Capturing the diurnal changes in renin activity and blood pressure to streamline drug therapy of Renin-Angiotensin-Aldosterone-related disorders in dogs.

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Introduction

Though daily variations in renin activity (RA) and blood pressure (BP) have been extensively characterized in human beings (Kunita *et al.,* 1976; Cugini *et al.,* 1985), little is actually known about the periodicity of these variables in dogs. Gordon & Lavie (1985) have reported nocturnal increases in RA with concomitant peaks in urine osmolality and potassium excretion in four adult, female mongrel dogs fed a regular diet. Tuning in to body's rhythms can have a substantial impact on the effectiveness of drug therapies. Investigations in human patients have specifically documented differences in efficacy and duration of action depending on the administration time of drugs interacting with the Renin-Angiotensin-Aldosterone System (RAAS). The present manuscript reports the results of a pioneer experiment investigating diurnal changes in RA and its relation to BP in dogs. Blood specimens for RA determination were collected once every 2 hours over a 24-hour span in eighteen healthy beagle dogs, while systolic and diastolic BP was recorded continuously from three healthy telemetered individuals[1].

[1] RA determination and telemetry recordings were performed in two separate groups of animals to prevent manipulation-related disturbances (*e.g.* sample collection) on BP (Baumgart, 1991).
Baumgart P (1991). Circadian rhythm of blood pressure: internal and external time triggers. *Chronobiol Int*, 8(6):444-50.

Problem list

- Tuning in to body's rhythms to adapt drug dosing schedules
- Do renin activity and blood pressure oscillate with a circadian periodicity in dogs?
- What is the contribution of RAAS peptides to the regulation of blood pressure?
- Does it matter when food is taken?

Tuning in to body's rhythms to adapt drug dosing schedules

Little attention is usually paid to the time at which medications should be given. A common thinking is that taking them once a day, in the morning, would improve drug compliance. Yet, recent investigations have pointed out the importance of administration time-dependent effects of treatment in the management of several diseases *e.g.* human rheumatoid arthritis, cancer and cardiovascular diseases. Sole & Martino (2009) have shown that heart and vessels growth and remodeling were dynamic and occurred more actively during the period normally allocated to sleep. According to the authors this piece of information provides a molecular rationale for the temporal targeting of remodeling by ACE inhibitors (ACEI). In mice, administrations of Captopril at sleeping hours significantly improved cardiovascular function and reduced adverse remodeling, while no effects were reported when Captopril was given during active hours of the day (Martino *et al.,* 2011).

A rich body of literature has established that RAAS peptides oscillate with a circadian periodicity in humans. During sleeping hours, renin secretion increases and is probably the main controller of blood pressure. Results from Hermida & Ayala (2009) in hypertensive patients indicate that administrations of Ramipril at night significantly decreased BP at all times of the day, whereas the reduction in BP was only transient when Ramipril was given in the morning. These differences may also depend on time-dependent changes in pharmacokinetics along the 24-hour span. Variations in gastric pH, motility, blood flow and liver enzyme activity can contribute to circadian stage-dependent change of the disposition of drugs. The understanding of circadian rhythms can thus have a substantial impact on the therapeutic management of RAAS-related diseases by determining the time of drug administration that would optimize efficacy while minimizing the occurrence of adverse effects.

Do renin activity and blood pressure oscillate with a circadian periodicity in dogs?

In the present experiment, dogs were exposed to natural daylight, in addition to fluorescent light from 06.00 am to 06.00 pm, and were fed a regular diet (BioMill Adult Medium®) early in the morning (07.00am). The area under the curve of day (AUCs $_{(7.00\text{-}19.00)}$) *versus* night (AUCs $_{(19.00\text{-}07.00)}$) observations were derived from individual time course profiles, averaged and compared by analysis of variance[2]. Two-tailed p-values < 0.05 were considered as significant.

As highlighted in Figure 1 RA, diastolic and systolic BP fluctuated over a 24-hour span, with low values in the morning followed by a substantial rise in the daytime, and a peak activity at late evening. AUCs $_{(19.00\text{-}07.00)}$ were 91%, 9% and 8% higher than AUCs $_{(7.00\text{-}19.00)}$ for RA (p-value: 0.0001), diastolic (p-value: 0.02) and systolic BP (p-value: 0.003), respectively (see Figure 2).

