**Endocrine,** *vol. 21, no. 2, 169–173, July 2003* 0969–711X/03/21:169–173/$25.00 

# Melatonin Effects on Metabolism Independent of Gonad Function

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**We previously demonstrated that daily melatonin administration to middle-aged rats, restoring nocturnal plasma melatonin to young adult levels, decreased body weight and suppressed visceral fat and plasma leptin. In some species, metabolic and some neuronal responses to melatonin are mediated or dependent at least in part on gonadal steroid levels. Thus, melatonin-induced changes in gonadal steroid secretion may have mediated the aging-dependent melatonin-induced metabolic responses in our previous studies. To address this issue, melatonin (0.4 µg/mL) or vehicle (0.01% ethanol) was administered for 10 wk in the drinking water of both castrate and sham-operated Sprague-Dawley male rats, starting 1 mo after surgery at 9 mo of age. Melatonin treatment decreased ($p < 0.05$) body weight in sham-operated rats by 7 ± 2% relative to control ($n = 7$/treatment), comparable to our previous results; melatonin likewise decreased ($p < 0.05$) body weight in castrate rats by 6 ± 2% relative to control ($n = 7$/treatment). Melatonin treatment also decreased both intraabdominal fat and plasma leptin levels in both intact and castrate rats, with no significant differences of percentage suppression in the intact versus castrate rats. These results demonstrate that suppression of body weight, visceral adiposity, and plasma leptin levels by daily melatonin administration to middle-aged rats was independent of gonadal function.**

**Key Words**: Melatonin; testosterone; gonadal steroids; visceral fat; leptin; insulin.

## Introduction

Body weight and visceral fat both commonly begin to increase at middle age and appear to be causally related to various pathologies such as glucose intolerance, insulin resistance, diabetes, dyslipidemia, hypertension, and cardiovascular disease (reviewed in *1*). Conversely, human and rat melatonin secretions commonly decrease by middle age and continue to decline throughout senescence *(2–4)*. Melatonin is secreted by the pineal gland into the bloodstream and cerebrospinal fluid predominately at night, entraining endogenous circadian rhythms and influencing other physiological functions such as reproduction and, in at least some species, metabolism *(3,4)*. We have demonstrated that daily melatonin administration to middle-aged male rats restored nocturnal plasma melatonin to young adult levels and decreased body weight, visceral fat, and plasma leptin (a hormone secreted by adipocytes) to youthful levels *(5–8)*. Furthermore, these metabolic responses to melatonin supplementation were exhibited by middle-aged and older rats, but not young rats, and thus were likely dependent on the aging-associated decline in endogenous pineal melatonin secretion *(7)*. These results suggest a role for declining melatonin levels in the weight gain, increased adiposity, and resultant metabolic syndrome commonly first evident at middle age.

In some species, including humans and rats, some responses to melatonin and/or changes in photoperiod are mediated or modulated, at least in part, by changes in gonadal steroid secretion. For example, neuronal and neuroregulatory responses to melatonin *(9,10)* as well as the number of melatonin binding sites in the brain *(11–14)* have been demonstrated to be dependent on gonadal steroid levels. In addition, melatonin levels have been reported to fluctuate with the menstrual cycle, and decreased melatonin concentrations have been associated with both puberty onset and menopause (reviewed in *15*). Thus, melatonin-induced changes in the daily pattern and/or amplitude of gonadal steroid secretion may have mediated or modulated the aging-dependent melatonin-induced metabolic responses in our previous studies *(5–8)*. To address this issue, we have now investigated the effect of prior castration versus sham surgery on the metabolic responses to melatonin supplementation to middle-aged male rats, using the same experimental paradigm as characterized in our previous studies *(5–7)*.

## Results

Pretreatment body weights of the sham-operated rats were greater than those of the castrated rats (523 ± 10 vs 495 ± 7 g, respectively, $p < 0.05$) (Fig. 1). The sham-operated rats continued to gain weight throughout the full 10 wk of control

Received January 29, 2003; Revised February 28, 2003; Accepted March 4, 2003.

