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Corticosterone tissue-specific response in Sprague Dawley rats under acute heat stress

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ABSTRACT

Our study evaluated the physiological responses to acute heat stress in rats via body temperature and tissue corticosterone levels, and investigated the relative tissue response to heat stress based on corticosterone. Body temperature of rats under 22 °C (control) and 42 °C for 30 (H30), 60 (H60) and 120 min (H120) was measured. Correspondingly, corticosterone was analyzed in 11 tissues (adrenal, brain, heart, kidney, liver, lung, leg muscle, blood, stomach, spleen and small intestine). Analysis of variance and correlations were conducted on body temperature and corticosterone levels. The receiver operating characteristic (ROC) analyzed the thermo-sensitivity via corticosterone. Body temperature of rats in H30, H60 and H120 groups were higher ($P < 0.05$) than the control. Compared to the control, corticosterone levels of heart, stomach and small intestine at H30, corticosterone levels in adrenal, leg muscle and stomach at H60, and corticosterone levels in adrenal, heart, lung, stomach and small intestine at H120 differed ($P < 0.05$). The corticosterone in lung tissue was an excellent indicator of acute heat stress, with an area under the curve (AUC) of 1.00 at H60 and H120. In order to improve the prediction of acute heat stress, models combining corticosterone levels of multiple tissues reached an AUC of 1.00 for H30, and the sensitivity increased to 100% for H60 and H120. In conclusion, changes in the patterns and thermosensitivity of corticosterone levels associated with the duration of heat stress across body tissues were evidenced. The single and multi-organizational corticosterone models serve as indicators for evaluating heat stress across different time periods.

1. Introduction

Heat stress is a major component among a variety of stressors affecting homeostasis and health status of animals, consequential to the demands of thermoregulation (Bocheva et al., 2008). The heat stress response depends mainly on the hypothalamic-pituitary-adrenal (HPA) axis, which induces a series of neuroendocrine reactions, including an increase in the release of corticosterone and adrenocorticotropic hormones (Jasnic et al., 2011; Nakahara et al., 2004). In turn, these hormones assist animals to support greater allostatic loads (McEwen, 1998) and reduce the negative repercussions associated with heat stress. Further research of how animals cope with heat stress in relation to the HPA-axis and other perspective body tissues is essential towards understanding thermo-tolerance, as well as in the discovery and validation of potential biomarkers.

When heat stress occurs in homeotherms body temperature increases, respiratory rate accelerates, and sweating ensues among other physiological manifestations (Ahmed et al., 2017; Cwynar et al., 2014). Additional changes provoked by heat stress induce spatiotemporal temperature distribution in different tissues (Rakesh et al., 2013) and fluctuations in neuroendocrine hormones (Koko et al., 2004; Leon and Helwig, 2010). These changes are related to the activation of the HPA-axis from heat stimulus, and therefore could be used as predictors for assessing the extent of heat stress (Wang et al., 2015).

Elevated corticosterone associated with stress plays a vital role in coordinating vertebrate responses for managing acutely stressful stimuli

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(Sapolsky, 2000). Increased corticosterone due to high intensity or sudden heat stress represents a short-term activation of responses; this contributes to the animal's capacity to cope with environmental changes, particularly in time of acute stress exposure (Pignatelli et al., 1996). Several studies have reported that alterations in corticosterone were influenced by the type of body tissue (e.g., blood and adrenal) (Ippolito et al., 2014) and the duration of acute stress exposure (Djordjevic et al., 2003; Mete et al., 2012). These findings suggest that corticosterone could be used as an indicator of acute heat stress. However, differences in the corticosterone levels comparing tissues, along with variability in the duration of time spent subject to heat stress, should be considered.

To the best of our knowledge, no study has reported an analysis integrating corticosterone levels across multiple tissue types under different heat stress conditions. We hypothesized that corticosterone levels across body tissues might vary according to time and body location under different heat stress conditions. Our research aimed to 1) perform a corticosterone mapping of tissues under different conditions of acute heat stress and; 2) identify the body tissues more sensitive to heat stress through changes in corticosterone levels.

