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Changes in ear-pinna temperature as a useful measure of stress in sheep (Ovis aries)

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Abstract

Activation of the sympathetic nervous system, with associated increases in heart rate and the redistribution of blood in preparation for 'fight or flight', is an integral part of the 'defence reaction'. In sheep, the defence reaction involves vasoconstriction in the ear-pinna. If decreases in ear-pinna temperature (T_p) can be used to indicate vasoconstriction, then it may be possible to use changes in T_p as a measure of the defence reaction. Ewe lambs were exposed to stressors including mustering into pens, moving between pens, isolation from conspecifics, and prolonged periods of exercise. Measurements of heart rate (HR), T_p, vaginal temperature (T_v), and salivary cortisol and urinary catecholamine concentrations were used to assess stress responses. A repeatable pattern of changes in HR, T_p and T_v was observed in response to stressors. Short-term disturbances resulted in increased HR, reduced T_p, and increased T_v. More sustained disturbances — for example, prolonged periods of exercise — resulted in a sustained elevation in HR, a sustained decrease in T_p, and a sustained elevation in T_v. The highest levels of cortisol and catecholamines were associated with the treatments that resulted in the longest periods of decreased T_p. We infer that changes in T_p occur largely in response to changes in sympathetic nervous activity, and that the potential exists to measure elements of stress responses by monitoring T_p in freely behaving animals. This is a minimally invasive measure that allows the monitoring of modest numbers of animals over prolonged periods with minimal handling.

Keywords: *animal welfare, defence reaction, ear-pinna, sheep, stress, temperature logger*

Introduction

In conducting initial studies on temperature regulation during exercise and transport in sheep (*Ovis aries*), we found that during exercise the ear-pinna temperature (T_p) shows a rapid relative decrease concurrent with an increase in core temperature (Ingram *et al* 2002). As changes in T_p are commensurate with vasoconstriction and vasodilation in the ear-pinna of sheep and rabbits (Bligh & Milton 1973; Li *et al* 1998), a likely explanation of these observations is the redistribution of blood flow away from the skin to the muscles in response to the onset of exercise (Bell *et al* 1983). Such rapid changes in cutaneous blood flow suggest mediation by the sympathetic nervous system, rather than by local control.

The importance of the sympathetic nervous system for effecting rapid blood-flow changes in preparation for exercise is well recognised, and if fully activated this causes immediate widespread changes including tachycardia, muscular vasodilation, and skin and splanchnic vasoconstriction (Cannon 1929; Abrahams *et al* 1960, 1962). This has been termed the 'defence reaction', and, as the original concept has evolved, it has become clear that a background of sympathetic nervous activity is continually imposed on conscious animals (Hilton 1982). More recent research has clearly demonstrated rapid blood-flow changes to the ear-pinna in rabbits as part of the defence reaction (Yu & Blessing 1997). In particular, it has been demonstrated for rabbits that cutaneous vasoconstriction of the ear occurs in response to the perception of both noxious and non-noxious stimuli (Yu & Blessing 1997). These initial changes in sympathetic vasomotor tone are considered the early 'alerting stage' of the defence reaction (Hilton 1982; Yu & Blessing 1997).

We hypothesise that changes in T_p can be used as a sensitive measure of activation of the defence reaction. The defence reaction is indicative of situations that an animal perceives as threatening, the response being changes in blood distribution in anticipation of exercise.

Our aim in this study was to measure changes in T_p in sheep in various stress situations, and to compare any changes in T_p with changes in other more standard physiological measures of stress. The stressors included visual isolation from conspecifics, exercise, and a combination of isolation and exercise. Physiological measures included T_p, vaginal temperature (T_v), heart rate (HR), urinary catecholamine concentration, and salivary cortisol concentration.

