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Daily Melatonin Administration to Middle-Aged Male Rats Suppresses Body Weight, Intraabdominal Adiposity, and Plasma Leptin and Insulin Independent of Food Intake and Total Body Fat*

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ABSTRACT

Pineal melatonin secretion declines with aging, whereas visceral fat, plasma insulin, and plasma leptin tend to increase. We have previously demonstrated that daily melatonin administration at middle age suppressed male rat intraabdominal visceral fat, plasma leptin, and plasma insulin to youthful levels; the current study was designed to begin investigating mechanisms that mediate these responses. Melatonin (0.4 μg/ml) or vehicle was administered in the drinking water of 10-month-old male Sprague Dawley rats (18/treatment) for 12 weeks. Half (9/treatment) were then killed, and the other half were submitted to cross-over treatment for an additional 12 weeks. Twelve weeks of melatonin treatment decreased ($P < 0.05$) body weight (BW; by 7% relative to controls), relative intraabdominal adiposity (by 16%), plasma leptin (by 33%), and plasma insulin (by 25%) while increasing ($P < 0.05$) locomotor activity (by 19%), core body temperature (by 0.5 C), and morning plasma corticosterone (by 154%), restoring each of these parameters toward more youthful levels. Food intake and total body fat were not changed by melatonin treatment. Melatonin-treated rats that were then crossed over to control treatment for a further 12 weeks gained BW, whereas control rats that were crossed to melatonin treatment lost BW, but food intake did not change in either group. Feed efficiency (grams of BW change per g cumulative food intake), a measure of metabolic function, was negative in melatonin-treated rats and positive in control rats before cross-over ($P < 0.001$); this relationship was reversed after cross-over ($P < 0.001$). Thus, melatonin treatment in middle age decreased BW, intraabdominal adiposity, plasma insulin, and plasma leptin, without altering food intake or total adiposity. These results suggest that the decrease in endogenous melatonin with aging may alter metabolism and physical activity, resulting in increased BW, visceral adiposity, and associated detrimental metabolic consequences. (*Endocrinology* **141:** 487–497, 2000)

THE PINEAL GLAND secretes melatonin into the circulation almost entirely at night in vertebrates (1, 2). This nocturnal secretion mediates entrainment of endogenous circadian rhythms and influences other physiological functions. Pineal melatonin biosynthesis and secretion decline with aging; levels are significantly decreased by middle age (2, 3) and tend to decline throughout old age.

Adiposity, especially visceral adiposity, increases with advancing age in humans (4), nonhuman primates (5, 6), and rats (7), as do plasma insulin and leptin levels. These changes in adiposity, insulin, and leptin levels are often associated with detrimental metabolic consequences, such as glucose intolerance, insulin resistance, diabetes, dyslipidemia, and hypertension (4, 5, 8). We have previously demonstrated that daily nocturnal melatonin administration to middle-aged male rats suppressed intraabdominal (commonly referred to as deep abdominal or visceral) fat, plasma leptin, and plasma insulin to youthful levels (9), suggesting that appropriate melatonin supplementation may potentially reverse some of the aging-associated metabolic changes. Melatonin administration did not alter the aging-related decline in plasma testosterone, T_3, or insulin-like growth factor I concentrations, nor were plasma T_4 levels significantly altered, suggesting that the actions of melatonin occurred independent of marked changes in gonadal, thyroid, or somatotropin regulation.

The current study was designed to begin investigating mechanisms that may mediate these responses, focusing on the effects of melatonin on food intake and energy expenditure (physical activity and body core temperature) as well as on body composition. In a subset of subjects, a cross-over design also allowed us to assess the reversibility of the melatonin-induced weight loss. Preliminary behavioral tests evaluated the possibility that the taste of melatonin in the drinking water was detectable or that effects of melatonin might induce a conditioned taste aversion.

Materials and Methods

Animals

Male Sprague Dawley rats from Harlan Sprague Dawley, Inc. (Indianapolis, IN), were individually housed in standard 10.5 × 19-in. clear plastic cages. The light cycle was 14 h of light and 10 h of darkness, with lights off at 1800 h. Laboratory Rodent Diet 5001 (Ralston Purina Co., St.

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Louis, MO) and water were available *ad libitum*. All procedures were performed under an institutionally approved protocol in accord with the NIH Guide for Care and Use of Laboratory Animals.

Two-bottle choice test

The presence of novel tastes can alter ingestive behavior in rats. To test whether the rats could detect concentrations of melatonin added to the drinking water in the current study, we performed a two-bottle choice test. On 2 sequential days, nine individually housed male rats (6 months of age) that had not previously received melatonin treatment were each provided with two identical Liquidiet Feeding Tubes (Bio-Serv, Frenchtown, NJ) placed side by side after removal of the standard cage-top water bottle. One tube contained 0.01% ethanol vehicle in water, and the other contained 0.01% ethanol vehicle and 0.4 μg melatonin/ml water. On the first day, five of the nine rats were presented melatonin solution in the drinking tube on the left, and the remaining four rats were presented melatonin in the tube on the right. On the second day, the left *vs.* right presentation of melatonin *vs.* vehicle control solutions was reversed for each rat. The drinking tubes were weighed at the start and end of both 24-h periods to determine consumption, and clean tubes and freshly prepared solutions were provided each day. Consumption of melatonin-containing water and vehicle control water on the 2 days were each averaged for each rat to provide indexes of consumption of melatonin-containing *vs.* vehicle control water independent of bottle position.

Alternating days consumption test

To further resolve whether melatonin acutely altered water consumption, we also performed an alternating days consumption test. Middle-aged (10 months of age; n = 24) male rats that had not previously received any melatonin treatment were housed two per cage; each cage was provided water in a standard cage-top bottle equipped with a ball-bearing sipper tube as the sole source of fluid. On the first day of a 3-day test, the water bottle for each cage contained 0.01% ethanol vehicle. After 24 h, this water bottle was exchanged for one containing 0.01% ethanol and 0.4 μg melatonin/ml water, and after another 24 h, this water bottle was again replaced with one containing only 0.01% ethanol vehicle. The fluid consumption on each of these 3 sequential days was determined by weighing the water bottles at the start and finish of each 24-h period. Clean water bottles and freshly prepared solutions were provided each day.

Conditioned saccharin taste aversion test

When consumption of a novel taste (conditioned stimulus) is paired with exposure to a toxin, rats will avoid consumption of that taste in the future. This learned response, known as conditioned taste aversion, can be used to determine whether a treatment (*i.e.* the unconditioned stimulus) is perceived as toxic. A 6-day, single pairing, two-bottle saccharin taste-conditioning procedure (10) was slightly modified for this study. The taste-conditioning apparatus consisted of a standard cage with standard cage-top bottles with ball-bearing sipper tubes containing water, 0.15% saccharin solution, or both; water and saccharin consumption in the conditioning apparatus was quantitated by weighing the bottles before and after each conditioning or test trial. Individually housed middle-aged male rats (10 months of age; n = 24) that had not previously received any melatonin treatment were maintained under reversed light/dark conditions (lights off at 0800 h and on at 1800 h) for 3 weeks before and then throughout the experiment. On day 1, the colony water bottles were removed from the home cages at 0900 h. On day 2, the water-deprived animals were each placed individually in a taste-conditioning apparatus at 0900 h and allowed 15-min access to drinking water. All rats were then returned to their home cages, and colony water bottles were replaced. The amount of water consumed during the 15-min access period on this day provided the basis for subsequently distributing the rats into two groups (a and b; n = 12/group; see below) with comparable initial water consumption. On day 3, the colony water bottles were again removed from the home cages at 0900 h. On day 4, after this second 24-h water deprivation, the rats were again each placed individually in a taste-conditioning apparatus at 0900 h and allowed 15-min access to 0.15% saccharin solution containing either 0.01% ethanol control vehicle (group a) or 0.01% ethanol and 0.4 μg melatonin/ml (group b). All rats were then returned to their home cages equipped with water bottles. On day 5, the colony water bottles were again removed from the home cages at 0900 h. On day 6, after a third 24-h water deprivation, the rats were again each placed individually in a taste-conditioning apparatus at 0900 h and presented with a 15-min choice between two identical bottles, counterbalanced for position upon the cage top, containing either control vehicle in water or a 0.15% saccharin solution in water containing vehicle. The measure of taste conditioning was the difference score between saccharin solution intake on day 6 after the pairing of melatonin/vehicle with saccharin *vs.* day 4 before the pairing. For example, if melatonin ingestion produced effects perceived as toxic, saccharin ingestion on day 6 would be decreased more in those rats for which saccharin taste was previously paired with melatonin than in those rats for which saccharin had been paired with vehicle alone.

