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ORIGINAL PAPER

Effect of a high-fat diet on 24-h pattern of circulating levels of prolactin, luteinizing hormone, testosterone, corticosterone, thyroid-stimulating hormone and glucose, and pineal melatonin content, in rats

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Abstract Circadian rhythmicity is affected in obese subjects. This article analyzes the effect of a high-fat diet (35% fat) on 24-h changes circulating prolactin, luteinizing hormone (LH), testosterone, corticosterone, thyroid-stimulating hormone (TSH) and glucose, and pineal melatonin content, in rats. When body weight of rats reached the values of morbid obesity, the animals were sacrificed at six different time intervals throughout a 24-h cycle, together with age-matched controls fed a normal diet (4% fat). Plasma hormone levels were measured by specific radioimmunoassays and glucose concentration by an automated glucose oxidase method. In rats under a high-fat diet, a significant disruption of the 24-h pattern of plasma TSH, LH, and testosterone and a slight disruption of prolactin rhythm were found. Additionally, high-fat fed rats showed significantly lower total values of plasma TSH and testosterone and absence of correlation between testosterone and circulating LH levels. Plasma corticosterone levels increased significantly in high-fat fed rats and their 24-h variation became blunted. In obese animals, a significant hyperglycemia developed, individual plasma glucose values correlating with circulating corticosterone in high-fat fed rats only. The amplitude of the nocturnal pineal melatonin peak decreased significantly in high-fat fed rats. The results underlie the significant effects that obesity has on circadian organization of hormone secretion.

Keywords Circadian · High-fat diet · Obesity · Hyperglycemia · Melatonin · Prolactin

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Introduction

Temporal organization is an important feature of the biological systems and its main function is to facilitate the adaptation of the organism to the environment [1, 2]. The daily variation of biological variables arises from an internal time-keeping system, and the major action of the environment is to synchronize this internal clock to a 24 h period. The light–dark cycle, food, ambient temperature, scents, and social cues have been identified as environmental synchronizers or "Zeitgebers" in mammals [1, 2].

There is a large body of evidence that links feeding regimens and food components with the circadian system [3]. A high-fat dietthat contributes to insulin resistance, impaired glucose metabolism, type 2 diabetes mellitus, stroke, and coronary artery disease [4–11] can feed back to influence the biological clock [12]. This could explain why the circadian oscillation of many hormones involved in metabolism, such as corticosterone, insulin, glucagon, adiponectin, leptin, and ghrelin, becomes disrupted in the development of the metabolic syndrome and obesity [3]. Because obesity is a phenotypic expression of energy imbalance [13], and the hypothalamic pituitary thyroid hormonal axis orchestrates metabolic processes like thermogenesis and energy expenditure [14,15], it is conceivable that obese individuals have altered hypothalamic–pituitary–thyroid axis activity, as shown by circadian studies on thyroid-stimulating hormone (TSH) secretion [16].

Food availability acts as a Zeitgeber resulting in, e.g. "food anticipatory activity" among other phenomena [17]. We previously examined this phenomenon by assessing the 24-h variation of pituitary-testicular function in young male Wistar rat animals submitted to food restriction for 4 weeks starting on day 35 of life [18]. Mean secretion of luteinizing hormone (LH) and testosterone decreased, and that of prolactin increased, in calorie-restricted rats along with significant changes in the 24-h secretory pattern of circulating LH, testosterone, and prolactin levels.

As a continuation of those studies, we undertook the present experiment to examine whether a high-fat diet affects the 24-h changes of pituitary-testicular function in rats, as assessed by the changes in LH, prolactin, and testosterone levels. The 24-h changes of two circadian endocrine signals like plasma corticosterone and pineal melatonin, as well as circulating glucose and TSH levels, were also measured. The results underline the significant effects that a high-fat diet has on circadian organization of hormone secretion.

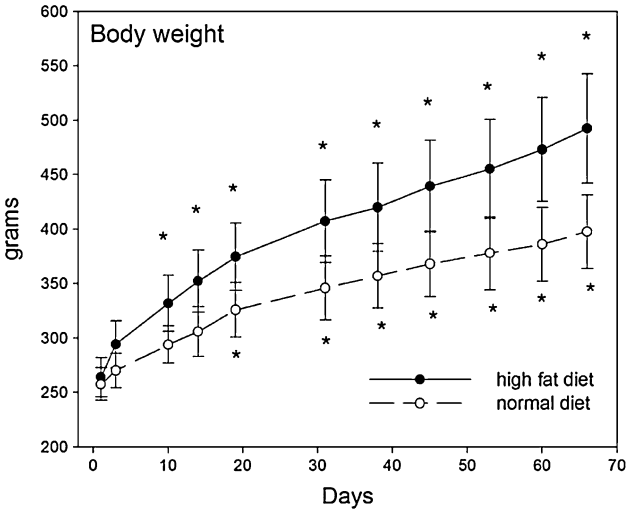


Fig. 1 Progression of body weight in Wistar male rats fed with normal or high-fat diet, as described in Sect. "Materials and methods" shown are the means ± SEM ($n = 48$ rats/group). * $P < 0.01$ vs. initial weight, one-way ANOVA followed by a Student–Newman–Keuls multiple comparisons test. For further statistical analysis, see text

