Metabolic Effects of Prolonged Melatonin Administration and Short-Term Fasting in Laboratory Rats

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Abstract

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The aim of this work was to evaluate the effect of prolonged administration of the pineal hormone melatonin and short-term fasting on metabolic variables in male and female Wistar:Han rats. Melatonin (MEL, 4μg/ml of tap water) was administered daily since the 5th week of age. The control group drank tap water. Rats were fed a standard type of diet *ad libitum* and were kept in the light regimen L:D – 12:12 h. The experiment was terminated after 11 (variant B) or 12 (variant A) weeks of MEL administration. The animals were sacrificed by quick decapitation following overnight fasting (variant A) or 48-h fasting (variant B). Selected organs and tissues were removed and weighed and selected metabolic variables in the serum and tissues were determined.

MEL decreased body mass independent of food and water intake in both sexes. In males (variant A) MEL increased the weight of the heart muscle, spleen and adrenals; it decreased the absolute weight of epididymal fat and increased serum corticosterone and phospholipids concentration in comparison with controls. In females, serum glucose decrease and liver triacylglycerols increase were found. After 48-h fasting (variant B) liver, spleen and adrenal weight increase in MEL-drinking females was found. In males MEL increased the thymus weight and decreased the epididymal fat weight. In both sexes MEL increased serum corticosterone and liver glycogen concentration; MEL increased serum glucose in males and serum cholesterol concentration in females. Changes in the evaluated variables were also related to fasting duration prior to decapitation.

A 48-h fasting at the end of the prolonged MEL intake (variant B vs. A) decreased the absolute liver weight in both sexes and the epididymal/periovarial fat weight, and increased thymus weight in males. In females it decreased the absolute heart muscle weight and increased the spleen weight. In males, 48-h fasting increased serum corticosterone and phospholipids concentration; it decreased the liver triacylglycerols content in females and the liver cholesterol content in males and females. In both sexes 48-h fasting increased glucose concentration in the serum and glycogen concentration in the liver and heart muscle as well as triacylglycerols and cholesterol concentration in the serum, phospholipids concentration in the liver and bone marrow and decreased malondialdehyde concentration in the liver. Forty-eight hour fasting after prolonged MEL administration resulted in a wider range of carbohydrate and lipid metabolism alterations of young rats of both sexes.

Melatonin, prolonged administration, metabolic variables, short-term fasting

Melatonin (MEL), the main pineal hormone, is a substance of numerous physiological effects. In addition to epiphysis, MEL is synthesized in other tissues too – e.g. in the retina, extraorbital lacrimal glands, Harderian's glands, gastrointestinal tract and in bone marrow cells (Vijayalaxmi et al. 2002). Receptors or high affinity MEL binding sites were found

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in many tissues, indicating that MEL regulates physiological functions through a direct effect on these tissues. MEL synchronizes biological rhythms and exerts antioxidant (Tan et al. 1993ab) and oncostatic (Hill and Blask 1988) properties; sedative, immunomodulative, analgetic, myorelaxing, cardioprotective and neuroprotective effects have been reported (Maestroni 1993; Reiter et al. 1998; Lagneux et al. 2000; Ebadi et al. 1998; Vijayalaxmi et al. 2002).

MEL inteferes with the carbohydrate and lipid metabolism; the precise mechanism remains to be revealed. The results of studies on the relationship between MEL and glucose metabolism regulation are inconsistent. Pinealectomy was shown to increase serum glucose concentration, MEL administration normalized serum glucose concentration (Rodriguez et al. 1989; Diaz and Blazquez 1986). Contrary to that, Milcu et al. (1971) recorded hypoglycemia after pinealectomy in rats. Yet, Bizot-Espiard et al. (1998) found no effects of either pinealectomy or MEL administration to pinealectomized rats on glycemia. Glucose intolerance and decreased sensitivity of adipocytes to insulin after glucose load was reported in pinealectomized rats (Lima et al. 1998). Alonso-Vale et al. (2004a) recorded insulin resistance of adipocytes after pinealectomy in vitro. Single MEL administration increased glycemia (Fabis et al. 2002), constant MEL infusion in rats had no effect on results of intravenous glucose tolerance test (Bailey et al. 1974). Effects of exogenous MEL in other experimental models were ambiguous, too. In birds (Zeman et al. 1993; Mahata et al. 1988) and rabbits (Dhar et al. 1983) the increase, decrease (Mahata et al. 1988) as well as no effect (Mahata et al. 1988; John et al. 1990) on glycemia were found. The inconsistence of the in vivo results may be due to the fact that the MEL effect depends on the dose, duration and time of administration. MEL may modulate serum glycemia indirectly through the hormones involved in the carbohydrate metabolism regulation like insulin, glucagon, catecholamines, or growth hormones. MEL receptors were found in pancreatic islet cells (Peschke et al. 2000), indicating that MEL interferes with insulin or other pancreatic hormones production and/or secretion. However, the results of the studies are inconsistent. Insulin secretion inhibition in vitro was reported in rodents (Peschke et al. 1997); other authors found no changes (Frankel and Strandberg 1991). In rats, however, insulin secretion increased after supraphysiological MEL dose administration (Fabis et al. 2002). Pinealectomy decreased (Bailey et al. 1974; Diaz and Blazquez 1986; Rodriguez et al. 1989) and MEL administration normalized isulinemia (Diaz and Blazquez 1986; Rodriguez et al. 1989). Contrary to that, Gorray et al. (1979) reported an increase of insulin secretion following pinealectomy. MEL may modulate insulin or other hormones receptor expression; Rodriguez et al. (1989) reported changes in insulin and glucagon liver receptor concentration following pinealectomy.

