J. Endocrinol. Invest. *25:* 716-723, 2002

Effects of continuous light and melatonin treatment on energy metabolism of the rat

A-M. Mustonen, P. Nieminen, and H. Hyvärinen
Department of Biology, University of Joensuu, Joensuu, Finland

ABSTRACT. Melatonin affects food intake, body mass and adiposity of several mammals, but the effects of melatonin on energy metabolism remain largely unknown. This study investigated subacute effects of persistent melatonin treatment and continuous light on carbohydrate and fat metabolism of rat liver and kidney. The male and female rats (no.=40) were maintained either in 12L:12D photoperiod or in constant light. Half the rats in both lighting conditions were treated with continuous-release melatonin implants. Liver lipid concentrations, liver and kidney glucose-6-phosphatase, glycogen phosphorylase and lipase esterase activities, glycogen contents as well as plasma T_4, T_3, insulin, glucose and melatonin concentrations were determined. There was clear sexual dimorphism in the responses to exogenous melatonin and constant light. Continuous light stimulated carbohydrate metabolism of rat liver. Exogenous melatonin enhanced utilization of liver carbohydrates but suppressed hepatic lipolysis. Changes in normal circulating melatonin concentrations led to enhanced utilization of kidney carbohydrates supporting a role for melatonin in renal function. Both exogenous melatonin and constant light seem to have a strong regulatory effect on rat energy metabolism. (J. Endocrinol. Invest. *25:* 716-723, 2002)

INTRODUCTION

Melatonin is mainly synthesized by the pineal gland during the scotophase (1). Melatonin secretion of the rat begins to increase slowly soon after light offset (2). Levels reach a peak at mid-darkness and during the second half of the night melatonin concentrations fall to reach the lower daytime levels near the time of light onset. The melatonin secretion rhythm is endogenous and it free-runs in continuous darkness (1). Constant light, on the other hand, obliterates the diurnal rhythm of N-acetyltransferase (3), the key enzyme for melatonin synthesis.

Melatonin is involved in various physiological functions such as seasonal reproduction (4), thermoregulation (5) and moulting (6). Melatonin binding sites have been localized in several peripheral tissues *e.g.* in the gastrointestinal tract (7), liver (8), kidneys (9) and pancreas (10). Although melatonin is known to affect body mass (BM), adiposity and energy intake of seasonal mammalian species (11-13), effects of melatonin on energy metabolism of mammals remain largely unknown.

Suggestions of a possible participation of the pineal gland in carbohydrate (14) and fat metabolism (15) are not new. Data concerning pineal influence on energy metabolism have, however, been controversial. We studied the effects of melatonin treatment and constant light on liver and kidney energy metabolism. As the liver functions as the center of the intermediate metabolism (16), the metabolic effects of melatonin most probably affect the liver. Melatonin may also have effects on energy metabolism of the kidneys, as it affects *e.g.* urine production (17), blood pressure (BP) (18) and glomerular filtration rates (19).

This study was a part of a research project on endocrine weight regulation in the seasonal adaptation of mammals. The aim of the study was to investigate subacute effects of persistent melatonin treatment and continuous light on mammalian energy metabolism. The laboratory rat (*Rattus norvegicus*) was selected as an experimental animal, because it is a marginally photoperiodic rodent serving as a reference species to more seasonal mammals. Both of the manipulations induced changes in liver and kidney energy metabolism supporting the involvement of melatonin in mammalian weight regulation.

Key-words: Glucose-6-phosphatase, glycogen, glycogen phosphorylase, lipase esterase, lipids, melatonin, rat, T_4, T_3.

Correspondence: Anne-Mari Mustonen, Department of Biology, University of Joensuu, P.O. Box 111, FIN-80101 Joensuu, Finland.

E-mail: ammusto@cc.joensuu.fi

Accepted April 5, 2002.

MATERIALS AND METHODS

Barrier-bred Wistar rats [Kuo:WH, 20 males (M) and 20 females (F)] were purchased from the National Laboratory Animal Center of the University of Kuopio (Kuopio, Finland) a month before the beginning of the experiment. This outbred stock of rats originated from Harlan Winkelman, Hannover, Germany (HsdBr:WH). The animals were conventionally maintained in a dark room with artificial illumination from 06:00 h to 18:00 h (12L:12D) at 20±1 C. They were housed singly in solid-bottomed plastic cages (Makrolon; 42 cm*22 cm*15 cm) with wood shavings for bedding and had free access to tap water and a pelleted commercial diet (R36; 18.5% raw protein, 4.0% raw fat, 1 260 kJ metabolizable energy 100 g^{-1}, Lactamin, Stockholm, Sweden). The intensity of lighting was about 150 lux inside the cages. All procedures were in accordance with institutional guidelines for animal care of the University of Joensuu as well as with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

The rats were 9 wk of age and weighed 163-203 g (F) and 240-287 g (M) at the beginning of the experiment. On day 1 half the animals were maintained in the photoperiod of 12L:12D, and the randomly assigned other half were moved to continuous light (24L) to suppress the melatonin synthesis of the animals (3). The rats of both lighting conditions were further divided into 2 randomly assigned groups. Half received sc melatonin implants, and the other half was sham-operated. We used PRIME-X®, melatonin implants containing 12 mg melatonin in a silastic matrix (Wildlife Pharmaceuticals Inc., Fort Collins, CO, USA). The capsules were administered with a sterile syringe into the interscapular sc tissue. The release of melatonin from the implant was known to be without any 24-h rhythm. It has, however, been documented in ewes that constant-release melatonin implants provide animals with a short day signal rather than a functional pinealectomy (20).

