Effects of risperidone on energy balance in female C57BL/6J mice

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Abstract

Objective—To investigate the effect of risperidone on energy expenditure and weight gain in female C57BL/6J mice.

Design and Methods—Body weight and composition, food intake, energy expenditure, and activity were determined weekly. mRNA expression of uncoupling protein 1 in brown adipose tissue, orexin and brain-derived neurotrophic factor in the hypothalamus were quantified by Real-Time PCR.

Results—Risperidone tended to induce a greater body weight gain (P=0.052) and significantly higher food intake (P=0.038) relative to the placebo-treated group. Risperidone-treated mice had a higher resting energy expenditure (P=0.001), and total energy expenditure (P=0.005) than the placebo group. There were no effects of treatment, time, and treatment by time on NREE between groups. Risperidone-treated mice showed a significantly lesser locomotor activity than placebo-treated mice over 3 weeks (P<0.001). Risperidone induced a higher UCP1 mRNA (P=0.003) and a lower orexin mRNA (P=0.001) than placebo.

Discussion—Risperidone-induced weight gain is associated with hyperphagia and a reduction in locomotor activity in C57BL/6J mice. Additionally, higher total and resting energy expenditure were accompanied by higher levels of UCP1 mRNA in BAT. The increased total energy expenditure could not offset the total intake of energy through risperidone-induced hyperphagia, therefore resulting in weight gain in female C57BL/6J mice.

Keywords

risperidone; energy expenditure; locomotor activity; UCP1; orexin; BDNF

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Introduction

Individuals experience significant weight gain when using atypical antipsychotic drugs (AADs) (1). Risperidone has been shown to induce a 2.1 kg weight gain during 10 weeks of treatment in patients, still less than the weight gain induced by other AADs such as clozapine and olanzapine (1). The mechanisms for this AAD-induced weight gain are currently unknown. The slow progress in understanding the mechanisms of AAD-induced weight gain is due, in part, to the absence of a reliable model to study the drug-specific body weight effects (2-5).

Although hyperphagia is an important component of AAD-induced weight gain (2, 4, 6, 7), the contribution of energy expenditure to AAD-induced weight gain has not been fully investigated. Human studies have reported that energy expenditure is lower than predicted in patients taking olanzapine or clozapine, suggesting that decreased energy expenditure might be an additional contributor to AAD-related weight gain (7-11). Coccurello *et al.* (3) assessed the effect of olanzapine on energy expenditure in CD-1 mice and found there was no effect on total energy expenditure (TEE) or REE . Likewise, risperidone treatment in rats showed no effects on total energy expenditure (2). The effects of AADs on non-resting (or activity-related) energy expenditure (NREE) in rodents are still inconsistent. Several studies have found that olanzapine or risperidone reduce activity in rodents (6, 12, 13), whereas another study showed that olanzapine administration had no effect on locomotor activity in mice (3). Therefore, understanding the contribution of the energy expenditure components (REE and NREE) to weight gain in AAD-treated animals may be helpful in clinical studies to reduce AAD-associated weight changes (8).

The contribution of thermogenesis to AAD-induced weight gain is unclear. Uncoupling protein 1 (UCP1) is involved in adaptive thermogenesis (14) and has been associated with the regulation of AAD-induced weight gain (5, 6, 15). However, the central regulation of energy expenditure is complex, involving a number of neuropeptides. Orexin and brain-derived neurotrophic factor (BDNF) are two candidates that are altered after AAD treatment and may participate in the regulation of energy metabolism (16, 17). Orexin plays an important function in thermoregulation via the activation of the sympathetic nervous system and interscapular BAT in rodents (16). Studies suggest that AADs influence orexin expression, but the relationship between thermoregulation and the participation of orexin is still unknown (6, 18). Hypothalamic BDNF is implicated in eating behavior and the control of body weight by regulating activity and energy expenditure (17, 19). BDNF administration induces a significant increase in energy expenditure and enhanced UCP1 mRNA and protein expression through activation of the sympathetic nervous system (17) by an uncharacterized mechanism. Several studies have shown that BDNF levels in the serum and the hypothalamus are associated with AAD-induced weight gain (20, 21). The role of these two hypothalamic neuropeptides, orexin and BDNF in AAD-induced weight gain requires further investigation.

The purpose of the present study was to investigate the effect of risperidone on body weight, food intake, energy expenditure, thermogenic potential through UCP1 in BAT, locomotor activity, and hypothalamic orexin and BDNF mRNA expression.

Methods

Mice

Eight-week-old female C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were maintained on a 12/12 hour light/dark cycle (lights on at 600h) at $22.0\pm1.0°C$. Animals were group housed for one week and then housed individually. Ten-week-old mice were acclimated to plain peanut butter pill administration (placebo; twice a day; 0900 and 1500 hours) for two weeks (4, 22). After two weeks, mice were weighed, matched for body weight, and assigned to one of two groups (n=12/group), risperidone (RISP) or placebo (PLA). Treatment began at 13-weeks of age. On day 6 and 7 of each week, mice were placed in an indirect calorimetry system. Standard mouse chow (Teklad Global 16% Protein Rodent Diet, Harlan, Madision, WI, USA) and autoclaved water were provided *ad libitum.* All procedures were approved by the UAB IACUC.