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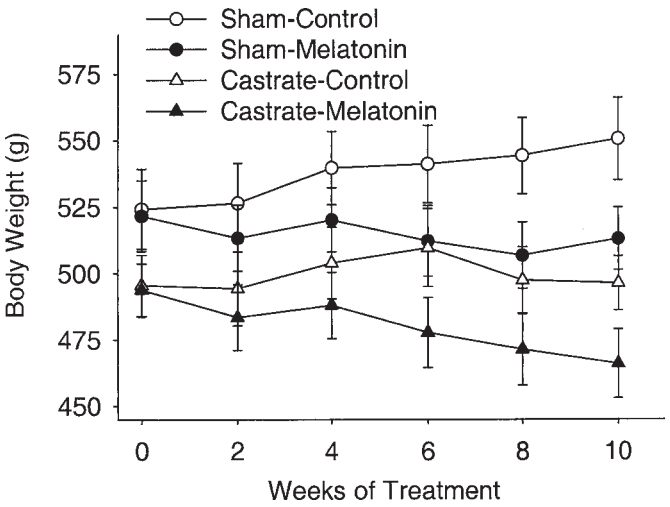


**Fig. 1.** Effect of 10 wk melatonin treatment on body weight of middle-aged castrate versus sham-operated male rats. Each data point represents the mean ± SEM of seven rats.

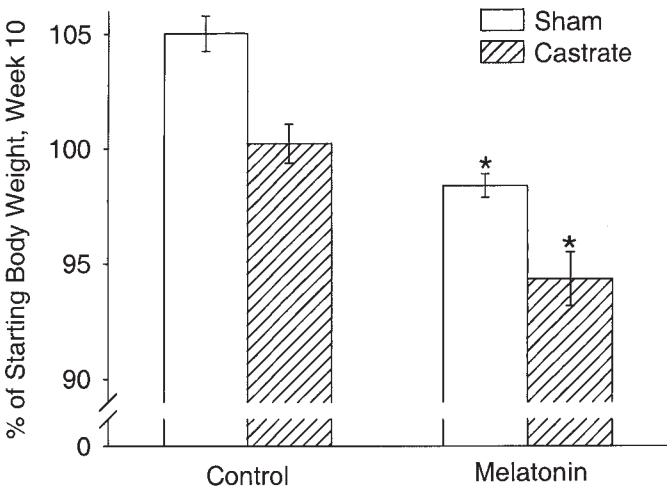


**Fig. 2.** Effect of 10 wk melatonin treatment on body weight of middle-aged male castrate versus sham-operated control rats, expressed as percentage of pre-treatment starting weight. Each bar represents the mean ± SEM of 7 rats. *$p < 0.01$ vs corresponding sham-operated or castrate control.

treatment, while the castrated rats did not (Figs. 1, 2), but melatonin treatment reduced body weight in both, with similar time courses (Figs. 1, 2). The melatonin-induced reduction of body weight relative to body weight of rats receiving control treatment was not significantly different between sham-operated and castrated rats (to 93.2 ± 2.1 vs 93.9 ± 2.6 % of starting body weight, respectively).

Melatonin treatment of the sham-operated rats did not significantly alter plasma testosterone concentrations at the time of decapitation (1.1 ± 0.2 vs 0.9 ± 0.2 ng/mL for the sham-control vs sham-melatonin rats, respectively; $p > 0.05$). Plasma testosterone levels in the castrated rats were below the minimum detection limit of the radioimmunoassay (i.e., < 0.04 ng/mL).