Results

Figure 1 depicts the progression of body weight in the two groups of animals. Weight at sacrifice was 397.5 ± 33.7 (normal diet) and 492.5 ± 50.2 g (high-fat diet). Both diet and time of day were identified as significant factors in the factorial one-way analysis of variance (ANOVA) ($F = 600$ and 201, $P < 0.00001$, respectively). A significant interaction "diet × time" was also found ($F = 12.4$, $P < 0.0001$), indicating the expected cumulative effect of high-fat diet with time (Fig. 1).

The effect of a high-fat diet on circulating levels of prolactin, TSH, LH, testosterone, corticosterone and glucose, and of pineal melatonin content, is depicted in Figs. 2–6. Cosinor analysis of the data is summarized in Table 1.

Figure 2 depicts the daily changes of plasma prolactin, TSH, LH, and testosterone.

As shown in Fig. 2 and Table 1, the changes in prolactin were slight and only limited to a marginally significant interaction "diet × time of day" in the factorial ANOVA ($F = 2.44$, $P < 0.05$) which were not reflected in a significant difference in acrophase in Cosinor, possibly because of the percent of variation explained by the cosine function was significantly less in high-fat fed rats (Table 1). A high-fat diet decreased mean TSH levels by 32% ($F = 15.7$, $P < 0.0001$, factorial ANOVA) and changed significantly acrophases from early morning (08:13 h) to the evening (17:26 h, Table 1). Amplitude of plasma TSH rhythm as described by Cosinor also decreased significantly (Table 1).

Both LH and testosterone daily variations exhibited in controls two peaks (at 13:00 and 21:00 h) and therefore the data did not fit a cosine function (Table 1). The factorial ANOVA indicated that LH and testosterone daily pattern was disrupted by high-fat feeding ($F = 3.25$, $P < 0.02$ and 7.51, $P < 0.0001$ for the interaction "diet × time of day", respectively). In high-fat fed rats, there was a significant dissociation in the acrophases of plasma LH and testosterone values (i.e., 20:21 and 04:30 h, respectively, Table 1). Additionally, high-fat fed animals showed significantly lower mean plasma testosterone levels ($F = 3.61$, $P < 0.04$) (Fig. 2). The correlation between circulating LH and testosterone found in rats fed a normal diet disappeared among high-fat fed rats (Fig. 3).

The effect of the high-fat diet on corticosterone daily secretory pattern is shown in Fig. 4. Both diet and time of day were identified as significant factors in the factorial ANOVA ($F = 28.5$, $P < 0.0001$ and $F = 3.93$, $P < 0.005$, respectively). A high-fat diet increased mean plasma corticosterone by 61%. While in rats fed a normal diet, the pattern found was characterized by low levels of corticosterone during the day, increasing to peak values at night (acrophase at 22:40 h, Table 1), in rats fed a high-fat diet, time of day variation of plasma corticosterone became suppressed (Fig. 4).

As shown in Fig. 4, a significant hyperglycemia developed in high-fat fed rats ($F = 45.1$, $P < 0.00001$). Additionally, time of day changes were significant in both groups of animals with acrophase values of 12:33 (normal diet) and 15:10 h (high-fat diet, Table 1). Individual

Table 1 Cosinor analysis of 24-h changes in plasma prolactin, TSH, LH, testosterone, corticosterone, and glucose levels, and in pineal gland melatonin content, in rats fed with normal or high-fat diet

