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Methyl donor supplementation alters serum leptin levels and increases appetite but not body weight in cross-fostered male Syrian hamster offspring (*Mesocricetus auratus*)

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Abstract

A pregnant hamster's exposure to changes in environmental factors, such as light, temperature, and nutrition, may influence behavioral and physiological changes in offspring. In this study, dietary methyl donor supplementation was employed to examine the role of maternal diet on appetite, body weight, serum leptin levels, and locomotor activity in male Syrian hamster offspring. Dams were fed a standard control (SC) or methyl donor supplemented (MDSD) diet through pregnancy and lactation. At birth, offspring were cross-fostered to dams fed an SC or MDSD diet (SC-MDSD and MDSD-SC) or remained with their birth mothers (SC-SC and MDSD-MDSD). At weaning, offspring were fed a SC or MDSD diet until 60 days of age. Food intake, serum leptin levels, and locomotor activity were measured from 30-60 days of age. Offspring fed a MDSD diet post-weaning (MDSD-MDSD and SC-MDSD) consumed more than double the amount of food daily compared with offspring fed a SC diet postweaning (SC-SC, MDSD-SC). Interestingly, there were no observed differences in body weight among all four groups. Serum leptin levels at 60 days of age were depressed in offspring fed a MDSD diet postweaning (MDSD-MDSD and SC-MDSD). There were no observed differences in wheel running activity between the SC-SC and MDSC-SC groups. Wheel running activity was at least twice the amount in offspring fed a MDSD diet postweaning (SC-MDSD and MDSD-MDSD). Taken together, these results indicate that the timing of methyl donor supplementation appears to be an important factor during the development of offspring.

KEYWORDS: hamster, leptin, methyl donor supplementation, maternal transfer

1 INTRODUCTION

Genes, environmental factors, and their interactions may contribute to the health or development of diseases in organisms. One important environmental factor is nutrition (Kauwell, 2005). Nutrition is a strong player not only for its influence on gene expression but more importantly, because early alterations in nutrition could contribute to later development of chronic diseases through epigenetic mechanisms. Early life nutritional exposures, such as maternal protein restriction in pregnancy and methyl donor nutrients including folate, methionine, and some B-vitamins, may leave long-lasting changes in DNA methylation (Peter

et al., 2016; Choi & Friso, 2010), which is a biological process where methyl groups are added to DNA. Numerous studies have focused on the link between diet and DNA methylation in mammals to elucidate the dietary exposures that may have lifelong consequences on epigenetic marks (Jaenisch & Bird, 2003; Waterland & Michels, 2007). Different types of *in vitro* and *in vivo* studies demonstrated the relationship between nutrition and DNA methylation, including during prenatal and postnatal periods, and indicate that diets deficient in methyl donors and proteins may cause global DNA hypomethylation. Furthermore, they also demonstrated that high-fat diet consumption may result in changes in DNA methylation (Zhang, 2015; Amarasekera et al., 2014; Yu et al., 2015; Altmann et al., 2013). Because DNA methylation affects gene expression and is passed down from generation to generation, nutrients can have a variety of effects on gene regulation. For example, folate, vitamin B12, choline, betaine and methionine may be methyl group donors. One of the most popular models, the 'yellow agouti (Avy) mice' model, examined the link between diet and DNA methylation. The agouti gene is responsible for the regulation of brown/black (eumelanin) and yellow (pheomelanin) pigmentation in the mammalian coat (Waterland & Jirtle, 2003). It has also been shown that if the mothers are provided some nutrients (e.g., methionine, betaine, folate, and vitamin B12) during their pregnancy, the agouti gene is suppressed and obesity does not develop in mice (Wolff, Kodell, Moore, & Cooney, 1998).

Environmental factors can also alter or disrupt the circadian rhythms of many physiological parameters in organisms. Studies show that nutrient availability affects the circadian control of locomotor activity. In a study in rats, it was observed that locomotor activity increased in anticipation of daily feding (Richter, 1922). In addition, most food restricted hamsters displayed increased locomotor activity (Bae, Larkin & Zucker, 2003). No study showing the relationship between early methyl donor nutrition and locomotor activity has been done to date.

