J. Dairy Sci. 97:6955–6963
http://dx.doi.org/10.3168/jds.2014-8327

Cardiac responses to palpation per rectum in lactating and nonlactating dairy cows

L. Kovács,*†[1] J. Tőzsér,* O. Szenci,† P. Póti,* F. L. Kézér,* F. Ruff,‡ Gy. Gábriel-Tőzsér,‡ D. Hoffmann,§ M. Bakony,# and V. Jurkovich‖
*Institute of Animal Husbandry, Faculty of Agricultural and Environmental Science, Szent István University, Páter Károly utca 1, Gödöllő 2100, Hungary
†Hungarian Academy of Sciences-Szent István University (HAS-SZIU) Large Animal Clinical Research Group, Üllő-Dóra major 2225, Hungary
‡Institute of Economics, Law and Methodology, Faculty of Economics and Social Sciences, Szent István University, Páter Károly utca 1, Gödöllő 2100, Hungary
§Bóly Co., Ady Endre utca 21, Bóly 7754, Hungary
#Rumino-Vet Bt, Csillés utca 2, Érd 2030, Hungary
‖Department of Animal Hygiene, Herd Health and Veterinary Ethology, Faculty of Veterinary Science, Szent István University, István utca 2, Budapest 1078, Hungary

ABSTRACT

Interest in the monitoring of heart rate variability (HRV) has increased recently, as it gives more detailed and immediate information about the level of stress than traditional behavioral or hypothalamus-pituitary-adrenal measures. In this study, we evaluated heart rate (HR) and parasympathetic HRV parameters to monitor cardiac stress responses to palpation per rectum (PPR) in lactating (LACT; n = 11) and nonlactating (NLACT; n = 12) dairy cows. Heart rate and HRV were recorded from 40 min before PPR until 120 min after it was completed. Heart rate, the root mean square of successive differences (RMSSD), and the high-frequency component (HF) of HRV were analyzed by examining 5-min time windows. To compare cardiac responses to PPR between groups, changes in HR and HRV parameters were calculated as area under the curve (AUC) for LACT and NLACT cows. An immediate increase in HR was detected during PPR in both LACT (+21.4 ± 2.4 beats/min) and NLACT cows (+20.6 ± 2.3 beats/min); however, no differences were found between groups on the basis of parameters of AUC. The increase in HR in both groups along with a parallel decrease in RMSSD (LACT cows: −5.2 ± 0.4 ms; NLACT cows: −5.1 ± 0.4 ms) and HF [LACT cows: −10.1 ± 0.8 nu (where nu = normalized units); NLACT cows: −16.9 ± 1.2 nu] during PPR indicate an increase in the sympathetic, and a decrease in the parasympathetic tone of the autonomic nervous system. The increase in RMSSD (LACT cows: +7.3 ± 0.7 ms; NL cows: +17.8 ± 2.2 ms) and in HF (LACT cows: +24.3 ± 2.6 nu; NLACT cows: +32.7 ± 3.5 nu) immediately after PPR indicated a rapid increase in parasympathetic activity, which decreased under the baseline values 10 min following PPR. The amplitude and the maximum RMSSD and HF values were greater in NLACT cows than in LACT animals, suggesting a higher short-term cardiac responsiveness of NLACT cows. However, the magnitude and the duration of the stress response were greater in LACT cows, as indicated by the analysis of AUC parameters (area under the HRV response curve and time to return to baseline). Cow response to the PPR was more prominent in parasympathetic HRV measures than in HR. Based on our results, the effect of PPR on the cows' cardiac stress responses may have an impact on animal welfare on dairy farms, and investigating the effect of lactation on the cardiac stress reactions could prove useful in modeling bovine stress sensitivity. Further research is needed to find out whether the differences due to lactation are physiological or management related.

Key words: heart rate, heart rate variability, palpation per rectum, dairy cow

INTRODUCTION

On commercial dairy farms, stressors are prevalent in a wide variety and intensities. Transrectal examination [e.g., palpation of the uterus per rectum (**PPR**)] is a frequent procedure performed by veterinarians (Baillie et al., 2005). Palpation of the uterus per rectum is used for the detection of postpartum uterine diseases (LeBlanc et al., 2002; Sheldon et al., 2006), during AI, and as the most common method, it is used for early pregnancy diagnosis in dairy cattle (Youngquist, 1997; Romano et al., 2007). The procedure is usually quickly done by skilled veterinarians, yet it can last up to 5 to 10 min for inexperienced students in training. Palpation of the uterus per rectum is a nontraumatic

---

Received May 5, 2014.
Accepted August 3, 2014.
[1] Corresponding author: Kovacs.Levente@mkk.szie.hu

procedure; however, it can result in physiological stress reactions (Nakao et al., 1994; Waiblinger et al., 2004; Cingi et al., 2012).

It has been demonstrated that merely the presence of humans can cause discomfort in dairy cows (Hagen et al., 2004). In farm animals, an increasing number of studies have used a combination of physiological and behavioral measures for the assessment of pain as a stressor. Behavioral responses are useful for the qualitative description of the sensory experiences and nervous processes (Mellor et al., 2000; Pilz et al., 2012); however, interpretation of behavior is often observer dependent (Mason and Mendl, 1993).

Measures of hypothalamus-pituitary-adrenal axis activity (e.g., plasma cortisol concentrations) have extensively been used to evaluate pain in cattle (Stafford and Mellor, 2011; Coetzee, 2013). However, measures of the activity of the autonomic nervous system (**ANS**) may have advantages over measuring hypothalamus-pituitary-adrenal activity when investigating responses to acute stress, as they provide more immediate and detailed information (Stewart et al., 2010; Ledowski et al., 2012; reviewed by Kovács et al., 2014).