Exogenous MEL effects on lipid metabolism variables are better defined. Acute MEL administration increased total, free and esterified cholesterol concentration and decreased non-esterified fatty acids concentration in the rat blood (Fabis et al. 2002); the effects of prolonged administration were somewhat different. In rats fed a high-cholesterol diet, MEL administration decreased serum cholesterol concentration (Mori et al. 1989; Hoyos et al. 2000) and reduced liver fat infiltration (Mori et al. 1989); no changes were found in the concentration of serum triacylglycerols. MEL reduced LDL-cholesterol increase and prevented HDL-cholesterol decrease after high-cholesterol diet feeding (Hoyos et al. 2000). MEL administration, however, did not influence serum triacylglycerols concentration in rats fed a standard type of diet (Mori et al. 1989; Hoyos et al. 2000). MEL administration in genetically hypercholesterolemic rats decreased cholesterol concentration and prevented fat liver (Ayoma et al. 1988). MEL did not prevent age-related hypercholesterolemia in Syrian hamsters (Vaughan et al. 1982).

MEL interferes with energy metabolism, too. Leptin regulation (adipocyte cells hormone regulating food consumption) by MEL was reported in numerous studies. Leptin level is positively correlated to BMI (Maffei et al. 1995). Exogenous as well as endogenous MEL decreased leptin concentration in rodents (Rasmussen et al. 1999; Wolden-Hanson et al. 2000; Canpolat et al. 2001; Gündüz et al. 2002; Puchalski et al. 2003). MEL administration decreased body mass in male rats independent of food consumption (Wolden-Hanson et al. 2000; Marková et al. 2003, 2004) as well as in male rats fed a high-fat diet (Prunet-Marcassus et al. 2003); in female rats the body mass was not influenced (Marková et al. 2003), or it increased after 6 months of administration respectively (Marková et al. 2004). These differences may result from different intersexual leptin level regulation. On the other hand, Mustonen et al. (2002) found no changes of body mass and food consumption in rats receiving MEL or kept in continuous light. The relation between exogenous/endogenous MEL and leptin level needs to be verified in other studies.

In this study, the metabolic effect of exogenous melatonin alone and in combination with short-term fasting was evaluated. Starving could be one of the factors involved in carcinogenesis. These nutritional conditions should be considered in the use of exogenous melatonin in experimental and clinical oncology; therefore we used a 48-h model of fasting in our experiments.

Materials and Methods

Male and female Wistar:Han rats (Central vivarium, Faculty of Medicine, P. J. Šafárik University, Košice, Slovak Republic) aged 5 weeks (variant A – 37-40 days, variant B – 40-42 days; body mass : variant A – females 113 ± 2g, males 126 ± 2 g; variant B – females 127 ± 2 g, males 176 ± 5 g) were used in the experiment. The animals were adapted to standard vivarium conditions (temperature 23 ± 3 °C, relative humidity 60-70%) and artificial light:dark regimen LD 12:12, lights at 07:00 h with 150 lux intensity per cage (TESLA fluorescent lamps, 40W). Four to five animals were housed per cage. The animals were fed a MP diet (Top-Dovo, Dobrá Voda, Slovak Republic) and drank tap water and MEL solution, respectively, *ad libitum*.

MEL (Sigma, Deisenhofen, Germany) was administered in tap water at a concentration of 4μg/ml discontinuously for the period of 11 (variant B) or 12 weeks (variant A) daily from 15.00 h to 08.00 h (from 08.00 h to 15.00 h the rats drank tap water). The onset of MEL administration was at the 5th week of age. Twenty mg of MEL were dissolved in 0.4 ml of 30% ethanol and mixed with tap water to the desired concentration. The MEL solution was freshly prepared three times a week. The control group was given water containing 0.03 g ethanol/dm^3.

