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# **Performance, acute health symptoms and physiological responses during exposure to high air temperature and carbon dioxide concentration**

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**Short running title:** Effects of high temperature and CO<sub>2</sub> concentration

**Competing financial interests:** The authors declare they have no financial interests.

## **Abstract**

Human subjects were exposed for 3 hours in a climate chamber to the air temperature of 35°C that is an action level, at which the working time needs to be diminished in China. The purpose was to put this action level to test by measuring physiological responses, subjective ratings and cognitive performance, and compare them with responses at temperature of 26°C (reference exposure). Moreover, CO<sub>2</sub> was increased to 3,000 ppm (CO<sub>2</sub> exposure) at 35°C to further examine, whether this change will have any effect on the measured responses. Compared with the reference exposure, exposure to 35°C caused subjects to report feeling uncomfortably warm, to rate the air quality as worse, to report increased sleepiness and higher intensity of several acute health symptoms. Eardrum temperature, skin temperature, heart rate and body weight loss all increased significantly at this exposure, arterial oxygen saturation decreased significantly, while the percentage of adjacent inter-beat cardiac intervals differing by >50 ms (pNN50) decreased significantly, indicating elevated stress. The performance of addition and subtraction tasks decreased significantly during this exposure, as well. Increasing CO<sub>2</sub> to 3,000 ppm at 35°C caused no significant changes in responses. Present results reaffirm the selection of 35°C as an action level, and show that concurrently occurring high CO<sub>2</sub> levels should not exacerbate the hazards.

*Key words: Temperature; Carbon Dioxide; Physiological Responses; Acute Health Symptoms; Work Performance*

# 1. Introduction

High temperatures create major health and safety risks especially to individuals working outdoors, where the effects are difficult to mitigate, e.g. craftsmen on construction sites [1,2] and motorbike riders [3]. Driver vigilance is greatly reduced even at moderate compartment temperatures [4]. Extreme heat events are becoming more frequent, severe and long lasting due to global climate change [5]. People working outdoors will thereby face a severe adverse challenge due to increased, and at times extremely high, outdoor temperatures especially in the summer months.

In China an outdoor temperature of 35°C is the action level, at which a high temperature yellow warning is issued; at this temperature outdoor workers are required to reduce their working time and to protect themselves from heat stroke ([www.cma.gov.cn](http://www.cma.gov.cn)). It is relevant to examine physiological reactions and cognitive performance, as well as other human responses at this temperature. It is also valid to examine, whether other environmental factors interact with the selected action level for temperature and exacerbate these responses.

Temperatures higher than 35°C result in a significant increase in core temperature, heart rate and sweat rate. These effects were observed, when subjects were exposed to temperatures ranging from 35 to 50°C for 80 ~ 120 min [6-10]; it is worth mentioning that during the 2 hour exposures to 50°C, the air relative humidity was kept as 40% and the subjects were at complete rest [6]. The exposure to high temperatures decrease also both systolic and diastolic blood pressure. These effects were observed during 4-hour exposures in experiments, which examined the effects of increasing temperature from 20°C to 34 ~ 42°C [11]. Lu and Zhu [11] showed that oral temperature of 38°C and dehydration causing body weight loss of 1% should be considered as the physiological limits for the health safety of persons exposed to heat. Temperatures close to 35°C and above cause additionally changes in subjective responses.

Holland et al. [12] found that the rise of core temperature to 39°C (by immersion in 41°C water) induced a significant decrease in alertness and increased irritability as judged by subjects exposed for 20 min to 35°C in air. In a study by Tamm et al. [10], subjectively rated fatigue and exertion increased during 1 hour exposures, when the temperature increased from 22°C to 42°C. Finally, high temperatures reduce also mental performance. Wilkinson et al. [13] observed a significant decrease in the performance of two-digit addition and an improvement in the performance of a vigilance test (subjects listened to a series of tones), when body temperature was maintained at 38.5°C with ambient temperature at 37°C for 2 hours. Epstein et al. [6] found that percentage of errors in a shooting TV game rose gradually, when the air temperature increased from 24°C to 37°C and then to 50°C; at each temperature the exposure lasted 2 hours. The effect observed in their study indicated a deterioration in psychomotor functions caused by the heat load. Hocking et al. [7] found that thermal stress at 35°C induced deficits in working memory, information retention and information processing, and there was a marked difference in the electrical responses of the brain when subjects were thermally strained. Mohr et al. [8] examined the effect of high air temperature on physical performance during a 90-minute football game. The total distance run by the players and high intensity running (>14 km/h) declined, but the success rate for passes and crosses and peak sprint speed increased at 43°C compared to 21°C. The latter was most likely caused by the lower distance of high intensity running and reduced pressure from the opposition on the player in possession. On the other hand, Holland et al. [12] were not able to show that 20-minute exposure to 35°C after raising the body temperature to about 39°C (by immersion in water at 41°C) had a significant effect on the accuracy with which tests examining long-term and short-term memory were performed, or on the accuracy of reasoning tests such as logic problems and two-digit subtraction. This exposure was actually associated with a significant increase in the speed, at which reasoning tests were performed. This result may be

explained by a direct effect of temperature on neuronal processes and by the 20-minute exposure at which subjects can maintain a high level of motivation.

Carbon dioxide is gradually increasing in ambient air and there is a new evidence that the concentrations below occupational level set at 5,000 ppm [14] may influence the ability to make decisions [15,16] although these levels have not been shown to cause negative effects on health or comfort, or result in measurable physiological responses [17,18]. One isolated experiment suggested that exposure to CO<sub>2</sub> at 3,000 ppm increased fatigue and reduced wellbeing [19], but no other study supports this observation. Whether levels of CO<sub>2</sub> below 5,000 ppm would impair mental performance is not completely clear at the moment. Some studies show that the performance of cognitive tests and tasks examining subjects' ability to perform office work are not affected during exposures to CO<sub>2</sub> (dosed from cylinder to otherwise clean environment with low exposures) below 5,000 ppm [20-22] and some studies have shown that negative effects are first seen at levels as high as 12,000 ppm [23] and even higher, up to 60,000-70,000 ppm [24]. On the other hand, some recent studies have shown that the performance of proof reading is negatively affected by exposure to CO<sub>2</sub> at 4,000 ppm [19] and that exposure to CO<sub>2</sub> at levels as low as 1,000 ppm can reduce the ability to take decisions [15,16]. In certain cases, e.g., in a welding factory, green house and in mines, elevated CO<sub>2</sub> can occur together with high temperatures. Consequently, it is important to determine whether exposure to high CO<sub>2</sub> levels would modify the effects of exposure to high temperature, and whether it would intensify the observed effects. This question has not yet been examined: the Authors of the present paper were not able to find any study in the published literature that examined the combined effects of temperature and pure CO<sub>2</sub>.

The objective of the present study was to supplement the existing evidence on the effects of elevated air temperatures on humans in particular by extending the experimental protocols by adding measurements of oxygen saturation, end-tidal CO<sub>2</sub>, stress biomarkers and

neurobehavioral tests of cognitive performance, as well as by examining whether increased concentration of CO<sub>2</sub> at elevated air temperature would have any modifying effect on the measured responses. The results are expected to facilitate decisions and recommendations aiming to protect the workers during events with high temperatures.

## **2. Methods**

### *2.1 Approach*

Twelve subjects, two persons at a time, were exposed for 3 hours in the climate chamber to the air temperature of 26°C and 35°C at CO<sub>2</sub> level of 380 ppm and to the air temperature of 35°C at CO<sub>2</sub> level of 3,000 ppm. The three exposure conditions were experienced in balanced order. They performed different cognitive tests, rated their acute health symptoms and assessed the environmental conditions. Their physiological reactions were monitored.

### *2.2 Facilities*

The experiment was conducted in a climate chamber (Figure 1), one of the twin climatic chambers located at Technical University of Denmark (DTU) [25,26]. The size of the chamber is 3.6x2.5x2.5 m<sup>3</sup>; the volume of the chamber with its recirculation ducts is 30 m<sup>3</sup>. The chamber is ventilated by 100% outdoor air through a perforated floor and the air is exhausted through outlets in the ceiling. The air temperature and humidity are controlled by the specially designed air-conditioning system. To achieve good mixing, the air in the chamber is recirculated at an exchange rate of >10 h<sup>-1</sup>.

Three workstations were set up in the chamber. They were used by the subjects and the

experimenter during exposures. Each consisted of a table, a chair, a desk lamp and a laptop.

### *2.3 Experimental conditions*

Three indoor environmental conditions were established.

In Condition 1 (reference condition designated T26), the air temperature in the chamber was set at 26°C. The temperature of 26°C was selected because this is the lower limit of air-conditioning temperature in public buildings as specified by the Chinese government during summer, if both thermal comfort requirements and energy conservation are taken into account.

In Condition 2 (exposure condition designated T35), the air temperature in the chamber was set to 35°C. A temperature of 35°C was selected because this is the threshold for high temperature yellow warning in China (see Introduction).