Different methods exist for evaluating ANS function. Heart rate (**HR**) mainly reflects the activity of the sympathetic nervous system (**SNS**; Hopster et al., 1995; Sgoifo et al., 1999); however, it is difficult to draw conclusions about underlying control mechanisms related to ANS activity from HR measurements exclusively (Sayers, 1973; Hainsworth, 1995; Malliani, 1995). Measuring HR variability [**HRV**; i.e., the short-term fluctuations in the variability of successive cardiac interbeat intervals (**IBI**)] is the most effective and least invasive method for the assessment of ANS regulatory activity in dairy cattle (von Borell et al., 2007; Kovács et al., 2014). An increasing number of studies have used HRV indices as indicators for the response of the ANS to stress. Using the IBI and calculating parameters in time and frequency domains, it is possible to measure the prevailing balance between SNS and parasympathetic nervous system (**PNS**) activity (von Borell et al., 2007). To our knowledge, only a few studies exist that describe HRV changes in response to pain in adult farm animals [horse (Rietmann et al., 2004); sheep (Stubsjøen et al., 2009)].

Acute stress activates the SNS; however, defining HRV as a measure of SNS activity remains a subject of debate (Akselrod et al., 1985; Houle and Billman, 1999). It is generally agreed that the root mean square of successive differences (**RMSSD**) between the consecutive IBI is the primary time domain measure of HRV that represents vagal regulatory activity (von Borell et al., 2007). The high-frequency component (**HF**) of HRV is strongly associated with the baroreceptor input, which varies with the breathing cycle and also reflects the changes in PNS tone (Akselrod et al., 1981; Malliani, 1995). Both parameters have been studied in dairy cattle welfare research (Kovács et al., 2014). Reduced vagal tone was found in cows subjected to waiting after parlor milking with nonvoluntary exit (Kovács et al., 2013) or during milking in a novel milking environment (Sutherland et al., 2012), and in calves exposed to external stress or pathological loads (Mohr et al., 2002).

In cattle, short-term cardiac responses to pain-evoking procedures have been investigated only in calves. In these experiments, animals were subjected to hot-iron disbudding (Stewart et al., 2008) or surgical castration (Stewart et al., 2010) to test the efficiency of local anesthesia. Authors have reported contradictory findings. In the case of castration without local anesthesia, an increase in PNS parameters was found after surgery, whereas in their earlier study, RMSSD and HF decreased following disbudding without local anesthesia, suggesting that the procedure was painful. Those authors suggested that this was due to the varying nature of the pain (somatic vs. visceral), which resulted in higher vagal response to castration, as the PNS is highly involved in carrying noxious impulses from the testes.

In the present work, we monitored HR and HRV parameters in lactating and nonlactating dairy cows before, during, and following PPR in a field study. As severe pain is characterized by a propensity to evoke strong autonomic responses (Ness and Gebhart, 1990) and the rectum is considered as a visceral organ and has only autonomic innervation, we expected that ANS indices of HRV would be useful to study stress reactions of cows. We hypothesized lower cardiac responses to PPR in lactating cows than in nonlactating ones, as this procedure is usually done during the postpartum period, whereas nonlactating animals are not exposed to PPR during the late prepartum period.

MATERIALS AND METHODS

Animals and Housing

A total of 23 multiparous [11 lactating (**LACT**) and 12 nonlactating (**NLACT**)], clinically healthy Holstein-Friesian cows were selected from a large-scale herd (2,000 lactating cows) of Bóly Co. (Csípőtelek, Hungary). The LACT and NLACT group averages were similar in age (mean ± SD; 3.8 ± 1.3 vs. 3.5 ± 1.1 yr), parity (3.1 ± 0.9 vs. 2.6 ± 0.7 lactations), and BCS (3.1 ± 0.6; vs. 3.3 ± 0.8). Cows were housed in modern freestall barns bedded with sand. The barns were equipped with self-locking headlocks (head gates and headrails; Arntjen Germany GmbH, Rastede, Germany) along the full length of the feeding area. Cows locked themselves in place when putting their heads in the stanchions to eat, after being milked. It was a routine practice on the farm that regular checkups and treatments were carried out while the animals remained restrained and standing after feeding. Such a feeding and restraint system has been used for years. Total mixed ration was fed once per day at 800 h and animals had free access to water. Lactating cows (mean ± SD; DIM = 112 ± 21.5 d; daily milk yield = 47.8 ± 8.6 kg) were milked 3 times per day in a 72-stall rotary milking parlor (BouMatic Xcalibur 360; BouMatic, Madison, WI). Nonlactating animals were pregnant and at least 10 d after drying off and 20 d before expected calving. Lactating animals were nonpregnant and had recent experience with PPR.

Experimental Design

The experiment was carried out in November 2013, during a 4-d period. Three cows from each group were involved each day. The Polar Equine RS800 CX mobile recording system (Polar Electro Oy, Kempele, Finland) was used, with 2 integrated electrodes and a specific transmitter. After soaking the body surface under the electrodes with tap water, transmitters and the 2 electrodes were positioned on the thoracic region, as advised by von Borell et al. (2007) in their review. Electrode sites were covered with ample ultrasound transmission gel (AquaUltra Blue; MedGel Medical, Barcelona, Spain) without shaving the skin. The devices were fitted while the animals were standing restrained in the headlocks after feeding, 18 h before the start of recordings to allow enough time to acclimatization to wearing the equipment (Mohr et al., 2002; Gygax et al., 2008).