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Materials and methods

Animals and experimental design

The use of animals in this study was approved by the Ruakura Animal Ethics Committee, New Zealand. Romney-cross ewe lambs (n = 20) were used, aged approximately 11 months, with body weights of 37.5–48.0 kg (mean ± standard error, 41.7 ± 0.6 kg). Animals were purchased from a commercial farm and maintained at the Ruakura Agricultural Centre, Hamilton, New Zealand (37°46′S, 175°20′E) in outdoor paddocks with *ad libitum* access to pasture and water for three weeks prior to the experiment. The experiment was conducted during the southern hemisphere autumn. The lambs were randomly divided into four treatment groups of five animals, with each group monitored on the day of the experiment from 0600h until 1000h–1400h, dependent on treatment. Immediately following experimental treatments, the lambs were slaughtered (as they were intended for further use in meat science experiments). Lambs were stunned using a captive bolt and then immediately exsanguinated by throat cutting.

All treatment groups were brought into the yards at 0620h for saliva sampling and then remained in the yards until their respective treatments began. These treatments were:

Control — lambs were brought into the yards at 0620h where they remained until 1000h.

Exercise — lambs were walked at a moderate pace on a farm track from 0700h to 1100h, with a 5 min break in walking, on the hour, every hour.

Isolation — lambs were put into isolation pens from 0900h to 1300h.

Isolation–exercise — at 1000h lambs were separated into isolation pens for one hour, and were then walked for one hour. This was followed by a second hour of isolation and a second hour of exercise, finishing at 1400h.

The isolation pens had solid plywood walls and measured 1200 × 575 × 1200 mm (length × width × height). We define isolation as a single animal confined in an isolation pen without visual contact of conspecifics.

Collection of saliva and urine samples

Saliva was collected immediately before and after the experimental treatments. A spatula wrapped with cotton wool was placed in the animals' mouths and chewed. The saliva was then expressed from the cotton wool using a syringe and stored at –20°C until analysis. Urine samples were taken from the bladder within 5 min of slaughter using a needle and syringe. Each 4 ml urine sample was mixed with 50 µl of concentrated sulphuric acid to stabilise the catecholamines.

On-animal instrumentation

Three days prior to the experiment the animals were fitted with instrumentation to measure HR, T_v and T_p. Each animal was equipped with two temperature loggers (Temptag®, HortResearch, Hamilton, New Zealand) and a Free-Range Physiological Monitor (FRPM, HortResearch, Hamilton, New Zealand). The temperature loggers were recently developed by our group for long-term monitoring of temperature in freely behaving animals. The loggers can record 2000 measurements from each of two temperature sensors (thermistors), with an accuracy of 0.1°C and a resolution of 0.05°C, and can be programmed to record at different time intervals (between four seconds and several hours). The loggers are enclosed in a circular plastic case (diameter 45 mm, width 8 mm) and weigh a total of 17 g. In this study, the loggers were secured to the animal using velcro patches. The underside of each velcro patch was glued, with cyanoacrylate glue (Loctite 454 gel, Loctite Australia Pty Ltd, NSW, Australia), to wool below the ear (T_p) and on the rump (T_v). The temperature sensor for each of the temperature channels was connected to the logger by a lightweight flexible cable.

T_v was obtained from a sensor inserted into the vagina to a depth of approximately 100 mm. The sensor was secured by taping the external part of the lead to the vulva and rump. T_p was obtained from a sensor attached to the ventral surface of the ear pinna, located near the midline and approximately 40 mm from the tip. The sensor was attached directly to the ear using cyanoacrylate glue and then overlaid with thermally insulating foam tape (Microfoam surgical tape, 3M Health Care, USA).

The FRPM is an ambulatory physiological monitor, previously described in detail by Harris *et al* (1998), which can be configured to measure a range of physiological variables including the electrocardiogram (ECG). The device is enclosed in an aluminium case (260 × 120 × 30 mm) and weighs a total of 980 g. The FRPM is attached using a velcro patch glued to the animal's back and secured with an elastic girth strap. The ECG signal was recorded from two Ag/AgCl surface electrodes (Red Dot, 3M Health Care, USA) positioned on sites, which had been shaved and degreased with ethanol, just behind the left front leg over the axilla region and high on the left side immediately posterior to the scapula.

Climatic data were collected hourly from the Ruakura climate station (National Institute of Water & Atmospheric Research Ltd, Hamilton, New Zealand) located approximately 600 m from the experimental paddocks and yards.