In Condition 3 (CO<sub>2</sub> condition designated T35C3000), the air temperature in the chamber was kept at 35°C and the concentration of CO<sub>2</sub> in the chamber was increased to 3,000 ppm by adding pure CO<sub>2</sub> of a high quality (99.99%) from a pressurized cylinder. The concentration of 3,000 ppm was selected to match the concentration studied by Zhang et al. [20,21], and because it was higher than the concentration in the experiments by Satish et al. [15] and Allen et al. [16], while still lower than occupational exposure limit of 5000 ppm.

In all three exposure conditions, the outdoor air supply rate was 720 m<sup>3</sup>/h; this corresponds to > 60 L/s per person with 3 people in the chamber. At this rate CO<sub>2</sub> concentration (without adding CO<sub>2</sub> from cylinder) was close to the ambient level of 380 ppm and human bioeffluents emitted by the occupants in the chamber and any other residual pollutants in the chamber were very low. The relative humidity was not controlled but remained <35% while the air velocity was on average less than 0.2 m/s. The level of illumination (62 lux) and the sound

level (48 dB(A)) were similar in all three conditions.

The experiment was carried out during May and June 2014, when the daily mean outdoor temperatures were  $14 \pm 3^\circ\text{C}$ .

#### *2.4 Subjects*

Six male and six female college-age subjects (mean  $\pm$ SD age:  $24.8 \pm 2.6$  years, height:  $172.4 \pm 8.5$  cm, weight:  $68.8 \pm 18.3$  kg) were recruited for the experiment. To reduce bias and potential over-reporting of symptoms, it was decided to select subjects who repeatedly experienced high summer temperatures and for extended periods earlier in life; it was an essential recruitment criterion. Consequently, Chinese were selected to participate in the experiments rather than Danes who only seldom experience the high temperature chosen as one exposure condition. The selected Asian subjects lived in Denmark for at least 3 months prior to the experiments and were not heat acclimated.

The subjects had sufficiently good skills in English. They were non-smokers, not chronically ill, did not take any medication during the experiments, did not have any history of cardiovascular disease, and were not colour blind. This information was obtained from a questionnaire completed by the subjects upon recruitment and was not verified by examining medical records. The subjects received financial compensation for participating in the experiments.

#### *2.5 Measurements*

Air temperature, relative humidity and CO<sub>2</sub> concentration were continuously measured and recorded by HOBO data loggers (Onset, USA) at two workstations in the chamber. Each

HOBO data logger contained built-in temperature, humidity and light intensity sensors. It was also connected to a CO<sub>2</sub> monitor (VAISALA, FI). The measured physiological parameters comprised skin temperature at six sites (left chest, right upper arm, left forearm, left hand, anterior thigh and anterior calf) [27], eardrum temperature in the right ear (to estimate the core temperature), heart rate (inter-beat intervals), blood pressure, end-tidal partial CO<sub>2</sub> (ETCO<sub>2</sub>), arterial blood oxygen saturation (SpO<sub>2</sub>) and body weight. The instruments used to perform the measurements were calibrated. Their accuracy and other specifications are listed in Table S1 in Appendix 1.

Mean skin temperature was calculated based on the measurements taken at four sites [27], using the following formula:

$$T_{msk} = 0.2T_{AC} + 0.2T_{AT} + 0.3T_{LC} + 0.3T_{RUA} \quad (1)$$

where  $T_{msk}$  is mean skin temperature.  $T_{AC}$ ,  $T_{AT}$ ,  $T_{LC}$  and  $T_{RUA}$  are skin temperatures at anterior calf, anterior thigh, left chest and right upper arm, respectively.

Based on the inter-beat intervals determined by the analysis software of the heart rate monitor (Firstbeat technologies Ltd., Jyväskylä, Finland), respiratory ventilation rate (the amount of air breathed per min) was calculated and the time-domain measure of heart rate variability (HRV) was determined using the percentage of adjacent inter-beat intervals differing by >50 ms (pNN50). pNN50 reflects parasympathetic modulation of the heart [28] and can be affected by stress.

Biomarkers in saliva (alpha-amylase and cortisol) were measured with a non-stimulated passive drool salivary sampling procedure [29]. Salivary alpha-amylase is a biomarker for stress-related changes in the body that reflect the activity of the sympathetic nervous system [30]. Subjects assessed their comfort and experienced acute health symptoms during exposures using a specially designed questionnaire; they were also asked to rate how well they performed the cognitive tests. The following responses were collected: thermal sensation,

thermal comfort, acceptability of the thermal environment, acceptability of the air quality, odour intensity, sleepiness, self-estimated work performance and the intensity of dry nose, dry throat, aching and dry eyes, and dry skin as well headache, difficulty in concentrating and thinking clearly, wellbeing, mood, fatigue and dizziness [21,29]. The scales are described in detail in Appendix 2.

During the exposure, the subjects performed different cognitive tests. The tests were included in a battery of neurobehavioral tests that has been used previously to evaluate the effects of temperature on performance [29]. The following tests were included: mental redirection, grammatical reasoning, digit span memory, visual learning memory, number calculation (one digit addition & subtraction), one digit multiplication, a Stroop test and visual reaction time. The Stroop test and number calculation were presented with and without feedback on performance [31]. In the former case, the subjects could not continue these tests until they had corrected the errors. It took subjects 40 minutes to complete the entire battery of tests. Accuracy and speed or response time were used as the measures of performance. Subjects performed also the d2-test, which is used to examine attention and concentration [32]. The total number of characters processed, accuracy and overall concentration, i.e. the total number of correctly identified d2 characters minus errors of commission, were used as the measures of performance [33]. Subjects performed also the Tsai-Partington test. This is a cue-utilization test providing an indication of arousal [34,35]. The number of correct links and errors were used as measures of performance. All cognitive tests are described in detail in Appendix 2.

In the case of gradual improvement of performance independently of the exposure conditions (learning), the following adjustments were made to the measures of performance [36]:

$$P'_{i,n} = \frac{\overline{P}_1}{\overline{P}_n} \times P_{i,n} \quad (2)$$

Where:  $n$  refers to the number of times the test was presented from 1 to  $n$  and  $i$  refers to the number of participants from 1 to  $i$ ,  $P'_{i,n}$  is the performance of the  $i$ th participant at the  $n$ th presentation after correction,  $\overline{P}_n$  and  $\overline{P}_1$  respectively refer to average performance of all subjects at the  $n$ th presentation and first presentation, and  $P_{i,n}$  means the performance of the  $i$ th participant at the  $n$ th presentation before correction.

## 2.6 Protocol

Subjects were exposed to three conditions in a design balanced for order of presentation to eliminate carry-over effects and systematic bias, as well as to reduce the potential gradual improvement of performance (learning). They were divided into 6 groups, each consisting of 1 male and 1 female. One group was exposed at a time. Each group participated in the experiment on three consecutive weekdays, from Tuesday to Thursday. If a public holiday fell on one of these days, the subjects participated either from Monday to Wednesday or from Wednesday to Friday. This happened on four occasions.

On a day prior to the first exposure the subjects attended a 1.5 hour practice session in the same group as during the actual exposures. During the practice session, the temperature was set at 26°C as in the reference exposure. The subjects received instructions and then they rehearsed all elements of the experimental procedures, i.e. performed cognitive tests and filled in questionnaires, and physiological measurements were made including the saliva sampling. The subjects wore their own clothing during the practice session including short sleeves, trousers and sport shoes. They were asked to adjust their clothing until they felt thermally neutral. They were then told to wear the same clothing ensemble during the actual

experiments. The insulation of the clothing ensemble worn by the subjects during experiments was estimated to be 0.42 [37].

The subjects were asked to avoid caffeine, alcohol, and intense physical activity for at least 12 hours prior to each experimental session.

Each experiment lasted 3.5 hours from 13:30 to 17:00, of which 3 hours was the exposure in the chamber (Figure 2). The 3-hour exposure at 35°C is unlikely to cause heat acclimation [38,39]. The subjects did not leave the chamber during each exposure. They were allowed to drink water after the saliva had been sampled during the 20 min pause in the middle of exposure, and the amount of water that remained in the bottle was measured.

The study conformed to the guidelines contained within the Declaration of Helsinki and was approved by the relevant Ethics Review Board of Technical University of Denmark (KA04741). Verbal and written informed consent was obtained from each subject prior to participation in the experiments.

## *2.7 Statistical analysis*

The mixed ANOVA model was used to examine the effects of exposures on the parameters measured. The exposure conditions (at 3 levels: T26, T35 and T35C3000), the time at which measurements were performed and the interaction between exposure condition and time were included in the model as fixed factors. The subjects were included as random factors. A post-hoc analysis using Bonferroni test was performed to compare differences between different levels of the factors. Two comparisons were made for the effect of exposure condition at the same time during exposure: the outcomes at T26 and T35 were compared to reveal whether there were any statistically significant differences as a result of increased temperature from 26°C to 35°C, and the outcomes at T35 and T35C3000 were compared to

examine whether increasing CO<sub>2</sub> to 3,000 ppm at an air temperature of 35°C caused changes in the outcomes.