Melatonin treatment decreased ($p < 0.05$) retroperitoneal fat in both sham-operated and castrate rats; melatonin treatment likewise decreased ($p < 0.05$) relative retroperitoneal fat (percentage of body weight) in sham-operated rats and tended to decrease ($p = 0.052$) relative retroperitoneal fat in castrated rats (Table 1). Melatonin treatment also decreased ($p < 0.05$) both total intraabdominal fat and relative total intraabdominal fat in castrated rats, and also tended to decrease total intraabdominal ($p = 0.09$) fat in sham-operated rats, relative to their respective controls (Table 1). Melatonin treatment did not produce a significant change ($p = 0.16$) in relative intraabdominal fat in sham-operated rats relative to sham-operated controls.

There were also nonsignificant trends ($p = 0.10$) for melatonin to decrease plasma leptin concentrations in both sham-operated and castrated rats at the time of sacrifice, 1 h after lights-on (Table 1). Melatonin treatment did not alter plasma insulin levels at this time point in either sham-operated or castrate rats (Table 1).

## Discussion

Administration of melatonin for 10 wk reduced body weight and tended to decrease plasma leptin levels and intraabdominal fat of sham-operated middle-aged control rats, consistent with our previous studies *(5–8)*. Castrated rats weighed less than sham-operated rats at the start of treatment, likely due to removal of not only the testes but also the associated large epididymal fat pad during the castration surgery. Nonetheless, castration did not alter the time course or relative amplitude of the melatonin-induced reduction of body weight. Furthermore, melatonin treatment also decreased intraabdominal fat and tended to decrease plasma leptin levels in castrated rats. Thus, reduction of body weight, visceral adiposity, and plasma leptin levels by daily melatonin administration to middle-aged male Sprague-Dawley rats appears to be independent of gonadal function.

Plasma melatonin levels were not determined in this study. We previously demonstrated *(5–8)* that provision of this dosage of melatonin (i.e., 0.4 μg/mL) in the drinking water of middle-aged male Sprague-Dawley rats produced nocturnal plasma melatonin levels of approx 150 pg/mL (i.e., slightly supraphysiological), with nightly melatonin consumption of approx 30–35 μg/kg of body weight, or about 12–16 μg/rat. However, it should be noted that plasma melatonin levels produced in the castrated rats in the current study may have differed from those produced in intact rats.

Testosterone has been reported to be critical for maintenance of the nocturnal melatonin peak in male rats *(16)*, to

**Table 1**
Effect of 10 wk Melatonin Treatment on Intraabdominal Fat, Plasma Leptin, and Plasma Insulin Levels of Middle-Aged Castrated vs Sham-Operated Rats at Time of Sacrifice, 1 h After Lights On

| | Retroperitoneal fat (g) | Relative retroperitoneal fat (g/kg BW) | Total intraabdominal fat (g) | Relative intraabdominal fat (g/kg BW) | Leptin (ng/mL) | Insulin (ng/mL) |
|---|---|---|---|---|---|---|
| Sham-control | 4.7 ± 0.3 | 8.5 ± 0.5 | 11.0 ± 0.8 | 20.0 ± 1.0 | 6.7 ± 0.7 | 1.5 ± 0.1 |
| Sham-melatonin | 3.7 ± 0.2* | 7.0 ± 0.4* | 9.1 ± 0.7 | 17.4 ± 1.2 | 5.0 ± 0.7 | 1.6 ± 0.1 |
| Castrate-control | 4.4 ± 0.4 | 8.9 ± 0.8 | 10.7 ± 0.7 | 21.6 ± 1.3 | 5.1 ± 0.7 | 1.5 ± 0.1 |
| Castrate-melatonin | 3.1 ± 0.2* | 6.8 ± 0.6 | 7.9 ± 0.6* | 16.9 ± 1.1* | 3.8 ± 0.4 | 1.6 ± 0.1 |

Intraabdominal fat = retroperitoneal + omental + mesenteric + perirenal fat pads. Each value represents the mean ± SEM of seven rats. *$p < 0.05$ vs corresponding sham-operated or castrate control.

