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EFFECTS OF MELATONIN ADMINISTRATION ON PLASMA LEPTIN CONCENTRATION AND ADIPOSE TISSUE LEPTIN SECRETION IN MICE

Y.-M. SONG[1] and M.-D. CHEN[2]*

[1] Division of Endocrinology and Metabolism, Taichung Veterans General Hospital, Taichung City 407, Taiwan, Republic of China
[2] General Education Center, Overseas Chinese Institute of Technology, Taichung City 407, Taiwan, Republic of China

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Both melatonin and leptin show a circadian variation in circulating levels and participate in energy metabolism. An interrelationship between these two hormones has thus been proposed. In addition, melatonin has been shown to be capable of influencing circulating leptin concentration. However, whether melatonin will increase or decrease leptin production is still uncertain. This study was undertaken to examine the effect of melatonin on leptin production using male C57BL/6 adult mice treated with or without daily melatonin supplements (10 μg/mL) in drinking water for 1 month. In addition, *in vitro* experiments using adipose tissue fragments derived from epididymal fat pads of adult mice incubated with or without melatonin (1 nM) administration were also conducted. The results showed that melatonin-supplemented mice had significantly higher plasma leptin levels than control mice. However, melatonin incubation did not cause any marked changes in the amount of leptin secreted from adipose tissue fragments. Our findings from this study indicate that melatonin does not affect leptin secretion via mouse adipose tissue. Nevertheless, melatonin could still influence leptinemia indirectly via regulatory effects in intact animals.

Keywords: Adipose fragments – epididymal fat deposit – leptin – melatonin – mice

INTRODUCTION

Melatonin, a pineal hormone mainly secreted in the dark phase of the day, is well known for its regulatory effects on circadian rhythms [15]. Melatonin also has a variety of other physiological effects, such as regulation of reproductive and immune functions [26]. In addition, with its anti-oxidant and free-radical scavenger property and the frequently found reduced secretion in the elderly, melatonin has been pro-

* Corresponding author; e-mail: mdchen@ocit.edu.tw

posed to have anti-aging potential, although this awaits more evidence to prove [30]. Furthermore, melatonin has been found to participate in energy metabolism and to be capable of reducing body fat mass of diet- and aging-induced increase in adiposity [27–29, 32], the mechanisms through which melatonin might exert such effects are yet to be clarified.

Leptin, an adipokine secreted by adipocytes, plays a role in mediating satiety and energy expenditure [14]. In addition, a physiological nocturnal rise in the circulating leptin level has been noted and this may contribute to body fat gain [18]. Although leptin is secreted in proportion to and correlates closely with the amount of body fat mass, a variety of nutritional manipulations and hormonal factors could also influence circulating leptin levels as well as leptin secretion by adipocytes [18].

Since both leptin and melatonin participate in mediating energy metabolism and adiposity, and both hormones have elevated circulating levels at midnight, an interrelationship between leptin and melatonin has been proposed. Indeed, animal studies have shown that pinealectomy (melatonin deficiency) or melatonin supplementation can cause alterations in circulating leptin concentrations independent of the change in adiposity [27–29, 32]. However, the finding of the effect of melatonin administration on leptin production is inconclusive. Many studies have indicated that pinealectomy increases and melatonin supplementation decreases circulating leptin concentrations [7, 10, 25, 27–29, 32]. On the other hand, some investigators have reported contradictory findings whereby pinealectomy reduces circulating leptin levels and exogenous melatonin administration increases both blood levels and secreted amounts of leptin [1–4, 6, 22]. Furthermore, melatonin has also been shown to have no effect on altering circulating leptin concentrations [9, 23]. In order to clarify the discrepant findings described above, the present study was undertaken to examine the effects of melatonin administration on changes in plasma leptin concentration and leptin secretion in intact adult mice and their adipose tissue fragments, respectively.

