Long-term melatonin treatment attenuates body weight gain with aging in female mice

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Abstract

Women usually experience body weight gain with aging, which can put them at risk for many chronic diseases. Previous studies indicated that melatonin treatment attenuates body weight gain and abdominal fat deposition in several male animals. However, it is unclear whether melatonin affects female animals in the same way. This study investigated whether long-term melatonin treatment can attenuate body weight gain with aging and, if it does, what the mechanism is. Ten-week-old female ICR mice were given melatonin-containing water (100 μg/mL) or only water until 43 weeks. Melatonin treatment significantly attenuated body weight gain at 23 weeks (control; 57.2 ± 2.0 g vs melatonin; 44.4 ± 3.1 g), 33 weeks (control; 65.4 ± 2.6 g vs melatonin; 52.2 ± 4.2 g) and 43 weeks (control; 66.1 ± 3.2 g vs melatonin; 54.4 ± 2.5 g) without decreasing the amount of food intake. Micro-CT analyses showed that melatonin significantly decreased the deposition of visceral and s.c. fat. These results suggested that melatonin attenuates body weight gain by inhibiting abdominal fat deposition. Metabolome analysis of the liver revealed that melatonin treatment induced a drastic change in the metabolome with the downregulation of 149 metabolites, including the metabolites of glucose and amino acids. Citrate, which serves as a source of de novo lipogenesis, was one of the downregulated metabolites. These results show that long-term melatonin treatment induces drastic changes in metabolism and attenuates body weight gain and fat deposition with aging in female mice.

Introduction

Human obesity has become a global epidemic. Obesity increases the risk of many health problems, including diabetes mellitus, metabolic syndrome, cardiovascular diseases and cancer and hence leads to higher mortality (Flegal et al. 2013, Aune et al. 2016, Global et al. 2016). Therefore, obesity has been recognized as a public health problem. People usually experience body weight gain in middle age. This owes to the concurrence of different factors, such as inactivity, poor nutritional habits, and a decrease of basal metabolism, which increase abdominal fat deposition, particularly in visceral sites (Inelmen et al. 2003). Women especially experience weight gain around menopause (Guthrie et al. 1999). In fact, the prevalence of obesity in the elderly is higher in women than in men (Perissinotto et al. 2002). One of the likely mechanisms is the reduction in estrogen levels due to progressive loss of ovarian function (Mauvais-Jarvis et al. 2013).

Recently, obesity has been increasing not only in adults but also in children and adolescents worldwide (Wang & Lim 2012). Children and adolescents are likely...
to maintain their obese status into adulthood and are at high risk for developing chronic diseases in adulthood such as hypertension, dyslipidemia, diabetes mellitus, cardiovascular diseases and cancers (Wang & Lim 2012, Cote et al. 2013). Therefore, treatment and prevention of obesity should begin in childhood (Cunningham et al. 2014, Gortmaker & Taveras 2014). The prevalence of overweight and obesity in young adult women has dramatically increased in recent years (Wane et al. 2010). Compared with men, young adult women with obesity have risks associated with reproduction. Overweight and obesity in women are associated with infertility (Lainez & Coss 2019) and pregnancy complications, such as pre-eclampsia (Hurter et al. 2019), gestational diabetes mellitus (Chatzakis et al. 2019) and fetal macrosomia (Gaudet et al. 2014). Therefore, it is important to control body weight in women throughout life.