A paired-t test was used to examine whether there were any differences on performance of the tasks taken by the subjects at two times during the same exposure.

The statistical analysis was performed with SPSS 19.0 software (SPSS Inc., Chicago, USA). The significance level was set at  $p=0.05$ .

### **3. Results**

All measured physical parameters describing the conditions in the climate chamber during different exposures are listed in Table 1. The measured air temperature was 27°C, i.e. 1°C higher than the intended temperature of 26°C. CO<sub>2</sub> concentration was kept close to the intended level.

Thermal responses are shown in Table 2. The thermal sensation votes indicate that the subjects felt neutral at T26 and warm at T35; the difference was statistically significant ( $p<0.01$ ). Thermal sensation votes did not change at T35C3000, when CO<sub>2</sub> concentration was 3,000 ppm. Similar results were observed for the thermal comfort votes and the votes of thermal acceptability ( $p<0.01$ ): the subjects voted that the thermal environment was comfortable and acceptable at T26, and that they felt slightly uncomfortable and rated the thermal condition unacceptable at T35. The percentage dissatisfied with the thermal environment increased from 0% at T26 to 64% at T35. The thermal comfort and thermal acceptability votes did not change significantly when CO<sub>2</sub> level was 3,000 ppm at T35C3000 compared with T35.

As shown in Table 2, after 5-10 min of exposure, the subjects assessed the air quality to be less acceptable at T35 compared with T26 ( $p<0.01$ ). The percentage dissatisfied with air

quality at T35 and T26 were 53% and 0% respectively. The subjects also rated the air to be stuffier and drier at T35 than at T26 ( $p<0.01$ ). No significant change of the air quality votes, air freshness votes and humidity votes were observed when CO<sub>2</sub> was higher at T35C3000 compared with T35. In all experimental conditions, the subjects rated the odour intensity to be low.

The subjects indicated that they were significantly more sleepy at T35 compared to T26 (Table 3). No significant change of the sleepiness votes was seen at T35C3000 compared with T35.

Table 4 shows the acute health symptoms reported by the subjects. Dry nose, dry eyes, dry throat and dry skin were rated to be significantly higher at T35 compared with T26 ( $p<0.05$ ). The ratings of these symptoms did not change at T35C3000, when CO<sub>2</sub> was higher, compared with T35. Similarly, difficulty in concentrating and thinking clearly, fatigue, wellbeing, dizziness, headache and mood were significantly worse at T35 than at T26 ( $p<0.05$ ). No change in these ratings was observed at T35C3000 compared with T35. The difficulty in thinking clearly and the intensity of fatigue increased along the course of exposure at T35 ( $p<0.01$ ) and T35C3000 ( $p<0.05$ ), while they remained unchanged along the course of exposure at T26.

The heart rate was significantly higher at T35 than at T26 ( $p<0.01$ ), especially after 90 min of exposure (Figure 3). At T35C3000, when CO<sub>2</sub> was at 3,000 ppm, the heart rate slightly reduced compared with T35, but the change was not statistically significant. The mean skin temperature at T35 was significantly higher compared with T26 ( $p<0.01$ ). (Figure 3).

ETCO<sub>2</sub> was available for only 8 subjects. During exposures it was lower at T35 than at T26 and it tended to decrease in the course of each exposure at each experimental condition (Figure 3). The differences were systematic but not statistically significant. A systematic difference was also observed in the case of respiration rate for which the data were also

available only for 8 subjects. It was higher at T35 compared with T26 but again not significantly. The respiratory ventilation rate became higher at T35 than at T26, and the difference reached statistical significance during two middle periods ( $p < 0.01$ ): 100~107 min and 120~160 min (Table 5).  $\text{ETCO}_2$ , respiration rate and respiratory ventilation rate did not change significantly when  $\text{CO}_2$  was 3,000 ppm at T35C3000 compared with T35.

The eardrum temperature and loss of the body weight were higher at T35 than at T26 (Figure 3).  $\text{SpO}_2$  was lower at T35 and significantly lower near the end of the exposure compared with T26 (Figure 3). The measured eardrum temperature, body weight loss and  $\text{SpO}_2$  did not change at T35C3000 compared with T35.

The percentage of adjacent inter-beat intervals differing by  $>50$  ms (pNN50) measured during the experimental conditions were  $0.21 \pm 0.13$  at T26,  $0.12 \pm 0.11$  at T35, and  $0.14 \pm 0.11$  at T35C3000. pNN50 decreased significantly at  $35^\circ\text{C}$  compared with  $26^\circ\text{C}$  ( $p = 0.01$ ) but no further change in pNN50 was observed at T35C3000 when  $\text{CO}_2$  was high. The alpha-amylase measured tended to be higher at T35C3000 compared with the other two conditions (Table 6).

Neither speed nor reaction time nor accuracy changed in the course of exposure and therefore the analysis of the performance of cognitive tests was performed on the average accuracy and speed or reaction time for each exposure condition. It showed that the accuracy of addition and subtraction decreased significantly ( $p < 0.05$ ) at T35 ( $0.973 \pm 0.017$ ) compared with T26 ( $0.989 \pm 0.009$ ) but that no significant difference was seen between T35C3000 and T35. For other tests no significant differences in the accuracy and speed or reaction time were observed between the exposure conditions. The speed at which all cognitive tests were performed showed a clear tendency for a gradual increase in the course of the experiments, independently of the conditions. The measured speed was therefore adjusted using Equation (2). This adjustment did not change the results described above (Table S2 in Appendix 3).

## 4. Discussion

The effects observed as a result of changing the temperature between 26°C and 35°C are consistent with the results reported by the previous studies summarised in the Introduction section, both as regards physiological responses and subjective ratings of comfort and acute health symptoms. The observed physiological responses were most likely the result of thermoregulation at 35°C, which maintained a body temperature at a normal level of 37°C. This is further explained in Figure 4, which shows the hypothetical mechanism describing the effects observed based on the present results.

At a high ambient temperature metabolic rate increases [40] and more oxygen is consumed. The physiological indicators of this process are raised heart rate [41] and the decreased oxygen content in the blood [42]. Both effects were observed during exposure to 35°C. Lan et al. [29] also observed that SpO<sub>2</sub> decreased when their subjects were exposed to 30°C compared with exposure to 22°C.

To remove the excessive CO<sub>2</sub> due to increased oxygen consumption at high ambient temperature, the respiratory ventilation rate must increase. As a consequence, the end-tidal partial pressure of CO<sub>2</sub> (ETCO<sub>2</sub>) will decrease [43]. In the present experiment ETCO<sub>2</sub> tended to decrease after 30 minutes at 35°C and remained lower at this temperature compared with 26°C. A similar result was obtained by Kitazawa et al. [44], who showed that ETCO<sub>2</sub> began to fall, when the air temperature was higher than 29°C. In the study of Lan et al. [29] it was observed that ETCO<sub>2</sub> increased at 30°C. Since skin temperature and thermal sensation were similar in the study of Lan et al. [29] and in the present study, the increase in ETCO<sub>2</sub> could be due to differences in the respiratory ventilation rate. In the present experiment, the respiratory ventilation rate increased by about 20% at 35°C compared to 26°C, while only by 10% in the study by Lan et al. when the temperature increased from 22°C to 30°C.

High temperature is expected to increase arousal [45,46]. However, no significant change in the level of salivary alpha-amylase or cortisol or in the performance of the Tsai-Partington test were observed at 35°C, which may suggest that there was no significant effect of increased temperature on arousal. These results are consistent with the findings obtained by Lan et al. [29]. However, the percentage of adjacent inter-beat intervals differing by >50 ms (pNN50) decreased at 35°C compared with 26°C. This may suggest parasympathetic withdrawal due to the heat stress induced by the high temperature [47]. Shin [48] reported gradually reduced pNN50 as the air temperature increased from 17°C to 25°C and further to 38°C during a 10-min exposure. A reduction in parasympathetic nervous activity was also indicated in the present experiments by the higher heart rate at 35°C. The significant change of pNN50 and heart rate suggests higher physiological stress caused by the effort to remove heat from body at the high temperature, which is somewhat inconsistent with no changes in the other stress indicators i.e. biomarkers in the saliva, diastolic blood pressure and performance of the Tsai-Partington test. Too few observations could be the reason why no effects on other indicators of arousal were seen. It should also be noted that more errors in addition and subtraction can also imply higher arousal at 35°C.

High air temperature elevated thermal sensation and caused thermal discomfort, as expected. It caused the air to be rated as less fresh and the air quality as less acceptable, although the odour intensity and the actual air quality did not change in the chamber. As shown by Fang et al. [49], the perception of air quality is reduced by increasing enthalpy of air even if the load of pollution and ventilation are unchanged; this effect was seen on acceptability but not on odor intensity which is compatible with present findings. The effect on perceived air quality is believed to occur due to the reduced capacity of warm air to cool the nasal mucosa.

