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Melatonin and Diet-induced Metabolic Syndrome in Rats. Impact on the Hypophysial-Testicular Axis.

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Melatonin and the Metabolic Syndrome

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 diet impact 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, with absence of significant changes in circulating follicle-stimulating hormone (FSH) or in 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 i.p. 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 the diet-induced MS in rats and with the partial effectiveness of melatonin to counteract the metabolic but not the testicular sequels of rodent MS.

Keywords: metabolic syndrome; melatonin; fructose; high fat diet; LH; FSH; testosterone; hypertension; dyslipidemia; glucose tolerance; uric acid.

Abbreviations: ANOVA: analysis of variance; AUC: area under the curve; BMI: body mass index; BP: blood pressure; FSH: follicle-stimulating hormone; HDL-c: high-density lipoprotein-cholesterol; IPGTT: i.p. glucose tolerance test; LDL-c: low-density lipoprotein-cholesterol; LH: luteinizing hormone; MS: metabolic syndrome; RIA: radioimmunoassay.

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]. The MS is a major clinical challenge with a prevalence of 15-30% depending on the world region considered, its presence increasing overall cardiovascular mortality by 1.5- to 2.5-fold. Indeed, the 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 of fat mass and visceral fat [7], which increases susceptibility to the MS and cardiovascular disease. Adipocytes actively secrete leptin and proinflammatory cytokines and activate a vicious cycle leading to additional weight gain largely in the form of fat [8,9].

One of the factors that contributes 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 (that 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 employed for decades to model obesity, dyslipidemia and insulin resistance in rodents [13].

The increase in body weight after a high fructose or 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]. On the other hand, obesity is associated with an altered hormonal milieu that can affect the reproductive system, as shown by the association of an 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 hypophysial-testicular axis after giving to rats 10% fructose as a drinking solution or a high (35%) fat diet for 10 weeks. The possible corrective effect of melatonin on MS sequels was examined because among several substances with the capacity to curtail the MS, melatonin has received increasing attention due to its very low or absent toxicity that turns it potentially appropriate for human use [2,24-28].

Material and methods

Animals and experimental design

Male Wistar rats (60 days of age) were kept under standard conditions of controlled light (12:12 h light/dark schedule; lights on at 08:00 h) and temperature (22 ± 2 °C).

In a first experiment the effect of fructose or high fat administration on plasma LH, testosterone and follicle-stimulating hormone (FSH) levels and reproductive organ weight was measured. In addition a number of somatic and metabolic components employed clinically to monitor the MS, i.e. body weight increase, systolic BP, i.p. glucose tolerance test (IPGTT) and several circulating analytes including triglycerides, total cholesterol, high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c), creatinine, urea and uric acid were also measured. For the fructose experiment groups of 8 rats had ad libitum access for 10 weeks to one of the following drinking solutions: (i) a 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 less than 0.4% fructose) providing a total caloric content of 2.9 Kcal/g. For the high fat diet experiment groups of 8 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 while the normal chow provided a total caloric content of 2.9 Kcal/g.

In a second experiment the efficacy of melatonin to counteract the hypophysial-testicular sequels of the 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. Since ethanol was used as a melatonin's vehicle, drinking solutions in groups (i) and (iv) were added 0.015 % ethanol. The activity of the hypophysial-testicular axis and somatic and metabolic components of the 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. Drinking solutions in group (i) was added 0.015 % ethanol. The activity of the hypophysial-testicular axis and somatic and metabolic components of the MS were measured as in experiment 1.

Chow and water consumption were measured weekly. Caloric intake for fructose-fed rats was calculated as sum of calories ingested as food on the basis of 2.9 kcal per gram of chow consumed and on that each ingested gram of fructose corresponds to 4.0 kcal. Caloric intake for high fat-fed rats was calculated as sum of calories ingested as food on the basis of 5.4 kcal per gram of chow consumed.