Drug treatment

Mice were treated orally twice a day (0900 and 1500 hrs) with peanut-butter pills containing risperidone (total daily dose, ~4mg/kg body weight, each pill contained ½ the daily dose) or placebo (4, 22).

Body weight, body composition, and food intake

Body composition was determined by quantitative magnetic resonance (QMR, Echo 3-in-1, Echo Medical System, Houston, TX); the measurement was taken the day before drug treatment started and after the indirect-calorimetry measurements. QMR scans were conducted between 1200-1300 hours as previously described (23). Weekly food intake was calculated by subtracting the amount of food remaining from the amount added to the hopper. Bedding was visually inspected for spilled food and was collected and weighed if found.

Energy expenditure, RER and activity

Energy expenditure, respiratory exchange ratio (RER) and locomotor activity were acquired using an indirect-calorimetry system (Labmaster; TSE Systems, Bad Homburg, Germany). During the entire experiment, mice were individually housed in indirect-calorimetry chambers, fresh air was supplied at 0.42L/min, and O_2 consumption and CO_2 production were measured for 1 min every 9 minutes. TEE (total energy expenditure) was determined by calculating the average hourly energy expenditure over 46 hrs multiplied by 24. REE (resting energy expenditure) as previously described (24). Non-resting energy expenditure (NREE) was calculated as the difference between TEE and REE. Locomotor activity was determined with infrared beams for horizontal (x, y) activity.

UCP1, orexin, and BDNF mRNA expression

Mice were killed by decapitation and trunk blood collected. Brown adipose tissue (BAT) and hypothalamus were collected, frozen in liquid N_2, and stored at $-80°C$. Total RNA was extracted using Trizol reagent (Life Technologies, Gathiersburg, MD). cDNA was synthesized using a SuperScript III kit (Invitrogen, Carlsbad, CA). Multiplex RT-PCR was

carried out using LUX-labeled oligonucleotide primers (Invitrogen, Carlsbad, CA) on a Chromo4 Instrument (MJ Research, Ramsey, Minnesota) with Opticon Monitor™ software version 3.0. Primers for mouse UCP1 and orexin as described previously (6), and for BDNF as follows: BDNF sense: 5′CGC CTA TAC TTC GGT TGC ATG AAG G-3′, BDNF antisense: 5′ TTG CCT TGT CCG TGG ACG TTT A 3′.

Statistical analyses

All statistical analyses used SAS (Version 9.1; SAS Institute Cary, NC). Repeated measures analysis of variance was used to determine significant differences in body weight gain, food intake, body composition, RER, and activity over time. Proc Mixed model was used for the analysis of TEE, REE, and NREE considering treatment, time, and treatment by time as variants, and lean mass as the covariate. The post-hoc *Bonferroni* correction was used for multiple comparisons. UCP1, orexin, and BDNF expression were analyzed by Student's t-test. Data are reported as mean ± SEM. The criterion for statistical significance was $P<0.05$.

Results

Body weight, composition and food intake

Body weight increased over time in both groups ($P<0.001$); treatment had a tendency to affect weight gain ($P=0.052$, Fig. 1a). There were no significant effects of treatment ($P=0.687$), time ($P=0.676$), or treatment by time ($P=0.800$) on changes in fat mass (Fig. 1b). Changes in lean mass were significantly affected by time ($P<0.001$), mice increased their lean mass over 3 weeks ($P<0.001$). There was no effect of treatment on lean mass between groups even though lean mass in RISP mice tended to be higher than PLA mice over 3 weeks ($P=0.094$, Fig. 1c). Treatment ($P=0.038$) and time ($P<0.001$) had significant effects on food intake; RISP mice increased their food intake over 3 weeks ($P<0.001$), and food intake was significantly higher than PLA mice at week 2 ($P=0.005$) and 3 ($P=0.001$, Fig. 1d).

There was no difference in diurnal rhythm of food intake at baseline and week 3 between RISP and PLA mice (baseline, $P=0.469$, week 3, $P=0.240$; Fig. 2a, 2b). Risperidone had no effect on food intake during the light phase ($P=0.251$; Fig. 2c), whereas food intake was significantly higher in the RISP mice than PLA mice during the dark phase ($P=0.037$; Fig. 2d).

Energy expenditure and RER

During the 46-hour baseline measurement, there was no difference in TEE between groups ($P=0.859$; Fig. 3a). However, there were significant effects of time ($P<0.001$) and time by treatment ($P=0.015$) on TEE at week 3; RISP induced an increase in TEE relative to PLA ($P=0.055$, Fig. 3b). There were no group differences in RER during the 46-hour baseline measurement ($P=0.292$; Fig. 3c), however, there were significant effects of treatment ($P=0.020$) and time by treatment ($P<0.001$) on RER at week 3; RISP induced an increase in RER relative to PLA ($P=0.055$, Fig. 3d).