	Mesor	Amplitude	Acrophase (h, min)	Percent of rhythm
Plasma prolactin				
Normal diet	10.9 ± 2.1	4.1 ± 0.5	01:57 ± 00:20	94.2 ± 10.1
High-fat diet	11.4 ± 1.9	3.9 ± 0.4	02:20 ± 01:57	52.5 ± 7.2**
Plasma TSH				
Normal diet	2375 ± 278	595 ± 85	08:13 ± 02:05	39.3 ± 5.1
High-fat diet	1615 ± 202*	321 ± 62*	17:26 ± 02:52*	43.8 ± 7.2
Plasma LH				
Normal diet	653 ± 82	–	–	–
High-fat diet	732 ± 90	402 ± 58	20:21 ± 02:07	69.1 ± 9.1
Plasma testosterone				
Normal diet	1.30 ± 0.21	–	–	–
High-fat diet	0.81 ± 0.12*	0.28 ± 0.10	04:30 ± 01:16	69.9 ± 12.1
Plasma corticosterone				
Normal diet	283 ± 35	97 ± 11	22:40 ± 01:17	87.3 ± 7.1
High-fat diet	456 ± 42**	n.s.	n.s.	n.s.
Plasma glucose				
Normal diet	98.7 ± 1.2	8.3 ± 1.2	12:33 ± 01:39	72.1 ± 12.1
High-fat diet	114.2 ± 1.3**	7.9 ± 2.1	15:10 ± 03:15	44.1 ± 5.9**
Pineal melatonin				
Normal diet	4,408 ± 512	3,634 ± 512	21:37 ± 01:19	51.6 ± 4.5
High-fat diet	3,087 ± 469*	–	–	–

Shown are the means ± SEM (n = eight per group). Mesor and amplitude values are expressed as ng/ml plasma (prolactin, TSH, LH), ng/ml plasma (testosterone, corticosterone), mg/dl plasma (glucose), or pg/pineal (melatonin). Percent of rhythm defines the part of variation that could be explained by a cosine function in Cosinor. Asterisks designate significant differences (* $P < 0.05$, ** $P < 0.01$) as compared to normal diet, Student's t-test. n.s., not significant daily changes in a one-way ANOVA; (–): not significant changes in Cosinor

plasma glucose values correlated with circulating corticosterone in high-fat fed rats only (Fig. 5).

The high-fat diet brought about a 30% decrease in mean value levels ($F = 13.1$, $P < 0.001$ for diet as main factor, factorial ANOVA) and about a 50% decrease in amplitude of pineal melatonin peak, occurring at the beginning of scotophase (Fig. 6 and Table 1).

Discussion

Foregoing results indicate a significant alteration of circadian rhythmicity in rats fed a high-fat diet, as shown by the disruption of plasma corticosterone rhythm and the decreased of amplitude of daily pineal melatonin rhythm, two important circadian signals. Obesity disrupted plasma TSH rhythmicity by decreasing its mean levels and blunting the daily maximum of TSH seen at the end of scotophase. Both LH and testosterone daily rhythms under a normal diet were also disrupted by high-fat feeding while plasma prolactin rhythmicity was only slightly affected. Additionally, high-fat fed animals showed significantly lower plasma testosterone and absence of correlation of testosterone with circulating LH, thus underlining the disrupting role of obesity on the hypophysial–gonadal axis. Plasma corticosterone increased significantly in high-fat fed rats and, as a consequence, a hyperglycemia developed, as indicated by the significant correlation of plasma glucose values with circulating corticosterone concentration found in these animals.

The key involvement of the adrenocortical axis in obesity in animals is well recognized, since most obese animals are hypercorticoid and many of the metabolic and endocrine impairments are normalized or attenuated by adrenalectomy or by preventing glucocorticoid action [19–21]. Indeed, high-fat feeding alters both basal and stress-induced hypothalamic–pituitary–adrenal activity in the rat [22]. In genetically obese Zucker rats, as well as in other rodent models of obesity [23, 24]. a major observation is that the return of ACTH and corticosterone to initial values was delayed, indicating resistance to glucocorticoid feedback [25]. In a study to assess whether dietary fat-induced increase in corticosterone was due to an altered regulation of hypothalamic–pituitary–adrenal axis, rats fed a high-fat diet exhibited significantly elevated levels of plasma ACTH, corticosterone, fatty acid, and glucose before, during, and after the termination of a restraint stress [22].