The animals were divided into 4 groups:

1. MEL-drinking females (MEL-females), n (the number of animals) = 14 (variant A) or 10 (variant B), respectively
2. control females (intact animals, CONT-females), n = 14 (variant A) or 10 (variant B), respectively
3. MEL-drinking males (MEL-males), n = 14 (variant A) or 8 (variant B), respectively
4. control males (intact animals, CONT-males), n = 14 (variant A) or 10 (variant B), respectively.

The food and water intake was monitored on the 6th and 10th week of the experiment (since MEL administration). The rats were weighed twice a week. After 11 weeks (and 48-h fasting in variant B) or 12 weeks (and overnight fasting in variant A) the animals were sacrificed by quick decapitation (MEL solution was administered until decapitation). The liver, heart muscle, spleen, thymus, adrenals, periovarial and epididymal fat were removed and weighed. Concentrations of glucose, triacylglycerols, total cholesterol, phospholipids, corticosterone and insuline were determined in the serum from mixed blood; the concentrations/contents of glycogen, triacylglycerols, total cholesterol, phospholipids and malondialdehyde were determined in the liver; concentrations of triacylglycerols, phospholipids and malondialdehyde were determined in the bone marrow (femur); the concentration/content of glycogen was determined in the heart muscle. Glucose and triacylglycerols were measured using commercial sets (Lachema, Brno, Czech Republic), insulin was determined using the commercial set of Linco Research (St Charles, MO, USA), phospholipids were measured from lipid phosphorus according to Bartlett (1959), cholesterol according to Zlatkis et al. (1953), glycogen according to Roe and Dailey (1966), malondialdehyde was measured in reaction with thiobarbituric acid according to Satch (1978), corticosterone was measured using fluorimetry according to Guillemin et al. (1958). Results were evaluated by one way analysis of variance and Kruskal-Wallis test, respectively; the criterion for the choice of the relevant test was the value of Bartletts' number. Data are presented as means ± standard error of the mean (S.E.M.), significant differences between groups are expressed as **a** for $p \leq 0.05$, **b** for $p \leq 0.01$, **c** for $p \leq 0.001$.

Results

MEL administration decreased body mass from the 3rd week until the end of the experiment in comparison with controls (Fig. 1); this decrease was more prominent in males than in females. The food intake in MEL-females decreased in the 6th week; no other changes in food and water intake were recorded.

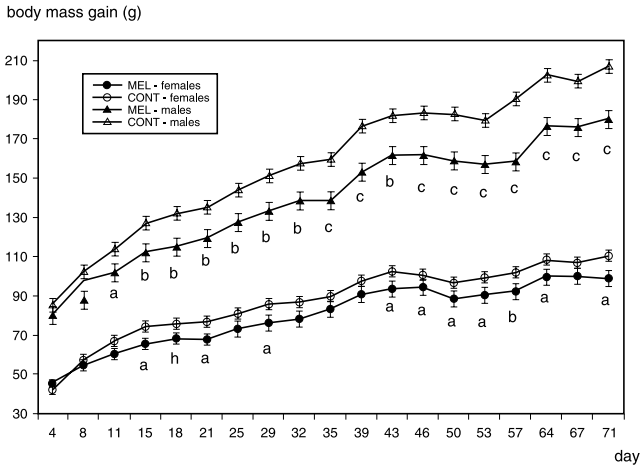

Fig. 1. The effect of prolonged MEL administration on body mass gain of male and female Wistar:Han rats.
Data are expressed as means ± S.E.M. Significant differences between groups are designated as: **a** for $p \leq 0.05$; **b** for $p \leq 0.01$; **c** for $p \leq 0.001$, **h** for $p =$ (0.051-0.055). Abbreviations: MEL–melatonin, CONT–control

Variant A (overnight fasted rats): MEL increased the relative heart muscle, spleen and adrenal weight and decreased the absolute epididymal fat weight in males; in females no changes of organ weights were recorded. In females glycemia decreased; the concentration/content of triacylglycerols in the liver increased. In males concentrations of phospholipids and corticosterone increased. Malondialdehyde concentration in the liver increased in both sexes (Table 1, 3).

Variant B (48-h fasted rats): After 48-h fasting, the relative liver, spleen and adrenal weight increase and the absolute heart muscle weight decrease were recorded in females; in males, MEL increased the relative thymus weight and reduced the absolute/relative epididymal fat weight. MEL increased serum corticosterone concentration and concentration/content of liver glycogen in both sexes. MEL increased glycemia in males and serum cholesterol concentration in females (Table 2, 4).