Heart rate recordings started on the next day, approximately 10 min after the animals had finished feeding, having returned from the morning milking. After a baseline period of 40 min, the PPR was performed. For the accuracy of HRV analysis (see Analysis of HRV section), the examination lasted 5 min, which was longer than what is usual in typical practice. The PPR was done with care. After removing part of the feces, examiners tried to localize the reproductive organs (the cervix and horns of the uterus, and the ovaries), the arteria uterina media, and in the case of pregnant cows, the approachable cotyledons. The examiners were unfamiliar to the cows. Heart rate recordings continued for 120 min after the PPR had finished. To avoid the influence of moving on HR, the experimental animals remained standing throughout the whole length of the recording period. To avoid the influence of separation during this time, neighboring cows on each side were also left restrained. All other cows were released at the time the PPR were finished. Any other kinds of disturbance (e.g., sudden noise and presence of people) or any unnecessary contact with animals throughout the whole experimental period was avoided.

Analysis of HRV

The IBI recorded from 40 min before the examination to 120 min afterward were used in the analysis. The HRV analysis was performed using Kubios 2.1 HRV software (Niskanen et al., 2004). A custom filter was applied to correct for any artifacts. Interbeat intervals differing from the previous IBI by more than 30% were identified as artifacts. An average error rate of 5% was accepted for analysis. In addition, a visual inspection of the corrected data was performed to edit out any artifacts still existing. Only one 5-min time window from the baseline measurements and four 5-min time windows from the post-PPR recordings were excluded, due to artifacts. Due to equipment failure, IBI data between 100 and 120 min after PPR was not recorded for 1 cow.

For the interpolation of IBI time series before analysis using fast Fourier transformation (**FFT**), segments of 512 IBI were examined (ESC-NASPE Task Force, 1996; von Borell et al., 2007) and then HRV was analyzed in the following periods: (1) in the 40 min before PPR (**PrePPR**); (2) during the 5-min of PPR, and (3) during the 120 min following PPR (**PostPPR**). As baseline, the mean values in the 15 min before the examination were used. The analyzed time domain measures were mean HR and RMSSD. As a frequency domain parameter, HF was chosen, presented in normalized units, calculated by FFT. Recommendations of von Borell et al. (2007) were fulfilled by setting the limits of the spectral components (low frequency = 0.05–0.20 Hz and HF = 0.20–0.58 Hz). This frequency band width for the HF power was also used by earlier reports on dairy cattle for HRV analysis (Mohr et al., 2002; Hagen et al., 2005; Kovács et al., 2013).

Statistical Analysis

Heart rate, RMSSD, and HF were analyzed with a generalized linear model procedure (SPSS 18.0; SPSS Inc., Chicago, IL) with penalized quasi-likelihood. The residuals of the model were inspected graphically for distribution and homogeneity of variances with the Kolmogorov-Smirnov test. Because data were not normally distributed, HR, RMSSD, and HF parameters (as dependent variables) were subjected to logarithmic transformations before analysis. Covariates (parity, condition, and age) were added to the model as fixed factors. The Bonferroni adjustment was used for post hoc comparisons of HR and HRV values within the groups. A value of $P < 0.05$ was considered significant.

For reducing the number of statistical comparisons between groups during the PostPPR period, changes in HR and HRV parameters were calculated as area under the curve (**AUC**). The AUC represents both the magnitude and the changes over time of the response (Fekedulegn et al., 2007) and simplifies the statistical analyses by transforming the multivariate data into univariate space, especially when the numbers of repeated measurements are high and a need exists to summarize the information (Watamura et al., 2004). The evaluated parameters included baseline and maximum values of HR, RMSSD, and HF; amplitude (the maximal alteration compared with baseline) of the HR, RMSSD, and HF response; and long-term measures of cardiac responses to the PPR (AUC response and time to return to baseline). The area under the response curve ($\mathrm{AUC_{RESP}}$) was determined for the period of time to return to baseline using a method described by Lay et al. (1996):

$$\mathrm{AUC_{RESP}} = \Sigma[(\mathrm{C_n} + \mathrm{C_{n+1}})/2 \times \mathrm{h} - \mathrm{baseline}],$$

where C is the cardiac parameter (HR, RMSSD, or HF) at a given time point, h is the time in hours between the 2 C values, and baseline is the mean value over the final 15 min of recorded HR and HRV data before PPR. The AUC 40 min before PPR was also calculated for both LACT and NLACT groups ($\mathrm{AUC_{PRE}}$). As there was no response above baseline values of either HR or HRV parameters to measure, the area was simply calculated as follows:

$$\mathrm{AUC_{PRE}} = \Sigma[(\mathrm{C_n} + \mathrm{C_{n+1}})/2 \times \mathrm{h}].$$

The $\mathrm{AUC_{PRE}}$ and $\mathrm{AUC_{POST}}$ parameters are presented in absolute values. The nonparametric Friedman test was used for the comparison of AUC parameters between groups because of substantial non-normality in several parameters (evaluated by Levene's test). The level of significance was set at $P < 0.05$.

RESULTS

Changes in HR showed a similar pattern in both LACT and NLACT cows throughout the experiment (Figure 1a). In the PrePPR period, HR did not change significantly in either group. During PPR, HR increased ($P < 0.001$, in both groups), and in the first 5 min after PPR it decreased and then returned back to the normal (baseline) values.

Although baseline and maximum HR were significantly higher ($P < 0.001$, in both cases) in LACT than NLACT group, the amplitude of HR did not differ between groups (Table 1). The AUC analysis did not detect any effect of the lactation either in the PrePPR or in the PostPPR period. After returning to baseline, HR was relatively stable in both studied groups, with an average slightly below the physiological level in LACT and slightly above that in NLACT cows (Figure 1a).