Study design

The principal experiment in the current investigation employed a cross-over design for a subset of the subjects. Before the start of the experiment, 37 individually housed middle-aged (10 months) male Sprague Dawley rats were weighed and divided into 2 groups of 18–19 each, maintaining similar distribution of weights between the 2 groups. Both groups were switched from pelleted to powdered food (Laboratory Rodent Diet 5001, powdered formulation, Ralston Purina Co.) 2 weeks before initiating the experiment and then maintained on powdered food throughout. At the start of the experiment, one of the groups (MELATONIN) began receiving melatonin (0.4 μg/ml) in ethanol vehicle (0.01%) in their daily drinking water, whereas the other group (CONTROL) received vehicle alone. An additional group of 18 young rats (YOUNG; 3 months of age at the start of the experiment) was individually housed in the same room as the middle-aged rats and received powdered food and ethanol vehicle in the drinking water. After 12 weeks of treatment, 9 rats from each of the 3 groups (YOUNG, MELATONIN, and CONTROL) were killed. At this point the remaining 9 young rats (6 months of age) were removed from the study. At week 14, the treatments for the remaining middle-aged MELATONIN and CONTROL groups were crossed over, *i.e.* the rats that had received melatonin during the first 14 weeks then received control treatment (0.01% ethanol vehicle in their drinking water) and the rats that had received control treatment then received melatonin treatment (0.4 μg/ml melatonin and 0.01% ethanol). After an additional 12 weeks, these remaining middle-aged rats were killed.

Melatonin administration

Melatonin (Sigma, St. Louis, MO) was dissolved in 100% ethanol and stored in aliquots at −70 C. Fresh solutions of drinking water containing 0.01% ethanol with or without 0.4 μg melatonin/ml were prepared twice weekly. The water bottles were covered with aluminum foil; clean bottles with fresh solutions were provided twice weekly.

In a recent study using this same model of melatonin administration via drinking water, we determined that both control and melatonin-treated middle-aged male Sprague Dawley rats maintained in a 14-h light, 10-h dark cycle in our facility (*i.e.* animals and conditions essentially identical to the current study) drank more than 90% of their total daily water during the dark period (9). Assuming that the rats in the current study exhibited a comparable pattern and drank a consistent amount during each hour of the dark period, melatonin consumption in the current study (14–19 μg melatonin/day; 30–35 μg melatonin/kg BW·day) would correspond to approximately 1.3–1.7 μg/h (2.8–3.2 μg/kg BW·h) through the 10-h night.

Body weights (BW)

All rats were weighed twice weekly, on Mondays and Fridays, between 0900–1100 h. These two weights were averaged to yield a weekly weight for each animal.

Food consumption

Each rat was provided with approximately 40 g fresh powdered chow between 1100–1200 h daily in a 6-oz powdered food feeder jar (PFF6D,

Allentown Caging, Allentown, NJ) secured in a stainless steel hanging jar holder (HJH6D, Allentown Caging) modified by addition of a stainless steel spring that anchored the feeder jar to the hanging jar holder (preventing it from being tilted or dislodged). Approximately 10–15 g food remained when the feeder jar was removed and another jar provided at the same time on the subsequent day. Providing only slightly more food than that consumed each day reduced the amount of food in the jar (*i.e.* the jar was well under half full when initially provided each day), minimizing and usually eliminating spillage. Food consumption was measured by weighing the jar plus food before feeding and then subtracting the weight of the jar plus food after 24-h consumption on Mondays, Wednesdays, and Fridays. These three values were averaged as an index of daily consumption for the week. Data from days when spillage was detectable were not included (this occurred on <2% of days of monitored food consumption). Cumulative food intake was calculated by multiplying average daily food intake by 7, then summing the calculated weekly averages throughout the treatment period. Relative food intake was calculated as grams of daily food intake per g BW. Feed efficiency was calculated as grams of BW change per g food eaten throughout that time period. Bulk powdered chow was stored in a room temperature covered bin to minimize the variability in weight of daily aliquots due to condensation and/or evaporation that can occur with refrigerated storage.

Locomotor activity

Home cage spontaneous ambulatory locomotor activity was measured by sequential infrared beam breaks using an Opto-Varimex Mini animal activity monitoring system (Columbus Instruments, Columbus, OH). In this system, an array of 15 infrared emitters is placed along 1 side of the home cage, and an array of 15 receivers is placed along the other; movement of the rat interrupts the infrared beams, generating digital signals that are tabulated by computer. For this study only sequential breaks of different beams (primarily corresponding to ambulatory locomotion) were tabulated, excluding sequential repetitive breaks of the same beam (commonly associated with grooming or eating). Activity was monitored continuously during the last 4 h of the light period, the entire 10-h dark period, and the first 6 h of the next light period, for a total of 10 h of light and 10 h of darkness. One hour before and then throughout this time, the colony room door remained closed, and the rats were not disturbed. Two rats were independently monitored each day during weeks 7–10 of treatment.

Core body temperature

Body temperature was measured with a digital Precision thermometer (model 4600) with 400 series small semiflexible vinyl rectal/esophageal Thermistor probe (YSI, Inc., Yellow Springs, OH), providing 0.01 C resolution and a time constant of 1.4 sec. Each rat was removed from the cage at 1000–1100 h and held on an investigator's arm; another investigator inserted the lubricated Thermistor probe 5 cm into the colon, and temperature was recorded after 30 sec. The measurement caused no apparent distress and was completed less than 45 sec after removing the rat from the home cage. The temperature of each rat was determined once per week during weeks 7–9 of the experimental trial, alternating the order of determinations in sequential weeks; the three estimates for each rat were averaged to provide a single value for core body temperature.

Tail blood collections

On each of the 3 days preceding blood sample collection, each rat was transferred in its home cage to the adjoining blood-sampling room, exposed to a mock blood-sampling procedure, and returned to the colony. On the day of sample collection, the distal tip (<1 mm) of the tail was snipped with sharp surgical scissors, and blood was gently "milked" into a heparinized capillary tube; plasma was then separated by centrifugation and stored at −70 C. Spontaneous bleeding stopped immediately after sample collection; subsequent removal of the coagulate allowed an additional sample to be collected on the same day without an additional cut. The rats were unrestrained; they remained in their home cages throughout the blood collection procedures and did not appear distressed.

Collection of 350-μl blood samples for characterization of plasma melatonin levels at the midpoint of the light period and at the midpoint of the dark period was performed during week 6. The samples at the midpoint of the light period and those at the midpoint of the dark period were both collected on the same day, with the light period sample collected first. For samples collected during the dark period, lights remained off in the colony room; each rat was exposed to dim light (60–100 lux) in the blood-sampling room for less than 3 min.

Collection of 40-μl blood samples for characterization of plasma corticosterone levels in the early morning (1–2 h after lights on) and late afternoon (0–1 h before lights off) was performed during week 10 of the cross-over study. The early morning and late afternoon samples were both collected on the same day, with the morning sample collected first.

Decapitation, preparation for DEXA

The rats were killed by decapitation 2–3 h after the start of the light period; blood was collected, and plasma was stored at −70 C. The carcasses were rapidly frozen, ventral side down, on large blocks of dry ice and buried under powdered dry ice, then stored in sealed plastic storage bags at −20 C. The carcasses were positioned during freezing to provide consistent dual energy x-ray absorptiometry (DEXA) analysis (see below); the front legs were extended rostrally, the rear legs were extended caudally, and the tail was turned rostrally to lie along the body.

