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Maternal high-fat diet effects on adaptations to metabolic challenges in male and female juvenile nonhuman primates

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Abstract

OBJECTIVE—Determine whether maternal high-fat diet (HFD) consumption in nonhuman primates alters the ability of offspring to adapt metabolically to nutrient and caloric challenges.

METHODS—Offspring from Japanese macaque dams fed either a control diet or a HFD were weaned onto a control diet creating two groups: maternal HFD (mHFD, n=18) and maternal control diet (mCTR, n=12). Male and female offspring were exposed to a 5-day 30% calorie restriction and to a 35-day high-fat diet (HFD) challenge, at 16 and 24 months of age respectively. Caloric intake, body weight and energy expenditure were measured.

RESULTS—Offspring from both groups showed similar body weight, food intake and metabolic adaptations to a 5-day calorie restriction. mHFD offspring demonstrated increased food intake and early weight gain in response to a 35-day HFD challenge; however, group differences in weight dissipated during the challenge. Unlike mCTR animals, the mHFD group had a significant increase in fasting insulin after acute HFD exposure.

CONCLUSIONS—The current findings indicate that offspring exposed to a maternal HFD show largely similar metabolic adaptations to calorie restriction as offspring exposed to a maternal control diet, but delayed adaptation upon exposure to an acute HFD challenge.

Keywords

obesity; metabolism; high-fat diet

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Introduction

Childhood obesity has a long-term detrimental impact on metabolic health. The development of obesity at an early age indicates these individuals will likely suffer from metabolic dysfunction for a significant portion of their lives. In addition, the majority of weight loss interventions have a low efficacy, and weight regain is common over the long-term (1–3), creating a life-long struggle with obesity. Numerous factors contribute to the rising prevalence of childhood obesity, including the increasing availability of high-fat and high-sugar diets and decreases in physical exercise (4). Maternal obesity has also been implicated as a factor contributing to childhood obesity in offspring (5–10). Given that 38% of women in the United States are obese (11), maternal obesity likely represents a significant and prevalent risk factor for future generations.

It remains a challenge to determine the contributions of a maternal diet in clinical studies since children will likely consume a similar diet to their mother's postnatally. This confound makes it difficult to separate the impact of maternal obesity versus postnatal diet on the development of childhood obesity. Studies in rodents have demonstrated significant metabolic impairments, including increased weight, adiposity and decreased activity in offspring born to obese mothers (12–15). However, development in rodents is significantly different from humans, with late development occurring postnatally in rodents and thus potentially less influenced by maternal environment. The nonhuman primate demonstrates similar gestational development to humans but retains the advantage of allowing for separation of pre- and postnatal diet consumption. Our group has spent many years characterizing the effect of maternal HFD exposure on nonhuman primates through one year of life (16–20). These studies were all carried out in basal metabolic conditions to assess differences in growth curves in the unchallenged state. However, metabolic adaptation to changing nutrient conditions is a fundamental aspect of homeostatic control and we hypothesize that maternal HFD may alter the ability of offspring to metabolically adapt to changing nutrient and caloric environments, which could underlie the vicious cycle of obesity. The current study examined the effects of maternal HFD consumption on offspring metabolism between 1 and 2 years of life, extending findings on the overall physiology of animals from this model, and their ability adapt to both a calorie restriction and a high-fat diet challenge.

Methods

Animals, maternal/postnatal diet, and body weight

All animal procedures were approved by the Oregon National Primate Research Center (ONPRC) Institutional Animal Care and Use Committee. Adult dams (*Macaca fuscata*) were assigned to the study, and offspring from a total of 32 dams are included in the current dataset, with 13 dams contributing more than one offspring. The average number of previous pregnancies for the dams was 2.76 ± 0.3 (mean $\pm$ SE), with a range from 0 to 7 previous pregnancies (n=46). The average maternal age at conception was 8.71 ± 2.6 years (mean $\pm$ SE) with a range of 4.5–13.5 years (n=46). The average weight at the time of conception was 9.36 ± 0.29 kg (mean $\pm$ SE) with a range of 6.5 – 15.1 kg (n=45). Dams were either maintained on a control diet (CTR, 15% calories from fat primarily from soybeans and corn;

