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SHORT COMMUNICATION

Effects of adaptation to handling on the circadian rhythmicity of blood solutes in *Mongolian gerbils*

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Abstract

The *Mongolian gerbil* has been widely used in many research fields and has been reported to be a diurnal laboratory animal. The circadian rhythmicity of these gerbils was investigated in the present study by measuring two hormones that show daily oscillations, cortisol and ACTH, in serum using ELISA kits. The levels of the two hormones were highest at 8:00 AM and their rhythmic changes were similar to those in humans. In addition, the influence of stress of handling and blood collection on the physiological parameters of the gerbils was examined. After adaptation to handling for 1 week, some serum parameters in the animals changed. Handling and blood collection did not impact significantly on the following parameters: creatine kinase (CK), lactate dehydrogenase (LD), alanine aminotransferase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), and albumin (ALB). However, blood glucose (GLU), total protein (TP) and globulin (GLB) significantly increased while creatinine (CRE) and albumin/globulin (A/G) significantly decreased after adaptation. This work further confirms that the *Mongolian gerbil* is a diurnal animal and also indicates that a suitable adaptation procedure is necessary for getting reliable results when performing experiments using these animals.

KEYWORDS
circadian rhythmicity, diurnal animals, handling adaptation, *Mongolian gerbils*, physiological parameters, stress

1 | INTRODUCTION

Circadian rhythmicity exists in almost every biological process,[1] and many animals display obvious differences in physiology between day and night. Unfortunately, the animals used most widely laboratory in biomedical research—rats, mice, guinea pigs, rabbits, etc.—are nocturnal (active at night).[2,3] As we all know, humans are diurnal (active during the day), so researchers should consider the importance of time of day in their experimental procedure designs,[4] and a diurnal animal model would be more suitable for some experiments than the traditional nocturnal models.

The *Mongolian gerbil* has been widely used as laboratory animal for about 80 years.[5] It has been called a multi-function laboratory animal.[6-9] The first report to record the gerbil as a diurnal animal appeared in 1983.[10] Subsequent reports confirmed that gerbils are diurnal, and they have also been identified as ideal laboratory

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animals in neurological and pathological research.[11,12] However, more recent studies have indicated that the activity rhythms of the gerbil are weaker than those of other two diurnal animals, the antelope ground squirrel and the African grass rat.[3] Thus, there is a need to confirm the suitability of the gerbil as a diurnal laboratory animal model.

Circadian rhythm research routinely uses hormones in serum as biomarkers for activity cycles. For instance, two hormones, cortisol and adrenocorticotropic hormone (ACTH), have been shown to be reliable indicators of circadian rhythmicity in many studies.[13-15] Thus, in the current study, we tested these two hormones to verify the circadian rhythmicity of gerbils and to see if it is similar to human rhythms.

There are many factors such as temperature, humidity, light intensity, noise, handling, cage movement, dietary schedules and transportation that may affect experimental results by producing stress in laboratory animals.[16,17] If the animals have not adapted to the new environment at the procedure of experiment, their physiological state may not have returned to normal, and the biomedical research results obtained may be influenced by even quite small physiological changes.[18,19] Handling and blood collection are two of the commonest operations in animal experiments and may influence physiological parameters for a period of time during the experiment. However, it is impossible to establish a completely stress-free environment and so avoid such perturbations. It has been reported that some kinds of stresses, such as transportation stress, can be decreased by acclimatization,[20,21] but there are few reports describing whether handling and blood collection stress significantly affects the results during animal experiments. Therefore, we hypothesized that training the animals, by handling them prior to blood collection several times during the days before the experiments, would enable them to adapt to the experimental environment and minimize the influence of the stress, while also improving the reliability of the research results and the animals' welfare.[22] In the present study, we tested whether the gerbils can adapt to such training by handling them every day for a week and measuring the serum parameters before and after adaptation.

2 | METHODS

2.1 | Animals

The study was carried out on a total of 10 adult inbred gerbils (five females, five males), aged around 3.5 months with body weights of 55-65 g. All animals were provided by the Experimental Animal Center at Capital Medical University and maintained in controlled conditions, with a room temperature of 22 ± 2°C, humidity of 55 ± 10%, and a 12 hour light/dark cycle. The gerbils were allowed ad libitum access to food and water and were housed in this environment for 1 month prior to the experiments. All experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee of Capital Medical University (Permit No. AEEI-2016-154).

