M. I. C. Alonso-Vale
C. N. Borges-Silva
G. F. Anhê
S. Andreotti
M. A. Machado
J. Cipolla-Neto
F. B. Lima

Light/Dark Cycle-dependent Metabolic Changes in Adipose Tissue of Pinealectomized Rats

Abstract

We investigated the effects of pinealectomy on adipose tissue metabolism at different times of day. Adult male Wistar rats were divided into two groups: pinealectomized and control (sham-operated). Eight weeks after surgery, the animals were killed at three different times (at 8.00 a.m., at 4.00 p.m. and 11.00 p.m.). We collected blood samples for glucose, insulin, corticosterone, and leptin determinations, and periepididymal adipocytes for *in vitro* insulin-stimulated glucose uptake, oxidation, and incorporation into lipids. Pinealectomy caused insulin resistance as measured by 2-deoxyglucose uptake (a fall of ~ 40% in the maximally insulin-stimulated rates) accompanied by hypercorticosteronemia at the three time points investigated without changes in plasma insulin an or leptin levels. Furthermore, pinealectomy increased the insulin-induced glucose incorporation into lipids (77%) at 4.00 p.m. and insulin-induced glucose oxidation in the morning and in the afternoon, while higher rates were observed in the evening and in the morning in control rats. In conclusion, cell responsiveness to insulin was differentially affected by pineal ablation and time of day, and persistent insulin resistance was obtained in pinealectomized rats. We hypothesize that pinealectomy exposes the animal to an inadequate match between energy requirements and fuel mobilization.

Key words
Pinealectomy · Adipocyte metabolism · Corticosterone · Insulin resistance · Daily metabolic variations

Introduction

A diurnal variation in blood glucose level has been clearly demonstrated in mammals. Plasma concentrations of glucose and insulin in rats are higher during the dark than during the light period [1]. Counterregulatory hormones such as corticosterone present a circadian variation whereby concentrations are lowest in rats just before lights are turned on and at their peak just before lights are turned off [2].

The pineal gland synthesizes and releases melatonin into the circulation almost entirely during the night period in vertebrates [3,4]. Since the profile of melatonin in plasma accompanies that of the environmental darkness, it is able to signal daily and seasonal timing events to the internal milieu [5]. This internal synchronization role of melatonin has been shown to be crucial for the wide range of physiological systems that exhibit diurnal rhythmicity [4–7], such as glucose tolerance and insulin sensitivity [8–11].

Pinealectomy reduces glucose tolerance, induces insulin resistance, decreases hepatic and muscular glycogenesis, and increases the blood pyruvate concentrations in rats [11–13]. These effects on metabolism can partly be explained by changes in insulin secretion and/or changes in peripheral insulin sensitivity. In addition, melatonin's direct inhibition of adrenal ACTH-induced cortisol release [14] and glucose-induced insulin secretion in pancreatic islets [9], enhancing adipocyte sensitivity to insulin after 4 h *in vitro* treatment [15], have been demonstrated. In addition, we have already demonstrated that pinealectomy in-

Affiliation
Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of Sao Paulo, Brazil

Correspondence
Dr. F. B. Lima, M.D., Ph.D. · Department of Physiology and Biophysics · Institute of Biomedical Sciences · University of Sao Paulo · 1524 Prof. Lineu Prestes Ave. · 05508-900 · Sao Paulo · SP · Brazil · Phone: 55/11/3091-7248 · Fax: 55/11/3091-7248 · E-Mail: fabio@fisio.icb.usp.br

Received 22 September 2003 · **Accepted after revision** 9 February 2004

Bibliography
Horm Metab Res 2004; 36: 474–479 ·
DOI 10.1055/s-2004-825723 · ISSN 0018-5043

duced a reduction in insulin-stimulated glucose uptake by promoting a fall in adipocyte GLUT-4 content [8]. However, there is little information on the role of melatonin in the metabolism of adipose tissue throughout the day.

For a better understanding of this subject, the present investigation aimed to analyze the effects of pinealectomy on glucose uptake, glucose oxidation, lipogenesis and insulin receptor binding at distinct time points of the light-dark cycle (8.00 a.m., 4.00 p.m. and 11.00 p.m.). Plasma levels of leptin, corticosterone, insulin, and glucose were assessed in parallel.