Metabolic changes after prolonged MEL administration were also related to fasting duration prior to decapitation (variant B vs. A). Forty-eight hour fasting reduced the absolute liver weight and the absolute/relative epididymal/periovarial fat weight in both sexes; the absolute/relative thymus weight increased in males; in females the absolute heart muscle weight was reduced and the absolute/relative spleen weight increased (Table 4). In males, 48-h fasting increased serum corticosterone and phospholipids concentration and decreased the liver cholesterol concentration/content; in females, 48-h fasting decreased the liver triacylglycerols concentration/content and the cholesterol content. In both sexes, 48-h fasting increased glycemia (by 14% in males and 29% in females), glycogen concentration

Table 1. The effect of 12-week MEL administration on selected metabolic variables in variant A - overnight fasted rats

	CONT-females n = 14	MEL-females n = 14	CONT-males n = 14	MEL-males n = 14
SERUM				
GLU (mmol/l)	4.83 ± 0.20	3.75 ± 0.13 **c**	4.68 ± 0.25	5.05 ± 0.21
TAG (mmol/l)	0.21 ± 0.03	0.23 ± 0.04	0.33 ± 0.03	0.31 ± 0.03
CH (mmol/l)	0.85 ± 0.06	0.89 ± 0.06	0.85 ± 0.02	0.91 ± 0.02
PL (mmol/l)	1.26 ± 0.07	1.31 ± 0.06	1.00 ± 0.04	1.16 ± 0.03 **b**
CTS (ng/ml)	345.35 ± 47.33	395.90 ± 58.31	56.95 ± 11.46	132.37 ± 16.83 **b**
INS (ng/ml)	0.28 ± 0.04	0.25 ± 0.04	0.39 ± 0.05	0.41 ± 0.04
LIVER				
GLY (µmol/g)	8.01 ± 2.03	3.98 ± 0.54	5.50 ± 0.61	8.43 ± 1.73
GLY (µmol)	36.94 ± 8.88	19.51 ± 1.82	49.83 ± 6.05	83.44 ± 17.78
TAG (µmol/g)	21.24 ± 1.97	28.92 ± 2.09 **b**	17.09 ± 2.16	18.14 ± 1.67
TAG (µmol)	119.65 ± 10.93	170.75 ± 15.14 **b**	152.64 ± 18.45	157.67 ± 17.42
CH (µmol/g)	20.19 ± 0.88	21.18 ± 0.49	20.06 ± 0.35	19.94 ± 0.43
CH (µmol)	114.39 ± 5.28	117.87 ± 3.66	179.37 ± 5.63	169.44 ± 6.08
PL (µmol/g)	33.99 ± 0.81	35.61 ± 1.02	34.94 ± 0.96	35.95 ± 0.29
PL (µmol)	192.46 ± 5.07	201.09 ± 6.12	313.14 ± 12.61	306.33 ± 10.44
MDA (nmol/g)	37.45 ± 1.84	55.38 ± 5.70 **b**	16.99 ± 1.39	21.88 ± 1.69 **a**
MDA (nmol)	214.01 ± 13.28	305.31 ± 29.97 **b**	151.49 ± 12.15	187.75 ± 18.01
BONE MARROW				
TAG (µmol/g)	12.27 ± 2.37	13.54 ± 5.16	16.16 ± 2.99	9.95 ± 2.09
PL (µmol/g)	12.10 ± 0.56	11.73 ± 0.59	10.41 ± 0.48	10.57 ± 0.41
MDA (nmol/g)	81.48 ± 11.66	66.05 ± 7.25	45.34 ± 2.95	38.69 ± 3.35
HEART MUSCLE				
GLY (µmol/g)	16.47 ± 1.13	14.62 ± 1.02	18.34 ± 1.12	19.52 ± 1.28
GLY (µmol)	9.50 ± 0.73	8.62 ± 0.61	15.27 ± 0.92	15.95 ± 1.06

Data are expressed as means ± S.E.M. Significant differences between MEL group and CONT group are designated as **a** for $p \leq 0.05$, **b** for $p \leq 0.01$, **c** for $p \leq 0.001$. Abbreviations: MEL - melatonin, CONT - control, GLU - glucose, GLY - glycogen, TAG - triacylglycerols, CH - cholesterol, PL - phospholipids, MDA - malondialdehyde, CTS - corticosterone, INS - insulin, n - number of animals.

in the liver (by 343% in males and 398% in females) and in the heart muscle (by 38% in males and 76% in females), triacylglycerols (by 107% in males and 78% in females) and cholesterol concentration (by 25% in males and 26% in females) in the serum, phospholipids concentration in the bone marrow (by 42% in males and 34% in females) and in the liver (by 43% in males and 61% in females) and decreased malondialdehyde concentration in the liver (by 46% in males and 64% in females) (Table 2).

