ORIGINAL ARTICLE

10.2478/AMB-2025-0074

INFRARED THERMOGRAPHY REVEALS ALTERATIONS IN SURFACE BODY TEMPERATURE IN STREPTOZOTOCIN-INDUCED DIABETIC MICE

M. Hristov, P. Andreeva-Gateva

Department of Pharmacology and Toxicology, Faculty of Medicine, Medical University – Sofia, Bulgaria

Abstract. *Diabetes mellitus is associated with impaired thermoregulation, yet the extent of these alterations remains insufficiently understood. This study aims to evaluate changes in surface body temperature in streptozotocin-induced diabetic mice using infrared thermography, a non-invasive technique for assessing body temperature regulation. Experimental studies were conducted on male BALB/c mice. Type 1 diabetes was induced with a single intraperitoneal injection of streptozotocin (150 mg/kg). Infrared imaging was performed 20 days after streptozotocin injection at 11:00 AM (light phase) and 7:00 PM (transition to the dark phase). The analysis focused on key body regions, including the head, interscapular area, and tail base. The results revealed a significant reduction in surface body temperature in diabetic mice compared to controls at both time points, with a more pronounced decrease observed in the evening. The interscapular region exhibited lower surface temperatures in diabetic mice, suggesting impaired thermogenesis in brown adipose tissue. Additionally, reduced tail base temperature indicated increased vasoconstriction, further supporting the hypothesis of compromised thermoregulation. These findings highlight the impact of diabetes on body temperature regulation and suggest a potential circadian component to these alterations. The study underscores the utility of infrared thermography as a valuable method for assessing thermoregulatory dysfunction in metabolic disorders. Future research should explore the progression of these temperature changes over time and their response to different environmental conditions and pharmacological interventions.*

Key words: *infrared thermography, diabetes, streptozotocin, body temperature, thermoregulation, mice*

Corresponding author: *Milen Hristov, MD, PhD, Department of Pharmacology and Toxicology, Faculty of Medicine, Medical University – Sofia, Bulgaria, tel: +359 2 9 515 652, email: mhristov@medfac.mu-sofia.bg*

ORCID: *0000-0002-6185-2157 – Milen Hristov*

ORCID: *0000-0002-3791-8654 – Pavlina Andreeva-Gateva*

Received: *25 February 2025;* ***Accepted:*** *07 May 2025*

INTRODUCTION

Diabetes mellitus is one of the most prevalent chronic diseases worldwide, posing a significant public health challenge [1]. Chronic hyperglycemia has profound effects on the quality of life of affected individuals, as it leads to various macrovascular complications, such as ischemic heart disease, stroke, and peripheral artery disease, as well as microvascular complications, including neuropathy, nephropathy, and retinopathy [2]. Numerous factors influence the complexity of diabetic pathology in humans. To address these challenges, the rodent streptozotocin (STZ) model is widely used, enabling controlled investigations into abnormalities associated with insulin deficiency [3].

Several studies have reported alterations in body temperature regulation in STZ-induced diabetic animal models. For example, using a digital thermistor thermometer inserted rectally, researchers observed a decrease in body temperature in diabetic rats between 9 and 11 hours within a 24-hour cycle on the 12th day post-STZ injection [4]. Another study, which employed surgically implanted transmitter devices in the peritoneal cavity of rats, found a rapid decline in body temperature occurring 3–5 days after STZ administration [5]. While rectal thermometry provides direct contact measurements, it is associated with handling stress that may artificially elevate body temperature and pose potential health risks due to probe insertion. Telemetry implantation allows for continuous and accurate core body temperature monitoring but requires invasive surgery, which can induce physiological alterations, cause stress, and demand substantial time and technical expertise from researchers [6]. Given these limitations, further research employing non-invasive methods is necessary to better characterize temperature changes in experimental models of diabetes while minimizing stress and physiological disruptions in animal subjects.