2. Materials and methods

2.1. Animal experimentation

The experiments were performed at the College of Animal Science and Technology, China Agricultural University. The institutional Animal Care and Use Committee approved the experimental procedures, which complied with the guidelines of the China Physiological Society for research involving animals. Fifty-eight female Sprague-Dawley rats were used (Beijing Vital River Laboratory, Animal Technology Co, Ltd, Beijing, China), at eight weeks of age and weighing 205 ± 7.16 g (mean ± standard deviation). Individual rats were identified by ink-labeled numbers on their tails and were segregated into training and validation subsets as part of the experimental design. In the training set, 48 rats were assigned to either the control group (room temperature: 22 °C, relative humidity (RH): 50%, n = 24) (Gordon et al., 2004), or one of three groups (n = 8) under heat treatment at 42 °C (Nonaka et al., 2015) and RH 50% for either 30 (H30), 60 (H60), or 120 min (H120). In the validation set, another 10 rats of the same gender, age and body condition were randomly allocated to either the control (n = 4) or H120 (n = 6) groups to verify the accuracy of the corticosterone prediction model obtained from the trial test. The maintenance feed (Beijing KeaoXieli Feed Co, Ltd, Beijing, China) composed of 226 g/kg crude protein, 45 g/kg crude fat, 39 g/kg crude fibre, 92 g/kg moisture, 12.2 g/kg calcium, 7.4 g/kg phosphorus and 64 g/kg crude ash was provided ad libitum with water. Rats were housed in Nalgene polycarbonate cages (40 by 30 by 180 cm, Beijing Vital River Laboratory, Animal Technology Co, Ltd, Beijing, China) bedded with soft woodchips. The floor-standing artificial climate incubator (BIO250, Boxun Medicine Instrument Co, Shanghai, China) was used to conduct the heat treatments. The body temperature of rats was measured rectally with a thermometer (precision of ± 0.1 °C, MC-347, Omron Corporation, Kyoto, Japan) before and after exposure to heat stress. The procedure for collecting body temperature measurements began by grabbing the back skin of the rat to position the abdomen upward, then the calibrated thermometer thermistor was inserted 1 cm into the rectum. The rectal temperature was obtained from the display on the thermometer 10 s after insertion. All animals were acclimatized to the laboratory conditions (room temperature: 22 °C, RH 50%, light hours: 12 h with lights on at 6:00 and off at 18:00) for one week prior to the experiment. During this acclimatization period, the body temperature of rats was measured a few times daily. Throughout the experiment, the rats were free in the cages and unnecessary human interference was circumscribed.

2.2. Collection of body tissues and corticosterone determination

Forty-two rats were sampled, including 32 rats in the training set (8 rats per group) and 10 rats in the validation set. Rats were anesthetized with 1.2 ml pentobarbital sodium (1%, 40 mg/kg of body weight) after the body temperature measurement. The rats were sacrificed within 2 min from the end of heat treatment exposure. Cardiac puncture was performed on each rat using a needle (1 cm by 22 G, BD Vacutainer® EclipseTM, Becton, Dickinson and Company, USA) to obtain an approximate 3 ml blood sample, which was then stored in heparin blood collection tubes (BD Vacutainer® EclipseTM, Becton, Dickinson and Company, USA). Blood samples were centrifuged at 3500 rpm for 5 min, then blood plasma (blood) was harvested and stored at −20 °C until further assays. The adrenal, brain, heart, kidney, liver, lung, leg muscle (muscle), stomach, spleen and small intestine (intestine) were harvested in the training set, and adrenal and muscle were collected in the validation set. Tissue samples were washed in ice-cold phosphate buffer solution (PBS) and snap frozen immediately in liquid nitrogen, before being stored at −80 °C until further analysis.

Tissues were processed prior to corticosterone analysis. Briefly, frozen tissues were placed into 2 ml polypropylene test tubes (MCT-200-C, Axygen Co, 33210 Central Avenue Union City, California, USA) containing 1 ml of ice-cold PBS (proportion of 9 ml PBS per gram of body tissue). The samples were homogenized (35 Hz for 5 min) with a high throughput tissue lapping instrument (Scientz-48, Scientz Biotechnology Co., Ltd. Ningbo, China), then centrifuged at 3000 rpm for 10 min. The supernatants were harvested and placed into 1.5 ml polypropylene test tubes (MCT-200-C, Axygen Co, 33210 Central Avenue Union City, California, USA), and stored at −20 °C for further corticosterone analysis. The tissue corticosterone was determined in duplicate by a radioimmunoassay (RIA) technique using rat commercial rCorticosterone ^{125}IRIA kits (IZOPOT, Institute of isotopes Ltd, Budapest, Hungary) according to the manufacturer's instructions. Intra- and inter-assay coefficients of variation were measured by running standards in each assay, which fell in accordance with manufacturer recommendations. Intra-assay variation was below 9.5%, and inter-assay variation was below 7.5%.