A distorted cosine model was used to characterize the rhythmicity of RA, systolic and diastolic BP, as follows:

$$f(\theta i, tij) = \Gamma i \; x \; (1 + ai \; x \;\; cos((\, tij - \lambda i) \; x \; (\frac{2\pi}{\phi i})))$$

Where Γi is the mesor or baseline (daily average of rhythm) for the i^{th} individual, ai is the amplitude of the cosine, λi is the acrophase (phase shift), and ϕi is the period for that individual *i* (see Figure 3).

Nonlinear mixed effects modeling was performed using NONMEM version 7.2[3]. The first order conditional estimation method was used for all analyses. Graphical assessment was performed using the R-based software Xpose version 4.1[4]. Basic goodness-of-fit plots, including population and individual predictions *versus* observed concentrations and the distribution of the weighted residuals over time were used for diagnostic purposes (see Figure 4). Similar to Kawasaki *et al.* (1990) a single cosine

[2] Under the assumptions that the observations were log-normal distributed, which seemed reasonable based on available literature (*e.g.* Bodey *et al.*, 1996).
Bodey AR, Michell AR. (1996). Epidemiological study of blood pressure in domestic dogs. J Small Anim Pract, 37(3):116-25.
[3] Icon Development Solutions, Ellicott City, Maryland, USA.
[4] Jonsson and Karlsson (1999) in R version 2.8.1 (The R Foundation for Statistical Computing, Vienna, Austria).

model was found to describe the dynamics of renin activity and blood pressure well. Altogether these results indicate that RA and BP oscillate with a circadian periodicity in dogs. Individual plots are available in Figure 5.

What is the contribution of RAAS peptides to the regulation of blood pressure?

Our data showed that RA and BP variables oscillate in parallel along the 24-hour span. There are many ways by which components of the RAAS participate in the control of systemic BP. As part of the *juxtaglomerular apparatus*, the macula densa (MD) is an area of specialized cells that respond to changes in volume or sodium chloride concentration in the tubular fluid by mediating renin release from granular cells of the kidneys. In a study by Passo *et al*. (1971) the increase in renin secretion was associated with a substantial rise in arterial BP in 16 dogs. Additional investigations have shown that renin by itself had little influence on BP. Instead, it converts inactive forms of angiotensins into angiotensin I with subsequent formation of angiotensin II (AII) that leads to increased aldosterone secretion from the adrenals. The main contribution of the RAAS to BP regulation is mediated by the Na-retaining effects of aldosterone, and the powerful vasoconstrictor action of AII. The role of RAAS activation in the development of congestive heart failure (CHF) and hypertension has long been recognized. Hypertension has been reported as highly prevalent in dogs and cats with kidney diseases (Ross, 1992). In a renal failure model by Mishina & Watanabe (2008), the RAAS was significantly activated in association with apparent increases in BP, indicating that the RAAS was involved in the development of nephron loss-associated hypertension in those dogs.

Does it matter when food is taken?

To determine the effect of feeding time on the periodicity of RA and BP, dogs were fed a regular diet at 07.00pm in the second part of the study. Measurements were performed using the same experimental procedure as described before. As reported in Figure 6, when food was offered at 07.00pm instead of 07.00am, diurnal variations in RA and BP disappeared. Similar conclusions on RA were drawn by Kunita *et al*. (1976) in five healthy men where circadian changes in RA disappeared when meals were taken at night instead of the usual times of the day. The authors' conclusions were that feeding time modified the circadian rhythm of RA due to differences in the timing of sodium ingestion. The influence of sodium intake on the periodicity of RAAS peptides have been assessed by several chronobiological studies (*e.g.* Cugini *et al.,* 1985). According to Cugini *et al*. cells of the MD act as an oscillator capable of modulating the function of the RAAS in a rhythmic manner. In that context, changes in the administration time of food (*i.e.* sodium) could have disrupted the circadian periodicity of RA, as observed in our experiment.