Purina Mills Monkey Diet no. 5052) or placed on a HFD (37% calories from fat; Purina Mills TAD Primate Diet no. 5LOP, diet details provided previously (21, 22)), provided *ad libitum* for 8 months to 9 years prior to offspring delivery and throughout lactation. Dams were solely separated into their diet groups independent of obesity. Female and male offspring were born naturally and remained on dam's diets until weaning (ranging from 6–10 months of age). Before weaning, animals were housed in groups of 4–12 adults and their offspring (1–2 males per 3–10 females) in indoor/outdoor enclosures. At weaning, offspring were group housed in enriched indoor environments with 6–10 similarly aged juveniles and 1–2 unrelated adult females per group. Offspring from both maternal diet groups were assigned to a postnatal CTR diet yielding two groups mCTR (n=12, 7 females and 5 males) and mHFD (n=18, 9 females and 9 males). An additional cohort of offspring was switched to a HFD at weaning yielding two additional groups mCTRpHFD (n=6, 2 females and 4 males) and mHFDpHFD (n=9, 5 females and 4 males). Sample sizes vary for individual measurements and are therefore reported in all figure legends.

Energy expenditure

Indirect calorimetry was performed before and during calorie restriction and HFD challenges, as well as 7 days following calorie restriction as described previously (17, 23, 24). Monkeys were acclimated for 15 hours, then metabolic measurements were collected for 24 hours. Monkeys were fed a banana in the morning (0900h) and had unrestricted access to their normal diet in the afternoon (1400h); water was available *ad libitum*. A familiar monkey was housed across from the monkey undergoing metabolic testing to prevent social isolation. Oxymax software calculations of heat (kcal/hr) and respiratory quotients (RQ, VC_{O_2}/VO_2) were used for analysis.

Calorie Restriction Challenge—Animals underwent calorie restriction at approximately 16 months of age. Prior to the experiment, animals were sedated for an overnight fasted blood draw and weighed. Animals first underwent basal indirect calorimetry assessment and were immediately restricted to roughly 30% of their individual baseline caloric intake for the next five days. On the fifth and final day indirect calorimetry testing was repeated. Animals were then returned to *ad libitum* access to food and retested for indirect calorimetry 7 days later representing the “rebound” time point.

HFD Challenge—At roughly 24 months of age animals underwent baseline tests including glucose tolerance tests and indirect calorimetry. Five days of baseline CTR food intake were measured then the animals were switched to the HFD and food consumption was measured for the first 15 days of a 35-day challenge. Between days 15–35 of the HFD challenge, metabolic measures were collected again including indirect calorimetry in the metabolic chamber at approximately 16–24 days into the HFD challenge and glucose tolerance tests at approximately 30–35 days into the HFD challenge, performed as described previously (20).

Automated food intake measurements

Food intake during the HFD challenge was measured using an automated food dispenser system (Med Associates, Inc., St. Albans, VT) in group-housed offspring as previously described (17). Animals were identified by AVID microchips previously implanted in their

wrist (Avid Identification Systems, Inc., Norco, CA). The number of lever pulls and the animal associated with each pull were recorded and used to calculate caloric intake. This method has been previously validated in group-housed NHPs (25), but does represent an approximation as animals may not consume each pellet that is dispensed. Offspring were presented with a CTR diet [(TestDiet 5052, Primate Tablet #1814687, 5AP7, Richmond, IN) or HFD (TestDiet TAD 5L0P, Primate Tablet #1814863, 5ASN)] provided *ad libitum*.

Hormone Assays

Plasma concentrations of insulin and leptin were measured by the Endocrine Technologies Support Core (ETSC) at the Oregon National Primate Research Center (ONPRC) from fasted blood samples collected at GTTs. Insulin concentrations were determined using a chemiluminescence-based automatic clinical platform (Roche Diagnostics Cobas e411, Indianapolis, IN); this assay was used previously in nonhuman primates (26). The range of insulin was 0.2 – 1000 uIU/ml, with intra- and inter-assay variations less than 7%. Leptin concentrations were measured by radioimmunoassay (Millipore HL-81K, Billerica, MA) as described previously for the nonhuman primate (27). The assay range was 0.78–100 ng/ml and intra- and inter-assay variations were 13.9% and 24.7%, respectively.