The daily melatonin dosage used varied from 1.9 to 3.2 mg/kg, the higher values corresponding to rats drinking fructose. The human equivalence dose, calculated by 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 by using a manometer-tachometer (Rat Tail NIBP System; ADInstruments Pty Ltd., Sydney, Australia) employing an inflatable tail-cuff connected to a MLT844 Physiological Pressure Transducer (ADInstruments) and PowerLab data acquisition unit (ADInstruments). Rats were placed in a plastic holder mounted on a thermostatically controlled warm plate that was maintained at 35°C during measurements. An average value from three BP readings (that differed by no more than 2 mm Hg) was determined for each animal after they became acclimated to the environment. All BP measurements were made 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 following the collection of an unchallenged sample (time 0), a glucose solution of 2 g/kg body weight was administered i.p. 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 the Accu-Check Compact kit (Roche Diagnostics, Indianapolis, Indiana, USA). The area under the curve (AUC) for glycemia was calculated by 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 (ICLAS). Trunk blood was collected and plasma samples were obtained by centrifugation of blood at 1,500 x g for 15 min. EDTA (6 g/100 mL) was used as an 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 NIDDK's National Hormone and Pituitary Program and by Dr. A. Parlow (Harbor UCLA Medical Center, 1000 West Carson Street, Torrance, CA, USA), as described elsewhere [19]. The intra- and inter-assay coefficients of variation were 6 and 8%, respectively. Sensitivity of the RIA was 97.5 pg/ml using the NIDDK rat

appropriate standard. Plasma testosterone concentration was measured by a specific RIA obtained from DAsource ImmunoAssays S.A. Rue de l'Industrie, 8, B-1400, Nivelles, Belgium. The intra- and inter-assay coefficients of variation were 6 and 8%, respectively. Sensitivity of the RIA was 0.1 ng of testosterone /ml.

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

Statistical analysis

After verifying normality of distribution of data, the statistical analysis of the results was performed by a one-way or a two-way factorial analysis of variance (ANOVA) followed by Bonferroni's multiple comparison or Student's t tests, as stated. P values lower than 0.05 were taken as evidence of statistical significance.

Results

In fructose studies, chow consumption (g/rat/day) was similar for controls (16 ± 2) and fructose-overloaded rats (15 ± 2). Water consumption (ml/rat/day) was 25 ± 4 (controls) and 39 ± 5 (10 % fructose) ($P < 0.02$, Student's t test). Therefore, the individual total caloric intake (kcal/day) was 44 ± 3 (controls) and 61 ± 4 (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 affect significantly 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 of 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 fructose or a

high fat diet had significantly higher plasma levels of LH and significantly lower plasma levels of testosterone, with absence of significant changes in plasma FSH. Only the weight of 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 sequels in the fructose-induced MS is summarized in Fig. 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 (Fig. 1). Neither testicular nor epididymal weight were affected by treatment, while seminal vesicle weight augmented significantly in rats drinking the 10% fructose solution (Fig. 1). Melatonin significantly blunted the body weight and systolic BP increase found in rats drinking a 10% fructose solution (Table 3). The increase in the AUC of glycemia after an IPGTT found in fructose-fed rats was also prevented by melatonin, as well as the changes in lipid profile and uric acid levels (Table 3). When analyzed as a main factor in a factorial ANOVA, melatonin decreased uric acid levels significantly ($P < 0.01$).

The efficacy of melatonin to counteract the changes in the hypophysial-testicular axis and somatic and metabolic changes seen in rats fed a high fat diet is summarized in Fig. 2 and Table 4. Diet manipulation produced a significant inhibition of testosterone secretion and a significant stimulation of LH release with absence of effects on FSH release (Fig. 2). Melatonin administration normalized the abnormally high LH levels without affecting the inhibited testosterone secretion found in high fat fed rats. A significant inhibition of testosterone levels was found in rats administered with melatonin alone (Fig. 2). Neither testicular, epididymal nor seminal vesicle weight were affected by any treatment.

Rats fed with a high fat diet showed significantly higher body weights and systolic BP after 10 weeks. Melatonin effectively counteracted these changes (Table 4). Additionally, in high fat fed rats circulating LDL-c, cholesterol and triglyceride concentration augmented significantly, melatonin being effective to counteract the changes in lipid profile observed. Melatonin did not affect the increase in plasma HDL-c found in high fat fed rats nor modified the circulating lipid profile when given alone (Table 4). Melatonin prevented the increase in uric acid found in high fat fed rats and when analyzed as a main factor in a factorial ANOVA, melatonin decreased uric acid levels significantly ($P < 0.01$) (Table 4).