Melatonin (N-acetyl-5-methoxytryptamine) is a molecule secreted by the pineal gland in a circadian manner. In addition to regulating circadian rhythms, melatonin has a significant role in the regulation of metabolism, such as lipid metabolism and glucose metabolism (Tamura et al. 2008a, Cipolla-Neto et al. 2014, Szewczyk-Golec et al. 2015). Melatonin treatment reduced the body weight and abdominal fat deposition in male animals in several species (Prunet-Marcassus et al. 2003, Agil et al. 2011, Nduhirabandi et al. 2011, Favero et al. 2015). It is interesting that pineal melatonin secretion decreases with aging, which may contribute to weight gain with aging (Rasmussen et al. 2001, Cipolla-Neto et al. 2014). These observations suggested that melatonin could be a therapeutic reagent for the prevention of body weight gain with aging. However, studies of the effect of melatonin on body weight were done in male animals of several species. Thus, little is known about the inhibitory effect of melatonin on body weight gain in female animals. In addition, most studies on the effect of melatonin on weight were done on animals that were fed high-fat diets (Prunet-Marcassus et al. 2003, Rios-Lugo et al. 2010, Agil et al. 2011, Nduhirabandi et al. 2011). It remains unclear whether melatonin has similar effects in animals fed a normal diet. Furthermore, the mechanisms by which melatonin attenuates weight gain have not been fully clarified.

We have reported that melatonin works as an antioxidant within the ovarian follicle and protects oocytes and granulosa cells against oxidative stress, and that melatonin treatment improves fertilization and pregnancy rates in women with infertility (Tamura et al. 2008b, Tamura et al. 2009, 2012, 2013, 2014, 2020). In addition, we recently established a mouse model for examining the effects of long-term melatonin treatment on ovarian aging (Tamura et al. 2017). In this model, 10-week-old (corresponding to the adolescence of human) female mice were treated with melatonin until the age of 43 weeks (corresponding to the perimenopausal period of human). This long-term melatonin treatment delayed ovarian aging. Interestingly, in that study, we noticed that long-term melatonin treatment attenuated body weight gain with aging.

In this study, we examined the effect of long-term melatonin treatment on body weight gain with aging in normal female mice. We also analyzed the metabolome to examine how melatonin affects metabolisms and attenuates body weight gain.

Materials and methods

Animals

ICR mice were purchased from Japan SLC (Hamamatsu, Japan). The mice were housed at 24°C under controlled conditions (lights on from 5:00 to 19:00 h) under specific pathogen-free conditions and fed standard chow according to our published protocol (Tamura et al. 2017). All experimental protocols were approved by the Committee for Ethics on Animal Experimentation and performed under the guidelines for animal experiments at Yamaguchi University Graduate School of Medicine in accordance with Law No. 105 and Notification No. 6 of the Japanese government.

Experimental procedures

We first examined the effect of melatonin on body weight gain during aging as a pilot study. We performed the experiment according to the previous report (Nava et al. 2003). Ten female ICR mice (10 weeks old) were randomly allocated to two groups. Half were housed together and given only water to drink and the other half were given water supplemented with 100 μg/mL melatonin until 43 weeks of age. Melatonin was provided in the drinking water during the day as well as at night. The previous report showed that the 100 μg/mL of melatonin in drinking water increased serum melatonin levels up to 1–3 ng/mL (Nava et al. 2003). Body weights were measured at 10, 23, 33 and 43 weeks. To confirm reproducibility and to examine the possible mechanisms for the effect of melatonin on body weight gain, 16 female ICR mice (10 weeks old) were randomly allocated to two
groups and were treated in the same way. The amount of food intake was checked every day per cage by subtracting the remaining food before feeding. The average daily food intake per one mouse was calculated in each group. At 43 weeks, these mice were subjected to the experiments described subsequently.

Quantification of abdominal fat deposition by micro-computed tomography imaging

Mice were anesthetized with 2% isoflurane (Wako Pure Chemical Industries), and CT images were acquired by 3D micro-CT (R_mCT2; Rigaku, Tokyo, Japan) as reported previously (Kina-Tanada et al. 2017). The CT images were visualized and analyzed using CTAtlas Metabolic Analysis Ver. 2.03 software (Rigaku). Abdominal fat was measured from the base of the enisiform cartilage to the pelvic floor and was divided into visceral and s.c. fat. Fat mass was expressed as a percentage of the body volume scanned.