Infrared thermography enables the precise measurement of infrared radiation, allowing surface temperature to be determined based on fundamental physical principles. This technique is particularly valuable due to its non-invasive nature and its ability to measure temperature at distances of less than 1 meter, facilitating the examination of specific sites of heat loss. Infrared thermography provides detailed surface temperature variations without the need for numerous temperature sensors and avoids inaccuracies that conventional solid sensors may introduce when measuring mammal coats [7]. Therefore, in the present study, we utilized infrared thermography as a non-invasive method to investigate surface temperature changes in STZ-induced diabetic mice.

MATERIALS AND METHODS

Animals

Male BALB/c mice (32±3 g, 13-15 weeks old) were used in the experiment. The mice had free access to standard chow and water and were housed in a temperature-controlled room (22 ± 1°C) under a 12:12-hour light-dark cycle (lights on at 07:00 and off at 19:00). All procedures were conducted in accordance with Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. The Ethical Council of the Bulgarian Food Safety Agency approved the study protocol.

Experimental Protocol

Type 1 diabetes was induced in the mice by a single intraperitoneal injection of streptozotocin (150 mg/kg, dissolved in citrate buffer, pH 4.5) (S0130, Sigma-Aldrich, Germany) [8]. Control mice received an equivalent volume of citrate buffer alone. After returning the mice to their cages, food and a 10% glucose solution were provided ad libitum. Three days later, the glucose solution was replaced with fresh water. On the fifth day after STZ injection, blood glucose levels were measured using a standard glucometer (Wellion CALLA Light, Med Trust, Austria). One drop of tail blood was analyzed, and mice with blood glucose levels >16.7 mmol/L were considered diabetic [3]. Infrared imaging was performed 20 days after STZ injection at 11:00 AM (light phase) and 7:00 PM (transition to the dark phase). Each experimental group consisted of six animals.

Infrared Imaging

Infrared imaging was performed as described previously [9]. A FLIR infrared thermographic camera (FLIR T530, Teledyne FLIR, USA) (Infrared resolution: 320 × 240 pixels; field of view: 24° × 18°; thermal sensitivity/NETD: <40 mK at 30°C; object temperature range: -20°C to 120°C) was used to record the temperature of freely moving mice for 1 min. Infrared imaging was conducted in a room with a stable ambient temperature of 22 ± 1°C and a relative humidity of ~50%. Radiometric images were captured from a distance of 80 cm [9], with the camera's emissivity set to 0.95 [10]. Infrared thermographic recording was performed from above the testing box, which had no lid. Infrared thermal profiles were saved and analyzed using FLIR Tools 6.4 image processing software (Teledyne FLIR, USA). Three regions of interest (ROIs) (Fig. 1) were selected for thermal analysis: 1) Head: This ROI covered a substantial portion of the frontal and parietal regions. 2) Back: This ROI extended from the ears and encompassed the interscapular region. 3) Tail base: This ROI was

defined as the region where the tail base connects to the rump. For each animal, five frames were selected for analysis, ensuring that the ROIs were consistently positioned across all samples. The maximum and minimum surface temperatures were recorded within each selected ROI, and the average values of these temperatures from the five frames were used for analysis. Identical ROI sizes were applied across all frames and animals. Measurements were taken when the animals were positioned with all four limbs on the ground, ensuring that all relevant regions were within the designated ellipse radius.

Statistical analysis

Statistical analysis was performed using SigmaPlot 12.5 (Systat Software GmbH, Erkrath, Germany). Data normality was assessed using the Shapiro-Wilk test, confirming a normal distribution. Comparisons between groups were made using a two-tailed Student's t-test, with statistical significance set at $p < 0.05$. Results are expressed as mean ± standard error of the mean (SEM).

