Melatonin and diet-induced metabolic syndrome in rats: impact on the hypophysial-testicular axis

Abstract: Combinations of fructose- and fat-rich diets in experimental animals can model the human metabolic syndrome (MS). In rats, the increase in blood pressure (BP) after diet manipulation is sex related and highly dependent on testosterone secretion. However, the extent of the impact of diet on rodent hypophysial-testicular axis remains undefined. In the present study, rats drinking a 10% fructose solution or fed a high-fat (35%) diet for 10 weeks had higher plasma levels of luteinizing hormone (LH) and lower plasma levels of testosterone, without significant changes in circulating follicle-stimulating hormone or the weight of most reproductive organs. Diet manipulation brought about a significant increase in body weight, systolic BP, area under the curve (AUC) of glycemia after an intraperitoneal glucose tolerance test (IPGTT), and plasma low-density lipoprotein cholesterol, cholesterol, triglycerides, and uric acid levels. The concomitant administration of melatonin (25 μg/mL of drinking water) normalized the abnormally high LH levels but did not affect the inhibited testosterone secretion found in fructose- or high-fat-fed rats. Rather, melatonin per se inhibited testosterone secretion. Melatonin significantly blunted the body weight and systolic BP increase, the increase in the AUC of glycemia after an IPGTT, and the changes in circulating lipid profile and uric acid found in both MS models. The results are compatible with a primary inhibition of testicular function in diet-induced MS in rats and with the partial effectiveness of melatonin to counteract the metabolic but not the testicular sequelae of rodent MS.

Keywords: dyslipidemia; follicle-stimulating hormone (FSH); fructose; glucose tolerance; high-fat diet; hypertension; luteinizing hormone (LH); metabolic syndrome; melatonin; testosterone; uric acid.

Introduction

The cluster of cardiovascular disease risk factors including obesity, hypertension, hyperinsulinemia, glucose intolerance, and dyslipidemia is known as the metabolic syndrome (MS) [1–4]. MS is a major clinical challenge with a prevalence of 15%–30% depending on the world region considered, and its presence increases overall cardiovascular mortality by 1.5- to 2.5-fold. Indeed, MS and the aging of the population are the two greatest public health concerns of the 21st century [5, 6]. Each of these trends has important effects on body composition, functional disability, and mortality. An important change in body composition with aging is the increase in fat mass and visceral fat [7], which increases susceptibility to MS and cardiovascular disease. Adipocytes actively secrete leptin and proinflammatory cytokines and activate a vicious cycle that leads to additional weight gain largely in the form of fat [8, 9].

One of the factors that contribute to the increase in MS incidence is poor eating habits, which are mainly characterized by a large increase in fructose and fat consumption [1–4]. In the case of fructose, an impending increase in intake, primarily in the form of sucrose (which contains 50% fructose) and corn syrup (55% fructose content), has been documented in the last 25 years [4]. High fructose intake has been commonly modeled in rats [10] and lately in non-human primates [11]. In both types of animals, fructose feeding induces hypertension, hyperinsulinemia, insulin resistance, and hypertriglyceridemia [12]. In the case of high-fat diets, they have been used for decades to model obesity, dyslipidemia, and insulin resistance in rodents [13].
The increase in body weight after a high-fructose or high-fat diet is accompanied by increased systolic blood pressure (BP) and endothelial dysfunction [14–16]. This effect is sex related and needs the presence of testosterone to become apparent [17]. Meanwhile, obesity is associated with an altered hormonal milieu that can affect the reproductive system, as shown by the association of increased body mass index (BMI) in men with low testosterone and sex hormone-binding globulin levels [18].

In rat models of diet-induced MS, diet manipulation brought about a significant decrease in total plasma testosterone levels [19–21] and a loss of correlation between circulating testosterone and luteinizing hormone (LH) levels [19]. Other studies, however, failed to observe such an effect of diet on testosterone secretion [22, 23]. The aim of the present experiments was to examine the impact of diet on the activity of the hypothalamic-pituitary-gonadal axis and the somatic and metabolic components of MS that can affect the reproductive system, as shown by the association of increased BMI in men with low testosterone and sex hormone-binding globulin levels [18].

**Materials and methods**

**Animals and experimental design**

Male Wistar rats (60 days old) were kept under standard conditions of controlled light (12:12 h light/dark schedule; lights on at 08:00 h) and temperature (22°C±2°C). In the first experiment, the effect of fructose or high-fat administration on plasma LH, testosterone, and follicle-stimulating hormone (FSH) levels and on reproductive organ weight was measured. In addition, a number of somatic and metabolic components were used clinically to monitor MS, i.e., body weight increase, systolic BP, intraperitoneal glucose tolerance test (IPGTT), and several circulating analytes including triglycerides, total cholesterol, high- and low-density lipoprotein cholesterol (HDL-c and LDL-c, respectively), creatinine, urea, and uric acid were also measured. For the fructose experiment groups, eight rats had ad libitum access for 10 weeks to one of the following drinking solutions: (i) 10% fructose solution (in which fructose accounted for 48%–57% of total caloric intake [29]); (ii) tap water. Normal rat chow was given ad libitum; it contained 3% fat, 16% protein, and 60% carbohydrate (mainly as starch with <0.4% fructose), providing a total caloric content of 2.9 kcal/g. For the high-fat-diet experiment groups, eight rats had ad libitum access for 10 weeks to tap water and one of the following diets: (i) high-fat chow; (ii) normal rat chow. The high (35%)-fat chow contained 35% carbohydrates and 20% proteins, providing a total caloric content of 5.4 kcal/g, whereas the normal chow provided a total caloric content of 2.9 kcal/g.

In the second experiment, the efficacy of melatonin to counteract the hypophysial-testicular sequelae of MS seen in rats fed a 10% fructose solution was examined. Animals were randomly divided into four groups (n=8/group) and had free access to chow and one of the following drinking solutions for 10 weeks: (i) 10% fructose; (ii) 10% fructose plus 25 μg/mL of melatonin; (iii) 25 μg/mL of melatonin; (iv) tap water. Because ethanol was used as a melatonin vehicle, drinking solutions in groups i and iv were added 0.015% ethanol. The activity of the hypophysial-testicular axis and the somatic and metabolic components of MS were measured as in experiment 1.

The aim of experiment 3 was to examine the efficacy of melatonin to counteract the changes in the hypophysial-testicular axis seen in rats fed a high-fat diet. Animals were randomly divided into four groups (n=8/group) and had free access to high-fat or control chow and one of the following drinking solutions for 10 weeks: (i) tap water; (ii) 25 μg/mL of melatonin. In group i, 0.015% ethanol was added to the drinking solutions. The activity of the hypophysial-testicular axis and the somatic and metabolic components of MS were measured as in experiment 1.