Analysis of samples

Saliva samples were frozen at –20ºC until assay (enzyme-linked immunosorbent assay [ELISA]). Inter- and intra-assay coefficients of variation (CVs) were determined using commercially available controls (Salimetrics, USA) containing 1 and 10 ng ml^{-1} of cortisol in artificial saliva: inter-assay CV using 1 ng ml^{-1}, 10.3%; intra-assay CV using 1 ng ml^{-1}, 5.0%; inter-assay CV using 10 ng ml^{-1}, 5.1%; intra-assay CV using 10 ng ml^{-1}, 2.8%. Assay sensitivity was 0.047 ng ml^{-1}.

Noradrenaline and adrenaline concentrations were determined using high-performance liquid chromatography (HPLC) and electrochemical detection. Noradrenaline and adrenaline were extracted from 0.1 ml urine using

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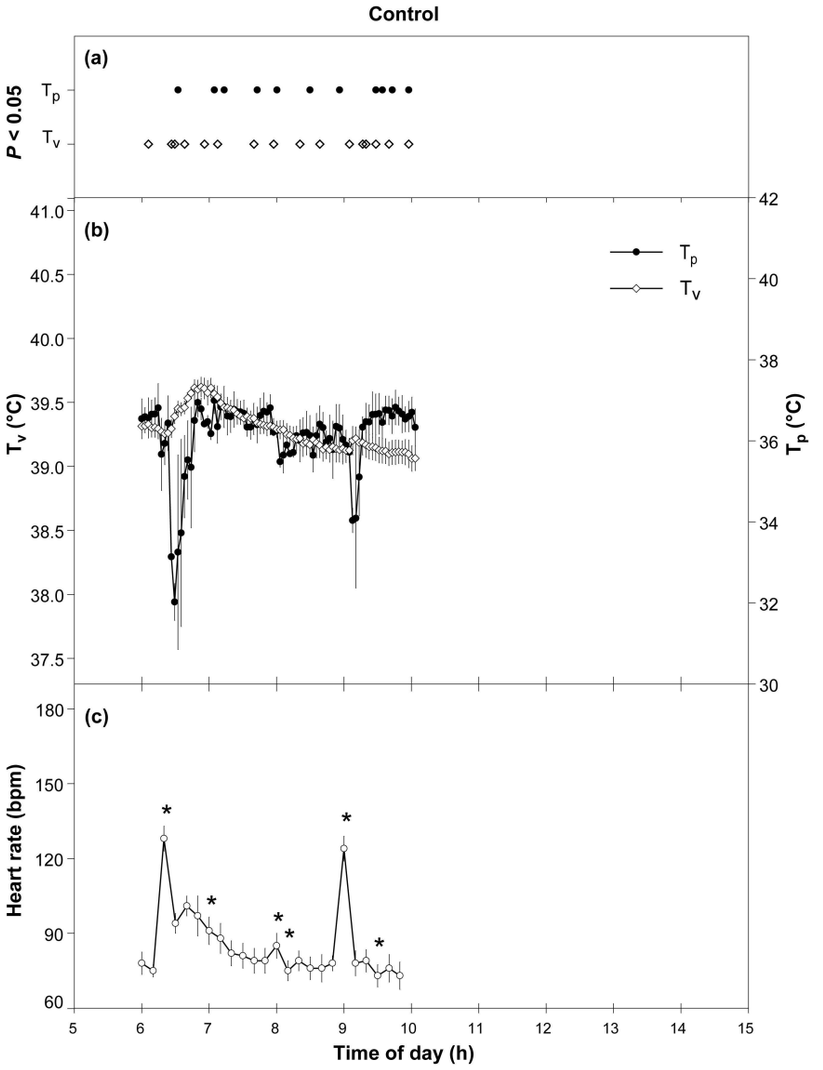
Figure 1



Control treatment. Changes in ear-pinna temperature (T_p), vaginal temperature (T_v), and heart rate with time of day. Sheep were mustered at 0620h and held in yards until 1000h. Figure 1a indicates when significant changes in T_p and T_v were detected for successive measurements ($P < 0.05$). Values are mean ± SEM; n = 3.

aluminium oxide, as described by He *et al* (1997). Urinary values for noradrenaline and adrenaline were all adjusted for glomerular filtration rate, by expressing the results as ng μM creatinine^{-1}. Creatinine is an endogenous marker of glomerular filtration rate, and was measured by HPLC (Thienpont *et al* 1995).