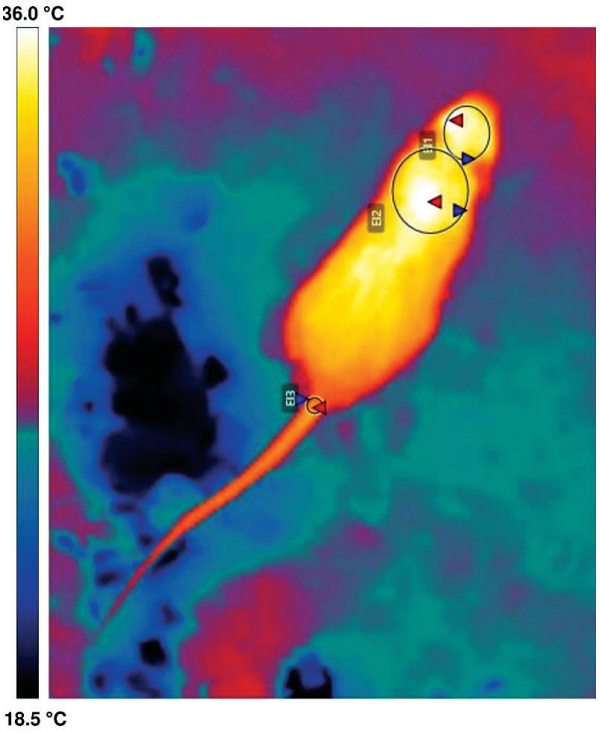

Fig. 1. A representative top-down infrared thermographic image of a freely moving control mouse captured with a FLIR camera. The camera was positioned 80 cm above the animal, allowing continuous tracking of surface temperature changes. Thermographic imaging highlights three regions of interest: the head, back (interscapular region), and tail base, used for surface temperature assessments. Red and blue triangles indicate the maximum and minimum surface temperatures within each region, respectively

RESULTS

Infrared thermographic measurement at 11:00 AM

Infrared thermography revealed a decrease in surface body temperature in diabetic mice compared to controls at 11:00 AM (Fig. 2A). Specifically, in the head region, diabetic mice exhibited a significantly lower maximum recorded temperature compared to controls (Figure 2B, $p = 0.016$). The surface temperature in the head region was approximately 1.3°C lower in diabetic animals. Regarding the minimum surface temperature, a decreasing trend was observed in the diabetic group; however, statistical significance was not reached (Figure 2E, $p = 0.404$).

A similar pattern was observed in the back region. The maximum surface temperature was significantly lower in diabetic mice (Figure 2C, $p < 0.001$). Diabetic animals exhibited an approximately 1.4°C lower temperature in the back region compared to controls. As for the minimum surface temperature in this region, a decreasing trend was noted in diabetic mice, but statistical significance was not reached (Figure 2F, $p = 0.154$).

At the tail base, both the maximum and minimum temperatures were significantly lower in the diabetic group by approximately 1.4°C compared to controls (Figure 2D, maximum temperature: $p = 0.007$; Figure 2G, minimum temperature: $p = 0.009$).

Infrared Thermographic Measurement at 7:00 PM

Infrared thermography revealed a decrease in surface body temperature in diabetic mice compared to controls at 7:00 PM (Fig. 3A). Specifically, in the head region, diabetic mice exhibited significantly lower maximum and minimum recorded temperatures compared to controls (Figure 3B; maximum temperature: $p < 0.001$; Figure 3E; minimum temperature: $p = 0.024$). The maximum surface temperature in the head region was approximately 2°C lower in diabetic animals, while the minimum temperature was about 1°C lower.

Interestingly, regarding the maximum surface temperature in the back region, only a decreasing trend was observed in diabetic mice, but statistical significance was not reached (Figure 3C, $p = 0.136$). However, for the minimum surface temperature in the back region, diabetic mice had a significantly lower temperature compared to controls (Figure 3F, $p < 0.001$). The minimum temperature in the back region was approximately 1.4°C lower in diabetic animals than in controls.

At the tail base, both maximum and minimum temperatures were significantly lower in the diabetic group by approximately 2.2°C compared to controls (Figure 3D; maximum temperature: $p = 0.021$; Figure 3G; minimum temperature: $p = 0.014$).