Chow and water consumption were measured weekly. The caloric intake for fructose-fed rats was calculated as the sum of calories ingested as food based on 2.9 kcal/g of chow consumed, with each ingested gram of fructose corresponding to 4.0 kcal. The caloric intake for high-fat-fed rats was calculated as the sum of calories ingested as food based on 5.4 kcal/g of chow consumed.

The daily melatonin dosage used varied from 1.9 to 3.2 mg/kg, with the higher values corresponding to rats drinking fructose. The human equivalence dose, calculated using the body surface area normalization method [30], was 0.31–0.52 mg/kg (i.e., 21–35 mg/day for a 70-kg adult).

**BP measurement**

Systolic BP was measured using a manometer-tachometer (Rat Tail NIBP System; ADInstruments, Sydney, NSW, Australia) with an inflatable tail-cuff connected to a MLT844 Physiological Pressure Transducer (ADInstruments, Sydney, NSW, Australia) and PowerLab data acquisition unit (ADInstruments, Sydney, NSW, Australia). Rats were placed in a plastic holder mounted on a thermostatically controlled warm plate that was maintained at 35°C during measurements. The average value from three BP readings (which differed by no more than 2 mm Hg) was determined for each animal after they became acclimated to the environment. All BP measurements were done between 09:00 and 12:00 h.

**Biochemical assays**

The IPGTT was performed at 09:00 h after a 2-h fast. Rats were anesthetized, and after the collection of an unchallenged sample (time 0), a glucose solution of 2 g/kg body weight was intraperitoneally administered. During the test, blood was collected by lateral tail bleeding at 30, 60, and 120 min after glucose administration to
measure glucose concentration. Glycemia was measured using an Accu-Check Compact kit (Roche Diagnostics, Indianapolis, IN, USA). The area under the curve (AUC) for glycemia was calculated using the trapezoidal method test [31].

The rats were euthanized by decapitation under conditions of minimal stress. All experiments were conducted in accordance with the guidelines of the International Council for Laboratory Animal Science. Trunk blood was collected, and plasma samples were obtained by centrifugation of blood at 1500 g for 15 min. EDTA (6 g/100 mL) was used as anticoagulant. Samples were stored at −70°C until further analysis.

Plasma LH and FSH levels were measured by a homologous-specific double antibody radioimmunoassay (RIA), using materials kindly supplied by the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and by Dr. A. Parlow (Harbor UCLA Medical Center, Torrance, CA, USA), as described elsewhere [19]. The intra- and inter-assay coefficients of variation were 6% and 8%, respectively. The sensitivity of the RIA was 975 pg/mL using the NIDDK rat appropriate standard. Plasma testosterone concentration was measured by a specific RIA obtained from DIAsource Immunoassays (Nivelles, Belgium). The intra- and inter-assay coefficients of variation were 6% and 8%, respectively. The sensitivity of the RIA was 0.1 ng of testosterone/mL.

The plasma lipid profile was determined by measuring the triglyceride, total cholesterol, HDL-c, and LDL-c content using commercially available reagent kits per manufacturer instructions (BioSystems, Buenos Aires, Argentina). Creatinine, urea, and uric acid were measured by standard enzymatic procedures (BioSystems, Buenos Aires, Argentina).

Statistical analysis

After verifying the normality of distribution of data, the statistical analysis of the results was performed by a one- or two-way factorial analysis of variance (ANOVA) followed by Bonferroni multiple comparison test or Student’s t-test, as stated, with statistical significance at p<0.05.

Table 1  Changes in body weight, systolic BP, IPGTT, plasma levels of reproductive hormones, and testicular, epididymal, and seminal vesicle weight in rats receiving either a 10% fructose drinking solution or tap water (fructose study) or a high-fat or normal diet (high-fat study) for 10 weeks.

|                      | Control (tap water) | 10% Fructose | t   | p-Value | Control (4% fat) | High fat (35% fat) | t   | p-Value |
|----------------------|---------------------|--------------|-----|---------|------------------|-------------------|-----|---------|
| Initial body weight, g | 263±15             | 287±12       | 1.25| NS      | 253±19           | 267±19            | 0.51| NS      |
| Final body weight, g  | 311±28             | 406±34       | 2.16| 0.049   | 376±32           | 492±40            | 2.26| 0.040   |
| Systolic BP, mm Hg    | 107±8              | 132±8        | 2.21| 0.044   | 110±8            | 132±9             | 2.20| 0.045   |
| IPGTT, mg/dl 120 min  | 7623±631           | 10,042±898   | 2.20| 0.045   | 8567±828         | 15,892±1603       | 4.06| 0.001   |
| Plasma LH, pg/mL      | 34±6               | 98±21        | 2.93| 0.011   | 47±9             | 93±15             | 2.63| 0.020   |
| Plasma FSH, pg/mL     | 199±74             | 290±46       | 1.04| NS      | 245±56           | 335±45            | 1.25| NS      |
| Plasma testosterone, ng/mL | 1.56±0.22 | 0.9±0.1     | 2.73| 0.016   | 1.69±0.42         | 0.93±0.13         | 2.26| 0.040   |
| Testicular weight, g  | 1.55±0.04          | 1.63±0.03    | 1.60| NS      | 2.11±0.33         | 2.53±0.56         | 0.65| NS      |
| Epididymal weight, g  | 0.54±0.08          | 0.50±0.02    | 0.24| NS      | 0.43±0.11         | 0.65±0.23         | 0.83| NS      |
| Seminal vesicle weight, g | 0.79±0.03 | 1.06±0.08   | 3.16| 0.007   | 0.56±0.07         | 0.65±0.12         | 0.65| NS      |

For experimental details, see Methods. Data are shown as means±SEM (n=8/group), with Student’s t-test and corresponding p-values. NS, not significant.

Results

In the fructose studies, chow consumption was similar for controls (16±2 g/rat/day) and fructose-overloaded rats (15±2 g/rat/day). Water consumption was 25±4 mL/rat/day (controls) and 39±5 mL/rat/day (10% fructose) (p<0.02, Student’s t-test). Therefore, the individual total caloric intake was 44±3 kcal/day (controls) and 61±4 kcal/day (fructose) (p<0.03, Student’s t-test).

