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Original article

APPLICATION OF INFRARED THERMOGRAPHY TO THE DETECTION OF CHANGES IN INTERSCAPULAR BROWN ADIPOSE TISSUE TEMPERATURE AND ANALYSIS OF SOME BLOOD BIOCHEMICAL AND BEHAVIOURAL PARAMETERS IN BALB/C AND C57BL/6 MOUSE STRAINS

J. ZYMANTIENE, V. OBERAUSKAS, R. ZELVYTE, K. MUSAYEVA, A. SEDEREVICIUS, I. MONKEVICIENE & U. SPANCERNIENE

Department of Anatomy and Physiology, Faculty of Veterinary Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania

Summary

Zymantiene, J., V. Oberauskas, R. Zelvyte, K. Musayeva, A. Sederevicius, I. Monkeviciene & U. Spancerniene, 2026. Application of infrared thermography to the detection of changes in interscapular brown adipose tissue temperature and analysis of some blood biochemical and behavioural parameters in BALB/c and C57BL/6 mouse strains. *Bulg. J. Vet. Med.,* **29**, No 1, 37–52.

Brown adipose tissue (BAT) has received renewed interest as a potential treatment target for obesity and comorbidities due to its thermogenic activity capacity and contribution to energy expenditure. Murine models are among the commonest preclinical models for studying human disease, including BAT studies. C57BL/6 and BALB/c are the two most commonly used mouse strains, however, different strains manifest significant behavioural differences, levels of sociability, emotionality, exercise response, respond differently to severe spinal cord injury, show differences in susceptibility to diet-induced obesity and insulin resistance, etc. To gain a broader understanding of the peculiarities, this study aimed to investigate the effect of age, strain and gender on interscapular BAT temperature changes and to analyse some blood biochemical and behavioural parameters in the aforementioned strains. The age of the mice (5–8 weeks) did not influence the interscapular BAT area temperature. The mean temperatures of the maximum (T_{max}) and the average temperatures (T_{isoth}) in the BALB/c mice were by 0.46 °C ($P<0.001$) and by 0.37 °C ($P<0.001$) higher than in the C57BL/6 mice. In addition, the gender of the mice also modulated the BAT area temperature, causing an increment of 0.29 °C ($P<0.001$) and 0.21 °C ($P<0.05$) in T_{max} and T_{isoth} temperatures of males, respectively. The difference in mean glucose, cholesterol and triglyceride levels between the BALB/c and C57BL/6 strains was not substantial. Behavioural analyses disclosed a statistically insignificant distribution of grooming activities in male BALB/c and C57BL/6 mice and significant ($P<0.001$) differences in female mice. Collectively, our findings revealed that infrared thermography can be successfully used to measure interscapular BAT temperature in mice *in vivo*, and that mouse strain and gender can alter BAT measurements.

Key words: brown adipose tissue, infrared thermography, mouse, temperature

INTRODUCTION

Brown adipose tissue (BAT) is a thermogenic tissue that dissipates energy as heat and an essential organ for thermoregulation in small and hibernating mammals due to its mitochondrial uncoupling capacity (Halpern *et al*., 2014; Cypess *et al.,* 2025). BAT also plays a relevant role in glucose homeostasis and triglyceride clearance (Virtue & Vidal-Puig, 2013; Oiwa *et al*., 2020). In the last years, BAT has also emerged as a secretory organ that produces batokines which can influence the activity of other metabolic organs (Villarroya *et al*., 2019). There is mounting evidence suggesting that BAT mass/ activity negatively correlates with body mass index, total and visceral adipose tissue, fasting glucose levels, and insulin resistance in rodents and humans (Wang *et al*., 2015; Soundarrajan *et al*., 2020; Maliszewska & Kretowski, 2021). In newborns of precocial species such as deer and lamb BAT is predominantly found in the perirenal fat deposit, while in rodents and human newborns, it is mainly found in the interscapular area, as well as in cervical, para-aortal and subcostal area (Himms-Hagen, 1985; Moonen *et al*., 2019). It was considered that BAT suffered progressive involution until its total disappearance during early childhood (Lee *et al*., 2013) but accumulating research evidence supports the presence of functional BAT in adult humans (Cypess *et al*., 2009; Virtanen *et al*., 2009) and its critical role in energy expenditure and whole-body metabolism (Shamsi *et al*., 2020). Therefore, there has been resurgence in interest in BAT, as a potential target for management of obesity, diabetes, non-alcoholic fatty liver disease, Alzheimer's disease (Bartelt *et al*., 2011; Khedoe *et al*., 2015; Liu *et al*., 2015; Poekes *et al*., 2019; Kordic *et al*., 2022; da Rosa *et al*., 2023; Li *et al*., 2024), other metabolic disorders, dyslipidaemias or cardiovascular diseases (Raiko *et al.*, 2020; Becher *et al*., 2021) especially when rodents (in this study mice) are used as animal model in biomedical research.

There are many different (direct and indirect) imaging modalities (positron emission tomography-computed tomography, computed tomography, magnetic resonance imaging and magnetic resonance spectroscopy, contrast-enhanced ultrasound, infrared thermography, optical techniques, etc.) of assessing BAT mass and/or activity (Ang *et al*., 2017; Law *et al*., 2018; Oelkrug & Mittag, 2021; Yang *et al*., 2021) although which method is optimal remains controversial. The large amount of ongoing studies and recent publications involving biomedical imaging of BAT emphasises for the importance of high-quality non-invasive imaging. Thermal imaging, also known as infrared thermography (IRT), as a potentially safe, rapid and relatively inexpensive non-invasive *in vivo* technique that lacks ionising radiation and offers the possibility of large cohort studies. Another benefit of the method is that it causes minimal physiologic discomfort in tested animals allowing the animals to move freely without any disturbance during temperature measurement, which makes this method completely animal-friendly. Imaging signals can be affected by a variety of factors, including changes in environmental temperature, exposure to cold, pharmacological agents, diet, hormones, animal state of the sympathetic nervous system, social defeat, handling, maternal diet, or the presence of an intruder (Rothwell & Stock, 1997; Zhang *et al*. 2010; Whittle *et al*., 2013; Kataoka *et al*., 2014; Mohammed *et al*., 2014; Oiwa *et al*., 2020; San-

thanam *et al*., 2021; Yang *et al*., 2021). Elaborating the factors that may influence BAT function is beneficial because it can improve the quality of scientific outcomes and help narrow down which mouse model is most appropriate for your research question. Therefore, the aim of our study was to investigate whether age, gender and strain of mice can affect temperature measurements of interscapular BAT, blood parameters and behavioural patterns in mice.