Materials and Methods

Animals

Six-week-old male adult Wistar rats from the Institute of Biomedical Sciences Animal Resource of the University of Sao Paulo weighing 100 to 135 g were initially anesthetized with sodium pentobarbital (Hypnol®, 4.0 mg/100 g body wt. ip.) and subjected to pinealectomy or to sham operation (PINX and CONTROL groups, respectively). Since preliminary studies from our laboratory did not show any differences between the data obtained for sham-operated or for intact animals (data not shown) in the following sections, the sham-operated group was designated as the CONTROL group. After recovery from surgery (according to procedures described elsewhere [16]), the rats were housed in cages (3 – 4 animals per cage) with food (Nuvital® balanced chow pellets) and water *ad libitum* in a temperature-controlled room (25 °C) under a 12/12 h light-dark cycle (lights on at 7.00 a.m.). Body weight as well as food and water consumption were measured weekly. Eight weeks post-surgery, the animals (CONTROL and PINX) were killed by decapitation at 8.00 a.m. and 4.00 p.m. (under white light) and at 11.00 p.m. (under red light), respectively, 1 h after lights on, 3 h before lights off and 4 h after lights off once the pinealectomy was verified as complete. Blood samples were collected into heparinized tubes for determinations of plasma insulin, leptin, corticosterone, and glucose. We opened the abdominal wall, excised the periepididymal fat pads, and processed them for adipocyte isolation and metabolic studies – insulin-stimulated 2-deoxy-D-[^{3}H]glucose uptake, incorporation of D-[U-^{14}C]-glucose into lipids and conversion of D-[U-^{14}C]-glucose to $^{14}CO_2$, and insulin binding. All procedures followed a protocol approved by the Institute of Biomedical Sciences Ethics Committee for Animal Research (CEEA) (No. 032/99).

Adipocyte isolation

Epididymal fat pads were minced with fine scissors and added to a flask containing 4.0 ml of EHB buffer (Earle's salts-25 mM HEPES-4% BSA), 5 mM glucose and 1.25 mg/ml collagenase type II, pH 7.4 at 37 °C [17]. The mixture was incubated for 30 min at 37 °C in an orbital shaker (New Brunswick Scientific, Edison, NJ) at 150 rpm. The isolated adipocytes were filtered through a fine plastic mesh, washed three times with 25.0 ml EHB (Earle's salts, 20 mM HEPES, 1% BSA, no glucose, 2 mM Na pyruvate, and 4.8 mM $NaHCO_3$), pH 7.4, at 37 °C, and resuspended to a final cell concentration of 20% (vol/vol, corresponding to a $7 – 9 \times 10^5$ cells/ml). This cell suspension was maintained in a water bath for 30 min before initiating the biological tests. Adipocyte viability was tested using Trypan blue, and cell number was determined as previously described. Briefly, we measured the isolated adipocytes' (100 cells) diameters under a light microscope equipped with an ocular micrometer. Assuming that the isolated adipocyte is spherical, the mean volume and surface area were determined according to the method by Di Girolamo et al. [18].

Insulin-stimulated 2-deoxy-D-[^{3}H]glucose uptake (2DGU)

Isolated adipocytes (a 20% cell suspension in EHB buffer) were incubated in the presence or absence of a maximally stimulating insulin concentration (10 nM). At the end of the incubation period (30 min), the basal and maximal rates of 2DGU were determined according to protocols described elsewhere [19].

Insulin binding to receptors

From the same 20% adipocyte suspension, 450 µl aliquots in EHB, pH 7.8, were transferred to a 12 × 75 mm polypropylene test tube prepared with a 10 µl mixture of A_{14}-monoiodo-^{125}I-labeled insulin (10 000 counts/min/tube, Amersham International) in the presence or absence of "cold" insulin (0 and 1 µM) in a 500 µl final reaction volume. This mixture was incubated for 180 min in a water bath at 16 °C. The assay was stopped by the centrifugation of 200 µl aliquots through silicone oil, and the radioactivity trapped in the cell pellets was measured as described elsewhere [19]. Specific insulin binding was determined; this is reported as percent total insulin bound per 10^6 cells.

Incorporation of D-[U-^{14}C]-glucose into lipids and conversion of D-[U-^{14}C]-glucose to $^{14}CO_2$

A 450 µl aliquot from a 20% adipocyte suspension in Krebs/Ringer/Phosphate buffer with 1% BSA and 2 mM glucose, pH 7.4, at 37 °C and saturated with a gas mixture of CO_2 5%/O_2 95% was pipetted into polypropylene test tubes (17 × 100 mm) previously containing D-[U-^{14}C]-glucose (0.1 µCi/tube) in the presence or absence of insulin (10 nM), and incubated (500 µl – final volume) for 1 h at 37 °C in an orbital shaking water bath (New Brunswick) (150 rpm). The tubes had an upper isolated well containing a loosely folded piece of filter paper moistened with 0.2 ml of ethanolamine. After incubation, the medium was acidified with 0.2 ml of H_2SO_4 (8N) and the tubes were incubated for a further 30 min period. At the end of incubation, the filter paper was removed and plunged into scintillation vials for the measurement of adsorbed radioactivity and the remaining reaction mixture was treated with 2.5 ml Dole's reagent (isopropanol: n-heptane: H_2SO_4, 4:1:0.25, v/v/v) for lipid extraction [17]. The results are expressed as nmol glucose incorporated into lipids/(10^6 cells/h).