2.2 | Sample collection before and after handling

After 14 hours fasting, all gerbils were handled and blood was collected from the orbital venous plexus by capillary tube after topical anesthesia using lidocaine hydrochloride (0.5%) applied to the canthus, firstly at 08:00, then at 16:00 and 24:00, making a total of three time points. After this first collection of blood samples, the gerbils were handled every morning for 1 minute for 7 days. Then the blood samples were collected again using exactly the same procedures as previously, including manipulation by the same technicians at the same time points, using the same materials. All the blood samples were collected in capillary tubes, with about 200 μL blood being collected at each time point. The serum was extracted by centrifugation at 3000 g for 20 minutes at 4°C and stored at −80°C until analysis.

2.3 | Blood parameter measurements

Before the measurements, the serum was diluted twice with normal saline as required for the tests. The cortisol and ACTH levels in serum were determined using a commercial ELISA kit (Mouse Cortisol Elisa kit and Mouse ACTH Elisa kit, Mecen Unicreate Biotech Corporation, Ltd) according to the manufacturer's instructions. The other 11 biomedical parameters—blood glucose (GLU), total protein (TP), globulin (GLB), creatinine (CRE), creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), albumin (ALB), and albumin/globulin (A/G)—were analyzed using Synchron cx5 (Beckman, USA) and MEK-7222K (Nihon Kohden, Japan) analyzers at the Youanmen Clinical Testing Center of Capital Medical University. All measurement data were doubled because the serum samples had already been diluted twice.

2.4 | Statistics

The data are presented as means ± standard error of the mean (SEM). Comparisons to determine the impact of adaptation and the two hormone levels were made using an independent *t* test. Statistical analysis was performed with SPSS® software or Graph Pad Prism® 5.00 software. Differences were considered significant when the *P* value was less than 0.05.

3 | RESULTS

To confirm that the gerbil is a diurnal animal, we tested the circulating cortisol and ACTH levels at three time points: 08:00, 16:00, and 24:00. The results showed that before adaptation, the cortisol level was much lower at 08:00 than at 16:00 and 24:00, whereas ACTH levels exhibited no statistical differences among the three time points although the level was higher at 08:00 than at other two time points (Figure 1). However, after handling adaptation for a week, the cortisol level in the serum varied significantly. The level was highest

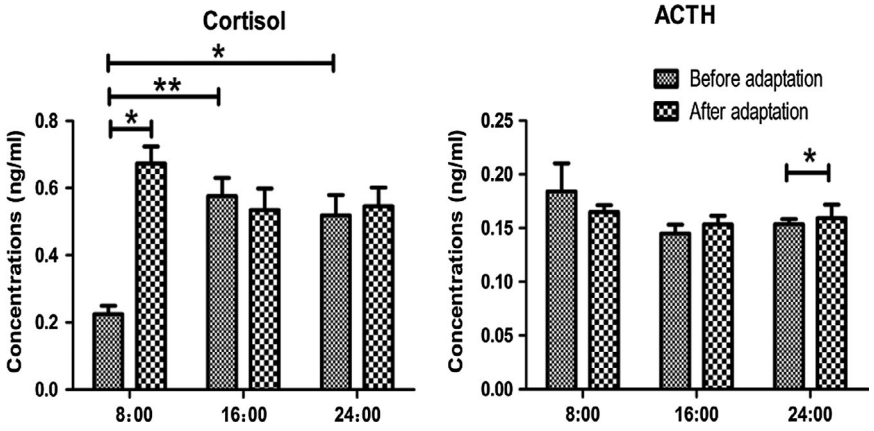


FIGURE 1 Serum cortisol and ACTH concentrations (ng/ml) measured before and after adaptation. Serum cortisol concentration was significantly increased at 08:00 after adaptation compared to the level before adaptation. There were significant differences in cortisol levels among the three time points—08:00, 16:00, and 24:00—before adaptation. Plasma ACTH concentration was significantly increased at 24:00 after adaptation. Both hormones displayed a downward trend after adaptation. *$P < 0.05$, **$P < 0.01$