MATERIALS AND METHODS

Ethical statement

All experimental procedures were conducted in compliance with EU Directive 2010/63/EU for animal experiments. Ethical approval to perform experiment was issued by the State Food and Veterinary Service (13/11/2019, protocol No G2-127).

Animals and housing conditions

A summary of experimental design of this study is presented on Fig. 1. A total of 353 BALB/c and C57BL/6 mice, aged 5–8 weeks, were initially enrolled in the study and housed in the vivarium of the Lithuanian University of Health Sciences under controlled conditions (22±24°C, humidity 55%±5%, 12-hour light/12-hour dark cycle (lights on at 07:00), with food and water provided *ad libitum* throughout the experiment). All animals were housed in standard Tecniplastic (Italy) polycarbonate mouse cages (floor area/animal – 100 cm^3, minimum enclosure height – 12 cm). The mice were fed a commercial rodent diet containing 19.90% crude protein, 12.50% crude fat, 1.70% crude fiber, cereals, trace elements and vitamins.

Thermal measurements and animal grouping

The surface temperature of interscapular BAT in mice was measured using a handheld infrared thermal imaging camera (model FLIR T640, FLIR Systems AB, Sweden) placed 70 cm above the freely moving animal to minimise stress. The camera has a thermal sensitivity of <0.035°C at 30°C and a resolution of

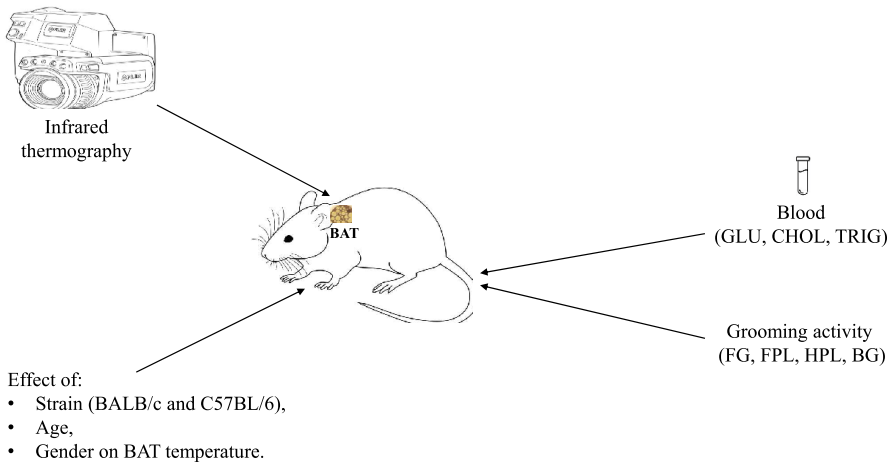

Fig. 1. Illustration of study procedures in the *in vivo* experiment. BAT – brown adipose tissue, GLU – glucose, CHOL – cholesterol, TRIG – triglyceride, FG – nose/face grooming, FPL – front paw licking, HPL – hind paw licking, BG – all body grooming/scratching.

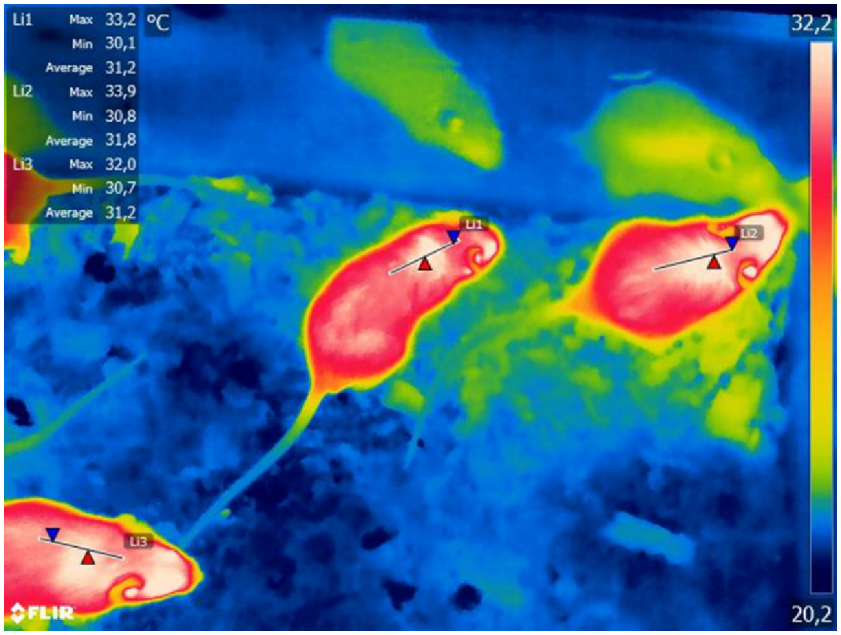

Fig. 2. Dorsal view of a unrestrained mouse captured by infrared thermography, demonstrating heterogeneity in temperature measurements of interscapular brown adipose tissue by colour coding (isotherms Li1, Li2, Li3; the red triangle represents the point of maximum recorded temperature, and the blue triangle represents the minimum recorded temperature). Measurements are presented as maximum (T_{max}), minimum (T_{min}) or isothermal average temperatures (T_{isoth}) .