2.3. Statistical analyses

Variables were tested to confirm normality based on the Shapiro Wilk test, kurtosis and skewness, prior to means comparison. One-way analysis of variance (ANOVA) with Bonferroni's multiple comparison tests using the SAS version 9.4 (SAS, Statistical Analysis System Institute, Cary, NC, USA) were used to test differences among least square means of the body temperature and corticosterone levels in the body tissues. Data were presented as mean plus or minus standard error of the mean (SEM). The Pearson's correlation coefficients among the 12 continuous variables (body temperature and the corticosterone levels of 11 tissues) across all groups (standardized within group before pooling) was calculated using the correlation procedure of SAS version 9.4 (SAS, Statistical Analysis System Institute, Cary, NC, USA). Additionally, the Pearson's correlation was determined within each group using the correlation procedure in R package (version 3.5.0, https://cran.r-project.org/src/base/R-3/), where the Pearson's correlation significance was computed using the Hmisc procedure. The logistic regression model of SAS version 9.4 (SAS, Statistical Analysis System Institute, Cary, NC, USA) was performed to explore the value of tissue corticosterone in the prediction of heat stress. The heat stress and non-heat stress states of rats were defined as “1” and “0”, respectively. A standard logistic regression model for heat stress can be described as: $logit\ (P_i) = \alpha + \beta X_i$, where P_i is the probability the rat is heat stressed; α is an intercept parameter; β is a vector of the coefficients of the tissue corticosterone and estimated by the method of maximum likelihood estimation (MLE); X_i means a vector of the tissue corticosterone that affects the response to heat stress of the rat i. Consequently, the P_i value

can be calculated with substitution of selected tissue corticosterone levels of rats into these equations, and then classified according to the standard value (default value: 0.5) to determine whether it is correct or not.

The receiver operating characteristic (ROC) analysis, which is a widely practiced methodology for diagnostic performance evaluations in this type of research, was used to evaluate the predictive power of the regression model (Hua et al., 2015). The area under the curve (AUC), 95% confidence interval (CI), sensitivity, specificity and the optimal cut-off value for assessing the predictive power of ROC was calculated using SAS version 9.4 (SAS, Statistical Analysis System Institute, Cary, NC, USA). The utility of thermosensitivity predictions through corticosterone levels were evaluated based on AUC as follows: 0.9–1.0 = excellent; 0.8–0.9 = good; 0.7–0.8 = fair, whereas an AUC value of 0.5 corresponded to a poor predictive model (Chan et al., 2009). In the present study, the AUC value in the validation study was re-assessed. Rats in the control and H120 groups were regrouped based on the cut-off values of adrenal and muscle obtained from the training set, and the new regression models of corticosterone levels in adrenal and muscle were established (Nishiumi et al., 2012). The probability obtained from these two regression models were used to determine whether the rats were from the heat stress group. Moreover, the sensitivity, specificity, and accuracy of the predictive model were re-estimated to evaluate the quality of the ROC. The roccontrast statement of the logistic regression procedure was used to conduct the combined prediction analysis for the multi-index model, and produced the probabilistic models of multi-tissue corticosterone for predicting the occurrence of acute heat stress response. For least square mean comparisons and correlations analysis, results were considered statistically significant when $P < 0.05$.

3. Results

3.1. Body temperature of rats

Fig. 1 shows the body temperature of rats in relation to their heat exposure. The body temperature of heat-treated rats was higher ($P < 0.05$) than the control and increased with extended heat exposure. Compared with the control (37.58 ± 0.44 °C), the body temperature of rats in H30, H60, and H120 increased by 2.73, 3.46, and 3.89 °C, respectively. Moreover, rats suffered more severely under H120 heat stress, where 3/8 rats had body temperatures exceeding 42 °C. The body temperature in H60 (41.03 ± 0.46 °C) and H120 (41.46 ± 0.47 °C) were higher ($P < 0.05$) than in H30 (40.31 ± 0.40 °C). There was no difference ($P \geq 0.05$) in body temperature between H60 and H120.