Each randomly assigned study group consisted of five individuals of the same sex. The study groups and their abbreviations were as follows: group 1; control males in 12L:12D (CM), group 2; control females in 12L:12D (CF), group 3; control males in 24L (CM+L), group 4; control females in 24L (CF+L), group 5; melatonin-treated males in 12L:12D (MM), group 6; melatonin-treated females in 12L:12D (MF), group 7; melatonin-treated males in 24L (MM+L) and group 8; melatonin-treated females in 24L (MF+L) (Table 1).

The BM and food intake of the rats were recorded throughout the study. A 4-wk study period was considered to be long enough for being able to observe possible changes in BM and food intake induced by the treatments. At the end of the experiment, the rats were sacrificed at 10:00-14:00 h by diethyl ether. Blood samples were obtained by cardiac puncture with aseptic needles into test tubes containing EDTA and centrifuged at 1000 *g* to obtain plasma. The livers and kidneys were dissected and all the samples were immediately frozen in liquid nitrogen and stored at -40 C.

The liver and kidney samples were weighed and homogenized. Homogenization was carried out in cold citrate buffer for the glucose-6-phosphatase (G-6-Pase) (pH 6.5) and glycogen phosphorylase measurements (pH 6.1) (21). The activity of G-6-Pase was measured using glucose-6-phosphate as substrate in the presence of EDTA after an incubation time of 30 min at 37.5 C. Glycogen phosphorylase activity was measured in the presence of glucose-1-phosphate, glycogen, sodium fluoride and AMP (21). The homogenization was carried out in cold 0.85% sodium chloride for the lipase esterase measurement. The lipase esterase activity was measured using 2-naphtyl-laurate without taurocholate as substrate (22). Also the glycogen concentrations of the livers and kidneys were measured spectrophotometrically (23).

For lipid chromatography, the total lipids of liver were extracted according to the method of Folch *et al*. (24). The recovered chloroform extracts were separated and quantified using a thin layer chromatography-flame ionization detection system (TLC-FID, IATROSCAN new Mark V, Iatron Laboratories Inc., Tokyo, Japan). The chromatorods were developed with chloroform/methanol/water (50/20/2.5 by vol.) ad 5 cm and *n*-hexane/diethyl ether/formic acid (65/5/0.15) ad 10 cm. The detector responses were calculated with authentic lipid standards (Sigma Ltd., St. Louis, MO, USA).

The plasma melatonin concentrations were determined with the Melatonin RIA kit (DLD Diagnostika GmbH, Hamburg, Germany). Plasma T_4 and T_3 levels were measured with the Spectria T_4 and T_3 [^{125}I] Coated Tube RIA kits of Orion Diagnostica (Espoo, Finland). The plasma glucose levels were determined spectrophotometrically with the UV method for the determination of D-glucose (Boehringer Mannheim, Mannheim, Germany). The plasma insulin levels were determined with the Coat-A-Count Insulin kit (DPC, Los Angeles, CA, USA).

The BMIs reflecting the amount of fat in the body were calcu-

Table 1 - *Lighting conditions and melatonin levels of the study groups.*

Group	No.	Sex	Abbreviation	Lighting condition	Melatonin levels
1	5	M	CM	12L:12D	Endogenous
2	5	F	CF	12L:12D	Endogenous
3	5	M	CM+L	24L	Endogenous
4	5	F	CF+L	24L	Endogenous
5	5	M	MM	12L:12D	Endogenous+Exogenous
6	5	F	MF	12L:12D	Endogenous+Exogenous
7	5	M	MM+L	24L	Endogenous+Exogenous
8	5	F	MF+L	24L	Endogenous+Exogenous

CF: control females; CM: control males; F: females; +L: 24-h light; M: males; MF: melatonin-treated females; MM: melatonin-treated males. 12L:12D: artificial illumination from 06:00 h to 18:00 h; 24L: continuous light.

lated by the formula: weight (kg) length^3 (m)$^{-1}$. The same formula is used for human infants and toddlers, as their body surface in relation to BM is greater than in adult humans [weight (kg) length^2 (m)$^{-1}$]. Multiple comparisons were performed with the SPSS-program using the one-way analysis of variance (ANOVA) followed by the *post hoc* Duncan's test. Paired comparisons were performed with the *t* test for unpaired data. Part of the data was logtransformed to obtain homogeneity of variances and the normality of distribution, which were tested with the Levene test and Kolmogorov-Smirnov test, respectively. For non-parametric data the Mann-Whitney U test was performed. Correlations were tested with the Spearman correlation coefficient (r_s). *P* value less than 0.05 was considered to be statistically significant.