Discussion

Overweight and insulin resistance, which are paramount components of the MS, affect the endocrine system, alter the hypothalamo-hypophyseal-gonadal hormonal axis and depress testosterone secretion [32]. In a metaanalysis of clinical studies on the effect of BMI on testicular function [18] 18 out of 20 studies measuring testosterone reported negative relationships between BMI and circulating testosterone. Circulating total testosterone, and in particular free testosterone, were negatively correlated with BMI [18]. In rodents a decrease in plasma testosterone has been reported in experiments involving diet-induced models of the 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, with absence of 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.

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 regulation of the synthesis of vasoconstrictor eicosanoids, is essential for the development of endothelial dysfunction and 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]. Since gonadectomy was effective to prevent 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 hereby examined 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 reported in high-fat diet fed rats a significant decrease in total plasma testosterone levels and a loss of correlation between testosterone with circulating LH levels [19], findings which were coincident with other published observations [20,21]. Since saturated fatty acid treatment decreases LH-stimulated adenylate cyclase activity [34] and testosterone levels [35] in rat testis 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 several substances with the capacity to curtail the MS, melatonin has received increasing attention because of its very low or absent toxicity that turns 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 ref. see [37]). In addition, melatonin treatment induces regeneration/proliferation of β -cells in 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 amelioration of insulin resistance [40].

In high-fat/high sucrose-fed rats giving an i.p. injection of 4 mg/kg melatonin every morning for 8 weeks, starting after 20 weeks of feeding, weight gain inhibition occurred together with improved insulin sensitivity [41]. Rats fed a diet containing 60% fructose exhibited an inhibition of melatonin secretion and turned hypertensive unless a daily supplementation of melatonin (30 mg/kg in drinking water) was given [42]. In another study the melatonin activity on the 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 i.p. 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 to improve 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 circulating lipid and uric acid profile found in two diet-induced models of rodent MS. Collectively, the present and previous results are compatible with the view that melatonin can effectively reduce adiposity in several rodent models of hyperadiposity [44-52]. Remarkably this effect of melatonin is exerted in the absence of significant differences in food intake. To what extent the weight-loss-promoting effect of melatonin is attributable to an increase in energy expenditure by brown adipose tissue deserves further exploration (see for ref. [53]).

At the initial phase of the MS induced in rats by fructose overload, hypertriglyceridemia and fatty liver without modifying or even increasing plasma glucose tolerance to a glucose load have been reported [54,55]. Recently we observed in rats at this initial stage of the 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 [56]. The administration of melatonin, although unable to modify the increased tolerance to glucose, was effective to normalize the altered BP and lipid profile found at this early stage of the MS. Again the data support the possible therapeutical role of melatonin in the MS, both at an initial and at the 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 found; rather it had an inhibitory effect on testosterone when given alone. The results support a lack of effectiveness of melatonin to counteract the testicular sequels of rodent MS. Indeed information has accumulated for decades on a direct inhibitory effect on testosterone production in mammalian and non-mammalian testicular tissue [57-60]. Such an effect of melatonin on circulating testosterone levels appears to be absent in humans [61-65].

In the laboratory rat a number of physiological parameters display seasonal changes even under constant conditions of temperature, lighting and food availability (see for ref. [66]). Since the administration of melatonin in drinking water is an equivalent to expose the animals to short daily photoperiod in terms of a prolonged duration of the melatonin signal [67,68], a possible interpretation on 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 the 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 [66].

Hyperuricemia is considered a true cardiovascular and renal risk factor in MS. Hyperuricemia predicts the development of hypertension, diabetes, stroke and cardiovascular events [69]. Mild hyperuricemia in normal rats induces systemic hypertension, renal

vasoconstriction, glomerular hypertension and hypertrophy, as well as tubulointerstitial injury independent of intrarenal crystal formation [70,71]. Lowering uric acid in fructose-fed rats ameliorates much of the MS, including a reduction in BP, serum triglycerides, hyperinsulinemia, and weight gain [69]. In the present study melatonin besides counteracting the changes in plasma LDL-c, triglyceride and cholesterol, decreased plasma uric acid levels. This last effect could be of a potential therapeutic value in human MS [69].