Data collection and statistical analysis

Temptag loggers were configured to record temperature measurements every 3 min throughout the experiment. At the end of the treatment period, data were downloaded directly to a PC.

Electrocardiograms were recorded for 1 min, every 10 min, from 0600h on the morning of the experiment until the end of the treatment period. Data were then downloaded directly to a PC. The data were analysed to detect HR using an in-house peak-detection programme. HR is expressed as beats per min (bpm).

Temptag data (T_p and T_v) are shown as the mean ± standard error of the mean (SEM). Complete data sets were not obtained for every animal and the sample size is indicated in figures. The data were analysed using multiple paired *t*-tests to compare consecutive time points. Significance was set at $P < 0.05$. Urinary catecholamine concentrations were tested using one-way ANOVA. Salivary cortisol concentrations before and after treatments were compared using two-way repeated-measures ANOVA. All statistical analyses were performed using Sigmastat version 2.03.

Results

Control treatment

Brief elevations were observed in HR at 0620h (Figure 1). This coincided with mustering the sheep into the yards and was common to all treatments. A second large increase in HR occurred at 0900h in the control, isolation and isolation–exercise treatments, which coincided with the

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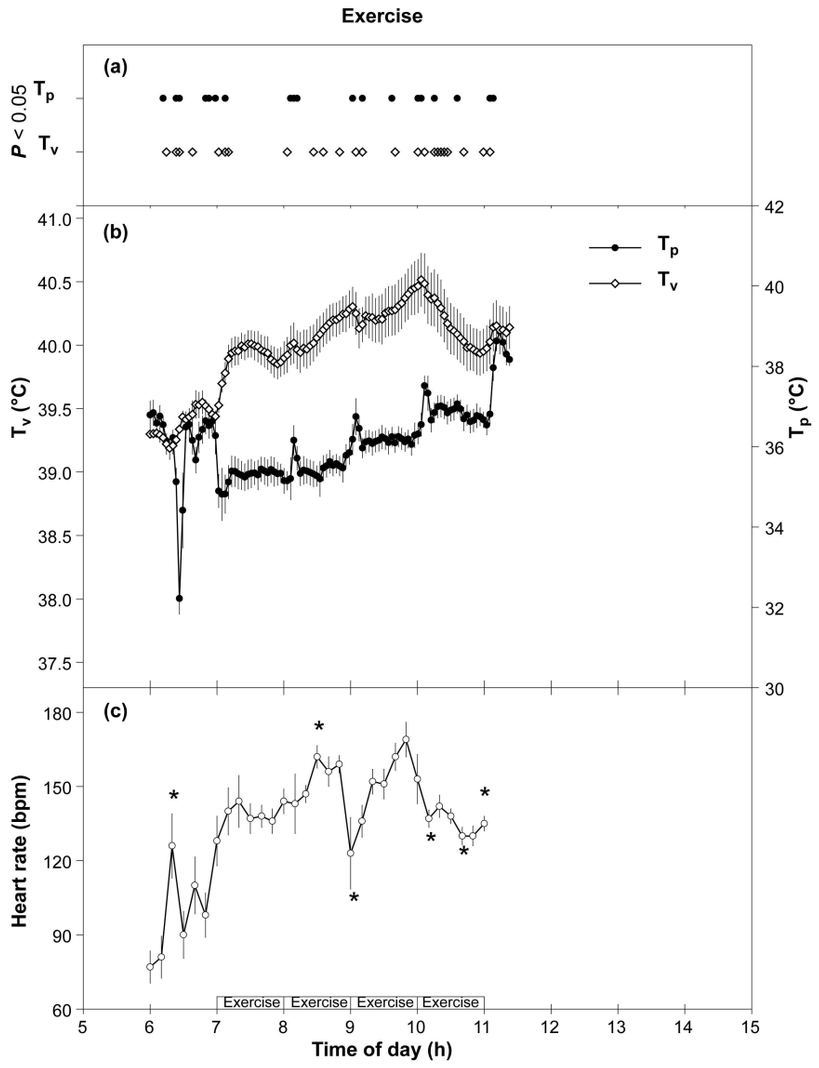
Figure 2



Exercise treatment. Changes in ear-pinna temperature (T_p), vaginal temperature (T_v), and heart rate with time of day. Sheep were mustered at 0620h and exercised from 0700h to 1100h. Figure 2a indicates when significant changes in T_p and T_v were detected for successive measurements ($P < 0.05$). Values are mean ± SEM; n = 4.

moving of all the animals in these groups within the yards. HR then returned to pre-mustering levels in the control group for the remainder of the experiment.