RESULTS

The BM, food intake or BMIs of the rats were not affected by constant illumination or melatonin treatment (Table 2). The male rats had higher BMs and they consumed more food than the females (ANOVA, $p<0.05$), but there was no sexual dimorphism in the BMIs (Table 2). The liver glycogen concentrations were decreased by constant light (*t* test, $p<0.0004$) (Table 2). Melatonin treatment decreased the liver glycogen content of the female rats maintained in 12L:12D (ANOVA, $p<0.05$) (Table 2). Neither constant light nor exogenous melatonin had influence on the hepatic G-6-Pase activities of the rats (Table 2). The liver glycogen phosphorylase activities were suppressed by constant light (*t* test, $p<0.021$) and increased by melatonin treatment (*t* test, $p<0.003$) (Table 2). The liver G-6-Pase (*t* test, $p<0.0004$) and glycogen phosphorylase activities (*t* test, $p<0.003$) were higher in the male rats (Table 2).

Constant light increased the hepatic lipase esterase activities of the control males, but decreased the activity levels of the melatonin-treated males (ANOVA, $p<0.05$) (Table 2). The lipase esterase activities of the 12L:12D maintained males were increased but in 24L kept males suppressed by exogenous melatonin (ANOVA, $p<0.05$) (Table 2). Melatonin treatment decreased the lipase esterase activity levels of the female rats (*t* test, $p<0.0004$) (Table 2). When all the melatonin-treated rats were compared to the controls, melatonin significantly suppressed the lipase esterase activities (*t* test, $p<0.0004$).

The liver diacylglycerol (DG) levels of the control rats were increased (Mann-Whitney U test, $p<0.007$), but the levels of the melatonin-treated rats decreased by constant light (Mann-Whitney U test, $p<0.034$) (Table 3). In 12L:12D melatonin treatment increased the DG concentrations (Mann-Whitney U test, $p<0.023$), while the opposite was observed in 24L (Mann-Whitney U test, $p<0.003$) (Table 3). The cholesteryl ester (CE) concentrations of the control rats were increased by

Table 2 - *The body mass of the rats at the beginning[1] and at the end[2] of the study, cumulative food intake, the BMIs, the activities of liver and kidney glucose-6-phosphatase, glycogen phosphorylase and lipase esterase and the concentrations of liver and kidney glycogen (mean±SE).*

		CM 12L:12D	CF 12L:12D	CM 24L	CF 24L	MM 12L:12D	MF 12L:12D	MM 24L	MF 24L
Body mass[1] (g)		262±9.2^{c}	187±4.4	265±7.8^{c}	186±3.8	264±7.4^{c}	188±4.5	265±5.5^{c}	183±5.7
Body mass[2] (g)		333±13.7^{c}	221±6.6	337±9.8^{c}	208±4.4	338±11.4^{c}	217±5.6	333±10.8^{c}	209±5.1
Cum. food intake (g)		552±28.7^{c}	404±16.6	560±11.1^{c}	396±7.6	560±22.7^{c}	382±8.4	529±19.7^{c}	394±2.8
BMI kg ((m)^3)$^{-1}$		28.1±0.73	28.9±0.57	27.7±0.48	28.0±0.19	28.8±0.46	27.7±0.83	28.1±0.34	27.3±0.78
Glucose-6-phosphatase	L	39.1±2.08^{c}	32.2±1.43	39.9±1.20^{c}	31.4±0.69	38.4±2.78^{c}	36.1±1.98	40.8±1.17^{c}	32.0±3.79
(µg P mg^{-1} h^{-1})	K	50.7±1.00^{c}	37.1±2.30	38.6±2.33^{bc}	35.0±1.13^{b}	35.7±2.27^{ac}	29.8±1.02^{a}	31.3±3.39^{abc}	29.5±1.49^{ab}
Glycogen phosphorylase	L	43.2±1.49^{c}	33.6±2.29	35.4±2.52^{bc}	31.3±2.65^{b}	47.7±1.81^{ac}	39.9±2.37^{a}	40.6±0.67^{abc}	38.5±1.49^{ab}
(µg P mg^{-1} h^{-1})	K	6.1±0.32^{c}	7.5±0.40	4.5±0.12^{cl}	7.4±0.30	6.5±0.15^{c}	7.7±0.34	5.3±0.65^{cl}	7.1±0.54
Lipase esterase	L	47.6±1.30	61.8±3.00	65.0±2.60^{d}	57.7±3.78	55.7±2.62^{e}	43.0±0.73^{f}	42.0±2.15^{gh}	44.8±2.15^{f}
µg 2-naphtol	K	24.5±1.60	24.5±0.95	23.1±1.02	23.6±1.00	23.3±1.14	20.6±0.58^{k}	27.0±0.94^{ig}	28.2±1.19^{ij}
(mg^{-1} h^{-1})									
Glycogen	L	33.8±4.07	36.2±5.20	18.1±2.07^{b}	14.0±1.49^{b}	35.4±3.26	20.6±4.72^{k}	19.7±5.69^{b}	11.8±2.30^{b}
(µg mg^{-1})	K	0.6±0.05	0.6±0.05	0.5±0.07^{b}	0.5±0.01^{b}	0.4±0.07^{a}	0.4±0.04^{a}	0.3±0.02^{ab}	0.3±0.04^{ab}