Discussion

In the present experiment, the metabolic effect of prolonged MEL administration and 48-h fasting was studied in young Wistar:Han rats. This work is related to our previous experiments where MEL was administered to 5-week-old Sprague-Dawley (SD) rats for 2,5 months or 6 months, respectively (Marková et al. 2003, 2004). MEL was administered in tap water in the concentration of 4µg/ml daily during afternoon and night, i.e. in the period of the highest tissue sensitivity to MEL. Prolonged administration reduced adrenal, liver, heart muscle and epididymal fat weight in males; in females, the heart muscle and periovarial fat weight increased (Marková et al. 2003). Changes in organ weights in MEL-

Table 2. The effect of 11-week MEL administration and fasting on selected metabolic variables in variant B - 48-h fasted rats

	CONT-females n = 10	MEL-females n = 10	CONT-males n = 10	MEL-males n = 8
SERUM				
GLU (mmol/l)	5.08 ± 0.21	4.84 ± 0.19 ↑**z**	4.96 ± 0.15	5.74 ± 0.21 **b,** ↑**x**
TAG (mmol/l)	0.32 ± 0.04 ↑**x**	0.41 ± 0.02 ↑**y**	0.55 ± 0.08 ↑**y**	0.64 ± 0.10 ↑**z**
CH (mmol/l)	0.94 ± 0.03	1.12 ± 0.08 **a,** ↑**x**	1.05 ± 0.04 ↑**z**	1.14 ± 0.04 ↑**z**
PL (mmol/l)	1.34 ± 0.05	1.45 ± 0.17	1.24 ± 0.06 ↑**y**	1.37 ± 0.05 ↑**y**
CTS (ng/ml)	317.84 ± 66.25	577.58 ± 79.31 **a**	185.74 ± 23.84 ↑**z**	305.31 ± 55.24 **a,** ↑**y**
INS (ng/ml)	-	-	-	-
LIVER				
GLY (µmol/g)	13.21 ± 1.66	19.74 ± 2.87 **a,** ↑**z**	12.48 ± 1.23 ↑**z**	37.36 ± 5.59 **c,** ↑**z**
GLY (µmol)	64.06 ± 7.92 ↑**x**	98.59 ± 13.33 **a,** ↑**z**	103.03 ± 9.88 ↑**z**	276.11 ± 44.63 **c,** ↑**z**
TAG (µmol/g)	20.92 ± 1.82	20.63 ± 1.80 ↓**y**	12.30 ± 0.82	15.37 ± 1.90
TAG (µmol)	102.44 ± 10.54	99.90 ± 6.83 ↓**y**	102.03 ± 7.15	116.76 ± 14.96
CH (µmol/g)	20.34 ± 0.48	19.91 ± 0.68	18.08 ± 0.40 ↓**z**	18.29 ± 0.49 ↓**x**
CH (µmol)	99.05 ± 4.23 ↓**x**	99.40 ± 4.55 ↓**y**	148.68 ± 7.79 ↓**y**	136.46 ± 7.24 ↓**y**
PL (µmol/g)	56.96 ± 1.87 ↑**z**	57.16 ± 2.93 ↑**z**	53.61 ± 1.57 ↑**z**	51.29 ± 1.74 ↑**z**
PL (µmol)	275.17 ± 10.65 ↑**z**	281.78 ± 11.20 ↑**z**	432.42 ± 23.28 ↑**z**	380.63 ± 14.91 ↑**z**
MDA (nmol/g)	21.32 ± 2.31 ↓**z**	19.76 ± 3.20 ↓**z**	11.68 ± 0.98 ↓**y**	11.90 ± 2.16 ↓**y**
MDA (nmol)	103.73 ± 11.35 ↓**z**	99.88 ± 15.44 ↓**z**	73.45 ± 13.19 ↓**z**	90.68 ± 18.32 ↓**y**
BONE MARROW				
TAG (µmol/g)	5.80 ± 1.47 ↓**x**	6.21 ± 1.38	7.95 ± 1.81 ↓**x**	3.86 ± 0.95
PL (µmol/g)	14.49 ± 1.10 ↑**x**	15.74 ± 1.30 ↑**y**	15.50 ± 0.89 ↑**z**	15.03 ± 1.66 ↑**y**
MDA (nmol/g)	49.44 ± 3.25 ↓**x**	59.54 ± 10.46	32.02 ± 2.91 ↓**y**	30.67 ± 5.01
HEART MUSCLE				
GLY (µmol/g)	25.43 ± 2.64 ↑**y**	25.79 ± 2.08 ↑**z**	25.58 ± 2.48 ↑**y**	26.90 ± 3.20 ↑**x**
GLY (µmol)	14.53 ± 1.77 ↑**y**	13.71 ± 1.06 ↑**z**	20.80 ± 2.00 ↑**y**	22.76 ± 2.59 ↑**y**

Data are expressed as means ± S.E.M. Significant differences between MEL group and CONT group are designated as **a** for $p \leq 0.05$, **b** for $p \leq 0.01$, **c** for $p \leq 0.001$. Significant differences between variant B group and variant A group are designated as follows: **x** for $p \leq 0.05$, **y** for $p \leq 0.01$, **z** for $p \leq 0.001$, symbol ↑ indicates the increase and symbol ↓ indicates the decrease of a particular variable in group B when compared to group A. For abbreviations see Table 1.

