *Food Restriction*

Male (n=8) and female (n=8) wild-type and male (n=8) and female (n=8) mutant mice were individually housed. We previously determined the absolute weight of chow consumed by a mouse in a 24-hour period. A subset of each sex and genotype were restricted to 25 % of total daily food intake of control mice. Food restriction spanned a 3-day period. Body weights were measured everyday. After the 3-day food restriction phase animals were allowed to re-feed to *ad lib* chow for an additional 3-day period. Body weights were measured daily.

## **Results:**

### **Experiment 1**

*Body weight and food intake:* As depicted in figure 1, panels A and C, no differences in body weight change were observed in mice maintained on chow for 3 months. Further, as seen in panel B of that figure, no difference was found between female wild-type and female mutant mice given access to high fat diet for 3 months. However, male mutant mice had a greater increase in body weight, relative to wild-type males, when maintained on high fat diet. Panel D depicts this difference. A two-tailed t-test revealed that this difference was statistically reliable. [ $t(20) = 5.61, p < 0.001$ ]. Three-month caloric intake did not depend on sex, genotype or diet. Figure 2, panels A and B depict caloric intake for male mice with access to chow and high fat diet. Female data is similar to the male data and is not shown. Two-tailed t-tests on each comparison yielded no significant effects (high fat, [ $t(5) = 2.19, p = 0.079$ ] and chow, [ $t(5) = 1.09, p = 0.0326$ ]).

*Carcass analysis:* Results from the carcass analysis are depicted in Table 1. As seen in that table, mutant males on high fat diet had increased body fat, lean tissue and water compared to wild-type mice on either diet. Further, male mutant mice on high fat diet had increased water, lean tissue, and fat, relative to mutant male mice on chow. The statistical validity of these conclusions was assessed with ANOVA on each dependent variable (water, lean tissue, and fat content) and LSD post-hoc tests. Results of these tests are represented in Table 1. Figure 3, panel A, depicts fat-content data for male mutant mice maintained on either chow or high fat diets. ANOVA revealed a significant 2-way interaction of genotype and diet [ $F(1,42) = 30.23, p < 0.01$ ]. The basis for the interaction was high fat diet caused increased body fat in mutant but not wild-type mice. A two-tailed t-test revealed this difference is statistically reliable [ $t(20) = 5.19, p < 0.001$ ]. Female mutant mice, on the other hand, demonstrated an overall higher body fat content than wild-type mice when given access to chow and high fat diet. Figure 3, panel B, demonstrates the mutant mice have a greater percent body fat than wild-types when maintained on chow [ $t(20) = 3.13, p < 0.005$ ] or high fat diet [ $t(16) = 4.66, p < 0.001$ ].

*Plasma leptin and insulin:* Figure 4 depicts data from analysis of plasma leptin and insulin. As predicted, plasma insulin levels were increased after three months access to high fat diet, relative to maintenance on chow (Figure 4, panels A and C). However, the effect of diet was similar in both sexes and genotypes. The validity of this conclusion was assessed with a 2-way ANOVA, which revealed a significant main effect of diet [ $F(1,29) = 23.65, p < 0.01$ ], but neither a main effect of genotype nor an interaction.

Plasma leptin levels were also increased by high fat diet. However, this effect was dependent on both sex and genotype. Female mice of both genotypes on high fat diet had increased leptin levels, relative to females on chow (Figure 4, panel B). ANOVA yielded a main effect of diet ( $F(1,32) = 32.9, p < 0.01$ ). In males however, the high fat diet increased plasma leptin only in mutant mice (Figure 4, panel C). ANOVA revealed a significant 2-way interaction of diet and genotype ( $F(1,29) = 5.17, p < 0.01$ ) and subsequent LSD post-hoc tests revealed that male mutant mice on high fat diet had increased leptin levels relative to all other conditions.

## **Experiment 2:**

*Body weight and food intake:* During the month-long experiment, it appeared that mutants had greater increases in body weight than wild-types mice, regardless of diet. Figure 5 depicts the 1-month change in body weight. As seen in that figure, both male and female mutants appeared to gain more weight than wild-types on each of the three diets. The statistical validity of these conclusions was assessed with three 2-tailed  $t$  tests between mutant and wild-type male mice (on test on each diet). These tests revealed a significant difference only between mutants and wild-types maintained on high fat diet [ $t(14) = 3.34, p < 0.005$ ]. Mutant males maintained on chow or low fat diet did not differ significantly from wild-types. In the case of the females, these conclusions was assessed with three 2-tailed  $t$  tests between mutant and wild-type male mice (on test on each diet). These tests revealed a significant difference between mutants and wild-types maintained

on low fat and high fat diet [ $t(14) = 2.03, p < 0.05$ ], [ $t(14) = 3.20, p < 0.003$ ]. Mutant females maintained on chow did not differ significantly from wild-types.