Conclusions

In view of the present results RA and BP oscillate with a circadian periodicity in healthy beagle dogs. Diurnal variations in RA disappeared when dogs were fed in the late afternoon (07.00 pm), indicating that food intake may drive the oscillations of renin in dogs. This work will be followed by investigations on RA and BP in disease dogs under ACEI to determine whether it is possible to improve drug therapy of CHF or hypertension by selecting the appropriate time of treatment.

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Figure 1: Geometric mean plasma renin activity (pg/mL/h) *vs*. time profiles in dogs fed a normal-sodium diet at 07.00 am. Vertical bars indicate +/-one standard error of the geometric mean. Time starts at 07.00 am clock time (*e.g*. 0: 07.00 am, 12: 07.00 pm). Limit of quantitation: 30 pg/mL/h.

The data show that RA oscillates with a circadian periodicity in fed dogs. Measured levels of RA were low in the morning, rose during daytime and peaked in the evening (09.00 pm).

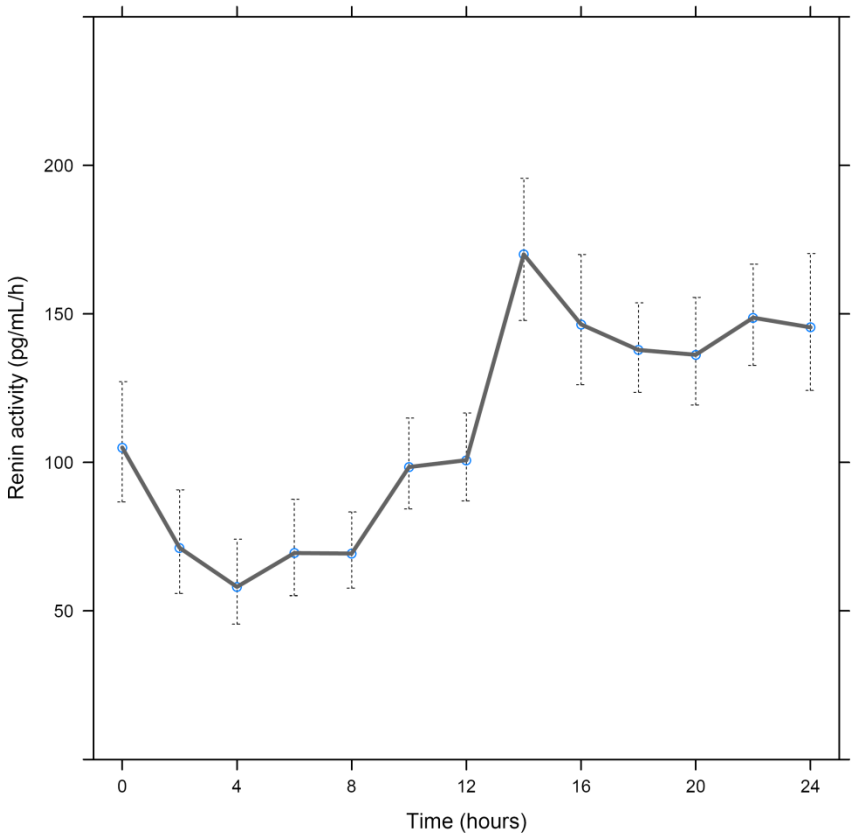

Figure 1 (cont'd): Geometric mean systolic (upper pane) and diastolic blood pressure (mmHg) (lower pane) in dogs fed a normal-sodium diet at 07.00 am. Vertical bars indicate +/-one standard error of the geometric mean. Time starts at 07.00 am clock time (*e.g*. 0: 07.00 am, 12: 07.00 pm).

Systolic and diastolic BP oscillated in parallel, and in parallel to RA over the observation span. A significant trend could be identified whereby BP values are higher at night compared to daytime.