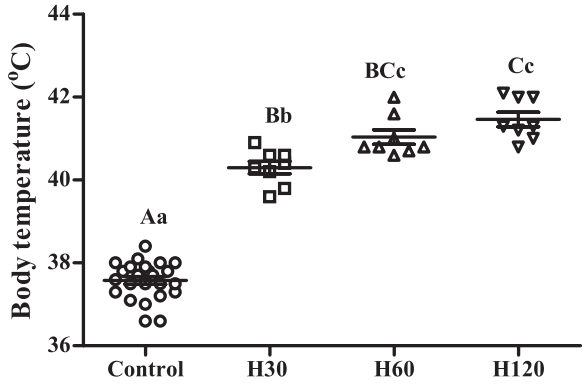

Fig. 1. Least square mean ± standard error of the mean of rats' body temperature (°C) kept under control or heat stressed for different durations. Control (n = 24) was kept at room temperature (22 °C), ; heat stressed were acclimated to 42 °C for 30 (H30), 60 (H60) and 120 min (H120) (n = 8 in each group). The lower- and upper-case letters represent $P < 0.05$ and $P < 0.01$ differences between groups, respectively.

3.2. Corticosterone profile of body tissues

Comparative analysis revealed that corticosterone levels were spatially and temporally specific (Fig. 2). Corticosterone levels in the adrenal, heart, lung, muscle, stomach and intestine were greatly impacted by different heat stress durations (Fig. 2a). Compared with the control, the corticosterone concentration decreased ($P < 0.05$) 14.4, 40.33 and 13.69 ng/mg in the heart, stomach and intestine in H30, respectively. When comparing H60 to the control, the corticosterone increased ($P < 0.05$) 30.86% in both the adrenal and muscle tissues, and decreased ($P < 0.05$) 48.08% in the stomach. In the comparison between H120 with the control, lower ($P < 0.05$) corticosterone in the heart (H120, 19.09 ± 1.78 vs. control, 37.28 ± 5.11 ng/mg), lung (H120, 20.94 ± 1.99 vs. control, 38.20 ± 1.57 ng/mg), stomach (H120, 22.99 ± 4.44 vs. control, 66.77 ± 9.77 ng/mg) and intestine (H120, 13.61 ± 1.45 vs. control, 31.37 ± 6.58 ng/mg) were observed. While an increase ($P < 0.05$) in corticosterone levels of the adrenal (H120, 26.11 ± 1.78 vs. control, 20.64 ± 1.03 ng/mg) and muscle (H120, 48.42 ± 2.37 vs. control, 23.43 ± 5.15 ng/mg) were observed between H120 and the control. Additionally, corticosterone levels of the muscle in H120 (48.42 ± 2.37 ng/mg) was higher than in H30 (18.81 ± 2.19 ng/mg, $P < 0.05$) and H60 (30.66 ± 3.28 ng/mg, $P < 0.05$). The varying corticosterone levels in the brain, kidney, liver, blood and spleen under the heat treatments were shown in Fig. 2b. Despite the fluctuations observed on corticosterone levels across these tissues, no differences were observed in relation to the control group ($P \geq 0.05$).

3.3. Correlations of body temperature and body tissue corticosterone content

Table 1 presents the correlations of body temperature and corticosterone levels across the 11 tissues evaluated. We found that the body temperature was negatively correlated with corticosterone levels in the adrenal, and positively correlated to corticosterone levels in the heart, lung, stomach and intestine. The corticosterone levels in the adrenal were negatively correlated with corticosterone levels in the kidney, while the corticosterone levels in the lung were positively correlated to corticosterone levels in the stomach and intestine. Additionally, the corticosterone levels in the stomach were correlated with corticosterone levels in the intestine. Pearson's correlation between body temperature and corticosterone levels and the 11 body tissues within each treatment group were provided in the Supplementary file (Fig. S1).

3.4. Thermal sensitivity analysis of corticosterone levels

In the training set, AUC values of the corticosterone levels from the 11 tissues for the three heat stress treatments were compared with the control (Table 2). In H30, the stomach corticosterone levels showed a relatively high AUC, potentially serving as an excellent predictor (AUC > 0.90). The AUC for corticosterone levels in the lung were higher than 0.80, representing a relatively high indicative power. The sensitivity of corticosterone in the kidney, liver, lung and stomach were greater than 70%, supporting the possibility to infer about heat stress based on corticosterone levels in these organs.

In H60, the lung corticosterone levels had the highest thermosensitivity (85%) with AUC equal to 1.00, supporting a strong indication of power (AUC > 0.90). Corticosterone levels of the adrenal and stomach were also highly useful in determining heat stress, with AUC greater than 0.80 and relatively high sensitivity. While, only corticosterone levels of the adrenal and lung had very high predictive accuracy (Table 2).