There were significant effects of treatment on TEE (*P*=0.005) and REE (*P*=0.001) after normalizing for lean mass (Fig. 4d, 5d), similar to the data without normalization (TEE, *P*=0.003; REE, *P*=0.000, Fig 4a, 5a). RISP mice increased their TEE and REE compared with PLA mice over 3 weeks (absolute values: TEE, *P*=0.009; REE, *P*=0.000; after normalization for lean mass: TEE, *P*=0.060; REE, *P*=0.008), and RISP mice had significantly higher TEE at week 1 (absolute values, *P*<0.001; after normalization for lean mass, *P*<0.001) and 2 (absolute values, *P*<0.001; after normalization for lean mass, *P*=0.001), and REE at week 1 (absolute values, *P*<0.001; after normalization for lean mass, *P*=0.001), 2 (absolute values, *P*<0.001; after normalization for lean mass, *P*<0.001), and 3 (absolute values, *P*=0.020; after normalization for lean mass, *P*=0.031). TEE was significantly increased in RISP mice in both light phase (*P*=0.001) and dark phase (absolute values; *P*=0.050; Fig. 4b, 4c). After normalization with lean mass, TEE was significantly increased in RISP mice in the light phase (*P*<0.001), but not in the dark phase (*P*=0.197; Fig. 4e, 4f). RISP mice had significantly higher REE than PLA mice in both light (*P*=0.001) and dark phase (*P*=0.011) after normalization with lean mass (Fig 5e, 5f), which was similar to the results without normalization (light phase, *P*<0.001; dark phase, *P*=0.001, Fig 5b, 5c)). There were no significant (*P*<0.05) effects of treatment, time, and treatment by time on NREE.

Locomotor activity

There was no difference in locomotor activity during the baseline measurement between groups (*P*=0.986; Fig. 6a). Treatment (*P*=0.006) and time (*P*<0.001) had significant effects on activity at week 3, and significant differences were detected at 4.5h, 13.5h, 17h, 28.5h, 30h, and 37.3h (*P*<0.05, Fig. 6b).

Treatment (*P*=0.002), time (*P*<0.001), and treatment by time (*P*=0.008) had significant effects on locomotor activity. RISP mice decreased activity over 3 weeks relative to PLA mice (*P*<0.001). Locomotor activity in RISP mice was lower than PLA mice from week 1 (*P*=0.002) to the end of experiment (*P*=0.009). By the end of the experiment, activity in RISP mice was 27.4% lower than PLA mice (*P*=0.009, Fig. 6c). There was a trend (*P*=0.099; Fig. 6d) for the effect of treatment on activity during the light phase, whereas activity was significantly decreased in the RISP mice during the dark phase (*P*=0.001; Fig. 6e).

Gene expression

RISP mice had a 54.8% higher mRNA expression of UCP1 (*P*=0.003) and a 57.0% lower mRNA expression of orexin than PLA mice (*P*=0.001, Fig. 7). There was no significant difference in mRNA expression of BDNF between groups (*P*=0.210; Fig. 7).

Discussion

The present data show that treatment with risperidone causes weight gain accompanied by hyperphagia and reduced locomotor activity in C57BL/6J mice. Additionally, increased TEE and TEE, both absolute and after normalization by lean mass, are accompanied by higher

levels of UCP1 in BAT. The increased TEE could not offset the total intake of energy through risperidone-induced hyperphagia, therefore resulting in weight gain.

In the present study, risperidone treatment was associated with weight gain and hyperphagia in female C57BL/6J mice, findings that agree with our previous work (6), other animal models (2, 5) and human studies (1). . However, risperidone failed to induce the accumulation of body fat mass in C57BL/6J mice associated with weight gain, a result that is similar to our previous studies (6). In fact, the effect of AADs on body composition and its association with weight gain are still discrepant. Weight gain in patients after olanzapine treatment is mainly attributed to an increase in body fat (7). Similar results were observed in female Wistar rats, hooded-Lister rats, and CD-1 mice (2, 3, 12). However, our previous study in mice found olanzapine and risperidone induced a significant increase in lean mass, but not fat mass (22). The potential explanation was based on the age of the mice, as overfeeding young animals leads to an increase of lean mass, accounting for 80% of weight gain relative to adults, whose lean mass comprised only 15-30% of weight gain (22, 25, 26). Additionally, the increase in lean mass may be due to increases in insulin-like growth factor 1 and Akt, both of which are increased by risperidone (27). In addition to the inconsistent results in lean mass, reductions in body fat have been demonstrated; oral administration of risperidone to 3-week-old C57BL/6J mice caused a reduction of fat mass (28). To date, there is still no clear answer to **t**he varied effects of AADs on body composition .

Although hyperphagia is an important component of AAD-induced weight gain, the role of energy expenditure remains unclear. Studies with clozapine have shown that patients with mental illness decrease their REE after long-term drug treatment (6 months) (9, 10). However, the effect of olanzapine on energy expenditure has been inconsistent, as patients with bipolar disorder had reduced REE after 6 months of treatment (11), whereas no effect was found in patients after 4 weeks of treatment (7). Two animal studies investigated the contribution of energy expenditure to AAD-induced weight gain (3, 15). In CD-1 mice and Sprague Dawley rats, 24-30 days of olanzapine administration did not alter energy expenditure (3, 15). However, one study found that 24 days of olanzapine treatment (6mg/kg/day) decreased BAT UCP1 expression by 42% in female Sprague Dawley rats (15). In addition BAT temperature was reduced in the dark phase implying that reduced BAT thermogenic potential may contribute to olanzapine-induced weight gain (15). The present study showed that risperidone-treated mice significantly increased their TEE and REE, even after normalizing for lean mass, and that the increase in TEE is greater during the day than at night. Unlike placebo-treated mice, risperidone-treated mice did not show the expected drop in TEE during the daylight hours. The lack of a decline in TEE during the day is partially due to the elevated REE of the risperidone - treated mice, and is not due to a decrease in locomotor activity. We have shown previously (6) that core body temperature of risperidone-treated mice does not decrease during the daylight hours. Both placebo- and risperidone-treated mice have body temperatures that average around 37.6°C at night, whereas during the day, placebo-treated mice show a drop to approximately 36.6°C and risperidone-treated mice do not lower their core body temperatures below 37°C (6).