Leptin, a protein secreted by the adipocytes after feeding, mirrors body fat content and signals the status of energy stores to the brain (i.e., inhibits food intake). In this regard, leptin receptors have been reported in the suprachiasmatic nuclei (SCN) (Guan et al., 1997). The release of leptin exhibits a 24 h rhythm that varies in timing between different laboratory rodent species (Ahima et al., 1996). In mice and rats, serum leptin concentrations and mRNA content of leptin in adipose tissue decrease during the light phase and increase in the dark phase. (Ahima, Prabakaran & Flier, 1998; Pickavance, Tadayyon, Williams & Vernon, 1998; Shimokawa & Higami, 1999). In Syrian hamsters (*Mesocricetus auratus*), however, serum

leptin concentrations decrease during the dark phase and increase in the light phase (Gündüz, 2002).

The Syrian hamster is an animal that exhibits strong seasonality and photoperiodism. Although this species is used extensively as an animal model to study the physiological mechanisms that underlie the photoperiodic control of circadian rhythms, little information is available about the interactions between methyl donor nutrition, leptin levels, and locomotor activity in this hamster species. Utilizing a cross-fostering experimental design, we tested the hypothesis that early exposure (gestation) to a methyl donor supplemented diet would alter feeding behavior, serum leptin levels, body weight, and locomotor activity in male Syrian hamster offspring.

2 MATERIALS AND METHODS

All animals were treated and cared for in accordance with the guidelines of Canakkale Onsekiz Mart University. The protocol (permit no: 2014/07-08) was approved by the Institutional Animal Care and Use Committee (08/14/2014). Animals were maintained in plastic cages (16 x 31 x 42 cm) with pine shaving used as bedding. Animals had *ad libitum* access to food pellets and tap water. Studies were conducted under long photoperiod 14L (14 h light, 10 h dark; lights off between 20.00–06.00 h). All lighting was provided by cool-white fluorescent tubes controlled by automated, programmable timers. Ambient temperatures in the animal facilities were held constant at 22 ± 2 ^{o}C in air-ventilated rooms. All animals were fed a control diet (Purina Rodent Chow; diet formula contained 57% carbohydrates, 13% lipids, 30% proteins, and caloric density=3.35 kcal/g) prior to breeding (two weeks before). From the onset of breeding, through pregnancy (16-18 days) and lactation (20 days), dams (n=5/group) were fed one of two diets: 1) a standard control (SC) diet; and 2) a methyl donor-supplemented (MDSD) diet, which was designed to provide substantially increased amounts of cofactors and methyl donors for methyl metabolism. Methyl donor supplementation involved addition of the following nutrients per kg of feed: 5 g choline chloride; 5 g betaine; 5 mg folic acid; and 0.5 mg vitamin B12 (Wolff et al., 1998). The MDSD diet was pelleted after addition of the supplements. Both SC and MDSD diets were fed *ad libitum* to dams.

Cross-Fostering Experiment: After dams were fed either an SC or MDSD diets for two weeks, ten dams were mated. At parturition, two groups of cross-fostered offspring were generated: offspring exposed to a SC diet during pregnancy who were cross-fostered to a MDSD-fed dam during lactation (SC-MDSD, n=10); or offspring exposed to a MDSD diet during pregnancy and cross-fostered to a SC-fed dam during lactation (MDSD-SC, n=10).