Plasma hormones and glucose measurements

Plasma glucose was determined by the enzymatic glucose-oxidase/peroxidase method [20] using a commercial kit (Glicose SL-e, CELM, Sao Paulo, Brazil). Plasma leptin and insulin levels were quantified using rat leptin and insulin radioimmunoassay (RIA) kits from Linco Research, Inc. (St. Charles, MO). Total corticosterone was also quantified using a commercial RIA kit from Amersham Biosciences (Biotrak Rat Corticosterone, Buckinghamshire, England).

Statistical analysis

Data were analyzed statistically by two-way ANOVA followed by Bonferroni post-tests for multiple comparisons among groups, and p-values less than 0.05 were considered statistically signifi-

cant. Data are presented as the mean ± SEM. The statistical software package used was the GraphPad PRISM version 3.1 from GraphPad Software Inc.

Results

Growth profiles of PINX and CONTROL rats

Body weights (101 ± 4 g and 102 ± 5 g at surgery and 274 ± 5 and 270 ± 4 g at sacrifice for PINX and CONTROL animals, respectively, n = 12, p > 0.05) were similar. No significant differences were detected in mean daily food intake (PINX = 21.7 ± 0.6 g *vs.* CONTROL = 21.9 ± 0.5 g per rat, n = 12, p > 0.05) or water consumption (PINX = 36.8 ± 1.1 ml *vs.* CONTROL = 36.2 ± 0.9 ml per rat, n = 12, p > 0.05) or in mean adipocyte size (239 ± 21 and 223 ± 21 pl of cell volume for PINX and CONTROL, respectively, n = 12, p > 0.05).

Insulin-stimulated 2DGU and insulin binding studies

Fig. **1 a** shows the effect of pinealectomy on 2DGU. The adipocytes of PINX animals showed a fall in maximally insulin-stimulated 2DGU (around 40 %, p < 0.0001) at the three time points investigated compared to CONTROL. The increase in basal 2DGU observed in CONTROL at 11.00 p.m. (p = 0.001) was abolished by pinealectomy. The difference between maximal and basal (Δ_{MX-BS}) 2DGU rates was significantly reduced in PINX at all times investigated (p = 0.001). However, a decrease in Δ_{MX-BS} was detected in both groups during the dark period. Although there was no significant difference in total insulin binding to the adipocytes (p > 0.5) between the two groups at any time, an increase in this parameter was seen in CONTROL at 11.00 p.m. (p = 0.003), but not in PINX animals (Fig. **1 b**).

Conversion of D-[U-^{14}C]-glucose to $^{14}CO_2$ and incorporation of D-[U-^{14}C]-glucose into lipids

As observed in Fig. **2 a**, CONTROL animals showed the highest level of basal and maximally insulin-induced glucose oxidation at 8.00 a.m. followed by a decrement (63 %, p = 0.0001) at 4.00 p.m. and a later recovery at 11.00 p.m. Conversely, PINX rats showed the highest oxidative rates at 8.00 a.m., followed by a progressive decline (55 %, p = 0.002), reaching the lowest level at 11.00 p.m. PINX rats presented a higher $\triangle_{MX-BS}$ of glucose oxidation during the light hours, whereas this increase occurred during the dark period in CONTROL. In addition, at 8.00 a.m. and 4.00 p.m., both the maximally insulin-stimulated and Δ_{MX-BS} responses were higher in PINX than in CONTROL animals (p < 0.05).

Fig. **2 b** shows both basal and maximal rates of insulin-stimulated glucose incorporation into lipids. There was no variation in this parameter in the CONTROL animals throughout the day. In PINX, a 77 % higher rate of maximally insulin-induced glucose incorporation into lipids and a 113 % higher $\triangle_{MX-BS}$ response than in CONTROL (p = 0.0001) were observed at 4.00 p.m. No differences in the basal rates were detected between the two groups.

