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Diurnal rhythms in plasma cortisol, insulin, glucose, lactate and urea in pigs fed identical meals at 12-hourly intervals

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Abstract

Diurnal rhythms in plasma cortisol, insulin, glucose, lactate and urea concentrations were investigated in eight catheterized pigs of ~35 kg BW. Pigs were fed isoenergetic/isoproteinic diets at a restricted level (2.5×maintenance requirement for energy) in two daily rations (06:00 and 18:00 hours) in order to obtain equal intervals between feed intake. Preprandial plasma cortisol concentration was 22±3 ng/mL in the morning and 14±2 ng/mL in the evening ($p<0.025$), whereas the concentrations of insulin, glucose, lactate, and urea were similar. In the postprandial period in the morning (06:00–09:00 hours) plasma cortisol, insulin and lactate concentrations (expressed as the total area under the curve) were greater ($p<0.001$) compared to the evening (18:00–21:00 hours) by 100%, 42%, and 24%, respectively, while postprandial plasma glucose and urea concentrations were not affected by time of the meal. When postprandial plasma concentrations were expressed as a response over preprandial concentrations (decremental or incremental area under the curve), the diurnal rhythm was not observed for cortisol and glucose, persisted for insulin and lactate, and appeared for urea with a smaller postprandial urea response ($p<0.05$) in the morning compared to the evening. We conclude that the diurnal rhythm in plasma cortisol is independent of feeding whereas the diurnal rhythms in plasma insulin, lactate and urea are unveiled by the morning/evening meals in pigs. At equal 12-h intervals between meals, the postprandial responses of lactate and urea show diurnal variations, each in a specific manner, which suggest decreased postprandial efficiency of carbohydrate metabolism and increased postprandial efficiency of protein metabolism in the morning compared to the evening.

Keywords: Diurnal; Circadian; Prandial; Fasting; Cortisol; Insulin; Lactate; Urea; Pigs

1. Introduction

The activity of the pituitary–adrenocortical system shows cyclic changes over a 24-h diurnal/nocturnal period, which is manifested in variations of the cortisol concentration in plasma. This circadian periodicity is described in diurnal species like man [1,2] and pigs [3,4] with high cortisol concentrations in the morning, and in nocturnal species like rats [5,6] with high cortisol concentrations in the evening. The cortisol rhythm is mainly driven by the autonomic nervous system [7] and persists after fasting [8]. In contrast to cortisol, the daily rhythm in plasma insulin concentrations is mainly driven by a circadian pattern in feed intake [9]. However, a direct circadian control of insulin secretion by the autonomic nervous system can not be excluded as shown in rats [10,11].

Cortisol and insulin are antagonists [12] in their functional ability to regulate carbohydrate [13,14] and protein metabolism [15,16] since cortisol regulates catabolic, and insulin anabolic processes. This implies that an interaction may exist between cortisol and insulin depend-

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ing on the time of day and in relation to the pattern of feed intake. Insulin is secreted postprandially to stimulate the uptake of feed-derived glucose and amino acids from blood into tissues. It is hypothesized that the plasma insulin concentration is not only in proportion to the consumed meal, but also in proportion to counteract the catabolic pressure of cortisol on metabolism. The latter depends on the time of day and thus on the plasma cortisol concentration. Although it is known that a circadian pattern in feeding behaviour exists, the feed driven effect and the direct circadian effect of the autonomic nervous system on rhythms in cortisol, insulin and metabolism are still not well distinguished. Rhythms in carbohydrate metabolism can be detected by variations in concentrations of glucose and lactate (index of glycolysis) in plasma and a rhythm in protein metabolism can be identified by variations in plasma urea (index of amino acid oxidation) concentrations [17]. Previous studies have shown the existence of circadian rhythms in both carbohydrate [1,9–11] and protein metabolism [18–20].

