Circadian Profile of Peripheral Hormone Levels in Sprague-Dawley Rats and in Common Marmosets (*Callithrix jacchus*)

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Abstract. *Aim: In the present study, we report the circadian profiles of a wide panel of hormones measured in rats and common marmosets (Callithrix jacchus), under physiological conditions, paying special attention to minimising the stress imposed on the animals. Materials and Methods: Blood collections were performed over a 24-hour period for the analysis of stress and pituitary hormones, metabolic markers and cytokines from male cannulated rats connected to a fully automatic system, and healthy marmosets in which gender differences were also evaluated. Results: In rats, a significant time effect was observed for corticosterone, prolactin (PRL), thyroid stimulating hormone (TSH), growth hormone, follicle-stimulating hormone, brain-derived neurotrophic factor, total ghrelin, insulin, leptin, insulin-like growth factor-1, adiponectin and interleukin-10. In marmosets, a significant time effect for cortisol, adrenocorticotropic hormone (ACTH), PRL and TSH, with gender effect for ACTH and PRL only, was observed. On the contrary, luteinizing hormone in the rat and active ghrelin, peptide YY, pancreatic polypeptide and gastric inhibitory polypeptide in the marmoset did not show any significant circadian variation. Conclusion: The present work confirmed that, due to time-of-day dependent modulation of hormones, circadian rhythmicity is relevant in physiological studies and should also be taken into consideration when performing pharmacological studies.*

A circadian rhythm is a roughly 24-h cycle in the biochemical, physiological or behavioral processes of living organisms, including mammals, plants, fungi and cyanobacteria. Circadian rhythms dictate patterns of brain wave activity, hormone production, cell regeneration and other biological activities (1). Moreover, they can influence sleep/wake cycles, body temperature and other key physiological functions (2, 3).

The daily variation of biological variables arises from an internal time-keeping system, and the major influence of the environment is to synchronize this internal clock to an exactly 24-h period. Environmental entrainment is mainly achieved by the light-dark cycle, although other external cues, such as food, temperature, scents, and stress, also play a role (4). In addition, almost all diurnal rhythms that occur under natural conditions continue to cycle under laboratory conditions (5).

Due to the great importance that biomarkers are assuming in physiology studies, in medical research and in the drug discovery process, the investigation of the circadian modulation of peripheral hormones under laboratory conditions is becoming increasingly relevant, particularly in the species most adopted in the preclinical evaluations.

In the drug discovery process, the quantification of peripheral bioanalytes can support the exploration of targets for therapeutic intervention, as well as validate mechanisms of drug action. In addition, these bioanalytes may function as pharmacodynamic indicators of drug activity, response and toxicity for subsequent use in clinical development (6). A thorough understanding of the physiological circadian baseline of the bioanalyte under investigation is essential for the optimal design of any biomarker study in a preclinical setting and for the accurate interpretation of variations in the levels of potential biomarkers.

Rats and non-human primates, a nocturnal and diurnal species respectively, play important roles as models in pharmacological and translational studies aimed at developing and testing novel treatments for human diseases. Recent evidence shows that due to their close kinetic and metabolic similarities to humans (7), the use of marmosets is extremely valuable in biomedical research, particularly for preclinical drug development programs and the analysis of complex

behaviors, and provide one of the most valid paradigms for the transition from the preclinical to the clinical research setting. Although the common marmoset (*Callithrix jacchus*) is increasingly used as a model in the study of neuroscience, reproductive biology and infectious diseases, and extensive background information is currently available regarding this species' anatomy and behaviour (8, 9), knowledge about its hormone profile remains very limited.

The aim of the present investigation was to analyse the circadian profile of a wide panel of hormones and cytokines in both rats and common marmosets, paying special attention to the reduction of stress imposed on the animals, in order to work as closely as possible under physiological conditions. Hence, experimental studies were designed in order to reduce stress by using permanently cannulated rats to perform automated blood sampling, and by daily manipulation and spacing blood collection in marmosets over time. Moreover, a multiplex analysis technique was exploited to reduce the volume of sample collected, especially because of the animals' small size.

Materials and Methods

The protocols used in this study were in accordance with the European directive 86/609/EEC governing animal welfare and protection, which is acknowledged by Italian Legislative Decree no. 116, 27 January 1992, and consistent with internal reviews performed by GlaxoSmithKline Committee on Animal Research and Ethics (CARE) and with the company Policy on the care and use of laboratory animals. Efforts were made to reduce animal numbers and minimize their suffering.

Rats. Upon arrival in the animal facility, Sprague Dawley adult male rats (n=16, 275-290 g; CRL:CD Charles River, Italy) were housed in groups of 4-5. They were allowed to acclimatise for 5-7 days in a room controlled for temperature (22±1˚C), humidity (60%), and lighting (12-h light-dark cycle; lights on from 0600 h to 1800 h) with free access to standard food pellets (Altromin R®; Rieper, Germany) and filtered tap water, until they were subjected to surgery for the implantation of femoral vein catheters. On the day of surgery, rats were anaesthetised with 2.5% isofluorane in 100% O$_2$ (Forane, Abbott, Italy). Each rat underwent analgesic (Rimadyl® 5 mg/kg, Pfizer, Italy) and antibiotic (Rubrocillina Forte Veterinaria®, 0.1 ml/rat; Intervet, Italy) treatment after the induction of anaesthesia. A polyvinylchloride catheter (0.5 mm inner diameter, 0.9 mm outer diameter; Instech Solomon, PA, USA) was inserted in the left femoral vein and tunnelled to the interscapular region, where it was exteriorised and then filled with a lock solution (glucose 50% and heparin 500 UI/ml; Sigma Aldrich, MO, USA, and Eparina Vister, Pfizer, Italy, respectively) in saline (Freseinus Kabi, Italy). To protect the catheter, rats were dressed with a Covance harness (Instech Solomon). After surgery, rats were housed singly and allowed to recover at least 5 days before the study. Twice a week, catheters were checked for patency, and the body weight of animals recorded.

