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Susceptibility of Male Wild Type Strains to Antipsychotic-induced Weight Gain

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Abstract

While both men and women gain weight as a side effect of antipsychotic treatment, studies in mice have found only female mice are susceptible to weight gain. Therefore, to we set out to identify a strain of male mice that gain significant weight in response to APs which could better model AP-induced weight gain observed in humans. These studies determined that male Balb/c mice developed late onset olanzapine-induced weight gain. Patients often take APs for many years and thus understanding AP-mediated changes in energy expenditure and body weight regulation is particularly important.

Keywords

Antipsychotic; Olanzapine; Side-effects; Weight gain; Food intake; Obesity; Energy expenditure

Introduction

Approximately four million people in the USA are currently prescribed antipsychotic (AP) medications to manage a range of illnesses including schizophrenia, bipolar disorder, depression, anxiety, dementia, insomnia and post-traumatic stress disorder [1–6]. Despite their therapeutic efficacy, APs cause serious side effects of excessive weight gain [7–11] and associated metabolic disease [12]. Both men and women gain significant amounts of weight in response to APs [13–16]. However, in contrast to the clinical data, rodent studies show highly reproducible sex-specific differences in weight gain in repsonse to APs. While olanzapine treatment consistently results in significant weight gain in female rodents,[17–30] male rodents appear to be protected from AP-induced weight gain [17,28,29,31,32]. To date, the underlying reasons for this sexual dimorphism in rodent's response to APs are not yet known [33–35].

Genetic background of mice plays a significant role in the susceptibiliy to of many metabolic traits ranging from obesity [36], non-alcoholic hepatosteotosis [37] and cardiometabolic diseases [38]. For example, high fat diet feeding results in significant weight

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gain in male C57BL/6 [36,39] DBA/2 [36,39] 129X1[39], and FVB/N[39] mice while male Balb/c [39], I/STN [36], SWR/J [36] and SJL/J [36] are notably protected from diet-induced obesity. To develop a consistent model for AP-induced weight gain in mice, Morgan et al., tested the effect of olanzapine treatment in female mice from eight inbred strains (A/J, C57BL/6J, 129S1SvlmJ, NOD/ShiLtJ, NZO/HlLtJ, CAST/EiJ, PWK/PhJ, WSB/EiJ) [40]. This study found that C57BL/6J mice were highly susceptible to olanzapine-induced weight gain and thus C57BL/6J mice have been widely used to model for AP-induced weight gain in numerous studies [29,30,35,40]. However, this female C57BL/6J model is often criticized for lack of translational relevance due to the finding male C57BL/6J are protected from AP-induced weight gain [29] while both men and women gain weight in repsonse to APs [13–16]. Therefore, to we set out to identify a strain of male mice that gains significant weight in response to APs which could provide a better model of AP-induced weight gain observed in humans.

Materials and Methods

All procedures were approved by the University of California San Diego IACUC.

Male mouse strains were purchased from The Jackson Laboratory (Sacramento, CA) at 9 weeks of age: C57BL6/J (000664), Balb/cJ (000651), DBA/1J (000670), CBA/J (000656), AKR/J (000648), SJL/J (000686), C3H/HeJ (000659), 129X1/SvJ (000691). Mice were acclimatized to the laboratory conditions for 7 days. Animals were maintained in a 12-hour light:dark cycle with a humidity between 60-70%. Three days before the start of the study, mice were switched from normal chow (Rodent chow, 5001, Labdiet, CA) to high fat diet (HFD, 45% calories from fat, D09092903, Research Diets) and singly housed in standard laboratory cages. At the start of the study, mice were randomized (n=6/group) to receive either the 45% HFD with or without olanzapine (54mg/kg, D161110301, Research Diets). This robust model of administering olanzapine in 45% HFD to initiate weight gain has been widely used in similar studies[29,30,40]. Careful dosing studies established this dose of olanzapine in the diet resulted in a plasma concentrations (10–25 ng/mL) corresponding with the therapeutic range in humans [40]. Food and water were provided ad libitum. Food intake was measured daily. Mice were housed in cages with highly absorbent paper to facilitate accurate determination of any spilled food (Kimberly Clark Wypal X60). Body weight was measured every other day for up to 28 days.