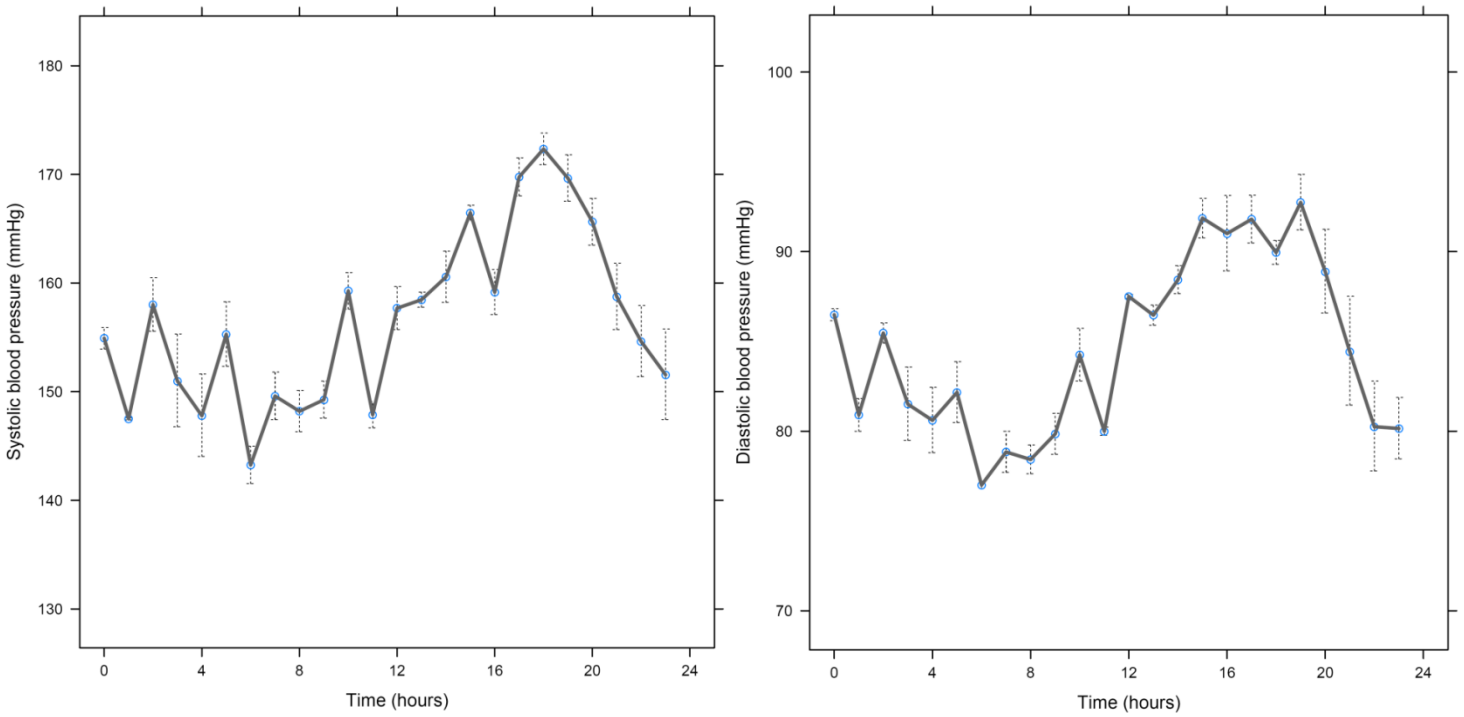

Figure 2: Area under the curve of night (AUCs $_{(19.00\text{-}07.00)}$) *versus* day (AUCs $_{(7.00\text{-}19.00)}$) observations derived from individual time course profiles. Differences from AUC $_{(7.00\text{-}19.00)}$ are expressed in percentage. Vertical bars indicate one standard error. Significant differences are indicated by an asterisk (*).

The data show that AUCs $_{(19.00\text{-}07.00)}$ were 91%, 9% and 8% higher than AUCs $_{(7.00\text{-}19.00)}$ for RA (p-value: 0.0001), diastolic (p-value: 0.02) and systolic BP (p-value: 0.003), respectively.