administered male Wistar:Han rats decapitated after overnight fasting in this experiment (variant A) were different from those observed in SD rats (Marková et al. 2003): increased heart muscle, spleen and adrenal weights in male rats were recorded due to specific characteristics of rat strains. Epididymal fat weight was reduced in both strains. In Wistar:Han females, organ weights and periovarial fat (as observed in SD rats) were not changed. In the present work, MEL reduced body mass gain in both sexes without prominent changes in food and water intake. In the study of Marková et al. (2003) body mass gain reduction was recorded in males only. Because we found no permanent changes in food consumption, the body mass reduction could be related to altered insulin and leptin signaling: further experiments in this area are needed. Metabolic variables in the present work slightly changed with regard to carbohydrate metabolism; glycemia decreased in females. More pronounced changes were recorded with regard to lipid metabolism: increased liver triacylglycerol concentration in females and increased serum corticosterone and phospholipids concentration in males were recorded. Liver malondialdehyde concentration was found increased in both sexes, which is surprising since MEL is a well-known antioxidant. However, Sakano et al. (2004) found the oxidative effect of

Table 3. The effect of 12-week MEL administration on the weight of selected organs and tissues in variant A - overnight fasted rats

	CONT-females	MEL-females	CONT-males	MEL-males
LIVER				
- absolute (g)	5.67 ± 0.10	5.56 ± 0.10	8.95 ± 0.20	8.49 ± 0.25
- relative (%)	2.55 ± 0.03	2.55 ± 0.04	2.49 ± 0.03	2.58 ± 0.05
HEART MUSCLE				
- absolute (mg)	585.00 ± 8.27	598.64 ± 14.02	835.50 ± 14.39	821.14 ± 17.52
- relative (%)	0.26 ± 0.005	0.27 ± 0.004	0.23 ± 0.003	0.25 ± 0.007 **b**
SPLEEN				
- absolute (mg)	632.43 ± 24.19	638.50 ± 29.48	808.50 ± 35.05	830.21 ± 35.84
- relative (%)	0.28 ± 0.008	0.28 ± 0.01	0.22 ± 0.008	0.25 ± 0.01 **a**
THYMUS				
- absolute (mg)	268.29 ± 13.90	253.71 ± 13.37	230.93 ± 16.93	241.21 ± 8.08
- relative (%)	0.12 ± 0.006	0.12 ± 0.004	0.07 ± 0.005	0.08 ± 0.003
ADRENALS				
- absolute (mg)	56.36 ± 1.52	58.57 ± 2.53	46.46 ± 2.00	49.50 ± 1.86
- relative (%)	0.026 ± 0.001	0.027 ± 0.002	0.012 ± 0.001	0.016 ± 0.001 **a**
PERIOVARIAL FAT				
- absolute (g)	4.64 ± 0.44	3.76 ± 0.21		
- relative (%)	2.09 ± 0.20	1.77 ± 0.11		
EPIDIDYMAL FAT				
- absolute (g)			3.84 ± 0.27	2.92 ± 0.19 **a**
- relative (%)			1.07 ± 0.08	0.89 ± 0.06

Data are expressed as means ± S.E.M. Significant differences between MEL group and CONT group are designated as **a** for $p \leq 0.05$, **b** for $p \leq 0.01$.

6-hydroxymelatonin, a MEL metabolite on DNA in vitro. The increase of liver malondialdehyde concentration after MEL administration might arise from higher availability of 6-hydroxymelatonin as a prooxidant.

There are only few reports on the metabolic effects of MEL administered during longer periods. MEL administration to young Sprague-Dawley rats had no effect on metabolic and hormonal variables. However, in middle-aged animals (10 months) 10 weeks of administration (0.2μg/ml) and 12 weeks of administration (0.4 and 4μg/ml) in tap water decreased body mass, intraabdominal fat relative weight and serum insulin and leptin concentration to youthful levels (Rasmussen et al. 1999, 2001). Similar effects as found by the above mentioned authors, of exogenous MEL administered to rats for 3 months at a concentration of 0.4 μg/ml was also reported by Wolden-Hanson et al. (2000). MEL administration from the 10th month of age decreased body mass, serum insulin and leptin concentration and increased serum corticosterone concentration, body core temperature and locomotory activity to youthful levels. Food/water intake, total body fat and glycemia did not differ from the controls (Wolden-Hanson et al. 2000). These results indicate that a natural substitute of the endogenous MEL decrease in older age by exogenous MEL can ameliorate adverse metabolic and functional changes related to ageing. In young animals (3 months of age), exogenous MEL was ineffective (Rasmussen et al. 2001), so it seems that there is an age-related responsiveness to MEL. However, our results of MEL administration to young 5-weeks old animals do not support this hypothesis.