Two non-cross fostered groups included offspring exposed to a SC diet during pregnancy and reared by the same dam fed on a SC diet (SC-SC, n=10) and offspring exposed to a MDSD diet during pregnancy and reared by the same dam on a MDSD diet (MDSD-MDSD, n=10). The dams were carefully removed from their home cage and temporarily placed in empty cages equipped with pine shaving and some food pellets. Using clean gloves to avoid smell of the birth dam,
offspring were then removed from the cage. The litters were placed in artificial nests while the bedding in home cages was changed. The litters were placed in the cage of their foster mothers, and the dams were returned to their home cages. The cross-fostering procedure did not take longer than 5 min. After some reorganization of the offspring and the nest, the dams calmed down and began to lactate. Litter size was normalized to 10 offspring as necessary to equalize access to nutrition throughout lactation. Dams continued to be fed a SC or MDSD diet during lactation. The cross-fostering procedure thus produced two non-cross fostered groups (SC-SC and MDSD-MDSD) and two cross-fostered groups (SC-MDSD and MDSD-SC) (Figure 1).

At weaning (20 days of age), dams and female offspring were returned to the hamster colony. At weaning, male offspring were single-housed in cages. The male offspring continued to be fed diets that their birth mothers or foster mothers were fed. Taking a dam's own offspring from it immediately after birth and providing another dam's offspring was a source of stress, but only one dam rejected the offspring in this experiment. This did not affect the number of male offspring obtained for the study.

2.1 Measurement of body weight

Body weight measurements of male offspring were taken at birth, at weaning, and initiated starting at 30 days of age. Weight measurements after 30 days of age were taken at the same time every 10 days between the hours of 19.00-20.00 h. Before individual animals were placed on a digital scale, food pieces in their mouths were checked (excluding newborn and weaned offspring), if any, in order not to affect their weight. All measurements were completed within 15-20 min.

2.2 Measurement of food intake

Male offspring continued to be fed diets that their birth mothers or foster mothers were fed. Male offspring were given *ad libitum* access to a known amount (~ 10 g/d) of SC or MDSD pellets daily from 20-60 days to age. Food was placed in cages before the lights turned off (20.00 h). Food in each wire mesh food hopper was weighted daily and an equal amount

of fresh food was added to replace the amount consumed. Obvious food particles that had dropped through the mesh hopper were removed and weighed as well. Because Syrian hamsters also store their food in their mouths, possible food pieces were also collected from here. The difference between these two measurements was calculated as a measure of daily food consumed per animal. Food intake was measured every day but were taken as the average of five-day intervals.

Daily food intake in dams was measured prior to breeding (10 days before), during pregnancy (16-18 days) and lactation (20 days). All measurements are represented as an avarage of each period. The same technique for measuring food intake in offspring was applied to adults.

2.3 Measurement of locomotor activity

Locomotor activity of male offspring from 30-60 days of age was measured by running-wheel activity (Lafayetta Instrument Acitiviy Wheel System; IN, USA). The number of wheel revolutions per ten minute interval was automatically recorded and stored on a computer hard drive. The stored results were analyzed by the AWM-Data Management Program (Microsoft Excel Add-In) and Actogram software. Activities are represented as double-plotted actograms.

2.4 Measurement of serum leptin levels

For leptin measurements, approximately 1.0 ml of blood was collected from animals every week at midday (between 12.00 and 13.00 h) and midnight (between 00.00 and 01.00 h) from the orbital sinus under light ether anesthesia. The first sample was taken at 30 days of age. Samples taken during the dark phase were taken under a dim red light. To prevent the loss of circulating plasma volume, a 0.9% NaCl solution was injected intraperitoneally immediately after each blood collection in the same volume as drawn. The NaCl replacement solution was sterilized and warmed to body temperature prior to replacement. Blood samples were centrifuged at 4 °C for 30 min at 1000 × g. Serum aliquots were aspirated and frozen at –20 °C. Leptin was measured with a commercial ELISA kit according to the manufacturer's instructions (ICN, Costa Mesa, CA, USA). Serum concentrations of hamster leptin were measured in duplicate, with a lower detection limit of 0.5 ng/ml. Both the intra- and inter-assay coefficients of variation (CV) were less than 10% (Gündüz, 2002).