Root mean square of successive differences—similarly to HR—did not change considerably in the PrePPR period in either group (Figure 1b). Baseline RMSSD was lower in the LACT group, compared with the NLACT group ($P < 0.001$); $\mathrm{AUC_{PRE}}$ was similar in both groups (Table 1). During PPR, RMSSD decreased in both LACT and NLACT cows ($P < 0.001$ and $P < 0.01$, respectively), reflecting the sudden decrease in the PNS activity. The RMSSD in the first 5 min of PostPPR highly exceeded the baseline in both groups ($P < 0.001$). The rate of the increase was, on average, 24.4 and 41.7% in the LACT and NLACT groups, respectively. The maximum and amplitude of RMSSD were lower in the LACT group, compared with the NLACT group ($P < 0.001$ and $P < 0.01$, respectively). For LACT cows, RMSSD took longer to return to baseline ($P < 0.001$) and $\mathrm{AUC_{RESP}}$ was also greater in LACT cows ($P < 0.01$; Table 1).

Similarly to RMSSD, baseline HF was lower in LACT than NLACT cows ($P < 0.001$). During PPR, HF decreased in both groups ($P < 0.001$; Figure 1c). The rate of decline was, on average, 28.5% in LACT and 38.1% in NLACT cows, respectively. Area under the curve parameters of HF also differed between groups. A higher maximum in the 5 min following PPR ($P < 0.001$) and higher amplitude ($P < 0.05$) was found in NLACT cows, whereas LACT cows showed a greater $\mathrm{AUC_{RESP}}$ ($P < 0.001$) and longer time to return to baseline ($P < 0.001$) measured in HF (Table 1).

DISCUSSION

Baseline HR differences between LACT and NLACT cows are consistent with that found earlier (Mohr et al., 2002), yet those authors did not find the difference (83 vs. 74 beats/min) relevant. We suggest that this difference can primarily be attributed to the increased energy demands of lactation, as higher milk production is correlated with higher heart rate (Weiss et al., 2004). Such effect of milk production on ANS activity is also reflected in baseline RMSSD and HF differences between LACT and NLACT cows. The findings of Hagen et al. (2005) suggest that other factors, such as housing, management, and breed, could also be influential, as

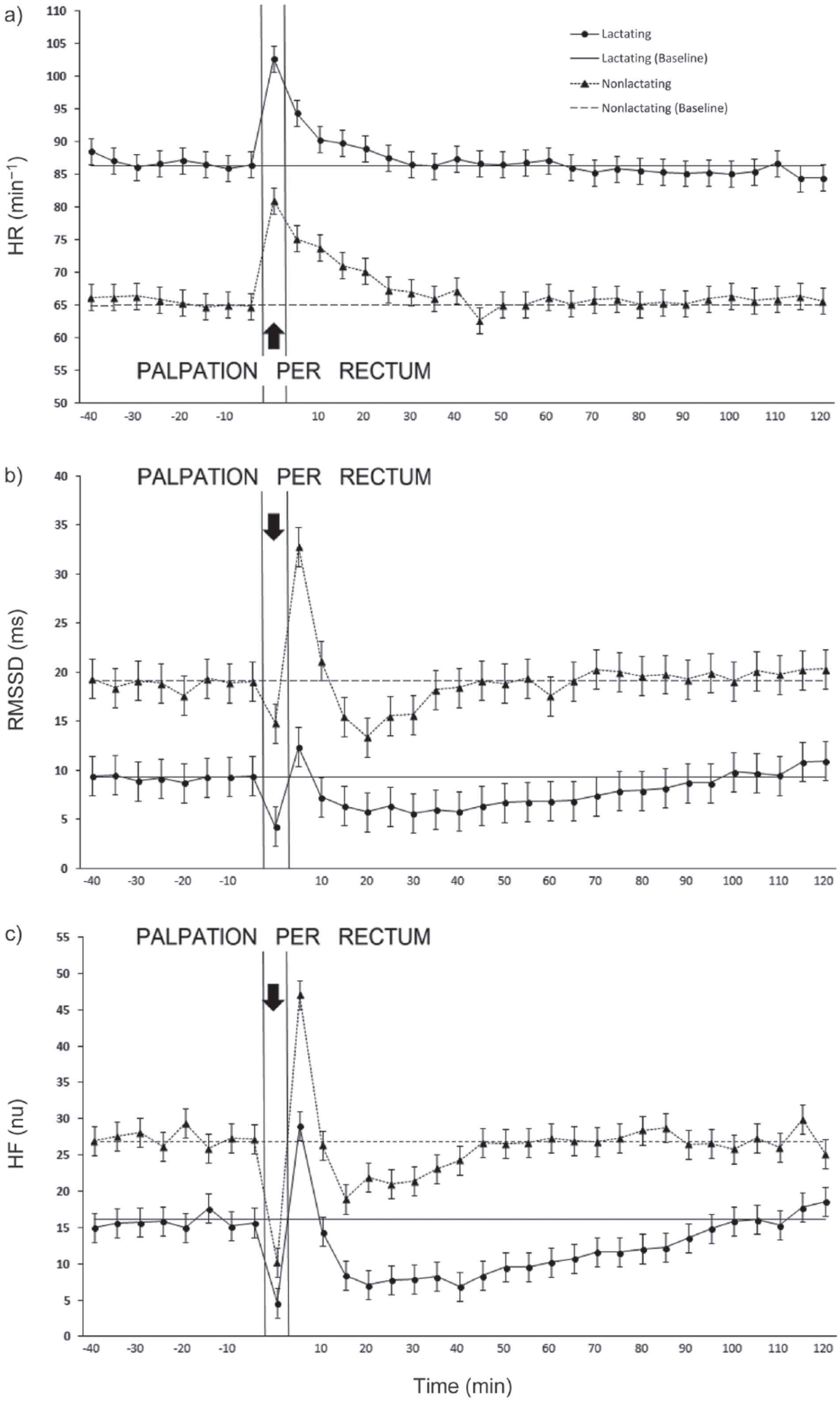

Figure 1. Changes in (a) heart rate (HR; beats/min), (b) the root mean square of successive differences (RMSSD; ms), and (c) the high-frequency component [HF; nu (where nu = normalized units)] of heart rate variability (HRV) in nonlactating (▲; n = 11) and lactating cows (●; n = 12) before, during, and after palpation per rectum (PPR). For significant differences between groups for cardiac response, parameters are calculated as area under the curve (AUC). Mean baseline HR, RMSSD, and HF values are represented by the horizontal lines. Values are means ± SEM.