Marmosets. Four vasectomized males and four normal cycling females (3-6 years old) were kept in pairs made up of a female and a male, housed in large cages (93×74×168 cm) in the same colony

room. The stage of the menstrual cycle of the females was not known. Temperature was set at 25±1˚C and relative humidity at 55±10%. The photoperiod was set at 12:12 h, with the daylight period between 0530 h-1800 h and simulation of sunrise and sunset occurring between 0530 h-0600 h and 1730 h-1800 h, respectively.

The diet provided was Teklad Global 25% Protein Primate Diet (Harlan, IN, USA), given in the morning (0900 h-0930 h) either as dry pellets or as a mash, mixed with powdered sugar milk and water. This was followed in the afternoon with eggs, cereals and a selection of fruits, such as bananas, apples or pears. They had free access to water supplemented with vitamin C (Cebion; Merck, Darmstadt, Germany).

Circadian rhythms in rats. The experiment was split into two consecutive sessions in order to reduce the total amount of blood collected from each animal within the 24-h period. In each session, each member of one group of adult male rats (n=8) was connected to an Accusampler® station (DiLab, Lund, SE) two hours before the first sampling. Blood collection was performed at 4-h intervals starting at 1000 h and 1200 h for session 1 and session 2, respectively, in order to obtain a final collection of blood at 2 h intervals for a 24-h period. At each time point, 300 μl blood (2×150 μl, with a 5 minute gap) were automatically collected into refrigerated micronic tubes (Thermo Scientific, Waltham, MA, USA). Serum was separated by centrifugation at 1800× *g* for 15 min at 4˚C and split into aliquots in the presence of a protease inhibitor mix (GE Healthcare Biosciences, NJ, USA). Serum aliquots were frozen on dry ice and stored at −80˚C until hormone quantification.

Circadian rhythms in marmosets. Marmosets (n=4/sex) were randomly assigned to two experimental groups (2 males and 2 females/group). Blood was collected at 0000, 0400, 0800, 1200, 1600 and 2000 h. For each group of animals, blood collection for a single time point was performed on alternate weeks in order to reduced stress induced by sampling. For this reason, in order to collect 6 time points/group, 12 weeks were required. Marmosets were previously habituated to manipulation and bleeding procedures. For each blood sampling, the marmoset was caught in its home cage and conveyed to an adjacent procedure room. In all cases, the procedure was completed within 3 minutes from catch and the marmoset was then released back into its home cage. At each time-point, 300 μl blood were collected from the femoral vein into refrigerated tubes containing K3EDTA, aprotinin (500 kIU/ml; Sigma Aldrich) and a protease inhibitor mix (GE Healthcare Biosciences). Blood was centrifuged for 10 min at 1800× *g* at 4˚C, plasma was split into aliquots and samples were stored at −80˚C until hormone quantification.

Quantification of bioanalyte levels. In rats, corticosterone was measured by radioimmunoassay (RIA; MP Biomedicals, CA, USA; the intra- and inter-assay coefficients of variation were respectively <10.5% and <7.5%); prolactin (PRL), thyroid stimulating hormone (TSH), growth hormone (GH), follicle-stimulating hormone (FSH), brain-derived neurotrophic factor (BDNF), adrenocorticotropic hormone (ACTH) and luteinizing hormone (LH) (rat pituitary Milliplex™ MAP; Millipore, MA, USA; the intra- and inter-assay coefficients of variation were respectively <15% and <9.5%); amylin, glucagon, insulin, leptin and glucagon-like peptide-1 (GLP-1) (rat endocrine Milliplex™ MAP; Millipore; the intra- and inter-assay coefficients of variation were between 3.8-10.6% and

4.8-20.7% respectively) and cytokines (interleukin (IL)-1α, IL1-β, IL-2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)-α, interferon (IFN)-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Bio-plex Rat cytokines 9-plex; Bio-Rad, CA, USA; the intra- and inter-assay coefficients of variation were <10%) were measured by Luminex technology on a Bio-plex instrument (Bio-Rad) following the manufacturer's instructions.

Total ghrelin (rat/mouse total ghrelin; Millipore; the intra- and inter-assay coefficients of variation were respectively <2% and <5%); adiponectin (B-Bridge International, CA, USA; the intra- and inter-assay coefficients of variation were <10%) and insulin-like growth factor-1 (IGF-1; Quantikine Mouse Immunoassay, R&D systems, MN, USA; the intra- and inter-assay coefficients of variation were <10%) were measured by ELISA.