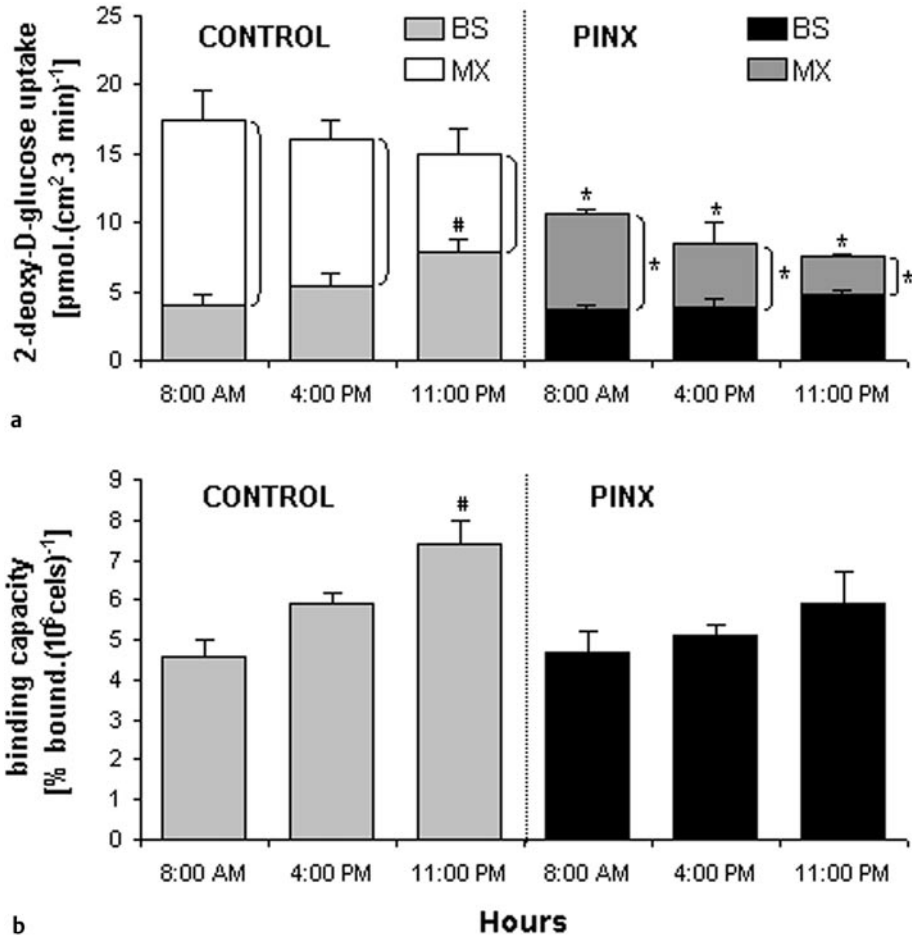

Fig. **1** Influence of pinealectomy and time of day on (**a**) [^{3}H]-2-deoxy-D-glucose uptake and (**b**) binding of insulin to its receptors in isolated adipocytes. The differences between maximal (MX) and basal (BS) rates (corresponding to the area limited by the maximal and basal bars) illustrate the responsiveness to insulin and are represented by the brackets on the right side of the bars. Values are mean ± SEM, n = 7. (*) p < 0.05, PINX *vs.* CONTROL at the same time point; (#) p < 0.05, 11.00 p.m. *vs.* 8.00 a.m. within the same group.