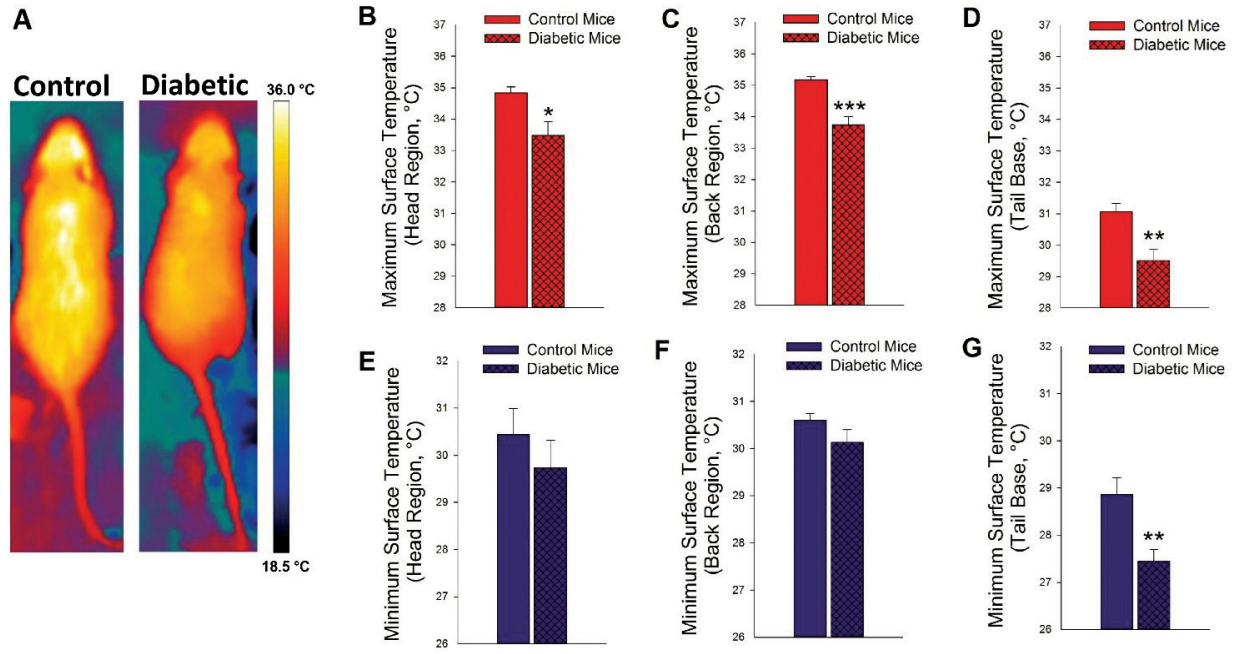

Fig. 2. Infrared thermographic measurements at 11:00 AM. (**A**) Representative top-down infrared thermographic images of a control mouse and a diabetic mouse. (**B, E**) Bar graphs depicting the maximum and minimum surface temperatures, respectively, measured in the head region of control and diabetic mice. (**C, F**) Bar graphs depicting the maximum and minimum surface temperatures, respectively, measured in the back (interscapular) region of control and diabetic mice. (**D, G**) Bar graphs depicting the maximum and minimum surface temperatures, respectively, measured at the base of the tail in control and diabetic mice. Data are presented as mean ± SEM (n = 6 mice per group). *p < 0.05; **p < 0.01; ***p < 0.001 vs. control mice