Statistics—Data was analyzed in SPSS, except for area under the curve which was calculated in Prism GraphPad. Data was checked for normality and log₁₀ transformed to correct for normality when possible. For single time-points a linear model analysis was performed to examine the main effect of maternal diet. Longitudinal data was analyzed by mixed models repeated measure analysis and analyzed with main effects of maternal diet, time and their interaction. To examine the effect of dam age and weight at conception as well as offspring sex on the association between maternal diet and outcome variables, these variables were evaluated as potential covariates in both analysis types. The final parsimonious model was determined by backward selection. Any covariate with a main effect p-value less than 0.1 was left in the model and reported in the text for significant findings. All graphs were generated in Prism GraphPad.

Results

Baseline Metabolic Physiology at 16 and 24 months of age

Initial body weights at 16 months were similar between offspring from mothers consuming a HFD (mHFD) compared to offspring from mothers that consumed a control diet (mCTR; Fig. 1A). Average food intake at 16 months of age was also similar between the two groups (Fig. 1B). Indirect calorimetry did not find any differences in resting metabolic rate (RMR, average kcal/hr between 0100–0500), total energy expenditure (TEE, kcal/day) or average daytime (07:00–19:00) respiratory quotient (RQ, Fig. 1C and D). Animals from the mHFD group did show significant elevations in nighttime (19:00–7:00) RQ compared to the mCTR group (mCTR: 0.86, n=12; mHFD: 0.90, n=14, p<0.01; Fig. 1D). All measurements were repeated at 24 months of age, but no group differences in body weight, food intake, RMR, TEE, daytime or nighttime RQ were observed (Fig. 1E–H).

Calorie Restriction Challenge

At 16 months of age animals from both groups were exposed to a 5 day 30% calorie restriction protocol, with indirect calorimetry performed at baseline, on the 5th day of calorie restriction and 7 days following resumption of *ad libitum* food intake (rebound). The change in body weight at the end of the 5-day calorie restriction was not different between the groups (Fig. 2A). The average daily caloric intake was similar between mCTR and mHFD animals during the calorie restriction and rebound period (Fig. 2B). The average daily caloric intake during the rebound period was elevated compared to calorie restriction in both groups (mCTR: calorie restriction 280 kcal, rebound 393 kcal, $n=12$, $p<0.01$; mHFD: calorie restriction 287, rebound 438 kcal, $n=18$, $p<0.01$; mixed model adjusted for the covariate sex $p=0.02$).

Measures of indirect calorimetry demonstrated metabolic adaptations to acute calorie restriction, but relatively few differences between the two animal groups (Fig. 3). In both groups the RMR decreased as expected during CR (mCTR: baseline 5.42, calorie restriction 4.03 kcal/hr, $n=10$ $p<0.01$; mHFD: baseline 5.51 kcal/hr, calorie restriction 4.24 kcal/hr, $n=14$, $p<0.01$) and increased again during the rebound (mCTR: rebound 4.99 $n=10$, $p<0.01$; mHFD: rebound 5.63 $n=14$, $p<0.01$, Fig. 3A, C). TEE did not significantly change between baseline and calorie restriction (Fig. 3A and D); however, both groups had a significant increase in total energy expenditure between calorie restriction and rebound (mCTR: calorie restriction 2.29 log kcal/day, rebound 2.35 log kcal/day, $n=10$ $p=0.03$; mHFD: calorie restriction 2.37 log kcal/day, rebound 2.46 log kcal/day, $n=14$, $p<0.01$; Fig. 3B and D). Both mCTR and mHFD groups increased daytime RQ with calorie restriction (mCTR: baseline 0.83, calorie restriction 0.88, $n=12$, $p<0.01$; mHFD: baseline 0.84, calorie restriction 0.87, $n=14$, $p<0.01$; Fig. 3E and G) and daytime RQ remained elevated over baseline during the rebound period (mCTR: baseline 0.83, rebound 0.87 $n=12$ $p<0.01$, Fig. 3F and G; mHFD: baseline 0.84, rebound 0.87, $n=14$ $p=0.03$; Fig. 3F and G). Average nighttime RQ revealed a significant interaction with maternal diet across the three timepoints ($F_{2,48}=3.42$, $p=0.04$; Fig. 3E, F and H; mixed model adjusted for the covariate sex $p=0.08$). In addition to baseline differences in nighttime RQ (Fig. 1D and Fig. 3H), there were differences in the magnitude of response. The mCTR group demonstrated elevated nighttime RQ from baseline to calorie restriction (mCTR: baseline 0.86, calorie restriction 0.90, $n=12$, $p<0.01$), and from calorie restriction to rebound (mCTR: calorie restriction 0.90, rebound 0.94, $n=12$, $p=0.02$). mHFD animals did not demonstrate any differences in nighttime RQ between baseline and calorie restriction, but rebound nighttime RQ was higher than baseline (mHFD: baseline 0.90, rebound 0.93, $n=14$, $p=0.04$).