A large decrease in T_p was seen briefly at approximately 0620h (Figure 1). This decrease was seen in all groups (Figures 1–4). A second decrease in T_p was observed at 0900h in the control, isolation and isolation–exercise groups, when these groups were all moved within the yards. A sustained increase in T_v was observed, beginning at 0620h and finishing at approximately 0715h, followed by a second smaller peak at 0900h.

Exercise

Exercise began at 0700h and finished at 1100h (Figure 2). Over this time, mean HR ranged between 122 and 170 bpm. At the beginning of exercise, T_p decreased, and this decrease was sustained during the periods of exercise in the 4 h experimental period. Within this period there were three small increases during the 5 min breaks from walking that the sheep had every hour. The most marked changes in T_v occurred during mustering and at the end of each hour of exercise. The decrease in T_v during the fourth hour of exercise was associated with a lowering of exercise intensity, as demonstrated by a corresponding decrease in HR at this time.

Isolation

When the lambs were first placed in isolation they bleated for several minutes (all lambs were in adjacent pens and could hear each other) and they were clearly physically agitated. As isolation continued they appeared to settle.

HR increased at 0900h to 110 bpm, but within 20 min had decreased to approximately 78 bpm where it remained until the end of isolation at 1300h (Figure 3). There were only two small increases of HR after separation into isolation pens, at 1120h and 1210h.

T_p showed a transitory decrease at 0900h, and then steadily increased until 1300h, with some variation from 1200h to

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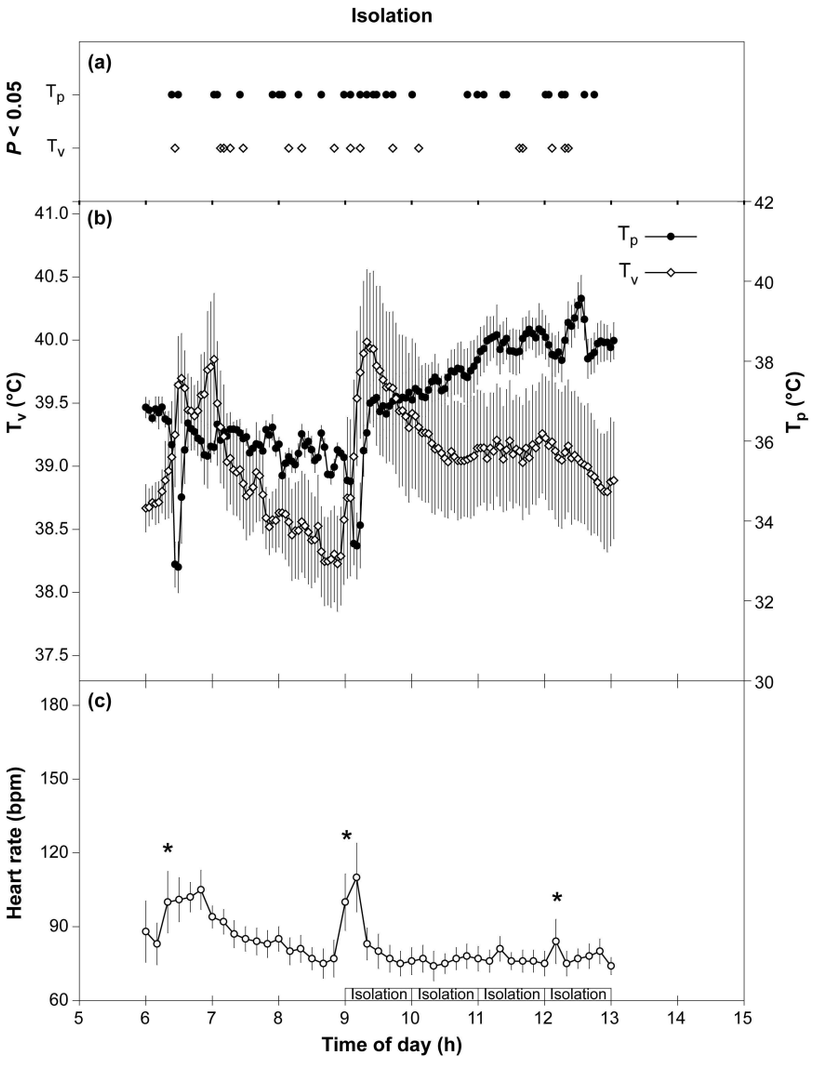
Figure 3