2.5 Statistical analysis

Data were expressed as the means ± SEM and analyzed using the statistical software package IBM SPSS for Windows version 22.0. Differences in body weight, food intake, locomotor activity, serum leptin levels were examined using two-way ANOVA (maternal diet

x methyl supplementation). Interactions and main effects are described in Results, and significant Duncan's post-hoc comparison tests, where required, are indicated in the figures. Results are considered statistically significant at a two-tailed α level of 0.05.

Justification of sample size: The accessible population of this study is new-born pups of hamsters. Inclusion criteria is new-born, male, Syrian hamster pups, without any physical deformities and able to drink milk from their dam. Exclusion criteria is sick pups and drop out criteria is dead male pups during the experimental period. The sample size used in this study was determined with a statistical power of 0.8 by following the procedures described by Charan and Kantharia (Charan & Kantharia, 2013).

Using the formula of $(t-1)(r-1) \geq 15$ where t = number of experimental group, r = repetitions in this study, t = 4, written. Thus, the equation is written as: $(4-1)(r-1) \geq 15$.

Using the formula resulted in r = 6, therefore, the minimal sample size for this study was 6x4=24 male hamsters. On the other hand, specific to this study, 10 animals were used for each group, considering the losses that may occur after birth, during lactation and during experimental procedures. These numbers were established by carefully considering the possibility that the dams can eat their offspring due to stress, especially after birth, and that the dam can also reject the pups during cross-fostering.

3 RESULTS

3.1 Food intake

The effects of SC and MDSD diets on food intake on male hamster offspring is shown in Figure 2. There were no observed differences in food intake between the SC-SC and MDSD-SC groups where offspring consumed SC diet post-weaning ($p > 0.05$). Significant increases in food intake of more than double the amount were observed in SC-MDSD and MDSD-MDSD groups compared with the SC-SC and MDSD-SC groups, where offspring consumed a MDSD diet post-weaning ($p < 0.01$). Moreover, significant incremental increases in food consumption was observed in the MDSD-MDSD group compared with the SC-MDSD group starting from 50 days of age ($p < 0.01$). This difference was statistically more significant at 60 days of age ($p < 0.001$).

The effects of SC and MDSD diets on daily food intake in dams is shown in Figure 3. There were no observed differences in average daily food intake between the SC and MDSD groups prior to breeding. The average daily food intake significantly increased during pregnancy in both SC and MDSD groups ($p < 0.05$), although there were no differences detected between these group ($p > 0.05$). Similarly, there were no differences detected in daily

food intake in all dams (SC-SC, SC-MDSD, MDSD-MDSD, MDSD-SC) during lactation, although the amount consumed was significantly lower following pregnancy ($p > 0.05$).

3.2 Body weights

Analysis of this data set revealed no significant differences detected in body weight among all four SC and MDSD groups ($p > 0.05$) (Figure 4).

3.3 Serum leptin levels

Serum leptin levels in the four groups of offspring did not show any significant differences until 60 days of age (Figure 5). In all four groups, serum leptin levels were signficantly higher in the light phase as compared to the dark phase. In addition, serum leptin levels in offspring fed a MDSD diet postweaning (MDSD-MDSD and SC-MDSD groups) exhibited significantly lower leptin levels compared with the SC-SC group in both the light and dark phases. The trend was reversed in the MDSD-SC group where serum leptin levels were significantly higher compared with the MDSD-MDSD group in light and dark phases.

3.4 Locomotor activity

Representative actograms showing locomotor activities of individual animals from the SC-SC and MDSD-MDSD groups are shown in Figure 6. There were no differences detected in the period length of locomotor activity among the four groups of offspring (SC-SC: 22.50 ± 0.21 h; MDSD-MDSD: 22.56 ± 0.21 h; MDSD-SC: 23.10 ± 0.18 h; SC-MDSD: 23.05 ± 0.20 h). Because the actogram of the MDSD-SC group from the groups in the cross-fostering experiment is similar to Figure 5A and the actogram of the SC-MDSD group in the cross-fostering experiment is similar to Figure 5B, these data are not presented.