640×480 pixels. Measurements were taken from the interscapular BAT area (between the shoulder blades, Fig. 2) under the same environmental and measurement time conditions (9:00-13:00). For subsequent data analysis, the mice were divided into different groups according to age, gender and strain. All 353 mice were allotted into four age groups (n=47 at 5 weeks, n=91 at 6 weeks, n=95 at 7 weeks and n=120 at 8 weeks). The total gender and strain distribution was as follows: n=169 males, n=184 females and n=310 BALB/c and n=43 C57BL/6, respectively. For the influence of strain and gender analysis, mice from each strain were divided as follows: n=159 males and 151 females in the BALB/c strain and n=10 males and n=33 females in the C57BL/6 strain. The maximum (T_{max}), minimum (T_{min}), isotherm (the lines connecting locations with the same average temperature) and average temperature (T_{isoth}) of the interscapular BAT were determined using FLIR Tools version 5.1 software (FLIR, Wilsonville) with the Rainbow high contrast thermal palette set.

Determination of some blood biochemical parameters

Some biochemical blood parameters were evaluated in randomly selected 8-week-old BALB/c (n=6) and C57BL/6 (n=6) mice. Mice were manually restrained using a restraining device (TV-150 SM-Tailveiner Restrainer for Mice, Braintree Scientific) to ensure proper collection of blood samples from the tail vein, a common vascular access route that does not require anaesthesia. In addition, the mice

were habituated to the restraint device to avoid unnecessary stress and were returned to their home cage immediately after blood collection. Blood glucose (GLU), total cholesterol (CHOL) and triglyceride (TRIG) values were measured using the portable multiparameter diagnostic device "MULTICAREIN" (Biochemical Systems International S.p.A. (BioSys), Italy, 2021) using two different measurement technologies, amperometric for GLU and reflectometric for CHOL and TRIG detection.

Analysis of mouse grooming behaviour

Forty BALB/c and C57BL/6 mice (n=10 at 6 weeks and n=10 at 7 weeks) were divided into four equal groups of 10 (in female and male mice, respectively). Behavioural testing was conducted in cages between 09:00 and 13:00 using video cameras. The following grooming patterns were recorded for each bout: nose/face grooming (FG), front paw licking (FPL), hind paw licking (HPL), and all body grooming/scratching (BG).

Statistical analyses

Data were analysed using R software, version 4.3.2. After confirming the normal distribution of the variables (T_{max}, T_{min} and T_{isoth} temperature data) (Shapiro-Wilk test) and homogeneity of variances (F-test), descriptive statistics including mean and standard deviation (SD), median and interquartile range were used to present the data. Wilcoxon and Kruskal-Wallis tests were used to compare two groups and more than two groups, respectively. In addition, one-way analysis of variance (ANOVA) for repeated measures followed by Tukey's post hoc test was used. Student's t-test was used to compare two groups. Pearson correlation analyses were used to assess the correlation between mouse age group and T_{max}, T_{min} and T_{isoth} of interscapular BAT. 95% confidence interval (CI) was calculated for the analysis of blood parameters. The percentage distributions of behavioural components in both groups were compared using the chi-square test. Results were considered statistically significant when the probability (P) values were less than 0.05.

RESULTS

The effect of age in mice

Normal distribution ($P>0.05$) of T_{max}, T_{min} and T_{isoth} temperatures was observed in all age groups (5, 6, 7 and 8 weeks). In addition, one-way ANOVA test showed no statistically significant difference in T_{max} (Fig. 3A), T_{min} (Fig. 3B) and T_{isoth} (Fig. 3C) temperatures between different age groups. The differences in T_{max} temperature between all age groups were not statistically significant. When the mean temperatures of T_{min} and T_{isoth} were evaluated, it was also established that the differences between all age groups were not statistically significant, except for the comparisons between 5 and 8 weeks of age, where the temperature differences were statistically significant ($P<0.05$). It was noticed that the mean temperature of T_{min} was by 0.27°C higher and the mean temperature of T_{isoth} was by 0.23°C higher in 5-week-old mice than in 8-week-old mice.

The correlation between the age of the mice and the temperature indicators was weak and negative (with T_{max} – coefficient of correlation (r)=0.058 (P=0.274), with T_{min} – r=0.115 (P=0.029) and with T_{isoth}– r=0.079 (P=0.139).