An additional set of animals were weaned onto a HFD (between 6–10 months of age, pHFD), creating two additional groups: mCTRpHFD ($n=6$) and mHFDpHFD ($n=9$). Overall pHFD groups show similar weight curves to pCTR groups from 16–23 months of age (Supplemental Fig. 1) and no baseline body weights differences were observed at 16 months of age prior to the calorie restriction (Fig. 4A). In addition, both metabolic rate and RQ appeared similar between groups at baseline (Fig. 4D and G). The effect of calorie restriction of a HFD was also examined in these animals, and revealed that unlike animals weaned onto a CTR diet, pHFD animals did not experience significant weight loss (Fig. 4B)

despite the reduction in caloric intake (mCTR: baseline 461 kcal/day, calorie restriction 325, n=6, $p<0.01$; mHFD: baseline 445 kcal/day, calorie restriction 320 kcal/day, n=8, $p<0.01$; Fig. 4C). In addition, there were no apparent elevations in nighttime RQ in pHFD groups during calorie restriction (Fig. 4G), whereas increases in nighttime RQ were noted during CR and rebound in the post-weaning CTR groups (Fig. 3). Daytime heat appeared modestly lower in the mHFDpHFD group during calorie restriction (Fig. 4E); however, missing data points prevented repeated measures statistical analysis of differences in TEE and RMR. This cohort showed a significant interaction of maternal diet across the three timepoints for average daytime RQ (maternal diet $F_{1,11}=4.2$, $p=0.07$, maternal diet*time $F_{2,10}=4.7$, $p=0.04$, mixed model adjusted for dam weight $p=0.02$). Specifically, mHFDpHFD demonstrated an elevated daytime RQ compared mCTRpHFD group during calorie restriction (mCTRpHFD 0.84 n=6, mHFDpHFD 0.89 n=9, $p<0.01$; Fig. 4H), as well as to their own baseline (mHFDpHFD: baseline 0.82, n=9, $p<0.01$; Fig. 4G and H). No differences in nighttime RQ were observed across the groups or time points.

HFD Challenge

At approximately 24 months of age, post-weaning CTR offspring were exposed to HFD for 35-days to determine whether maternal HFD consumption increases susceptibility to the detrimental effects of HFD exposure later in life as observed in rodents (28, 29). Automated food intake measurements in group-housed animals during the first 15 days revealed a significant effect of maternal HFD to increase HFD calorie consumption (maternal diet $F_{1,26}=8.02$, $p<0.01$; mCTR: average 753 kcal/day n=12, mHFD average 1060 kcal/day, n=18; mixed model adjusted for dam age at conception $p<0.01$ and dam weight at conception $p<0.01$; Fig. 5A). Average daily food intake was calculated for days when measurements from all animals were available and similarly revealed a significant increase in HFD consumption in the mHFD group (mCTR: 2.85 log kcal/day n=12, mHFD 3.01 log kcal/day n=18, $p<0.01$; mixed model adjusted for covariates dam age at conception $p<0.01$ and dam weight at conception $p<0.01$; Fig. 5B). Consistent with an initial modest increase in caloric consumption in the mHFD group, there was increased weight gain at Day 8 of the challenge (mCTR: 2.4%, n=12; mHFD: 6.7%, n=18, $p=0.02$; Fig. 5C) and a trend for an overall maternal diet effect (maternal diet $F_{1,28}=4.02$, $p=0.06$; mixed model adjusted for dam weight at conception $p<0.01$). However, the change in weight converged for the two groups at 15 and 23 days into the HFD challenge and was not significantly different between the two groups.