In marmosets, cortisol was analyzed by RIA (human GammaCoat™ cortisol RIA kit; DiaSorin, MN, USA). The intra- and inter-assay coefficients of variation were <8% and <10%, respectively. The pituitary (ACTH, PRL, TSH, GH) and the gastrointestinal [active ghrelin, gastric inhibitory polypeptide (GIP), pancreatic polypeptide (PP), peptide-YY (PYY)] hormones were analysed exploiting Luminex technology on a Bio-plex instrument, following the manufacturer's instructions (human pituitary and human gut Milliplex™ MAP, Millipore). The intra- and inter-assay coefficients of variation were <11% and <15% for the pituitary panel and <11% and <19% for gastrointestinal panel, respectively.

Statistical analysis. In the experiments, the samples were randomized on different plates to be analyzed with a Bioplex instrument. In rats, for each analyte separately, a linear mixed effects analysis was applied (SAS version 9.1.3; Sas Institute, NC, USA) on the logged analyte expressions (to improve compliance to assumptions of normality), with time and experimental plate as fixed effects and rat as random effect.

Similarly, in marmosets for each analyte separately, a linear mixed effects analysis was applied (SAS version 9.1.3; Sas Institute) on the logged biomarker expressions, with time and sex (and their interaction) as fixed effects and marmoset as random effect.

In the graphs, data are expressed as estimates of analyte levels at each time point and the corresponding 95% confidence intervals have been constructed. We considered 0.05 as *p*-value threshold to assess statistical significance.

Results

Analysis of circadian rhythms in rats. Most of the measured bioanalytes showed a statistically significant circadian variation during the 24-h cycle. A significant time effect was observed for corticosterone ($F_{(11,62)}$=7.03; $p<0.0001$). As reported in Figure 1, corticosterone levels peaked at 1600 h, while the nadir was at 0600 h, with a 17.13-fold change between maximum and minimum serum concentrations. A significant time effect was observed in the following pituitary hormones: PRL ($F_{(11,58)}$=3.33, $p<0.01$), TSH ($F_{(11,55)}$=11.98, $p<0.0001$), GH ($F_{(11,54)}$=6.68, $p<0.0001$) and FSH ($F_{(11,58)}$=3.80, $p<0.001$) (Figure 2). PRL levels were relatively low in the morning and rose to a maximum at 1800 h, with a 6.42-fold change between minimum and maximum (Figure 2A). A strong increase in TSH levels was
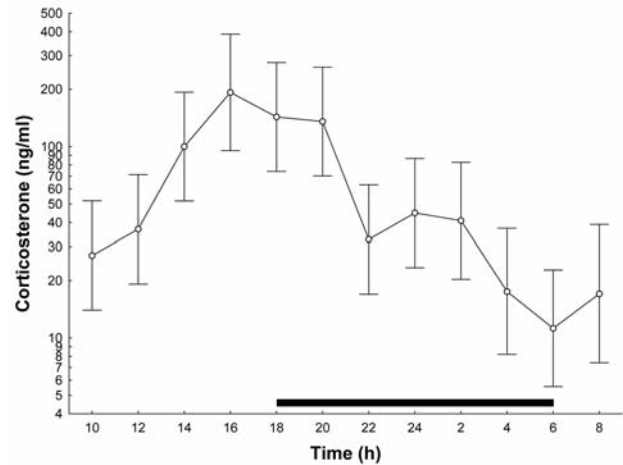
Figure 1. *Circadian profile of corticosterone levels in rat serum (n=8). Data are shown as point estimates ±95% confidence intervals. The period of darkness is indicated by a black line.*

observed at the beginning of the light phase, with a maximum level at 0800 h, and a gradual decrease in the afternoon, with a 9.22-fold change between nadir and peak levels (Figure 2B). Analysis of GH data revealed several peaks during the 24-h. The broad range of data measured at several time points throughout the 24-h period resulted in an 895-fold change between minimum and maximum GH levels (Figure 2C). The FSH profile presented a slight increase (1.94-fold change) at the beginning of the light phase, with a profile resembling TSH, but showing fluctuating levels between 10 and 15 ng/ml during the dark phase (Figure 2D). In the same panel, we also analysed ACTH and LH. ACTH levels were undetectable, while no significant time effect was observed for LH levels ($F_{(11,58)}$=1.46, p=0.17), suggesting a minimal daily variation (data not shown). Analysis of BDNF levels revealed a significant time effect ($F_{(11,58)}$=6.35, $p<0.0001$) with high levels early in the morning, peaking at 0800 h, and a 7.12-fold change from lowest levels was observed during the dark phase (Figure 3).

Serum levels of gastrointestinal hormones, including amylin, glucagon, GLP-1 and total ghrelin showed a significant time effect for total ghrelin only ($F_{(11,62)}$=8.51, $p<0.0001$), with a 2.12-fold change from nadir to peak level. The total ghrelin level reached its maximum at 0600 h, corresponding to the transition from the dark to the light phase (Figure 4). Amylin and GLP-1 were undetectable. Glucagon levels were below the limit of quantification in several samples, thus a sound statistical analysis could not be performed (data not shown).