correlate with melatonin concentrations in hypogonadic men *(17)*, and to normalize melatonin secretion in hypergonadotropic hypogonadal men *(18)*. In female rats, melatonin secretion appears to be moderated by the elevated levels of gonadal steroids during proestrus *(16)*. In addition, exogenous restoration of plasma gonadal steroid levels has been demonstrated to reverse the decreased ability of the pineal to secrete melatonin in castrated or ovariectomized rats *(14–16)*. Thus, gonadal steroids can affect melatonin secretion. Furthermore, circulating gonadal steroid levels have been demonstrated to regulate melatonin-binding-site density in the brain *(12,13)* (i.e., decreased testosterone or estradiol levels resulted in decreased density, and increased levels resulted in increased density), with castration also increasing the density of melatonin binding sites in the anterior pituitary *(14)*. However, our results demonstrate that any changes in melatonin or melatonin receptor mechanisms associated with complete removal of gonadal steroid production did not compromise the metabolic response to exogenous melatonin administration. This suggests that (a) putatively altered melatonin receptor responsiveness due to the removal of gonadal steroids was nonetheless sufficient to mediate the metabolic response to exogenous melatonin; (b) the potentially altered receptor responsiveness was selective for cell populations not required for the metabolic response; or (c) the metabolic response was not mediated by melatonin receptor binding. In this context, it is interesting to note that although overall melatonin receptor density in the rat brain has been demonstrated to decrease with aging *(19)*, we have demonstrated that the metabolic response to melatonin supplementation (decreased body weight, decreased intraabdominal adiposity, and decreased plasma leptin levels) nonetheless occurs in middle-aged and older rats, but not young rats *(5,7)*.

Previous studies have suggested that increased melatonin levels can inhibit gonadal steroid secretion in humans *(17,18)*, rats *(20)*, and hamsters *(21)*. In the present study there was no significant difference between circulating testosterone levels in the sham-operated rats receiving melatonin treatment vs control, consistent with other reports suggesting that melatonin has no effect on testosterone levels *(22, 23)* or secretory pattern in normal men *(24)*. However, testosterone levels reported in the present study reflect analysis at a single time point based on a relatively small number of animals. Further characterization at more time points in the diurnal pattern of daily testosterone secretion may reveal changes.

Melatonin treatment suppressed the body weight of both sham-operated and castrated rats by about 6%. This response amplitude is consistent with responses in our previous studies *(5–7)*, including preliminary results from a study in which melatonin treatment reduced weight gain in middle-aged rats consuming a high-fat diet *(8)*. Other investigators have also reported suppression of body weight by melatonin treatment; in one study, rats injected with melatonin 1 h before the dark period for 4 wk gained less weight than controls in both reproductively stimulatory and inhibitory photoperiods *(20)*.

Melatonin treatment decreased retroperitoneal fat in both sham-operated and castrated rats, consistent with our previous demonstration that this dosage of melatonin decreased retroperitoneal fat in intact middle-aged Sprague-Dawley rats *(5)*. Melatonin treatment also decreased total intraabdominal fat content in castrated rats and likewise tended ($p = 0.09$) to decrease total intraabdominal fat in sham-operated control rats. That the suppression of total intraabdominal fat in the sham-operated controls was only a trend and did not achieve statistical significance may be related to the fact that the large epididymal fat pads (which comprised about 35% of total intraabdominal fat and were consistently reduced by melatonin treatment in our previous studies *[5–8]*), were not included in the current analysis because they were removed during castration. Nonetheless, the clear trend of changes in the remaining smaller fat pads (retroperitoneal + perirenal + omental + mesenteric) was consistent with results of our previous studies.