Body composition and adiposity measurements

Body composition (fat and lean mass) was measured in frozen rat carcasses by DEXA (QDR 1500, Hologic, Inc., Waltham, MA), using the Rat Whole Body software package (version 5.67, Hologic, Inc.). DEXA provides a reliable method for quantification of rat whole body composition, as we (7) and others (11, 12) have previously described. The system is based on the differential attenuation of low (70 keV) and high (140 keV) energy x-rays by the tissues in the scan area. Fat mass detected by DEXA consists primarily of adipose tissue, but lean mass includes organs, tendons, cartilage, blood, and body water in addition to skeletal muscle. A tissue calibration scan, using calibration phantoms, was performed each half-day, and measurement of a separate frozen rat carcass was repeated each day as a control for scanning variations. Intrascan coefficients of variation were 5.0% for fat mass and 0.6% for lean mass. The interscan coefficients of variation for fat and lean masses were 6.0% and 1.0%, respectively. The sum of individual animals' fat mass plus lean mass plus bone mineral content was highly correlated ($r = 0.999$) with direct measurement of BW.

After DEXA, the carcasses were thawed to 4 C, and all intraabdominal fat depots were dissected. An incision was made at the ventral midline, exposing the abdominal cavity, and the gastrointestinal tract was severed above the stomach and at the base of the colon and removed, exposing the internal fat pads. The omental fat depot was carefully separated from the pancreas, and the mesenteric fat depot was removed from the length of the gut, with blood vessels and connective tissue included. Paired retroperitoneal, perirenal, and epididymal fat pads were then removed, with kidneys, adrenals, testes, and epididymis dissected away. The fat pads were weighed immediately after dissection to avoid evaporative weight loss. Estimates of intraabdominal and peripheral fat were calculated by summing intraabdominal fat pad (omental plus mesenteric plus retroperitoneal plus perirenal plus epididymal) weights. This figure was subtracted from the total body fat determined by DEXA analysis to provide an estimate of peripheral fat.

Adrenal and thymus weights

At the time of dissection of fat pads, the thymus gland and both adrenal glands were also removed and weighed.

Glucose measurement

Plasma glucose concentrations were determined by the hexokinase enzymatic method with spectrophotometric quantitation, using the glucose (HK) kit (procedure 16-UV) from Sigma. All samples were quantitated in a single assay. The sensitivity of the assay was 1.0 mg/dl, and the intraassay coefficient of variation was 2.0%.

RIA

Melatonin was quantitated as previously described (13), using the Guildhay antiserum, which has been well characterized for determination of melatonin concentrations in rat plasma (14). Leptin and insulin were quantitated with rat leptin and insulin RIA kits from Linco Research, Inc. (St. Charles, MO). Total corticosterone was quantitated as previously described (15). For each hormone, all samples were assayed in a single RIA. Intraassay coefficients of variation were all less than 10%. Limits of detection for the melatonin, leptin, insulin, and corticosterone assays were 11.5 pg/ml, 0.5 ng/ml, 0.1 ng/ml, and 5 ng/ml, respectively.

Statistical analyses

Behavioral tests were analyzed by statistical tests appropriate to each; these are described in *Results*. Other comparisons were made by one-way ANOVA, except as noted. For the cross-over study, results were compared between treatment groups by one-way ANOVA, and compared within treatment group before *vs.* after the cross-over by one-way ANOVA. Results are reported as the mean ± SEM, with the level of significance set at $P < 0.05$. Data from the YOUNG group are reported for reference, but were not included in the statistical analyses of melatonin treatment effects. The statistical software package used was StatView version 4.57 for Windows (Abacus Concepts, Inc., Berkeley, CA; and SAS Institute, Inc., Cary, NC).

Results

Two-bottle choice test

When presented with 24-h choices between water containing 0.4 μg/ml melatonin plus 0.01% ethanol vehicle *vs.* water containing vehicle alone, nine previously untreated rats consumed 50.5 ± 2.5% of their total daily water consumption as water with vehicle alone and 49.5 ± 2.5% as water containing melatonin. These relative amounts were not significantly different by Student's *t* test. Thus, addition of melatonin to the drinking water did not alter preference relative to water that did not contain melatonin.

Alternating days consumption test

During the first 24 h of the alternating days consumption test, 24 previously untreated rats each drank 36.1 ± 1.0 ml water containing 0.01% ethanol vehicle. When the water bottles contained both ethanol vehicle and 0.4 μg/ml melatonin for the next 24 h, the rats drank 37.8 ± 1.0 ml. When the water bottles were again replaced with bottles containing only vehicle in water for 24 h, the rats drank 36.8 ± 0.7 ml. The volumes consumed on these 3 consecutive days were not significantly different by repeated measures ANOVA. Thus, addition of melatonin to the sole source of drinking water at a dosage of 0.4 μg/ml did not alter the volume of drinking water consumed over a 24-h period. Furthermore, the addition of melatonin to the drinking water for 24 h did not alter the volume of drinking water consumed over the subsequent consecutive 24-h period after removal of the melatonin.

Conditioned saccharin taste aversion test

On the conditioning day (day 4) of the conditioned taste aversion test, the 12 rats allowed access to saccharin solution containing control vehicle (group a) each consumed 11.0 ± 0.8 ml in 15 min, whereas the 12 rats allowed access to a saccharin solution containing vehicle plus 0.4 μg melatonin/ml (group b) each consumed 11.9 ± 0.8 ml in 15 min (providing an acute oral dosage of 4.8 ± 0.3 μg melatonin, or 8.7 ± 0.5 μg melatonin/kg BW). The rats that drank saccharin solution containing vehicle alone on the conditioning day (group a) each drank 2.4 ± 1.0 ml less saccharin solution when allowed access to a choice between saccharin solution *vs.* water on the test day (day 6); the rats that drank saccharin solution containing melatonin on the conditioning day (group b) drank 2.1 ± 1.2 ml less saccharin solution on the test day. These difference scores between saccharin solution intake on the conditioning day *vs.* that on the subsequent test day were not significantly different between the melatonin-conditioned *vs.* vehicle control-conditioned rats by Student's *t* test. Thus, addition of 0.4 μg/ml melatonin to the drinking water did not alter the volume of acute water plus saccharin consumption by water-deprived rats (on day 4), and pairing of melatonin consumption with the taste of saccharin did not induce conditioned taste aversion.

Plasma melatonin levels

Daytime plasma levels of melatonin were low for both middle-aged groups (CONTROL, 11.5 ± 0.00 pg/ml; MELATONIN, 14.1 ± 2.6 pg/ml). Melatonin treatment resulted in significantly ($P < 0.001$) increased levels during the dark period (150.5 ± 19.2 pg/ml), whereas nighttime melatonin was not increased in the CONTROL group (24.1 ± 8.8 pg/ml) relative to daytime levels. In the YOUNG untreated rats, low levels of plasma melatonin during the daytime (20.0 ± 6.3 pg/ml) were significantly increased at night (50.0 ± 4.7 pg/ml).

BW, weight change, and food consumption

The BW of MELATONIN animals (Fig. 1 and Table 1) decreased 3% (16 ± 2 g weight loss) over the initial 12 weeks of study compared with a 3.6% increase (19 ± 3 g weight gain) in CONTROL rats. There were no significant effects of melatonin treatment on relative food intake (grams of daily food intake per g BW; Fig. 1), daily food intake (Table 1), or cumulative food intake (Table 1). Feed efficiency (grams of BW change per g cumulative food intake) was negative in MELATONIN and positive in CONTROL animals (Table 1).

Physical activity and core body temperature

Total 20-h locomotor activity (Fig. 2) of the MELATONIN group was 19% greater than that of CONTROL rats ($P < 0.05$). Compared with YOUNG animals, activity was 35% lower for CONTROL and 23% lower for MELATONIN rats.

Core body temperature was significantly ($P < 0.01$) higher in MELATONIN (37.9 ± 0.1 C) compared with CONTROL (37.3 ± 0.1 C) rats. The core body temperature of MELATONIN animals was equivalent to that of YOUNG rats (38.0 ± 0.1 C).