In H120, the AUC of corticosterone in the lung was 1.00, and the sensitivity was 2.5% lower than the specificity. Meanwhile,

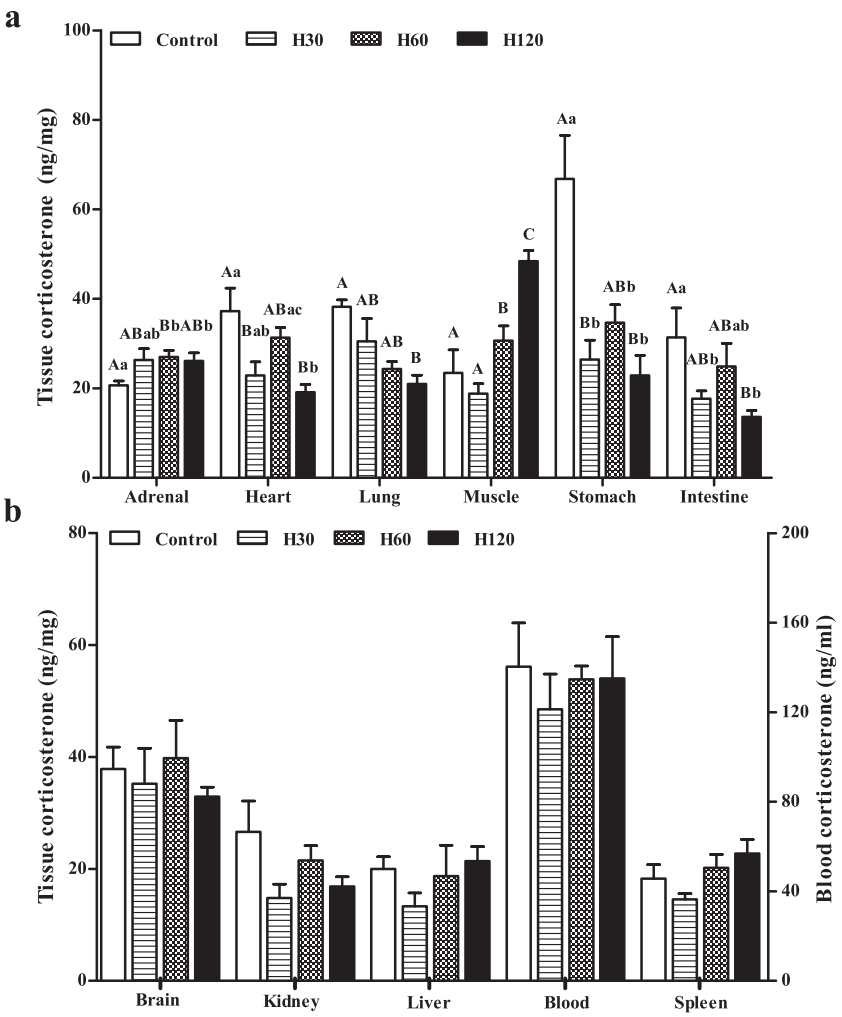

Fig. 2. Least square mean ± standard error of the mean of corticosterone levels in body tissues of rats (n = 8 per group). a) and b) Corticosterone levels across body tissues. Control was kept at 22 °C and heat stressed rats were kept at 42 °C for 30 (H30), 60 (H60) and 120 min (H120). Columns with differing lower or upper case letters, within the same body tissues, indicate $P < 0.05$ and $P < 0.01$ according to Bonferroni multiple comparison test.

corticosterone in the adrenal, stomach and intestine were also excellent predictive indices of acute heat stress (AUC > 0.9). The predictive efficacy of stomach corticosterone was superior to that of the adrenal and intestine, although they had the same sensitivity (87.5%). Corticosterone levels in the heart and muscle also had significant predictive power. In addition, the predictive accuracy of corticosterone in the adrenal, lung, muscle and stomach was greater than 80%. Corticosterone in other tissues also showed different thermosensitivity under diverse thermal stimulation conditions (Table 2). The results from the validation subset showed that the AUC, sensitivity, specificity, and accuracy values of corticosterone in adrenal and muscle tissues reached 100%, indicating the cut-off values of the training set could accurately predict whether the rats were exposed to heat stress.

The combined predictive analyses of tissue corticosterone in different treatments are presented in Table 3. Based on a regression analysis, probabilistic models of tissue corticosterone for predicting the

Table 1
Correlations among corticosterone levels and body temperature across body tissues in non- and heat stressed rats.