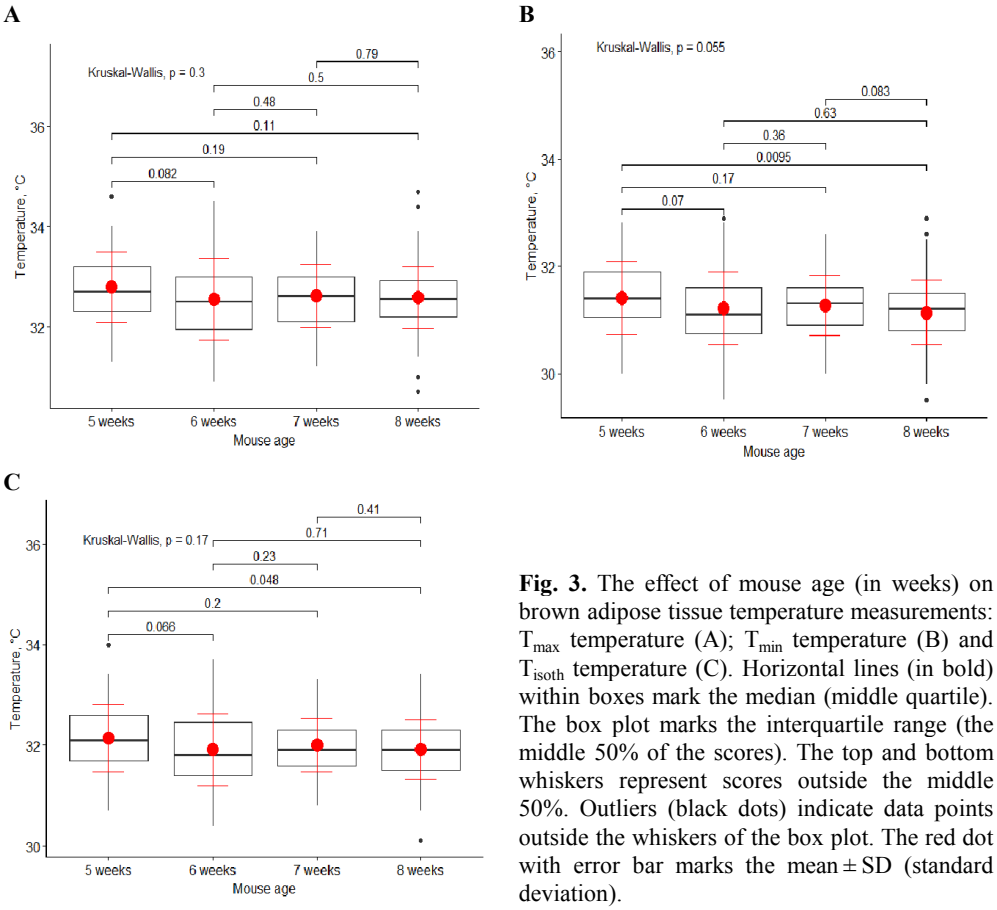

Fig. 3. The effect of mouse age (in weeks) on brown adipose tissue temperature measurements: T_{max} temperature (A); T_{min} temperature (B) and T_{isoth} temperature (C). Horizontal lines (in bold) within boxes mark the median (middle quartile). The box plot marks the interquartile range (the middle 50% of the scores). The top and bottom whiskers represent scores outside the middle 50%. Outliers (black dots) indicate data points outside the whiskers of the box plot. The red dot with error bar marks the mean ± SD (standard deviation).

The effect of mouse gender

The mean T_{max} and T_{isoth} were by 0.29 °C ($P<0.001$) and by 0.21 °C ($P<0.05$) higher in males than in females. The gender (as an independent variable) had a strong effect on T_{max} (Fig. 4A, $P<0.001$) and T_{isoth} (Fig. 4C, $P<0.001$) temperatures, but did not affect T_{min} (Fig. 4B, $P>0.05$) measurements.

The effect of the mouse strain

The BALB/c mice exhibited higher (Fig. 5A–C) T_{max}, T_{min} and T_{isoth} values compared to C57BL/6 mice. The mean T_{max} and T_{isoth} in BALB/c mice were by 0.46°C ($P<0.001$) and by 0.37°C ($P<0.001$) higher than in C57BL/6 mice. The strain (as an independent variable) had a strong effect on T_{max} (Fig. 5A, $P<0.001$) and T_{isoth} (Fig. 5C, $P<0.001$) temperatures, but a less important effect on T_{min} (Fig. 5B, $P>0.05$) measurements.

The effect of mouse strain and gender

The T_{max} temperature of male mice of the BALB/c and C57BL/6 strains was by 0.28°C ($P<0.001$, Fig. 6A) and by 0.55°C ($P<0.05$, Fig. 6B) higher than respective temperatures of female mice. The greater

difference in T_{max} of male mice was observed in the C57BL/6 strain. The mouse gender influenced T_{max} by 4.2% (P<0.001) and by 14.4% (P=0.012) in the BALB/c and C57BL/6 strains, respectively.

Analysing the T_{min} results, it was observed that the gender of BALB/c and C57BL/6 mice had no statistically significant effect (P>0.05, Fig. 6C–D). However, when studying the T_{isoth} (Fig. 6E–F), the gender of the mice had a statistically significant effect only on BALB/c mice (Fig. 6E). The mean average temperature of BALB/c males was by 0.2°C (P<0.01) higher than that of females. In addition, the gender of the mice influenced the mean T_{isoth} by 2.8% (P<0.01) in the BALB/c strain.

Blood biochemical parameters

Mean blood glucose, was 7.85±0.50 mmol/L and 6.95±0.96 mmol/L for BALB/c mice and C57BL/6, respectively (Table 1). The difference in the mean glucose (0.905 mmol/L) level between the BALB/c and C57BL/6 groups was insignificant (P=0.0796). A similar trend (no significant differences between strains) was observed in mean blood total cholesterol (0.407 mmol/L, P=0.0132) and triglycerides (0.068, P=0.5074) levels between BALB/c and C57BL/6 mice.