RNA extraction and quantitative PCR: Mice were sacrificed (non-fasted), and the hypothalamus, gonadal white and interscapular brown adipose tissues were collected, frozen in liquid nitrogen and stored at −80°C until further analyses. Total RNA was extracted using Trizol (Invitrogen) and RNeasy Extraction Kit (Qiagen). RNA concentration and quality were assessed using Nanodrop. cDNA was synthesized from 500 ng of RNA using High Capacity cDNA transcription kit (Thermo Fisher). qPCR was performed using Step One Plus (Applied Biosciences). Gene expression was normalized to housekeeping genes hypoxanthine-guanine phosphoribosyltransferase (Hprtl) and phosphoglycerate kinase 1 (Pgk1) for the hypothalamus and Hprtl and ATP synthase F1 subunit epsilon (Atp5e) for the adipose tissues. Primer sequences are detailed in Table 1.

Results

Treatment of C57BL6/J female mice with olanzapine resulted in significant increase in food intake (Fig. 1A) and body weight gain (Fig. 1B). In contrast, treatment of C57BL6/J male mice with olanzapine did not result in increased food intake or weight gain compared with control treated mice (Fig 1C–D).

A strain survey of response to olanzapine revealed AKR/J (Fig. 2A), CBA/J (Fig. 2B), C3H/HeJ (Fig. 2C), DBA/1J (Fig. 2D), SJL/J (Fig. 2E) and 129X1/SvJ (Fig. 2F) male mice did not gain additional weight in response to olanzapine. In addition, olanzapine did not significantly change food intake in these strains (Fig. 2A–F). Interestingly, we observed that olanzapine treatment of male Balb/cJ mice resulted in significant increase in body weight and weight gain (Fig. 3A–B, supplemental Fig.1A) that became more pronounced after ~14-28 days of treatment. However, this weight gain does not appear to be driven by increased food intake (Fig. 3C, supplemental Fig. 1B) and in agreement with this no significant differences in appetite regulating hypothalamic neuropeptides, including Agouti-related protein (Agrp), Neuropeptide Y (Npy), Orexin (Hcrt), Cocaine- and amphetamine-regulated transcript (Cart) and Pro-opiomelanocortin (Pomc), were observed between olanzapine and control treated mice (Fig. 3D). Olanzapine treatment did not significantly change expression of lipogenic genes Acetyl-CoA Carboxylase alpha (Acaca) and Fatty acid synthase (Fasn), lipolytic genes carnitine palmitolytransferase (Cpt1a) Hormone-sensitive lipase Lipe or metabolic genes leptin (Lep) and adiponectin (Adipoq) in the gonadal white adipose tissue (Fig. 3E). However, olanzapine treatment resulted in reduction in expression of genes implicated in energy metabolism[41] including peroxisome proliferator-activated receptor gamma coactivator 1-alpha (Ppargc1a) and peroxisome proliferator activated receptor alpha (Ppara) in brown adipose tissue while no significant differences were observed in Fasn, Sirtuin 3 (Sirt3) and uncoupling protein 1 (Ucp1) (Fig. 3F). Female Balb/cJ treated with olanzapine gained a similar amount of weight (Fig. 3G) and had no differences in food intake (Fig. 3H) compared with control treated mice.

Discussion

AP drugs have the unwanted side effect of significant weight gain[7] that often leads to the development of metabolic disease. While both men and women gain weight in response to APs, rodent studies have found only females gain significant weight [29,30,40,42,43], while male rodents appear to be resistant to AP-induced weight gain [43]. Because genetic background plays a significant role in the susceptibiliy of mice to a wide array of metabolic traits [37–39], we explored the effect of AP treatment on weight gain in seven strains of male mice.

In these studies, we determined that male Balb/c mice developed late onset susceptibility to olanzapine-induced weight gain. Balb/c male mice are protected from high-fat diet induced obesity [39], and as expected, we observed a similar effect in our control HFD fed mice in our study. However, olanzapine treatment of male Balb/c mice resulted in significant weight gain compared with control treated mice. No significant differences in food intake were observed, suggesting that these late onset weight differences may be mediated through olanzapine-induced changes in energy expenditure. While we did not directly measure energy