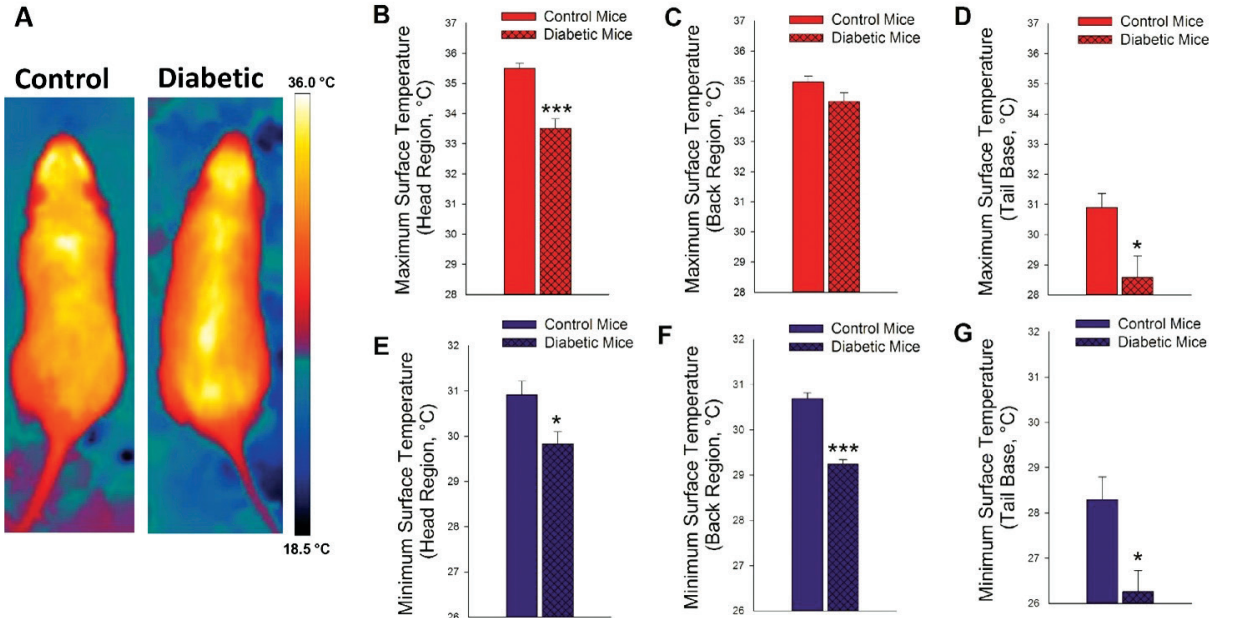

Fig. 3. Infrared thermographic measurements at 7:00 PM. (**A**) Representative top-down infrared thermographic images of a control mouse and a diabetic mouse. (**B, E**) Bar graphs depicting the maximum and minimum surface temperatures, respectively, measured in the head region of control and diabetic mice. (**C, F**) Bar graphs depicting the maximum and minimum surface temperatures, respectively, measured in the back (interscapular) region of control and diabetic mice. (**D, G**) Bar graphs depicting the maximum and minimum surface temperatures, respectively, measured at the base of the tail in control and diabetic mice. Data are presented as mean ± SEM (n = 6 mice per group). *p < 0.05; ***p < 0.001 vs. control mice.

DISCUSSION

Thermal imaging provides a non-invasive method for recording body temperature in freely moving, undisturbed laboratory mice, eliminating the confounding effects of handling stress [6]. However, this method measures surface (skin) temperature rather than deep body temperature [11]. Recent research has optimized and validated an algorithm that enables the estimation of relative changes in core temperature based on continuous, non-invasive infrared measurements of skin temperature in mice housed at room temperature [6]. Additionally, infrared thermography may provide insight into deep body temperature by measuring the radiative temperature of the eyes in mice [12]. Therefore, infrared thermography is a valuable method with the potential to provide important insights into body temperature changes under experimental conditions [11].

In our study, infrared thermographic measurements at both 11:00 AM and 7:00 PM consistently demonstrated reduced surface body temperature in diabetic mice compared to controls. At 11:00 AM, significant decreases in maximum surface temperature were observed in the head, back, and tail base regions, with a trend toward lower minimum temperatures. By 7:00 PM, both maximum and minimum temperatures in the head and tail base regions were significantly lower in diabetic mice, while in the back region, only the minimum temperature showed a statistically significant reduction. These findings suggest that diabetes is associated with impaired thermoregulation, leading to a sustained decrease in surface body temperature. Moreover, the temperature reductions observed in the evening (7:00 PM) were generally more pronounced than those in the morning (11:00 AM), suggesting potential diurnal variations in thermoregulatory responses in diabetic mice. Our findings align with those of Ramos-Lobo et al., who demonstrated that STZ-induced diabetes leads to a significant reduction in core body temperature and a disruption of circadian temperature rhythms in rats. Using telemetry implants to measure core body temperature, the authors found that temperature regulation became unstable, with shifts in acrophase timing, lower MESOR values, and increased amplitude fluctuations [13]. One possible explanation for these disturbances is melatonin deficiency associated with diabetes [14]. Melatonin follows a circadian pattern and plays a crucial role in maintaining stable body temperature rhythms [15]. Studies suggest that STZ-induced diabetes interferes with melatonin synthesis, which may contribute to the observed irregularities in temperature regulation [13, 16].