*Carcass analysis:* Mutants of both sexes had increased body fat, relative to wild-types, regardless of maintenance diet. Figure 6 depicts percent body fat for male mutant and wild-type mice. As seen in that figure, maintenance on high fat diet increased body fat content for both genotypes. Data from females is similar and not shown here. The mutants had increased body fat content, relative to wild-types, on each of the three diets. The statistical validity of these conclusions was assessed with two separate ANOVAs (one for each sex). For both sexes, ANOVA revealed reliable main effects of diet and genotype [ $F(1,19) = 19.84, p < 0.001$ ]. Subsequent post-hoc tests revealed that the low-fat diet increased body fat relative to chow, while high fat diet increased body fat relative to low fat diet ( $p$ 's  $< 0.05$ ). Further, in both sexes and all diets, mutants had increased body fat content relative to wild-types.

### **Experiment 3:**

*Energy expenditure:* Figure 7 depicts energy expenditure data in males, expressed as kcal expended per hour per gram body weight. Female data is similar to the males and is not shown here. Energy expenditure was not dependent on sex or genotype. That is, all mice appeared to expend similar kcal / kg body weight regardless of sex or genotype. These conclusions were supported by overall ANOVA, which yielded no reliable main effects or interactions (all  $F$ 's  $< 1.0$ ).

*Food restriction:* Additionally, neither the loss nor regain of body weight was dependent on sex or genotype (Figure 8). All mice lost approximately similar amounts of body weight during the 72-hr food restriction period. And, all mice regained that lost weight at approximately equal rates. Again, these conclusions were supported by the overall ANOVA, which yielded no reliable main effects or interactions (all  $F$ 's  $<1.0$ ).

## **Discussion**

Of course, increases in overall body weight can be the result of changes in lean tissue, water or caloric stores in the form of body fat. Consequently, we did a complete body composition analysis and found that, regardless of housing condition or diet, mutant mice had increased body fat, relative to wild types.

In an attempt to determine whether the increased body fat was the result of increased caloric consumption or decreased caloric expenditure, we measured both food intake and energy expenditure. First, we found no reliable differences in food intake between mutant and wild type mice. This was true for both types of housing and both types of diet. Second, we failed to observe any differences in energy expenditure between mutant and wild type mice. However, negative data on these measures, needs to be interpreted cautiously. Male mutant mice exposed to the high-fat diet for 3 months

demonstrated on average a 5.5 g difference in body weight. Based upon caloric content, differences in body composition and differences in body weight can be accounted for by 56 calories consumed and/or expended between mutant and wild-type mice over the 3-month period. The possibility exists that subtle changes in either expenditure or intake that could account for the difference in body fat are within the error of measurement for both of these endpoints.

Plasma insulin and leptin were also measured in group-housed animals after 3-months of exposure to chow and high-fat diet. Male mutants with access to high-fat diet demonstrated a reliable increase in circulating leptin after exposure to high fat diet. Female mutant mice demonstrated hyperleptinemia to both chow and high-fat diet. This result is consistent with previous reports indicating circulating levels of leptin reflect increased adiposity (11); (21). These data reinforce the finding that mutant mice demonstrate an increased adiposity without reflecting differences in overall body weight.

An alternate explanation for the increased body weight and adiposity of the D3-R<sup>-/-</sup> mice is that they have altered locomotor activity. It is well established that dopaminergic signaling plays a substantial role in regulating rodent locomotion (5); (13). Thus, it may be proposed that the mutant mouse gains body weight without demonstrating hyperphagia because of decreased locomotion in comparison to the wild-type mice. However, investigation into the role of the D3-R in locomotor behavior using antisense injections (33) and agonists delivered directly into the NAcc (36) have demonstrated a direct inhibitory effect on locomotion. Consequently, the D3-R mutant

mouse would be predicted to be hyperlocomotive compared to wild-type mice and therefore leaner. Consistent with locomotor analysis performed on the mutant mouse used in these studies (53) analyses with D3-R mutants from other laboratories demonstrate no reliable differences in locomotion between wild-type mice and mutant mice (1); (6).

One interesting possibility is that the D3-R is involved in a yet undiscovered metabolic pathway, which causes the mutant to store additional fat without hyperphagia as has been reported for melanocortin 3 receptor knockout mice (8). It is known that the D3-R has been reported to be involved in thermoregulation (2); [Kurashima, 1995 #3757}. The results of these studies indicate the D3-R agonist, 7-OH-DPAT, may cause a dose-dependent reduction in body temperature. However, these results appear inconsistent with our findings from the D3-R mutant mice. That is, the predicted body temperature of the mutant would be elevated as a result of the deletion of the D3-R. Metabolically this would result in a leaner phenotype. Although body temperature was not measured in this study, we did not observe any increase in energy expenditure that would result from increased heat production and thus our findings are consistent with results which suggest the D3-R does not play an exclusive role in dopamine-induced hypothermia (37).