expenditure, we observed decreased gene expression of Ppargc1a and Ppara in the brown adipose tissue associated with lower energy expenditure [44] AP-induced weight gain in rodents and humans is initially driven by increased food intake [45,46], but later in the course of treatment, body weight gain persists [47,48] even when changes in food intake are no longer present [46,49]. This suggests that changes in energy expenditure may play a significant role in AP-induced weight gain in the later course of treatment [50]. The small number of studies and isolated observations on human patients treated with APs, where energy expenditure has been measured, have, to date, yielded equivocal results [8,51–54] Our studies suggest male Balb/c mice may provide a model of the late onset changes in energy expenditure observed after chronic AP treatment. However, olanzapine treatment in female Balb/c did not significantly affect weight gain as noted in other studies [55]. Therefore, these studies suggest a further sexual dimorphic effect of APs on energy expenditure in Balb/c mice. Future studies will be needed to delineate the effect of APs on energy expenditure in male and female Balb/c mice.

Amphetamine-induced hyperlocomotion is a standard test used to test the preclinical efficacy of AP drugs whereby amphetamine treatment results in hyperactivity that is blunted by AP treatment[56]. Interestingly, both Balb/c [57,58], and C57BL6 mice are commonly used in these tests [29,59] suggesting multiple strains of mice can model the therapeutic efficacy of APs in humans. However, the metabolic side effects of APs are strain and sex dependent in mice suggesting they are not directly linked to the therapeutic effect of APs.

While both men and women are both susceptible to gain weight in response to APs, there is a growing body of evidence that females have a higher risk of weight gain [60–62, 63–66]. The most compelling evidence comes from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) where women were more likely than men to have metabolic syndrome compared with the general male population [67]. Therefore, while male rodents are more resistant to AP-induced weight gain, this may reflect aspects of body weight observations in clinical studies after AP treatment.

In summary, there is a lack of understanding of the molecular mechanisms underlying the sexual dimorphism of AP-induced weight gain in rodent and human studies [68–70]. While we have identified male Balb/c mice are susceptible to late onset weight gain in response to APs, further studies are needed to identify a robust model of AP-induced food intake and weight gain that more closely models the effect of APs in humans. This is an important area of research as patients often take APs for many years and understanding AP-mediated changes in energy expenditure and body weight regulation warrants further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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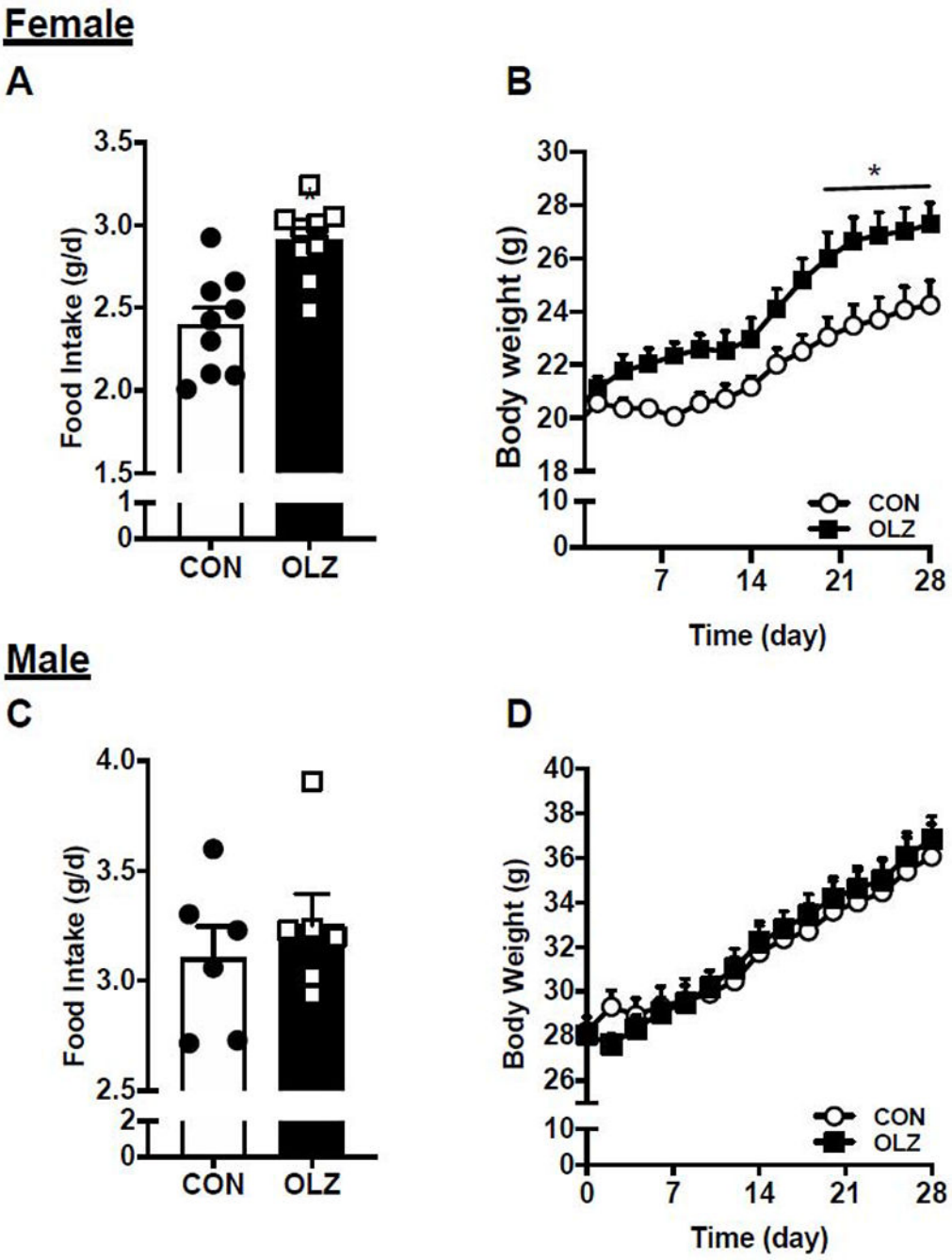