	Adrenal	Brain	Heart	Kidney	Liver	Lung	Muscle	Blood	Stomach	Spleen	Intestine
T_b[1]	− 0.48*	0.06	0.41*	0.33	0.19	0.47*	− 0.06	0.12	0.67*	− 0.13	0.39*
Adrenal	–	− 0.09	− 0.23	− 0.37*	0.00	0.02	0.26	0.08	− 0.16	− 0.13	− 0.32
Brain		–	0.18	0.03	− 0.18	0.16	− 0.18	0.00	− 0.04	0.09	0.26
Heart			–	0.18	− 0.07	0.03	− 0.14	− 0.06	0.22	0.26	0.19
Kidney				–	− 0.06	0.25	− 0.05	0.33	0.07	− 0.21	0.19
Liver					–	0.04	− 0.05	0.03	0.04	0.03	0.00
Lung						–	− 0.13	0.05	0.36*	− 0.23	0.47*
Muscle							–	0.03	− 0.02	− 0.04	− 0.34
Blood								–	0.00	− 0.04	0.02
Stomach									–	− 0.18	0.45*
Spleen										–	0.09

[1] Body temperature (T_b = 48) and corticosterone levels of body tissues (n = 32) of rats kept at 22 or 42 °C for 30, 60 and 120 min.
* denotes $P < 0.05$.

Table 2
Effect of heat stress duration (TIME) on corticosterone measured at different body tissues in rats.

TIME*	Tissues	AUC[1]	CL[2]	SEN[3] (%)	SPE[4] (%)	ACC[5] (%)	Cut-off[6]
H30	Adrenal	0.73	0.47–0.99	50.00	62.50	56.30	25.05
	Brain	0.63	0.32–0.93	0.00	0.00	0.00	31.01
	Heart	0.79	0.57–1.00	62.50	62.50	62.50	21.06
	Kidney	0.79	0.55–1.00	74.10	42.90	57.10	18.88
	Liver	0.77	0.48–1.00	75.50	75.50	75.00	13.42
	Lung	0.83	0.60–1.00	75.00	75.00	75.00	28.27
	Muscle	0.55	0.24–0.86	50.00	12.50	31.30	23.69
	Blood	0.61	0.30–0.92	50.00	37.50	43.80	101.54
	Stomach	0.91	0.76–1.00	75.00	75.00	75.00	25.26
	Spleen	0.69	0.40–0.97	62.50	62.50	62.50	15.32
	Intestine	0.75	0.48–1.00	62.50	62.50	62.50	25.72
H60	Adrenal	0.89	0.63–1.00	85.70	75.00	80.00	25.05
	Brain	0.52	0.16–0.81	0.00	0.00	0.00	38.18
	Heart	0.64	0.32–0.96	50.00	62.50	56.30	18.05
	Kidney	0.53	0.23–0.83	42.90	42.90	42.90	17.98
	Liver	0.67	0.36–0.98	0.00	0.00	0.00	19.51
	Lung	1.00	1.00–1.00	87.50	100.00	93.80	37.27
	Muscle	0.75	0.47–1.00	37.50	75.00	56.30	19.90
	Blood	0.56	0.10–0.78	0.00	0.00	0.00	113.56
	Stomach	0.86	0.67–1.00	75.00	62.50	68.80	39.66
	Spleen	0.59	0.29–0.89	37.50	25.00	31.30	18.50
	Intestine	0.60	0.30–0.91	62.50	25.00	43.80	18.57
H120	Adrenal	0.92	0.76–1.00	87.50	75.00	81.30	24.28
	Brain	0.63	0.31–0.94	62.50	50.00	56.30	40.32
	Heart	0.88	0.69–1.00	75.00	62.50	68.80	32.57
	Kidney	0.65	0.32–0.96	16.70	62.50	42.90	16.70
	Liver	0.55	0.12–0.79	0.00	0.00	0.00	21.08
	Lung	1.00	1.00–1.00	87.50	100.00	93.80	29.58
	Muscle	0.89	0.67–1.00	87.50	87.50	87.50	35.37
	Blood	0.55	0.21–0.89	0.00	0.00	0.00	170.95
	Stomach	0.94	0.81–1.00	87.50	87.50	87.50	25.05
	Spleen	0.72	0.42–1.00	50.00	75.00	62.50	18.49
	Intestine	0.92	0.79–1.00	87.50	62.50	75.00	14.09

* The H30, H60, and H120 represent the acclimation of rats to 42 °C for 30, 60 and 120 min, respectively (n = 8 in each group).
[1] AUC: area under the curve.
[2] CI: 95% confidence interval for the AUC determination.
[3] SEN: sensitivity.
[4] SPE: specificity.
[5] ACC: accuracy; and.
[6] cut-off values.