In high-fat-diet studies, individual daily chow and water consumption were similar for controls (17±3 g and 27±3 mL) and high-fat-fed rats (16±3 g and 29±2 mL). Individual total caloric intake (kcal/day) was 44±3 (controls) and 71±4 (high-fat diet) (p<0.01, Student’s t-test). Melatonin administration did not significantly affect chow or water consumption.

The results of experiment 1 are summarized in Tables 1 and 2. The administration of a 10% fructose drinking solution or a high-fat chow to rats brought about significant increases in body weight, systolic BP, and AUC of glycemia after an IPGTT (Table 1). Rats fed with fructose or a high-fat diet had significantly higher plasma levels of LH and significantly lower plasma levels of testosterone, without significant changes in plasma FSH. Only the weight of the seminal vesicles of fructose-fed rats was significantly higher than that of controls (Table 1). The experimental manipulation of diet in rats brought about significant changes in circulating analytes in fructose- and high-fat-fed rats, with increases of LDL-c, cholesterol, triglycerides, and uric acid (Table 2).

The effectiveness of melatonin to counteract the hypophysial-testicular sequelae in the fructose-induced MS is
summarized in Figure 1 and Table 3. Melatonin administration normalized the abnormally high LH levels but did not affect the inhibited testosterone secretion found in fructose-fed rats. Rather, a significant inhibition of testosterone levels was found in rats administered with melatonin alone (Figure 1). Neither testicular nor epididymal weight was affected by the treatment, but the weight of the seminal vesicle was significantly augmented in rats drinking the

| Lipid profile       | Control (tap water) | 10% Fructose | t  | p-Value | Control (4% fat) | High fat (35% fat) | t  | p-Value |
|---------------------|---------------------|--------------|----|---------|------------------|--------------------|----|---------|
| LDL-c, mg/dL plasma | 46±6                | 89±10        | 3.68| 0.002   | 35±5             | 63±6               | 3.58| 0.003   |
| HDL-c, mg/dL plasma | 64±5                | 55±6         | 1.15| NS      | 69±9             | 94±7               | 2.19| 0.046   |
| Cholesterol, mg/dL plasma | 65±7           | 119±24       | 2.16| 0.049   | 169±29           | 307±32             | 3.19| 0.006   |
| Triglycerides, mg/dL plasma | 182±32        | 345±63       | 2.31| 0.037   | 1.3±0.1          | 1.2±0.2            | 0.35| NS      |
| Creatinine, mg/dL plasma | 1.3±0.2       | 1.2±0.2      | 0.35| NS      | 1.3±0.1          | 1.2±0.2            | 0.45| NS      |
| Urea, mg/dL plasma  | 64±5                | 56±6         | 1.02| NS      | 49±6             | 41±7               | 0.86| NS      |
| Uric acid, mg/dL plasma | 1.2±0.3       | 2.2±0.4      | 2.36| 0.034   | 1.3±0.1          | 1.8±0.2            | 2.23| 0.041   |

For experimental details, see Methods. Data are shown as means±SEM (n=8/group), with Student’s t-test and corresponding p-values. NS, not significant.
**Discussion**

Overweight and insulin resistance, which are paramount components of MS, affect the endocrine system, alter the hypothalamo-hypophysial-gonadal hormonal axis, and depress testosterone secretion [32]. In a meta-analysis of clinical studies on the effect of BMI on testicular function [18], 18 of 20 studies measuring testosterone reported negative relationships between BMI and circulating testosterone. Circulating total testosterone, in particular, free testosterone, negatively correlated with BMI [18]. In rodents, a decrease in plasma testosterone has been reported in experiments involving diet-induced models of MS [19–21], but not in all cases [22, 23].

Our foregoing results indicate that rats drinking a 10% fructose solution or fed a high-fat diet for 10 weeks had higher plasma levels of LH and lower plasma levels of testosterone, without significant changes in plasma FSH, thus indicating a primary effect of diet on testosterone production at the testicular level. This endocrine profile came along with the expected alterations of the experimental MS induced, i.e., significant increases in body weight and systolic BP, impaired glucose tolerance, and increased circulating levels of LDL-c, cholesterol, triglycerides, and uric acid.

---

**Table 3** Effect of melatonin on body weight, systolic BP, IPGTT, and plasma levels of several analytes in rats drinking a 10% fructose solution for 10 weeks.

|                       | Control       | Fructose     | Fructose+Melatonin | Melatonin   | F   | p-Value |
|-----------------------|---------------|--------------|--------------------|-------------|-----|---------|
| Initial body weight, g| 245±28        | 267±24       | 258±27             | 262±30      | 0.12| NS      |
| Final body weight, g  | 381±21        | 520±35*      | 380±42             | 330±16      | 7.25| <0.001  |
| Systolic BP, mm Hg    | 108±4         | 128±3*       | 112±4              | 100±4       | 9.73| <0.001  |
| IPGTT, AUC, mg/dL 120 min | 8624±631    | 13,442±1003*| 7563±823           | 6678±567    | 15.1| <0.001  |

For experimental details, see Methods. Data are shown as means±SEM (n=8/group), with F-values in ANOVA and corresponding p-values. NS, not significant. Letters indicate significant differences between the experimental groups after a one-way ANOVA followed by a post hoc Bonferroni test: *p<0.02 vs. the remaining groups; †p<0.01 vs. control and melatonin-alone groups; p<0.04 vs. fructose+melatonin group: ′p<0.01 vs. the remaining groups; ′′p<0.02 vs. control and melatonin-alone groups. For further statistical analysis, see text.
Endothelial dysfunction and increased BP following insulin resistance play an important role in the development of secondary cardiovascular complications in MS. The presence of testosterone, possibly via the regulation of the synthesis of vasoconstrictor eicosanoids, is essential for the development of endothelial dysfunction and

Table 4 Effect of melatonin on body weight, systolic BP, IPGTT, and plasma levels of several analytes in rats fed a high-fat diet for 10 weeks.