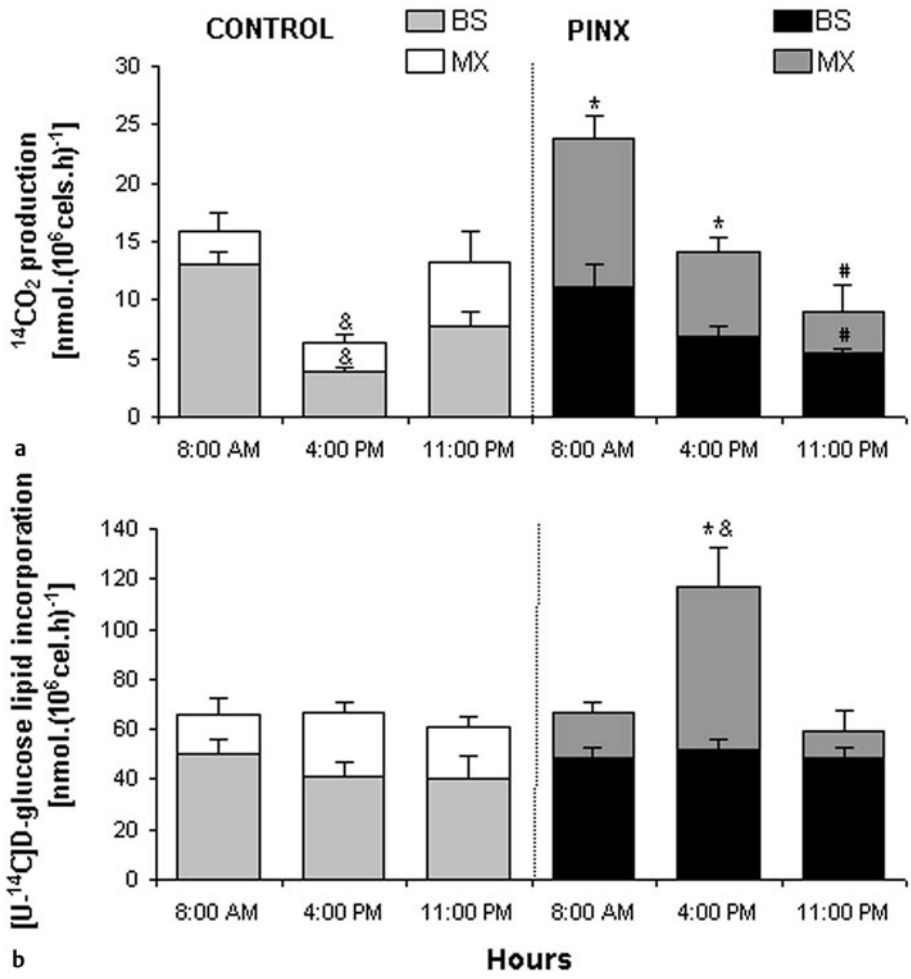

Fig. **2** Influence of pinealectomy and time of day on (**a**) CO_2 production from [U-^{14}C] glucose and (**b**) on [U-^{14}C] glucose incorporation into lipids in isolated adipocytes. Values are mean ± SEM, n = 7. (*) $p < 0.05$, PINX *vs.* CONTROL at the same time point; (#) $p < 0.05$, 11.00 p.m. *vs.* 8.00 a.m. within the same group; (&) $p < 0.05$, 4.00 p.m. *vs.* 8.00 a.m. and 11.00 p.m. within the same group.

Plasma hormones and glucose measurements

Plasma glucose levels (Fig. **3a**) were not significantly altered by pinealectomy; however, an increase was observed at 11.00 p.m. ($p < 0.05$) in both experimental groups. Similar results were obtained for plasma insulin and leptin levels (Fig. **3b** and Fig. **3c**, respectively). Fig. **3d** shows that both groups presented an increase ($p < 0.05$) in corticosterone levels at 4.00 p.m. in comparison to the preceding time (8.00 a.m.). The glucocorticoid levels were higher (± 2 fold, $p < 0.05$) in PINX than in CONTROL at any time investigated.

Discussion

Even though *in vitro* melatonin treatment has been previously demonstrated to inhibit lipolysis and complete glucose oxidation and its incorporation into lipids in adipose tissue of rodents [21–23], these studies have not investigated the effects of pinealectomy on such metabolic parameters or on their modulation over the day. Thus, this is the first study that correlated adipose tissue metabolism at different times of day with the absence of the pineal gland.

Pinealectomy promoted an impressive 2-fold increase in plasma corticosterone levels at all times investigated. Classical studies have shown that melatonin and pinealectomy have a modulatory effect on rat adrenal glands [11, 24, 25]. In addition, a recent study demonstrated the presence of melatonin receptors in primate adrenals, which inhibited ACTH-stimulated cortisol production [14]. These data agree with the hypercorticosteronemia observed in our PINX rats.

Assuming that there was a daily variation in insulin receptor number in CONTROL animals and that pinealectomy disrupted it, and considering that pinealectomized rats develop insulin resistance [8], it is possible that this disturbance somehow contributed to the development of resistance in PINX rats. However, since there was no difference in the absolute amount of receptors between the groups, it is evident that post-receptor defects were triggered by pinealectomy and worked to evoke insulin resistance. Some experimental observations have shown that melatonin potentiates several points of the insulin-transduction pathway in an action mediated by membrane receptors [26]. Thus, the absence of melatonin due to pinealectomy might have reduced the intensity of insulin intracellular signaling contributing to the observed insulin resistance.

In addition, our data showed that pinealectomy caused a persistent increase in glucocorticoid levels, apparently without disturbing its putative daily variation. Hypercorticism elicits insulin resistance, impairs GLUT4 translocation to the plasma membrane, and reduces the transporter content in adipocytes [27, 28]. On the other hand, the lack of melatonin leads to similar results as we have shown here and elsewhere [8]. Therefore, we propose