Table 3
Analytics of the best joint predictive models for assessing the degree of heat stress duration (TIME) in rats based on corticosterone in different body tissues.

TIME*	Tissues	SEN[1] (%)	SPE[2] (%)	ACC[3] (%)	AIC[4]	SC[5]
H30	Heart, Lung, Stomach	75.0	75.0	75.0	24.18	24.95
H60	Adrenal, Lung, Stomach	100.0	87.5	93.3	22.73	23.44
H120	Muscle, Stomach, Intestine	100.0	75.0	87.5	22.73	23.44

* H30, H60, and H120 represent the acclimation of rats to 42 °C for 30, 60 and 120 min, respectively (n = 8 in each group).
[1] SEN: sensitivity.
[2] SPE: specificity.
[3] ACC: accuracy.
[4] AIC: Akaike information criterion and.
[5] SC: Schwarz criterion.

occurrence of acute heat stress response under specific thermal stimuli are listed as follows: $logit\ (P)_{H30} = 110.50 - 0.65 * heart - 1.08 * lung - 1.39 * stomach$; $logit\ (P)_{H60} = 17.02 + 1.14 * adrenal - 1.31 * lung - 0.17 * stomach$; and $logit\ (P)_{H120} = 0.16 + 0.42 * muscle - 0.25 * stomach - 0.30 * intestine$. In the current study, the predictive value of the joint prediction model of corticosterone in the heart, lung and stomach was 100%, and the sensitivity, specificity, and accuracy were 75% in H30. The combined model of corticosterone in the adrenal, lung and stomach greatly improved the sensitivity of the prediction of heat stress in H60. Moreover, in H120, the combination of corticosterone in the muscle, stomach and intestine had 100% better thermal sensitivity than a single index.

4. Discussion

The present study tested the influence of heat stress on the body temperature of rats, which is critical for interpreting the extent of heat stress (Poole and Stephenson, 1977). In order to avoid the detection of behaviour affecting changes in body temperature and corticosterone, the body temperature of all animals was measured regularly for a week prior to the experiment. Therefore, the effect of measuring body temperature on changes in corticosterone contents were minimized in this study. In the current study, the rats suffered from mild heat stress when exposed to 42 °C for 30 min, 60 min and 120 min (Fig. 1), showing a range of body temperature changes (38.4–42.1 °C) which were consistent with previous reports. For instance, Leon et al. (2005) observed a maximum body temperature of 42.4 °C in rats exposed to the ambient temperature of 39.5 ± 0.2 °C for 240 min. Both body temperature and corticosterone levels are useful indices to infer about heat stress in rats (Chauhan et al., 2017; Judelson et al., 2007; Mete et al., 2012). However, the correlation between body temperature and tissue corticosterone was not reported previously. Correlations between the body temperature and tissue corticosterone levels herein (Table 1) support the association of body temperature and heat stress at the tissue level. Pearson correlations among the 12 continuous variables were also calculated based on the original data within each group (Fig. S1).

Different relationships among variables within each group, in terms of magnitude, direction and significance were noted. However, these should be considered with caution due to the reduced number of experimental units to calculate the proposed relationships.

The evidence of variation for corticosterone levels across different body tissues indicates a spatial-time variation in response to heat stress. This may be the result of complex interactions between physical structure, organ properties, as well as internal and external heat transfer regulatory mechanisms (Bouchama and Knochel, 2002; Islam et al., 2013; Tansey and Johnson, 2015; Rakesh et al., 2013; Leon et al., 2006). The changes of corticosterone levels in the adrenal were expected due to its role as a primary organ in response to stressful stimuli. Corticosterone synthesis occurs in the zona fasciculata of the adrenal cortex and is simultaneously regulated by the HPA-axis and suprachiasmatic nucleus (Son et al., 2008; Spiga et al., 2014). The HPA-axis promotes acute synthesis of corticosterone via genomic and non-genomic mechanisms during the onset of stress (Conway-Campbell et al., 2007). In addition, the brain-body reaction that appears clearly under stress (de Kloet et al., 2005) can activate the HPA-axis and subsequent release of corticosterone in the adrenal, which further modulates neurotransmitter release in the brain (McEwen et al., 1968). The strongest effects of acute heat stress on muscle were observed in H120 (Fig. 2), however an increase in corticosterone in the muscle may be beneficial for the conversion of proteins into an energy source for managing heat stress (Sapolsky et al., 2000).