|                      | Control      | High-fat diet | High-fat diet+Melatonin | Melatonin | F   | p-Value |
|----------------------|--------------|---------------|-------------------------|------------|-----|---------|
| Initial body weight, g | 254±32       | 289±32        | 267±15                  | 260±23     | 0.35| NS      |
| Final body weight, g  | 351±30       | 479±36*       | 370±32                  | 371±30     | 3.29| 0.035   |
| Systolic BP, mm Hg    | 102±8        | 129±6*        | 103±4                   | 100±8*     | 4.18| 0.015   |
| IPGTT, AUC, mg/dL 120 min | 9666±731      | 17,729±1435*  | 7867±866                | 8629±465   | 23.4| <0.001  |
| Lipid profile         |              |               |                         |            |     |         |
| LDL-c, mg/dL plasma   | 35±5         | 69±7*         | 39±4                    | 25±4       | 13.5| <0.001  |
| HDL-c, mg/dL plasma   | 64±8         | 59±8          | 42±7                    | 68±5*      | 3.61| 0.025   |
| Cholesterol, mg/dL plasma | 65±6         | 88±4*         | 67±5                    | 71±4       | 4.71| 0.009   |
| Triglycerides, mg/dL plasma | 175±23       | 302±26*       | 215±19                  | 164±13     | 9.04| <0.001  |
| Creatinine , mg/dL plasma | 1.1±0.1       | 1.2±0.2       | 1.1±0.1                 | 1.3±0.1    | 0.52| NS      |
| Urea, mg/dL plasma    | 44±5         | 40±6          | 38±3                    | 42±4       | 0.31| NS      |
| Uric acid, mg/dL plasma | 1.4±0.1       | 1.9±0.2*      | 1.2±0.1                 | 1.1±0.1    | 7.23| <0.001  |

For experimental details, see Methods. Data are shown as means±SEM (n=8/group), with F-values in ANOVA and corresponding p-values. NS, not significant. Letters indicate significant differences between the experimental groups after a one-way ANOVA followed by a post hoc Bonferroni test: *p<0.05 vs. control; **p<0.03 vs. high-fat diet; ***p<0.01 vs. the remaining groups; ****p<0.03 vs. high-fat diet+melatonin group; *****p<0.03 vs. control and high-fat diet+melatonin groups; ******p<0.02 vs. the remaining groups; *******p<0.01 vs. high-fat diet+melatonin and melatonin-alone groups. For further statistical analysis, see text.
increased BP [14–16, 22]. Moreover, testosterone treatment of fructose-fed female rats increased BP [17]. There is also information that a similar testosterone effect is seen in high-fat-fed rats [33]. Because gonadectomy was effective in preventing endothelial dysfunction and increased BP in fructose-fed male rats [14–16, 22], the low amounts of testosterone secreted after diet manipulation in the two models of MS examined herein are presumably sufficient to provoke the vascular changes typically reported in these animals. Further studies using gonadectomized male rats could be useful to define this point, particularly in the case of high-fat-fed rats in which such information is lacking.

In a previous study, one of us (D.P.C.) reported a significant decrease in total plasma testosterone levels and a loss of correlation between testosterone with circulating LH levels in high-fat-diet-fed rats [19], findings that were coincident with other published observations [20, 21]. Because saturated fatty acid treatment decreases LH-stimulated adenylate cyclase activity [34] and testosterone levels [35] in rat testes and induces apoptosis of Leydig cells [36], the previous and present results are compatible with a deleterious effect of high-fat diet on testicular function.

Among the several substances that can curtail MS, melatonin has received increasing attention because of its very low or absent toxicity, making it potentially appropriate for human use. A number of studies indicate that melatonin has the ability to reduce type 2 diabetes and liver steatosis (for references, see [37]). In addition, melatonin treatment induces regeneration/proliferation of β-cells in the pancreas, which leads to a decrement in blood glucose in streptozotocin-induced type 1 diabetic rats [38]. Loss of circulating melatonin via pinealectomy results in marked hyperinsulinemia and accumulation of triglycerides in the liver [39]. Long-term administration of melatonin improves lipid metabolism in type 2 diabetic rats through the amelioration of insulin resistance [40].

In high-fat/high-sucrose-fed rats, intraperitoneal injection of 4 mg/kg melatonin every morning for 8 weeks, starting after 20 weeks of feeding, led to weight gain inhibition together with improved insulin sensitivity [41]. Rats fed a diet containing 60% fructose exhibited an inhibition of melatonin secretion and became hypertensive unless a daily supplementation of melatonin (30 mg/kg in drinking water) was given [42]. In another study, the melatonin activity on MS induced by a diet containing 60% fructose was examined [43]. This diet increased serum insulin, triglyceride, total cholesterol, free fatty acids, uric acid, leptin, and lipid peroxide concentrations as well as hepatic triglyceride and cholesterol concentrations. Insulin resistance, relative intra-abdominal fat, and an augmented liver weight were also apparent. The daily intraperitoneal administration of melatonin (1 or 10 mg/kg body weight), starting at 4 weeks of feeding, attenuated all these changes, underlining the efficacy of melatonin in improving a fully developed MS [43]. The present results indicate that the administration of melatonin significantly blunted the body weight and systolic BP increase and normalized glucose tolerance and the circulating lipid and uric acid profile found in two diet-induced models of rodent MS.

At high doses, melatonin can protect against several comorbidities of experimental MS, including diabetes and concomitant oxyradical-mediated damage, inflammation, microvascular disease, and atherothrombotic risk [25, 28, 44]. Indeed, hyperglycemia leads to vascular disease through many intertwined intracellular events linked to oxidative stress. Vascular production of both excessive reactive oxygen species and excessive reactive nitrogen species contribute to endothelial dysfunction by directly damaging macromolecules and activating several cellular stress-sensitive pathways, e.g., nuclear factor κB, which play a key role in the development of type 1 and type 2 diabetes complications as well as in insulin resistance and impaired insulin secretion occurring in type 2 diabetes [45]. Because melatonin provides both in vivo and in vitro protection at the level of cell membranes, mitochondria, and nucleus due to its free-radical-scavenging and antioxidant properties [44], the involvement of these mechanisms in melatonin’s prevention of vascular sequelae and insulin resistance in the two diet-induced models of rodent MS examined herein seems warranted.

Collectively, the present and previous results are compatible with the view that melatonin can effectively reduce adiposity in several rodent models of hyperadiposity [46–55]. Remarkably, this effect of melatonin is exerted in the absence of significant differences in food intake. Further exploration is needed to learn the extent to which the weight loss-promoting effect of melatonin is attributable to an increase in energy expenditure by brown adipose tissue (for references, see [56]).

At the initial phase of MS induced in rats by fructose overload, hypertriglyceridemia and fatty liver without modification or even increase in plasma glucose tolerance to a glucose load have been reported [57, 58]. Recently, we observed in rats at this initial stage of MS similar body weights and a greater tolerance to glucose than controls, together with a significant increase in systolic BP and changes in the circulating lipid profile [59]. The administration of melatonin, although unable to modify the increased tolerance to glucose, was effective normalizing...
the altered BP and lipid profile found at this early stage of MS. Again, the data support the possible therapeutic role of melatonin in MS, both at initial and established phases.