Previous studies have used infrared imaging of the interscapular area to assess brown adipose tissue activity in conscious mice [11, 17]. Crane et al. demonstrated a novel method for measuring brown adipose tissue thermogenesis using infrared imaging following beta-3 adrenoreceptor stimulation in mice. Their findings showed that the increase in body surface temperature observed with this technique is solely due to uncoupling protein-1-mediated thermogenesis and that this method can distinguish differences in brown adipose tissue activity between mice acclimated to 23°C and those maintained at thermoneutrality (30°C) [18]. In our study, we observed a decreased surface temperature in the interscapular region of diabetic mice, suggesting reduced brown adipose tissue thermogenesis during STZ-induced diabetes. These results are consistent with previous studies demonstrating decreased interscapular brown adipose tissue activity in STZ-induced diabetic animals [19-21].

The tail plays a key role in thermoregulation in mice, acting as an important site for heat dissipation [22]. Changes in tail surface temperature, which reflect vasoconstriction or vasodilation, can serve as an indirect measure of heat loss. By assessing tail temperature, infrared thermography can be used to evaluate vasomotor tone in mice [11, 17, 23]. In our study, we observed a significantly lower tail base temperature in diabetic mice compared to controls, suggesting increased tail vasoconstriction. Our results are in good agreement with the study by Katovich et al., who also reported altered thermoregulatory responses in tail skin temperature in STZ-induced diabetic rats [24]. Administration of the beta-adrenergic agonist isoproterenol (25 μg/kg, s.c.) caused a significant increase in tail skin temperature in the control group, but not in rats that had been diabetic for four weeks. A similar but more pronounced response was observed in controls following subcutaneous administration of 40 μg/kg and 100 μg/kg of isoproterenol. However, in the four-week diabetic rats, the tail skin temperature response remained negligible even at the higher doses [24]. Interestingly, the authors also reported that baseline tail skin temperature did not differ between control and diabetic groups. These differences may be attributed to variations in the methodology used to measure tail surface temperature. Katovich et al. measured tail skin temperature using a copper-constantan thermocouple attached to the dorsal region of the tail with porous adhesive tape, ensuring direct contact with the skin near the base of the tail [24]. In contrast, our study employed infrared thermography in freely moving mice, which may account for the observed discrepancies.

CONCLUSION

Our study demonstrates that infrared thermography is a valuable tool for assessing surface body temperature in STZ-induced diabetic mice. We observed a significant reduction in surface body temperature in diabetic mice compared to controls at both 11:00 AM and 7:00 PM, with more pronounced reductions in the evening, suggesting that STZ-induced diabetes is associated with impaired temperature regulation, leading to a reduction in surface body temperature. Further studies are needed to investigate how these changes in body temperature evolve with the progression of diabetes and how thermoregulatory responses are affected by different environmental temperatures or pharmacological interventions. By providing a reliable and non-invasive method for measuring surface body temperature, infrared thermography may serve as a useful tool for further research on diabetes-related alterations in body temperature and potential therapeutic strategies.

Funding: *This work was supported by the Bulgarian National Science Fund, Ministry of Education and Science, Contract КП-06-Н63/9 from 13.12.2022.*

Competing interests: *The authors have declared that no competing interests exist.*

Ethical statement: *All procedures were conducted in accordance with Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. The Ethical Council of the Bulgarian Food Safety Agency approved the study protocol.*