MATERIALS AND METHODS

Animals

Weaned male mice (C57BL/6J) were obtained from the National Laboratory of Animal Breeding and Research Center (NSC/ROC, Taipei, Taiwan). Throughout the study, animals were kept at a constant temperature (22 ± 1 °C) and a humidity (50 ± 5 °C) controlled room with a 12-h light/12-h dark cycle. Mice were housed in plastic nongalvanized cages and given standard laboratory chow (Fuso, Taichung, Taiwan) and double-distilled water *ad libitum.* This study was approved by the Animal Care and Research Committee of Taichung Veterans General Hospital. All chemicals, unless specifically indicated, were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

In vivo *study*

Young adult mice (3 months of age) were housed individually and separated into 2 groups based on melatonin supplementation (Calbiochem, La Jolla, CA, USA): water containing melatonin was given to melatonin-treated mice every day, but only from 1900 to 0700 hours during the dark cycle. Mice in the control group received double-distilled water throughout the 1-month treatment period. Each group contained 8 mice. The final concentration of melatonin in drinking water was 10 μg/mL and prepared as previously described [11]. Drinking water was changed every day, and the amounts of water and food intake were recorded. The mean treatment dosage of melatonin was calculated from water intake and was about 35 μg per day per mouse. Body weight was recorded twice a week. After 1 month of experimentation, mice (at 4 months of age) were sacrificed under anesthesia after overnight fast and their trunk blood samples were collected. Bilateral epididymal fat pads of the mice were removed.

In vitro *study*

Based on the facts that epididymal fat is the largest deposit and most available in comparison to other intra-abdominal fat locations (omental, mesenteric, retroperitoneal, perirenal) in mouse and in order to compare our observations with previous data obtained by the other investigators, epididymal fat pad was selected to be used in this study. Excised epididymal fat pads (about 0.6–0.8 g per mouse) were immediately placed into ice-cold Krebs-Ringer-HEPES buffer. After removing the blood vessels and connective tissues, epididymal adipose tissues were washed and cut into small pieces [12, 13]. Adipose tissue fragments from each mouse of the control group were weighed and equally separated into 4 tubes (about 150 mg tissue per tube) and flushed with 1.5 mL of Dulbecco's Modified Eagle medium (DMEM, containing 25 mM glucose, into which 0.5% fetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate were added), which contained melatonin (1 nM) or not. For each tested variable, the adipose tissue from a mouse was assessed in duplicate. Although DMEM is zinc-free, the incubation medium in this study contained about 100 nM zinc derived from fetal calf serum. After incubation for 24 hours at 37 °C under 95% air-5% CO_2, the medium in each tube was removed and leptin concentrations were measured. The administered dose of melatonin was selected in agreement with that in a previous study [3]. In addition, the prepared adipose tissue fragments had greater than 95% viability (assessed by trypan blue exclusion) at least 24 hours after the addition or not of melatonin.

Measurements

Body fat percentage was determined by measurement of total conductivity index with a body composition analyzer (EM-SCAN Inc., Springfield, IL, USA). Plasma glucose level was measured using the glucose oxidase method with an automated glucose analyzer (A&T Inc., Tokyo, Japan). Measurements of insulin (Crystal Chem Inc., Chicago, IL, USA) and leptin (R&D Systems, Minneapolis, MN, USA) were performed using enzyme-linked immunoabsorbent assay, following the manufacturer's instructions. Because some of melatonin's physiological effects are known to be closely associated with zinc [8, 20, 21], at least in immune functions, plasma zinc concentration was determined in this study using a flame atomic absorption spectrophotometer (Instrumenation Lab., Wilmington, MA, USA) and by following the method described elsewhere [11].

Statistical analyses

The data are presented as the mean ± SD. Statistical analyses of the results were conducted by ANOVA and Student's *t*-test using a commercial package, StatWorks 1.2 for Macintosh. The difference was considered to be significant when the P value was less than 0.05.

RESULTS

The data in the *in vivo* experiment showed that a 1-month melatonin supplementation in young adult mice caused no significant changes in body weight gain and body fat content, either in diet or water intake compared to control mice (Table 1). Melatonin supplementation also had no marked effect on fasting plasma glucose and insulin levels. However, plasma leptin concentrations were significantly elevated in mice receiving melatonin supplementation (Fig. 1). In addition, plasma zinc levels tended to be higher in melatonin-supplemented mice than those in control mice (P = 0.084).