In the present study, melatonin given simultaneously with a 10% fructose solution or a high-fat diet normalized the abnormally high LH levels but did not affect the inhibited testosterone secretion; rather, it had an inhibitory effect on testosterone when given alone. The results support a lack of effectiveness of melatonin in countering the testicular sequelae of rodent MS. Indeed, information has accumulated for decades on the direct inhibitory effect of melatonin on testosterone production in mammalian and nonmammalian testicular tissue [60–63]. Such an effect of melatonin on circulating testosterone levels appears to be absent in humans [64–68].

In the laboratory rat, a number of physiological parameters display seasonal changes even under constant conditions of temperature, lighting, and food availability (for references, see [69]). Because the administration of melatonin in drinking water is equivalent to exposing the animals to short daily photoperiod in terms of a prolonged duration of melatonin signal [70, 71], a possible interpretation of the changes in testosterone and LH secretion after melatonin is that they reflect the gonadal inhibition found in the natural environment for wild Rattus norvegicus during winter. In a recent study, it was reported that male rats receiving melatonin in drinking water (3 µg/mL) exhibited a profound inhibitory effect on pituitary PRL gene expression and circulating PRL levels as well as a significant decrease in plasma LH and testosterone concentration [69].

Hyperuricemia is considered a true cardiovascular and renal risk factor in MS. Hyperuricemia predicts the development of hypertension, diabetes, stroke, and cardiovascular events [72]. Mild hyperuricemia in normal rats induces systemic hypertension, renal vasoconstriction, glomerular hypertension, and hypertrophy as well as tubulointerstitial injury independent of intrarenal crystal formation [73, 74]. Lowering uric acid in fructose-fed rats ameliorates much of MS, including a reduction in BP, serum triglycerides, hyperinsulinemia, and weight gain [72]. In the present study, melatonin, besides countering the changes in plasma LDL-c, triglyceride, and cholesterol, decreases plasma uric acid levels. This last effect could be of a potential therapeutic value in human MS [72].

There is considerable evidence that circadian misalignment is associated with increased epidemiological risk for obesity, diabetes, and cardiovascular disease [28, 75]. Lifestyle changes, such as nocturnality and overly rich diets, are followed by a disruption of the sleep/wake cycle and other circadian rhythms. Due to its effects on circadian rhythmicity, melatonin can provide the basis for a therapeutic strategy in MS. Melatonin has been therapeutically used for the treatment of age-related insomnia as well as of other primary and secondary insomnia. A consensus of the British Association for Psychopharmacology on evidence-based treatment of insomnia, parasomnia, and circadian rhythm sleep disorders concluded that melatonin is the first-choice treatment when a hypnotic is indicated in patients older than 55 years [76].

There are clinical results indicating that type 2 diabetic patients have low levels of circulating melatonin [77] with a concomitant and expected melatonin membrane receptor mRNA expression upregulation [37]. Recently, genomic studies uncovered a link between specific single-nucleotide polymorphisms (SNP) of the melatonin MT2 receptor (MTNR1B) locus and a prognostic risk of type 2 diabetes [78–80]. The SNP correlated with higher fasting glucose levels and a pathologically altered insulin secretion responses. These findings strongly bind melatonin to blood glucose homeostasis.

As well as in animal models, clinical studies have shown that melatonin provides benefits on lipid profiles. Melatonin treatment (1 mg/kg for 30 days) elevated HDL-c levels in perimenopausal and postmenopausal women [81]. In an open-label study, which included 33 healthy volunteers and 30 MS patients treated with melatonin, patients with MS had significantly higher values than controls in total cholesterol, LDL-c, triglycerides, systolic and diastolic BP, glycemia, fibrinogen, and erythrocyte thiobarbituric acid-reactive substrate levels [26]. They also had lower levels of HDL-c and reduced activities of catalase, glutathione peroxidase, and superoxide dismutase in erythrocytes. Melatonin (5 mg/day) significantly decreased hypertension and improved the serum lipid profile and antioxidative status [26]. In another open-label study comprising 100 elderly hypertensive patients, the simultaneous application of melatonin together with lisinopril or amlodipine had a normalizing effect on BP and metabolic parameters [82]. Collectively, the results suggest that melatonin therapy can be of benefit for patients with MS, particularly those with arterial hypertension.

It must be noted that melatonin has a high safety profile and is usually remarkably well tolerated. In some studies, melatonin has been administered to patients in very large doses. For example, 300-mg/day doses of melatonin for up to 3 years decreased oxidative stress in patients with amyotrophic lateral sclerosis [83]. Therefore, further studies using melatonin doses in the 50- to 100-mg/day range are needed to clarify its potential...
therapeutic implications on MS in humans. If one expects melatonin to be an effective cytoprotector, especially in older people, it is likely that the low doses of melatonin used so far are not very beneficial.

**Acknowledgments:** This research was supported by grants from the Agencia Nacional de Promoción Científica y Tecnológica, Argentina (PICT 2012 0984) and the Universidad de Buenos Aires (M048).

**Conflict of interest statement:** The author declares no conflict of interest.

Received February 19, 2013; accepted June 3, 2013; previously published online July 2, 2013