The analysis of insulin (Figure 5A) and leptin (Figure 5B) levels revealed significant time effects ($F_{(11,59)}$=7.09, $p<0.0001$ and $F_{(11,59)}$=8.44, $p<0.0001$), showing 4.01 and 2.40-fold changes from nadir to peak levels respectively.
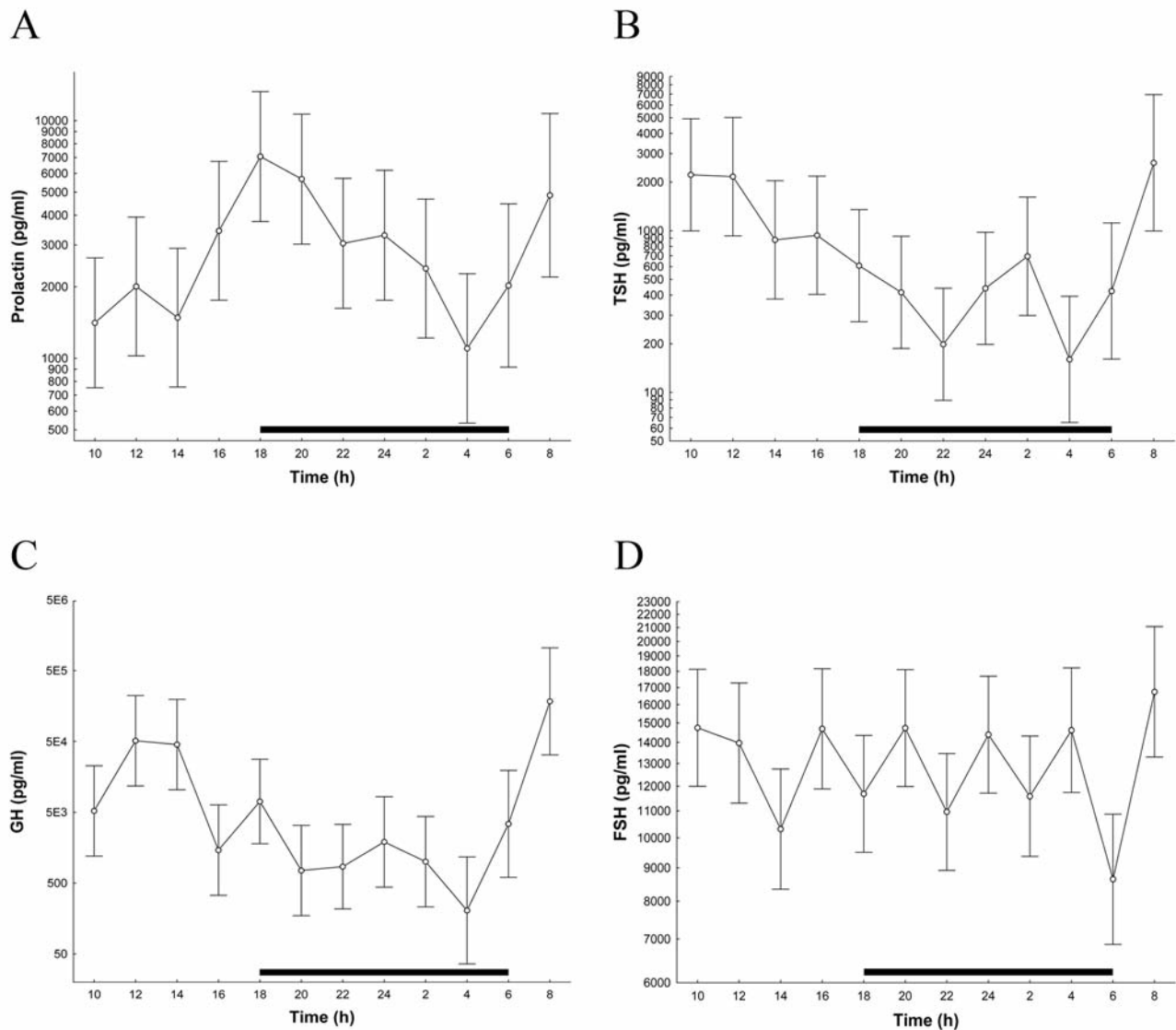
Figure 2. *Circadian profiles of PRL (A), TSH (B), GH (C) and FSH (D) levels in rat serum (n=8). Data are shown as point estimates ±95% confidence intervals. The period of darkness is indicated by a black line.*

They presented similar circadian profiles, with increasing serum concentrations approaching the dark phase, which remained high throughout the whole active period.

IGF-1 data analysis demonstrated a significant time effect ($F_{(11,58)}$=5.74, *p*<0.0001) and its 24-h profile showed high serum levels during the light phase, with a 2.00-fold change from lowest levels, as reported in Figure 6. A significant time effect was observed for adiponectin ($F_{(11,61)}$=2.37, *p*<0.05), with a fold change of 1.44 between minimum and maximum levels, fluctuating without clear peak and nadir in the 24-h period (Figure 7).

In healthy rats, we were not able to plot the 24-h profile for several circulating cytokines, such as IL-1a, IL-4, IL-6,

GM-CSF, IL-2, IL-1β, TNF-α and IFN-γ because they were undetectable in the majority of samples. Among measured cytokines, a significant time effect was described only for IL-10 ($F_{(11,60)}$=4.87, *p*<0.0001). IL-10 profile, reported in Figure 8, shows higher circulating levels during the dark phase compared to the light phase, with a 5.00-fold change between minimum and maximum concentrations.