There is considerable evidence that circadian misalignment is associated with increased risk for obesity, diabetes and cardiovascular disease [28,72]. Life style changes, such as nocturnality and overly rich diets, are followed by 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 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 over 55 years [73].

There are clinical results indicating that type 2 diabetic patients have low levels of circulating melatonin [74] with a concomitantly 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 [75-77]. 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 peri- and postmenopausal women [78]. 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) decreased significantly hypertension and improved the serum lipid profile and the antioxidative status [26]. In another open label study comprising 100 elderly hypertensive patients the simultaneous application of melatonin together with lisinopril or amlodipine had the normalizing effect on BP and metabolic parameters [79]. Collectively, the results suggest that melatonin therapy can be of benefit for patients with MS, particularly with arterial hypertension.

Hyperglycemia leads to vascular disease through many intertwined intracellular events linked to oxidative stress. Vascular production of both excessive reactive oxygen species (ROS) and excessive reactive nitrogen species (RNS) contribute to endothelial dysfunction by directly damaging macromolecules and by activating several cellular stress-sensitive pathways, e.g. nuclear factor kappa-beta, which play a key role in the development of type 1 and type 2 diabetes complications as well as in the insulin resistance and impaired insulin secretion occurring type 2 diabetes [80]. At high doses melatonin may protect against several comorbidities of the MS, including diabetes and concomitant oxyradical-mediated damage, inflammation, microvascular disease and atherothrombotic risk [25,28,81]. Since 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 [81], the involvement of these mechanisms in melatonin's prevention of vascular sequels and insulin resistance in the two diet-induced models of rodent MS herein examined seems warranted.

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 [82]. Therefore, further studies employing melatonin doses in

the 50-100 mg/day range are needed to clarify its potential therapeutical implications on the MS in humans. If one expects melatonin to be an effective cytoprotector, especially in aged people, it is likely that the low doses of melatonin employed so far are not very beneficial.

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Conflict of Interest

The authors declare no conflict of interest.

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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 .

	FRUCTOSE STUDY				HIGH FAT STUDY			
	Control (tap water)	10% Fructose	t	<i>P</i>	Control (4% fat)	High Fat (35% fat)	t	<i>P</i>
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 (mmHg)	107 ± 8	132 ± 8	2.21	0.044	110 ± 8	132 ± 9	2.20	0.045
IPGTT (AUC, mg/dL.120 min)	7623 ± 631	10042 ± 898	2.20	0.045	8567 ± 828	15892 ± 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. Shown are the means ± S.E.M (n= 8 per group). Student's t and the corresponding *P* values are quoted. NS: not significant.

Table 2. Changes in circulating lipid profile and plasma levels of creatinine, urea and uric acid 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 .

		FRUCTOSE STUDY				HIGH FAT STUDY			
		Control (tap water)	10% Fructose	t	P	Control (4% fat)	High Fat (35% fat)	t	P
Lipid Profile									
	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	59 ± 9	65 ± 9	0.47	NS
	Cholesterol (mg/dL plasma)	65 ± 7	119 ± 24	2.16	0.049	69 ± 9	94 ± 7	2.19	0.046
	Triglycerides (mg/dL plasma)	182 ± 32	345 ± 63	2.31	0.037	169 ± 29	307 ± 32	3.19	0.006
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. Shown are the means ± S.E.M (n= 8 per group). Student's t and the corresponding P values are quoted. NS: not significant.

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
Initial body weight (g)	245 ± 28	267 ± 24	258 ± 27	262 ± 30	0.12	NS
Final body weight (g)	381 ± 21	520 ± 35 ^a	380 ± 42	330 ± 16	7.25	<0.001
Systolic BP (mmHg)	108 ± 4	128 ± 3 ^b	112 ± 4	100 ± 4	9.73	<0.001
IPGTT (AUC, mg/dL.120 min)	8624 ± 631	13442 ± 1003 ^c	7563 ± 823	6678 ± 567	15.1	<0.001
Lipid Profile						
LDL-c (mg/dL plasma)	55 ± 6	99 ± 6 ^c	39 ± 5	32 ± 4	32.0	<0.001
HDL-c (mg/dL plasma)	58 ± 5	75 ± 6	82 ± 3 ^d	61 ± 5	5.47	0.004
Cholesterol (mg/dL plasma)	77 ± 4	138 ± 14 ^c	81 ± 10	65 ± 4	12.9	<0.001
Triglycerides (mg/dL plasma)	161 ± 12	372 ± 53 ^c	205 ± 32	180 ± 19	8.62	<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)	54 ± 5	60 ± 6	48 ± 5	42 ± 6	1.97	NS
Uric acid (mg/dL plasma)	1.1 ± 0.1	1.9 ± 0.2 ^a	1.3 ± 0.1	0.8 ± 0.1	12.3	<0.001