High air temperature also led to more severe acute health symptoms. This is also consistent

with previous studies [29,50]. It is worth considering, which physiological responses might be associated with the acute health symptoms reported by the subjects. It may be stipulated that the increase in core temperature could induce a significant increase in sleepiness [12]; the rise of skin temperature could increase thermal discomfort [7,27], and the decrease in blood oxygen saturation (SpO<sub>2</sub>) at high temperature could affect the intensity of some acute health symptoms, e.g. could increase fatigue [29]. More water loss by respiration through nose and diffusion through skin might cause higher intensity of dryness of these body parts at the higher temperature.

The accuracy at which the addition and subtraction tasks were performed was significantly lower at 35°C compared to 26°C. This implies that high temperature can impair the ability to perform some cognitive tasks and is also consistent with previous findings on this topic [6,7,13]. It can be stipulated that the increased thermal discomfort could result in reduced cognitive performance due to distraction [29]. Lower blood oxygen saturation at high temperature could also be expected to result in decreased cognitive function [51]. Some acute health symptoms experienced at 35°C, such as poor wellbeing and difficulty in thinking clearly and in concentration abilities could also be set forth as the reason why the performance of addition and subtraction was reduced.

The high temperature did not change the performance of other neurobehavioral tests in this experiment. One reason could be the nature of the tests and their short duration. It could also be that the subjects were motivated and could maintain performance at the higher temperature by exerting more effort. This explanation was suggested by Haneda et al. [52]. Higher effort exerted can result in increased fatigue and other neurobehavioral symptoms. It can then be expected that during extended exposures to high temperatures the cognitive performance is likely to reduce, as it was also postulated by Fang et al. [50], Tanabe and Nishihara [53] and Nishihara et al. [54].

There were no other differences in physiological responses, subjective ratings or cognitive performance when CO<sub>2</sub> was increased to 3,000 ppm at the temperature of 35°C. No effects of CO<sub>2</sub> at this level confirms the results reported by Zhang et al. [20-22] and is different from studies of Satish et al. [15] and Allen et al. [16]. The cumulative exposure to CO<sub>2</sub> in all these studies was about the same (around 6,200 to 10,000 ppmh) though slightly higher in the studies of Zhang et al. (around 12,000 ppmh); thus it cannot be attributed to the observed difference in results. Neither can the level of CO<sub>2</sub> be attributed to the differences because it was higher in the studies, where no effect on cognitive performance was observed. Similarly, exposure duration is not likely to explain the difference because it varied from 2.5 hours to 8 hours with no particular relationship between the length of exposure and the effects on performance. The difference could actually be due to the method of performance testing and the type of cognitive skills that are affected by CO<sub>2</sub>. This possibility was suggested by Zhang et al. [22]. They postulated that elevated stress during exposures to increased concentration of CO<sub>2</sub> can be a possible explanation for the discrepancy between their results and the results of Satish et al. [15] and Allen et al. [16], who used a battery of performance tests requiring a high level of cue-utilisation and showed that increasing CO<sub>2</sub> levels do reduce performance on tasks examining ability to take decisions. This explanation was proposed, because Zhang et al. observed a tendency of higher alpha-amylase level at 3,000 ppm, which suggests that the higher CO<sub>2</sub> level may increase mental stress. Increased alpha-amylase during exposures to high CO<sub>2</sub> was also seen in the present experiments. The explanation proposed by Zhang et al., although plausible, requires further examination in future experiments.

Fothergill et al. [55] suggested that if ET<sub>CO<sub>2</sub></sub> during exposure to CO<sub>2</sub> is between 5.3-6.3% the impaired cognitive performance is likely and only when ET<sub>CO<sub>2</sub></sub> is above 6.3% the impairment can occur systematically. In this study, the ET<sub>CO<sub>2</sub></sub> was < 5% and this could also explain why no effects of the increased CO<sub>2</sub> concentration on performance were observed.

The present findings indicate a possible work risk induced by the air temperatures at 35°C (and above) including increased heat stress and acute health symptoms. These effects can be detrimental to health [56-58] and cognitive performance [5,45]. In future, the protective safety standards for high temperature should take into full consideration of both human health and performance limits.

A limitation of the present work is that only young college students were recruited that were not heat acclimated but lived previously in regions, where the extended periods with elevated temperatures occur regularly. Outdoor workers, unless just recruited, experience high outdoor temperatures on regular basis and are normally exposed to high temperatures for much longer periods than the subjects examined in the present laboratory experiments. This can result in thermal adaption that modify thermal responses to a hot environment [59]. To extend the results of the present study the acclimatized subjects should be recruited in the future.

The present work examined only the impact of elevated temperature. To extrapolate present results to typical outdoor conditions and exposures it is necessary to consider the impact of other parameters including especially radiant temperature and relative humidity. Future experiments should examine to which extent these factors can add to the negative effects of elevated temperatures.

## **5. Conclusions**

(1) Increasing air temperature from 26°C to 35°C changed thermal sensation from neutral to warm or hot and caused subjects to report feeling thermally uncomfortable. The air quality (though exposure levels did not change) was assessed to be worse, the subjects felt more sleepy and the intensity of some acute health symptoms increased at 35°C. The measured

cardrum temperature, skin temperature, heart rate, respiratory ventilation rate and body weight loss increased at the higher temperature. The percentage of adjacent inter-beat intervals differing by >50 ms was lower, suggesting higher stress. Arterial oxygen saturation was also lower. The accuracy of addition and subtraction tasks decreased.

- (2) Increasing CO<sub>2</sub> concentration to 3,000 ppm at 35°C did not cause changes in any of the measured responses.

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**Table 1 Measured parameters in the climate chamber (mean  $\pm$  SD) at different exposure conditions**

Condition	Air temperature (°C)	Relative humidity (%)	CO <sub>2</sub> concentration (ppm)
T26	27.1 $\pm$ 0.1	31 $\pm$ 3	380 $\pm$ 9
T35	35.5 $\pm$ 0.2	25 $\pm$ 2	403 $\pm$ 41
T35C3000	35.5 $\pm$ 0.2	24 $\pm$ 3	3025 $\pm$ 92

**Table 2 Subjective ratings of thermal environment and air quality (mean ± SD) at different exposure conditions**

Time during exposure (min)	5~10	20~25	70~75	100~105	150~155	175~180
Thermal sensation <sup>1</sup>						
T26	<b>0.3±0.5</b>	<b>0.1±0.4</b>	<b>0.3±0.6</b>	<b>-0.2±0.6</b>	<b>-0.1±0.7</b>	<b>0.0±0.7</b>
T35	<b>2.2±0.8 **</b>	<b>2.0±1.0 **</b>	<b>2.5±0.4 **</b>	<b>2.4±0.5 **</b>	<b>2.1±0.8 **</b>	<b>2.4±0.6 **</b>
T35C3000	2.3±0.6	2.4±0.5	2.5±0.5	2.1±0.9	2.1±0.8	2.1±0.8
Thermal comfort <sup>2</sup>						
T26	<b>0.9±0.6</b>	<b>1.0±0.5</b>	<b>1.0±0.5</b>	<b>1.1±0.6</b>	<b>1.2±0.7</b>	<b>0.9±0.6</b>
T35	<b>-0.2±0.8 **</b>	<b>-0.2±0.6 **</b>	<b>-0.5±0.8 **</b>	<b>-0.5±0.7 **</b>	<b>-0.4±0.8 **</b>	<b>-0.1±0.6 **</b>
T35C3000	-0.0±0.7	-0.2±0.5	-0.3±0.8	-0.2±0.8	-0.1±0.7	-0.1±0.7
Thermal acceptability <sup>3</sup>						
T26	<b>0.6±0.2</b>	<b>0.6±0.2</b>	<b>0.7±0.2</b>	<b>0.6±0.3</b>	<b>0.6±0.2</b>	<b>0.6±0.2</b>
T35	<b>-0.1±0.4 **</b>	<b>-0.1±0.2 **</b>	<b>-0.2±0.4 **</b>	<b>-0.2±0.3 **</b>	<b>-0.2±0.4 **</b>	<b>-0.1±0.3 **</b>
T35C3000	-0.1±0.3	-0.1±0.3	-0.1±0.4	-0.1±0.4	-0.1±0.3	-0.1±0.3
Air quality acceptability <sup>4</sup>						
T26	0.5±0.2	<b>0.5±0.3</b>	<b>0.6±0.2</b>	<b>0.5±0.3</b>	<b>0.6±0.3</b>	<b>0.5±0.3</b>
T35	0.1±0.5	<b>-0.1±0.2 **</b>	<b>0.0±0.4 **</b>	<b>-0.1±0.3 **</b>	<b>0.0±0.4 **</b>	<b>-0.1±0.2 **</b>
T35C3000	0.0±0.3	0.0±0.3	-0.1±0.3	0.0±0.4	-0.1±0.4	-0.1±0.3

Bolded numbers indicate the pairs of responses that were significantly different.

\*\* (p<0.01)

<sup>1</sup>Thermal sensation: cold (-3), cool (-2), slightly cool (-1), neutral (0), slightly warm (1), warm (2) and hot (3).