Analysis of circadian rhythms in marmosets. Cortisol and pituitary (PRL, TSH, ACTH) hormones were measured in common marmosets.

The analysis of cortisol data showed a significant time effect ($F_{(5,30)}$=11.66, *p*<0.0001), without any statistically
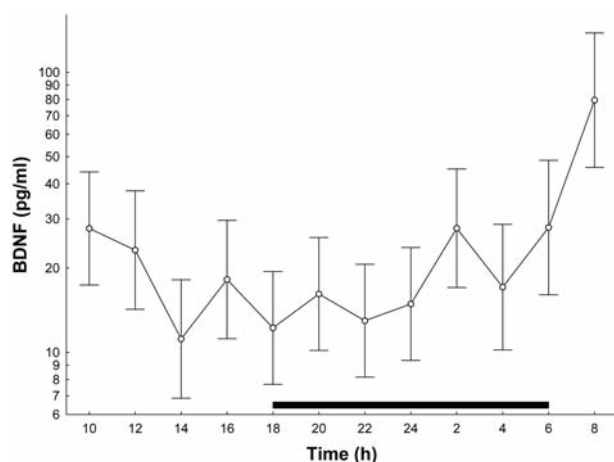
Figure 3. *Circadian profile of BDNF levels in rat serum (n=8). Data are shown as point estimates ±95% confidence intervals. The period of darkness is indicated by a black line.*
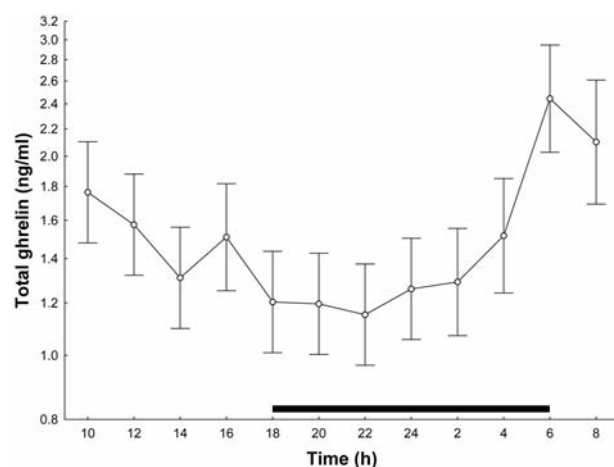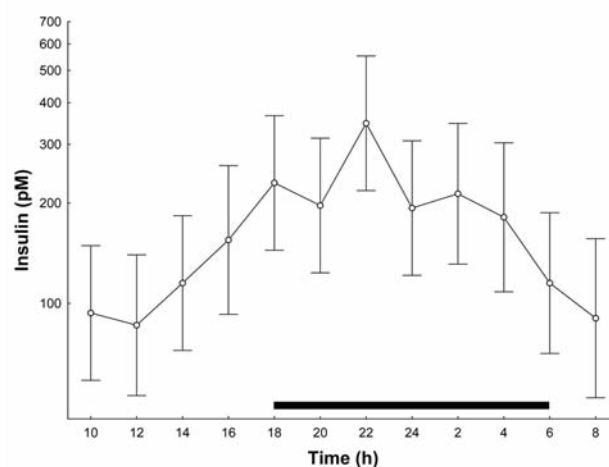

Figure 4. *Circadian profile of total ghrelin levels in rat serum (n=8). Data are shown as point estimates ±95% confidence intervals. The period of darkness is indicated by a black line.*

Figure 5. *Circadian profiles of insulin (A) and leptin (B) levels in rat serum (n=8). Data are shown as point estimates ±95% confidence intervals. The period of darkness is indicated by a black line.*

significant difference between genders ($F_{(1,30)}$=0.25, p=0.62). As shown in Figure 9, higher cortisol levels were observed at the onset of the active phase, peaking at 0800 h, with a 5.97-fold change from the lowest level (2000 h).