We found no source on fasting and MEL administration in laboratory animals. Short-term fasting in healthy men decreased (Rojdmark and Wetterberg 1989) and in

Table 4. The effect of 11-week MEL administration and fasting on the weight of selected organs and tissues in variant B - 48-h fasted rats

	CONT-females	MEL-females	CONT-males	MEL-males
LIVER				
- absolute (g)	4.86 ± 0.13 ↓**z**	5.00 ± 0.18 ↓**y**	8.19 ± 0.32 ↓**x**	7.49 ± 0.42 ↓**x**
- relative (%)	2.34 ± 0.06 ↓**y**	2.56 ± 0.07 **b**	2.38 ± 0.05 ↓**x**	2.39 ± 0.08 ↓**x**
HEART MUSCLE				
- absolute (mg)	564.20 ± 7.01	529.60 ± 15.24 ↓**y**	806.30 ± 14.28	771.63 ± 38.26
- relative (%)	0.27 ± 0.007	0.27 ± 0.009	0.24 ± 0.005	0.25 ± 0.01
SPLEEN				
- absolute (mg)	646.20 ± 35.05	754.70 ± 42.02 ↑**x**	761.50 ± 30.17	714.63 ± 45.22
- relative (%)	0.31 ± 0.02	0.39 ± 0.02 **b**, ↑**z**	0.22 ± 0.009	0.23 ± 0.02
THYMUS				
- absolute (mg)	256.40 ± 13.91	242.50 ± 8.45	256.80 ± 10.76	274.13 ± 16.11 ↑**x**
- relative (%)	0.12 ± 0.008	0.13 ± 0.007	0.08 ± 0.003	0.09 ± 0.004 **b**, ↑**x**
ADRENALS				
- absolute (mg)	55.50 ± 1.54	59.60 ± 3.57	45.20 ± 1.44	43.88 ± 2.24
- relative (%)	0.026 ± 0.001	0.030 ± 0.002 **a**	0.013 ± 0.0004	0.014 ± 0.0005
PERIOVARIAL FAT				
- absolute (g)	2.73 ± 0.30 ↓**y**	2.28 ± 0.35 ↓**z**		
- relative (%)	1.29 ± 0.13 ↓**y**	1.16 ± 0.16 ↓**y**		
EPIDIDYMAL FAT				
- absolute (g)			3.25 ± 0.24	2.00 ± 0.21 **b**, ↓**y**
- relative (%)			0.94 ± 0.06	0.64 ± 0.06 **b**, ↓**y**

Data are expressed as means ± S.E.M. Significant differences between MEL group and CONT group are designated as a for $p \leq 0.05$, b for $p \leq 0.01$. Significant differences between variant B group and variant A group are designated as follows: x for $p \leq 0.05$, y for $p \leq 0.01$, z for $p \leq 0.001$, symbol ↑ indicates the increase and symbol ↓ indicates the decrease of a particular variable in group B when compared to group A.

postmenopausal women increased endogenous MEL concentration in the serum (Breitins et al. 1985). In women 72-h fasting resulted in a MEL secretion phase-shift (Berga et al. 2001). In mice 24-h or 48-h fasting, respectively, increased MEL in the brain and gastrointestinal tissues (Bubenik et al. 1992). In male Wistar:Han rats fasted for 36 h, pinealectomy decreased serum leptin and increased corticosterone concentrations (Alonso-Vale et al. 2004b).

In our experiment (variant B vs. control group), 48-h fasting in rats of both sexes resulted in the expected serum corticosterone concentration increase; the increase of concentration/content of liver glycogen accompanied by the glycemia rise in males was unexpected. An increase in organ weights following 48-h fasting in MEL rats was recorded particularly in females, with the exception of the heart muscle. In males epididymal fat weight decreased. Mizrak et al. (2004) reported an increased heart muscle weight and myocardial fibrosis formation in pinealectomized female Wistar rats; exogenous MEL did not reverse these effects. We assume that the MEL treatment (4 mg/kg) was ineffective because it was short (3 days only). Decrease of the heart muscle weight in female rats in our experiment is probably the result of the antifibrotic effect of (elevated) corticosterone; the MEL-estrogen interaction could be of great importance too.