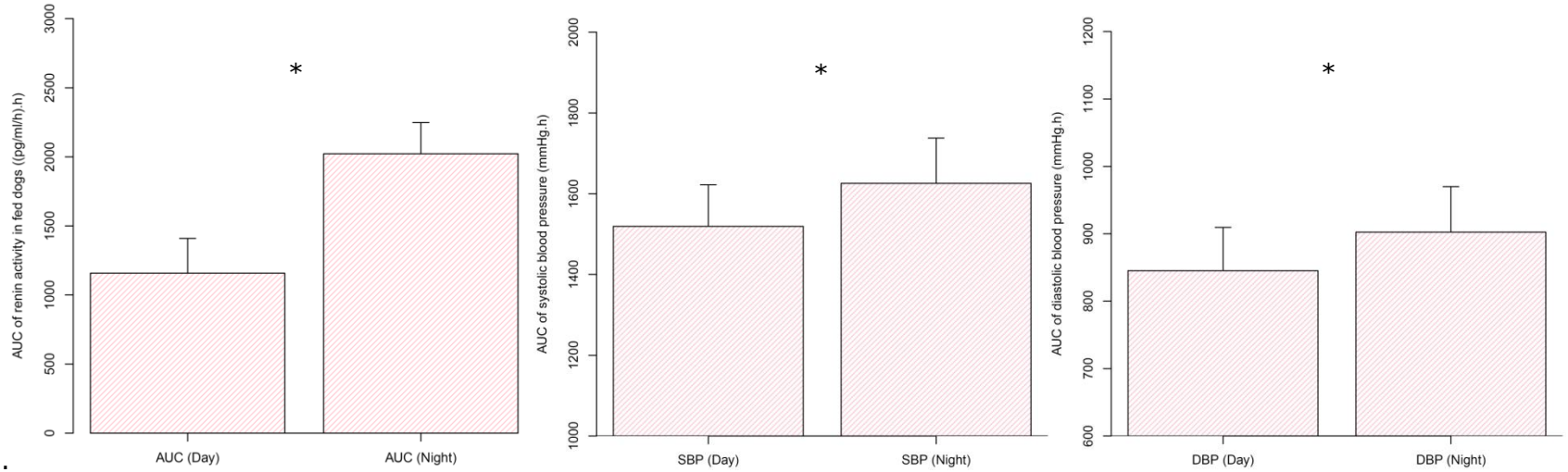

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Figure 3: Model of cosinor analysis. A distorted cosine model was used to characterize the rhythmicity of RA, systolic and diastolic BP, as follows:

$$f(\theta i, tij) = \Gamma i \; x \; (1 + ai \; x \;\; cos((\; tij - \lambda i) \; x \; (\frac{2\pi}{\phi i})))$$

Where Γi is the mesor or baseline (daily average of rhythm) for the i^{th} individual, ai is the amplitude of the cosine, λi is the acrophase (phase shift), and ϕi is the period for that individual *i* Similar to Kawasaki *et al.* (1990) a single cosine model was found to describe the dynamics of renin activity and blood pressure well. Altogether these results indicate that RA and BP oscillate with a circadian periodicity in dogs.