The analysis of pituitary hormones demonstrated a significant time effect for ACTH ($F_{(5,30)}$=3.05, $p<0.05$), PRL ($F_{(5,30)}$=8.72, $p<0.0001$) and TSH ($F_{(5,30)}$=4.55, $p<0.05$). ACTH showed a circadian variation with higher levels observed before the onset of the active phase, peaking at 0400 h, with a 2.03-fold change from the lowest levels (0800 h-1200 h) (Figure 10A). In addition, we identified a difference between males and females, with these being at higher concentrations in males ($F_{(1,30)}$=27.85, $p<0.0001$).
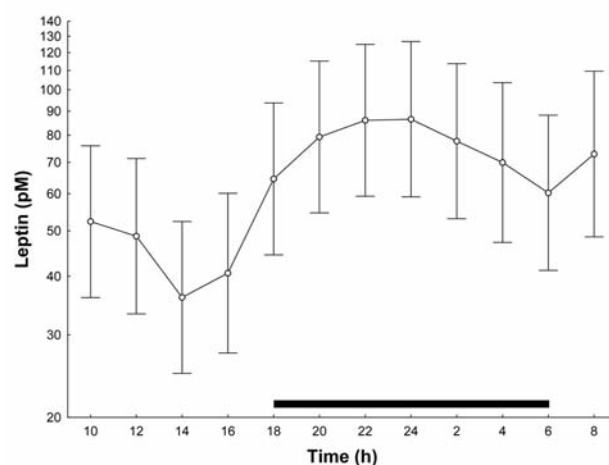
PRL plasma profiles (Figure 10B) were similar in males and females, with high constant levels during the active phase and a nadir just after the beginning of the resting phase. Females showed significantly higher prolactin levels when compared to males ($F_{(1,30)}$=5.95, $p<0.05$). As reported in Figure 10C, TSH plasma levels were constant during the 24-h period, with a sudden transitory decrease at 0800 h, representing a 2.36-fold change, without a gender effect ($F_{(1,30)}$=0.03, p=0.86). The analysis of gut hormone data (active ghrelin, GIP, PP, PYY) did not reveal any significant time or gender effect (data not shown), with the following plasma concentrations measured over the 24-h cycle: 350±41 pg/ml, 58±12 pg/ml, 310±34 pg/ml and 507±43 pg/ml, respectively (expressed as average±SE).
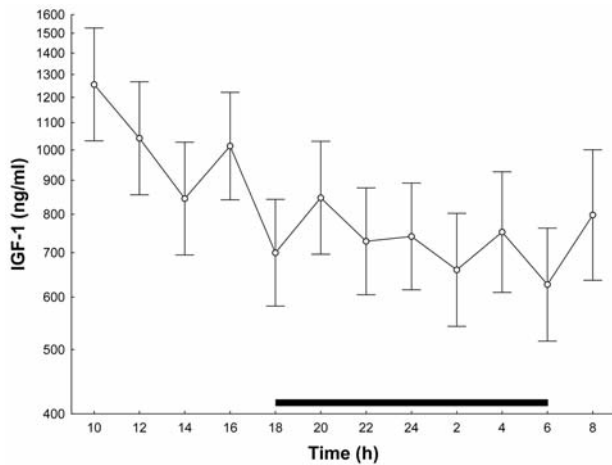
Figure 6. *Circadian profile of IGF-1 levels in rat serum (n=8). Data are shown as point estimates ±95% confidence intervals. The period of darkness is indicated by a black line.*

Figure 8. *Circadian profile of IL-10 levels in rat serum (n=8). Data are shown as point estimates ±95% confidence intervals. The period of darkness is indicated by a black line.*

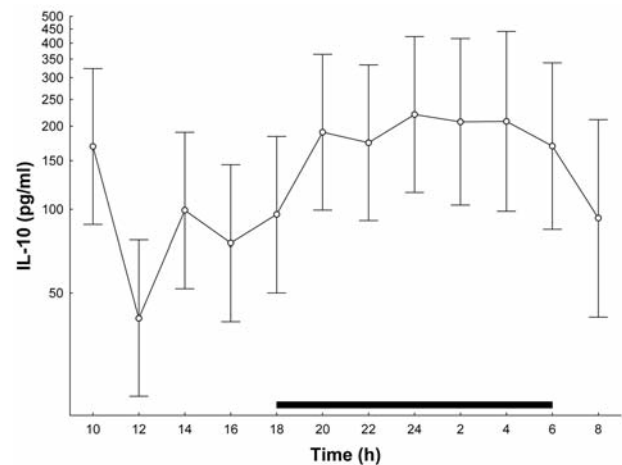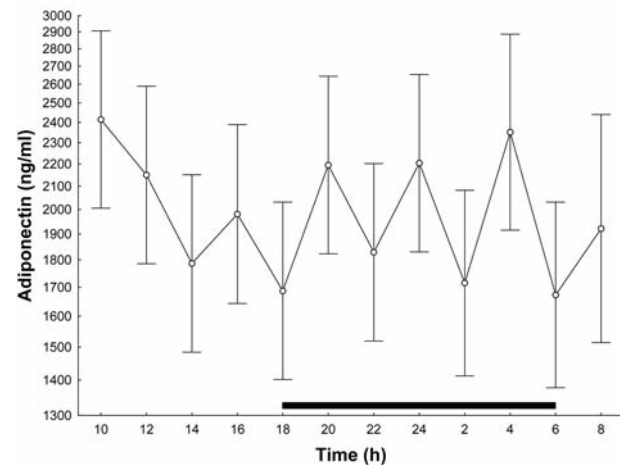Figure 7. *Circadian profile of adiponectin levels in rat serum (n=8). Data are shown as point estimates ±95% confidence intervals. The period of darkness is indicated by a black line.*
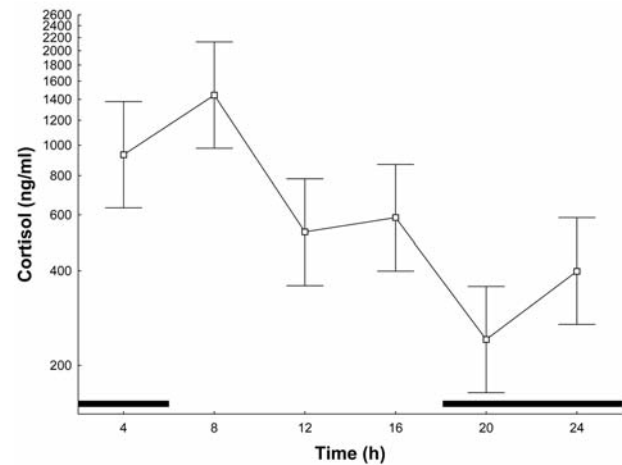

Figure 9. *Circadian profile of cortisol levels in marmoset plasma. The open squares represent levels in female and male marmosets (n=8). Data are shown as point estimates ±95% confidence intervals. The period of darkness is indicated by a black line.*

Furthermore, the interaction between time and sex was not statistically significant for any analyte.