Increased ambient temperature initiates the vasodilation response, while changes in blood corticosterone may represent an activation of the HPA-axis, thus indicating animals were subjected to heat stress (Gordon et al., 2002; Hernandez et al., 2014). Previous studies have reported that blood corticosterone levels increased when rats were exposed to a high ambient temperature of 40 °C for 90 min (Michel et al., 2007) or 38 °C for 4 h (Sinha, 2007). However, blood corticosterone has been shown to decrease with heat stress under 39 °C (1.3 ± 0.9) and 41 °C (1.09 ± 0.7) for 30 min (Mete et al., 2012). In the current study, no difference was observed between blood corticosterone levels in heat-stressed rats and those in the control (Fig. 2b). In addition, the predictive values of corticosterone in blood were lower than 0.70 (Table 2), thus, blood corticosterone levels cannot be used as an index to assess heat stress for 30, 60, and 120 min exposure times in rats. Factors such as the type of stressors, stress intensity and duration (Negrão et al., 2004; Waggoner et al., 2009) appear to compromise the use of blood corticosterone levels as a reliable indicator of stress, supported by our results.

The thermal sensitivity analysis of body tissues based on corticosterone levels, helps to predict the response upon exposure to heat stress conditions. In the present study, the sensitivity of various tissues showed significant differences across specific heat stress durations (Table 2). In recent studies, it was confirmed that the main factors that control corticosterone synthesis in vivo could lead to the interconversion of corticosterone between tissues (Bottoms and Goetsch, 1968; Kido et al., 2014), and induce increases in corticosterone levels within the brain, liver, and lung (Raul et al., 2004). It is expected that these changes might be due to the glucocorticoid and the mineralocorticoid receptors within the cytoplasmic compartment, which have an affinity for glucocorticoids (Reul et al., 1990). Moreover, the liver glucocorticoid receptor in rats has been shown to decrease after acute or chronic stress (Peijie et al., 2004).

Based on these predictions, there were several body tissues of which corticosterone levels were selected as potential biomarkers for heat stress across the heat stress duration treatments. However, some of these biomarkers showed relatively low sensitivity or specificity (Table 2), indicating that single tissue corticosterone was not practical for heat stress predicting. Conversely, the use of a multi-body tissue corticosterone combined prediction model might be more appropriate to identify candidates with high sensitivity and specificity. Although many approaches have been widely employed to identify single heat stress biomarkers (Rakesh et al., 2013; Lattin and Romero, 2014; Ippolito et al., 2014) or appropriate models for corticosterone studying (Russell et al., 2012; Fukasawa and Tsukada, 2010), these efforts cannot fully reflect the global heat stress process of the body. Based on the above, we recognize that the multi-body tissues corticosterone level models provide a more reliable predictive value compared to a single body tissue corticosterone model of heat stress under specific acute heat stress conditions (Table 3). Sensitivity and specificity are measures of predictive accuracy and the cut-off value refers to the value with maximized sensitivity. In the present study, the cut-off values for corticosterone levels in adrenal and muscle tissues for the training set regrouped the validation set rats 100% accurately, inherently supporting the accuracy of the corticosterone predictive model for these tissues.

In short, our study presented potential modifications based on the uniqueness of the heat stress response in tissue corticosterone levels under specific conditions. The main and novel findings of our study were: 1) the response of corticosterone to different heat stress durations was body tissue-specific; 2) the corticosterone in the lung and stomach were more sensitive to heat stress; 3) the appropriateness of individual body tissue in the multi-tissue corticosterone models to predict heat stress had their best-fit varying across each heat stress duration. Based on this evidence, further research on changes in corticosterone levels in other species using a similar approach is warranted. Our study could also be enhanced with an in-depth validation study at the levels of cellular and gene expression.

5. Conclusions

Collectively, we established a model for acute heat stress based on changes to body temperature and corticosterone levels in rats subject to varying durations of heat stress. We indicated that results for corticosterone mapping are unique to specific conditions of heat stress. In the present study, the stomach and lung were the most sensitive body tissues to heat stress, exemplified through changes in corticosterone levels. The joint analysis of multi-tissues for corticosterone levels improved the accuracy of the acute heat stress assessment.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2019.02.004.

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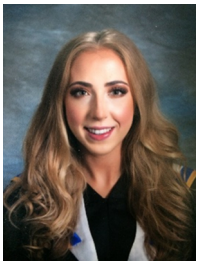

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