Finally, microdialysis studies have revealed the involvement of the mesolimbic dopaminergic system (MDS) and more specifically the nucleus accumbens (NAcc) during feeding (17); (38); (39); (50). The MDS and reward elicited by food have been

linked though numerous different studies (18); (20); (27); (28); (29). Compared to other DA receptors, the D3-R has a quite limited expression pattern, almost entirely within limbic structures (7); (32); (26); (15). Targeted disruption of the D3-R has been hypothesized to reduce reward associated with drugs of abuse and psychostimulants (52); (9). However, the current data do not reveal a significant difference in either chow or high-fat food intake. Thus it would appear that alterations in food reward that may be produced by disruption of the D3-R are not large enough to alter cumulative intake under these circumstances.

## PERSPECTIVES

Thus the current data reveal a novel role for the D3-R in the control of adiposity that would appear not to simply be a function of altered energy intake or energy expenditure. Given that adiposity is regulated via a complex and undetermined set of behavioral, neural and endocrine factors, future research will need to address the exact nature of the D3-R using other types of tissue specific genetic manipulations or site-specific pharmacological manipulations. Given that the rising tide of obesity is associated with changes in lifestyle including changes in diet in the developed world, the diet-sensitive role of D3-R signaling to influence body adiposity deserves further experimental attention.

### Acknowledgements

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Figure 1. Body weight change ( $\pm$ SEM) of group-housed male and female wild-type and mutant mice given access to chow and high fat diet over a 3-month period. \*Different from wild-type mice,  $P < 0.001$ .

Figure 2. Kilocalories ( $\pm$ SEM) of standard chow (panel A) and high fat diet (panel B) consumed by group-housed males over a 3-month period.

Figure 3. Percent body fat ( $\pm$ SEM) of male wild-type and mutant mice with access to chow and high fat diet (panel A) when group-housed. Percent body fat ( $\pm$ SEM) of female wild-type and mutant mice with access to chow and high fat diet (panel B) when group-housed. \*Different from wild-type mice,  $P < 0.001$ .

Figure 4. Plasma insulin ( $\pm$ SEM) (panel A) and leptin ( $\pm$ SEM) (panel B) in male wild-type and mutant mice given access to chow and high fat diet. Plasma insulin ( $\pm$ SEM)

(panel C) and leptin ( $\pm$ SEM) (panel D) in female wild-type and mutant mice given access to chow and high fat diet. \*Different from wild-type mice,  $P < 0.001$ . a,b,c and d in panel D indicate all bars different,  $P < 0.05$ .

Figure 5. Body weight change ( $\pm$ SEM) in individually-housed males (panel A) with access to chow, low fat, and high fat diet over the period of 1-month. Body weight change ( $\pm$ SEM) in individually-housed females (panel B) with access to chow, low fat, and high fat diet over the period of 1-month. \*Different from wild-type mice,  $P < 0.001$ .

Figure 6. Percent body fat ( $\pm$ SEM) of individually-housed males with access to chow, low fat, and high fat diet over the period of 1-month. \*Different from wild-type mice,  $P < 0.001$ .

Figure 7. Heat expenditure ( $\pm$ SEM) (kcal/hr/kg) of male wild-type and mutant mice, as determined by the indirect calorimeter, over a 24-hour period.

Figure 8. Body weight loss ( $\pm$ SEM) and gain of male wild-type and mutant mice after a 72-hour, 25 % of normal intake food restriction. Re-feeding indicates replacement of ad-lib chow.

Table 1. Body Composition

<b>3 Month Chow Diet</b>				
Sex	Genotype	Water	Lean Tissue	Fat
Male	+/+	16.7±0.44 <sup>a</sup>	9.68±0.42 <sup>a</sup>	1.39±0.11 <sup>a</sup>
	-/-	16.6±0.82 <sup>a</sup>	9.57±0.64 <sup>a</sup>	1.10±0.10 <sup>b</sup>
Female	+/+	12.1±0.30 <sup>c</sup>	7.27±0.14 <sup>c</sup>	0.89±0.11 <sup>c</sup>
	-/-	11.4±0.25 <sup>c</sup>	7.54±0.38 <sup>c</sup>	1.25±0.13 <sup>d</sup>
<b>3 Month High Fat Diet</b>				
Male	+/+	17.4±0.20 <sup>a</sup>	9.26±0.33 <sup>a</sup>	1.46±0.21 <sup>a</sup>
	-/-	18.4±0.18 <sup>b</sup>	11.6±0.29 <sup>b</sup>	4.80±0.48 <sup>b</sup>
Female	+/+	13.4±0.25 <sup>c</sup>	8.40±0.37 <sup>c</sup>	1.97±0.31 <sup>c</sup>
	-/-	12.8±0.19 <sup>c</sup>	7.85±0.40 <sup>c</sup>	3.11±0.43 <sup>d</sup>

Note: “+/+” = wild-type mice while “-/-” = mutant mice. ANOVAs on each dependent variable (Water, Lean Tissue, and Fat contents) were conducted followed by LSD post-hoc tests. Letters in superscript represent results from post-hoc tests. Values with different superscript letters are significantly different ( $p < 0.05$ ).