CF: control females; CM: control males; F: females; +L: 24-h light; M: males; MF: melatonin-treated females; MM: melatonin-treated males. 12L:12D: artificial illumination from 06:00 h to 18:00 h; 24L: continuous light; L: liver; K: kidney; ^{a}melatonin-treated rats (no.=20) differ from controls (no.=20); ^{b}24L rats (no.=20) differ from 12L:12D rats (no.=20); ^{c}male rats (no.=20) differ from females (no.=20); ^{d}CM 24L (no.=5) differs from CM 12L:12D (no.=5); ^{e}MM 12L:12D (no.=5) differs from CM 12L:12D (no.=5), ^{f}melatonin-treated females (no.=10) differ from control females (no.=10); ^{g}MM 24L (no.=5) differs from CM 24L (no.=5); ^{h}MM 24L (no.=5) differs from MM 12L:12D (no.=5); ^{i}24L melatonin-treated rats (no.=10) differ from 12L:12D melatonin-treated rats (no.=10); ^{j}MF 24L (no.=5) differs from CF 24L (no.=5); ^{k}MF 12L:12D (no.=5) differs from CF 12L:12D (no.=5); ^{l}24L males (no.=10) differ from 12L:12D males (no.=10) (ANOVA, *t*-test, Mann-Whitney U test, $p<0.05$).

Table 3 - *The liver lipid concentrations (μg mg^{-1}) of the rats (mean±SE). Due to the lack of sexual dimorphism, the values of the males and the females of a particular treatment have been pooled (10 rats per group).*

Liver lipids	C	C+L	M	M+L
Triacylglycerols	16.6±2.47	17.3±2.28	14.9±1.59	14.8±1.08
Diacylglycerols	1.1±0.11	2.4±0.40[b]	1.7±0.19[a]	1.1±0.13[ab]
Free fatty acids	5.3±0.43	6.3±0.80	6.0±0.64	6.3±0.71
Cholesterol	4.7±0.54	4.8±0.67	4.5±0.53	5.7±1.03
Cholesteryl esters	0.2±0.04	0.3±0.05[b]	0.2±0.05	0.2±0.05
Phosphatidylserine	0.3±0.06	0.4±0.07	0.3±0.05	0.3±0.05
Phosphatidyletanolamine	0.3±0.07	0.5±0.08	1.2±0.39[a]	0.9±0.12[a]
Phosphatidylcholine	10.9±1.89	12.2±2.63	11.0±1.85	13.8±2.47
Sphingomyeline	0.2±0.05	0.2±0.04	0.2±0.05	0.6±0.14[ab]
Lysophosphatidylcholine	0.1±0.05	0.1±0.02	0.1±0.03	0.3±0.09
Total lipids	39.7±4.12	44.5±5.74	40.1±3.17	44.0±5.09

C: control group; C+L: control group in 24L; M: melatonin-treated group; M+L: melatonin-treated group in 24L. [a]Melatonin-treated group differs from its control group of the same lighting condition; [b]control or melatonin-treated group kept in 24L differs from the control or melatonin-treated group kept in 12L:12D (Mann-Whitney U test, $p<0.05$).

constant light (Mann-Whitney U test, $p<0.049$) (Table 3). The phosphatidyletanolamine (PE) levels increased due to melatonin treatment in the both lighting conditions (Mann-Whitney U test, $p<0.0004$) (Table 3). Melatonin treatment increased the sphingomyeline (SM) levels in 24L (Mann-Whitney U test, $p<0.034$), and constant light elevated the SM concentrations of the melatonin-treated animals (Mann-Whitney U test, $p<0.028$) (Table 3).

The kidney glycogen content of the rats was suppressed by constant light (*t* test, $p<0.047$) and by melatonin treatment (*t* test, $p<0.0004$) (Table 2). Also the kidney G-6-Pase activities were suppressed by constant light (*t* test, $p<0.052$) as well as by exogenous melatonin (Mann-Whitney U test, $p<0.0004$) (Table 2). The activity levels of kidney G-6-Pase were higher in the males compared to the females (*t* test, $p<0.009$) (Table 2). The kidney glycogen phosphorylase activities of the male rats were decreased by constant light (ANOVA, $p<0.05$) (Table 2). The activity levels were higher in the female rats (*t* test, $p<0.0004$) (Table 2). Constant light increased the kidney lipase esterase activities of the melatonin-treated rats (*t* test, $p<0.0004$) (Table 2). Melatonin treatment increased the kidney lipase esterase activity levels of the male rats in 24L (ANOVA, $p<0.05$) (Table 2). In the females, the lipase esterase activities were increased in 24L but sup-

Table 4 - *Plasma melatonin, glucose, insulin, T_4 and T_3 levels and T_3T_4 ratios of the rats (mean±SE).*