Metabolome analysis

Mice were euthanized by cervical dislocation at 43 weeks. Liver tissues from five control and five melatonin-treated mice were used for metabolome analysis. Livers were harvested at the same time. Metabolome analysis was performed at Human Metabolome Technologies (Tsuruoka, Japan) as reported previously (Matsui et al. 2017). We analyzed 272 metabolites that were measurable in at least one out of ten mice. Statistical differences in single metabolites between two groups were evaluated with Welch’s two-sample t-test. Metabolites with a P-value less than 0.05 were considered as up- or downregulated metabolites by melatonin. The metabolomic data were subjected to hierarchical cluster analysis and principal component analysis using software (Human Metabolome Technologies). Data were also visualized on a metabolome-wide pathway map supported by VANTED software (https://immersive-analytics.infotech.monsash.edu/vanted/). Pathways associated with metabolites that are downregulated by melatonin were analyzed with ConsensusPathDB (Kamburov et al. 2013). The 272 measurable metabolites were used as a background list. Pathways with a P-value less than 0.05 were considered as enriched pathways.

Statistical analysis

Statistics were analyzed with SPSS for Windows 13.0 (SPSS Inc.). Differences between the two groups were analyzed with an unpaired t-test. Differences were considered significant at P < 0.05.

Results

Effects of long-term melatonin treatment on body weight and fat deposition with aging

We first examined whether long-term melatonin treatment attenuates body weight gain during aging in female mice. Compared with the control, melatonin treatment significantly attenuated body weight gain at 23 weeks (control; 57.2 ± 2.0 g vs melatonin; 44.4 ± 3.1 g, P = 0.008), 33 weeks (control; 65.4 ± 2.6 g vs melatonin; 52.2 ± 4.2 g, P = 0.03) and 43 weeks (control; 66.1 ± 3.2 g vs melatonin; 54.4 ± 2.5 g, P = 0.003) (Fig. 1A). We then examined several parameters associated with body weight gain at 43 weeks. Final body weight at 43 weeks (control; 72.4 ± 2.8 g vs melatonin; 60.4 ± 3.1 g, P = 0.02) and total weight gain from 23 to 43 weeks were significantly lower in melatonin-treated mice than control mice (Fig. 1B). The amount of food intake was not different between the two groups while it was higher in melatonin-treated mice when corrected for final body weight (Fig. 1B). Micro-CT measurements showed that the depositions of visceral and s.c. fat were significantly lower in the melatonin-treated mice (Fig. 1C and D), suggesting that long-term melatonin treatment attenuates body weight gain with aging by inhibiting abdominal fat deposition.

Effects of long-term melatonin treatment on the metabolome profiles of liver

The attenuation of body weight gain and fat deposition by melatonin led us to hypothesize that long-term melatonin treatment alters metabolism in the body. Many of the nutrients from food, such as glucose and amino acids, are metabolized in the liver. Therefore, we considered that the liver metabolome would reveal the effects of melatonin on the body’s metabolism. Among the 272 metabolites that were measurable, 149 (54.7%) were significantly downregulated by melatonin treatment whereas only 5 (1.8%) were upregulated (Fig. 2A and Supplementary Table 1, see section on supplementary materials given at the end of this article). A hierarchical cluster analysis of the 272 metabolites clearly separated the two groups, and the melatonin group had a number of downregulated metabolites (Fig. 2B). A principal component analysis also showed distinct clustering of metabolomic changes.
Melatonin attenuates weight gain with aging
I Tamura et al.

Changes in liver metabolisms by long-term melatonin treatment

Figure 3 shows the pathway maps in the metabolome analysis with the expression levels of various metabolites. Figure 3A is the pathway map of glucose and its related metabolites. During the first step of glycolysis, the absorbed glucose is metabolized into glucose 6-phosphate (G6P) and then converted to fructose 6-bisphosphate (F6P). These intermediates in the glycolysis pathway were significantly downregulated by melatonin treatment, suggesting that melatonin treatment inhibited glucose utilization by the attenuation of glucose uptake or inactivation of glycolytic enzymes.