Figure 1

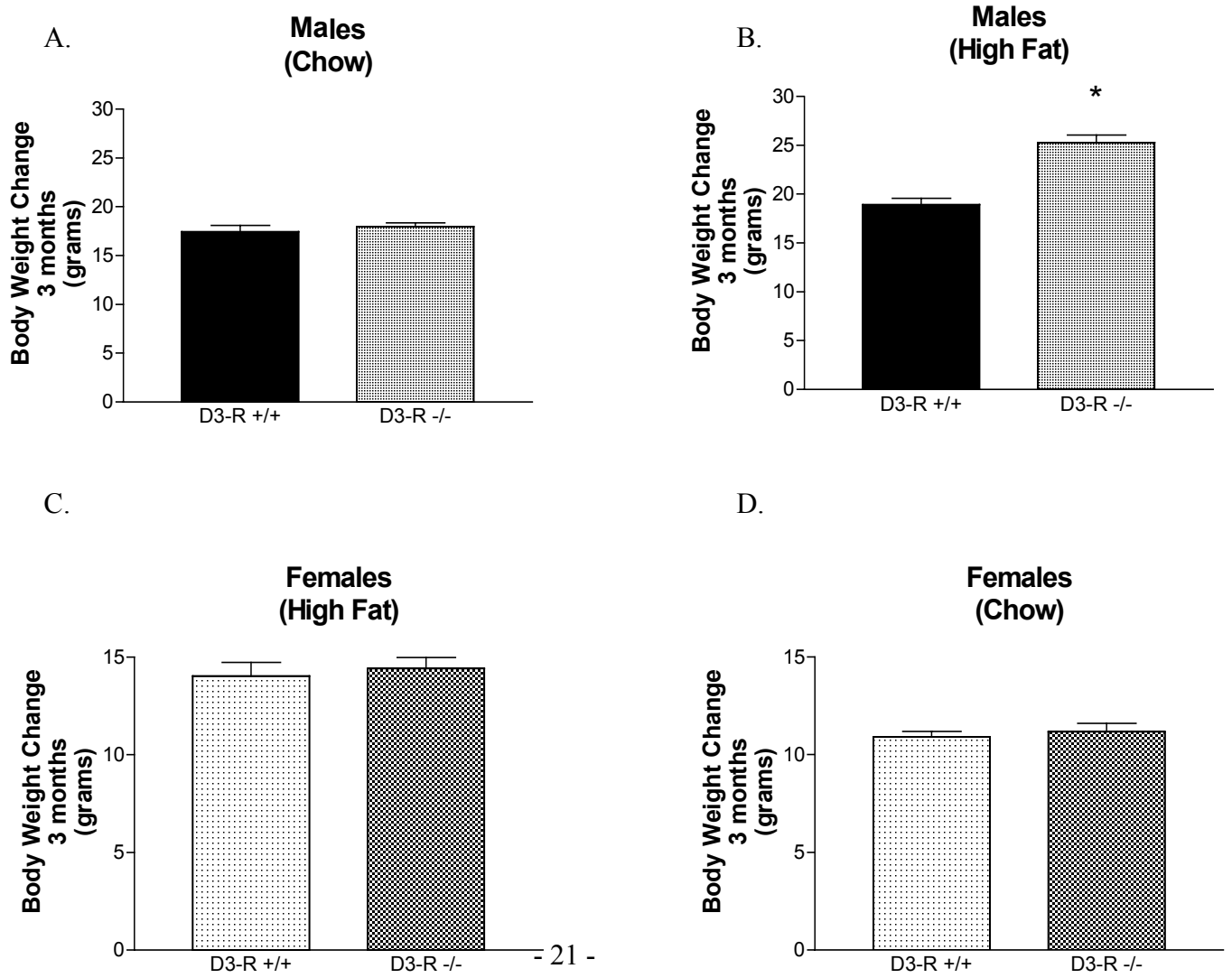
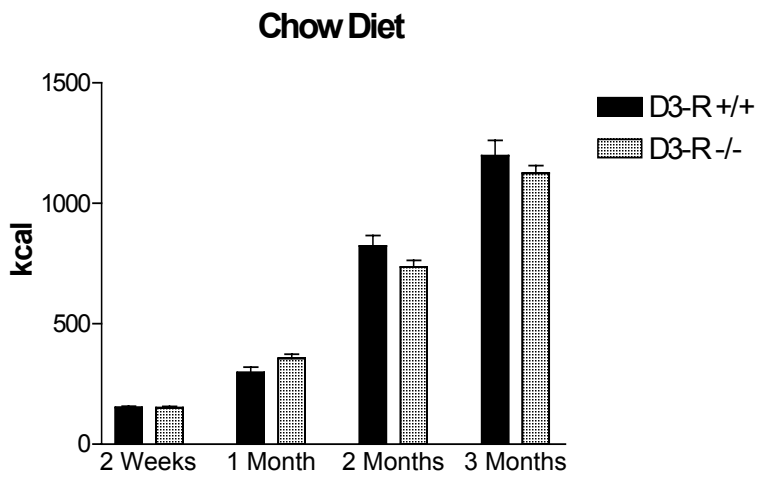




Figure 2

A.