Table 1. Heart rate (HR) response parameters and parameters of cardiac function [high frequency component (HF) of HR variability (HRV)] calculated as area under the curve (AUC) before, during, and following palpation per rectum (PPR) in nonlactating (n = 12) and lactating (n = 11) cows[1]

		Mean ± SD	
Item[2]	Units	Nonlactating	Lactating
HR response parameters			
AUC $_{\text{PRE}}$	beats	25.2 ± 18.4	13.9 ± 14.8
Baseline values	beats/min	64.9 ± 5.5***	86.3 ± 4.5
Maximum values	beats/min	81.0 ± 5.6***	102.7 ± 5.8
Amplitude of response	beats/min	16.1 ± 4.5	16.4 ± 3.4
AUC $_{\text{RESP}}$	beats	268.8 ± 110.1	183.0 ± 86.3
Time to return to baseline	min	37.5 ± 13.1	30.9 ± 21.2
RMSSD response parameters			
AUC $_{\text{PRE}}$	ms × min	22.1 ± 47.5	17.8 ± 24.6
Baseline values	ms	19.1 ± 3.3***	9.4 ± 1.2
Maximum values	ms	32.8 ± 14.8***	12.4 ± 5.5
Amplitude of response	ms	15.7 ± 13.1**	5.2 ± 3.7
AUC $_{\text{RESP}}$	ms × min	16.9 ± 206.3*	195.2 ± 93.6
Time to return to baseline	min	43.3 ± 14.2***	88.2 ± 17.9
HF response parameters			
AUC $_{\text{PRE}}$	nu × min	20.9 ± 39.2	44.3 ± 41.8
Baseline values	nu[3]	26.8 ± 5.4***	16.2 ± 3.3
Maximum values	nu	47.1 ± 6.5***	29.1 ± 6.4
Amplitude of response	nu	20.3 ± 4.5*	12.9 ± 6.4
AUC $_{\text{RESP}}$	nu × min	85.9 ± 181.7***	475.2 ± 250.4
Time to return to baseline	min	44.2 ± 17.6***	102.8 ± 7.9

[1] Descriptive statistics are based on mean ± SD of nontransformed data.

[2] AUC $_{\text{PRE}}$ = area under the curve during 40 min before PPR; baseline = the last 15 min before PPR; amplitude of response = the maximal alteration compared with baseline; AUC $_{\text{RESP}}$ = area under the response curve calculated for the period of time to return to baseline.

[3] nu = normalized units.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ [statistical significances for HR, root mean square of successive differences (RMSSD), and HF response parameters are based on logarithmically transformed data. *P*-values for differences between groups are based on results from the Friedman test].

they measured an average 6.6 ms of baseline RMSSD and 16.2 normalized units for baseline HF in their study on lactating Brown Swiss and Fleckvieh cows. During the baseline period, HR, RMSSD, and HF as well as AUC regarding any of the cardiac parameters did not change significantly in any of the groups, which suggests that restraint and the proximity of examiners did not eventuate considerable stress for the animals studied.

The elevation in HR during PPR is a good indicator of acute stress (Hopster et al., 1995; Sgoifo et al., 1999). The amplitude of the HR response did not differ between groups (average of 16 beats/min in both groups). Mialon et al. (2012) reported a similar rate of increase (80.9 vs. 96.9 beats/min) in adult cattle, in response to rumenocentesis (a rumen fluid collection procedure) without local anesthesia; however, baseline data were recorded after the restraint. When cows were restrained, they found no evidence to indicate that rumenocentesis was more stressful than handling the animals.

Waiblinger et al. (2004) found an average 7 beats/min increment in the HR of dairy cows during PPR performed by people unfamiliar to the animals. The animals were regularly examined as subjects of veterinary education, which might explain why the HR response was lower than what we observed. The difference could also be due to the short acclimatization period in their study, as the electrodes were attached only 35 min before PPR; therefore, baseline values (10–20 min before PPR) were likely to have been elevated. In the abovementioned studies, animals were fixed in restraining cages or insemination stalls during the experiment and they were isolated.

Baseline recordings may be biased in isolation or in surroundings that the animal associates with previous unpleasant experiences due to the triggering of moderate stress reactions, even without any manipulation (Boissy and Bouissou, 1994). In our study, cows were examined in their regular feeding place and were not isolated. Despite the confinement and the presence of experimenters during and after PPR, animals were relatively calm. We could conclude that the observed cardiac reactions are due to stress associated with the procedure of PPR.