Isolation treatment. Changes in ear-pinna temperature (T_p), vaginal temperature (T_v), and heart rate with time of day. Sheep were mustered at 0620h and held in yards until 0900h, then put into isolation pens from 0900h to 1300h. Figure 3a indicates when significant changes in T_p and T_v were detected for successive measurements ($P < 0.05$). Values are mean ± SEM; n = 5.

1240h. T_v increased rapidly at 0900h, then slowly decreased over the next hour and from 1000h remained constant until the end of the treatment at 1300h.

Isolation–exercise

The mean HR in the isolation–exercise treatment varied across the treatment phases (Figure 4). HR increased to approximately 125 bpm when the sheep were first placed in isolation at 1000h and then steadily decreased to approximately 75 bpm at 1050h. At 1100h the first period of exercise began, and HR rose to approximately 180 bpm. The second period of isolation resulted in a decrease in HR compared to the first exercise period. In the second exercise period HR rose to approximately 165 bpm.

T_p showed a brief decrease at the start of isolation at 1010h, and showed a sustained decrease during the first exercise period from 1110h to 1205h. T_p increased from exercise levels during the second period of isolation and decreased again during the second exercise period. T_v rapidly increased from 1000h at the beginning of isolation and then levelled for the remainder of the first isolation period. There was a rapid and large increase in T_v during the first exercise period, followed by a decrease during the second isolation period. The second exercise period caused T_v to increase again.

Ambient temperature

Ambient temperature began to increase from approximately 0800h (Figure 5). The expected thermoregulatory response to this increased heat load at rest is for increased vasodilation of the ear-pinna. This is reflected by an increase in T_p, as the gradient between ambient temperature and T_p decreases. The effect is seen in Figure 3 as T_p increases from approximately 0900h in the isolation treatment, but applies only when there is no apparent vasoconstriction of the ear.

Hormonal responses

Salivary cortisol concentration was significantly elevated in the exercise and isolation–exercise groups following their