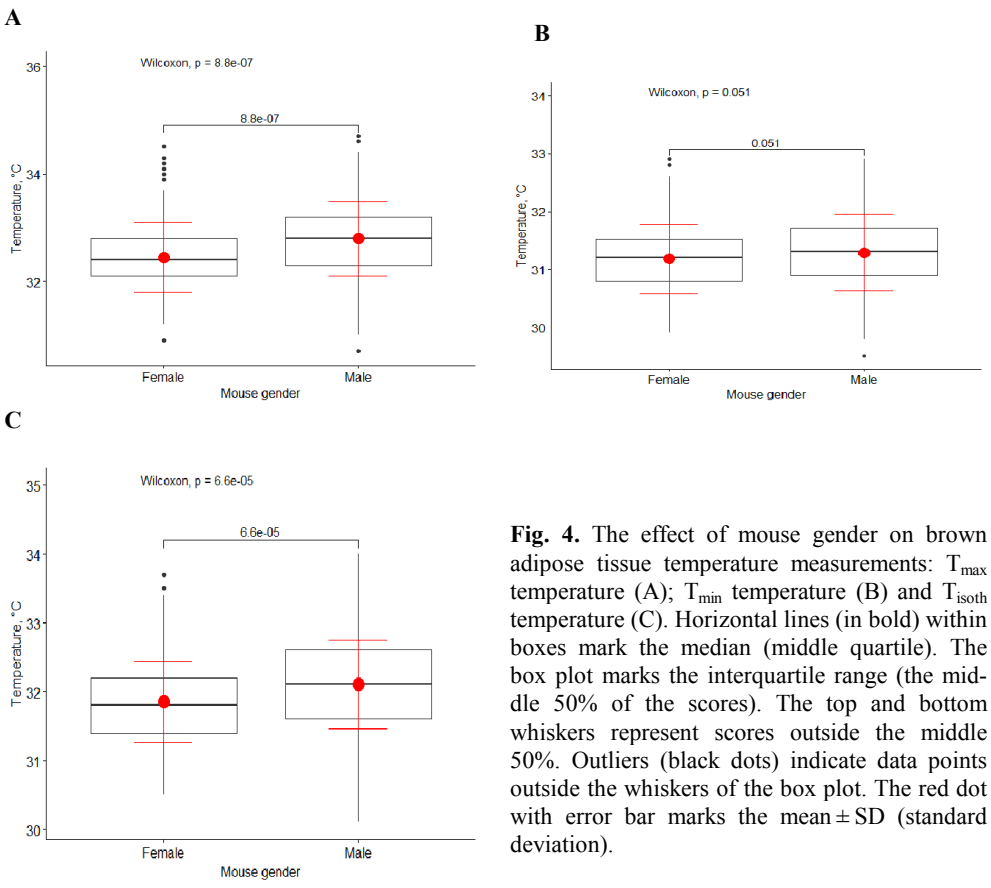

Fig. 4. The effect of mouse gender on brown adipose tissue temperature measurements: T_{max} temperature (A); T_{min} temperature (B) and T_{isoth} temperature (C). Horizontal lines (in bold) within boxes mark the median (middle quartile). The box plot marks the interquartile range (the middle 50% of the scores). The top and bottom whiskers represent scores outside the middle 50%. Outliers (black dots) indicate data points outside the whiskers of the box plot. The red dot with error bar marks the mean ± SD (standard deviation).

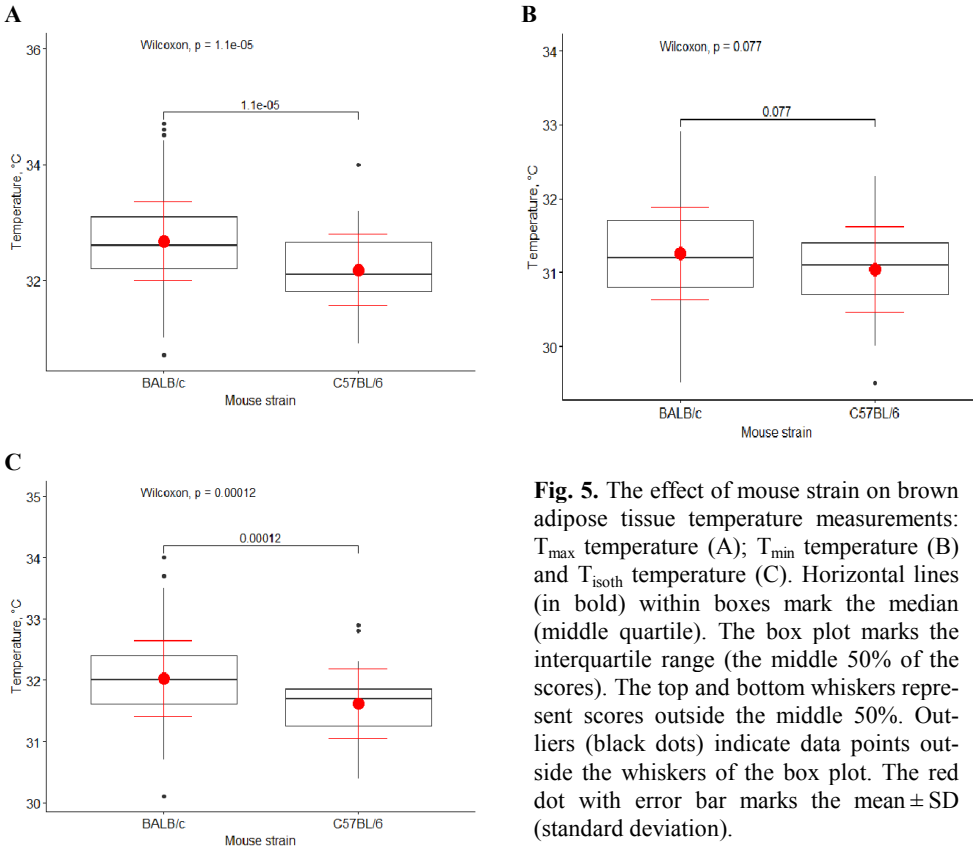

Fig. 5. The effect of mouse strain on brown adipose tissue temperature measurements: T_{max} temperature (A); T_{min} temperature (B) and T_{isoth} temperature (C). Horizontal lines (in bold) within boxes mark the median (middle quartile). The box plot marks the interquartile range (the middle 50% of the scores). The top and bottom whiskers represent scores outside the middle 50%. Outliers (black dots) indicate data points outside the whiskers of the box plot. The red dot with error bar marks the mean ± SD (standard deviation).

Analysis of mouse grooming behaviour

As can be observed from the mouse grooming results shown on Fig. 7, the nose/face grooming (FG) pattern was most common in BALB/c females (60%), all body grooming/scratching (BG) in BALB/c males and C57BL/6 females (40% each), front paw licking (FPL) in BALB/c females and males (40% each), and hind paw licking (HPL) in BALB/c males (30%). Grooming behaviour analyses revealed that the distribution of grooming activities in male BALB/c and C57BL/6 mice was not statistically significant ($P>0.05$), but was statistically significant in females ($P<0.001$). The distributions of FG, BG, FPL and HPL patterns in BALB/c females were 60%, 30%, 40% and 20% ($\chi2=37.3$, $P<0.001$), and in C57BL/6 females: 40%, 40%, 20% and 20% ($\chi2=19.05$, $P<0.001$), respectively.