	CM 12L:12D	CF 12L:12D	CM 24L	CF 24L	MM 12L:12D	MF 12L:12D	MM 24L	MF 24L
Melatonin (pg ml^{-1})	3.8±0.82	3.2±0.56	4.6±0.65	4.9±0.62	347.1±15.36[a]	375.6±76.37[a]	389.6±23.83[a]	428.7±72.40[a]
Glucose (g l^{-1})	1.8±0.27	1.5±0.19	1.7±0.19	1.6±0.15	2.2±0.47	1.7±0.31	2.0±0.12	1.9±0.29
Insulin (μIU ml^{-1})	11.3±0.80[b]	6.5±1.45	9.9±1.08[b]	9.4±2.21[c]	14.0±2.30[b]	6.2±1.74	10.3±0.92[b]	9.9±1.19[c]
T_4 (nmol l^{-1})	91.0±13.84[b]	38.4±3.07	59.9±5.54[b]	38.2±6.35	60.2±6.74[b]	68.7±13.06[d]	65.8±7.08[b]	56.6±11.21
T_3 (nmol l^{-1})	1.7±0.19[b]	2.3±0.10	1.8±0.16[b]	2.1±0.22	2.1±0.35[b]	2.7±0.36	1.6±0.11[b]	2.0±0.22
T_3T_4 ratio x100	1.9±0.18[b]	6.2±0.45	3.1±0.44[be]	6.1±0.12	3.5±0.31[bf]	4.3±0.58	2.5±0.28[b]	3.9±0.61

CF: control females; CM: control males; F: females; +L: 24-h light; M: males; MF: melatonin-treated females; MM: melatonin-treated males. 12L:12D: artificial illumination from 06:00 h to 18:00 h; 24L: continuous light. [a]Melatonin-treated rats (no.=20) differ from controls (no.=20); [b]male rats (no.=20) differ from females (no.=20); [c]24L females (no.=10) differ from 12L:12D females (no.=10); [d]MF 12L:12D (no.=5) differs from CF 12L:12D (no.=5); [e]CM 24L (no.=5) differs from CM 12L:12D (no.=5), [f]MM 12L:12D (no.=5) differs from CM 12L:12D (no.=5) (ANOVA, *t* test, Mann-Whitney U test, $p<0.05$).

pressed in 12L:12D by exogenous melatonin (ANOVA, $p<0.05$) (Table 2).

The plasma melatonin levels were significantly higher in the melatonin-treated rats compared to the controls (Mann-Whitney U test, $p<0.0004$) (Table 4). Photoperiod had no effect on the daytime melatonin levels (Table 4). Melatonin treatment elevated the T_4 concentrations of the 12L:12D kept females (ANOVA, $p<0.05$) (Table 4) (Fig. 1A). The T_4 levels were generally higher in the male rats (*t* test, $p<0.014$) (Table 4) (Fig 1A). The treatments did not affect the plasma T_3 levels, which were higher in the females (*t* test, $p<0.011$) (Table 4) (Fig. 1B). The T_3T_4 ratio of the control males was increased by constant light (ANOVA, $p<0.05$) (Table 4). Also melatonin treatment elevated the ratios of the 12L:12D kept males (ANOVA, $p<0.05$) (Table 4). The T_3T_4 ratio was higher in the females (*t* test, $p<0.004$) (Table 4). The plasma insulin concentrations of the female rats were significantly elevated by long photoperiod (*t* test, $p<0.04$) (Table 4). The insulin levels were higher in the male rats (*t* test, $p<0.004$, Table 4). Neither constant light nor melatonin treatment affected the plasma glucose levels of the rats (Table 4).

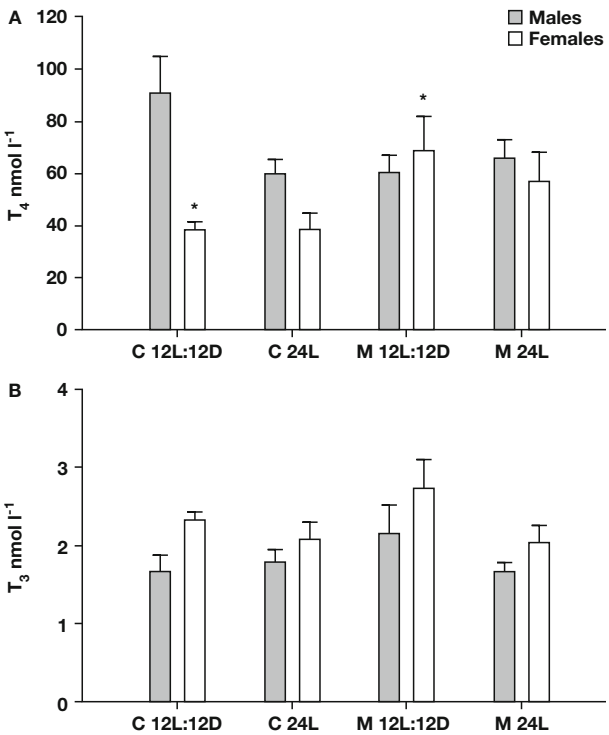

Fig. 1 - *Plasma T_4 (A) and T_3 (B) concentrations of the male and female rats (mean+SE). C: control; M: melatonin-treated; 12L:12D: artificial illumination from 06:00 h to 18:00 h; 24L: continuous light. Each group consists of 5 rats of the same sex. *CF 12L:12D differs from MF 12L:12D (ANOVA, p<0.05).*

DISCUSSION

Constant light or melatonin treatment did not affect the BM or the food intake of the rats, a phenomenon that has also previously been described (25). The liver glycogen concentrations of the rats were reduced by constant light, while melatonin had no clear effect on the parameter. Hepatic glycogen content of rats is minimal at the end of the light period, when animals are fasted, and at the initial hours of the dark period, when animals are active (26). Liver glycogen concentration increases around midnight, when digested carbohydrates are being deposited as glycogen. Locomotor activity of rats normally coincides with the scotoperiod. In continuous light the activity rhythms are usually disrupted and they dissociate into several recognizable periods (27). It is possible that the rats kept in constant light had several activity peaks and continuously utilized the glucose obtained from food, and for this reason the liver glycogen content was depressed.