For experimental details see Methods. Shown are the means ± S.E.M (n= 8 per group). F values in ANOVA and the corresponding *P* are quoted. NS: not significant. Letters indicate the existence of significant differences between the experimental groups after a one-way ANOVA followed by a post-hoc Bonferroni's test, as follows: ^a *P* < 0.02 vs. the remaining groups; ^b *P* < 0.01 vs. control and melatonin alone groups, *P* < 0.04 vs. fructose + melatonin group; ^c *P* < 0.01 vs. the remaining groups; ^d *P* < 0.02 vs. control and melatonin alone groups. For further statistical analysis, see text.

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
Initial body weight (g)	254 ± 32	289 ± 32	267 ± 15	260 ± 23	0.35	NS
Final body weight (g)	351 ± 30	479 ± 36 ^a	370 ± 32	371 ± 30	3.29	0.035
Systolic BP (mmHg)	102 ± 8	129 ± 6 ^a	103 ± 4	100 ± 8 ^b	4.18	0.014
IPGTT (AUC, mg/dL.120 min)	9666 ± 731	17729 ± 1435 ^c	7867 ± 866	8629 ± 465	23.4	<0.001
Lipid Profile						
LDL-c (mg/dL plasma)	35 ± 5	69 ± 7 ^c	39 ± 4	25 ± 4	13.5	<0.001
HDL-c (mg/dL plasma)	64 ± 8	59 ± 8	42 ± 7	68 ± 5 ^d	3.61	0.025
Cholesterol (mg/dL plasma)	65 ± 6	88 ± 4 ^e	67 ± 5	71 ± 4	4.71	0.009
Triglycerides (mg/dL plasma)	175 ± 23	302 ± 26 ^f	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 ^g	1.2 ± 0.1	1.1 ± 0.1	7.23	<0.001

For experimental details see Methods. Shown are the means ± S.E.M (n= 8 per group). F values in ANOVA and the corresponding P are quoted. NS: not significant. Letters indicate the existence of significant differences between the experimental groups after a one-way ANOVA followed by a post-hoc Bonferroni's test, as follows: ^a *P*< 0.05 vs. control; ^b *P*< 0.03 vs. high fat diet, ^c *P*< 0.01 vs. the remaining groups; ^d *P*< 0.03 vs. high fat diet + melatonin group; ^e *P*< 0.03 vs. control and high fat diet + melatonin groups; ^f *P*< 0.02 vs the remaining groups; ^g *P*< 0.01 vs. high fat diet + melatonin and melatonin alone groups. For further statistical analysis, see text.

Figure Legends

Figure 1.

Effect of melatonin on plasma LH, FSH and testosterone levels and reproductive organ weight in rats drinking a 10% fructose solution. Hormone levels were determined by specific RIA as described in Methods. Shown are the means \pm S.E.M (n= 8 per group). Letters indicate the existence of significant differences between the experimental groups after a one-way ANOVA followed by a post-hoc Bonferroni's test, ^a $P < 0.01$ vs. the remaining groups; ^b $P < 0.05$ vs. control and melatonin alone groups.

Figure 2.

Effect of melatonin on plasma LH, FSH and testosterone levels and reproductive organ weight in rats fed a high fat diet. Hormone levels were determined by specific RIA as described in Methods. Shown are the means \pm S.E.M (n= 8 per group). Letters indicate the existence of significant differences between the experimental groups after a one-way ANOVA followed by a post-hoc Bonferroni's test, ^a $P < 0.04$ vs. control; ^b $P < 0.01$ vs. the remaining groups.