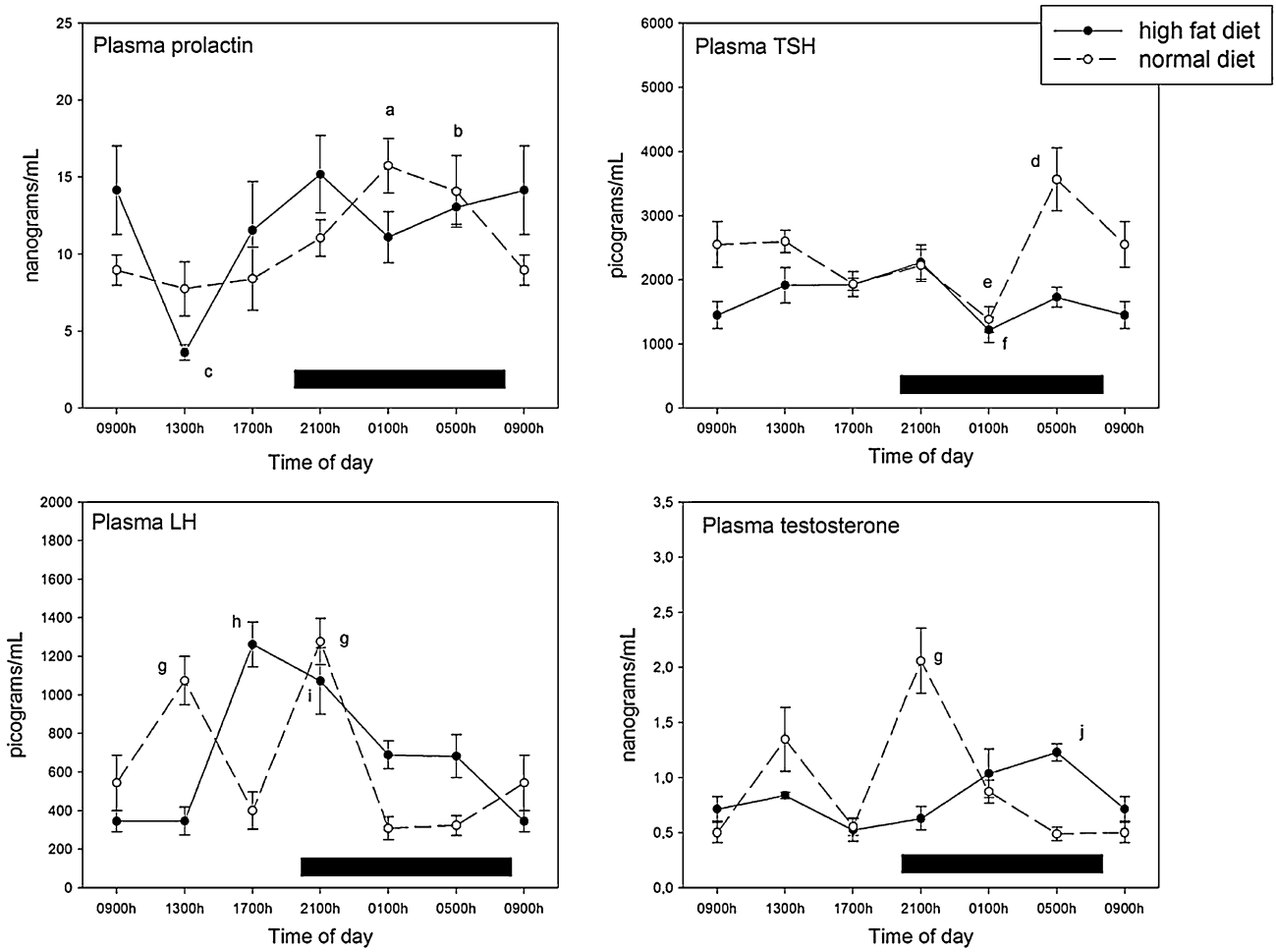


Fig. 2 Twenty-four hour changes in plasma prolactin, TSH, LH, and testosterone in Wistar male rats fed with normal or high-fat diet, as described in Sect. "Materials and methods." Groups of eight rats were killed by decapitation at six different time intervals throughout a 24-h cycle. Bars indicate scotophase duration. Shown are the means ± SEM. Letters indicate the existence of significant differences between time points within each age group after a one-way ANOVA followed by a Student–Newman–Keuls multiple comparisons test, [a] $P < 0.05$ vs. 09:00, 13:00, 17:00, and 21:00 h. [b] $P < 0.05$ vs. 09:00, 13:00, and 17:00 h. [c] $P < 0.05$ vs. 09:00 and 21:00 h. [d] $P < 0.05$ vs. 13:00, 17:00, 21:00, and 01:00 h. [e] $P < 0.05$ vs. 09:00, 13:00, and 21:00 h. [f] $P < 0.05$ vs. 13:00, 17:00, and 21:00 h. [g] $P < 0.01$ vs. 09:00, 17:00, 01:00, and 05:00 h. [h] $P < 0.01$ vs. 09:00, 13:00, 01:00, and 05:00 h. [I] $P < 0.01$ vs. 09:00 and 13:00 h. [j] $P < 0.01$ vs. 09:00, 13:00, 17:00, and 21:00 h. For further statistical analysis, see text