B.

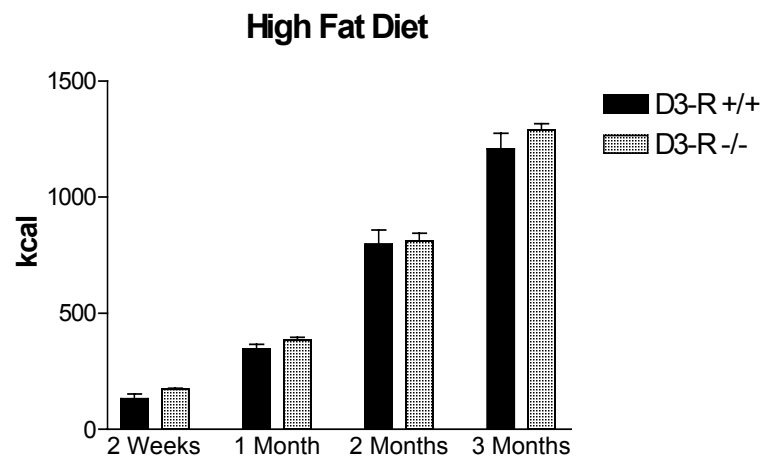
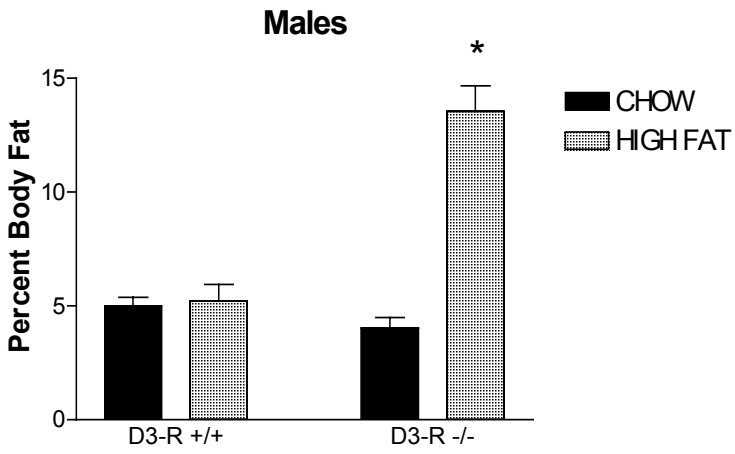


Figure 3

A.



B.