To eliminate the feed driven effect on circadian rhythmicity and thus to expose the direct effect of the autonomic nervous system on the circadian rhythm in plasma cortisol, insulin, glucose, lactate and urea, in the present study pigs were fed twice daily at equal 12-h intervals (at 06:00 and 18:00 hours) with equal amounts of feeds. Thus, a balanced metabolic status of the pig during 24-h periodicity could be achieved.

2. Materials and methods

Experimental protocols describing the management, surgical procedures, and animal care were reviewed and approved by the ASG-Lelystad Animal Care and Use Committee (Lelystad, The Netherlands).

2.1. Animals and housing

Eight crossbred barrows (Dutch Landrace×Yorkshir-Yorkshire×Finnish Landrace) with an average body weight of 35±2 kg (mean±SEM) were used in this study. Two weeks before surgery the pigs were housed in metabolism cages (1.15×1.35 m) and adapted to the light/dark cycle and the feeding regimen. Lights were on and off at 05:00 and 22:00 hours, respectively. Ambient room temperature was 20 °C.

2.2. Feed intake and surgery

The diet (Table 1) was mixed with water at a ratio of 1:4.0 (w/v) and the pigs were fed twice daily (at 06:00 and 18:00 hours) at a feeding level of 2.5 times maintenance requirements per day (maintenance requirement=293 kJ ME/kg $BW^{0.75}$). For a 35 kg pig this is 994 g of feed per day. Throughout the experiment, each meal was consumed within 15 min. The day before surgery, the 18:00 hours meal was skipped, the day of surgery the 06:00 and 18:00 hours meals were skipped. The day after surgery the pig was given 50% of its pre-surgery daily feed intake, and two days after surgery the pig returned to its preoperative feed intake.

Table 1
Diet composition

Calculated content of nutrients	Per kg feed	Per meal per kg $BW^{0.75}$
Metabolizable energy (MJ)	10.6	0.366
Ash (g)	55.3	1.91
Crude fat (g)	48.9	1.69
Crude fiber (g)	54.7	1.89
Crude protein (g)	158.6	5.48
Lysine (g)	9.5	0.323
Phosphorus (g)	4.4	0.152

Supplied per kg diet: Cu 130 mg, Vitamin A 7500 IU, Vitamin D3 1500 IU, Vitamin E 25 IU, avilamycin 20 mg.

Under general anaesthesia [21], polyethylene catheters (Tygon, i.d. 1.02 mm, o.d. 1.78 mm, length 1 m; Norton, Akron, Ohio, USA) were placed into the right carotid artery and the right external jugular vein according to a modified procedure [22,23]. The catheters were inserted and advanced until the tip of the catheter reached the aorta (carotid artery catheter) or the antrum (jugular vein catheter). The catheters were fixed firmly at the place of insertion and were tunneled subcutaneously to the back of the pig and exteriorized between the shoulder blades. The catheters were filled and sealed with physiological saline containing 50 IU heparin and 150.000 IU penicillin (Procpen; AUV, Cuijk, The Netherlands) per mL and kept in and protected by a back pack which was glued to the skin of the pig's back. During surgery the pig was given an intramuscular injection of antibiotic (300.000 IU procaïne penicilline G, Depocilline, Mycofarm Nederland B.V., De Bilt, The Netherlands) and anodyne (50 mg flunixine, Finadyne, Schering-Plough N.V./S.A., Brussels, Belgium).

After one week of postsurgical recovery, the pigs were used for the first series of blood sampling. The period between surgery and the first series of measurements, the pigs were habituated to the blood sampling procedure. The carotid artery was used for blood sampling and the jugular vein catheter was used as a back-up in case of a malfunctioning arterial catheter. The latter did not occur during the present experiment. During the blood sampling sessions, the catheters were flushed and filled with physiological saline containing 5 IU heparin per mL.

2.3. Blood sampling

Preprandial and postprandial measurements were carried out according to a cross-over design. Four pigs were first used in the morning phase (05:45–09:00 hours), and the other four were investigated in the evening phase (17:45–21:00 hours). Each series of blood sampling was conducted with a one week interval. Blood samples of 5 mL were taken