Table 1
Effects of 4 weeks melatonin supplementation on body weight gain, body fat content, and food and water intake in mice

	Control	Melatonin	P value
Body wt gain, g	1.8 ± 1.1	1.3 ± 0.8	0.567
Body fat, %	28.0 ± 6	22.0 ± 8	0.683
Diet, g/d	3.8 ± 0.6	3.6 ± 0.7	0.533
Water intake, mL/d	4.5 ± 0.9	5.0 ± 1.1	0.310

Data are given as mean ± SD of 8 mice.

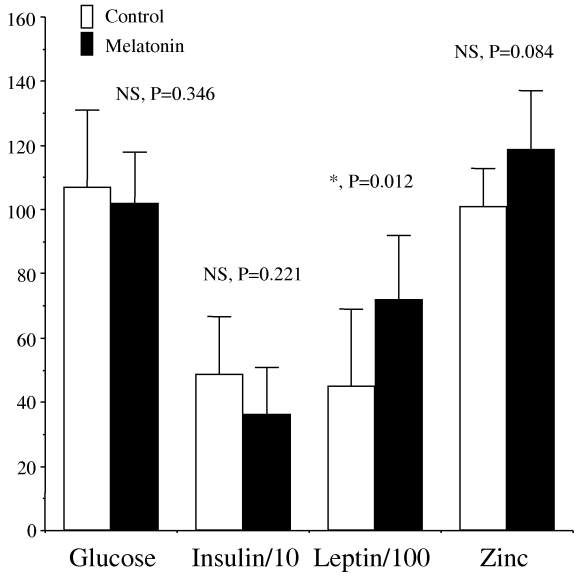

Fig. 1. Effects of 4 weeks melatonin supplementation on plasma values of glucose (mg/dL), insulin (pg/mL), leptin (ng/mL) and zinc (μg/dL) in mice. Data are given as mean ± SD of 8 mice. NS: non-significant, *P < 0.05

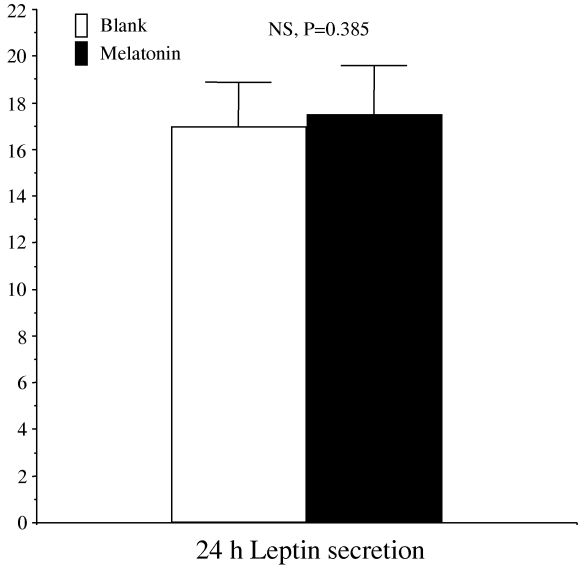

Fig. 2. Epididymal adipose tissue fragments obtained from mice were incubated for 24 h at 37 °C with 1 nM of melatonin or without any additive (blank). Twenty-four hours leptin secretion (ng/g tissue) into culture medium was measured. Data are given as mean ± SD of 16 observations. NS: non-significant

The results in the *in vitro* experiment indicated that 24-h-secretion amount of leptin by epididymal adipose tissue fragments derived from control mice did not differ from those treated with melatonin (Fig. 2, blank vs. melatonin = 17.0 ± 1.9 vs. 17.5 ± 2.1 ng/g, P = 0.385). However, leptin secretion by adipose tissue fragments taken from mice receiving 1-month oral melatonin supplementation (18.8 ± 3.3 ng/g) was greater, though not to a statistically significant extent, than that of control mice (18.8 ± 3.3 vs. 17.0 ± 1.9 ng/g, P = 0.080; 18.8 ± 3.3 vs. 17.5 ± 2.1 ng/g, P = 0.179, respectively).

DISCUSSION

The data from the present study shows that melatonin had no direct influence on adipose tissue leptin secretion. Nevertheless, melatonin could still affect circulating leptin values indirectly in intact animals, and this effect might be associated with melatonin-induced alterations in physiological functions of the intact animal, such as increased tissue availability of zinc.