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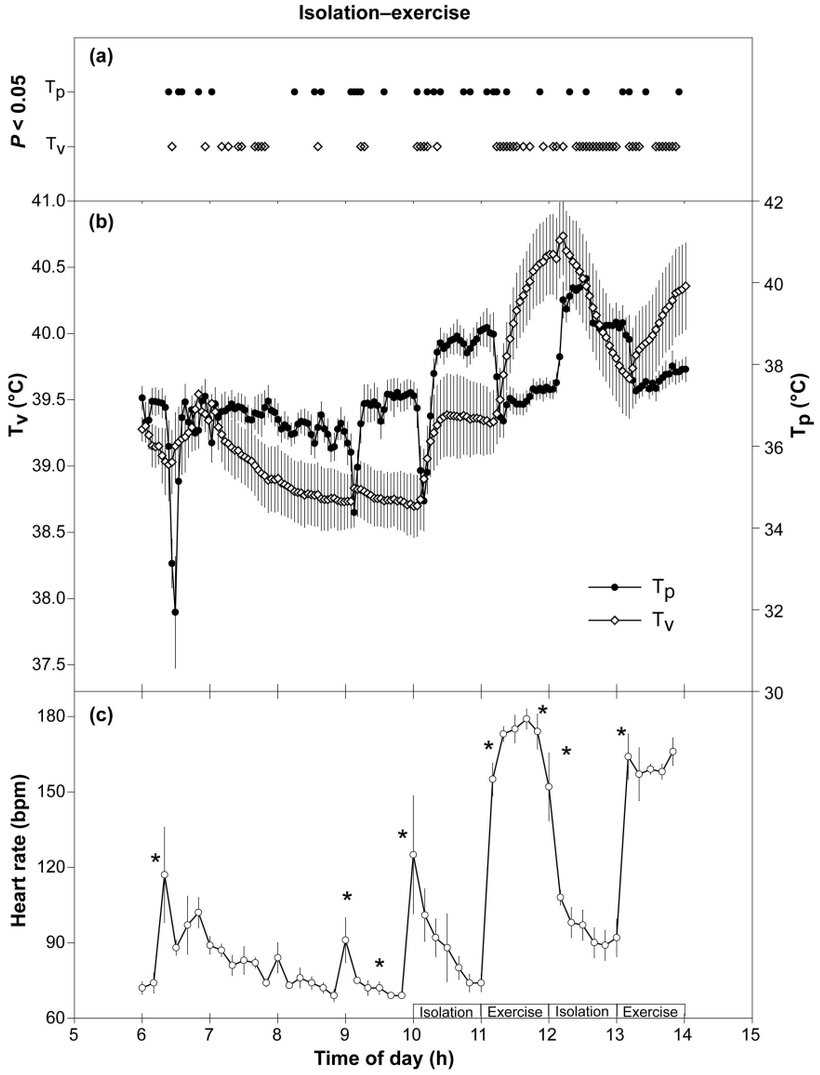
Table 1 Mean heart rate during the treatment period (mean ± SEM), mean concentrations of urinary noradrenaline and adrenaline (mean ± SEM), and concentrations of salivary cortisol immediately before and after treatment (salivary cortisol concentrations before and after treatment were compared using a two-way repeated-measures ANOVA where the SE of least-squared mean = 1.22). Values in the same row with the same superscript are not significantly different.

	Control	Exercise	Isolation	Isolation–exercise
Heart rate (bpm)	85.2 ± 3.0	144.0 ± 2.4	79.7 ± 1.7	125.8 ± 7.8
Urinary noradrenaline (ng μM creatinine^{-1})	2.16 ± 0.42^{a}	5.36 ± 0.50^{b}	1.88 ± 0.23^{a}	3.09 ± 0.45^{a}
Urinary adrenaline (ng μM creatinine^{-1})	1.20 ± 0.20^{a}	2.18 ± 0.27^{b}	1.05 ± 0.11^{a}	1.33 ± 0.29^{a}
Salivary cortisol before treatment (ng ml^{-1})	2.22^{a}	2.24^{a}	2.38^{a}	2.93^{a}
Salivary cortisol after treatment (ng ml^{-1})	2.3^{a}	9.52c	3.20^{ab}	7.02^{bc}

Figure 4



Isolation–exercise treatment. Changes in ear-pinna temperature (T_p), vaginal temperature (T_v), and heart rate with time of day. Sheep were mustered at 0620h and held in yards until 1000h, then alternatively put into isolation and then exercised every hour until 1400h. Figure 4a indicates when significant changes in T_p and T_v were detected for successive measurements ($P < 0.05$). Values are mean ± SEM; n = 4.

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respective treatments, compared to the control and isolation groups (Table 1). Urinary noradrenaline and adrenaline concentrations were elevated in the exercise group only (Table 1).

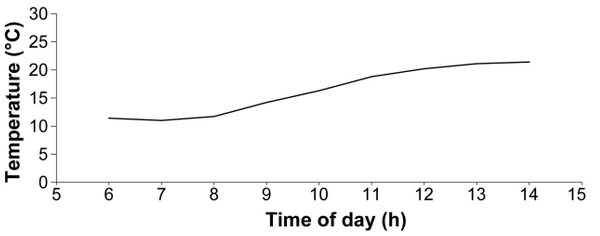
Discussion

In this study, a brief decrease in T_p in combination with increased HR was consistently seen upon mustering the sheep from pasture. An identical response pattern was also elicited during moving between pens in the yards and at the beginning of isolation (which initially involved physical agitation) and exercise. These changes are consistent with the sympathetic changes that occur in the 'defence reaction', which is generally regarded as preparation for muscular effort (Abrahams 1960, 1962; Hilton 1982).