The tricarboxylic acid (TCA) cycle and the energy carriers produced in this cycle were downregulated by melatonin treatment. Because the TCA cycle is downstream of the glycolysis pathway, we speculate that attenuation of glucose utilization by melatonin treatment would lead to the downregulation of intermediates in the TCA cycle. Citrate is one of the intermediates and is used as the source for de novo lipogenesis (Martinez-Reyes & Chandel 2020). Melatonin treatment significantly downregulated citrate, suggesting that de novo lipogenesis is inhibited by melatonin treatment through the downregulation of citrate.

The pentose phosphate pathway (PPP) produces energy carriers, such as NADPH, from G6P produced by glycolysis. PPP produces energy carriers, such as NADPH. It also produces the precursors for nucleotides synthesis, such as ribulose 5-phosphate (Ru5P) and ribose 5-phosphate (R5P). Melatonin downregulated the metabolites and energy carriers of the PPP, which in turn would have downregulated the metabolites in the nucleotide synthesis pathway (Supplementary Fig. 1).

Although the attenuation of glucose utilization leads to the downregulation of nucleotides and
energy carriers, how their downregulation is associated with the attenuation of body weight gain is unclear.

All non-essential amino acids (NEAAs) except cysteine (amino acids indicated by red dotted lines in Fig. 3B) and all essential amino acids (EAAs) (indicated by pink dotted lines in Fig. 3B) were significantly downregulated by melatonin treatment, and their downregulations were accompanied by decreases in a number of related metabolites. Because NEAAs are not only absorbed from the diet but are synthesized from the metabolites in the TCA cycle (Fig. 3B) (Akram 2014), it is speculated that the downregulation of NEAAs by melatonin treatment was due to the downregulation of the metabolites in the TCA cycle, possibly due to the inhibition of glucose utilization. In contrast to NEAAs, EAAs are not synthesized in the body but are absorbed from diet (Santana-Santos et al. 2008). Therefore, the downregulation of EAAs suggests that melatonin treatment inhibited the absorption of amino acids. Since amino acids are also catabolized and enter the TCA cycle, the decrease in the supply of amino acids by melatonin would also lead to the downregulation of intermediates in the TCA cycle including citrate.

Figure 3D shows the pathway map of ketone body metabolisms. 3-Hydroxybutyric acid (3-HBA) is one of the representative ketone bodies that increases under starvation (Cahill 2006). 3-HBA and its related metabolites were not upregulated by melatonin treatment, suggesting that melatonin treatment did not cause excessive energy deprivation or a disordered energy utilization.

**Pathway analysis of downregulated liver metabolites by long-term melatonin treatment**

We analyzed the pathways associated with 149 metabolites that were downregulated by melatonin. Of these, 22 pathways were identified as enriched pathways for the downregulated metabolites by melatonin treatment (Table 1). Among them, seven terms were associated with the transport of glucose or amino acids (shown in bold), suggesting that melatonin treatment induced a massive decrease of metabolites by attenuating the absorption and transport of glucose and amino acids.

**Discussion**

In the present study, we demonstrated that long-term melatonin treatment (from 10 weeks to 43 weeks of age) attenuated body weight gain during aging in female mice. We also revealed, using micro-CT, that melatonin treatment decreased the deposition of both visceral fat and s.c. fat. Furthermore, metabolome analysis revealed that melatonin treatment downregulated a number of metabolites involved in lipogenesis.