Significant differences in the number of average wheel turns were observed among the male hamster offspring (Figure 7). Both the SC-SC (21.08 ± 3.1 mean wheel turn (mwt)/d, n=10) and MDSD-SC (24.01 ± 2.2 mwt/d, n=10) groups exhibited the lowest rates of wheel running activity. Wheel running activity was twice as high in the SC-MDSD group (42.1 ± 0.18 mwt/d, n=10). The MDSD-MDSD group exhibited the highest rate of wheel running activity (51.3 ± 0.22 mwt/d, n=10) compared with the other three groups.

4 DISCUSSION

There is increasing evidence that maternal diet may have significant impacts on the long-term health of offspring. Underlying this association has been the development of a range of animal models that support detailed investigations into the mechanisms driving the maternal programming of offspring (Gündüz & Stetson, 2003). In the current study, results show that male offspring fed a SC or MDSD diet prenatally and postnatally display

significant differences in food intake, serum leptin levels, and average number of wheel turns/day. Interestingly, there was no observed differences in body weights of male hamsters among the groups from birth to 60 days of age. Taken together, these findings may provide new insight into the mechanisms linking maternal diet with physiological processes in hamsters, which may have implications for human health.

The transfer of information (e.g., homeostatic and environmental information) from mother to offspring occurs through maternal transfer. Maternal effects can have significant impacts on offspring via non-genetic factors (e.g., hormones, foods, antibodies) that mothers provide to their offspring (Mousseau & Fox, 1998). Environmental factors (e.g., light, food, temperature) to which a pregnant mother is exposed and subsequent effects on her offspring are also observed physiologically. It has been shown that offspring born to unhealthy mothers and fed methyl donors will turn out healthy (Wolff et al., 1998).

Changes in food intake and body weight differ from species to species. Siberian hamsters reduce their body weight during the winter (short day length) (Stebbins, 1978; Dark & Zucker, 1986; Reiter, 1993), while Syrian hamsters increase their body weight (Reiter, 1993; Bartness & Wade, 1984). In addition, short photoperiods cause a decrease in body weight in studies conducted on gerbil species (e.g., *Meriones crassus, Gerbillus dasyurus and Gerbillus henleyi*). Moreover, it has been shown that body weight development is directly proportional to the amount of food consumed (Karakaş, Çamsarı, Serin, & Gündüz, 2005).

Body weight regulation mechanisms in photoperiodic mammals have not yet been clearly elucidated, however, there are important findings about the roles of hormones, such as melatonin and leptin, related to this mechanism. The brain region that controls body weight are the suprachiasmatic nucleus (SCN) and arcuate nucleus (ArC) in the hypothalamus. Melatonin and leptin can cause changes in body weight depending on the species (Wolden-Hanson et al; 2000). In this study, serum leptin levels were significantly lower in hamster offspring groups (MDSD-MDSD and SC-MDSD) 60 days post partution, which suggests that the effects of dietary methyl donor supplmentation may occur after birth in this hamster species. Other studies, it has been demonstrated that methylating substances given during pregnancy do not affect body weight in the long term (Knopik, Marceau, Bidwell, & Rolan, 2019). Early exposure to methyl donor deficiency was not associated with increased or decreased body weight in Swiss mice (Cavalcante-Silva et al., 2016). Other studies in mice showed that leptin levels are affected by food intake, that is, its level decreases with short-term starvation and remains at a low level until food intake resumes (Ahima, Prabakaran, & Flier, 1998). In addition, body weight loss in rats and human causes a decrease in leptin

levels, and vice versa (Maffei et al., 1995; Considine & Caro, 1997). In mice, decreased leptin production is due to body weight loss (Ahima, Dushay, Flier, Prabakaran, & Flier, 1997). Body weight findings obtained from our study are in agreement with data obtained from other species (Knopik, Marceau, Bidwell, & Rolan, 2019; Cavalcante-Silva et al., 2016), namely, methyl donor feeding does not affect the body weight mechanism. Due to the lack of difference in daily food intake between the dam groups prior to breeding and during pregnancy and lactation, the effect of methyl donor supplementation does not appear to be confounded with an effect of food intake level on offspring and conclusions on the sole effect of the methyl donor supplementation can be drawn from our results.