Indirect calorimetry collected during days 16–24 of the HFDC did not reveal any differences between mCTR and mHFD groups during the baseline or HFD challenge (HFDC, Fig. 6). However the magnitude of changes in energy expenditure measures was slightly different between groups. RMR increased between baseline and HFDC in mHFD group (mHFD: baseline 0.77 log kca/hr, HFDC 0.87 log kcal/hr, n=18, $p<0.01$; Fig. 6A and B), but not in mCTR animals. Similarly, TEE increased between baseline and HFDC for mHFD group (mHFD: baseline 344 kcal/day, HFDC 387 kcal/day, n=18, $p=0.02$; mixed model adjusted for sex $p=0.01$; Fig. 6A and C) but not the mCTR group. During the HFDC both groups demonstrated a decrease in daytime RQ (mCTR: baseline 0.84, HFDC 0.81, n=12, $p<0.01$; mHFD: baseline 0.85, HFDC 0.81, n=18, $p<0.01$; Fig. 6D and E; mixed model adjusted sex

$p < 0.01$ and dam weight at conception $p = 0.01$) and nighttime RQ (mCTR: baseline $-0.05 \log$ RQ, HFDC $-0.08 \log$ RQ, $n = 12$, $p < 0.01$; mHFD: baseline $-0.06 \log$ RQ, HFDC $-0.09 \log$ RQ, $n = 18$, $p < 0.01$; mixed model adjusted for dam age $p = 0.01$; Fig. 6D and F).

Intravenous glucose tolerance tests (ivGTT) were performed immediately prior to the HFDC (baseline) and during the last five days of the 35-day HFDC (Fig. 7). Fasting glucose levels were not different between mCTR and mHFD groups at either baseline or at the end of the HFDC (Fig. 7A). Fasting insulin significantly increased between baseline and HFDC in the mHFD group (mHFD: baseline $0.45 \log \mu\text{g/mL}$, HFDC $0.78 \log \mu\text{g/mL}$, $n = 18$, $p < 0.01$; Fig. 7B), but not in the mCTR group, but no difference between the two groups was observed at either time point. HOMA-IR, an index of insulin resistance, significantly increased during the HFDC in both groups (mCTR: baseline $-0.35 \log$ HOMA-IR, HFDC $-0.11 \log$ HOMA-IR, $n = 12$, $p = 0.05$; mHFD: baseline $-0.45 \log$ HOMA-IR, HFDC $-0.01 \log$ HOMA-IR, $n = 18$, $p < 0.01$; Fig. 7C). No difference in HOMA-IR was observed between the two animal groups. Glucose area under the curve (AUC) from zero during the ivGTT was not significantly different between groups or time points (Fig. 7D). Insulin AUC was significantly increased between baseline and HFDC for both groups (mCTR: baseline $3.16 \log$ AUC, HFDC $3.25 \log$ AUC, $n = 12$, $p < 0.01$; mHFD: baseline $3.11 \log$ AUC, HFDC $3.25 \log$ AUC, $n = 18$, $p < 0.01$; Fig. 7E); however, there was no difference at either time point between the mCTR and mHFD group. Serum hormone levels of leptin ($\approx 2.5 \text{ ng/mL}$) were not affected by maternal HFD exposure or by HFDC (data not shown).

Discussion

The current study examined the effects of maternal HFD consumption on the ability of offspring to adapt to changes in calorie and nutrient availability. Although there were subtle differences in the metabolic responses to a 5-day calorie restriction and a 35-day HFD challenge, animals adapted to these metabolic challenges in a largely similar manner regardless of the diet consumed by their mothers. One difference observed between groups was that mHFD offspring showed increased food intake compared to mCTR offspring when challenged acutely with a HFD, which corresponded to greater weight gain during the first week of the challenge. In addition, basal insulin secretion significantly increased after acute HFD exposure in the mHFD group but not in the mCTR group. These findings could indicate that mHFD offspring are more sensitive to the negative metabolic effects of a HFD, an effect that may be more pronounced with prolonged exposure.