**References**

1. Brown T, Avenell A, Edmunds LD, Moore H, Whittaker V, Avery L, Summerbell C. Systematic review of long-term lifestyle interventions to prevent weight gain and morbidity in adults. Obes Rev 2009;10:627–38.
2. Garaulet M, Madrid JA. Chronobiology, genetics and metabolic syndrome. Curr Opin Lipidol 2009;20:127–34.
3. Maury E, Ramsey KM, Bass J. Circadian rhythms and metabolic syndrome: from experimental genetics to human disease. Circ Res 2010;106:447–62.
4. Tappy L, Le KA, Tran C, Paquot N. Fructose and metabolic diseases: new findings, new questions. Nutrition 2010;26:1046–9.
5. Mokdad AH, Bowman BA, Ford ES, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes in the United States. J Am Med Assoc 2001;286:1195–200.
6. Morley JE. Diabetes and aging: epidemiologic overview. Clin Geriatr Med 2008;24:395–405.
7. Riechman SE, Schoen RE, Weissfeld JL, Thaete FL, Kriska AM. Association of physical activity and visceral adipose tissue in older women and men. Obes Res 2002;10:1065–73.
8. Huffman DM, Barzilai N. Role of visceral adipose tissue in aging. Biochim Biophys Acta 2009;1790:1117–23.
9. Sepe A, Tchkonia T, Thomou T, Zamboni M, Kirkland JL. Aging and regional differences in fat cell progenitors – a mini-review. Gerontology 2011;57:66–75.
10. Tran LT, Yuen VG, McNeill JH. The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension. Mol Cell Biochem 2009;322:145–59.
11. Bremer AA, Stanhope KL, Graham JL, Cummings BP, Wang W, Saville BR, Havel PJ. Fructose-fed rhesus monkeys: a nonhuman primate model of insulin resistance, metabolic syndrome, and type 2 diabetes. Clin Transl Sci 2011;4:243–52.
12. Shimamoto K, Ura N. Mechanisms of insulin resistance in hypertensive rats. Clin Exp Hypertens 2006;28:543–52.
13. Speakman J, Hambly C, Mitchell S, Krol E. The contribution of animal models to the study of obesity. Lab Anim 2008;42:413–32.
14. Vasudevan H, Yuen VG, McNeill JH. Testosterone-dependent increase in blood pressure is mediated by elevated Cyp4A expression in fructose-fed rats. Mol Cell Biochem 2012;359:409–18.
15. Vasudevan H, Nagareddy PR, McNeill JH. Gonadectomy prevents endothelial dysfunction in fructose-fed male rats, a factor contributing to the development of hypertension. Am J Physiol Heart Circ Physiol 2006;291:H3058–64.
16. Vasudevan H, Xiang H, McNeill JH. Differential regulation of insulin resistance and hypertension by sex hormones in fructose-fed male rats. Am J Physiol Heart Circ Physiol 2005;289:H1335–42.
17. Kamary Y, Peleg E, Leibowitz A, Grossman E. Blunted blood pressure response and elevated plasma adiponectin levels in female Sprague Dawley rats. Am J Hypertens 2012;25:612–9.
18. MacDonald AA, Herbstorl P, Showell M, Farquhar CM. The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis. Hum Reprod Update 2010;16:293–311.
19. Cano P, Jiménez-Ortega V, Larrad A, Tosco CF, Cardinali DP, Esquifino AI. Effect of a high-fat diet on 24-h pattern of circulating levels of prolactin, luteinizing hormone, testosterone, corticosterone, thyroid-stimulating hormone, and pineal melatonin content, in rats. Endocrine 2008;33:118–25.
20. Ribeiro DL, Pinto MN, Maeda SY, Taboga SR, Goes RM. High fat-induced obesity associated with insulin-resistance increases FGF-2 content and causes stromal hyperplasia in rat ventral prostate. Cell Tissue Res 2012;349:577–88.
21. Viggers-Villaseñor RM, Rojas-Castaneda JC, Chavez-Saldana M, Gutierrez-Perez O, Garcia-Cruz ME, Cuevas-Alpuche O, Reyes-Romero MM, Zambrano E. Alterations in the spermatic function generated by obesity in rats. Acta Histochem 2011;113:214–20.
22. Song D, Arikawa E, Galipeau D, Battell M, McNeill JH. Androgens are necessary for the development of fructose-induced hypertension. Hypertension 2004;43:667–72.
23. Fernandez CD, Bellentani FF, Ferrandes GS, Perobelli JE, Favareto AP, Nascimento AF, Cigocna AC, Kempinas WD. Diet-induced obesity in rats leads to a decrease in sperm motility. Reprod Biol Endocrinol 2011;9:32.
24. Balliuzek MF, Grinenko TN, Kvetnai TV. The role of melatonin and the metabolic syndrome: association with chronodisruption, sleep disturbances, and therapeutical implications. Neuroendocrinology 2011;93:133–42.
25. Cardinali DP, Cano P, Jimenez-Ortega V, Esquifino AI. Melatonin and the metabolic syndrome: physiopathologic and therapeutic implications. Neuroendocrinology 2011;93:133–42.
26. Kozior M, Poliowczak AR, Duchnowicz P, Koter-Michalak M, Sikora J, Broncel M. Melatonin treatment improves blood pressure, lipid profile, and parameters of oxidative stress in patients with metabolic syndrome. J Pineal Res 2011;50:261–6.
27. Reiter RJ, Tan DX, Korkmaz A, Ma S. Obesity and metabolic syndrome: association with chronodisruption, sleep
110 Scacchi Bernasconi et al.: Melatonin and the metabolic syndrome

Deprivation, and melatonin suppression. Ann Med 2012;44:564–77.

28. Nduhirabandi F, Du Toit EF, Lochner A. Melatonin and the metabolic syndrome: a tool for effective therapy in obesity-associated abnormalities? Acta Physiol (Oxf) 2012;205:209–23.

29. Dai S, McNeill JH. Fructose-induced hypertension in rats is concentration- and duration-dependent. J Pharmacol Toxicol Methods 1995;33:101–7.

30. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J 2008;22:659–61.

31. Matthews JN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. Br Med J 1990;300:230–5.

32. Michalakis K, Mintziori G, Kaparra A, Tarlatzis BC, Goulis DG. The complex interaction between obesity, metabolic syndrome and reproductive axis: a narrative review. Metabolism 2013;62:457–78.

33. Uemura K, Mori N. Influence of age and sex on high-fat diet-induced increase in blood pressure. Nagoya J Med Sci 2006;68:109–14.

34. Gromadzka-Ostrowska J, Przepiorka M, Romanowicz K. Modulation of receptor-mediated gonadotropin action in rat testes by dietary fat. Am J Physiol 1988;254:E708–12.

35. Sebokova E, Garg ML, Clandinin MT. Modulation of receptor-mediated gonadotropin action in rat testes by dietary fat. Am J Physiol 1988;254:E708–12.

36. Shen Q, Su Z, Yin W. NEU-P11, a novel melatonin agonist, Delta-5 desaturase activity. J Pineal Res 2002;32:26–33.

37. Dukic A, Sharabi S, Shabat Z, Shami S, Grossman E. The role of melatonin in the pathogenesis of hypertension in rats with metabolic syndrome. Am J Hypertens 2008;21:348–51.

38. Kitagawa A, Ohta Y, Ohashi K. Melatonin improves metabolic syndrome induced by high fructose intake in rats. J Pineal Res 2011;52:403–13.

39. Hardeland R, Cardinalli DP, Srinivasan V, Spence DW, Brown GM, Pandi-Perumal SR. Melatonin – a pleiotropic, orchestrating regulator molecule. Prog Neurobiol 2011;93:350–84.