A high-fat diet contributes to insulin resistance, impaired glucose metabolism, type 2 diabetes mellitus, stroke, and coronary artery disease [4–11]. As far as glucose metabolism is concerned, dietary fat not only lowers glucose uptake but also stimulates inappropriate glucose production, resulting in elevations in both circulating insulin and glucose [26, 27]. High-fat diets decrease the number of insulin receptors in liver, skeletal muscle, and adipose tissue, decrease glucose uptake into skeletal muscle and adipose tissue, and decrease hepatic glycolysis and glycogen synthesis [8, 10]. One of the factors accounting for insulin resistance in high-fat fed animals is the elevation of glucocorticoid production that antagonizes most of insulin's actions. Indeed the effects of increased glucocorticoid levels mimic those of a high-fat diet, as suggested herein by the significant correlation between circulating corticosterone and glucose levels found in high-fat fed rats only.

In obese men, sex hormone-binding globulin as well as total testosterone levels are decreased [28, 29]. The present results in obese rats indicate a significant decrease in total plasma testosterone levels and a loss of correlation of testosterone with circulating LH levels. Since saturated fatty acid treatment decreases LH-stimulated adenylate cyclase activity [30] and testosterone levels [31] in rat testis, and induces apoptosis of Leydig cells [32], the present results are compatible with a deleterious effect of high-fat diet on testicular function. It must be noted, however, that interpretation of the total serum testosterone concentration is problematic because it is related directly to

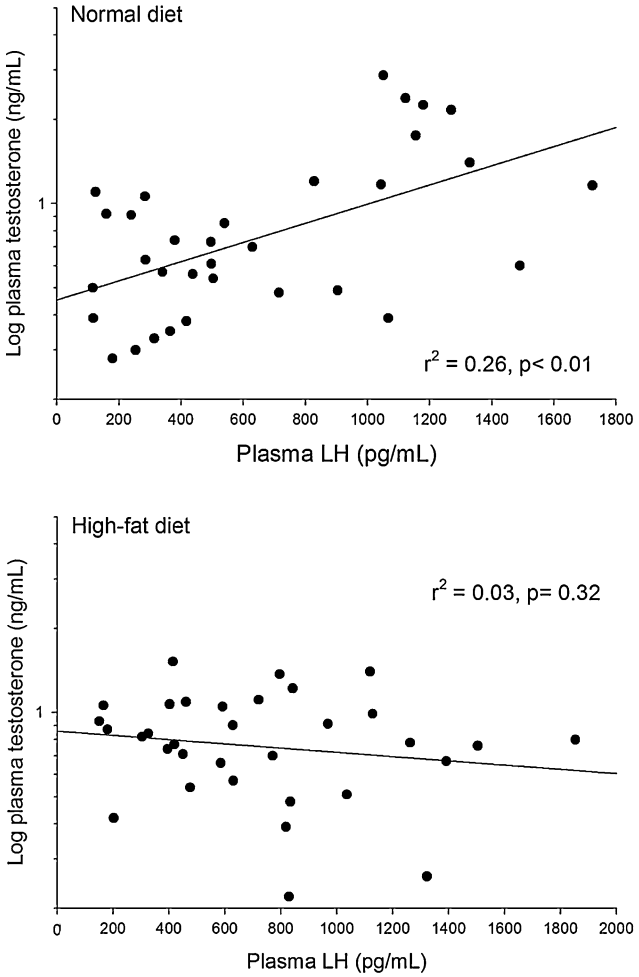


Fig. 3 Semi-log scatter diagrams of plasma testosterone levels plotted against plasma LH in rats fed with normal or high-fat diet

the serum sex hormone-binding globulin concentration. Further studies are needed to assess whether free testosterone are also decreased in high-fat fed rats. Indeed, an estimate of the serum-free testosterone concentration is frequently advised to better assess the clinical status of the obese patient [33], being decreased in some morbidly obese men only [34].