Figure 1. Olanzapine-induced food intake and weight gain in female C57BL/6 mice.

(A) Food intake and (B) body weight in female C57BL/6 mice. (C) Food intake and (D) body weight in male C57BL/6 mice. Average daily food intake was analyzed using student t-test while body weight was analyzed using two-way ANOVA with Sidak's multiple comparisons test. Values are expressed as mean ± SEM, *$p < 0.05$, n = 8 per group.

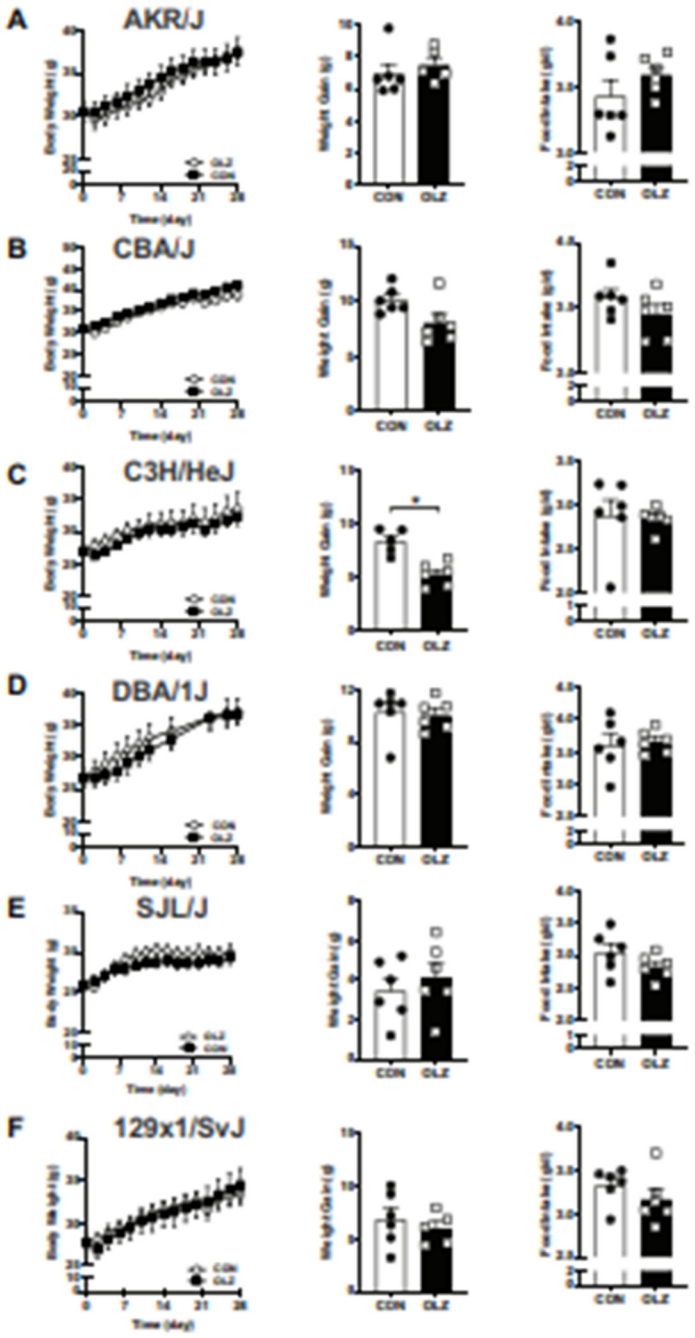

Figure 2. Effect of olanzapine on weight gain and food intake in different male mouse strains.
(A) AKR/J, (B) CBA/J, (C) C3H/HeJ, (D) DBA/1J, (E) SJL/J, (F) 129X1/SvJ. Body weight was analyzed using two-way ANOVA with Sidak's multiple comparisons test. Weight gain and average daily food intake were analyzed using student t-test. Values are expressed as mean ± SEM, n = 6 per group.