There may be other explanations with respect to risperidone-induced increases in REE and TEE. The activity of skeletal muscle may contribute to the increased energy expenditure, as

AAD treatment results in increased muscle tone in patients (29) and rats (30). Moreover, the automatic nervous system is deeply involved in the regulation of energy metabolism, and one study indicated that AADs induce instability in the automatic nervous system (31), which may increase firing rates of sympathetic nerves leading to increased energy expenditure (18, 30).

Physical activity is an important component of energy expenditure that may contribute to AAD-induced weight gain (15). Several, but not all (3), studies have found olanzapine or risperidone treatment leads to reduced activity in rodents (6, 12, 13, 15). A decrease in the energy expenditure associated with physical activity may partially explain AAD-induced weight gain (7, 15). In our present and previous studies, locomotor activity in mice was reduced after 3 weeks of risperidone (6). The decrease in activity was observed in the dark, but not the light phase; as has been shown by others (15). The decreased locomotor activity in the current model suggests that weight gain induced by risperidone may be influenced, at least in part, by the decreased activity as measured by beam break. However, there was no significant difference (P=0.661) in NREE between treated and control mice. Non-resting energy expenditure (NREE) in our system was determined as TEE minus REE, and REE accounts for 85-90% of TEE. Thus, the ability to determine significant difference in NREE, which accounts for a small portion of TEE, becomes problematic without larger sample sizes.

BAT is a significant contributor to adaptive thermogenesis in response to cold temperature or diet in small mammals, and UCP1 is the primary mediator of heat generation (14). To better understand the relationship of BAT thermogenic potential to AAD-induced weight gain, thermogenic capacity (UCP1 mRNA expression in BAT) was investigated. Risperidone induced an increase of UCP1 mRNA after 3 weeks treatment, which agrees with our previous work (6). Similarly, Ota *et al*. (2002) found increased expression of UCP1 after risperidone injection to male Sprague-Dawley rats (5). In contrast, olanzapine caused a decrease in UCP1 protein relative to placebo-treated mice, indicating the inhibition of thermogenic capacity contributed to olanzapine induced-weight gain (15). Analysis of the effects of AADs in UCP1 deficient mice, or in mice housed at thermoneutrality, would be two ways to further explore these findings. An important point is that UCP1 mRNA was measured only at the end of the experiment, thus further studies are needed to investigate the central regulation of UCP1 response to different AADs.

Orexin and BDNF have been shown to be associated with the regulation of feeding behavior, activity, and energy expenditure/thermogenesis (16, 19). Orexin enhances energy expenditure via stimulation of sympathetic nervous activity, increasing the firing rate of sympathetic nerves that innervate BAT (18). Moreover, orexin helps maintain energy expenditure by stimulating physical activity (16). Rats administrated orexin A increase core body temperature, which can be blocked by an injection of olanzapine (18). Our previous study showed that risperidone inhibits the expression of orexin in the hypothalamus (6), which is confirmed by the current results. Moreover, a higher body temperature in risperidone-treated mice was observed during the light phase (6) and was associated with higher TEE and food intake. The results of risperidone inhibited orexin expression with increased body temperature implied other neurotransmitter receptors such as the

noradrenergic system might play a role in antipsychotic modulation of energy expenditure (32). Alpha2C-adrenergic receptor mediates many physiological functions, including hypothermia and reduced activity by inhibiting neuronal firing rate and the release of norepinephrine (32, 33). Risperidone and other antipsychotic drugs may block alpha2C-adrenergic receptors, resulting in an increase firing rate and release of norepinephrine and other neurotransmitters that contribute to increased energy expenditure (32). Together, these results suggest that orexin may be involved in the regulation of activity and thermogenesis in risperidone-treated C57BL/6J mice

BDNF is associated with the regulation of feeding behavior, activity, and energy expenditure (19). Recent evidence suggests that centrally-released BDNF modulates feeding behavior and metabolism, and is responsible for body weight variations. BDNF administration induces higher energy expenditure and enhanced UCP1 expression through activation of the sympathetic nervous system (17). Several studies have shown that BDNF mRNA and protein content in the hippocamus are decreased during AAD-induced weight gain (20, 21). Risperidone has been shown to significantly decrease BDNF concentration in the frontal cortex, occipital cortex, and hippocampus of rats (21). However, neither the study by Angelucei *et al.* (20) nor the present study showed an effect of risperidone on BDNF in the hypothalamus of rats and mice, questioning whether BDNF expression in the hypothalamus is involved in the integrative regulation of energy metabolism during AAD treatment. Several studies have shown that the disturbance of wake and sleep cycle could have a significant effect on orexin and BDNF proteins in the brain (34, 35). Combined with the differential response of food intake, TEE, and activity in the light and dark phase, it is possible that the meal pattern of risperidone-induced hyperphagia results in the adjustment of energy expenditure and activity in mice.