<sup>2</sup>Thermal comfort: very uncomfortable (-2), uncomfortable (-1), just uncomfortable (-0.01) / just comfortable (0.01), comfortable (1), very comfortable (2).

<sup>3</sup>Thermal acceptability: clearly unacceptable (-1), just unacceptable (-0.01) / just acceptable (0.01), clearly acceptable (1).

<sup>4</sup>Air quality acceptability: clearly unacceptable (-1), just unacceptable (-0.01) / just acceptable (0.01), clearly acceptable (1).

**Table 3 Subjective ratings of sleepiness at different exposure conditions (mean  $\pm$  SD)**

Time (min)	Sleepiness <sup>1</sup>		
	20~25	100~105	175~180
T26	<b>1.6<math>\pm</math>1.2</b>	0.3 $\pm$ 1.6	0.7 $\pm$ 1.4
T35	<b>0.3<math>\pm</math>1.3</b> **	-0.2 $\pm$ 1.9	-0.3 $\pm$ 1.9
T35C3000	0.0 $\pm$ 1.5	0.0 $\pm$ 1.8	-0.5 $\pm$ 1.2

Bolded numbers indicate pairs of responses that were significantly different.

\*\* (p<0.01)

<sup>1</sup>Sleepiness: very sleepy (-4), sleepy, some effort to stay awake (-3), sleepy, no effort to stay awake (-2), some signs of sleepiness (-1), neither alert nor sleepy (0), rather alert (1), alert (2), very alert (3) and extremely alert (4)

**Table 4 Subjective assessment of acute health symptoms (mean  $\pm$  SD) at different exposure conditions**

Time during exposure (min)	20~25	100~105	175~180
Nose [running (0) – dry (100)]			
T26	47 $\pm$ 18	<b>42<math>\pm</math>17</b>	50 $\pm$ 20
T35	55 $\pm$ 24	<b>57<math>\pm</math>22</b> *	60 $\pm$ 28
T35C3000	56 $\pm$ 22	60 $\pm$ 21	63 $\pm$ 23
Eye [not dry (0) – dry (100)]			
T26	35 $\pm$ 19	<b>43<math>\pm</math>25</b>	46 $\pm$ 22
T35	50 $\pm$ 27	<b>60<math>\pm</math>26</b> *	58 $\pm$ 29
T35C3000	56 $\pm$ 22	51 $\pm$ 29	61 $\pm$ 30
Throat [not dry (0) – dry (100)]			
T26	<b>42<math>\pm</math>19</b>	48 $\pm$ 22	<b>54<math>\pm</math>19</b>
T35	<b>60<math>\pm</math>21</b> *	61 $\pm$ 25	<b>67<math>\pm</math>23</b> *
T35C3000	60 $\pm$ 22	61 $\pm$ 24	64 $\pm$ 26
Skin [not dry (0) – dry (100)]			
T26	<b>36<math>\pm</math>19</b>	47 $\pm$ 26	<b>43<math>\pm</math>23</b>
T35	<b>61<math>\pm</math>18</b> **	54 $\pm$ 22	<b>63<math>\pm</math>19</b> **
T35C3000	59 $\pm$ 20	51 $\pm$ 25	68 $\pm$ 16
Concentration [easy (0) – hard (100)]			
T26	<b>26<math>\pm</math>11</b>	45 $\pm$ 11	45 $\pm$ 18
T35	<b>44<math>\pm</math>26</b> *	61 $\pm$ 24	62 $\pm$ 16
T35C3000	54 $\pm$ 17	60 $\pm$ 22	61 $\pm$ 24
Thinking [clear (0) – difficult (100)]			
T26	<b>23<math>\pm</math>14</b>	39 $\pm$ 15	<b>34<math>\pm</math>20</b>
T35	<b>37<math>\pm</math>21</b> *	51 $\pm$ 19	<b>57<math>\pm</math>21</b> **
T35C3000	42 $\pm$ 19	54 $\pm$ 26	59 $\pm$ 27
Wellbeing [good (0) – bad (100)]			
T26	<b>25<math>\pm</math>10</b>	<b>38<math>\pm</math>15</b>	41 $\pm$ 23
T35	<b>49<math>\pm</math>22</b> **	<b>57<math>\pm</math>23</b> **	57 $\pm$ 21
T35C3000	57 $\pm$ 16	56 $\pm$ 25	62 $\pm$ 23
Mood [positive (0) – depressed (100)]			
T26	<b>23<math>\pm</math>12</b>	37 $\pm$ 14	38 $\pm$ 23
T35	<b>39<math>\pm</math>23</b> *	56 $\pm$ 23	54 $\pm$ 24
T35C3000	51 $\pm$ 21	52 $\pm$ 26	62 $\pm$ 25
Fatigue [rested (0) – tired (100)]			
T26	<b>32<math>\pm</math>15</b>	<b>41<math>\pm</math>16</b>	48 $\pm$ 26
T35	<b>48<math>\pm</math>21</b> *	<b>62<math>\pm</math>22</b> *	68 $\pm$ 16
T35C3000	54 $\pm$ 21	57 $\pm$ 23	64 $\pm$ 23
Dizzy [not dizzy (0) – dizzy (100)]			
T26	24 $\pm$ 16	39 $\pm$ 24	<b>30<math>\pm</math>24</b>
T35	39 $\pm$ 27	50 $\pm$ 30	<b>54<math>\pm</math>33</b> **
T35C3000	38 $\pm$ 26	46 $\pm$ 31	51 $\pm$ 27
Headache [no (0) – severe (100)]			
T26	25 $\pm$ 19	<b>29<math>\pm</math>20</b>	37 $\pm$ 26
T35	25 $\pm$ 23	<b>41<math>\pm</math>28</b> *	37 $\pm$ 33
T35C3000	28 $\pm$ 24	32 $\pm$ 33	40 $\pm$ 33

Bolded numbers indicate pairs of responses that were significantly different.

\* (p<0.05).

\*\* (p<0.01)

**Table 5 Respiration rate and respiratory ventilation rate (mean  $\pm$  SD) at different exposure conditions**

Time during exposure (min)	10~17	30~70	100~107	120~160	170~177
Respiration rate (breaths/min)					
T26	18.4 $\pm$ 1.7	17.5 $\pm$ 2.4	18.4 $\pm$ 1.5	17.3 $\pm$ 2.0	18.0 $\pm$ 1.8
T35	19.7 $\pm$ 2.4	17.9 $\pm$ 3.2	19.7 $\pm$ 3.1	18.3 $\pm$ 3.0	19.1 $\pm$ 2.7
T35C3000	19.3 $\pm$ 1.9	17.8 $\pm$ 3.3	18.5 $\pm$ 2.3	18.1 $\pm$ 3.0	18.3 $\pm$ 2.2
Respiratory ventilation rate (L/min)					
T26	10.6 $\pm$ 2.8	9.9 $\pm$ 3.0	<b>9.3<math>\pm</math>3.5</b>	<b>9.4<math>\pm</math>2.6</b>	8.6 $\pm$ 2.0
T35	11.6 $\pm$ 4.7	11.6 $\pm$ 5.4	<b>11.5<math>\pm</math>6.8</b> *	<b>11.6<math>\pm</math>3.9</b> **	10.5 $\pm$ 3.5
T35C3000	11.0 $\pm$ 3.6	11.7 $\pm$ 4.4	11.5 $\pm$ 4.9	11.4 $\pm$ 3.6	11.1 $\pm$ 3.5

Bolded numbers indicate the pairs of responses that were statistically significantly different.

\* (p<0.05)

\*\* (p<0.01)

**Table 6 Concentration (mean  $\pm$  SD) of salivary alpha-amylase and cortisol at different exposure conditions**

Time during exposure (min)	80~90	160~170
Alpha-amylase (U/ml)		
T26	98.3 $\pm$ 86.0	91.3 $\pm$ 49.1
T35	93.7 $\pm$ 72.8	94.1 $\pm$ 64.5
T35C3000	99.7 $\pm$ 50.5	100.9 $\pm$ 69.3
Cortisol ( $\mu$ g/dl)		
T26	0.44 $\pm$ 0.11	0.42 $\pm$ 0.12
T35	0.45 $\pm$ 0.14	0.43 $\pm$ 0.13
T35C3000	0.44 $\pm$ 0.13	0.45 $\pm$ 0.14