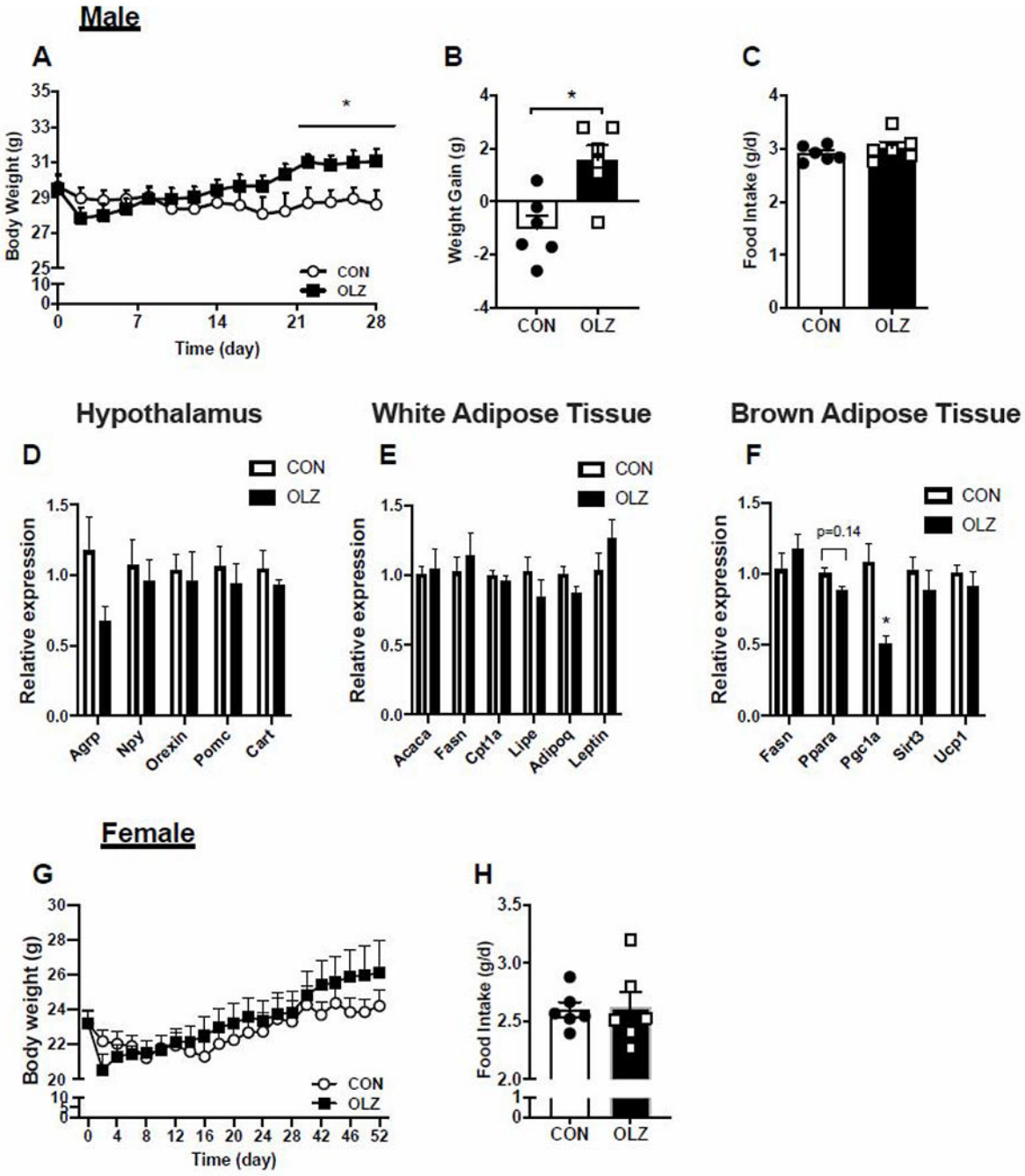

Figure 3. Effect of olanzapine on food intake and weight gain in Balb/c mice.

(A) Body weight, (B) weight gain, (C) food intake, gene expression in the (D) hypothalamus (E) gonadal white adipose tissue and (F) interscapular brown adipose tissue in male Balb/c mice in response to olanzapine treatment. (G) Body weight and (H) food intake in female Balb/c mice treated with olanzapine. Body weight and food intake were analyzed using two-way ANOVA with Sidak's multiple comparisons test. qPCR data were analyzed by students

t-test and corrected for multiple comparisons using the Holm-Sidak method, with alpha = 0.05. Values are expressed as mean ± SEM, *p < 0.05, n = 6 per group.

Table 1.

Primer sequences used for qPCR

Gene	Accession number	Sequence (5'-3')
Agrp	NM_001271806.1	F: TGACCAAATCCACCCCCTCC R: TTCCTGTAGCCAGGGCATGA
Npy	NM_023456.3	F: TAACAAGCGAATGGGGCTGT R: TTCAAGCCTTGTTCTGGGGG
Pomc	NM_001278581.1	F: GGCGACGGAAGAGAAAAGAGG R: TGTTCAGTCTCCTGCCTGTCG