Body composition and adiposity

The MELATONIN group had 20% less intraabdominal fat than CONTROL animals ($P < 0.05$; Table 2), whereas there was no significant effect of melatonin treatment on total or peripheral fat. The proportion of total fat located in the intraabdominal cavity was decreased by 16% ($P < 0.01$) in

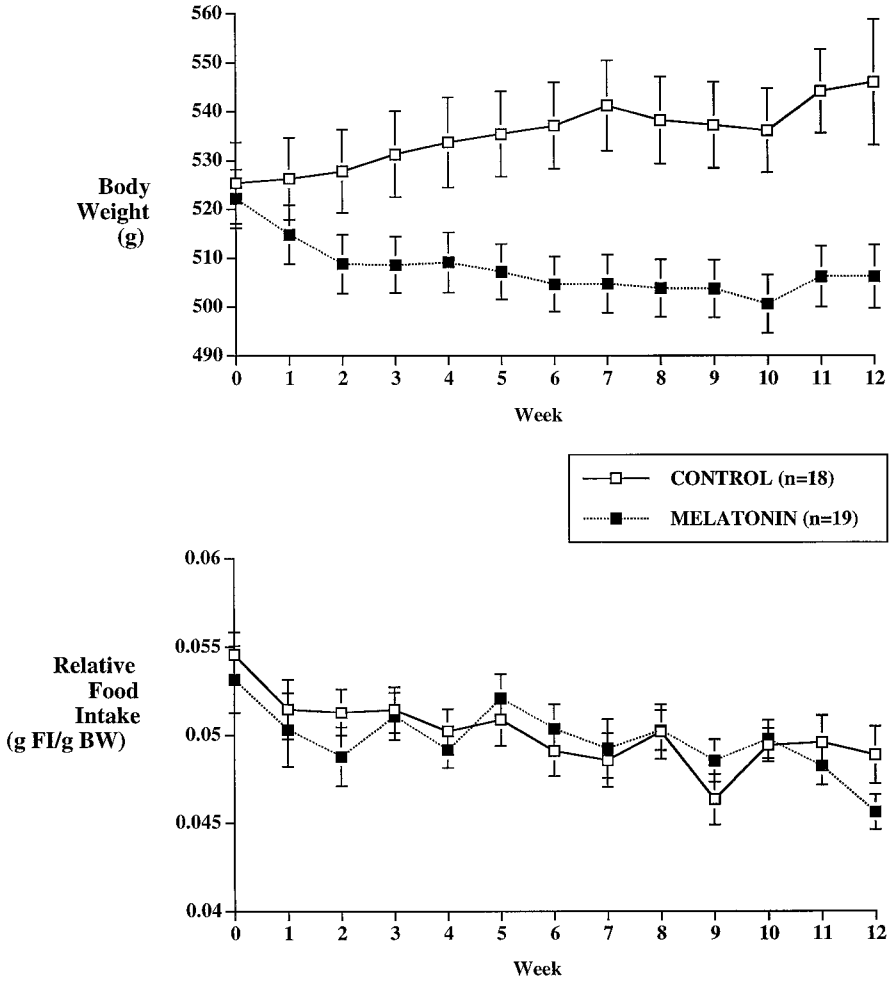

FIG. 1. Mean (±SEM) BW (grams; A) and relative food intake (FI; grams per g BW; B) over the initial 12 weeks of study of 18 CONTROL (*open symbols*) and 19 MELATONIN (*filled symbols*) male Sprague Dawley rats. Animals were 10 months old at the onset of the study.

TABLE 1. Body weight and food intake during initial 12-wk treatment

	Control (n = 18)	Melatonin (n = 19)
BW		
Initial BW (g)	525 ± 8	522 ± 6
Final BW (g)	546 ± 9	506 ± 6[a]
Wt change (g)	18.7 ± 2.5	−16.0 ± 2.4[a]
Wt change (%)	3.6 ± 0.6	−3.1 ± 0.5[a]
Food intake		
Daily average (g)	26.8 ± 0.7	25.2 ± 0.6
Cumulative (g)	2252 ± 58	2131 ± 48
Relative (g/day·g BW)	0.050 ± 0.001	0.050 ± 0.001
Feed efficiency	0.008 ± 0.001	−0.008 ± 0.001[a]

Calculation of average daily food intake is described in *Materials and Methods*. Cumulative food intake is calculated by multiplying average daily food intake by 7, then summing the calculated weekly averages throughout the treatment period. Relative food intake is calculated as grams of daily food intake per g BW. Feed efficiency is calculated as grams of BW change per g food eaten throughout that time period. Data are the mean ± SEM.

[a] $P < 0.0001$, by one-way ANOVA, comparing treatments.

MELATONIN compared with CONTROL animals (Table 2), consequently resulting in an increased proportion of fat located peripherally. Whereas melatonin treatment of middle-aged rats resulted in significantly less absolute lean body mass (grams), when lean mass was expressed relative to total BW (percent lean mass), there was no difference due to treatment (Table 2).

Each of the adipose depots in the MELATONIN group weighed less than those in the CONTROL group (Fig. 3), although these differences reached significance only for the large epididymal fat pad, which was 27% smaller ($P < 0.01$) after 12 weeks of melatonin treatment. Compared with YOUNG rats, middle-aged rats had nearly twice as much total body fat (~73 or 77 g compared with 40 g), whereas lean body mass was only 20–30% greater in MELATONIN and CONTROL groups, respectively.

Plasma hormone and glucose levels

Nonfasted plasma leptin and insulin concentrations (Table 2) were lower ($P < 0.05$) in MELATONIN than CONTROL rats (decreased by 33% and 25%, respectively). The plasma leptin and insulin levels in MELATONIN rats were not different from levels in the YOUNG group (leptin, 3.1 ± 0.4 ng/ml; insulin, 2.2 ± 0.2 ng/ml). Plasma glucose concentrations were not significantly altered by melatonin treatment (Table 2). Basal corticosterone concentrations (measured during the morning) were 2.5 times higher in MELATONIN compared with CONTROL rats ($P < 0.001$) and were not different from those in YOUNG animals (78.6 ±

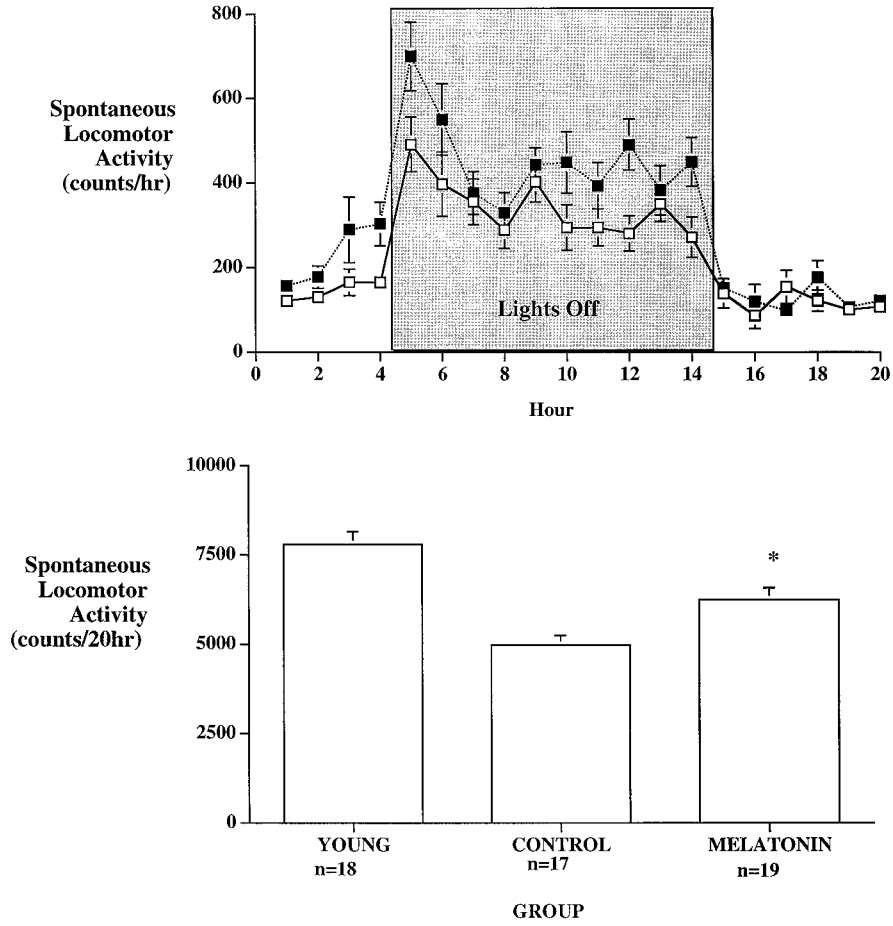

FIG. 2. Spontaneous locomotor activity of 17 CONTROL and 19 male Sprague Dawley rats. The activity of the animals was assessed while they were in their home cages for 20 h during weeks 7–10 of the study. Hourly averages (mean ± SEM) are shown in A for CONTROL (*open symbols*) and MELATONIN (*filled symbols*) groups. Twenty-hour averages (mean ± SEM) are shown in B, with activity in the YOUNG CONTROL group shown for comparison. Data from middle-aged animals were analyzed by one-way ANOVA, *, $P < 0.05$.