The activities of liver glycogen phosphorylase, the regulatory enzyme of glycogenolysis (16), were suppressed by constant illumination and increased by exogenous melatonin. The liver carbohydrate stores of the 24L maintained rats were relatively small. Possibly for this reason also the utilization of carbohydrates decreased in constant light. The high glycogen phosphorylase activities of the melatonin-treated rats without significant changes in the liver glycogen content indicated higher synthesis and turnover-rate of glycogen. Our findings do not support the recent results of Mazepa *et al.* (28), in which high melatonin administration increased liver glycogen contents of exercised and non-exercised rats.

The hepatic G-6-Pase activities, indicating the ability of the liver to release free glucose from glucose-6-phosphate into the blood stream (16), were neither affected by constant light nor by exogenous melatonin. Increased G-6-Pase activities are normally connected to periods of fasting with high rates of gluconeogenesis and glycogenolysis. We have previously reported a melatonin-induced increase in autumnal hepatic G-6-Pase activities of female minks (*Mustela vison*), possibly functioning as an adaptation to the wintertime scarcity of food (29). The rats of this experiment were, however, in good nutritional status. Although the liver glycogen stores of the 24L maintained rats were relatively small, food continued to be available *ad libitum*.

Melatonin increased the liver lipase esterase activ-

ities (hepatic lipolysis) of the male rats maintained in 12L:12D. In the 24L kept males as well as in the females of the both lighting conditions, melatonin treatment significantly decreased the lipase esterase activities. Instead of utilization, triacylglycerols (TG) were presumably transported and stored elsewhere in the body as energy reserve. A melatonin-induced decrease in lipase esterase activities has previously been observed in male minks preparing themselves for the winter by storing sc adipose tissue (29). Exogenous melatonin is known to stimulate accumulation of fat in several mammalian species such as the Syrian hamster (*Mesocricetus auratus*) (11) and the garden dormouse (*Eliomys quercinus*) (13). Also the rate of liver lipogenesis of chickens increases due to melatonin treatment and decreases in constant light (30). The melatonin-induced decrease in the lipase esterase activities of the laboratory rat may represent a remain of an adaptation to seasonal scarcity of food.

The only clear effect of exogenous melatonin on the liver lipid concentrations was the elevation of the PE levels in the both light regimes. Melatonin treatment has previously increased serum phospholipid levels of rats (31). Our results do not indicate increased levels of lipoprotein synthesis in the liver, as the other components of the lipid fraction of lipoproteins besides phospholipids [*i.e.* TG, CE, cholesterol (C)] were not affected by melatonin. Neither have Hoyos *et al.* (32) observed any effects of melatonin treatment on serum C and TG levels of rats fed a regular diet. Melatonin has been shown to participate in the regulation of cholesterol metabolism. Exogenous melatonin decreases circulating free C levels, which may be mediated by an augmentation of cholesterol esterification (31). In our experiment, neither melatonin nor constant light affected the liver C levels. The liver CE concentrations of the control rats were, however, increased by constant light. The effects of melatonin and constant light on the liver DG levels were confusing. DG is the key intermediate in the biosynthesis of PE (33), indicating that the melatonin-induced changes in these 2 lipid classes may be interrelated.

The insulin levels of the female rats were increased by constant light. Pinealectomy has previously caused hypersecretion of insulin from rat pancreatic islets (34). The glucose levels were not affected by melatonin treatment, as also previously described (35). The thyroid metabolism of the male and the female rats responded differently to melatonin. Exogenous melatonin increased the plasma T_4 levels of the females kept in 12L:12D photoperiod. Although melatonin administration usually lowers blood T_4 levels of rats and hamsters, in certain experimental conditions also a stimulatory effect can be demonstrated (36). In the male rats, both melatonin and constant light elevated the T_3T_4 ratio, indicating increased metabolic output perhaps trying to counteract the changes in circulating melatonin levels to either direction. Melatonin implants can also elevate the T_3T_4 ratio of the pigeon (37).

The kidney is essential in maintaining the balance of body water and inorganic ions (38). It is also an important endocrine organ regulating erythrocyte production, BP and calcium balance in mammals and birds. Some experiments have shown that melatonin affects *e.g.* water consumption, urine production and electrolyte concentration (17), BP (18) as well as glomerular filtration rates (19) of mammals. As melatonin has such diverse effects on renal function, it presumably affects kidney energy metabolism.

In fact, the kidney glycogen content and G-6-Pase activities of the rats were decreased by continuous light as well as by exogenous melatonin treatment. Kidney glycogenolysis of males was decreased by constant illumination, while melatonin treatment had no effect on the glycogen phosphorylase activities. The kidney lipase esterase activities were increased by constant light in the melatonin-treated rats. The effects of melatonin treatment on kidney lipid mobilization depended on the prevailing lighting condition. Exogenous melatonin increased the lipase esterase activities of both sexes in 24L. On the contrary, the activities were suppressed in the females maintained in 12L:12D. Our findings support a role for melatonin in renal function, as changes from the normal circulating melatonin concentrations to either direction seem to increase utilization of kidney carbohydrates.