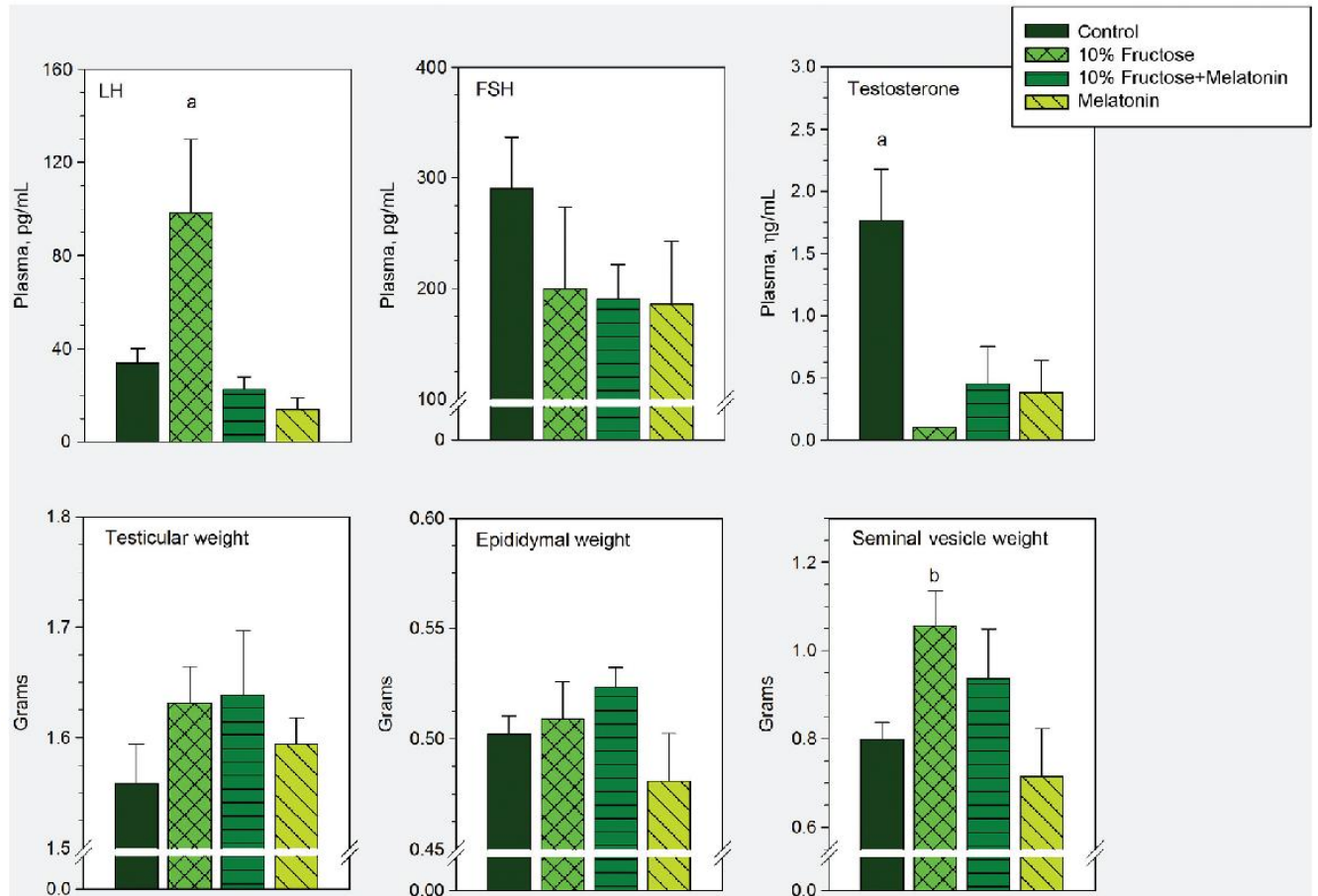


Figure 1 Effect of melatonin on plasma LH, FSH, and testosterone levels and on reproductive organ weight in rats drinking a 10% fructose solution. Hormone levels were determined by specific RIA as described in Methods. Data are shown as means \pm SEM (n=8/group). Letters indicate significant differences between the experimental groups after a one-way ANOVA followed by a post hoc Bonferroni test: ^ap<0.01 vs. the remaining groups; ^bp<0.05 vs. control and melatonin-alone groups.