Leptin is an important regulator of energy balance (reviewed in *25*), and plasma leptin levels are positively correlated with body mass index in humans *(26,27)* and rodents *(27)*. Thus, it was not surprising that melatonin treatment also tended to decrease ($p = 0.10$) plasma leptin concentrations in both sham-operated and castrated rats at the time

of sacrifice (1 h after lights on). This trend toward decreased plasma leptin concentrations is consistent with the significant decreases in plasma leptin levels demonstrated in our previous studies *(5–8),* although perhaps attenuated by variability associated with (a) the relatively small sample size ($n$ = 7/treatment) in the current study, and/or (b) the fact that rats were sacrificed closer to the start of the light period, when they may have eaten more recently. More unexpectedly, plasma insulin levels were not altered at the time of sacrifice in either sham-operated or castrated rats, in contrast to results in our previous studies in which melatonin treatment also suppressed plasma insulin levels *(5–8)*. However, we have presented preliminary results *(8)* demonstrating that melatonin-induced suppression of plasma insulin levels in middle-aged rats was apparent in "fasted" (e.g., at the end of the light period) but not "fed" (e.g., during the dark period) rats. Because the rats in the current study were sacrificed shortly after lights-on, their lack of insulin suppression may reflect the fact that they were not fasted.

In summary, these results demonstrate that exogenous melatonin supplementation to middle-aged male Sprague-Dawley rats produced metabolic responses independent of gonadal function. The mechanisms of these melatonin-induced changes in body weight, visceral adiposity, and plasma leptin levels remain to be resolved.

## Materials and Methods

Nine month old male Sprague-Dawley rats were shipped 4 d after either castration (which included removal of the associated epididymal fat pads) or sham-operation (with incisions but without removal of either the testes or epididymal fat pads) by Harlan Sprague-Dawley, Inc. (Indianapolis, IN) and then maintained in our facility for 3 wk before starting treatment. The rats were housed individually under 12/12-h light/dark photoperiod with lights off at 2000 h. Laboratory Rodent Diet 5001 (Ralston Purina Co., St. Louis, MO) and water were available *ad libitum.* All procedures were performed under an approved University of Washington Institutional Animal Care and Use protocol in accord with the NIH Guide for Care and Use of Laboratory Animals.

There were four treatment groups: sham-operated rats receiving melatonin, sham-operated rats receiving vehicle control, castrated rats receiving melatonin, and castrated rats receiving vehicle control, with $n$ = 7/treatment. The rats were treated with melatonin or vehicle for ten weeks.

Each rat was weighed on a Lume-O-Gram electronic balance (Ohaus, Florham Park, NJ) at 2-wk intervals, starting immediately before initiation of treatments. The weights were measured at between 1100 and 1200 h.

Melatonin (Sigma Chemical Co., St. Louis, MO) dissolved in 100 % ethanol was added to the drinking water of melatonin-treated rats at a final melatonin concentration of 0.4 μg/mL in bottles covered with aluminum foil, starting at 10 mo of age and continuing for 10 wk. The final ethanol concentration was 0.01% for both vehicle- and melatonin-treated animals. Fresh solutions were prepared twice weekly.

After 10 wk of treatment, the rats were decapitated 1 h after lights-on; because the rats had *ad libitum* access to chow and rats tend to eat during the dark period, it can be assumed that the rats were not fasted. Blood was collected and plasma stored at −70ºC until radioimmunoassay of the hormones noted below. The retroperitoneal, perirenal, omental, and mesenteric fat pads were dissected as described previously *(6)*, and immediately weighed.

Plasma testosterone was measured using a Testosterone Coated-Tube RIA kit from Diagnostic Systems Laboratories, Inc. (Webster, TX). Plasma leptin and insulin were measured with Rat Leptin and Insulin RIA kits, respectively, from Linco Research, Inc. (St. Charles, MO). Limits of detection for the testosterone, leptin, and insulin assays were 0.04, 0.5, and 0.1 ng/mL, respectively. For each hormone, all samples were measured in a single assay; intra-assay coefficients of variation were less than 10% for each hormone assayed.

Group comparisons were made by two way analysis of variance followed by post-hoc $t$-tests, with data presented as mean ± SEM; $p < 0.05$ was considered significant.

## Acknowledgments

This work was supported by the Department of Veterans Affairs and in part by NIH Grant AA10567 to DDR. The authors thank Dennis R. Mitton for excellent technical assistance.