Investigating short-term stress responses during castration without local anesthesia in bull calves, Stewart et al. (2010) observed an average 15 beats/min increase in HR. Those authors reported that HR started to decrease 2 min after the procedure had ceased. In our study, the decrease was more gradual, presumably due to the different severity or nature of pain. During PPR, RMSSD and HF decreased in both groups, which indicated the sudden decrease in PNS activity, which is consistent with the polyvagal theory of Porges (2003) that painful stimuli cause a decrease in vagal tone. A decrease in PNS activity was found in cows during waiting in the milking stall after milking (Kovács et al., 2013) and in cows milked for the first time in an unfamiliar milking parlor (Sutherland et al., 2012) by the changes in RMSSD and HF, and RMSSD, respectively. In calves, the HF was found to be suitable for the detection of acute pain caused by disbudding (Stewart et al., 2008), whereas in horses, RMSSD and HF were lower with higher levels of pain accompanied by laminitis (Rietmann et al., 2004). In healthy adults, studies investigating changes in HRV in response to experimentally induced acute pain also reported a decrease in PNS activity, as indexed by HF (Koenig et al., 2013). The decline in PNS activity in parallel with the short-term increase in HR measured in our study confirms that rapid changes in HR are always related to changes in the vagal function (Fritsch et al., 1986; Eckberg, 1991). However, merely HRV-based interpretation of pain associated with PPR is not well founded, as several physiological events are involved in pain. A tendency toward a decrease in RMSSD in response to a noxious ischemic stimulus by application of a forelimb tourniquet was found in ewes, suggesting that HRV is a sensitive method, which can detect mild to moderate pain (Stubsjøen et al., 2009). In their study, animals showed behavioral signs of aversion. The limitation of our study to fully investigate whether PPR in itself is painful is that no other measures of pain (e.g., behavioral signs or effect of analgesics) were measured along with HRV.

We described the magnitude and the duration of cows' cardiac responses by using the AUC method, which has been commonly performed in endocrinological studies on different species (Bhagwagar et al., 2005; Curley et al., 2008; Schmidt et al., 2010). Previously, HR and HRV (i.e., RMSSD) were presented as AUC in horses during road transport (Schmidt et al., 2010) and the method was found to be useful to distinguish between the levels of stress in horses in relation with different durations of transport. In our study, areas under the HR curve and time to return to baseline values did not reflect the differences in stress-responsiveness between LACT and NLACT animals; thus, we concluded that lactation has no effect on the magnitude and duration of the HR response. The reason for this might be that the complex interplay of the 2 branches of the ANS is not always comprehensible when cardiac activity is measured only by HR (Porges, 1995; Marchant-Forde et al., 2004). An increase in HR can occur due to an increase in SNS activity (Hainsworth, 1995), the decrease in vagal tone, or the simultaneous changes in both regulatory systems (von Borell et al., 2007).

As expected, short-term PNS responses were higher (higher maximum and amplitude of RMSSD and HF) in NLACT cows, compared with LACT animals. As the NLACT animals had been pregnancy checked 8 mo before the experiment, and LACT animals had likely undergone pregnancy testing 3 to 4 times in the preceding 2 mo, our results seem to confirm that habituation to a stressor can reduce the intensity of stress response when the stressor occurs repeatedly (Martí et al., 2001). In the first 5 min after PPR, the PNS measures of HRV peaked in both groups, indicating a compensatory increase in PNS activity. This phenomenon was also reported by Stewart et al. (2010), using RMSSD and HF parameters in the first 5 min following surgical castration without local anesthesia of bull calves. In our study, 10 min following PPR, PNS activity decreased below the baseline level.

Inconsistent with our hypothesis, the magnitude and the duration of the stress response were greater in the LACT group. $\mathrm{AUC}_{\mathrm{RESP}}$ following PPR and the time to return to baseline values of both RMSSD and HF demonstrate a prolonged ANS response in LACT animals. These differences were more pronounced in HF values. It is worth investigating whether these differences due to lactation are physiological or management related. One explanation for this prolonged decline in PNS tone during the PostPPR period in LACT cows could be the higher physiological load associated with lactation; however, in an earlier study, cardiac differences between LACT and NLACT animals were not observed (Mohr et al., 2002). It is important to note that the study by Mohr et al. (2002) was not designed to provide an evaluation of stress responsiveness; only HRV parameters at resting were compared. In our study, differences in short- or long-term stress response to PPR are precisely described by PNS indices of HRV.

CONCLUSIONS

The use of HRV parameters provides a noninvasive means to assess ANS responses to acute stress in adult cattle. Lactating and nonlactating cows differ in their basal cardiac activity measured in standing posture. Lactating cows exhibited lower short-term cardiac responsiveness to PPR than NLACT animals, whereas in terms of magnitude and duration, cardiac responses mirrored by PNS indices of HRV were more intensive in LACT cows than NLACT ones. Further research is needed to clarify these differences in ANS activity between NLACT and LACT animals. As PPR caused significant stress in both LACT and NLACT cows, the length and frequency of the transrectal examination should be as minimal as possible, especially during training.

ACKNOWLEDGMENTS

The authors thank Margit Kulcsár (Szent István University, Faculty of Veterinary Science, Department and Clinics of Reproduction, Budapest, Hungary) for her valuable technical assistance and the farm staff of Bóly Co. at Csípőtelek (Hungary) for taking care of the animals. Our work was partly supported by the GOP-1.1.1-11-2012-0153 project and the Research Centre of Excellence (Budapest, Hungary) 8526-5/2014/TUDPOL project.

REFERENCES

Akselrod, S., D. Gordon, J. B. Madwed, N. C. Snidman, D. C. Shannon, and R. J. Cohen. 1985. Hemodynamic regulation: Investigation by spectral analysis. Am. J. Physiol. 249:H867–H875.

Akselrod, S., D. Gordon, F. A. Ubel, D. C. Shannon, A. C. Berger, and R. J. Cohen. 1981. Power spectrum analysis of heart rate fluctuation: A quantitative probe of beat-to-beat cardiovascular control. Science 213:220–222.

Baillie, S., A. Crossan, S. A. Brewster, D. Mellor, and S. Reid. 2005. Validation of a bovine rectal palpation simulator for training veterinary students. Stud. Health Technol. Inform. 111:33–36.