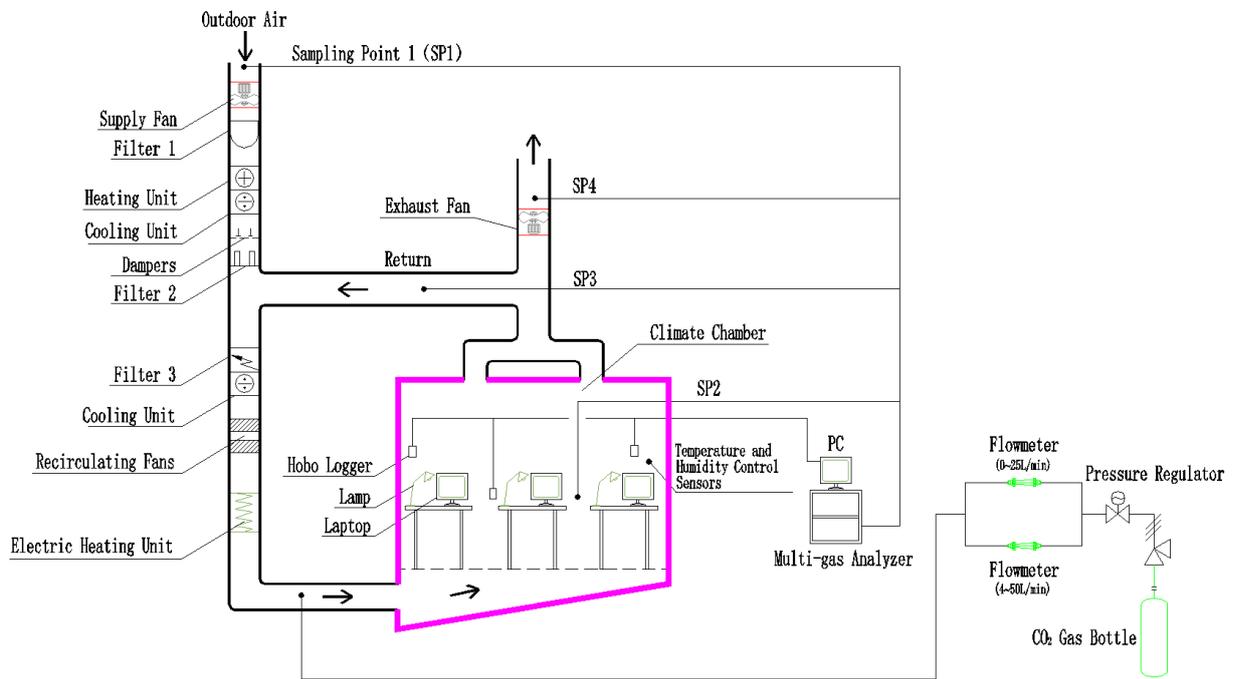
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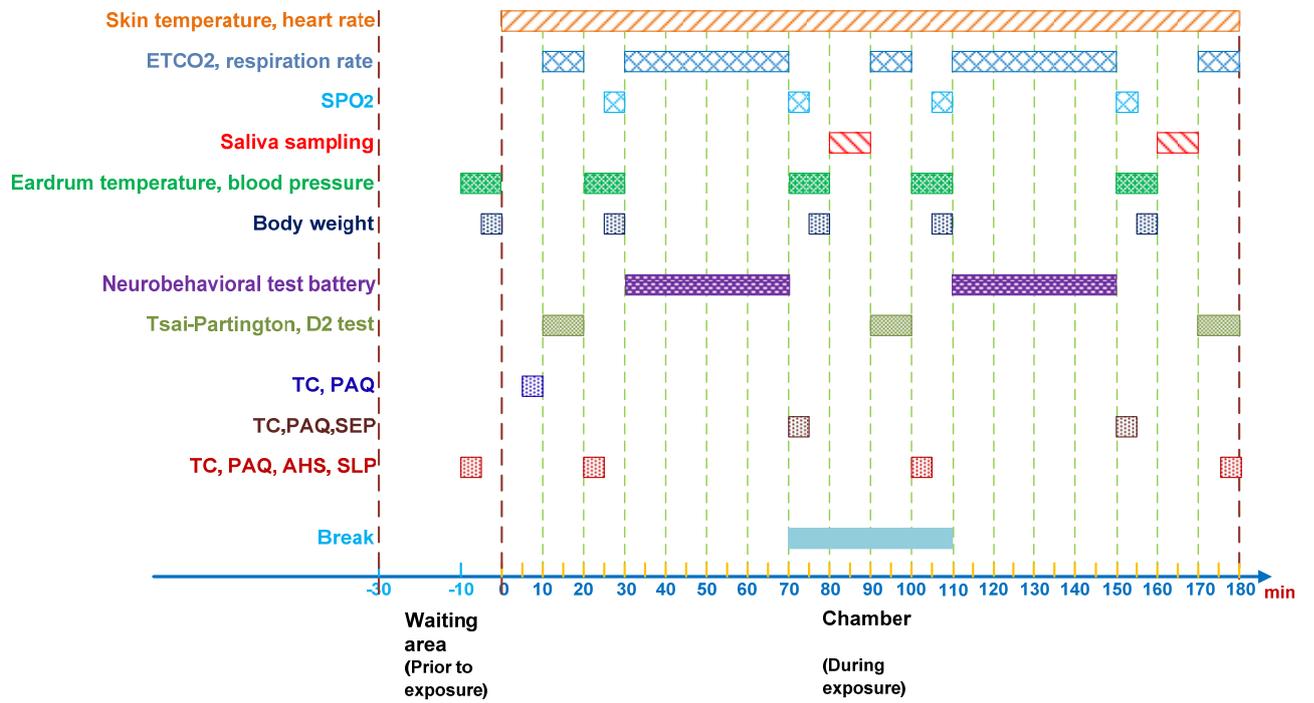
Figure 2 Experimental procedure. SpO<sub>2</sub> is arterial oxygen saturation and ETCO<sub>2</sub> is end-tidal partial CO<sub>2</sub> pressure. TC, PAQ, AHS, SLP and SEP are respectively the subjective ratings of thermal comfort, perceived air quality, acute health symptoms, sleepiness and self-estimated performance.

Figure 3 Physiological measurements (averages) at different exposure conditions and at different time during exposure. SpO<sub>2</sub> is arterial oxygen saturation. ETCO<sub>2</sub> is end-tidal partial CO<sub>2</sub> pressure.

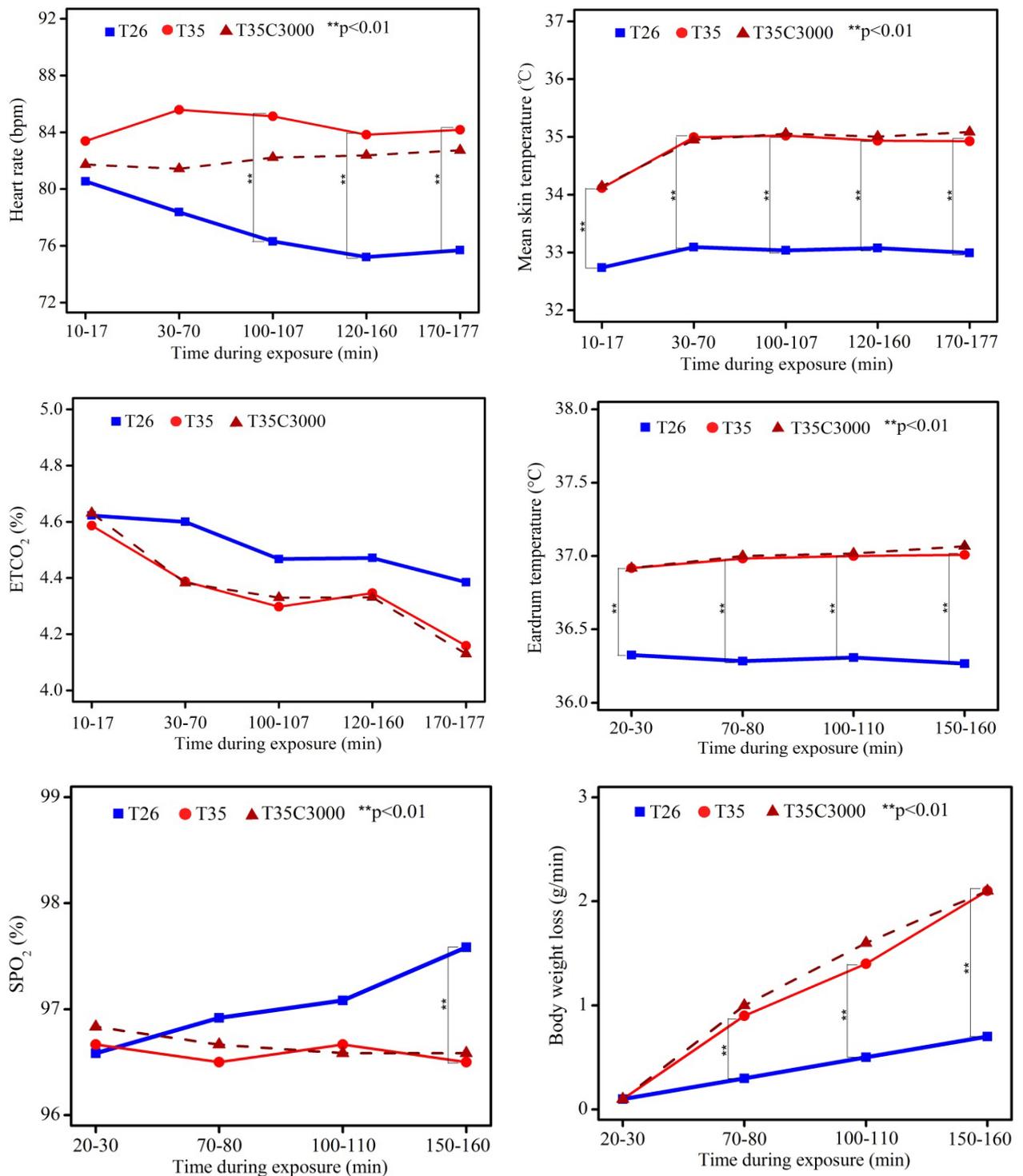
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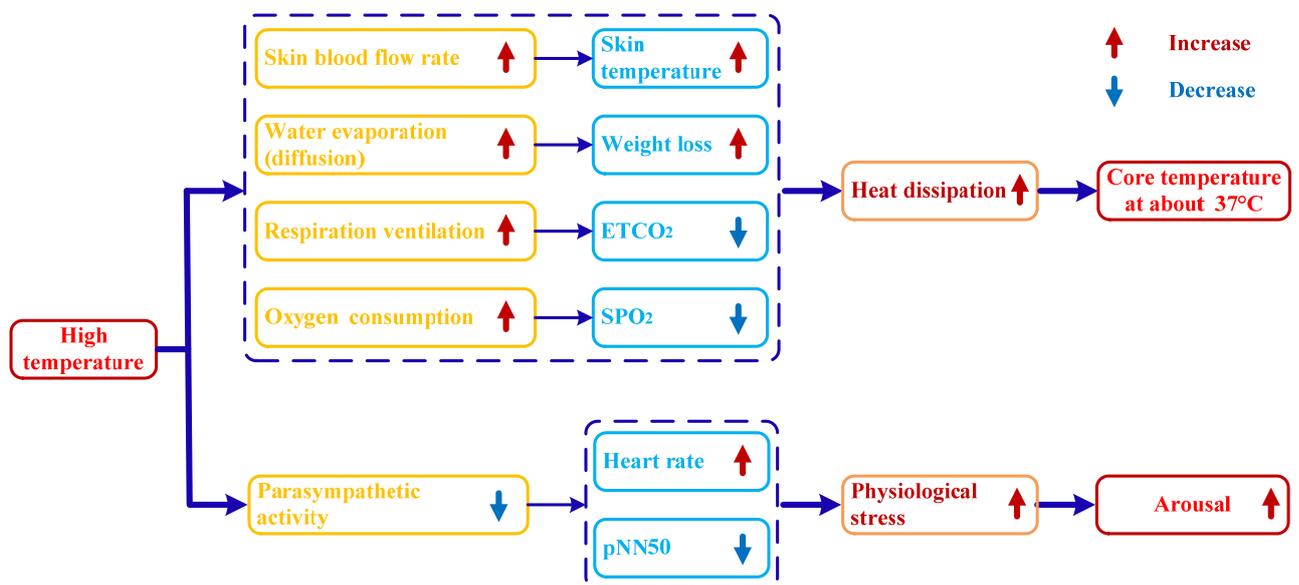
**Figure 1 Schematic diagram of the climate chamber**