The responses to constant light and persistent melatonin treatment were diverse, although the laboratory rat is normally considered to be only a marginally photoperiodic rodent. There were clear sex differences in the hormone levels (T_3, T_4, insulin) as well as in the enzyme activities of carbohydrate metabolism (G-6-Pase, glycogen phosphorylase). It is possible that the kidneys may compensate the lower activity levels of a particular enzyme in the liver. This is supported by the opposite sex differences in the liver and kidney glycogen phosphorylase activities of the rats. There was also clear sexual dimorphism in the responses to exogenous melatonin and constant light. This may have resulted from different rates of somatic growth and phases of reproductive cycles in the sexes. It is *e.g.* known that constant light can lead to a failure of ovulation

in female rats (39) but increase sperm production in males (40). It is possible that melatonin can directly affect peripheral tissues, as melatonin binding sites have been localized *e.g.* in the liver, kidneys and pancreas (8-10). The actions of melatonin could also be indirect and mediated by other hormones and molecules such as nitric oxide.

In summary, continuous light stimulated carbohydrate metabolism of rat liver while hepatic lipid metabolism remained stable. Exogenous melatonin enhanced utilization of liver carbohydrates but suppressed hepatic lipid mobilization. Changes from the normal circulating melatonin concentrations to either direction increased utilization of kidney carbohydrates. Both exogenous melatonin and constant light seem to have a strong regulatory effect on rat energy metabolism.

ACKNOWLEDGMENTS

We thank Ms. Leena Koponen for taking care of the rats. The technical assistance of Mrs. Anita Kervinen is highly appreciated. Financial support was provided by the Academy of Finland (Project # 57532).

REFERENCES

1. Arendt J. Melatonin and the mammalian pineal gland. Chapman & Hall, Cambridge, 1995, p. 72.
2. Reiter R.J. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocr. Rev. 1991, *12:* 151-180.
3. Reiter R.J., Klein D.C. Observations on the pineal gland, the Harderian glands, the retina, and the reproductive organs of adult female rats exposed to continuous light. J. Endocrinol. 1971, *51:* 117-125.
4. Tamarkin L., Baird C.J., Almeida O.F.X. Melatonin: a coordinating signal for mammalian reproduction? Science 1985, *227:* 714-720.
5. Saarela S., Reiter R.J. Function of melatonin in thermoregulatory processes. Life Sci. 1994, *54:* 295-311.
6. Allain D., Rougeot J. Induction of autumn moult in mink (Mustela vison Peale and Beauvois) with melatonin. Reprod. Nutr. Dev. 1980, *20:* 197-201.
7. Martin M.T., Azpiroz F., Malagelada J.R. Melatonin and the gastrointestinal tract. Thérapie 1998, *53:* 453-458.
8. Acuña-Castroviejo D., Reiter R.J., Menéndez-Peláez A., Pablos M.I., Burgos A. Characterization of high-affinity melatonin binding sites in purified cell nuclei of rat liver. J. Pineal Res. 1994, *16:* 100-112.
9. Song Y., Tam P.C., Poon A.M.S., Brown G.M., Pang S.F. 2-[^{125}I]Iodomelatonin-binding sites in the human kidney and the effect of guanosine 5´-O-(3-thiotriphosphate). J. Clin. Endocrinol. Metab. 1995, *80:* 1560-1565.
10. Williams L.M., Hannah L.T., Adam C.L., Bourke D.A. Melatonin receptors in red deer fetuses (Cervus elaphus). J. Reprod. Fert. 1997, *110:* 145-151.
11. Wade G.N., Bartness T.J. Seasonal obesity in Syrian hamsters: effects of age, diet, photoperiod, and melatonin. Am. J. Physiol. 1984, *247:* R328-R334.
12. Valtonen M., Vakkuri O., Blomstedt L. Autumnal timing of photoperiodic manipulation critical via melatonin to winter pelage development in mink. Anim. Sci. 1995, *61:* 589-596.
13. Le Gouic S., Delagrange P., Atgié C., *et al.* Effects of both a melatonin agonist and antagonist on seasonal changes in body mass and energy intake in the garden dormouse. Int. J. Obes. Relat. Metab. Disord. 1996, *20:* 661-667.
14. Milcu S.M., Milcu I. Über ein hypoglykämisch wirkendes Hormon in der Zirbeldrüse. Die Medizinische 1958, *17:* 711-715.
15. de Vlaming V.L., Sage M., Charlton C.B., Tiegs B. The effects of melatonin on lipid deposition in Cyprinodontid fishes and on pituitary prolactin activity in Fundulus similis. J. Comp. Physiol. 1974, *94:* 309-319.
16. Harris R.A. Carbohydrate metabolism I: major metabolic pathways and their control. In: Devlin T.M. (Ed.), Textbook of biochemistry with clinical correlations, 2nd ed. John Wiley & Sons, Singapore, 1986, p. 261.
17. Richardson B.A., Studier E.H., Stallone J.N., Kennedy C.M. Effects of melatonin on water metabolism and renal function in male Syrian hamsters (Mesocricetus auratus). J. Pineal Res. 1992, *13:* 49-59.
18. Kawashima K., Miwa Y., Fujimoto K., Oohata H., Nishino H., Koike H. Antihypertensive action of melatonin in the spontaneously hypertensive rat. Clin. Exp. Hypertens. 1987, *A9:* 1121-1131.
19. Tsuda T., Ide M., Iigo M. Influences of season and of temperature, photoperiod, and subcutaneous melatonin infusion on the glomerular filtration rate of ewes. J. Pineal Res. 1995, *19:* 166-172.
20. O'Callaghan D., Karsch F.J., Boland M.P., Roche J.F. What photoperiodic signal is provided by a continuous-release melatonin implant? Biol. Reprod. 1991, *45:* 927-933.
21. Hers H.G., van Hoof F. Enzymes of glycogen degradation in biopsy material. In: Colowick S., Kaplan N.O. (Eds.), Methods in enzymology. Academic Press, NY, 1966, p. 525.
22. Seligman A.M., Nachlas M.M. Lipase. In: Bermayer H.U. (Ed.), Methoden der Enzymatischen Analyse. Verlag Chemie GmbH, Weinheim, Germany, 1962, p. 776.
23. Lo S., Russell J.C., Taylor A.W. Determination of glycogen in small tissue samples. J. Appl. Physiol. 1970, *28:* 234-236.
24. Folch J., Lees M., Sloane Stanley G.H. A simple method for the isolation and purification of total lipides from animal tissues J. Biol. Chem. 1957, *226:* 497-509.
25. Dark J., Rayha L.L., Clark-Lane I., Kimler V. Melatonin and lighting condition: absence of long-term effects on food intake and body weight regulation in the albino rat. Physiol. Behav. 1980, *25:* 855-857.