Alterations in respiratory exchange ratio (RER) have been suggested as a pathophysiological index of AADs-inducing obesity and energy balance (3), and RER may reflect the metabolic fuel preference after AADs treatment (3, 36). One study has shown that acute oral gavage of olanzapine suppressed RER in both the light and dark phase in *ad libutum* fed rats, and suggested olanzapine-treated rats may shift their fuel utilization preference from carbohydrate to fat oxidation (36). In contrast, our present study showed that risperidone induced an increase in RER over 3 weeks of chronic treatment. Given that normal changes of RER are predominantly affected by energy balance (37, 38), the higher RER in risperidone-treated animals is likely the affect of chronic positive energy balance.

Conclusions

Risperidone-induced weight gain was associated with hyperphagia and a reduction in locomotor activity in female C57BL/6J mice. Additionally, enhanced total and resting energy expenditure, after normalization by lean body mass, are accompanied with higher levels of UCP1 in BAT. The increased total energy expenditure could not offset the total intake of energy through risperidone-induced hyperphagia, resulting in weight gain in female C57BL/6J mice.

Acknowledgements

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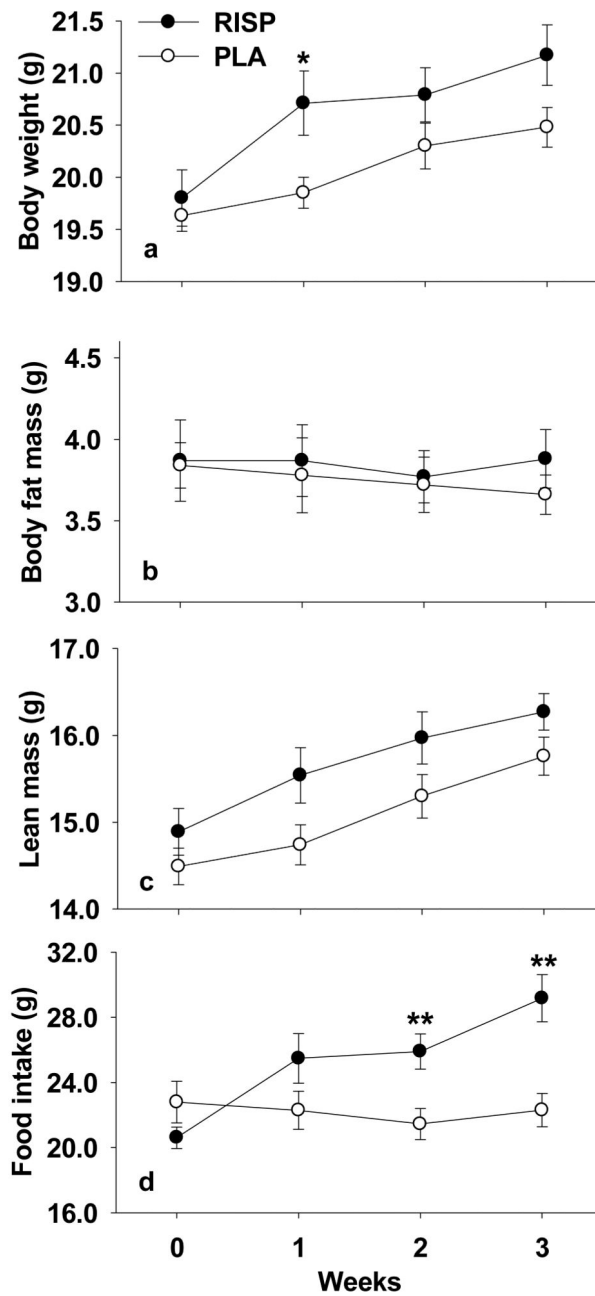
Figure 1.
Effect of risperidone (RISP) on (a) body weight; (b) body fat mass; (c) lean mass; (d) weekly food intake in female C57BL/6J mice treated for 3 weeks. Data are shown as mean ± SEM (n=12/group). *$P<0.05$, **$P<0.01$ compared with placebo (PLA)-treated mice at matched time point.
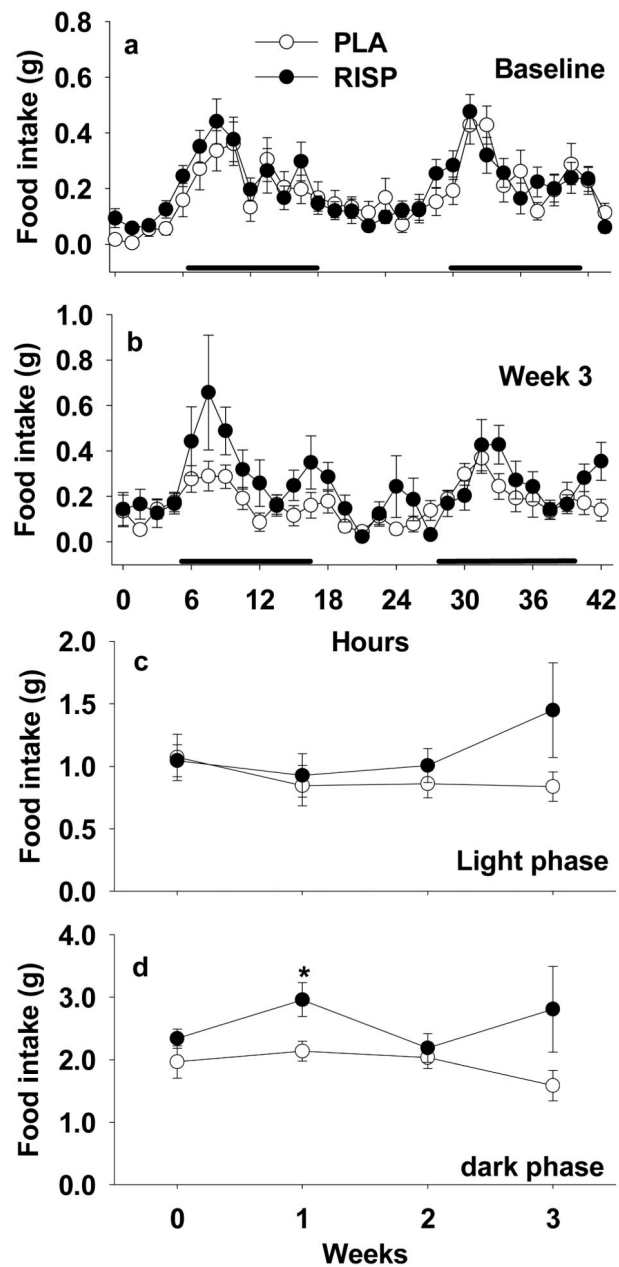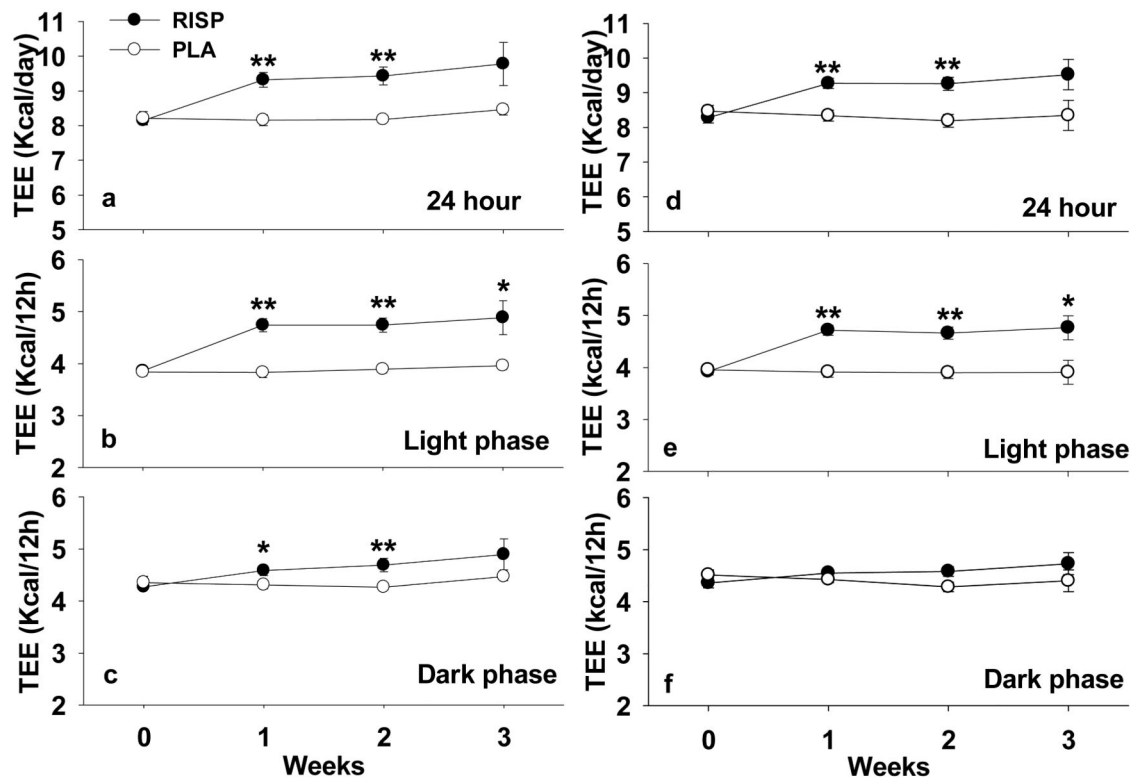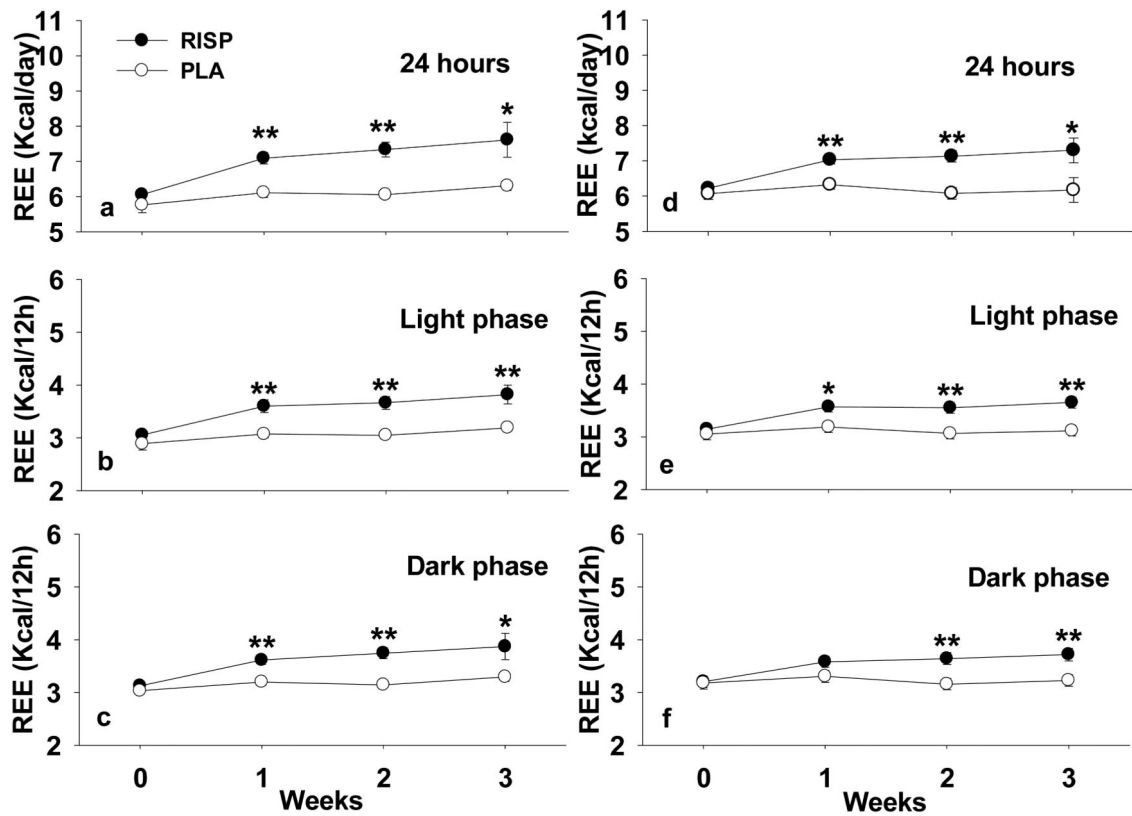
Figure 2.
Effect of risperidone (RISP) on circadian rhythm of food intake over 46 hours at baseline (a) and at week 3 (b), food intake during light phase (c) and food intake during dark phase (d) in female C57BL/6J mice treated for 3 weeks. Data are shown as mean ± SEM (n=12/group). * $P < 0.05$, ** $P < 0.01$ compared with placebo (PLA)-treated mice at matched time point.