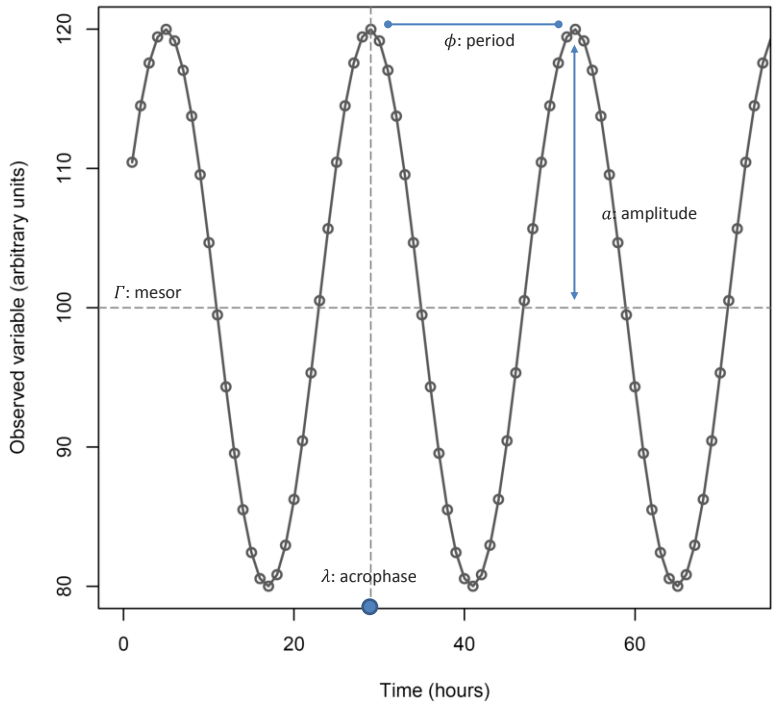
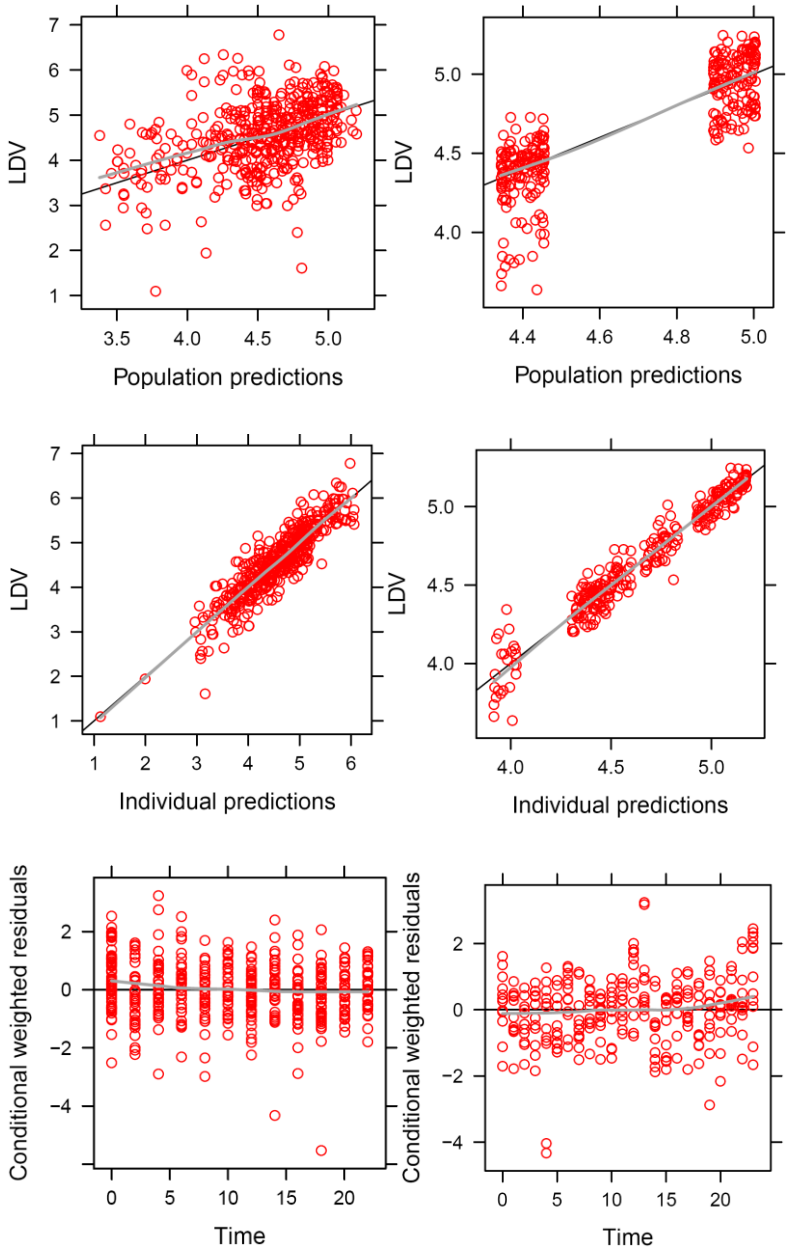


Figure 4: Standard goodness-of-fit diagnostics[5] for the nonlinear mixed effect model of renin activity (left panes) and blood pressure (right panes) dynamics over the 24-hour span. Solid black line: identity line, solid grey line: regression line. Upper panel: population predictions *vs*. observations (log scale LDV), middle panel: individual predictions *vs*. observations, lower panel: distribution of residues.



[5] An adequate mathematical model presents the following features: i) the line of identity should be aligned with the regression line (for both individual and population predictions), ii) the residues (conditional weighted residuals) should be centered on a mean value of 0, with iii) an homogeneous dispersion around the mean.

Figure 5: Individual renin activity (left panes) and blood pressure (right panes) predictions (log scale) *vs.* time (hours) based on individual parameters obtained as empirical Bayes estimates. Open circles: observations (LDV), solid line: individual predictions (IPRED), dashed line: population predictions (PRED). Out of clarity only a subset of 3 individuals per endpoint are represented herein (ordered in columns).