Bhagwagar, Z., S. Hafizi, and P. J. Cowen. 2005. Increased salivary cortisol after waking in depression. Psychopharmacology (Berl.) 182:54–57.

Boissy, A., and M. F. Bouissou. 1994. Effects of androgen treatment on behavioral and physiological-responses of heifers to fear-eliciting situations. Horm. Behav. 28:66–83.

Cingi, C. C., D. F. Baser, Y. S. Karafakioglu, and A. F. Fidan. 2012. Stress response in dairy cows related to rectal examination. Acta Sci. Vet. 40:1053.

Coetzee, J. F. 2013. Assessment and management of pain associated with castration in cattle. Vet. Clin. North Am. Food Anim. Pract. 29:75–101.

Curley, K. O., Jr., D. A. Neuendorff, A. W. Lewis, J. J. Cleere, T. H. Welsh Jr., and R. D. Randel. 2008. Functional characteristics of the bovine hypothalamic-pituitary-adrenal axis vary with temperament. Horm. Behav. 53:20–27.

Eckberg, D. L. 1991. Human respiratory-cardiovascular interactions in health and disease. Pages 253–258 in Cardiorespiratory and Motor Coordination. H. P. Koepchen and T. Huopaniemi, ed. Springer Verlag, Berlin, Heidelberg, Germany.

ESC-NASPE Task Force (Task Force of the European Society of Cardiology, North American Society of Pacing and Electrophysiology). 1996. Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. Circulation 93:1043–1065.

Fekedulegn, D. B., M. E. Andrew, C. M. Burchfiel, J. M. Violanti, T. A. Hartley, L. E. Charles, and D. B. Miller. 2007. Area under the curve and other summary indicators of repeated waking cortisol measurements. Psychosom. Med. 69:651–659.

Fritsch, J. M., D. L. Eckberg, L. D. Graves, and B. G. Wallin. 1986. Arterial-pressure ramps provoke linear increases of heart period in humans. Am. J. Physiol. 251:R1086–R1090.

Gygax, L., I. Neuffer, C. Kaufmann, R. Hauser, and B. Wechsler. 2008. Restlessness behaviour, heart rate and heart-rate variability of dairy cows milked in two types of automatic milking systems and auto-tandem milking parlours. Appl. Anim. Behav. Sci. 109:167–179.

Hagen, K., J. Langbein, C. Schmied, D. Lexer, and S. Waiblinger. 2005. Heart rate variability in dairy cows—Influences of breed and milking system. Physiol. Behav. 85:195–204.

Hagen, K., D. Lexer, R. Palme, J. Troxler, and S. Waiblinger. 2004. Milking of Brown Swiss and Austrian Simmental cows in a herringbone parlour or an automatic milking unit. Appl. Anim. Behav. Sci. 88:209–225.

Hainsworth, R. 1995. The control and physiological importance of heart rate. Pages 3–9 in Heart Rate Variability. M. Malik and A. J. Camm, ed. Futura Publishing, Armonk, NY.

Hopster, H., J. M. O'Connell, and H. J. Blokhuis. 1995. Acute effects of cow-calf separation on heart rate, plasma cortisol and behaviour in multiparous dairy cows. Appl. Anim. Behav. Sci. 44:1–8.

Houle, M. S., and G. E. Billman. 1999. Low-frequency component of the heart rate variability spectrum: A poor marker of sympathetic activity. Am. J. Physiol. 276:H215–H223.

Koenig, J., M. N. Jarczok, R. J. Ellis, T. K. Hillecke, and J. F. Thayer. 2013. Heart rate variability and experimentally induced pain in healthy adults: A systematic review. Eur. J. Pain http://dx.doi.org/10.1002/j.1532-2149.2013.00379.x.

Kovács, L., M. Bakony, J. Tőzsér, and V. Jurkovich. 2013. Short communication: The effect of milking in a parallel milking parlor with non-voluntary exit on the HRV of dairy cows. J. Dairy Sci. 96:7743–7747.

Kovács, L., V. Jurkovich, M. Bakony, O. Szenci, P. Póti, and J. Tőzsér. 2014. Welfare implication of measuring heart rate and heart rate variability in dairy cattle: Literature review and conclusions for future research. Animal 8:316–330.

Lay, D. C., Jr., T. H. Friend, R. D. Randel, O. C. Jenkins, D. A. Neuendorff, G. M. Kapp, and D. M. Bushong. 1996. Adrenocorticotropic hormone dose response and some physiological effects of transportation on pregnant Brahman cattle. J. Anim. Sci. 74:1806–1811.

LeBlanc, S. J., T. F. Duffield, K. E. Leslie, K. G. Bateman, G. P. Keefe, J. S. Walton, and W. H. Johnson. 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J. Dairy Sci. 85:2223–2236.

Ledowski, T., M. Reimer, V. Chavez, V. Kapoor, and M. Wenk. 2012. Effects of acute postoperative pain on catecholamine plasma levels, hemodynamic parameters, and cardiac autonomic control. Pain 153:759–764.

Malliani, A. 1995. Association of heart rate variability components with physiological regulatory mechanisms. Pages 173–188 in Heart Rate Variability. M. Malik and A. J. Camm, ed. Futura Publishing, Armonk, NY.

Marchant-Forde, R. M., D. J. Marlin, and J. N. Marchant-Forde. 2004. Validation of a cardiac monitor for measuring heart rate variability in adult female pigs: Accuracy, artefacts and editing. Physiol. Behav. 80:449–458.