DISCUSSION

Mouse models of disease continue to be a powerful tool in both the veterinary and biomedical research communities. A wide selection of mouse strains (BALB/c, C57BL/6, 129X1/SvJ, DBA/2, FVB/N, etc.) is currently in use, however strains can differ in their inherent propensities to develop disease. Some parameters can also effect each strain more than others and influence the final experimental outcomes.

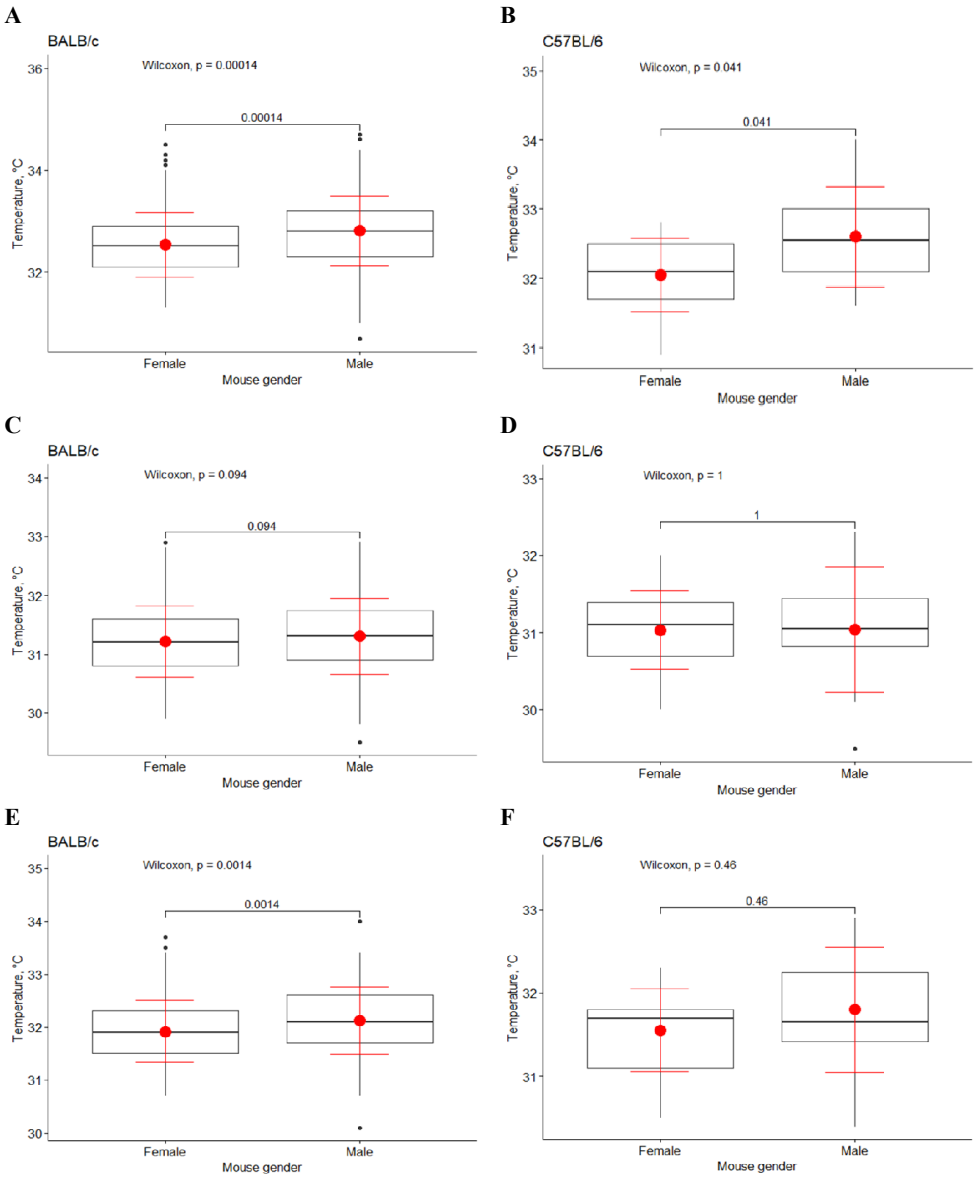

Fig. 6. The effect of mouse strain and gender on the measurement of brown adipose tissue temperature: A. T_{max} between gender groups of BALB/c mice; B. T_{max} between gender groups of C57BL/6 mice; C. T_{min} between gender groups of BALB/c mice; D. T_{min} between gender groups of C57BL/6 mice; E. T_{isoth} between gender groups of BALB/C mice; F. T_{isoth} between gender groups of C57BL/6 mice. Horizontal lines (in bold) within boxes mark the median (middle quartile). The box plot marks the interquartile range (the middle 50% of the scores). The top and bottom whiskers represent scores outside the middle 50%. Outliers (black dots) indicate data points outside the whiskers of the box plot. The red dot with error bar marks the mean ± SD (standard deviation).

Table 1. Results of some biochemical values in 8-week-old BALB/c and C57BL/6 mice strains (n=6)

Parameters	BALB/c			C57BL/6		
	Mean±SD	Range	95% CI	Mean±SD	Range	95% CI
GLU, mmol/L	7.85±0.50	7.1-8.62	7.32-8.38	6.95±0.96	5.92-8.43	5.94-7.95
CHOL, mmol/L	1.35±0.26	1.02-1.60	1.08-1.62	0.94±0.16	0.79-1.21	0.77-1.11
TRIG, mmol/L	0.95±0.21	0.72-1.28	0.73-1.17	1.02±0.13	0.87-1.20	0.89-1.15

GLU: glucose, CHOL: cholesterol, TRIG: triglyceride, SD: standard deviation, CI: confidence interval.