Cart	NM_013732.7	F: TGGATGATGCGTCCCATG R: TACTTCTTCTCATAGATCGGAAT
Orexin	NM_010410.2	F: TTCCTTCTACAAAGGTTCCCTGG R: CGTAGAGACGGCAGGAACAC
Adipoq	NM_009605.5	F: TGACGACACCAAAAGGGCTC R: CACAAGTTCCCTTGGGTGGA
Lep	NM_008493.3	F: CACACACGCAGTCGGTATCC R: ACTCAGAATGGGGTGAAGCC
Lipe	NM_010719.5	F: GGGTGACTCTAACGCGACTC R: CCTTTAATGGGTGGGGCTGA
Acaca	NM_133360.2	F: GAGAGGGGTCAAGTCCTTCC R: CTGCTGCCGTCATAAGACAA
Fasn	NM_007988.3	F: TGCTCCCAGCTGCAGGC R: GCCCGGTAGCTCTGGGTGTA
Ppara	NM_011144.6	F: ATGAAGAGGGCTGAGCGTAG R: AAACGCAACGTAGAGTGCTGT
Cpt1a	NM_013495.2	F: CAGCACCTGTACCGCCTCGC R: GCCGTCATCAGCAACCGGCC
Ucp1	NM_009463.3	F: ACTGCCACACCTCCAGTCATT R: CTTTGCCTCACTCAGGATTGG
Sirt3	NM_022433.2	F: TTTCTTTCACAACCCCAAGC R: ACAGACCGTGCATGTAGCTG
Pgc1a	NM_008904.2	F: TGCCATTGTTAAGACCGA R: GGTCATTTGGTGACTCTGG
Hprt1	NM_013556.2	F: CACAGGACTAGAACACCTGC R: GCTGGTGAAAAGGACCTCT
Pgk1	NM_008828.3	F: CTGACTTTGGACAAGCTGGACG R: GCAGCCTTGATCCTTTGGTTG
Atp5e	NM_025983.3	F: TGGCGACAGGCTGGACTCAG R: GCTGCCCGAAGTCTTCTCAGCG