When we compared 48-h fasted MEL rats and overnight fasted MEL rats (variant B vs. A), a wider range of changes in metabolic variables was found. Serum glucose concentration along with the liver glycogen content (in both sexes) and serum corticosterone concentration

(in males) increased, indicating a gluconeogenesis acceleration in fasting rats. Since the heart muscle glycogen concentration did not change in MEL rats of both variants A and B, the glycogen concentration increase (variant B vs. A) is the consequence of 48-h fasting in MEL and control rats. Ahlersová et al. (1982) reported increased heart muscle glycogen concentration during 6-day fasting in male Wistar:Han rats, indicating the metabolic specificity of this organ. Forty-eight hour fasting reduced liver malondialdehyde concentration; overnight fasting (variant A) increased it to the level of controls, both in males and females. In both males and females, 48-h fasting increased serum triacylglycerols and cholesterol concentration, phospholipids concentration in the bone marrow and liver, and decreased the liver triaclyglycerols content (in females) and the cholesterol content in both sexes. We explain the serum triacylglycerols and cholesterol increase in MEL-drinking rats as a consequence of hyperlipemia of the retention type, when plasmatic lipids entrance to peripheral tissues is reduced. The liver triacylglycerols decrease (significant only in MEL females) along with the total cholesterol content decrease in both MEL drinking and control rats indicate a lipogenesis attenuation in this organ in consequence of fasting. In both sexes, the liver and adipose tissue weights were reduced, the thymus (in males) and spleen weights (in females) increased and the heart muscle weight (in females) decreased.

In our experiment, changes in the carbohydrate and lipid metabolic variables after prolonged MEL administration were not prominent though we found some intersexual differences. MEL decreased the body mass gain in both sexes when compared to controls. Forty-eight hour fasting induced and increased metabolic changes, and changes in the organ weights. In MEL drinking rats, metabolic changes were more profound after 48-h fasting than after overnight fasting without any prominent intersexual differences.

When considering our results and the results of other experimental studies on the effect of long-term MEL administration, it is necessary to consider carefully MEL administration in clinical practice with regard to its unfavourable metabolic effects.

Metabolické účinky dlhodobého podávania melatonínu a krátkodobého hladovania u laboratórnych potkanov

Cieľom práce bolo vyhodnotenie účinku prolongovaného podávania pineálneho hormónu melatonínu a prídatného krátkodobého hladovania na metabolické parametre u samcov a samíc potkanov kmeňa Wistar:Han. Melatonín (MEL) bol podávaný od 5. týždňa veku zvierat v pitnej vode v koncentrácii 4 μg/ml denne počas 11 (variant B) resp. 12 týždňov (variant A) až do ukončenia experimentu. Kontrolná skupina pila vodu. Potkany boli kŕmené štandardnou diétou ad libitum a boli držané vo svetelnom režime L:D = 12:12h. Na konci experimentu boli zvieratá usmrtené rýchlou dekapitáciou po nočnom hladovaní (variant A) resp. po 48-hodinovom hladovaní (variant B). Boli stanovené hmotnosti zvolených orgánov a tkanív a základné metabolické parametre v sére a tkanivách.

Melatonín znížil telesnú hmotnosť u oboch pohlaví bez zmien v príjme potravy a vody. U samcov (variant A) MEL v porovnaní s kontrolami zvýšil hmotnosť myokardu, sleziny a nadobličiek, znížil absolútnu hmotnosť tukového tkaniva, zvýšil koncentráciu kortikosterónu a fosfolipidov v sére; u samíc bol zaznamenaný pokles glykémie a zvýšená koncentrácia triacylglycerolov v pečeni. Po 48-hodinovom hladovaní (variant B) bol zaznamenaný nárast hmotnosti pečene, sleziny a nadobličiek u samíc pijúcich MEL; u samcov MEL zvýšil hmotnosť týmusu a znížil hmotnosť tukového tkaniva. U oboch pohlaví MEL zvýšil koncentráciu kortikosterónu v sére a glykogénu v pečeni, u samcov zvýšil hodnoty glykémie, u samíc koncentráciu cholesterolu v sére. Zmeny sledovaných ukazovateľov po prolongovanom podávaní MEL záviseli aj od dĺžky hladovania pred dekapitáciou.

Hladovanie po dobu 48 hodín v závere podávania MEL (variant B vs A) znížilo absolútnu hmotnosť pečene a hmotnosť tukového tkaniva u oboch pohlaví, zvýšilo hmotnosť týmusu u samcov; u samíc znížilo absolútnu hmotnosť myokardu a zvýšilo hmotnosť sleziny. U samcov zvýšilo koncentráciu kortikosterónu a fosfolipidov v sére, u samíc znížilo koncentráciu triacylglycerolov v pečeni, u samcov a samíc znížilo obsah cholesterolu v pečeni. U oboch pohlaví zvýšilo glykémiu, koncentráciu glykogénu v pečeni a myokarde, koncentráciu triacylglycerolov a cholesterolu v sére, koncentráciu fosfolipidov v pečeni a v kostnej dreni a znížilo koncentráciu malondialdehydu v pečeni. 48-hodinové hladovanie po prolongovanom podávaní MEL mladým potkanom zväčšilo rozsah zmien sacharidového a lipidového metabolizmu u oboch pohlaví.

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