26. Kaminsky Y.G., Kosenko E.A., Kondrashova M.N. Analysis of the circadian rhythm in energy metabolism of rat liver. Int. J. Biochem. 1984, *16:* 629-639.
27. Chesworth M.J., Cassone V.M., Armstrong S.M. Effects of daily melatonin injections on activity rhythms of rats in constant light. Am. J. Physiol. 1987, *253:* R101-R107.
28. Mazepa R.C., Cuevas M.J., Collado P.S., Gonzáles-Gallego J. Melatonin increases muscle and liver glycogen content in nonexercised and exercised rats. Life Sci. 2000, *66:* 153-160.
29. Nieminen P., Käkelä R., Mustonen A-M., Hyvärinen H., Asikainen J. Exogenous melatonin affects lipids and enzyme activities in mink (Mustela vison) liver. Comp. Biochem. Physiol. 2001, *128C:* 203-211.
30. Osei P., Robbins K.R., Shirley H.V. Effects of exogenous melatonin on growth and energy metabolism of chickens. Nutr. Res. 1989, *9:* 69-81.
31. Esquifino A., Agrasal C., Velázquez E., Villanúa M.A., Cardinali D.P. Effect of melatonin on serum cholesterol and phospholipid levels, and on prolactin, thyroid-stimulating hormone and thyroid hormone levels, in hyperprolactinemic rats. Life Sci. 1997, *61:* 1051-1058.
32. Hoyos M., Guerrero J.M., Perez-Cano R., *et al.* Serum cholesterol and lipid peroxidation are decreased by melatonin in diet-induced hypercholesterolemic rats. J. Pineal Res. 2000, *28:* 150-155.
33. Vance D.E. Biosynthesis of membrane lipids. In: Zubay G.L. (Ed.), Biochemistry, 4th ed. Wm. C. Brown Publishers, Dubuque, 1998, p. 507.
34. Gorray K.C., Quay W.B., Ewart R.B.L. Effects of pinealectomy and pineal incubation medium and sonicates on insulin release by isolated pancreatic islets in vitro. Horm. Metab. Res. 1979, *11:* 432-436.
35. Aoyama H., Mori W., Mori N. Anti-glucocorticoid effects of melatonin in young rats. Acta Pathol. Jpn. 1986, *36:* 423-428.
36. Vriend J., Richardson B.A., Vaughan M.K., Johnson L.Y., Reiter R.J. Effects of melatonin on thyroid physiology of female hamsters. Neuroendocrinology 1982, *35:* 79-85.
37. John T.M., Viswanathan M., George J.C., Scanes C.G. Influence of chronic melatonin implantation on circulating levels of catecholamines, growth hormone, thyroid hormones, glucose, and free fatty acids in the pigeon. Gen. Comp. Endocrinol. 1990, *79:* 226-232.
38. Randall D., Burggren W., French K. Ionic and osmotic balance. In: Eckert animal physiology, mechanisms and adaptations, 4th ed. W.H. Freeman and Company, NY, 1997, p. 571.
39. Campbell C.S., Schwartz N.B. The impact of constant light on the estrous cycle of the rat. Endocrinology 1980, *106:* 1230-1238.
40. Rocha D.C.M., Debeljuk L., Franca L.R. Exposure to constant light during testis development increases daily sperm production in adult Wistar rats. Tissue Cell 1999, *31:* 372-379.