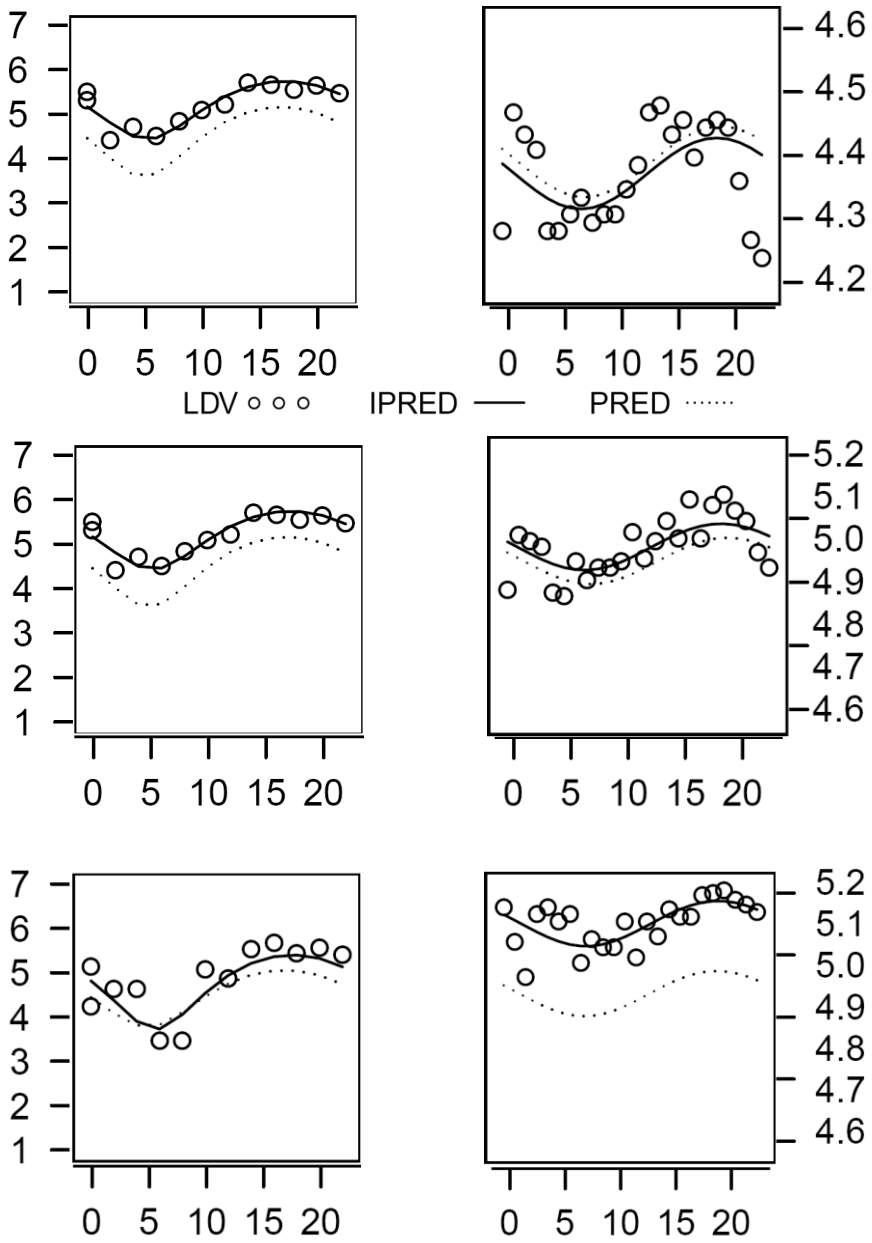


Figure 6: Geometric mean plasma renin activity (pg/mL/h) vs. time profiles in dogs fed a normal-sodium diet at 07.00 pm. Vertical bars indicate +/-one standard error of the geometric mean. Time starts at 07.00 am clock time (e.g. 0: 07.00 am, 12: 07.00 pm). Limit of quantitation: 30 pg/mL/h.

When food was offered at 07.00pm instead of 07.00am, diurnal variations in RA disappeared.