REFERENCES

1. Hossain MJ, Al-Mamun M, Islam MR. Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. Health Sci Rep, 2024, 7(3):e2004.
2. Paul S, Ali A, Katare R. Molecular complexities underlying the vascular complications of diabetes mellitus – A comprehensive review. J Diabetes Complications, 2020, 34(8):107613.
3. Motyl K, McCabe LR. Streptozotocin, type I diabetes severity and bone. Biol Proced Online, 2009, 11:296-315
4. O'Donnell JM, Banyasz T, Kovacs T. Altered thermoregulatory responses to clonidine in streptozotocin-diabetic rats. Br J Pharmacol, 1996, 117(5):938-942.
5. Howarth FC, Jacobson M, Naseer O, et al. Short-term effects of streptozotocin-induced diabetes on the electrocardiogram, physical activity and body temperature in rats. Exp Physiol, 2005, 90(2):237-245.
6. Van der Vinne V, Pothecary CA, Wilcox SL, et al. Continuous and non-invasive thermography of mouse skin accurately describes core body temperature patterns, but not absolute core temperature. Sci Rep, 2020, 10(1):20680.
7. Mccafferty DJ. The value of infrared thermography for research on mammals: previous applications and future directions. Mammal Review, 2007, 37(3):207-23.
8. Hayashi K, Kojima R, Ito M. Strain differences in the diabetogenic activity of streptozotocin in mice. Biol Pharm Bull, 2006, 29(6):1110-1119.
9. Faraji J, Metz GAS. Infrared Thermography Reveals Sex-Specific Responses to Stress in Mice. Front Behav Neurosci, 2020, 14:79.
10. Solon IG, Santos WS, Jesus AA, et al. Non-invasive evaluation of vascular permeability in formalin-induced orofacial pain model using infrared thermography. J Therm Biol, 2024, 119:103782.
11. Meyer CW, Ootsuka Y, Romanovsky AA. Body Temperature Measurements for Metabolic Phenotyping in Mice. Front Physiol, 2017, 8:520.
12. Vogel B, Wagner H, Gmoser J, et al. Touch-free measurement of body temperature using close-up thermography of the ocular surface. MethodsX, 2016, 3:407-416.
13. Ramos-Lobo AM, Buonfiglio DC, Cipolla-Neto J. Streptozotocin-induced diabetes disrupts the body temperature daily rhythm in rats. Diabetol Metab Syndr, 2015, 7:39.
14. Peschke E. Melatonin, endocrine pancreas and diabetes. J Pineal Res, 2008, 44(1):26-40.
15. Kräuchi K, Cajochen C, Pache M, et al. Thermoregulatory effects of melatonin in relation to sleepiness. Chronobiol Int, 2006, 23(1-2):475-484.
16. Amaral FG, Turati AO, Barone M, et al. Melatonin synthesis impairment as a new deleterious outcome of diabetes-derived hyperglycemia. J Pineal Res, 2014, 57(1):67-79.
17. Warner A, Rahman A, Solsjö P, et al. Inappropriate heat dissipation ignites brown fat thermogenesis in mice with a mutant thyroid hormone receptor α1. Proc Natl Acad Sci USA, 2013, 110(40):16241-16246.
18. Crane JD, Mottillo EP, Farncombe TH, et al. A standardized infrared imaging technique that specifically detects UCP1-mediated thermogenesis in vivo. Mol Metab, 2014, 3(4):490-494.
19. Seydoux J, Chinet A, Schneider-Picard G, et al. Brown adipose tissue metabolism in streptozotocin-diabetic rats. Endocrinology, 1983, 113(2):604-610.
20. Burcelin R, Kande J, Ricquier D, et al. Changes in uncoupling protein and GLUT4 glucose transporter expressions in interscapular brown adipose tissue of diabetic rats: relative roles of hyperglycaemia and hypoinsulinaemia. Biochem J, 1993, 291 (Pt 1):109-113.
21. Matsen ME, Thaler JP, Wisse BE, et al. In uncontrolled diabetes, thyroid hormone and sympathetic activators induce thermogenesis without increasing glucose uptake in brown adipose tissue. Am J Physiol Endocrinol Metab, 2013, 304(7):E734-E746.
22. Škop V, Liu N, Guo J, et al. The contribution of the mouse tail to thermoregulation is modest. Am J Physiol Endocrinol Metab, 2020, 319(2):E438-E446.
23. Fischer AW, Hoefig CS, Abreu-Vieira G, et al. Leptin Raises Defended Body Temperature without Activating Thermogenesis. Cell Rep, 2016, 14(7):1621-1631.
24. Katovich MJ, Sninsky CA. Altered tail-skin temperature responsiveness in streptozotocin-induced diabetic rats. Life Sci, 1987, 41(12):1529-1537.