Martí, O., A. García, A. Vallès, M. S. Harbuz, and A. Armario. 2001. Evidence that a single exposure to aversive stimuli triggers long-lasting effects in the hypothalamus-pituitary-adrenal axis that consolidate with time. Eur. J. Neurosci. 13:129–136.

Mason, G., and M. Mendl. 1993. Why is there no simple way of measuring animal welfare? Anim. Welf. 2:301–319.

Mellor, D. J., C. J. Cook, and K. J. Stafford. 2000. Quantifying some responses to pain as a stressor. Pages 171–198 in The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare. G. P. Moberg and J. A. Mench, ed. CABI Publishing, Wallingford, UK.

Mialon, M.-M., V. Deiss, S. Andanson, F. Anglard, M. Doreau, and I. Veissier. 2012. An assessment of the impact of rumenocentesis on pain and stress in cattle and the effect of local anaesthesia. Vet. J. 194:55–59.

Mohr, E., J. Langbein, and G. Nürnberg. 2002. Heart rate variability: A noninvasive approach to measure stress in calves and cows. Physiol. Behav. 75:251–259.

Nakao, T., T. Sato, M. Moriyoshi, and K. Kawata. 1994. Plasma cortisol response in dairy cows to vaginoscopy, genital palpation per rectum and artificial insemination. Zentralbl. Veterinärmed. A 41:16–21.

Ness, T. J., and G. F. Gebhart. 1990. Visceral pain: A review of experimental studies. Pain 41:167–234.

Niskanen, J. P., M. P. Tarvainen, P. O. Ranta-Aho, and P. A. Karjalainen. 2004. Software for advanced HRV analysis. Comput. Methods Programs Biomed. 76:73–81.

Pilz, M., C. Fischer-Tenhagen, G. Thiele, H. Tinge, F. Lotz, and W. Heuwieser. 2012. Behavioural reactions before and during vaginal examination in dairy cows. Appl. Anim. Behav. Sci. 138:18–27.

Porges, S. W. 1995. Cardiac vagal tone: A physiological index of stress. Neurosci. Biobehav. Rev. 19:225–233.

Porges, S. W. 2003. The polyvagal theory: Phylogenetic contributions to social behavior. Physiol. Behav. 79:503–513.

Rietmann, T. R., M. Stauffacher, P. Bernasconi, J. A. Auer, and M. A. Weishaupt. 2004. The association between heart rate, heart rate variability, endocrine and behavioural pain measures in horses suffering from laminitis. J. Vet. Med. A Physiol. Pathol. Clin. Med. 51:218–225.

Romano, J. E., J. A. Thompson, D. C. Kraemer, M. E. Westhusin, D. W. Forrest, and M. A. Tomaszweski. 2007. Early pregnancy diagnosis by palpation per rectum: influence on embryo/fetal viability in dairy cattle. Theriogenology 67:486–493.

Sayers, B. M. 1973. Analysis of heart rate variability. Ergonomics 16:17–32.

Schmidt, A., E. Möstl, C. Wehnert, J. Aurich, J. Müller, and C. Aurich. 2010. Cortisol release and heart rate variability in horses during road transport. Horm. Behav. 57:209–215.

Sgoifo, A., J. M. Koolhaas, E. Musso, and S. F. De Boer. 1999. Different sympathovagal modulation of heart rate during social and nonsocial stress episodes in wild-type rats. Physiol. Behav. 67:733–738.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65:1516–1530.

Stafford, K. J., and D. J. Mellor. 2011. Addressing the pain associated with disbudding and dehorning in cattle. Appl. Anim. Behav. Sci. 135:226–231.

Stewart, M., K. J. Stafford, S. K. Dowling, A. L. Schaefer, and J. R. Webster. 2008. Eye temperature and heart rate variability of calves disbudded with or without local anaesthetic. Physiol. Behav. 93:789–797.

Stewart, M., G. A. Verkerk, K. J. Stafford, A. L. Schaefer, and J. R. Webster. 2010. Noninvasive assessment of autonomic activity for evaluation of pain in calves, using surgical castration as a model. J. Dairy Sci. 93:3602–3609.

Stubsjøen, S. M., A. S. Flø, R. O. Moe, A. M. Janczak, E. Skjerve, P. S. Valle, and A. J. Zanella. 2009. Exploring non-invasive methods to assess pain in sheep. Physiol. Behav. 98:640–648.

Sutherland, M. A., A. R. Rogers, and G. A. Verkerk. 2012. The effect of temperament and responsiveness towards humans on the behavior, physiology and milk production of multi-parous dairy cows in a familiar and novel milking environment. Physiol. Behav. 107:329–337.

von Borell, E., J. Langbein, G. Després, S. Hansen, C. Leterrier, J. Marchant-Forde, R. Marchant-Forde, M. Minero, E. Mohr, A. Prunier, D. Valance, and I. Veissier. 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals: A review. Physiol. Behav. 92:293–316.

Waiblinger, S., C. Menke, J. Korff, and A. Bucher. 2004. Previous handling and gentle interactions affect behaviour and heart rate of dairy cows during a veterinary procedure. Appl. Anim. Behav. Sci. 85:31–42.

Watamura, S. E., B. Donzella, D. A. Kertes, and M. R. Gunnar. 2004. Developmental changes in baseline cortisol activity in early childhood: relations with napping and effortful control. Dev. Psychobiol. 45:125–133.

Weiss, D., S. Helmreich, E. Möstl, A. Dzidic, and R. M. Bruckmaier. 2004. Coping capacity of dairy cows during the change from conventional to automatic milking. J. Anim. Sci. 82:563–570.

Youngquist, R. S. 1997. Pregnancy diagnosis. Pages 295–303 in Current Therapy in Large Animal Theriogenology. R. S. Youngquist, ed. WB Saunders Co., Philadelphia, PA.