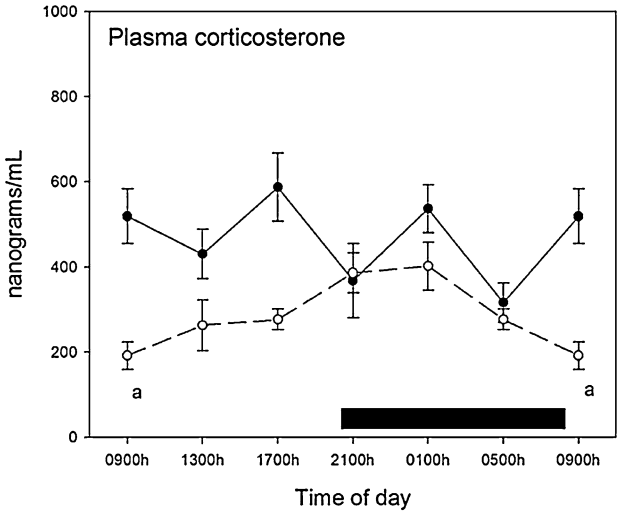
Besides any central effect on the circadian clock, overfeeding or dietary content or both may affect testosterone diurnal rhythm via changes in local autonomic balance. In rats, a high-fat diet increased sympathetic nervous system activation as assessed by urinary norepinephrine excretion and cardiac and renal norepinephrine spillover, while fasting acutely decreased sympathetic nervous system activity [35–37]. The increased local sympathetic activity by high-fat diet could explain both the decrease and rhythm changes in testosterone secretion as well as the dissociation of testosterone secretion from circulating LH levels. We previously reported a similar dissociation in young male Wistar rats animals submitted to a calorie restriction [18]. We do not have yet any plausible explanation on why both a high-fat diet and calorie restriction have a similar dissociating effect of plasma testosterone from plasma LH levels.

It is noteworthy that measurement of TSH secretion over 24 h in obese humans indicated a significant increase [16], while the present results in rats showed a depression of TSH secretion in high-fat fed rats. This observation points out species differences in the effect of obesity on TSH between rodents and humans. Further studies are needed to define to what extent the reduced TSH levels concomitant with weight gain found in rats are accompanied by changes in thyroid activity and an altered basal metabolic rate.

Melatonin has a role in energy expenditure and body mass regulation in mammals [38]. Daily melatonin administration has been found to inhibit age- and olanzapine-related gain in visceral fat [39–41], and to prevent the increase in body fat caused by oophorectomy in rats [42]. The latter study was recently confirmed by Sanchez-Mateos et al. [43] who demonstrated that in ovariectomized rats, melatonin treatment reduced food intake and partially prevented the increase in body weight and cholesterol, without changing leptin levels. Both effects of melatonin were amplified by food restriction.

In a model of diet-induced obesity, Sprague–Dawley rats fed a 39.7% high-fat diet and concomitantly administered with melatonin showed a 50% reduction in body weight increase [44]. Melatonin had no effect on plasma insulin level, but it decreased plasma glucose, leptin, and triglyceride levels significantly. Conversely, in pinealectomized high-fat fed rats, body weight gain and feed efficiency increased, an effect prevented by melatonin treatment [44]. These results, together with the significant reduction of melatonin synthesis in high-fat fed rats herein described, indicate that melatonin can act as a regulator of body weight in this model of obesity and can prevent some of the side effects on glucose homeostasis such as decreased insulin sensitivity.

Reduction in amplitude of circadian rhythms like that reported for melatonin in the present study has been attributed to fatness. For example, in rats susceptible to obesity (Osborne-Mendel rats) the amplitude of rhythm of leptin and insulin was about 50% that in rats relatively resistant to obesity [45]. Other 24 h rhythms are affected by high-fat diet in animals. For example, induction of obesity using a high-fat diet in rabbits causes hypertension by suppression of the nocturnal dip in blood pressure and heart rate and elimination of the day–night difference in both parameters [46]. Diurnal rhythms of blood pressure and heart rate were abolished as early as on day 1 of ad libitum high-fat feeding, before significant changes in body weight were evident [47]. Thus, food intake by itself