11.3 ng/ml). There was no effect of treatment on afternoon (peak) corticosterone levels.

Adrenal and thymus weights

There were no significant differences in adrenal or thymus weight between MELATONIN (0.030 ± 0.003 and 0.196 ± 0.017 g, respectively) and CONTROL (0.030 ± 0.004 g and 0.205 ± 0.015 g, respectively) rats. Adrenal weights of MELATONIN and CONTROL rats were not different from those of YOUNG rats (0.030 ± 0.002 g), but the average weight of the thymus gland was greater (0.377 ± 0.026 g) in the YOUNG group than in the MELATONIN or CONTROL group.

BW, weight change, and food consumption before and after cross-over

BWs were significantly different between treatment groups at cross-over ($P < 0.05$), but were not significantly different ($P < 0.07$) at the end of the study (Fig. 4). The nine rats initially treated with MELATONIN that were then crossed over to CONTROL treatment for an additional 12 weeks rapidly gained weight (+44 ± 3 g), whereas the nine CONTROL rats that were crossed over to MELATONIN rapidly lost weight (−24 ± 4 g), reversing their precross-over weight trends (Fig. 4). The final weights of rats that were crossed from MELATONIN to CONTROL treatment were

TABLE 2. Body composition, fat distribution, and hormones at 12 wk

	Control (n = 9)	Melatonin (n = 9)
Total fat mass (g)	76.7 ± 5.7	73.3 ± 5.3
Fat mass (% of carcass)	14.8 ± 0.9	15.4 ± 1.0
Lean mass (g)	428 ± 9	391 ± 7[a]
Lean mass (% of carcass)	83 ± 1	83 ± 1
Visceral fat mass (g)	18.2 ± 1.3	14.5 ± 1.1[b]
% of total fat mass	23.8 ± 0.7	19.9 ± 1.0[a]
% of carcass	3.5 ± 0.2	3.0 ± 0.2
Peripheral fat mass (g)	58.5 ± 4.5	58.8 ± 4.5
% of total fat mass	76.2 ± 0.7	80.1 ± 1.0[a]
% of carcass	11.3 ± 0.7	12.4 ± 0.9
Glucose (mg/dl)	179.3 ± 5.8	171.9 ± 6.5
Insulin (ng/ml)	2.7 ± 0.2	2.0 ± 0.2[b]
Leptin (ng/ml)	5.8 ± 0.5	3.9 ± 0.6[b]
Corticosterone (ng/ml)		
AM	25.1 ± 6.3	63.8 ± 6.0[c]
PM	78.5 ± 10.8	91.6 ± 6.3

Total fat and lean mass values were determined by DEXA. Visceral fat values are the sum of all five dissected visceral fat depots. Peripheral fat values are the difference between total fat (from DEXA) and visceral fat (from dissection). Plasma insulin and leptin were assessed by RIA from trunk blood of nonfasted animals; plasma cortisol was analyzed in tail blood samples. To convert insulin to microunits per/ml, divide by 0.037. Values are the mean ± SEM. Data were analyzed by one-way ANOVA, comparing treatments.

[a] $P < 0.01$.
[b] $P < 0.05$.
[c] $P < 0.001$.

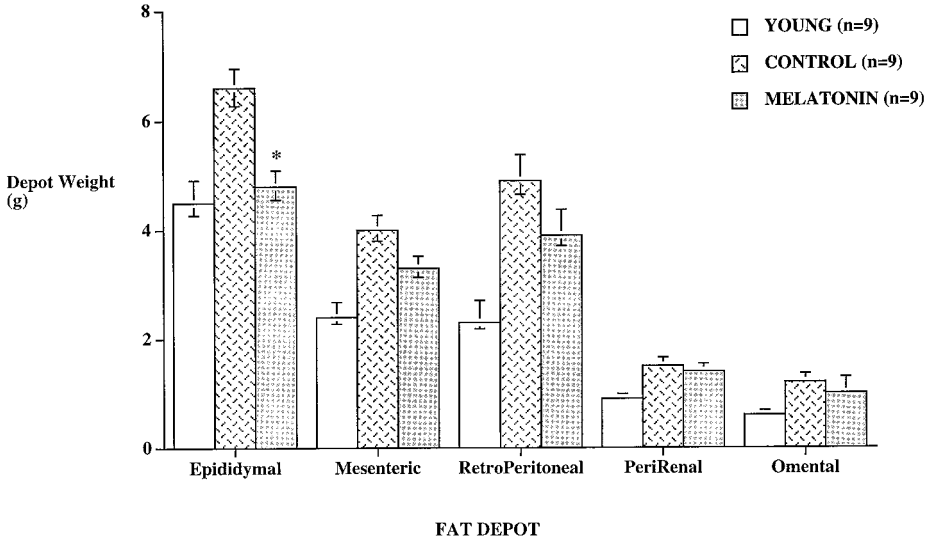

FIG. 3. Intraabdominal fat depot weights (grams; mean ± SEM) of nine CONTROL, nine MELATONIN, and nine YOUNG male Sprague Dawley rats at the end of 12 weeks of study. Data from the YOUNG group are reported for comparison. Data from middle-aged animals were analyzed by one-way ANOVA. *, $P < 0.01$.

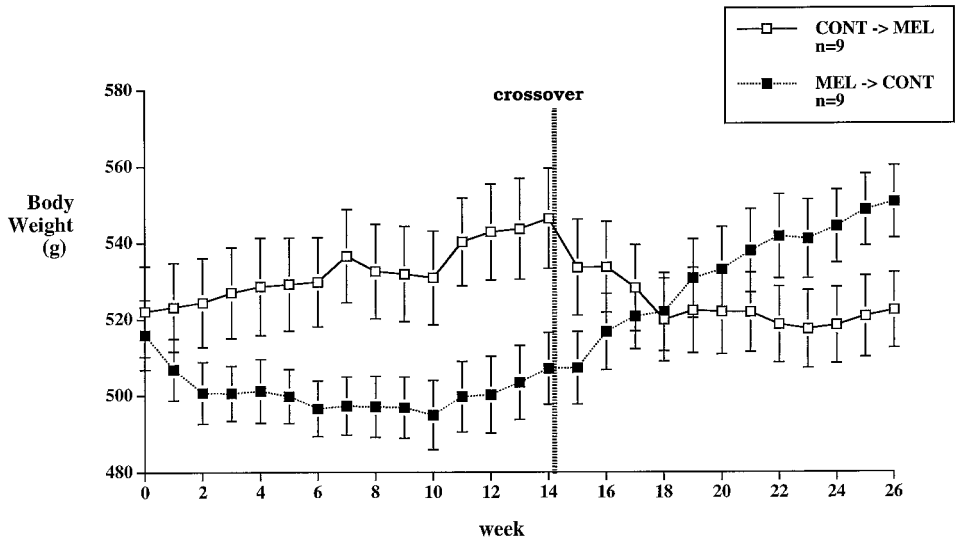

FIG. 4. Mean (±SEM) BW (grams) over the full 26 weeks of the study of nine CONTROL (*open symbols*) and nine MELATONIN (*filled symbols*) male Sprague Dawley rats. Treatments were crossed over at week 14 (*vertical dashed line*). Animals were 10 months old at the onset of the study.

similar to the weights of CONTROL rats before the cross-over (551 ± 9 and 546 ± 13 g, respectively); the final weights of rats that were crossed over from CONTROL to MELATONIN (522 ± 10 g) were similar to those of the MELATONIN group before cross-over (507 ± 9 g).