**Figure 2 Experimental procedure.** SpO<sub>2</sub> is arterial oxygen saturation and ETCO<sub>2</sub> is end-tidal partial CO<sub>2</sub> pressure. TC, PAQ, AHS, SLP and SEP are respectively the subjective ratings of thermal comfort, perceived air quality, acute health symptoms, sleepiness and self-estimated performance.



**Figure 3 Physiological measurements (averages) at different exposure conditions and at different time during exposure.** SpO<sub>2</sub> is arterial oxygen saturation. ETCO<sub>2</sub> is end-tidal partial CO<sub>2</sub> pressure. For continuous measurement of heart rate, mean skin temperature and ETCO<sub>2</sub>, the average was calculated for the different time periods.



**Figure 4 Physiological responses related to thermoregulation and heat stress at high temperature.** SpO<sub>2</sub> is arterial oxygen saturation. ETCO<sub>2</sub> is end-tidal partial CO<sub>2</sub> pressure. pNN50 is the percentage of adjacent inter-beat intervals differing by >50 msec. To enhance the heat dissipation from the body at the high temperature, skin blood vessels dilated and more blood was pumped from internal organs to the skin. This process increased heart rate and skin temperature. More water evaporated through the skin by diffusion to increase heat loss from the core which resulted in an increased loss of body weight. Since no significant decrease in the mean skin temperature was observed during exposure to 35°C (Figure 3), it can be inferred that no regulatory sweating occurred.

## Appendix 1 Accuracy of the measuring instruments

**Table S1 Specifications of measuring instruments and manufacturer data.** The CO<sub>2</sub> sensors were calibrated prior to experiments. The concentration of the calibration gas was 5,000 ppm. Temperature in the chamber was controlled by the calibrated Pt100 sensor. This sensor has not been calibrated before experiments but is regularly checked for accuracy. The other instruments were calibrated by their manufacturers.

Parameter	Instrument	Range	Accuracy
Air temperature	HOBO data logger	-20~70°C	±0.7°C
Relative humidity	HOBO data logger	0~95%	±5%
Light intensity	HOBO data logger	0.1~200000lux	±1 in last digit
CO <sub>2</sub> concentration	VAISALA	0~5000ppm	±(2.5% of range+3% of reading)
Arterial blood oxygen saturation	LifeSense monitor	0~100%	±2%
Blood pressure	Beurer BM 35	40~280mmHg	±3mmHg
Body weight scale	KERN DE 150K20D	0.02~150 kg	0.01kg
Eardrum temperature	Braun ThermoScan	34~42.2°C	±0.2°C
End-tidal partial CO <sub>2</sub>	LifeSense monitor	0~9.9kPa	±0.2kPa+6% of reading
Heart rate	Sunnto Dual belt	-	-
Skin temperature	iButton	-40~85°C	±0.125°C

## **Appendix 2 Description of measurements performed during experiments**

### Physiological parameters

Among the measured physiological parameters, the measurements of ET<sub>CO<sub>2</sub></sub>, SpO<sub>2</sub> and biomarkers in saliva provided new information on physiological responses at the high temperature, which were not measured in the previous studies.

*Skin temperature* was measured at six sites of human body using information buttons (iButton, USA) closely attached to the skin by the medical adhesive plaster. The measuring sites on the skin of human body included left chest, right upper arm, left forearm, left hand, anterior thigh and anterior calf.

*Eardrum temperature* was measured with the ear thermometer (Braun ThermoScan, GER). The eardrum temperature in the right ear was measured two times by the experimenter and the higher value was recorded if the reading was different. Before the measurement, every subject had been asked to clean his/her ear.

*Heart rate* was measured using the heart rate monitor. It consisted of a chest strap with electrodes (Sunnto Dual, FI). The heartbeats (bpm) and inter-beat intervals (msec) were continuously monitored and the data was transferred automatically to the computer for subsequent analysis.

*Blood pressure* was measured with the blood pressure monitor (Beurer BM 35, GER). The instrument is used for a non-invasive measurement and monitoring of arterial blood pressure (systolic and diastolic pressure) in adults. A cuff is connected by a hose with the instrument. For the measurement of blood pressure, the cuff was placed on the left upper arm so that the lower edge was 2 to 3 cm above the bend of the elbow and above the artery, and then it was fit around the bare left upper arm snugly, but not too tightly. During the measurement, the subject kept a correct sitting posture. The blood pressure was measured once by the subjects

and the reading was recorded by the experimenter.

*End-tidal partial CO<sub>2</sub> (ETCO<sub>2</sub>)* concentration was measured with a non-invasive capnographic monitor (LifeSense LS1, SWE). The measurement of ETCO<sub>2</sub> reflects the CO<sub>2</sub> concentration at the end of expiration and can be used to approximate arterial CO<sub>2</sub> noninvasively. The instrument also provided the measurement of *respiration rate* and *arterial blood oxygen saturation (SPO<sub>2</sub>)*, the latter using pulse oximetry, with a special finger probe attached to forefinger.

*Body weight* was measured using a high precision scale (KERN DE 150K20D, GER) to estimate body weight loss during exposure adjusted for the increment of liquids consumed during exposure (if any).

*Biomarkers in saliva (alpha-amylase and cortisol)* were measured with a non-stimulated passive drool salivary sampling procedure. Subjects were not allowed to drink for at least half an hour prior to the collection of saliva. During sampling, they accumulated and expelled saliva into a labeled sampling tube to provide a sample of about 4 ml. This took them about 5 to 10 minutes. The samples were then centrifuged for 15 min and stored in a fridge at the temperature lower than -20 °C. After 1 hour, the samples were centrifuged for 15 min again. They were then frozen and stored in the fridge at the temperature lower than -20 °C until analysis was performed by the external certified laboratory. Amylase assay was performed with Integra 400 plus (Roche Diagnostics Ltd.). Amylase samples were diluted 201 times before analysis to reduce the amylase level in saliva as the applied method was not capable for analyses of the levels as high as occurring in saliva. After dilution, the amylase level was determined and then the dilution factor applied to estimate the actual concentration in the saliva. Repeated analyses on the same samples using the dilution method described above returned similar results. The detection limit was 3 U/L while the analytical error of measurement was 5.7%, as provided by the external laboratory performing analyses. Cortisol

assay was performed with Cobas 6000/e601 (Roche Diagnostics Ltd.). The detection limit was 0.018 ug/dl while the analytical error of the measurement was 11.7%, as provided by the external laboratory performing analysis.