Discussion

The aim of the present study was to evaluate the circadian profile of a wide panel of hormones and cytokines both in rats and marmosets. Consequently, efforts were made to reduce the stress imposed on the animals and to work as closely as possible under physiological conditions. In rats, in order to minimize the stress due to the conventional bleeding procedures (tail blood sampling or decapitation) and to

reduce operator intervention, animals were connected to an Accusampler® apparatus. This procedure allowed serial sampling of peripheral blood in freely moving animals (10), with the reduction of the number of required animals, thus satisfying two of the 3Rs of experimentation (reduction and refinement) (11).

In marmosets, the number and volume of blood collections have to be carefully monitored because of the animal's small size and the risk of haematoma (7). For this reason, daily manipulated animals were used for sampling and blood collections were carried out throughout a period of 12 weeks, subjecting each animal to bleeding only once a week on
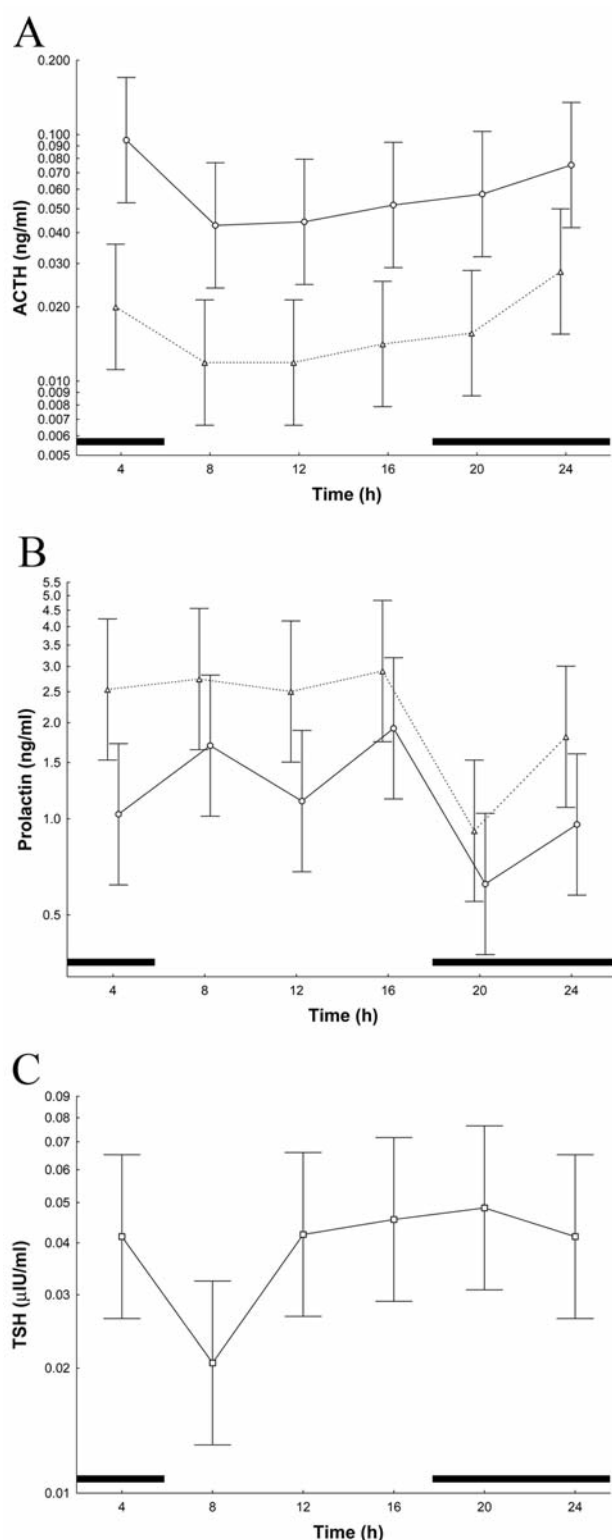
Figure 10. *Circadian profiles of ACTH (A), PRL (B) and TSH (C) levels in marmoset plasma. Open triangles and open circles represent females and males, respectively (n=4). Open squares represent levels in female plus male marmosets (n=8). Data are shown as point estimates ±95% confidence intervals. The period of darkness is indicated by a black line.*

alternate weeks. In conclusion, in both species, the experimental designs we used allowed the stress imposed on animals due to restraint during blood sampling to be minimised. Furthermore, in order to reduce the amount of blood collected, when possible, hormones were measured using Luminex technology, which allows the simultaneous quantification of multiplex analyte panels in a very low sample volume (lower than 30 μl).

In rats, as expected in nocturnal animals having an active phase during the night, and, as extensively described in literature (12-14), circulating corticosterone levels showed a clear circadian variation, with serum concentrations reaching a peak just before the onset of the dark phase.