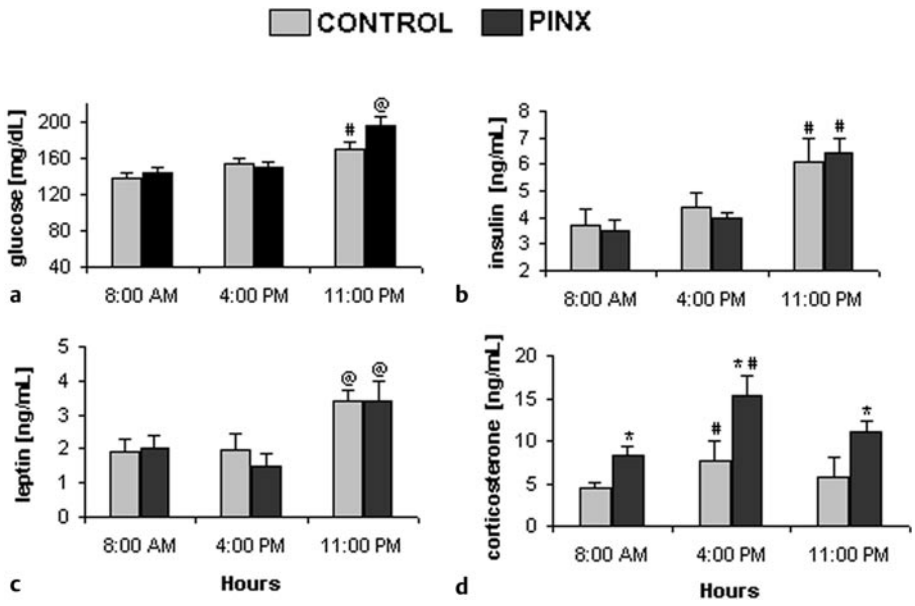

Fig. **3** Plasma glucose (**a**), insulin (**b**), leptin (**c**) and corticosterone (**d**) levels at different times of day for CONTROL and PINX rats. Two groups of animals were decapitated and blood was collected for determination of plasma glucose and hormone levels. Bars represent the mean ± SEM, n = 7. (*) $p < 0.05$, PINX *vs.* CONTROL at the same time point (#) $p < 0.05$, 11.00 p.m. *vs.* 8.00 a.m. within the same group; (@) $p < 0.05$, 11.00 p.m. *vs.* 8.00 a.m. and 4.00 p.m. within the same group.

that insulin resistance is a post-insulin receptor defect and both hypercorticism and lack of melatonin concur to produce the defect. Note, however, that this defect involves almost exclusively the glucose transport system since other actions of insulin (the stimulation of glucose oxidation and its incorporation into lipids) were not similarly regulated. This is the first time that the pineal gland has been shown to influence differential regulation of the pleiotropic actions of insulin *in vivo*.

We observed an important time-of-day-dependent oscillation in adipocyte ability to oxidize glucose in CONTROL rats, with higher values during the evening and morning and lower rates in the afternoon; this indicated that this metabolic activity was higher during the period of more intense energy expenditure. Conversely, PINX rats presented a higher $\triangle_{MX\text{-}BS}$ for glucose oxidation during the morning and afternoon, but not in the evening, which does not appear to match the animal metabolic needs since the rats are less active at these times of day. This effect is essentially similar to that observed by Picinato et al. [10], who showed that pinealectomized rats lose the ability to synchronize the glucose-stimulated insulin secretion during the dark period of the day. These two results show that pinealectomy generates a pathophysiological state that makes the animal metabolically unfit to deal with the daily period of activity. A recent review [29] emphasized that the nervous system and the adipose tissue had complex and reciprocal interactions. Here, we have shown that the pineal gland participates in and modulates such interactions.

Regarding the insulin-stimulated glucose incorporation into lipids, our data show an expressive increase in maximal responsiveness to insulin at 4.00 p.m. in PINX, a phenomenon not detected in CONTROL rats at any of the times investigated. An increase in adiposity with aging has been reported to occur [30–32], that is, opposite to the aging-associated decline in plasma melatonin concentrations [33,34]. We did not observe any significant increase in body weight since we monitored the body weight for only 8 weeks after surgery and in young animals. However, this particular increase in lipogenesis could explain future increases in adiposity such as those observed in middle-aged rats and the prevention of visceral fat accumulation by melatonin treatment as reported by Rasmussen et al. [35,36] and Wolden-Hanson et al. [37].

Leptin, one of the main hormonal products of adipocytes, is strictly related to body mass [38–41] and its release is potentially stimulated by glucocorticoids [42–45]. In the present study, higher increases in corticosterone levels detected in PINX were not accompanied by increased leptinemia. These results suggest that the pineal gland might play a crucial role in corticosterone-induced leptin secretion. On this basis, a direct action of melatonin should be considered since MT1 and MT2 functional receptors have been described in human and rat adipocytes [21,46].

In conclusion, our study emphasizes the crucial role of the pineal gland in the peripheral action of insulin by focusing on the metabolism of adipose tissue. Pinealectomy caused persistent insulin resistance, as measured by 2DG uptake. Taken together with the effects on glucose incorporation into lipids and oxidation, pineal ablation exposes the animal to an inadequate match between the energy requirements and fuel mobilization probably generated by the loss of the internal synchronization between metabolic rhythms and the daily activity-rest cycle. The lack of melatonin together with the upward displacement of the hypothalamus-pituitary-adrenal set point may be the main causative factors underling the mentioned changes.

Acknowledgements

Financial support for this study was provided by the *Fundação de Amparo a Pesquisa do Estado de São Paulo* (FAPESP), grant No. 99/04317-0, and by a fellowship from FAPESP (No. 98/15261-2). The authors would like to thank Melissa M. Zanqueta for the surgical procedures and Sidney Barnabé Peres, Solange M. Franzói-de-Moraes, Miriam H. Fonseca-Alaniz, Luciana C. Brito, Julie Takada and Cecília E. M. Costa for technical support.