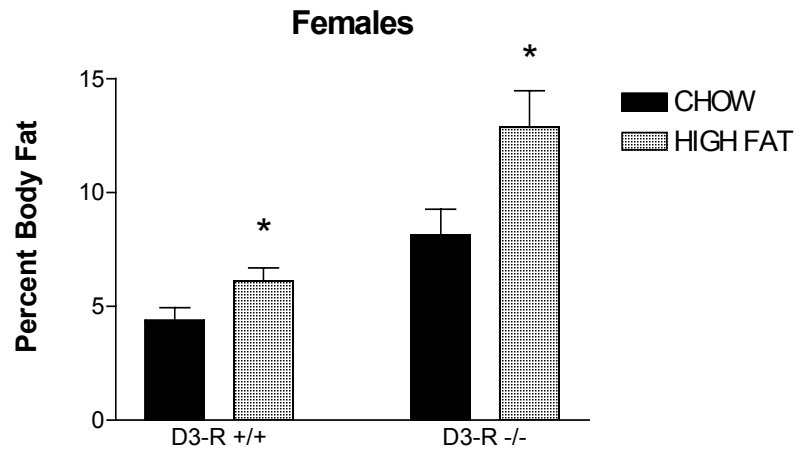


Figure 4

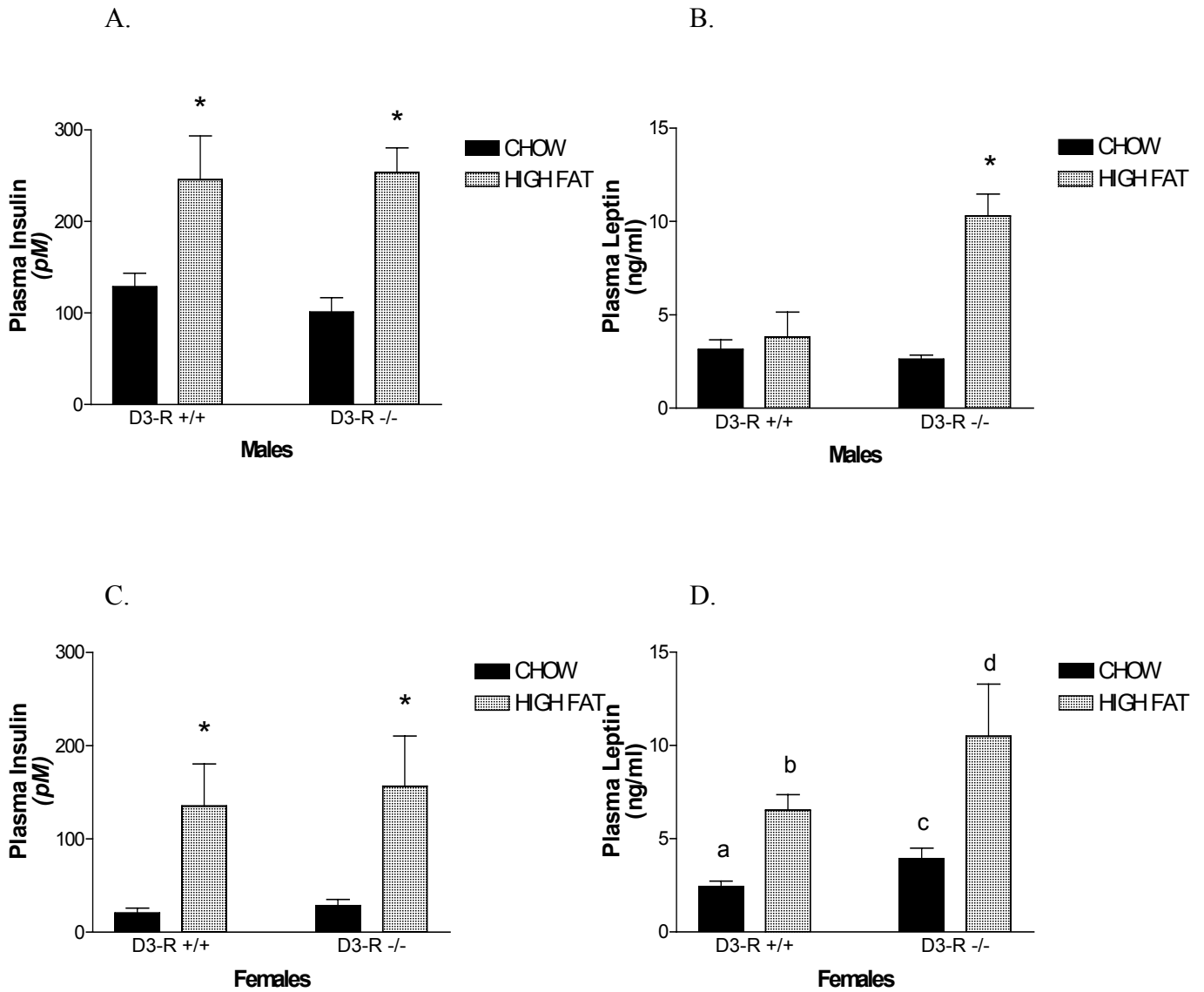


Figure 5