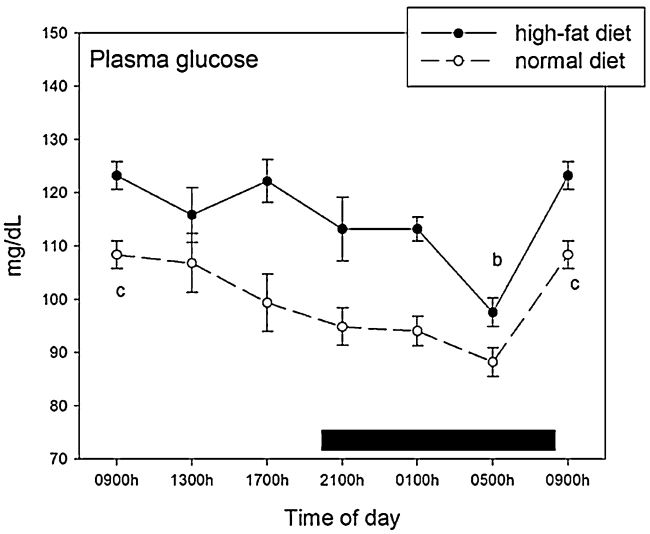


Fig. 4 Twenty-four h changes in plasma corticosterone and glucose levels in Wistar male rats fed with normal or high-fat diet, as described in Sect. "Materials and methods." Groups of eight rats were killed by decapitation at six different time intervals throughout a 24-h cycle. Bars indicate scotophase duration. Shown are the means ± SEM. Letters indicate the existence of significant differences between time points within each experimental group after a one-way ANOVA followed by a Student–Newman–Keuls multiple comparisons test, as follows: [a] $P < 0.02$ vs. 21:00 and 01:00 h. [b] $P < 0.01$ vs. 09:00, 17:00, and 01:00 h, $P < 0.05$ vs. 13:00 and 21:00 h. [c] $P < 0.01$ vs. 01:00 and 05:00 h. For further statistical analysis, see text

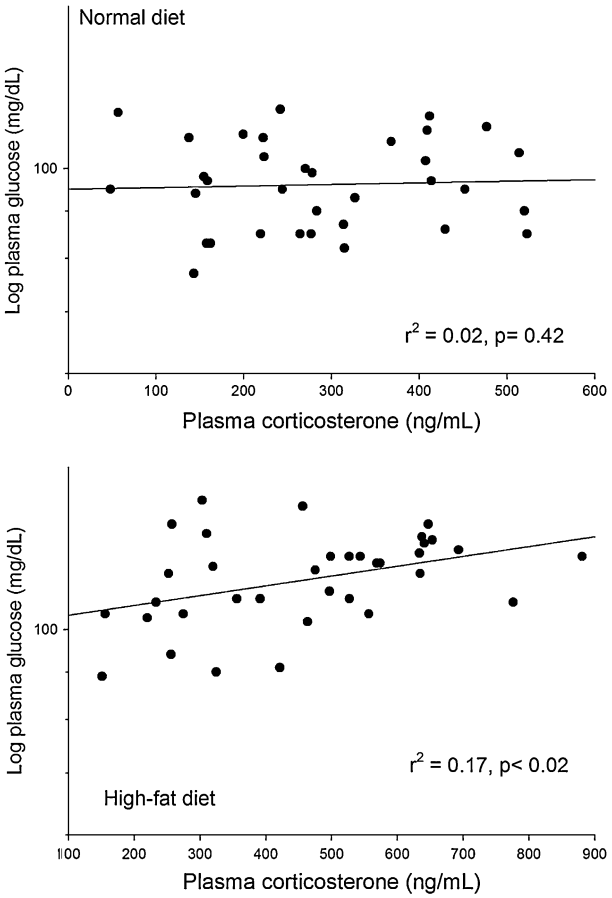


Fig. 5 Semi-log scatter diagrams of plasma glucose levels plotted against plasma corticosterone in rats fed with normal or high-fat diet

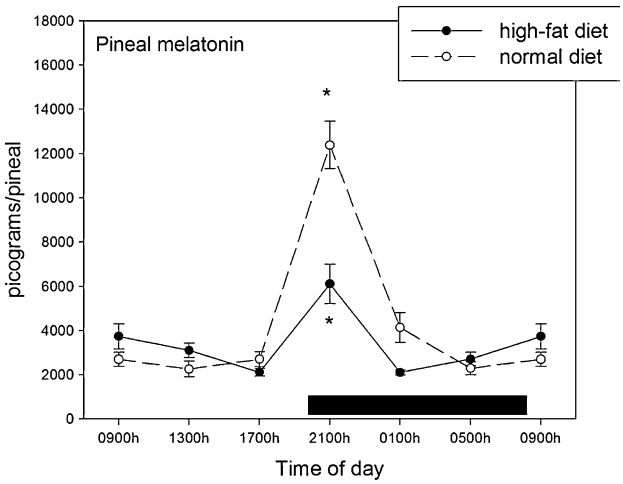


Fig. 6 Twenty-four h changes in pineal melatonin content of Wistar male rats fed with normal or high-fat diet, as described in Sect. "Materials and methods." Groups of eight rats were killed by decapitation at six different time intervals throughout a 24-h cycle. Bars indicate scotophase duration. Shown are the means ± SEM. * $P < 0.01$ vs. the remaining time points, one-way ANOVA followed by a Student–Newman–Keuls multiple comparisons test. For further statistical analysis, see text

appears to be a significant influence on the circadian apparatus.