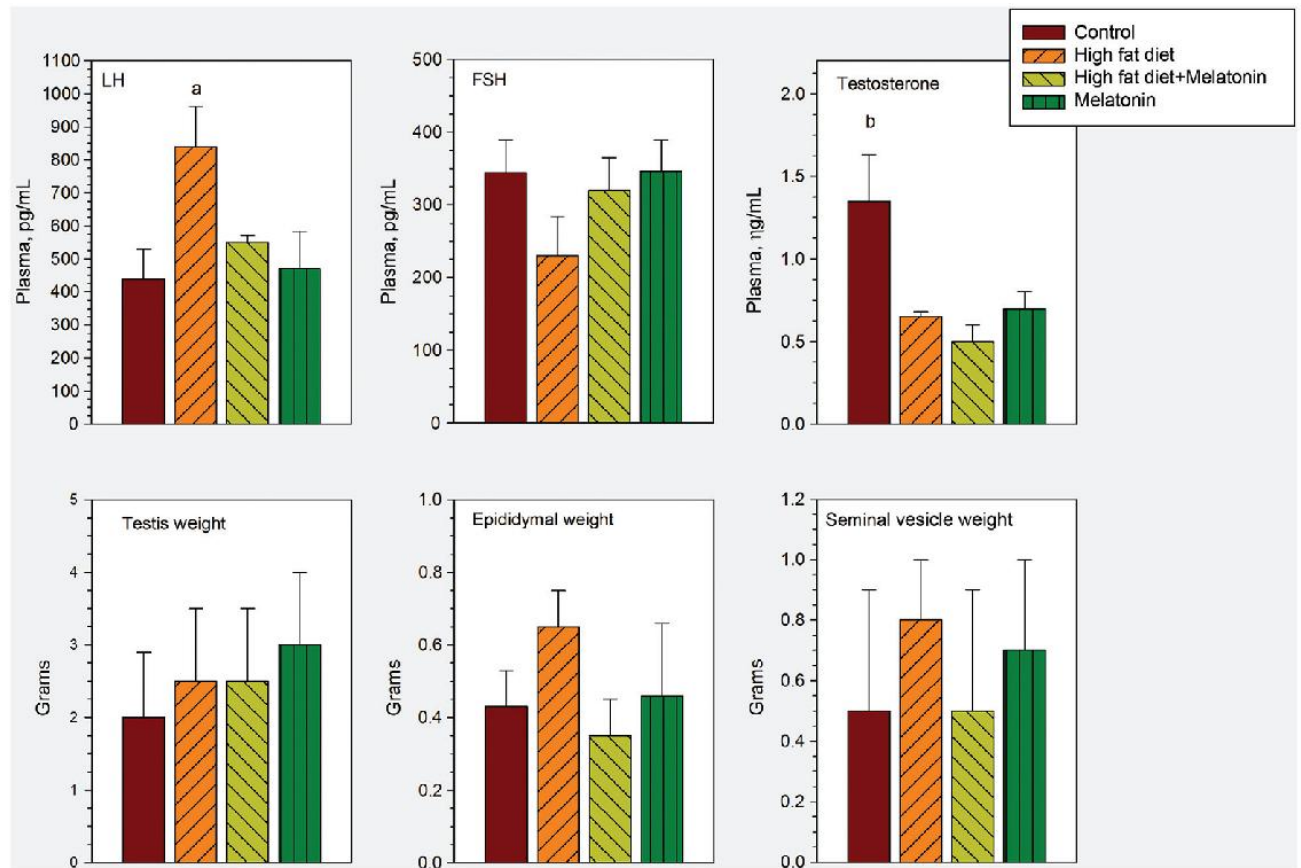


Figure 2 Effect of melatonin on plasma LH, FSH, and testosterone levels and on reproductive organ weight in rats fed a high-fat diet. Hormone levels were determined by specific RIA as described in Methods. Data are shown as means \pm SEM (n=8/group). Letters indicate significant differences between the experimental groups after a one-way ANOVA followed by a post hoc Bonferroni's test: ^ap<0.04 vs. control; ^bp<0.01 vs. the remaining groups.