Melatonin regulates not only circadian rhythms but also metabolism (Tamura et al. 2008a, Cipolla-Neto et al. 2014, Szewczyk-Golec et al. 2015). Previous reports showed that melatonin treatment reduced the body weight and abdominal fat deposition in several male animals (Prunet-Marcassus et al. 2003, Agil et al. 2011, Nduhirabandi et al. 2011).
Figure 3
Changes in liver metabolisms by long-term melatonin treatment. Expression levels of control group (blue) and melatonin group (red) are expressed as mean ± s.d. *P < 0.05, **P < 0.01 vs control; N.D., not detected. (A) Pathway map of glycolysis pathway, tricarboxylic acid (TCA) cycle and pentose phosphate pathway (PPP). The metabolites described in the text are underlined in red. (B) Pathway map of non-essential amino acids (NEAAs) metabolism. They can be synthesized from the TCA cycle. NEAAs significantly downregulated by melatonin treatment are circled with red dashed line. (C) Pathway map of essential amino acids (EAAs) metabolism. All EAAs (circled with pink dashed line) are significantly downregulated by melatonin treatment. (D) Pathway map of ketone body metabolisms. 3-hydroxybutyric acid (3-HBA), one of representative ketone bodies, is underlined in red.
Table 1  Pathways enriched in the downregulated metabolites by long-term melatonin treatment.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Translation</td>
<td>3.07E-04</td>
</tr>
<tr>
<td>Transport of inorganic cations/anions and amino acids/oligopeptides</td>
<td>1.95E-03</td>
</tr>
<tr>
<td>Amino acid transport across the plasma membrane</td>
<td>2.31E-03</td>
</tr>
<tr>
<td>tRNA aminoacylation</td>
<td>2.57E-03</td>
</tr>
<tr>
<td>Mitochondrial tRNA aminoacylation</td>
<td>2.57E-03</td>
</tr>
<tr>
<td>Cytosolic tRNA aminoacylation</td>
<td>2.57E-03</td>
</tr>
<tr>
<td>Amino acid and oligopeptide SLC transporters</td>
<td>3.95E-03</td>
</tr>
<tr>
<td>Na+/Cl--dependent neurotransmitter transporters</td>
<td>7.25E-03</td>
</tr>
<tr>
<td>Generic transcription pathway</td>
<td>0.0105</td>
</tr>
<tr>
<td>Gene expression (Transcription)</td>
<td>0.0155</td>
</tr>
<tr>
<td>Amine compound SLC transporters</td>
<td>0.0155</td>
</tr>
<tr>
<td>TP53 regulates metabolic genes</td>
<td>0.0192</td>
</tr>
<tr>
<td>Cellular responses to external stimuli</td>
<td>0.0215</td>
</tr>
<tr>
<td>Transcriptional regulation by TP53</td>
<td>0.0215</td>
</tr>
<tr>
<td>Transport of bile salts and organic acids, metal ions and amine compounds</td>
<td>0.0229</td>
</tr>
<tr>
<td>RNA polymerase II transcription</td>
<td>0.0299</td>
</tr>
<tr>
<td>Interconversion of nucleotide di- and triphosphates</td>
<td>0.0304</td>
</tr>
<tr>
<td>Histidine, lysine, phenylalanine, tyrosine, proline and tryptophan catabolism</td>
<td>0.0347</td>
</tr>
<tr>
<td>Cellular responses to stress</td>
<td>0.0426</td>
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<tr>
<td>Metabolism of RNA</td>
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<tr>
<td>SLC-mediated transmembrane transport</td>
<td>0.0427</td>
</tr>
<tr>
<td>Metabolism of proteins</td>
<td>0.0461</td>
</tr>
</tbody>
</table>

Twenty-two pathways were identified as enriched in the metabolites downregulated by melatonin. Seven terms associated with the transport of amino acids or glucose are shown in bold.

Favero et al. 2015). However, it should be noted that there have been no reports demonstrating the inhibitory effects of melatonin on body weight gain and fat deposition in female mice. Women experience the risk of weight gain throughout life. During the perimenopausal period, they tend to be obese due to the decrease of circulating estrogen levels (Mauvais-Jarvis et al. 2013). Previous reports have shown the inhibitory effect of melatonin treatment on body weight gain in postmenopausal women (Walecka-Kapica et al. 2014, Chojnacki et al. 2015, Amstrup et al. 2016). However, women should avoid becoming obese not only after menopause but also starting at a young age. Therefore, there has been a need to develop a new therapy controlling women’s body weight throughout life.