The findings of the current study are in contrast to findings in mouse models of maternal obesity, which have shown that offspring from obese dams gain more weight when exposed to a HFD later in life (28, 29). While mHFD animals ate more and gained more weight on a HFD initially, this effect appeared to dissipate towards the end of the challenge. This could indicate a short delay in the onset of adaptive responses to the higher fat consumption in mHFD offspring, but that the overall mechanisms are in place. In addition to the acute brief nature of the HFD exposure, it should also be noted that animals in the current study were still young and active and thus likely more resistant to the obesogenic effects of the HFD than adult animals. Therefore, it remains possible that maternal HFD consumption does impact the ability of offspring to adapt to HFD consumption, but that this may require long-

term exposure in older animals. Studies examining the metabolism of offspring in the current model closer to the time of puberty are ongoing.

Although direct comparison between mCTR and mHFD animals did not reveal differences either before or at the end of the HFD challenge, there were subtle differences in insulin secretion. While mHFD animals showed an increase in fasting insulin during HFD exposure over their own baseline, this was not observed in the mCTR group. It should be noted that the change in insulin secretion in the mHFD group was subtle and that overall fasting insulin and insulin AUC levels were not different between mCTR and mHFD groups during the HFDC. Although the elevation in insulin was small it occurred after a relatively short 35-day duration of the HFD challenge and indicates that offspring exposed to a maternal HFD may develop insulin resistance more quickly with longer exposure to a HFD. The current study confirms clinical findings of maternal obesity increasing the incidence of childhood insulin resistance (5, 8) and illustrates how rapid the changes in insulin secretion may develop upon acute exposure to a HFD.

The current model has been well characterized for the impact of maternal HFD exposure on early fetal health and more recently on early adolescent metabolic health (16, 17, 20, 21, 30), and demonstrates that fetal offspring from mHFD groups weigh less initially but demonstrate more rapid catch up growth during the first year of life, resulting in normalization of weight at 13 months of age in those groups consuming a control diet post-weaning. This growth pattern is strongly associated with worsened metabolic health during adulthood in humans (31, 32), indicating animals from the current model may be prone to obesity in adulthood. The current study examined metabolic health of these animals shortly after bodyweight normalized in groups consuming a postnatal CTR diet, and similar to past findings, demonstrated limited basal differences in metabolism at this developmental stage. However, it is important to note that these animals are still relatively young and prepubertal; current studies are ongoing to look at metabolic health at three years of age. Consistent with the hypothesis that these animals may be programmed for metabolic dysfunction later in life is previous data demonstrating changes in the hypothalamic melanocortin pathway regulating appetite in offspring exposed to a HFD *in utero*, which was observed in fetal offspring as well as one year old offspring (17, 18). The melanocortin pathway is a critical component of the homeostatic pathway regulating appetite; therefore, alterations in this pathway were expected to result in a decreased ability to adapt to metabolic challenges. The apparent lack of a substantial differences between the groups indicates that alterations in melanocortin levels do not prevent acute adaptation to metabolic challenge in the current model. However, consistent with the alteration of melanocortins and their role in appetite regulation, almost every measure of food intake in the current study found mHFD animals consumed slightly more than mCTR animals. Although these increases were so small as to not be significantly different from mCTR animals, it is possible that over the span of the animals' life such a modest increase in daily caloric intake could significantly increase the risk of obesity.

CONCLUSION

The current report finds that maternal HFD consumption results in mild changes in the adaptive responses to HFD exposure later in adolescence. The modest increase in basal insulin indicates that maternal HFD consumption may increase the risk for diabetes upon consumption of a HFD later in life. Future studies utilizing this model will continue to examine the underlying mechanisms by which maternal diet programs metabolic dysfunction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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STUDY IMPORTANCE QUESTIONS

- Relatively little is known about how maternal obesity alters the ability of offspring to adapt to metabolic challenges in primates.
- Our findings indicate that maternal high-fat diet consumption does not dramatically alter the ability of offspring to metabolically adapt to acute calorie restriction or placement onto a high-fat diet later in life.
- While weight and metabolic parameters appeared similar across groups, maternal HFD animals demonstrated increased food intake and a greater increase in fasting insulin in response to acute access to HFD, indicating they may be more susceptible to the diabetogenic effects of this diet.

Figure 1.

Metabolic profiles of mCTR and mHFD animals at 16 (A–D) and 24 (E–H) months of age. Body weight (A, E), food intake (B, F), heat (kcal/hr; C, G), and respiratory quotient (RQ, VCO_2/VO_2 ; D, H) were measured during indirect calorimetry at 16 months of age (upper panel) and 24 months of age (lower panel) just prior to metabolic challenges. * Denotes a statistically significant difference between mCTR and mHFD nighttime average RQ. mCTR n=12, mHFD n=16–18.