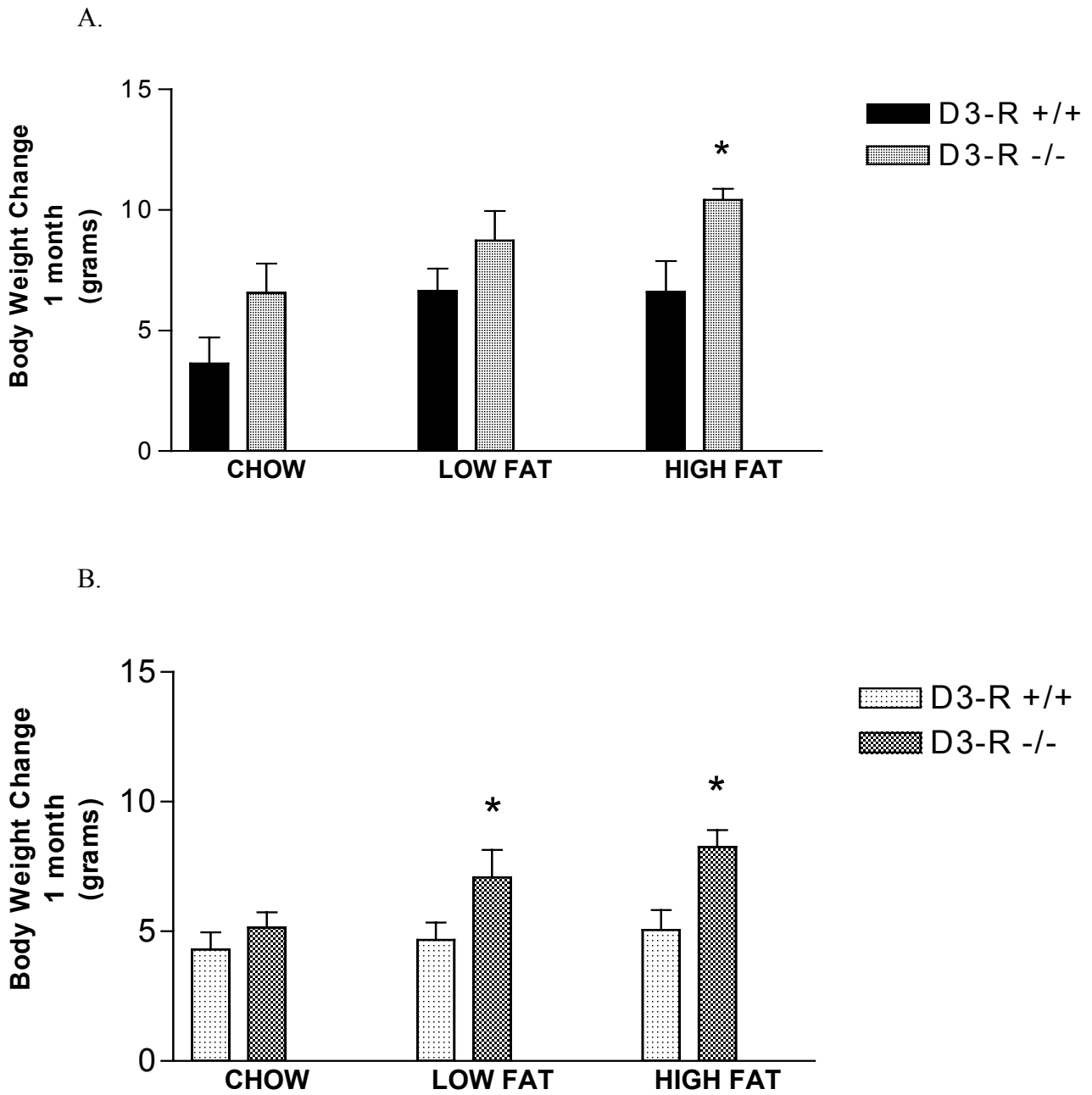


Figure 6

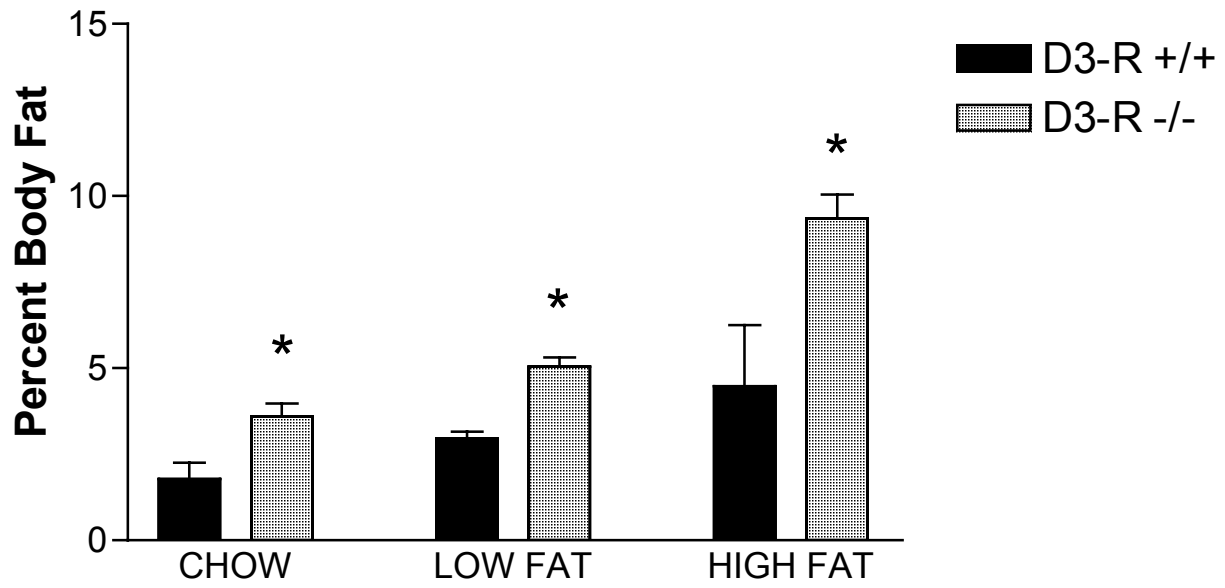


Figure 7

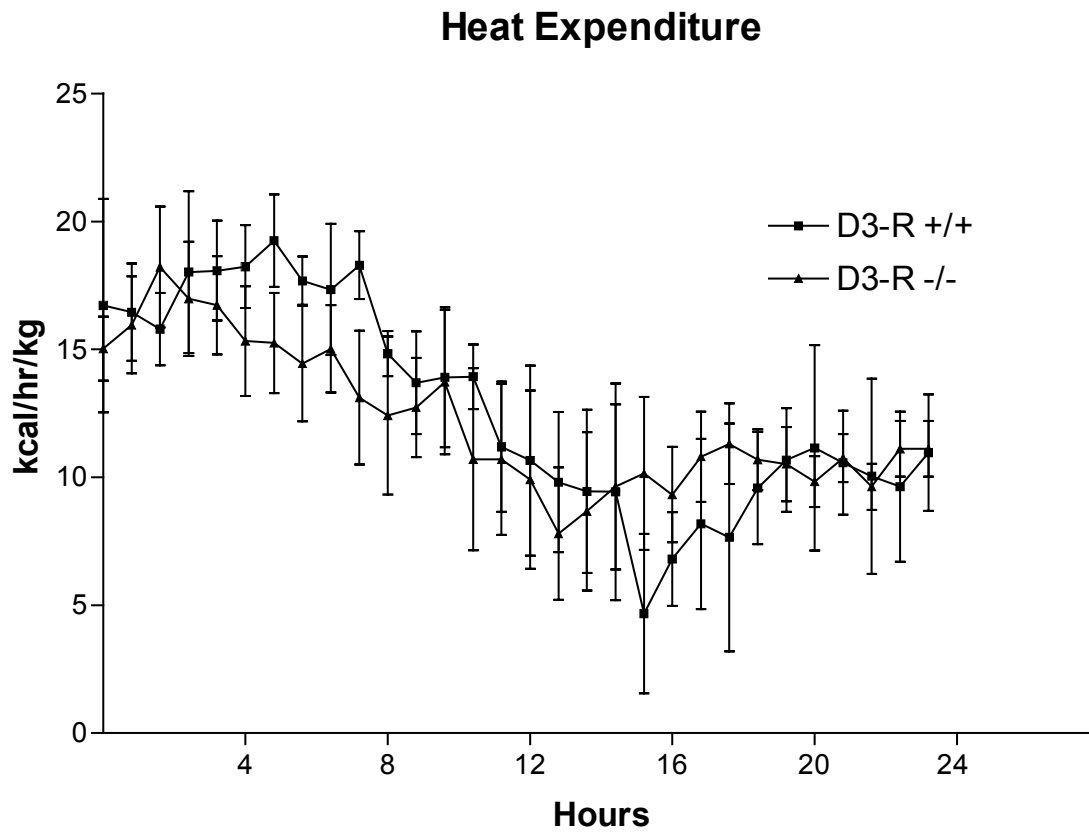