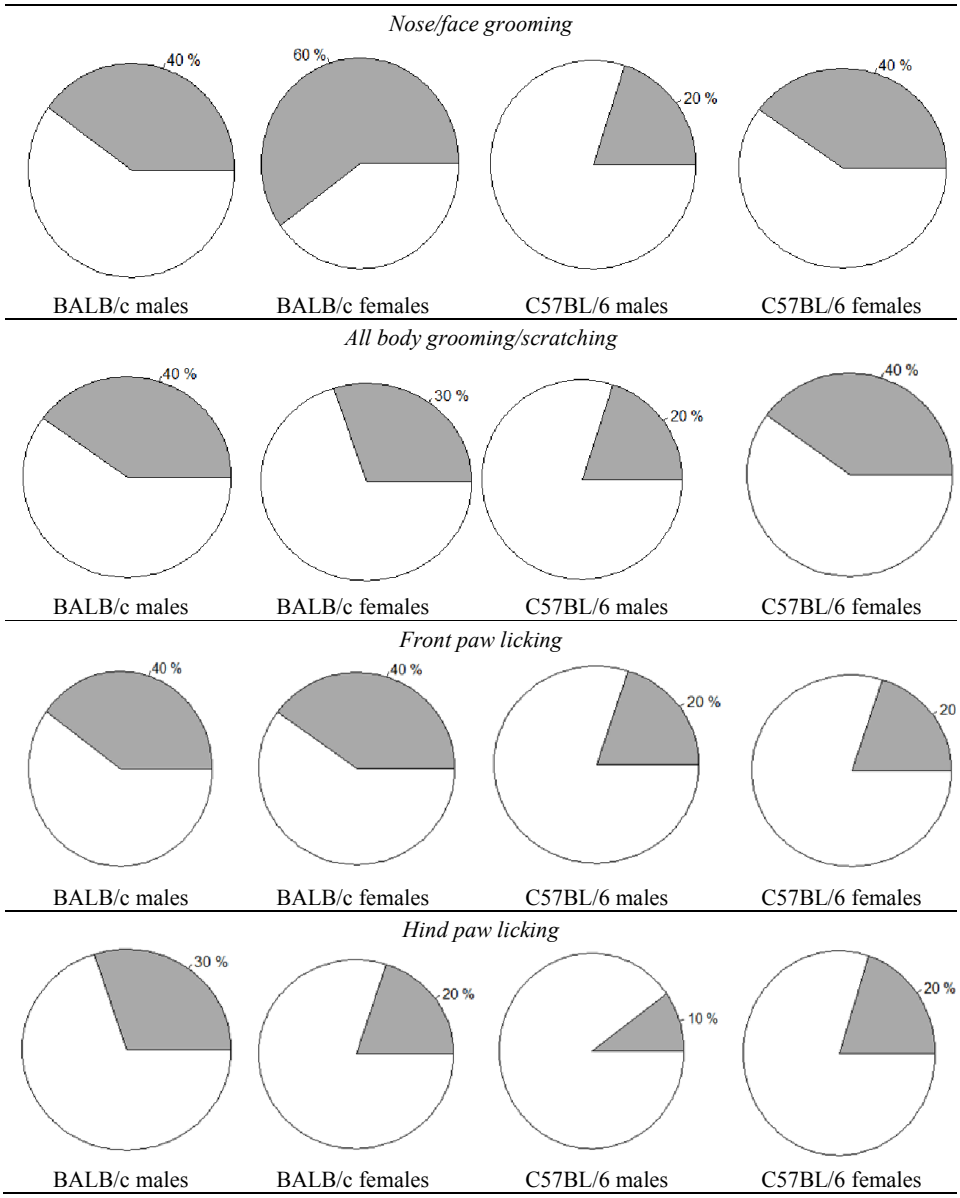

Fig. 7. Percent distribution of behavioural patterns in BALB/c and C57BL/6 mice from both genders; $\chi 2$ – chi-square test.

Therefore, it is useful to gain more knowledge about the possible variations and the factors (such as the effect of strain, sex, genetic background, specific behavioural response to environmental enrichment, experimental design, type of cage system and size, metabolic defects of the strain on a high-fat diet, etc.) that influence BAT (Zhang *et al*., 2010; Montgomery *et al*., 2013; Whittle *et al*., 2013; Kataoka *et al.*, 2014; Mohammed *et al*., 2014; Oiwa *et al*., 2020; Santhanam *et al*., 2021; Yang *et al*., 2021). The present study has characterised temperature measurements of BAT at 5–8 weeks of age in BALB/c and C57BL/6 strains of mice, contributing to their better understanding.

There are many methods for assessing the activity of BAT *in vivo*. IRT is the process of constructing an image of the temperature of an object (animal) by first measuring the infrared radiation emitted, then converting this radiometric data to a temperature, and finally displaying the temperature data as a colour coded image (Law *et al*., 2018). IRT has been successfully used in rodent studies to directly measure BAT thermogenesis (Marks *et al*., 2009; Carter *et al*., 2011; Meyer *et al*., 2017), however there is an ongoing debate whether to anaesthetise the animals and/or remove their fur (hairless mice had 19% more BAT than albinos; Heldmaier, 1974) during measurements to obtain more reproducible results (Akesson *et al*., 2007; Marks *et al*., 2009; Crane *et al*., 2014). For example, procedures like shaving and anaesthetising (Fiebig *et al.*, 2018) are invasive and cause distress to the animals. Therefore, seeking to provide research data of higher quality, also to adhere to the 3Rs (Replacement, Reduction and Refinement) guiding principle for a more ethical use of animals in BAT research, we did not shaved rodents prior testing and performed in vivo measurements on mice. Previous studies examining responses to stress with thermal imaging have shaved rodents prior to testing (Marks *et al.*, 2009; Carter *et al.*, 2011), and determined that it may alter the natural thermoregulatory state of the animals and influence subsequent experiments (Crane *et al.*, 2014). Fiebig *et al.* (2018), seeking to obtain accurate measurements, found that it was best to remove the animals' hair by shaving or use nude mice, as hair does not produce heat but can retain it. Furthermore, some uncontrollable factors, such as environmental noise or even stimuli caused by experimental handling of the animals, may potentially affect the nervous system of the mouse and thus the activity of the BAT (Wu *et al*., 2011). Therefore, we supported the notion of Wu *et al.* (2011) and Crane *et al*. (2014) and performed IRT of the free movement of mice in their natural environment and did not cause any potential increase in body temperature as a result of handling.