The activity of neuropeptide Y (NPY) and agouti-related protein (AgRP) neurons in relation to food intake is known to be regulated by leptin in the ArC (Kim et al., 2000). The increase in food intake associated with methyl donor diet supplementation post parturition suggests that methyl donors may cause changes in NPY and AgRP gene expression. The increase in NPY and AgRP activity associated with food intake may be related to low levels of leptin. A reduced or delayed leptin surge, which normally occurs during the second week of life in rodents, was observed in models of maternal transfer under nutrition and/or protein restriction, and associated with postnatal growth restriction (Coupe, Amarger, Grit, Benani, & Parnet, 2010; Palou, Priego, Sanchez, Palou, & Pico, 2010a; Palou, et al., 2010b). As mentioned earlier, the results from this study indicate that methyl donor diet supplementation has post parturition effects on food intake and serum leptin levels on male hamster offspring (e.g., MDSD-MDSD and SC-MDSD groups), who ate more food and exhibited reduced leptin levels compared with offspring (SC-SC and MDSD-SC groups) who received methyl donor diet supplementation during gestation. The observation that no significant differences in body weight were observed among all hamster offspring from birth to 60 days of age can be explained by the higher daily wheel running activity exhibited by hamster offspring (MDSD-MDSD and SC-MDSD groups) exposed to methyl donor diet supplement post partuition, who ate more food and were more active than the other two hamster offpring groups (SC-SC and MDSD-SC groups). In this experiment, because we did not measure leptin levels before 30 days of age, it is unclear when and if the leptin surge actually occurred. However, in a study by Giudicelli et al., maternal methyl donor supplementation was associated with lower leptin levels in the rat offspring at weaning (Giudicelli, Brabant, Grit, Parnet, & Amarger, 2013). Further studies are warranted to elucidate the mechanism(s) of methyl donor diet supplementation on post parturition effects in Syrian hamsters.

5 CONCLUSION

This study examined two behaviors in Syrian hamsters, locomotor activity and food consumption, to examine the effects of methyl donor diet supplementation in offspring from conception to 60 days of age. Methyl donor diet supplementation appears to have effects post partuition as indictated by suppressed leptin levels in hamster offspring at day 60 and increase in food consumption starting from 50 days of age. That the increased food consumption did not result in any differences in body weight is an interesting finding that warrants further investigation. Overall, the results from this cross-fostering study indicate that the timing of when nutritional factors are presented is important during the development of offspring as reflected in the observed physiological and behavioral changes in the Syrian hamster.

6 ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received (permit no: 2014/07-08). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

CONFLICT OF INTEREST

None.

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FIGURE LEGENDS

FIGURE 1. Cross-fostering experimental design of male Syrian hamster (*Mesocricetus auratus*) offspring examining the influence of methyl donor diet supplementation on body weight, food intake, locomotor activity, and serum leptin profiles.

FIGURE 2. The effects of a standard control (SC) and methyl donor-supplemented (MDSD) diets on food intake of male Syrian hamster offspring (*Mesocricetus auratus*) in a cross-fostering experiment from 30-60 days of age. Food intake was measured daily in single-housed animals although data shown here in the plot are represented at five-day intervals. Values are expressed as the mean ± SEM. Means with different letters are significantly different (Dunn's post hoc test, $p < 0.05$). Comparison between groups was made for the same day. SC-SC (n=10): offspring born to SC dams and reared by SC dams. MDSD-MDSD (n=10): offspring born to MDSD dams and reared by MDSD dams. MDSD-SC (n=10): offspring born to MDSD dams and reared by SC dams. SC-MDSD (n=10): offspring born to SC dams and reared by MDSD dams