There are several limitations to the present study. These results suggest that a high-fat diet had profound effects on several hormonal rhythms. However, it is not clear whether the altered hormone levels and circadian rhythms found are due to the high-fat content of the diet, to elevated caloric

intake, or to some indirect result of body weight gain, obesity and fat deposition. The study of rats pair-fed the high-fat diet but matched to the caloric input of the rats fed the control diet could yield valuable information on this point. Further examination is needed to assess whether acute overfeeding immediately disrupted diurnal rhythmicity, as shown for cardiovascular parameters in rabbits [47]. An earlier measurement of hormonal patterns at a time before body weights diverged significantly could be useful in this respect. Another limitation is given by the method of sampling. A pulsatile release exists for several of the hormones measured and such ultradian variations are totally loss with the experimental approach used. In addition, further experiments are needed to assess whether the changes in amplitude as well in timing of 24-h rhythms discussed herein can be attributed to an effect on the SCN or to a masking effect on some output(s) of the clock.

Materials and methods

Animals and experimental design

Male Wistar rats (45 days of age) were maintained under standard conditions with controlled light (12:12 h light/dark schedule; lights on at 08:00 h) and temperature (22 ± 2°C). Rats were divided into two groups: (a) normal diet ad libitum. (b) high-fat diet ad libitum. Both control (4% fat) and high-fat (35% fat) diets were obtained from Harlan interfauna IBERICA S.L., Barcelona, España. Diets were balanced for protein as a percentage of energy intake and for essential vitamins and minerals. The fat source in both the control and high-fat diets was corn oil. The high-fat diet contained 4.8 kcal/g and the 4% fat control diet, 4.0 kcal/g. In place of fat (corn oil), the 4% fat diet contained a slightly greater amount of cornstarch. Individual daily food intake was 17 ± 1 g (normal diet) and 13.5 ± 1 g (high-fat diet). Therefore, the caloric input was about 50% greater in high-fat fed rats. When animals under a high-fat diet reached morbid obesity (i.e., more than 80% weight increase), after 68 days of treatment, rats were sacrificed by decapitation under conditions of minimal stress at six different time intervals (eight rats per group), every 4 h throughout a 24-h cycle, starting at 09:00 h. All experiments were conducted in accordance with the guidelines of the International Council for Laboratory Animal Science. Trunk blood was collected and plasma samples were obtained by centrifugation of blood at 1,500*g* for 15 min and were stored at −20°C until further analysis. Immediately after sacrifice, the pineal glands were dissected out and were collected in 0.1 M acetic acid for further measurement of melatonin content.

Biochemical measurements

Plasma prolactin, TSH, and LH 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 [48]. The intra- and inter-assay coefficients of variation were 6 and 8%, respectively. Sensitivity of the RIA was 48.5 (prolactin), 97.5 (TSH), and 97.5 pg/ml (LH) using the NIDDK rat appropriate standards [48]. Plasma corticosterone and testosterone and pineal melatonin concentration were measured by specific RIAs obtained from ICN Pharmaceuticals, Inc., and Labor Diagnostika Nord GmbH & Co., Nordhorn, Germany. The intra- and inter-assay coefficients of variation were 6 and 8%, respectively. Sensitivity of the RIA was 25 (corticosterone) and 0.1 ng/ml (testosterone) and 6 pg/pineal (melatonin). Plasma glucose concentrations were measured by an automated glucose oxidase method with a Beckman Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA, USA).

Statistical analysis

Statistical analysis of results was performed by a one-way ANOVA or a two-way factorial ANOVA, as stated. For the factorial ANOVA, the analysis included assessment of the group effect (i.e., the occurrence of differences in mean values between normal and high-fat diet rats), of time-of-day effects (the occurrence of daily changes) and of the interaction between the two factors (diet and time, from which inference about differences in timing and amplitude could be obtained). Post hoc Student–Newman–Keuls multiple comparisons tests in a one-way ANOVA were then employed to show which time points were significantly different within each experimental group to define the existence of peaks. Cosinor analysis was used to analyze general rhythmic parameters, i.e., acrophase (the maximum of the cosine function fit to the experimental data), mesor (the statistical estimate of the 24-h time series mean), and amplitude (half the difference between maximal and minimal values of the derived cosine curve). Percent of rhythm defined the part of variation that could be explained by a cosine function. Statistical analysis of Cosinor parameters was carried out by standard procedures [49]. Statistical significance of the derived cosine curves was tested against the null hypothesis (i.e., amplitude = 0). Regression analysis was made on real data by using SPSS software, version 10.1 (SPSS Inc., Chicago, IL, USA), and the results being depicted in semi-log plots. *P*-values lower than 0.05 were considered as evidence for statistical significance.

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