## References

1. Bjorntorp, P. (1995). *J. Intern. Med.* **238,** 401–404.
2. Cagnacci, A. (1996). *J. Pineal Res.* **21,** 200–213.
3. Reiter, R. (1992). *Bioessays* **14,** 169–175.
4. Pang, S. F., Tsang, C. W., Hong, G. X., Yip, P. C., Tang, P. L., and Brown, G. M. (1990). *J. Pineal Res.* **8,** 179–192.
5. Rasmussen, D. D., Boldt, B. M., Wilkinson, C. W., Yellon, S. M., and Matsumoto, A. M. (1999). *Endocrinology* **140,** 1009–1012.
6. Wolden-Hanson, T., Mitton, D. R., McCants, R. L., et al. (2000). *Endocrinology* **141,** 487–497.
7. Rasmussen, D. D., Mitton, D. R., Larsen, S. A., and Yellon, S. M. (2001). *J. Pineal Res.* **31,** 89–94.
8. Puchalski, S., Green, J., Mitton, D. R., and Rasmussen, D. D. (2001). In: *Program of the 83rd Annual Meeting of the Endocrine Society,* 469 (Abstract).
9. Doolen, S., Krause, D. N., and Duckles, S. P. (1999). *A.J.P.—Heart and Circ. Physiol.* **276,** H1281–H1288.
10. Cagnacci, A., Arangino, S., Angiolucci, M., et al. (2000). *J. Pineal Res.* **29,** 152–158.
11. Zisapel, N., Shaharabani, M., and Laudon, M. (1987). *Neuroendocrinol.* **46,** 207–216.
12. Laudon, M. and Zisapel, N. (1987). *Brain Res.* **402,** 146–150.
13. Zisapel, N. and Anis, Y. (1988). *Mol. Cell. Endocrinol.* **60,** 119–126.
14. Vanecek, J., Kosar, E., and Vorlicek, J. (1990). *Mol. Cell. Endocrinol.* **73,** 165–170.
15. Reiter, R. J. (1998). *Ann. Med.* **30,** 103–108.
16. Alonso-Solís, R., Abreu, P., López-Coviella, I., et al. (1996). *Cell. Mol. Neurobiol.* **16,** 357–382.

17. Rajmil, O., Puig-Domingo, M., Tortosa, F., et al. (1997). *Eur. J. Endocrinol.* **137,** 48–52.
18. Luboshitzky, R., Wagner, O., Lavi, S., Herer, P., and Lavie, P. (1996). *J. Mol. Neurosci.* **7,** 91–98.
19. Laudon, M., Nir, I., and Zisapel, N. (1988). *Neuroendocrinol.* **48,** 577–583.
20. Heideman, P. D., Bierl, C. K., and Sylvester, C. J. (2001). *J. Neuroendocrinol.* **13,** 223–232.
21. Rudeen, P. K. (1981). *Hormone Res.* **15,** 99–108.
22. Pettorborg, L. J., Thalen, B. E., Kjellman, B. F., and Wetterberg, L. (1991). *Brain Res. Bull.* **27,** 181–185.
23. Terzolo, M., Piovesan, A., Puligheddu, B., et al. (1990). *J. Pineal Res.* **9,** 113–124.
24. Luboshitzky, R., Levi, M., Shen-Orr, Z., Blumenfeld, Z., Herer, P., and Lavie, P. (2000). *Human Reprod.* **15,** 60–65.
25. Hamann, A. and Matthaei, S. (1996). *Exp. Clin. Endocrinol. Diabetes* **104,** 293–300.
26. Havel, P. J., Kasim-Karakas, S., Mueller, W., Johnson, P. R., Gingerich, R. L., and Stern, J. S. (1996). *J. Clin. Endocrinol. Metab.* **81,** 4406–4413.
27. Maffei, M., Halaas, J., Ravussin, E., et al. (1995). *Nat. Med.* **1,** 1155–1161.