FIGURE 3. The effects of a standard control (SC) and methyl donor-supplemented (MDSD) diets on food intake of adult female Syrian hamsters (*Mesocricetus auratus*). Daily food intake in adult females dams was measured proior to breeding and during pregnancy and lactation. All measurements were taken as the average of the relevant period. Values are expressed as the mean ± SEM. Means with different letters are significantly different (Dunn's post hoc test, $p < 0.05$). SC: Dams fed a SC diet prior to breeding and through pregnancy. MDSD: Dams fed a MDSD diet prior to breeding and through pregnancy. SC-SC: Dams fed a

SC diet prior to breeding and during pregnancy through lactation. MDSD-MDSD: Dams fed a MDSD diet prior to breeding and during pregnancy through lactation: SC-MDSD: Dams fed a SC diet prior to breeding and during pregnancy followed by a MDSD diet from parturition through lactation. MDSD-SC: Dams fed a MDSD diet prior to breeding and during pregnancy followed by a SC diet from parturition through lactation

FIGURE 4. The effects of a standard control (SC) and methyl donor-supplemented (MDSD) diets on body weight of male Syrian hamster offspring (*Mesocricetus auratus*) in a cross-fostering experiment from birth to 60 days of age. Body weights of male offspring were measured at birth (Day 0), at weaning (Day 20) and then every 10 days. Values are expressed as the mean $\pm$ SEM. SC-SC (n=10): offspring born to SC dams and reared by SC dams. MDSD-MDSD (n=10): offspring born to MDSD dams and reared by MDSD dams. MDSD-SC (n=10): offspring born to MDSD dams and reared by SC dams. SC-MDSD (n=10): offspring born to SC dams and reared by MDSD dams.

FIGURE 5. Serum leptin levels in 60-day old male Syrian hamster offspring (*Mesocricetus auratus*) fed standard control (SC) and methyl donor-supplemented (MDSD) diets in a cross-fostering experiment. Blood was collected from animals at 12.00-13.00 h in the light (Day: A, C, E, G) and at 00.00-01.00 h in the dark (Night: B, D, F, H) every week starting from day 30 to 60 days of age. Values are expressed as the mean $\pm$ SEM. Means with different letters are significantly different (Dunn's post hoc test, $p < 0.05$). SC-SC (n=10): offspring born to SC dams and reared by SC dams. MDSD-MDSD (n=10): offspring born to MDSD dams and reared by MDSD dams. SC-MDSD (n=10): offspring born to SC dams and reared by MDSD dams. MDSD-SC (n=10): offspring born to MDSD dams and reared by SC dams.

FIGURE 6. Thirty day locomotor activities of male Syrian hamster offspring (*Mesocricetus auratus*) from 30-60 days of age fed standard control (SC) and methyl donor-supplemented (MDSD) diets in a cross-fostering experiment. A is a representative actogram from a SC-SC offspring. B is a representative actogram from a MDSD-MDSD offspring. Black bars represent the dark period (20.00-06.00 h), white bars represent the light period (06.00-20.00 h). Two consecutive days are represented horizontally and lines on the vertical axis represent successive days.

FIGURE 7. Average number of wheel turns per day in male Syrian hamster offspring (*Mesocricetus auratus*) from 30-60 days of age fed standard control (SC) and methyl donor-supplemented (MDSD) diets in a cross-fostering experiment. Values are expressed as the mean + SEM. Means with different letters are significantly different (Dunn's post hoc test, $p < 0.05$). SC-SC (n=10): offspring born to SC dams and reared by SC dams. MDSD-MDSD

(n=10): offspring born to MDSD dams and reared by MDSD dams. SC-MDSD (n=10): offspring born to SC dams and reared by MDSD dams. MDSD-SC (n=10): offspring born to MDSD dams and reared by SC dams.