References

[1] Pauly JE, Scheving LE. Circadian rhythms in blood glucose and the effect of different lighting schedules, hypophysectomy, adrenal medullectomy and starvation. Am J Anat 1967; 120: 627 – 636

[2] Fischman AJ, Kastin AJ, Graf MV, Moldow RL. Constant light and dark affect the circadian rhythm of the hypothalamic-pituitary-adrenal axis. Neuroendocrinology 1988; 47: 309 – 316

[3] Cagnacci A. Melatonin in relation to physiology in adult humans. J Pineal Res 1996; 21: 200 – 213

[4] Armstrong SM. Melatonin and circadian control in mammals. Experientia 1989; 45: 932 – 938

[5] Armstrong SM. Melatonin: the internal zeitgeber of mammals. Pineal Res Rev 1989; 7: 157 – 202

[6] Martins E Jr, Fernandes C, Bartol I, Cipolla-Neto J, Costa-Rosa LF. The effect of melatonin chronic treatment upon macrophage and lymphocyte metabolism and function in Walker-256 tumour-bearing rats. J Neuroimmunol 1998; 82: 81 – 89

[7] Blask DE, Dauchy RT, Sauer LA, Krause JA, Brainard GC. Light during darkness, melatonin suppression and cancer progression. Neuroendocrinol Lett 2002; 2: 52 – 56

[8] Lima FB, Machado UF, Bartol I, Seraphim PM, Sumida DH, Moraes SM, Hell NS, Okamoto MM, Saad MJ, Carvalho CR, Cipolla-Neto J. Pinealectomy causes glucose intolerance and decreases adipose cell responsiveness to insulin in rats. Am J Physiol 1998; 275: E934 – E941

[9] Picinato MC, Haber EP, Cipolla-Neto J, Curi R, de Oliveira Carvalho CR, Carpinelli AR. Melatonin inhibits insulin secretion and decreases PKA levels without interfering with glucose metabolism in rat pancreatic islets. J Pineal Res 2002; 33: 156 – 160

[10] Picinato MC, Haber EP, Carpinelli AR, Cipolla-Neto J. Daily rhythm of glucose-induced insulin secretion by isolated islets from intact and pinealectomized rat. J Pineal Res 2002; 33: 172 – 177

[11] Diaz B, Blazquez E. Effect of pinealectomy on plasma glucose, insulin and glucagon levels in the rat. Horm Metab Res 1986; 18: 225 – 229

[12] Mellado C, Rodriguez V, de Diego JG, Alvarez E, Blazquez E. Effect of pinealectomy and diabetes on liver insulin and glucagon receptor concentrations in the rat. J Pineal Res 1989; 6: 295 – 306

[13] Milcu SM, Nanu-Ionescu L, Milcu I. The effect of pinealectomy on plasma insulin in rats. In: Wolstenholme GEW, Night J (eds). Pineal Gland. Edinburgh, London: Churchill-Living-stone, 1971: 345 – 357

[14] Torres-Farfan C, Richter HG, Rojas-Garcia P, Vergara M, Forcelledo ML, Valladares LE, Torrealba F, Valenzuela GJ, Seron-Ferre M. mt1 Melatonin receptor in the primate adrenal gland: inhibition of adrenocorticotropin-stimulated cortisol production by melatonin. J Clin Endocrinol Metab 2003; 88: 450 – 458

[15] Lima FB, Matsushita DH, Hell NS, Dolnikoff MS, Okamoto MM, Cipolla-Neto J. The regulation of insulin action in isolated adipocytes. Role of the periodicity of food intake, time of the day, and melatonin. Braz J Méd Biol Res 1994; 27: 995 – 1000

[16] Hoffman RA, Reiter RJ. Rapid pinealectomy in hamsters and other small rodents. Anat Rec 1965; 153: 19 – 22

[17] Rodbell M. Metabolism of isolated fat cells. Effects of hormones on glucose metabolism and lipids. J Biol Chem 1964; 239: 357 – 380

[18] Digirolamo M, Medlinger S, Fertig JW. A simple method to determine cell size and number in four mammalian species. Am J Physiol 1971; 221: 850 – 858

[19] Lima FB, Bao S, Garvey T. Biological actions of insulin are differentially regulated by glucose and insulin in primary cultured adipocytes. Diabetes 1994; 43: 53 – 62

[20] Lott JA, Turner K. Evaluation of trinder's glucose oxidase method for measuring glucose in serum and urine. Clin Chem 1975; 21: 1754 – 1760

[21] Zalatan F, Krause JA, Blask DE. Inhibition of isoproterenol-induced lipolysis in rat inguinal adipocytes *in vitro* by physiological melatonin via a receptor-mediated mechanism. Endocrinology 2001; 142: 3783 – 3790

[22] Ng TB, Wong CM. Effects of pineal indoles and arginine vasotocin on lipolysis and lipogenesis in isolated adipocytes. J Pineal Res 1986; 3: 55 – 66

[23] Murthy GG, Modesto RR. Effects of melatonin and serotonin on 14C-glucose metabolism in rabbit adipose tissue. Experientia 1975; 31: 383 – 384

[24] Oxenkrug GF, McIntyre IM, Gershon S. Effects of pinealectomy and aging on the serum corticosterone circadian rhythm in rats. J Pineal Res 1984; 1: 181 – 185