In marmosets, a diurnal species commonly used in pharmaceutical research laboratories, we observed a 24-h variation of cortisol, the glucocorticoid corresponding to rodent corticosterone. Marmosets exhibited the classical pattern of cortisol variation observed in humans (15, 16), without gender differences, with an early morning acrophase, declining levels throughout the day, and a nocturnal nadir followed by a sudden rise in the second half of the night. In addition, the cortisol rhythm in marmosets showed a close temporal correspondence to the rhythm of plasma ACTH, with a 4 h delay as described for capuchin monkeys (17). In addition, a gender difference was also observed, with higher levels being found in male marmosets.

In rats, the distinctive PRL rhythm observed, with the acrophase concurrent with the onset of the dark phase, is in agreement with data reported in the literature (18, 19) and comparable with our data in marmosets, which also showed the highest levels during the active phase, similar to humans (20). Because PRL prepares the physiology of the female for lactation and maternal behavior, it is often referred to as 'the hormone of maternity'. Indeed, as expected, we detected higher PRL levels in females, as widely described in mammals (21, 22).

Rat TSH circadian rhythmicity, characterized by the presence of a peak between 0800 h and 1200 h, agrees with data reported in the literature (23, 24). In marmosets, we did not observe TSH circadian variation with nocturnal elevation as described in humans (25). However, our results are in agreement with data reported in male Rhesus monkeys: in this primate, TSH levels do not show any circadian variation and are not influenced by sleep wake cycles (26).

GH circadian analysis in rats reported several peaks within the 24-h cycle, thus confirming the peculiar ultradian rhythmicity of this hormone (27, 28). Due to the large individual variability observed at several time points, more frequent blood sampling is required to obtain a detailed profile of secretory GH episodes, as reported in the literature (28).

The rat FSH pattern observed in the present study is suggestive of slightly higher secretion levels between 0800 h-1200 h compared to the oscillating levels observed

throughout the remaining period. Contrasting data were found in the literature, showing no significant diurnal variations (29) or significant 24-h variations in FSH secretion (30, 31).

In the present study, in contrast with literature data presenting 24-h rhythmicity for rat LH (29), we observed low amplitude variations (2.72-fold change), without a significant LH circadian rhythm (data not shown). However, our results are in agreement with older data, reporting no significant LH fluctuations over the 24-h cycle in male rats (30, 32). The investigation of circadian rhythmicity of pituitary hormone levels has lead to contradictory results, possibly due to the influence of stress on their secretion (30). In the present study, circadian patterns of pituitary hormones were generated in freely moving rats, exploiting an automated blood sampling system, thus reporting physiological levels with minimal stress interference (10).

Despite methodological issues regarding the quantification of BDNF in blood (33), under our experimental conditions we were able to observe a significant BDNF circadian variation, with higher levels delayed towards the light phase with respect to the rhythm observed within the suprachiasmatic nucleus (34). Interestingly, rat BDNF showed the acrophase just after the onset of the resting phase (0800 h), with lowest levels between 1400 h–2400 h, opposed to what is observed in humans, where the peak serum BDNF level occurs at the beginning of the active phase with a nadir at 2200 h (35).

Under our experimental conditions, in agreement with the data reported by Bodosi *et al*. (36), highest levels of total ghrelin were measured during the light phase. On the contrary, we did not observe an increase at the beginning of the dark phase as described by Murakami *et al*. (37), except for a slight transient increase at 1600 h.

Exploiting Luminex technology with a human gut panel, we were able to measure active ghrelin, GIP, PP and PYY in marmosets. The statistical analysis did not reveal any significant variation during the 24-h cycle (data not shown).

In rats, insulin and leptin levels were lower during the light phase but started to increase a few hours before the onset of the dark phase concurrently with feeding time, in agreement with literature data (38, 39).

The analysis of IGF-1 levels showed an increase during the light phase in parallel with the increase in GH levels, but shifted 2 hours forward, due to the role of GH in influencing IGF-1 synthesis and secretion (40). In accordance with Oliver *et al*. (41), we did not find a circadian variation of adiponectin levels, but the observed significant time effect seems to reflect the ultradian pulsatility of this adipokine in serum. However, contrasting data are also present in the literature, which report that a daily adiponectin rhythm occurs both in rats (42) and humans (43, 44).

Within the cytokine panel, only anti-inflammatory IL-10 was detectable and showed a significant circadian pattern. Rat IL-10 serum level peaked during the active phase, with lowest concentrations during the resting period. Our results are in agreement with data reporting the immediate suppression of IL-10 production by sleep in humans (45, 46).

In conclusion, the present work extends the existing literature data on the circadian profile of peripheral bioanalytes in rats and marmosets, confirming their time-of-day dependent modulation. In addition, on one hand the use of the Accusampler® apparatus allowed the reduction in the number of animals used, providing high quality data while minimizing both stress and inter-animal variability. On the other hand, exploiting the Luminex technology, we were able to measure several analytes in a low sample volume that could be easily collected even in small-size animal species, such as rats and marmosets.

In the light of our results, obtained under physiological conditions, circadian rhythmicity of circulating hormones in preclinical species should be taken into consideration when designing experiments and evaluating data in pharmacological studies before the progression to human studies.

Declaration of Interest

All Authors were full-time GlaxoSmithKline employees when the study was performed.

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