BAT and/or its metabolic activity diminish with age in both rodents and humans (Halpern *et al*., 2014; Santhanam *et al*., 2021). Sellayah & Sikder (2014) showed that aging (3 weeks, 4, 6, 12 and 24 months of age) was associated with interscapular BAT morphological abnormalities and thermogenic dysfunction in mice. *In vitro* experiments revealed that brown adipocyte differentiation was defective in aged male C57BL/6 mice (Goncalves *et al*., 2017). Interscapular brown tissue in aged mice was progressively populated by adipocytes with white morphological features, the mice failed to mobilise intracellular fuel reserves from brown adipocytes and exhibited a deficiency in homeothermy (Sellayah & Sikder, 2014). Our results revealed that the age of the mice (5–8 weeks) did not alter

the temperature of the interscapular BAT area. This can be explained by the fact that more time is needed for age differences to become evident, thus the activity of BAT biochemical processes in mice remains the same between 5 and 8 weeks of age.

Goncalves *et al*. (2017), stated that the effects of ageing on BAT deserve attention, especially in female C57BL/6 mice, as they are less prone to age-induced weight gain and the effects remain largely unclear. In addition, Valle *et al*. (2008) showed a age-associated decline in T3, a hormone that promotes UCP1 synthesis and activity (Raasmaja, 1990) in female rats and suggested that the age decline in T3 and 17β-estradiol in female rats may, at least in part, mediate the effect of gender on the functional decline of the BAT with age, and this also seems to be the case in mice (not yet confirmed). Conversely, our findings indicated a higher maximum temperature in male BALB/C and C57BL/6 mice than in females. This fact may be partly explained by findings of McDonald & Horwitz (1999) that female rodents were more able to maintain their thermogenic capacity with age compared to males, although the overall gender distribution (females *vs* males) in our study was not equal between strains, a clear statement could not be made.

As well, the obtained results exhibited that the BAT surface temperature was strain- dependent (higher in BALB/C mice than in C57BL/6 mice). Comparisons are complicated by the lack of publicly available scientific articles in the area of strain. Interestingly, some authors (Montgomery *et al*., 2013) have noted that BALB/c mice did not accumulate excess lipid (triacylglycerols and diacylglycerols) in the liver, which may be related to lower fatty acid uptake rather than differences in lipogenesis or lipid oxidation. Also, BL6, 129X1, DBA/2 and FVB/N mouse strains were all susceptible to varying degrees to high-fat diet-induced obesity, glucose intolerance and insulin resistance, but BALB/c mice exhibited some protection from these adverse effects. This protection was not explained by differences in mitochondrial metabolism or oxidative stress in liver or muscle, or by inflammation in adipose tissue (Montgomery *et al*., 2013). The results of our study of strains need to be further investigated in mice of different age groups in order to make a definitive statement about the differences between the strains.

In addition, scientists have found that anaesthesia may alter blood glucose results, because it effects heart rate and blood flow and may induce hyperglycemia in mice and rats (Brown *et al*., 2005). Therefore, we carried out IRT of the mouse *in vivo*. Noteworthy, BAT has recently been identified as an important player in TRIG metabolism, but the mechanism by which BAT takes up fatty acid from TRIG-rich very-low-density lipoprotein and chylomicrons has not been fully established (Khedoe *et al*., 2015). Elevated plasma TRIG levels have been implicated in the pathology of various cardiovascular and metabolic diseases (Bartelt *et al.* 2011; Khedoe *et al.*, 2015). Our results demonstrated no statistically significant strain effect in mean blood GLU, CHOL and TRIG levels in BALB/c and C57BL/6 mice, which were relatively similar to data reported by other authors (Santos *et al*., 2016; Aigner, 2021).

Self-grooming is an innate, conserved, repetitive behaviour that plays multifaceted roles including hygiene maintenance, thermoregulation, de-arousal, stress reduction and social behaviour. It has been reported that adult male mice spend more

time self-grooming than females (Seiffe *et al*., 2021). Additionally, as mice age, they show a reduction in grooming behaviour, resulting in their fur becoming thicker and exposing more skin in the BAT region, making it difficult to interpret results from mice of different ages (Oelkrug & Mittag, 2021). However, our study results showed that the distribution of grooming activities was not statistically significant in male BALB/c and C57BL/6 mice, but was statistically significant in female mice. It should be noted that only a short period of time was studied (mice aged 5–8 weeks) and therefore no clear age-related tendency of changes in grooming could be outlined.

CONCLUSION

The age of the mice (5–8 weeks) did not alter the temperature of the interscapular BAT area. The strain of mice had a significant influence on the T_{max} and T_{isoth} temperatures of the interscapular BAT area. Furthermore, the gender of the mice also affected the BAT area temperature. Overall, our findings suggest that IRT is a completely animal-friendly and ethical method for the in vivo assessment of BAT temperature changes in mice. Furthermore, our study highlights the importance of further research to evaluate strain-specific cohorts of mice of different ages (weeks and months) for a comprehensive evaluation.

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Correspondence:

Ugne Spancerniene
Department of Anatomy and Physiology,
Faculty of Veterinary Medicine,
Lithuanian University of Health Sciences,
Tilzes str. 18, Kaunas, Lithuania,
phone: +370 37 363204
e-mail: ugne.spancerniene@lsmu.lt