[25] Heiman ML, Porter JR. Inhibitory effects of a pineal extract on adrenal cortex. Lack of competition with ACTH. Horm Res 1980; 12: 104 – 112

[26] Anhê GF, Caperuto LC, Souza LC, Cipolla-Neto J, Carvalho CRO. Melatonin induces tyrosine phosphorylation of IRS-1 and JAK-2 in the rat pre-optic hypothalamic area in vivo. Diabetes 2002; 51 (Suppl 2): A455

[27] Garvey WT, Huecksteadt TP, Monzon R, Marshall S. Dexamethasone regulates the glucose transport system in primary cultured adipocytes: different mechanisms of insulin resistance after acute and chronic exposure. Endocrinology 1989; 124: 2063 – 2073

[28] Carter-SU C, Okamoto K. Effect of glucocorticoids on hexose transport in rat adipocytes: evidence for decreased transporters in the plasma membrane. J Biol Chem 1985; 260: 11091

[29] Turtzo LC, Lane MD. Completing the loop: Neuron-adipocyte interactions and the control of energy homeostasis. Horm Metab Res 2002; 34: 607 – 615

[30] Wolden-Hanson T, Marck BT, Smith L, Matsumoto AM. Cross-sectional and longitudinal analysis of age-associated changes in body composition of male Brown Norway rats: association of serum leptin levels with peripheral adiposity. J Gerontol A Biol Sci Med Sci 1999; 54: B99 – B107

[31] Barzilai N, Banerjee S, Hawkins M, Chen W, Rossetti L. Caloric restriction reverses hepatic insulin resistance in aging rats by decreasing visceral fat. J Clin Invest. 1998; 101: 1353 – 1361

[32] Shimokata H, Tobin JD, Muller DC, Elahi D, Coon PJ, Andres R. Studies in the distribution of body fat: I. Effects of age, sex, and obesity. J Gerontol 1989; 44: M66 – M73

[33] Reiter R. The ageing pineal gland and its physiological consequences. BioEssays 1992; 14: 169 – 175

[34] Pang SF, Tsang CW, Hong GX, Vip PC, Tang PL, Brown GM. Fluctuation of blood melatonin concentrations with age: result of changes in pineal melatonin secretion, body growth and aging. J Pineal Res 1990; 8: 179 – 192

[35] Rasmussen DD, Mitton DR, Larsen SA, Yellon SM. Aging-dependent changes in the effect of daily melatonin supplementation on rat metabolic and behavioral responses. J Pineal Res 2001; 31: 89 – 94

[36] Rasmussen DD, Boldt BM, Wilkinson CW et al. Daily melatonin administration at middle age suppresses male rat visceral fat, plasma leptin, and plasma insulin to youthful levels. Endocrinology 1999; 140: 1009 – 1012

[37] Wolden-Hanson T, Mitton DR, Mccants RL et al. Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. Endocrinology 2000; 141: 487 – 497

[38] Skurk T, van Harmelen V, Lee YM, Wirth A, Hauner H. Relationship between IL-6, leptin and adiponectin and variables of fibrinolysis in overweight and obese hypertensive patients. Horm Metab Res 2002; 34: 659 – 663

[39] Heo YR, Claycombe K, Jones BH et al. Effects of fatty (fa) allele and high-fat diet on adipose tissue leptin and lipid metabolism. Horm Metab Res 2002; 34: 686 – 690

[40] Ahima RS, Flier JS. Leptin. Annu Rev Physiol 2000; 62: 413 – 437

[41] Campfield LA, Smith FJ, Burn P. The ob protein (Leptin) pathway – A link between adipose tissue mass and central networks. Horm Metab Res 1996; 28: 619 – 632

[42] Dagogo-Jack S, Umamaheswaran I, Askari H, Tykodi G. Leptin response to glucocorticoid occurs at physiological doses and is abolished by fasting. Obes Res 2003; 11: 232 – 237

[43] Bradley Rl, Cheatham B. Regulation of ob gene expression and leptin secretion by insulin and dexamethasone in rat adipocytes. Diabetes 1999; 48: 272 – 278

[44] Russell CD, Petersen RN, Rao SP, Ricci MR, Prasad A, Zhang Y, Brolin RE, Fried SK. Leptin expression in adipose tissue from obese humans: depot-specific regulation by insulin and dexamethasone. Am J Physiol 1998; 275: 507 – 515

[45] Halleux CM, Servais I, Reul BA, Detry R, Brichard SM. Multihormonal Control of *ob* gene expression and leptin secretion from cultured human visceral adipose tissue: increased responsiveness to glucocorticoids in obesity. J Clin Endocrinol Metab 1998; 83: 902 – 910

[46] Brydon L, Petit L, Strosberg AD, Strosberg AD, Jockers R. Functional expression of MT2 (Mel1b) melatonin receptors in human PAZ6 adipocytes. Endocrinology 2001; 142: 4264 – 4271