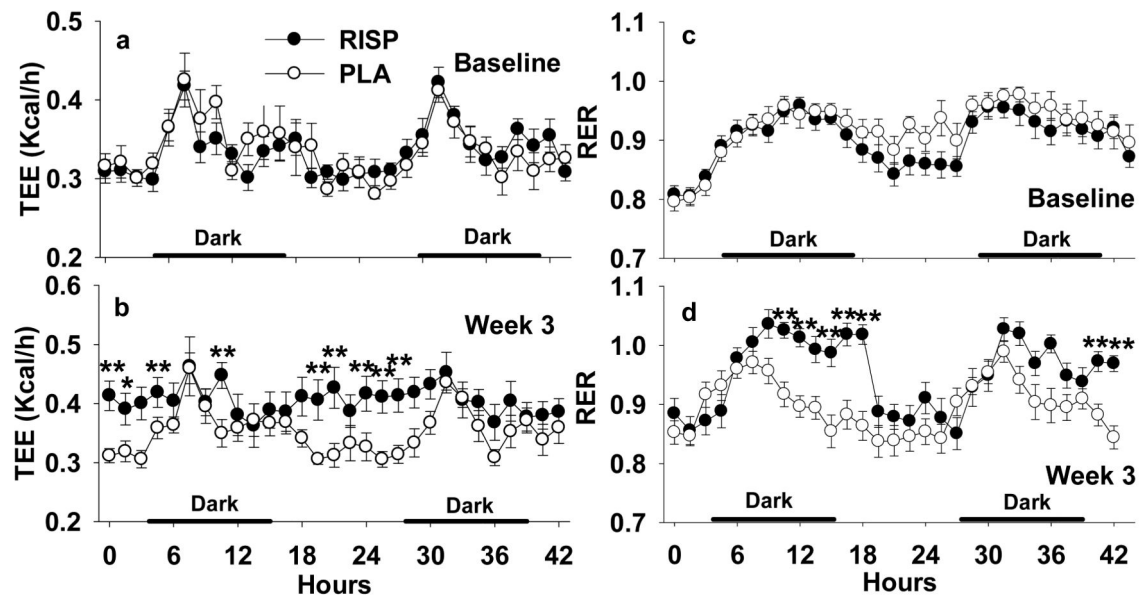
Figure 3.
Effect of risperidone (RISP) on total energy expenditure and respiratory exchange ratio (RER) over 46 hours (TEE, Kcal/h) at baseline (a, c) and at week 3 (b, d) in female C57BL/6J mice treated for 3 weeks. Data are shown as mean ± SEM (n=12/group). * P <0.05, ** P <0.01 compared with placebo (PLA)-treated mice at matched time point.
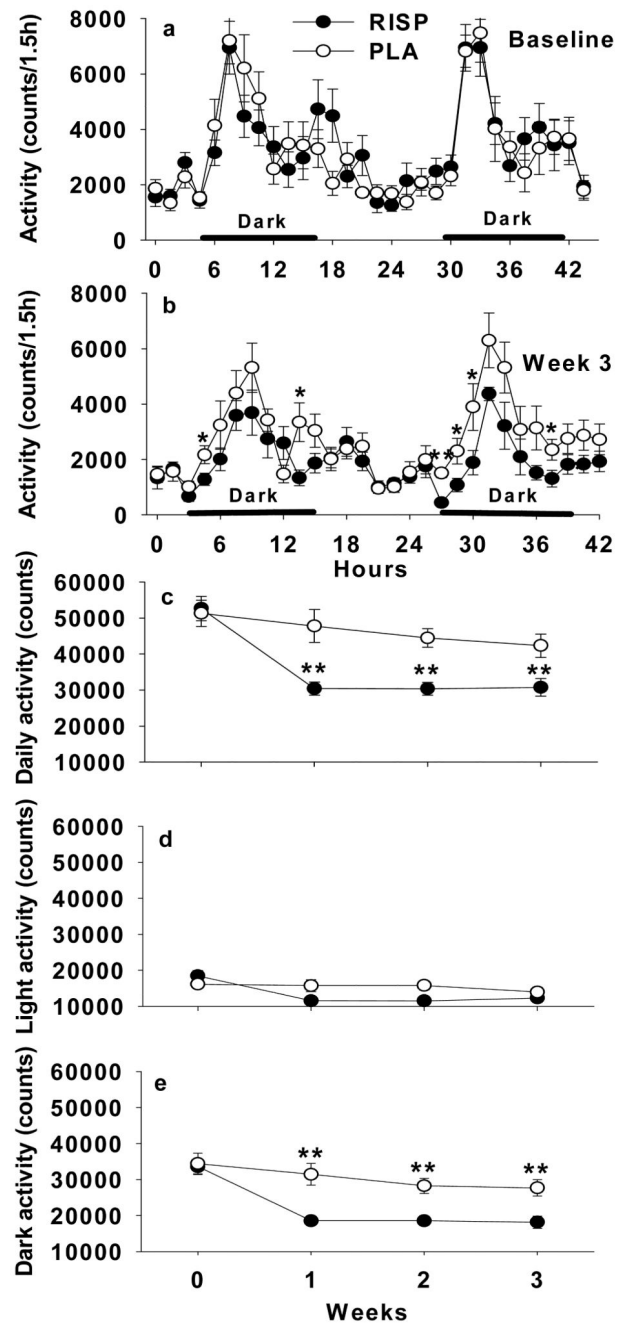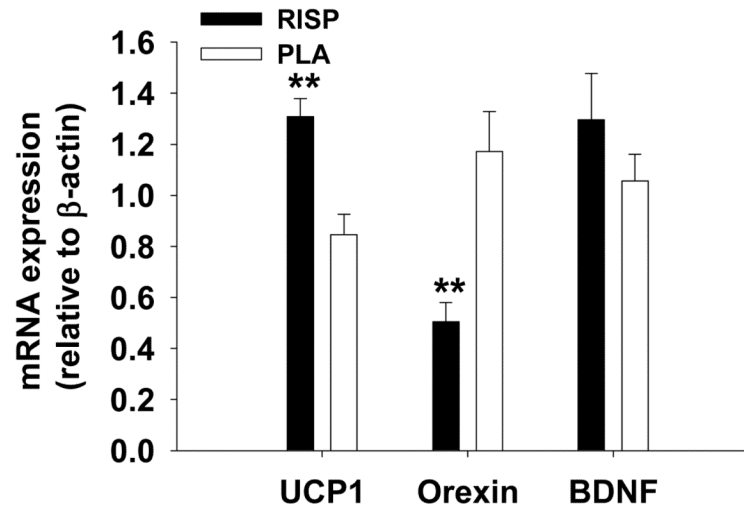
Figure 4.
Effect of risperidone (RISP) on total energy expenditure (TEE, Kcal/day) over 24 hours (a, d), TEE during the light phase (b, e), TEE during the dark phase (c, f) in female C57BL/6J mice treated for 3 weeks. Data are shown as mean ± SEM (n=12/group). TEE in d, e, and f are normalized for lean mass. * P <0.05, ** P <0.01 compared with placebo (PLA)-treated mice at matched time point.

Figure 5.
Effect of risperidone (RISP) on resting energy expenditure (REE, Kcal/day) over 24 hours
(a, d), REE during the light phase (b, e), REE during the dark phase (c, f) in female
C57BL/6J mice treated for 3 weeks. Data are shown as mean ± SEM (n=12/group). REE in
d, e, and f are normalized for lean mass. * P <0.05, ** P <0.01 compared with placebo
(PLA)-treated mice at matched time point.

Figure 6.
Effect of risperidone (RISP) on locomotor activity over 46 hours at (a) baseline; (b) week 3; and (c) daily activity; (d) daily light activity; (e) daily dark activity in female C57BL/6J mice treated for 3 weeks. Data are shown as mean ± SEM (n=12/group). * P <0.05, ** P <0.01 compared with placebo (PLA)-treated mice at matched time point.

Figure 7.
Effect of risperidone (RISP) on mRNA expression of uncoupling protein 1 (UCP1) in brown adipose tissue, orexin, and brain derived neurotrophic factor (BDNF) in the hypothalamus in female C57BL/6J mice treated for 3 weeks. Data are shown as mean ± SEM (n=12/group). * $P <0.05$, ** $P <0.01$ compared with placebo (PLA)-treated mice.