Food intake (relative, daily, and cumulative; Table 3) did not change for either group. Feed efficiency (grams of BW change per g cumulative food intake) was negative in MELATONIN and positive in CONTROL animals before cross-over, and this relationship was reversed after cross-over (Table 3).

Discussion

These experiments were designed to extend our previous work (9) by investigating the mechanisms through which melatonin treatment decreases visceral adiposity in middle-aged male rats. The results demonstrate that 12 weeks of melatonin treatment in middle age decreased BW, relative intraabdominal adiposity, and plasma leptin and insulin levels in male Sprague Dawley rats while increasing physical activity, body temperature, and basal plasma corticosterone concentrations toward youthful levels, without associated changes in food consumption. Melatonin-treated rats that were subsequently crossed over to control treatment for an additional 12 weeks gained weight, whereas control rats that were crossed to melatonin treatment lost weight, thus reversing their precross-over weight trends. As with the initial responses to melatonin, these weight changes after cross-over occurred without significant changes in food intake for either group. These results thus suggest that the decrease in endogenous melatonin secretion with aging may alter metabolism and physical activity independent of changes in food intake, resulting in the increased BW and visceral adiposity that are associated with glucose intolerance, insulin resistance, diabetes, dyslipidemia, and cardiovascular disease. This hypothesis is consistent with preliminary results of a more recent study (16) in which we have demonstrated that 10 weeks of daily melatonin administration to middle-aged (10-month-old) male Sprague Dawley rats again suppressed intraabdominal fat, plasma leptin, and plasma in-

TABLE 3. Food intake before and after cross

	Before cross[a]	After cross[b]	*P* (before *vs.* after)
Daily food intake (g)			
CONT → MEL	27.1 ± 0.9	25.5 ± 1.0	NS
MEL → CONT	24.5 ± 0.9	24.9 ± 0.8	NS
P (between treatment)	NS (0.063)	NS	
Cumulative food intake (g)			
CONT → MEL	2290 ± 75	2145 ± 81	NS
MEL → CONT	2090 ± 82	2090 ± 71	NS
P (between treatment)	NS (0.063)	NS	
Relative food intake (g/g BW)			
CONT → MEL	0.051 ± 0.002	0.049 ± 0.002	NS
MEL → CONT	0.049 ± 0.002	0.047 ± 0.002	NS
P (between treatment)	NS	NS	
Feed efficiency			
CONT → MEL	0.009 ± 0.002	−0.005 ± 0.002	<0.0001
MEL → CONT	−0.004 ± 0.002	0.021 ± 0.002	<0.0001
P (between treatment)	<0.0001	<0.0001	

Calculation of average daily food intake is described in *Materials and Methods*. Cumulative food intake is calculated by multiplying the average daily food intake by 7, then summing the calculated weekly averages throughout the treatment period. Relative food intake is calculated as grams of daily food intake per g BW. Feed efficiency is calculated as grams of change in BW per g food eaten throughout that time period. Animals received initial treatment for 14 wk, followed by opposite treatment for 12 wk. Values (mean ± SEM for nine animals per treatment group) from before cross ([a]) are differences between week 1 and week 14; after cross ([b]) are differences between week 14 and week 26. Data were analyzed by one-way ANOVA, comparing treatments, and by one-way ANOVA comparing results before *vs.* after the treatment cross-over. MEL, Melatonin.

sulin, whereas identical melatonin administration to young adult (3-month-old) rats did not change significantly any of these parameters, suggesting that the response to exogenous melatonin was dependent upon the aging-associated decrease in endogenous melatonin secretion.

The two-bottle choice test demonstrated that rats exhibited no altered preference for water containing 0.4 μg/ml melatonin relative to water that did not contain melatonin. Work originally conducted by Garcia and colleagues (17) on the concept of bait shyness has demonstrated that rats exhibit innate aversion to novel tastes. The results of the choice test thus suggest that the rats could not taste the melatonin present in the drinking water. The alternating days consumption test demonstrated that addition of this dosage of melatonin to the drinking water also had no effect on the volume of water consumed over a 24-h period as well as no rebound or delayed effect on the volume of water consumed over the 24-h period after removal of the melatonin. Finally, the conditioned saccharin taste aversion test demonstrated that acute (15-min) oral consumption of a relatively large dose of melatonin (*i.e.* an amount comparable to ~33% of the melatonin consumed over an entire 24-h period during the experimental trial) did not induce a conditioned taste aversion and thus was not perceived as toxic. The complementary results of these three experiments thus suggest that addition of melatonin to the drinking water in this study did not directly alter water consumption, could not be tasted by the rats, and did not produce effects perceived by the rats as toxic.

Melatonin supplementation via the drinking water increased nocturnal plasma melatonin levels, but did not alter basal plasma melatonin levels during the daytime, consistent with melatonin's short (20-min) biological half-life (18) and with our previous demonstration (9) that middle-aged rats drank more than 90% of their water plus melatonin during the dark phase. The melatonin treatment employed in this experiment thus reestablished a nocturnal pattern of melatonin exposure. The nocturnal melatonin levels resulting from this treatment protocol were higher than those in untreated young animals, although the single sample at a single time point chosen for nocturnal tail bleeding may have missed the spontaneous melatonin peak in the young animals. Preliminary results from a follow-up study reveal that a 50% lower melatonin dose produced nocturnal plasma melatonin levels in middle-aged male rats that were identical to nocturnal plasma melatonin levels in untreated young rats, and the effects on intraabdominal fat and plasma insulin and leptin levels were similar to those in the current study (16).

Melatonin treatment restored morning plasma corticosterone levels to those of young animals, although afternoon peak levels were not changed. This apparent time of day effect may be due to the fact that most of the daily melatonin exposure occurred at night, immediately before the time of the morning blood sample collection. The increased morning plasma corticosterone levels may have been due to either increased secretion or decreased clearance. The restoration of youthful morning corticosterone levels was achieved without affecting adrenal and thymus weights, sensitive indicators of chronic stress or glucocorticoid excess. Thus, it appears that melatonin supplementation may have altered some aspects of hypothalamo-pituitary-adrenal regulation in a manner not associated with a chronic stress response.

The ability of melatonin to decrease BW was observed within 2 weeks of treatment, after which weights tended to stabilize at the lower level rather than continuing to decline. In previous studies (9) we have demonstrated that this weight loss in response to melatonin treatment of middle-aged rats was sustained when melatonin treatment was continued for 1 yr, into old age (22 months). In the current study we have demonstrated that this weight loss was achieved despite the apparent lack of effect of melatonin treatment on food intake. These patterns were repeated in the cross-over portion of the study; the BWs of animals initially receiving

the melatonin treatment rapidly began to increase when switched to the control treatment, and the BWs of control animals rapidly decreased in response to melatonin treatment, without corresponding changes in food intake. Animals that initially received melatonin regained weight aggressively when switched to control treatment, gaining almost twice the amount of weight lost before the cross-over, whereas animals initially receiving control treatment and then crossed over to melatonin lost the same amount of weight as that lost by the opposite group during the initial melatonin treatment. This similarity between BWs and weight changes in each group at the end of the melatonin (or control) treatment may suggest that these treatments influenced the animals to defend different BW set-points.

Feed efficiency, a measure of metabolic function (19, 20), was negative in the melatonin-treated animals and positive in controls; this effect was immediately reversed when the treatments were crossed over. These results suggest that melatonin may alter either metabolism or the ability to store excess energy as body fat, and that this effect is readily reversible. This finding is consistent with the observation that melatonin injections increased the norepinephrine-stimulated metabolic rate in mice (21).

Aging is associated with decreased levels of physical activity (22, 23). We found that melatonin increased the spontaneous physical activity of middle-aged rats by 19%, toward levels in young animals. In a follow-up study (to be reported elsewhere) we recently determined that similar melatonin treatment again increased ($P < 0.05$) spontaneous locomotor activity in middle-aged male rats by 18%. Although a specific mechanism for this effect of melatonin is not clear, melatonin treatment has been found to reverse the decreased physical activity of old mice (24), of rats with suppressed endogenous melatonin secretion due to continuous light exposure (25), and of mice infected with murine retrovirus (26). Recent work (20) has shown that small changes in physical activity (*e.g.* fidgeting) can have large effects on the amount of weight gained due to caloric excess in humans. It is thus reasonable to speculate that similar small increases in physical activity of melatonin-treated middle-aged rats in the present study contributed to the decreased BW of these animals.