In contrast to some previous data [27–29, 32] but consistent with others [6], our data showed that a 1-month oral melatonin supplementation given to young adult mice with a relatively high dose (35 μg/d/mouse) caused no significant changes in body weight gain and body fat content. These discrepant results in melatonin effect on body weight gain and body fat deposit might be attributed to differences in the type (rat or mouse, normal or high-energy diet) and age (middle-age or young adult) of experimental animals used, or differences in the method of administration (from drinking water or injection), dosage (from 0.2 to 10 μg per mL drinking water or 30 mg/kg/d) and duration (from 3 wks to 12 months) of melatonin administration. Furthermore, based on the data described above, it seems reasonable to presume that the effect of melatonin on decreasing adiposity is only effective in pinealectomized and aged subjects because of their reduced pineal melatonin secretion and responsiveness.

In this study, we also found that plasma leptin levels were significantly elevated after oral melatonin supplementation. This observation supported the finding of a previous study [6], although a much higher dose (3 mg/kg/d) and longer duration (6 months) of melatonin administration to adult rats were different from our study design. It is interesting to note that in our *in vitro* data melatonin incubation did not affect 24-h leptin secretion from mouse adipose tissue fragments. This observation adds evidence to previous studies which reported that a melatonin-induced increase in leptin secretion by rat adipocytes can only be observed in the presence of insulin [3, 4]. In other words, melatonin alone does not have a direct effect on adipocyte's leptin secretion. But melatonin could still influence circulating leptin values indirectly via regulatory effects in intact animals.

There is a close interrelationship between melatonin, zinc and leptin [5, 8, 11–13, 16–17, 20–21, 24, 31], and we therefore suspected that alteration in zinc availability to adipose tissue might contribute to melatonin-induced changes in plasma leptin lev-

els. Zinc is known to take part in the synthesis and physiological responsiveness of melatonin [8]. On the other hand, melatonin could enhance zinc absorption in the gastric-intestinal tract [11, 20–21]. Previous studies also have indicated that subjects with zinc deficiencies have hypoleptinemia which can be recovered from after zinc supplementation [5, 12, 16–17]. In this study, plasma zinc values were elevated after melatonin supplementation, which was compatible with previous reports [6, 11, 20–21]. Since zinc can mediate leptin production [5, 12–13, 16–17, 24, 31], it seems reasonable to propose that melatonin-induced hyperleptinemia might be partly attributed to increased zinc availability to adipose tissue and the resultant zinc-induced augmentation in glucose uptake and lipogeneis, which in turn enhances leptin production [12–13]. This deduction was supported by the finding that leptin production by fat pads derived from oral melatonin-supplemented mice was greater than that of control mice in this study.

Nevertheless, our data in the *in vitro* experiment, as in previous studies [3–4], also showed that melatonin incubation alone had no direct effect on leptin secretion by mouse adipose tissue fragments taken from control mice. Intracellular zinc is not easily available, and a high exogenous zinc administration is required to produce significant changes in the intracellular concentration over short incubation periods [13, 19, 24, 31]. A possible explanation for the discrepant findings for melatonin's effect on leptin levels between *in vivo* experiments and *in vitro* experiments in this study might be a lesser amount of available zinc in the culture medium (about 100 nM) than in the circulation (about 15 μM). On the other hand, Tallman and Taylor [31] have shown that plasma leptin values are similar among mice regardless of whether they are receiving a zinc-deficient, zinc-adequate, or high-zinc content diet, which goes against our hypothesis. They also have shown a negative correlation between circulating leptin and adipose tissue zinc levels. The *in vitro* effect of zinc on increasing leptin production has also been argued by Ott and Shay [24]. We consider the inconsistencies described above could be related not only to the given dose but also to the period of time of zinc administration in intact animals and to adipose tissue fragments (pre- or during incubation). Unfortunately, because we did not measure zinc levels or zinc turnover rates in adipose tissue in this study, whether the mechanism(s) of melatonin-induced changes in leptinemia and leptin secretion are exerted through an alteration in zinc availability to adipose tissue thus affecting leptin production remains unclear and needs to be studied further.

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