40. Nishida S, Segawa T, Murai I, Nakagawa S. Effect of pinealectomy on plasma levels of insulin and leptin and on hepatic lipids in type 2 diabetic rats. J Pineal Res 2003;35:251–6.

41. Nishida S, Sato R, Murai I, Nakagawa S. Effect of pinealectomy on plasma levels of insulin and leptin and on hepatic lipids in type 2 diabetic rats. J Pineal Res 2003;35:251–6.

42. Gromadzka-Ostrowska J, Przepiorka M, Romanowicz K. Influence of dietary fatty acids composition, level of dietary fat and feeding period on some parameters of androgen metabolism in male rats. Reprod Biol 2002;2:277–93.

43. Lu ZH, Mu YM, Wang BA, Li XL, Lu JM, Li JY, Pan CY, Yanase T, Nawata H. Saturated free fatty acids, palmitic acid and stearic acid, induce apoptosis by stimulation of ceramide generation in rat testicular Leydig cell. Biochem Biophys Res Commun 2003;303:1002–7.

44. Peschke E, Stumpf I, Clandinin MT. Modulation of receptor-mediated gonadotropin action in rat testes by dietary fat. Am J Physiol 1988;254:E708–12.

45. Vincent HK, Innes KE, Vincent KR. Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. Diabetes Obes Metab 2007;9:813–39.

46. Agil A, Navarro-Alarcon M, Ruiz R, Abuhmadah S, El Mir MY, vazquez GF. Beneficial effects of melatonin on obesity and lipid profile in young Zucker diabetic fatty rats. J Pineal Res 2011;50:207–12.

47. Agil A, Rosado I, Ruiz R, Figueroa A, Zen N, Fernandez-Vazquez G. Melatonin improves glucose homeostasis in young Zucker diabetic fatty rats. J Pineal Res 2012;52:203–10.

48. Prunet-Marcussus B, Desbazeille M, Bros A, Louche K, Delagrange P, Renard P, Castella L, Penicaud L. Melatonin reduces body weight gain in Sprague Dawley rats with diet-induced obesity. Endocrinology 2003;144:5347–52.

49. Puchalski SS, Green JN, Rasmussen DD. Melatonin effect on rat body weight regulation in response to high-fat diet at middle age. Endocrine 2003;21:163–7.

50. Sartori C, Dessen P, Mathieu C, Monney A, Bloch J, Nicod P, Scherrer U, Duplain H. Melatonin improves glucose homeostasis and endothelial vascular function in high-fat diet-fed insulin-resistant mice. Endocrinology 2009;150:5311–7.

51. Rios-Lugo MJ, Cano P, Jimenez-Ortega V, Fernandez-Mateos MP, Scacchi PA, Cardinalli DP, Esquifino AL. Melatonin effect on plasma adiponectin, leptin, insulin, glucose, triglycerides and cholesterol in normal and high fat-fed rats. J Pineal Res 2010;49:342–8.

52. Ladizesky MG, Boggio V, Albornoz LE, Castrillón P, Mautalen CA, Cardinalli DP. Melatonin increases oestradial-induced bone formation in ovariectomized rats. J Pineal Res 2003;34:143–51.

53. Sanchez-Mateos S, Alonso-Gonzalez C, Gonzalez A, Martinez-Campa CM, Mediavilla MO, Cos S, Sanchez-Barcelo El, Melatonin and estradiol effects on food intake, body weight, and leptin in ovariectomized rats. Maturitas 2007;58:91–101.

54. Hussein MR, Ahmed OG, Hassan AF, Ahmed MA. Intake of melatonin is associated with amelioration of physiological changes, both metabolic and morphological pathologies associated with obesity: an animal model. Int J Exp Pathol 2007;88:19–29.

55. Raskind MA, Burke BL, Crites NJ, Tapp AM, Rasmussen DD. Olanzapine-induced weight gain and increased visceral adiposity is blocked by melatonin replacement therapy in rats. Neuropsychopharmacology 2007;32:284–8.

56. Tan DX, Manchester LC, Fuentes-Broto L, paredes SD, Reiter RJ. Significance and application of melatonin in the regulation of brown adipose tissue metabolism: relation to human obesity. Obes Rev 2011;12:167–88.

57. Roglans N, Sanguino E, Peris C, Alegret M, Vazquez M, Adzet T, Diaz C, Hernandez G, Laguna JC, Sanchez RM. Atorvastatin treatment induced peroxisome proliferator-activated receptor alpha expression and decreased plasma nonesterified fatty acids and liver triglyceride in fructose-fed rats. J Pharmacol Exp Ther 2002;302:232–9.

58. Park J, Lemieux S, Lewis GF, Kuksis A, Steiner G. Chronic exogenous insulin and chronic carbohydrate supplementation increase de novo VLDL triglyceride fatty acid production in rats. J Lipid Res 1997;38:2529–36.