Aging is also associated with decreased body core temperature and blunted circadian rhythms of body temperature (27–30). Melatonin treatment of middle-aged rats in this study increased daytime body core temperature to a level not different from that in young adult rats. The increased body temperature of the melatonin-treated rats may have been a consequence of increased physical activity in this group, and although great care was taken to minimize stress during the recording of body temperatures, it is possible that temperature changes may have been mediated by physical activity and/or stress-related responses specifically associated with the handling of the animals. Treatment with melatonin or an analog has been shown to restore blunted circadian rhythms of body temperature in old rats (27) or rats exposed to continuous light (25) and to acutely increase body core temperature in intact rats (31). The effects of acute and chronic daily melatonin treatment on thermoregulation have been investigated most frequently in experiments using photoperiodic animals or pinealectomy (see Ref. 29 for review). Melatonin has been shown to both increase and decrease body temperature; these inconsistencies may be explained by differences in species, age, and mode or time of day of melatonin treatment. In the present study the decreased body temperature of control middle-aged animals might be related to either decreased physical activity or decreased metabolism, highly interrelated variables. It is significant that decreased activity and/or metabolism have been shown to precede development of obesity and increased intraabdominal adiposity in both older rats and older humans (22, 23, 28, 32, 33).

Melatonin treatment decreased the amount of intraabdominal fat in middle-aged male rats, with no apparent changes in the level of total adiposity. There are a number of possible explanations for this observation, all speculative. One may be related to the increased core body temperature induced by melatonin. Lipoprotein lipase (an enzyme required for the uptake of fatty acids into adipocytes) has been shown to have increased activity when incubated at lower temperatures (34). This observation has led to the hypothesis that accumulation of visceral adipose tissue may be increased when body core temperatures are decreased (35). Observations of decreased core temperature in genetically obese mice (36) are compatible with this largely untested hypothesis. An alternate hypothesis, supported by human experimental trials, suggests that moderate increases in physical activity and exercise selectively decrease visceral adipose tissue (8, 37–39). Thus, the 19% increase in physical activity observed with melatonin treatment in the current study may have been at least in part responsible for the 20% decrease in intraabdominal fat stores. Alternatively, the increased activity and body temperature may simply reflect increased autonomic activity, which directly regulates metabolism (especially thermogenesis in brown fat, which is increased by sympathetic stimulation). A final potential explanation for the selective decrease in intraabdominal fat is suggested by studies in which peripheral leptin administration to rats was shown to selectively decrease visceral fat and improve insulin sensitivity (40); it is plausible that melatonin administration to the middle-aged rats in the current study may have increased sensitivity to endogenous leptin, resulting in selectively decreased intraabdominal fat and decreased plasma insulin.

Both leptin and insulin levels tend to parallel adipose tissue mass (5, 7, 41, 42), although they may be regulated differently by diet, exercise, age, and treatment. Although we have no direct measures of leptin or insulin sensitivity, it is reasonable to assume that the tissue responsiveness to these hormones increased, consistent with the decreases in circulating levels. Pinealectomy has recently been reported to cause glucose intolerance and a reduction in adipose tissue glucose transporter content (43) in young rats, although it should be noted that in another study pinealectomy and melatonin treatment were reported to have no effect on glucose homeostasis and insulin responsiveness (44). Overall, our results suggest that insulin and leptin sensitivity may be decreased by the gradual decline in endogenous circulating melatonin with aging, and that appropriate replacement with exogenous melatonin may reverse these declines.

The demonstrated dissociation between melatonin-induced BW and intraabdominal fat changes *vs.* the lack of corresponding changes in food intake, both during the initial

treatment period and in response to treatment cross-over, is consistent with evidence that peripheral administration of leptin at a dosage that did not alter food intake did decrease visceral adiposity and BW (40). Although the current results suggest that the effects of melatonin treatment are independent of effects on food intake, it should nonetheless be noted that facilitation of satiety may have prevented a compensatory increase in eating that would presumably have maintained BW changes similar to those in control rats.

Intracerebroventricular melatonin administration has been shown to acutely decrease plasma insulin levels and increase insulin sensitivity in rats (45), suggesting potential central mediation of the demonstrated metabolic effects of melatonin supplementation during aging. However, a recent preliminary report (46) suggests that direct peripheral effects of melatonin on adipocytes may also have a role. *In situ* perfusion of a sc fat pad with low, physiological levels of melatonin was reported to block fatty acid transport via a melatonin receptor-mediated mechanism (46). As leptin is produced in adipocytes in response to nutrient cycling (47), it is reasonable to suggest that melatonin's action to block fatty acid transport into the adipocytes could not only suppress lipogenesis but also acutely decrease leptin release into the circulation. If the sensitivity to leptin is increased by melatonin treatment, as suggested above, then even these lowered leptin levels could potentially facilitate energy expenditure, a response to leptin that can be dissociated from leptin's actions to suppress food intake (40).

In summary, we have shown that supplementation of melatonin in middle-aged male rats mimics some youthful energy regulatory responses, decreasing BW, intraabdominal adiposity, and plasma insulin and leptin concentrations while increasing core body temperature, physical activity, and plasma corticosterone levels. These results suggest that aging-associated decreases in endogenous melatonin secretion may alter energy regulation in middle age, resulting in increased BW and adiposity and their associated detrimental metabolic consequences. However, it is important to note that extrapolating from these findings to species other than the rat is not currently warranted, as melatonin effects on BW and adiposity have been found to be variable even in distinctly photoperiodic species (48–50), and the effects of daily melatonin administration on energy balance in nonphotoperiodic diurnal animals or humans have not been determined. Finally, although several potential mediating mechanisms have been suggested here, it is also important to note that melatonin, which is both water soluble and lipophilic, has access to essentially all cells and intracellular compartments in the body and has been demonstrated to exert effects not only at the cell membrane but also at the intracellular and genomic levels (51). Accordingly, the mechanisms by which melatonin administration may reverse or prevent aging-associated metabolic changes are under further investigation.