Figure 2.

Changes in body weight (BW) and food intake following a 5-day 30% calorie restriction. Body weight on the final day of the calorie restriction was compared to body weight measured four days prior to the challenge to determine the change in BW (A). The average daily food intake was measured in the indirect calorimetry chamber on the final day of the calorie restriction and 7 days later (Rebound; B). * denotes a statistically significant difference from the mCTR calorie restriction, # denotes a statistically significant difference from mHFD calorie restriction. mCTR n=10–12, mHFD n=14–18.

Figure 3.

Energy expenditure and fuel oxidation during calorie restriction and 7 days following resumption of *ad libitum* food intake (rebound). Hourly figures for heat during calorie restriction and rebound (A, B) and analysis of the resting metabolic rate (avg kcal/hr between 0100–0500, C) and total energy expenditure (sum of kcal/hr between 0800–0700, D). Hourly figures for RQ during calorie restriction and rebound (E, F) and calculation of the average daytime (G) and nighttime RQ (H). * denotes a statistically significant difference from mCTR baseline, # denotes a statistically significant difference from mHFD baseline, \$ denotes a statistically significant difference from mCTR calorie restriction, + denotes a statistically significant difference from mHFD calorie restriction, ~ denotes a statistically significant difference between mCTR and mHFD at baseline. mCTR n=12, mHFD n=14–18.

Figure 4.

Effects of calorie restriction on offspring consuming a HFD. A separate cohort of offspring were exposed to either mCTR or mHFD conditions then all offspring were weaned onto a HFD in the post-weaning period (pHFD). Initial body weight prior to calorie restriction was measured (A), as well as body weight on the final day of the HFD calorie restriction to determine the change in BW (B). The average daily food intake was measured in the indirect calorimetry chamber immediately prior to calorie restriction (baseline), on the final day of the calorie restriction and 7 days later (Rebound; C). Indirect calorimetry was performed at baseline, during calorie restriction and during rebound and hourly graphs for heat (kcal/hr, D–F) and respiratory quotient (RQ, G–I) are presented. * denotes a statistically significant difference from mCTR baseline, # denotes a statistically significant difference from mHFD baseline. mCTRpHFD n=5–6, mHFDpHFD n=5–9.

Figure 5.

Daily and average food intake and change in body weight during an acute HFD challenge. Daily food intake was measured in group housed animals using an automated monitoring system for 5 days on a chow diet prior to introduction of the HFD. Food intake was measured daily for the first 15 days of the challenge and then for 5 days following the reversal back to the control diet (A). Average daily food intake was calculated for all days within the first 15-day period of HFD consumption when data was available for all animals (B). Percent weight change from the first day of the challenge was calculated from weights obtained at 8, 15 and 23 days into the challenge (C). * denotes a statistically significant difference from mCTR. mCTR n=8–12, mHFD n=14–18.

Figure 6.

Energy expenditure and fuel oxidation during the HFD challenge (HFDC). Hourly figure for heat during the HFD challenge (A) and resting metabolic rate (avg kcal/hr between 0100–0500; B) and total energy expenditure (sum of kcal/hr between 0800–0700; C). Hourly figure for RQ during HFDC (D) and calculation of the average daytime (E) and nighttime RQ (F). * denotes a statistically significant difference from mCTR baseline, # denotes a statistically significant difference from mHFD baseline. mCTR n=12, mHFD n=18.

Figure 7.

Glucose and insulin sensitivity measurements before and during the HFDC. Intravenous glucose tolerance tests (ivGTT) were performed immediately before HFD exposure (baseline) and at days 30–35 of the HFD challenge. Initial fasting values of glucose (A) and insulin (B) were used to calculate the HOMA-IR ((glucose mg/dL $\times$ insulin)/405; C). ivGTT area under the curve (AUC) calculations for glucose (D) and insulin (E) were calculated from zero at baseline and during the HFDC. * denotes a statistically significant difference from mCTR baseline, # denotes a statistically significant difference from mHFD baseline. mCTR n=12, mHFD n=18.