We recently showed that long-term melatonin treatment in mice from 10 to 43 weeks of age (corresponding to adolescence to the perimenopausal period in humans) delayed ovarian aging (Tamura et al. 2017). The present study clearly demonstrated that long-term melatonin treatment is also effective for controlling weight gain during aging. In addition, unlike most previous reports, this study used a normal diet to mimic naturally occurring body weight gain with aging. It is also interesting to note that that excessive light-at-night situation could also be a weight gain risk factor through the disruption of circadian rhythm (Park et al. 2019). Therefore, long-term melatonin treatment may be particularly effective in preventing weight gain in women who are under the excessive light-at-night situation, such as night-shift workers. Taken together, our results suggest that long-term melatonin treatment may be a promising way for women to control their body weight gain throughout life. It seems that melatonin does not decrease body weight but can inhibit weight gain in obesogenic situations. In fact, melatonin is effective in preventing weight gain not only in women experiencing postmenopausal weight gain (Amstrup et al. 2016) but also in women experiencing side effects of antipsychotics (Modabbernia et al. 2014).

Interestingly, the present results showed that long-term melatonin treatment decreased the depositions of both visceral fat and s.c. fat, which is consistent with previous reports showing that melatonin inhibits fat deposition in high-fat diet model animals (Jimenez-Aranda et al. 2013, de Farias et al. 2019). To elucidate the mechanisms, we performed a metabolome analysis and found that long-term melatonin treatment remarkably attenuated metabolism in the liver, and this was accompanied by a decrease in the number of metabolites, including metabolites of glucose and amino acids. Metabolites from the carbohydrates, proteins and lipids in our diet enter the TCA cycle where they are used to produce energy molecules, such as ATP (Akram 2014). The TCA cycle is a central pathway that connects almost all metabolic pathways. Citrate is an intermediate in the TCA cycle and is
a source for de novo lipogenesis (Martinez-Reyes & Chandel 2020). When the nutrient supply is sufficient, surplus citrate is converted to triglycerides and transferred to fat tissue for storage, which increases fat mass (Alves-Bezerra & Cohen 2017). In this study, long-term melatonin treatment decreased the level of citrate in the liver. This may have contributed to the attenuation of de novo lipogenesis and fat deposition and the following weight gain. In fact, melatonin decreases plasma levels of triglycerides in rats with diet-induced obesity (Prunet-Marcassus et al. 2003, Rios-Lugo et al. 2010).

Several potential mechanisms by which melatonin attenuates body weight gain have been proposed (Tan et al. 2011, Cipolla-Neto et al. 2014, Genario et al. 2021). One is that melatonin inhibits appetite. Since the amount of food intake was not decreased by melatonin in this study, the effect of melatonin treatment is unlikely due to the loss of appetite. Considering that melatonin treatment attenuated weight gain independently of food intake, another possible mechanism is that melatonin treatment may have increased energy expenditure or energy loss, although we did not check them. Melatonin increases brown adipose tissue (BAT) in animals as well as humans (Tan et al. 2011, Halpern et al. 2019). BAT can convert extra energy into heat by oxidative phosphorylation in mitochondria (Porter 2017). Therefore, BAT can attenuate body weight gain by increasing energy expenditure via heat production. Therefore, it is possible that long-term melatonin treatment attenuated body weight gain through the increase of BAT, although we did not examine the effect of long-term melatonin treatment on the amount of BAT or thermogenesis. Further studies are needed to clarify the involvement of this mechanism. Another possible mechanism is that melatonin directly activated lipolysis of fat tissue. If lipolysis is activated with the increase of energy expenditure, body weight can be decreased. Under such a situation, ketogenesis should be generally activated and result in the increase of ketone bodies. However, as shown in Fig. 3D, ketone bodies were not increased by melatonin treatment. Therefore, the effect of melatonin treatment on body weight is unlikely due to the activation of lipolysis. As for energy loss, melatonin may have induced energy loss by increasing physical activities although this idea has been debated (Wilden-Hanson et al. 2000, Isobe et al. 2002, Raskind et al. 2007). We did not check the physical activities in this study. However, if physical activities were increased by melatonin treatment, energy-producing cycles, such as the TCA cycle, would be activated and result in the increase of ATP production in the liver. Because ATP was not increased by melatonin treatment in this study, it is unlikely that attenuation of body weight gain is due to the increase in physical activities.