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References

1. **Cagnacci A** 1996 Melatonin in relation to physiology in adult humans. J Pineal Res 21:200–213
2. **Reiter R** 1992 The ageing pineal gland and its physiological consequences. BioEssays 14:169–175
3. **Pang SF, Tsang CW, Hong GX, Vip PC, Tang PL, Brown GM** 1990 Fluctuation of blood melatonin concentrations with age: result of changes in pineal melatonin secretion, body growth and aging. J Pineal Res 8:179–192
4. **Bjorntorp P** 1995 Neuroendocrine ageing. J Intern Med 238:401–404
5. **Bodkin NL, Nicolson M, Ortmeyer HK, Hansen BC** 1996 Hyperleptinemia: relationship to adiposity and insulin resistance in the spontaneously obese rhesus monkey. Horm Metab Res 28:674–678
6. **Hudson JC, Baum ST, Frye DM, Roecker EB, Kemnitz JW** 1996 Age and sex differences in body size and composition during rhesus monkey adulthood. Aging 8:197–204
7. **Wolden-Hanson T, Marck BT, Smith L, Matsumoto AM** 1999 Cross-sectional and longitudinal analysis of age-associated changes in body composition of male Brown Norway rats: association of serum leptin levels with peripheral adiposity. J Gerontol A Biol Sci Med Sci 54:B99–B107
8. **Buemann B, Tremblay A** 1996 Effects of exercise training on abdominal obesity and related metabolic complications. Sports Med 21:191–212
9. **Rasmussen DD, Boldt BM, Wilkinson CW, Yellon SM, Matsumoto AM** 1999 Daily melatonin administration at middle age suppresses male rat visceral fat, plasma leptin, and plasma insulin to youthful levels. Endocrinology 140:1009–1012
10. **Heinrichs SC, Klaassen A, Koob GF, Schulteis G, Ahmed S, De Souza EB** 1998 Corticotropin-releasing factor receptor blockade enhances conditioned aversive properties of cocaine in rats. Psychopharmacology (Berl) 136:247–255
11. **Rikans LE, Venkataraman PS, Cai Y** 1993 Evaluation by dual-energy x-ray absorptiometry of age-related changes in body composition of male rats. Mech Age Dev 71:155–158
12. **Jebb SA, Garland SW, Jennings G, Elia M** 1996 Dual-energy x-ray absorptiometry for the measurement of gross body composition in rats. Br J Nutr 75:803–809
13. **Yellon SM, Hilliker S** 1994 Influence of acute melatonin treatment and light on the circadian melatonin rhythm in the Djungarian hamster. J Biol Rhythms 9:71–81
14. **Webley GE, Mehl H, Willey KP** 1985 Validation of a sensitive direct assay for melatonin for investigation of circadian rhythms in different species. J Endocrinol 106:387–394
15. **Rasmussen DD, Bryant CA, Boldt BM, Colasurdo EA, Levin N, Wilkinson CW** 1998 Acute alcohol effects on opiomelanocortinergic regulation. Alcohol Clin Exp Res 22:789–801
16. **Mitton DR, McGillivray SA, Yellon SM, Rasmussen DD** 1999 Aging-associated changes in the effect of melatonin supplementation on energy regulation in rats. Soc Neurosci Abstr 25:2071 (Abstract)
17. **Garcia J, Ervin F, Koelling R** 1966 Learning with prolonged delay of reinforcement. Psychonom Sci 5:121–122
18. **Yeleswaram K, McLaughlin LG, Knipe JO, Schabdach D** 1997 Pharmacokinetics and oral bioavailability of exogenous melatonin in pre-clinical animal models and clinical implications. J Pineal Res 22:45–51
19. **Stock MJ, Rothwell NJ** 1986 The role of brown fat in diet-induced thermogenesis. Int J Vitam Nutr Res 56:205–210
20. **Levine JA, Eberhardt NL, Jensen MD** 1999 Role of nonexercise activity thermogenesis in resistance to fat gain in humans. Science 283:212–214
21. **Hall E, Lynch G** 1985 Two daily melatonin injections differentially induce nonshivering thermogenesis and gonadal regression in the mouse (*Peromyscus leucopus*). Life Sci 37:783–788
22. **Poehlman ET** 1992 Energy expenditure and requirements in aging humans. J Nutr 122:2057–2065
23. **Viidik A, Skalicky M** 1997 Influence of physical exercise on old rats: changes in patterns of spontaneous activity and connective tissues. Aging 9:64–72
24. **Araghi-Niknam M, Lane L, Watson RR** 1999 Modification of physical movement in old C57BL/6 mice by DHEA and melatonin supplementation. Proc Soc Exp Biol Med 221:193–197
25. **Depres-Brummer P, Metzger G, Levi F** 1998 Pharmacologic restoration of suppressed temperature rhythms in rats by melatonin, melatonin receptor agonist, S20242, or 8-OH-DPAT. Eur J Pharmacol 347:57–66
26. **Araghi-Niknam M, Lane L, Watson RR** 1998 Physical inactivity of murine retrovirus infected C57BL/6 mice is prevented by melatonin and dehydroepiandrosterone. Proc Soc Exp Biol Med 219:144–148
27. **Koster-van Hoffen GC, Mirmiran M, Bos NP, Witting W, Delagrange P, Guardiola-Lemaitre B** 1993 Effects of a novel melatonin analog on circadian rhythms of body temperature and activity in young, middle-aged, and old rats. Neurobiol Aging 14:565–569
28. **Rothwell NJ, Stock MJ** 1983 Effects of age on diet-induced thermogenesis and brown adipose tissue metabolism in the rat. Int J Obes 7:583–589
29. **Saarela S, Reiter RJ** 1994 Function of melatonin in thermoregulatory processes. Life Sci 54:295–311
30. **Ueno N, Oh-ishi S, Segawa M, Nishida M, Fukuwatari Y, Kizaki T,**

Ookawara T, Ohno H 1998 Effect of age on brown adipose tissue activity in the obese (*ob/ob*) mouse. Mech Age Dev 100:67–76
31. **Padmavathamma K, Joshi BN** 1994 Thermotrophic effect of melatonin in adrenalectomized and thyroidectomized rats. Biol Signals 3:53–58
32. **Vaughan L, Zurlo F, Ravussin E** 1991 Aging and energy expenditure. Am J Clin Nutr 53:821–825
33. **Toth MJ, Gardner AW, Ades PA, Poehlman ET** 1994 Contribution of body composition and physical activity to age-related decline in peak VO_2 in men and women. J Appl Physiol 77:647–652
34. **Speake BK, Parkin SM, Robinson DS** 1985 Degradation of lipoprotein lipase in rat adipose tissue. Biochim Biophys Acta 840:419–422
35. **Kahn HS** 1996 The accumulation of visceral adipose tissue may be influenced by intra-abdominal temperature. Obes Res 4:297–299
36. **Harris RB, Zhou J, Redmann Jr SM, Smagin GN, Smith SR, Rodgers E, Zachwieja JJ** 1998 A leptin dose-response study in obese (*ob/ob*) and lean (+/?) mice. Endocrinology 139:8–19
37. **Despres JP, Tremblay A, Nadeau A, Bouchard C** 1988 Physical training and changes in regional adipose tissue distribution. Acta Med Scand [Suppl] 723:205–212
38. **Hunter GR, Kekes-Szabo T, Snyder SW, Nicholson C, Nyikos I, Berland L** 1997 Fat distribution, physical activity, and cardiovascular risk factors. Med Sci Sports Exercise 29:362–369
39. **Pescatello LS, Murphy D** 1998 Lower intensity physical activity is advantageous for fat distribution and blood glucose among viscerally obese older adults. Med Sci Sports Exercise 30:1408–1413
40. **Barzilai N, Wang J, Massilon D, Vuguin P, Hawkins M, Rossetti L** 1997 Leptin selectively decreases visceral adiposity and enhances insulin action. J Clin Invest 100:3105–3110
41. **Smith SR** 1996 The endocrinology of obesity. Endocrinol Metab Clin North America 25:921–942
42. **Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S** 1995 Leptin levels in human and rodent: measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. Nat Med 1:1155–1161
43. **Lima FB, Machado UF, Bartol I, Seraphim PM, Sumida DH, Moraes SM, Hell NS, Okamoto MM, Saad MJ, Carvalho CR** 1998 Pinealectomy causes glucose intolerance and decreases adipose cell responsiveness to insulin in rats. Am J Physiol 275:E934–E941
44. **Bizot-Espiard JG, Double A, Cousin B, Lesieur D, Guardiola-Lemaitre B, Delagrange P, Ktorza A, Penicaud L** 1998 Lack of melatonin effects on insulin action in normal rats. Horm Metab Res 30:711–716
45. **Shima T, Chun SJ, Niijima A, Bizot-Espiard JG, Guardiola-Lemaitre B, Hosokawa M, Nagai K** 1997 Melatonin suppresses hyperglycemia caused by intracerebroventricular injection of 2-deoxy-D-glucose in rats. Neurosci Lett 226:119–122
46. **Blask DE, Dauchy RT, Sauer LA,** Physiological melatonin inhibits lipogenesis and fasting-induced lipolysis in the inguinal fat pad perfused in situ in the rat. 81st Annual Meeting of The Endocrine Society, San Diego, CA, 1999 (Abstract P2–208, p 324)
47. **Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L** 1998 A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature 393:684–688
48. **Bartness TJ, Wade GN** 1985 Body weight, food intake and energy regulation in exercising and melatonin-treated Siberian hamsters. Physiol Behav 35:805–808
49. **Kriegsfeld LJ, Nelson RJ** 1996 Gonadal and photoperiodic influences on body mass regulation in adult male and female prairie voles. Am J Physiol 270:R1013–R1018
50. **Lincoln GA, Richardson M** 1998 Photo-neuroendocrine control of seasonal cycles in body weight, pelage growth and reproduction: lessons from the HPD sheep model. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 119:283–294
51. **Reiter R, Chang-Seok O, Fujimori O** 1996 Melatonin: its intracellular and genomic actions. Trends Endocrinol Metab 7:22–27