59. Cardinalli DP, Scacchi Bernasconi PA, Reynoso R, Reyes Toso CF, Scacchi P. Melatonin may curtail the metabolic syndrome: studies on initial and fully established fructose-induced metabolic syndrome in rats. Int J Mol Sci 2013;14:2502–14.
60. Kinson GA, Peat F. The influences of illumination, melatonin and pinealectomy on testicular function in the rat. Life Sci 1971;10:259–69.
61. Cardinali DP, Rosner JM. Effects of melatonin, serotonin and N-acetylserinotonin on the production of steroids by duck testicular homogenates. Steroids 1971;18:25–37.
62. Frungieri MB, Mayerhofer A, Zitta K, Pignataro OP, Calandra RS, Gonzalez-Calvar SI. Direct effect of melatonin on Syrian hamster testes: melatonin subtype 1a receptors, inhibition of androgen production, and interaction with the local corticotropin-releasing hormone system. Endocrinology 2005;146:1541–52.
63. Rossi SP, Matzkin ME, Terradas C, Ponzio R, Puigdomenech E, Levalle O, Calandra RS, Frungieri MB. New insights into melatonin/CRH signaling in hamster Leydig cells. Gen Comp Endocrinol 2012;178:153–63.
64. Wade AG, Ford I, Crawford G, McConnachie A, Nir T, Laudon M, Zisapel N. Nightly treatment of primary insomnia with prolonged release melatonin for 6 months: a randomized placebo controlled trial on age and endogenous melatonin as predictors of efficacy and safety. BMC Med 2010;8:51.
65. Wright J, Aldhous M, Frayn C, English J, Arnedt J. The effects of exogenous melatonin on endocrine function in man. Clin Endocrinol (Oxf) 1986;24:375–82.
66. Waldhauser F, Lieberman HR, Lynch HJ, Waldhauser M, Herkner K, Frisch H, Vierhapper H, Waldhausl W, Schemper M, Wurtman RJ. A pharmacological dose of melatonin increases PRL levels in males without altering those of GH, LH, FSH, TSH, testosterone or cortisol. Neuroendocrinology 1987;46:125–30.
67. Luboshitzky R, Levi M, Shen-Orr Z, Blumenfeld Z, Herer P, Waldhauser F, Lieberman HR, Lynch HJ, Waldhauser M, Wright J, Aldhous M, Franey C, English J, Arendt J. The effects of melatonin, serotonin and Acosta J. Mild hyperuricemia induces vasoconstriction and maintains glomerular hypertension in normal and remnant kidney rats. Kidney Int 2005;67:237–47.
68. Scheer FA, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. Proc Natl Acad Sci USA 2009;106:4453–8.
69. Wilson SJ, Nutt DJ, Alford C, Argyropoulos SV, Baldwin DS, Bateson AN, Britton TC, Crowe C, Dijk DJ, Espie CA, Gringras P, Hajak G, Idzikowski C, Krystal AD, Nash JR, Selsick H, Sharpley AL, Wade AG. British Association for Psychopharmacology consensus statement on evidence-based treatment of insomnia, parasomnias and circadian rhythm disorders. J Psychopharmacol 2010;24:577–601.
70. Tutuncu NB, Batur MK, Yildirir A, Tutuncu T, Deger A, Koray Z, Erbas B, Kabakci G, Aksoyek S, Erbas T. Melatonin levels decrease in type 2 diabetic patients with cardiac autonomic neuropathy. J Pineal Res 2005;39:43–9.
71. Dietrich K, Birkmeier S, Schleinitz D, Breitfeld J, Enigk B, Muller I, Bottcher Y, Lindner T, Stumvoll M, Tonjes A, Kovacs P. Association and evolutionary studies of the melatonin receptor 1B gene (MTNR1B) in the self-contained population of Sorbs from Germany. Diabet Med 2011;28:1373–80.
72. Prokopenko I, Langenberg C, Florez JC, Soranzo N, Soranzo N, Thorleifsson G, Loos RJ, Manning AK, Jackson AU, Aulchenko Y, Potter SC, Eerola M, Sanna S, Hottenga JJ, Wheeler E, Kaakinen M, Lyssenko V, Chen WM, Ahmadi K, Beckmann JS, Bergman RN, Bochud M, Bernstein LL, Buchwald U, Cao L, Cervino A, Collin FS, Crispiloni L, de Geus EJ, Depuokha A, Deloukas P, Doney AS, Elliott P, Freimer N, Gara V, Herder C, Hofman A, Hughes TE, Hunt S, Illig T, Inouye M, Isomaa B, Johnson T, Kong A, Krestyaninova M, Kuusisto J, Laakso M, Lim N, Lindblad U, Lindgren CM, McCann OT, Mohlke KL, Morris AD, Naiztas S, Orm C, Palmer C, Pouta A, Randall J, Rathmann W, Saramies J, Scheet P, Scott LJ, Scuteri A, Sharp S, Siibrands E, Smit JH, Song K, Steinthorsdottir V, Stringham HM, Tuom I, Tuomilehto J, Utterlinden AG, Voight BF, Waterworth D, Wichmann HE, Willemsen G, Wilmot P, Yuan X, Zhao J, Zeggini E, Schlessinger D, Sandhu M, Boomser DI, Madera S, Pardon DP, Penninx BW, Alshuler D, Vollenweider P, Jarvelin MR, Lakatta E, Wareham NJ, Meigs JB, Abecasis R. Genetic studies of the melatonin receptor 1B gene (MTNR1B) in the self-contained population of Sorbs from Germany. Diabet Med 2011;28:1373–80.
73. Prokopenko I, Langenberg C, Florez JC, Soranzo N, Thorleifsson G, Loos RJ, Manning AK, Jackson AU, Aulchenko Y, Potter SC, Eerola M, Sanna S, Hottenga JJ, Wheeler E, Kaakinen M, Lyssenko V, Chen WM, Ahmadi K, Beckmann JS, Bergman RN, Bochud M, Bernstein LL, Buchwald U, Cao L, Cervino A, Collin FS, Crispiloni L, de Geus EJ, Depuokha A, Deloukas P, Doney AS, Elliott P, Freimer N, Gara V, Herder C, Hofman A, Hughes TE, Hunt S, Illig T, Inouye M, Isomaa B, Johnson T, Kong A, Krestyaninova M, Kuusisto J, Laakso M, Lim N, Lindblad U, Lindgren CM, McCann OT, Mohlke KL, Morris AD, Naiztas S, Orm C, Palmer C, Pouta A, Randall J, Rathmann W, Saramies J, Scheet P, Scott LJ, Scuteri A, Sharp S, Siibrands E, Smit JH, Song K, Steinthorsdottir V, Stringham HM, Tuom I, Tuomilehto J, Utterlinden AG, Voight BF, Waterworth D, Wichmann HE, Willemsen G, Wilmot P, Yuan X, Zhao J, Zeggini E, Schlessinger D, Sandhu M, Boomser DI, Madera S, Pardon DP, Penninx BW, Alshuler D, Vollenweider P, Jarvelin MR, Lakatta E, Wareham NJ, Meigs JB, Abecasis R. Genetic studies of the melatonin receptor 1B gene (MTNR1B) in the self-contained population of Sorbs from Germany. Diabet Med 2011;28:1373–80.
81. Tamura H, Nakamura Y, Narimatsu A, Yamagata Y, Takasaki A, Reiter RJ, Sugino N. Melatonin treatment in peri- and postmenopausal women elevates serum high-density lipoprotein cholesterol levels without influencing total cholesterol levels. J Pineal Res 2008;45:101–5.

82. Shatilo VB, Bondarenko EV, Antoniuk-Shcheglova IA. [Metabolic disorders in elderly patients with hypertension and their correction with melatonin]. Adv Gerontol 2012;25:84–9.

83. Weishaupt JH, Bartels C, Polking E, Dietrich J, Rohde G, Poeggeler B, Mertens N, Sperling S, Bohn M, Huther G, Schneider A, Bach A, Siren AL, Hardeland R, Bahr M, Nave KA, Ehrenreich H. Reduced oxidative damage in ALS by high-dose enteral melatonin treatment. J Pineal Res 2006;41:313–23.