An alternative possibility is that melatonin attenuated body weight gain by inhibiting the absorption of nutrients, such as amino acids and glucose. In our study, melatonin treatment decreased the level of not only NEAAs but also EAAs. Because EAAs are not synthesized in the body and are absorbed from the diet (Santana-Santos et al. 2008), the melatonin-induced decrease in the levels of EAAs suggests that melatonin treatment attenuated the absorption of amino acids. In addition, melatonin treatment downregulated glucose-related metabolites, suggesting that melatonin attenuated glucose uptake. This is supported by recent reports that melatonin has an inhibitory effect on lipid absorption in the intestine (Hong et al. 2020), and that melatonin decreases serum levels of several EAAs in colitis model mice (Liu et al. 2017). Furthermore, melatonin treatment in sarcopenic elderly patients decreases serum albumin levels while cotreatment with EAA recovers it (Rondanelli et al. 2018). Further studies are needed to test the hypothesis that the absorption of the nutrients is inhibited by melatonin treatment.

There are some limitations to this study. We did not measure energy expenditure or fasting serum levels of glucose, insulin or non-esterified fatty acids. We also did not confirm whether melatonin treatment actually increased the serum melatonin levels. Melatonin was contained in the drinking water with a dose of 100 µg/mL. This method can increase serum melatonin levels up to 1–3 ng/mL (Navia et al. 2003), which is the serum level similar to humans who were administered the general dose of melatonin (1 mg/day) (our unpublished data). The dose of 3 mg/day was found to be effective for improving oocyte quality in infertile women. Therefore, the dose of 100 µg/mL in the drinking water may have an effect almost similar to the oral administration of melatonin 1–3 mg/day in humans.

In this study, melatonin was administered during the day as well as at night, as reported previously (Favero et al. 2015, Tamura et al. 2017). It was reported that melatonin administration during the day could have detrimental effects on circadian rhythm in the body (Cipolla-Neto et al. 2014). Therefore, we cannot completely exclude such negative effects of melatonin treatment, although mice are nocturnal animals and drink less water during the day. In addition, because a non-standard light/darkness cycle (lights on from 5:00 to 19:00 h) was used in this study, the effects of melatonin may differ when a standard light/darkness cycle (lights on from 7:00 to 19:00 h) is applied.

Although changing life habits is the key to success in maintaining body weight in the long term (Greaves et al. 2011), we cannot completely exclude such negative effects of melatonin treatment, although mice are nocturnal animals and drink less water during the day. In addition, because a non-standard light/darkness cycle (lights on from 5:00 to 19:00 h) was used in this study, the effects of melatonin may differ when a standard light/darkness cycle (lights on from 7:00 to 19:00 h) is applied. We did not check the physical activities in this study. However, if physical activities were increased by melatonin treatment, energy-producing cycles, such as the TCA cycle, would be activated and result in the increase of ATP production in the liver. Because ATP was not increased by melatonin treatment in this study, it is unlikely that attenuation of body weight gain is due to the increase in physical activities.
2017), several anti-obesity drugs are also available for the treatment of obesity at present (Franz et al. 2007). However, there are great concerns about their safety. Many drugs previously available in the market have been withdrawn due to the increased incidence of adverse effects, such as psychiatric and cardiac disorders (Li & Cheung 2011). On the other hand, few mild side effects of melatonin treatment, such as agitation, dizziness and sleepiness, have been reported in humans, even by long-term treatment (Andersen et al. 2016). Taken together, long-term melatonin treatment can be a novel effective and safe therapy for the prevention of body weight gain during aging. These encouraging findings in mice now need to be tested in a human trial.

Supplementary materials
This is linked to the online version of the paper at https://doi.org/10.1530/JOE-20-0462.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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