The stress-related decreases in T_p were typically followed by elevation of T_v for the duration of the stressful event. For example, lambs in the exercise treatment showed a rapid increase in T_v at the beginning of exercise, which continued until cooling mechanisms were apparently activated. This thermoregulatory lag between increases in core temperature and the onset of cooling is typical of exercising mammals (Sawka & Wenger 1988). If sheep are exercising in mild heat, then blood flow changes so that there is a net loss of blood to the ears and a large increase in blood flow to the nasal mucosa and turbinates (Bell *et al* 1983; Hales 1986). This pattern of blood-flow change explains why sustained vasoconstriction in the ear is consistent with heat loss while the lambs were exercising. The initial rise in T_v supports the idea that there is not any initial increase in heat dissipation and that elevation of core temperature is an important element of preparing an animal for exercise. Sheep are a panting species and achieve heat loss from respiration (Hales 1986). This contrasts with predominantly sweating species, such as humans, in which peripheral blood flow is important for cooling. Similar experiments in a sweating species could result in a different response based on these different cooling strategies.

An alternative or contributory mechanism for the elevation of core temperature is a psychogenic febrile response to stress, which is a rise in thermoregulatory set-point that has been reported for mammals, birds and reptiles (Moltz 1993; Parrott & Lloyd 1995; Cabanac & Seika 2000). Febrile increases in core temperature are caused by prostaglandin-dependent peripheral vasoconstriction and are initiated by some types of psychological stress (Kluger *et al* 1987). Febrile-like responses can be differentiated from hyperthermic increases, as, while hyperthermia from exercise is characterised by a rapid decrease in temperature following the end of exercise, febrile responses show more sustained increases (Parrott *et al* 1999). We have unpublished data showing that prostaglandin inhibitors can only partially suppress the initial temperature rise observed in the isolation treatment used in the present study. In addition, neither salivary cortisol nor urinary catecholamines were elevated in the isolated group. We conclude in the present study that the initial increases in T_v are predominantly a sympathetic vasomotor response as opposed to a febrile-induced response.

Figure 5



Ambient dry-bulb temperature on the day of the experiment.

In rabbits, changes in ear-pinna blood flow have been shown to be very sensitive indicators of noxious stimuli (ear pinch) and perceived non-noxious threats (noise, fur touch, drape removal), and these changes are typically of short duration. The 'defence reaction', whilst initially perceived as preparing the animal for fight and flight, is clearly graded and applicable to many non-stressful situations which involve modest physical activity. Non-noxious threats are emotionally mediated (and can be conditioned) and result from perception, whereas noxious stimuli always result in vasoconstriction and are not conditioned (Yu & Blessing 1997, 2001; Blessing 2003). The sampling frequency of T_p used in this study was not high enough to detect the subtle responses reported above for rabbits. Rather, in the present study, the instances in which T_p decreased correspond well with easily defined events that always involved a modest level of physical agitation.

The setting used for the loggers in this experiment was effective in detecting only prolonged vasoconstriction, and, as expected, the exercise and isolation–exercise treatments resulted in the longest periods of decreased T_p. This was associated with increased levels of cortisol and catecholamines. One particular area of interest to us has been the identification of stressors associated with the management and transport of cattle and sheep prior to slaughter. The association between elevated stress hormones and the potential for decreased meat quality has become firmly established in the literature (Tarrant 1988; Warriss 1990; Lowe *et al* 2004). Indeed, this association has created the perception that high quality meat equates with good animal welfare, which is not necessarily true. We believe that there is further scope in animal studies for the use of T_p as an indicator of significant sympathetic activity.

Animal welfare implications

This study suggests that the elements of the stress response that are mediated by the sympathetic nervous system (the defence reaction) can be monitored using T_p in freely behaving sheep. Decreases in T_p were good indicators of periods of physical activity in the animals, and prolonged periods of decreased T_p were associated with elevation of cortisol and catecholamines. The current sensitivity of the logger appears to be appropriate for detection of modest to

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high levels of stress. This type of measurement technology has several advantages for stress measurement: it is minimally invasive, it is applicable for prolonged monitoring of modest numbers of animals, and it eliminates the need for repeated experimenter intervention.

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