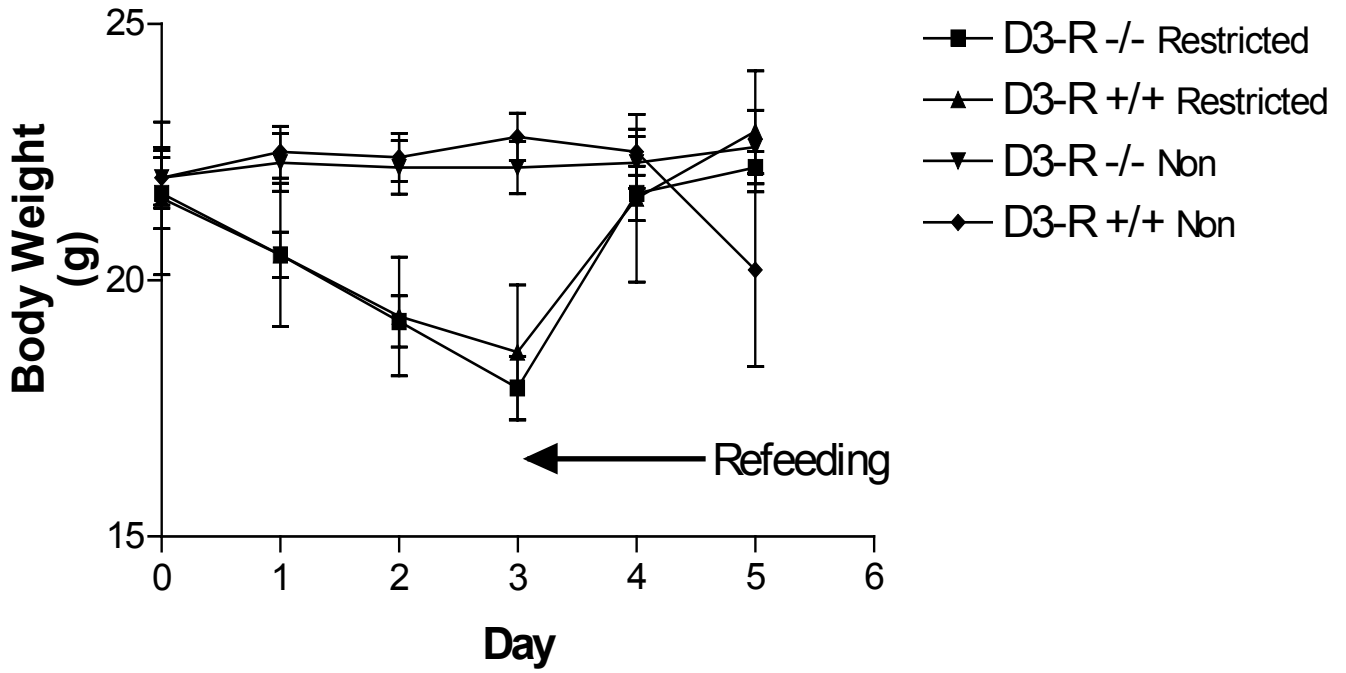


Figure 8



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