### Subjective ratings

Compared with the existing studies, this study used longer list of possible effects experienced by subjects. Following assessments were made by the subjects during exposure using specially designed questionnaire; the coding of the scales was not seen by the subjects and was used by experimenters when transferring the ratings from the scale into electronic databases:

- (1) Thermal sensation (TS) was evaluated using ASHRAE 7-point continuous scale coded as follows (reference): cold (-3), cool (-2), slightly cool (-1), neutral (0), slightly warm (1), warm (2) and hot (3).
- (2) Thermal comfort (TC) was assessed using a continuous scale broken in the middle point and coded as follows: very comfortable (2), comfortable (1), just comfortable (0.01) / just uncomfortable (-0.01), uncomfortable (-1) and very uncomfortable (-2).
- (3) Acceptability of thermal environment (TA) was assessed using the continuous scale coded as follows: clearly acceptable (1) and clearly unacceptable (-1) at the endpoints with the break point just acceptable (0.01) / just unacceptable (-0.01) in the middle.
- (4) Acceptability of air quality (PAQ) was assessed using the continuous scale coded as follows: clearly acceptable (1) and clearly unacceptable (-1) in the end points with break point just acceptable (0.01) / just unacceptable (-0.01) in the middle.
- (5) Odour intensity (OI) was assessed using the continuous scale coded as follows: no odour (0), slight odour (1), moderate odour (2), strong odour (3), very strong odour (4) and overwhelming overpowering odour (5).

- (6) Sleepiness (SLP) was evaluated on a continuous scale with nine points coded as follows: very sleepy (-4), sleepy, some effort to stay awake (-3), sleepy, no effort to stay awake (-2), some signs of sleepiness (-1), neither alert nor sleepy (0), rather alert (1), alert (2), very alert (3) and extremely alert (4).
- (7) Self-estimated work performance (SEP) was assessed on a continuous scale with end points marked as poor (0) and excellent (100).
- (8) Intensity of acute health symptoms (AHS) was assessed on a continuous scale with clearly marked endpoints (so called visual analogue scale) coded as follows: 0 (low intensity) and 100 (high intensity). They included the following symptoms: nose [running (0) – dry(100)], throat [not dry (0) – dry (100)], eyes [not aching (0) – aching (100)], eyes [not dry (0) – dry (100)], skin [not dry (0) – dry (100)], headache [no (0) – severe (100)], concentration [easy (0) – hard (100)], thinking [clear (0) – difficult (100)], wellbeing [good (0) – bad (100)], mood [positive (0) – depressed (100)], fatigue [rested (0) – tired (100)] and dizzy [not dizzy (0) – dizzy (100)].

#### Performance tests

During the exposure, the subjects performed different cognitive tests. Seven computerized tests were presented to subjects in the following order: mental redirection (a spatial orientation test) with 160 trials, grammatical reasoning (a logic reasoning task) with 30 trials, digit span memory (a test of verbal working memory and attention) with 12 trials, visual learning memory (a picture memory task measuring spatial working memory) with 4 trials, number calculation (one digit addition & subtraction: “number 1 + number 2 - number 3”) with 60 trials, and one digit multiplication (“number 1 \* number 2 \* number 3”) with 10 trials, Stroop (a test of attentional vitality and flexibility owing to perceptual/linguistic interference)

with 80 trials, and visual reaction time (a sustained attention task measuring response speed and accuracy to visual signals) with 200 trials. All the tests were presented to subjects on a PC and no feedback on their performance was given. Stroop test (80 trials) and number calculation (60 trials) were also presented with feedback on the performance: the subjects could not continue these tests until they corrected the errors. The tests were always performed in the same order independently of the condition. Four sets of tests with similar level of difficulty were randomly assigned to subjects. Accuracy (number of error free trials related to the total number of trials performed within each test) and speed (number of trials performed within the dedicated time) or response time (i.e., the time needed to respond to stimulus) were used as measures of performance; for the tests completed with feedback, only speed was used as a measure of performance. It took subjects ca. 40 minutes to complete these tests.

The subjects performed also d2-test used to examine attention and concentration. In this test, the subjects had to find and cancel out all target d2 characters ( “d” character with a total of two dashes placed above and/or below the character). The target d2 characters were placed among the nontarget characters (“d” characters with more or less than two dashes, and “p” characters with any number of dashes). Fourteen successive trials were made each lasting 20 seconds. In each trial, the subjects had to find 21-22 d2 target characters in the string of 47 characters. d2 test was presented to subjects on paper. Total number of characters processed, errors of omission (missed d2 characters), errors of commission (false positives), total number of errors, total number of characters minus total number of errors and concentration performance i.e. total number of correctly identified d2 characters minus errors of commission were used to examine the performance of d2 test.

Subjects performed also Tsai-Partington test. Twenty random numbers were randomly distributed on the paper. The task was to connect numbers in ascending sequence, beginning from the “start”. The time for completion of the test was set to 60 s. It was not possible to

complete the task in the indicated time. The number of correct links and errors were used as a measure of performance.

### Appendix 3 The results of performance

**Table S2 Performance (mean  $\pm$  SD) of the cognitive tests at different experimental conditions**

No.	Experiment condition	T26	T35	T35C3000
1	Mental Redirection			
	Accuracy (%)	97.2 $\pm$ 2.4	96.8 $\pm$ 3.5	97.2 $\pm$ 2.8
	Speed (Units/min)	48.5 $\pm$ 11.5	49.1 $\pm$ 12.6	50.0 $\pm$ 10.6
2	Grammatical Reasoning			
	Accuracy (%)	94.1 $\pm$ 4.8	91.8 $\pm$ 10.6	91.9 $\pm$ 9.2
	Speed (Units/min)	8.3 $\pm$ 2.2	9.1 $\pm$ 1.9	8.2 $\pm$ 0.8
3	Stroop			
	Accuracy (%)	98.1 $\pm$ 2.0	97.8 $\pm$ 4.0	97.9 $\pm$ 2.4
	Speed (Units/min)	24.0 $\pm$ 4.7	23.3 $\pm$ 5.6	23.1 $\pm$ 4.2
4	Addition & Subtract			
	Accuracy (%)	<b>98.9<math>\pm</math>0.9</b>	<b>97.3<math>\pm</math>1.7*</b>	97.8 $\pm$ 2.0
	Speed (Units/min)	21.1 $\pm$ 2.6	20.2 $\pm$ 3.0	19.7 $\pm$ 3.3
5	Multiplication			
	Accuracy (%)	93.3 $\pm$ 6.5	92.5 $\pm$ 5.4	90.8 $\pm$ 6.7
	Speed (Units/min)	5.7 $\pm$ 2.1	6.1 $\pm$ 1.9	5.7 $\pm$ 1.8
6	Visual Reaction Time			
	Accuracy (%)	97.9 $\pm$ 1.6	97.6 $\pm$ 1.8	98.1 $\pm$ 1.5
	Speed (Units/min)	92.9 $\pm$ 15.7	94.5 $\pm$ 15.7	93.4 $\pm$ 17.5
7	Visual Learning Memory			
	Accuracy (%)	89.3 $\pm$ 10.2	89.2 $\pm$ 10.0	87.0 $\pm$ 11.6
	Response time (s)	90.5 $\pm$ 14.4	90.2 $\pm$ 14.0	91.2 $\pm$ 14.9
8	Digit Span Memory			
	Span	9.90 $\pm$ 2.2	9.92 $\pm$ 2.1	9.90 $\pm$ 2.1
9	Stroop with feedback			
	Speed (Units/min)	27.6 $\pm$ 5.0	27.9 $\pm$ 3.8	27.0 $\pm$ 5.0
10	Calculation with feedback			
	Speed (Units/min)	15.3 $\pm$ 2.2	14.8 $\pm$ 2.3	15.0 $\pm$ 2.1
11	d2 test			
	Total processed (Units)	644 $\pm$ 18	634 $\pm$ 31	643 $\pm$ 22
	Accuracy (%)	97.1 $\pm$ 1.6	97.0 $\pm$ 1.3	97.2 $\pm$ 1.1
12	Tsai-Partington			
	Correct links (Units/min)	13.6 $\pm$ 3.7	13.8 $\pm$ 4.0	13.9 $\pm$ 3.9
	Error links (Units/min)	1.7 $\pm$ 1.9	1.3 $\pm$ 1.5	1.6 $\pm$ 1.9

Speed or response time was adjusted considering the learning effect.

Bolded numbers indicate the pairs of responses that were statistically significantly different.

\* (p<0.05)