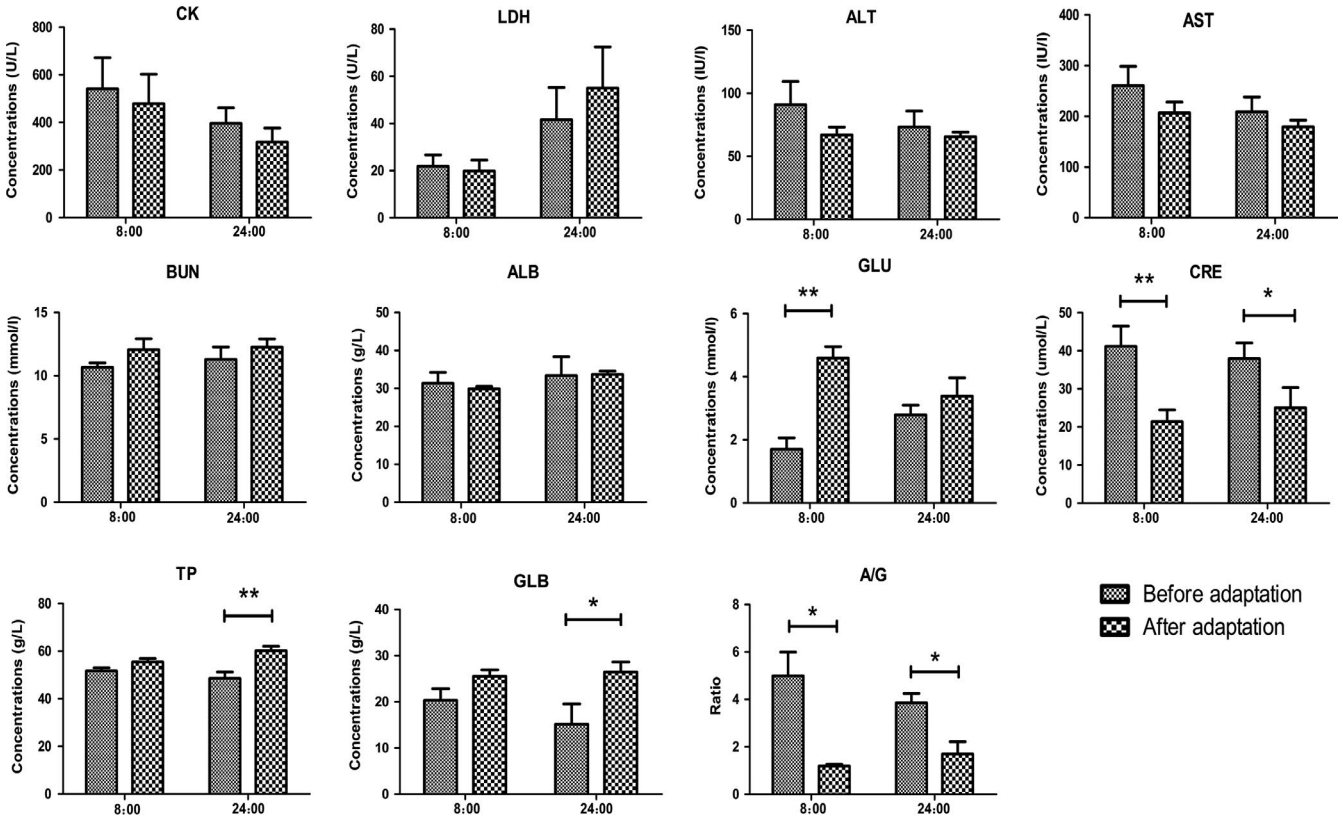


FIGURE 2 Effects of handling adaptation on the levels of CK, LDH, ALT, AST, BUN, ALB, GLU, CRE, TP, GLB, and A/G in the serum of gerbils. Circulating GLU (at 08:00, $P < 0.01$), TP (at 24:00, $P < 0.01$) and GLB (at 24:00, $P < 0.05$) levels showed significant increases after adaptation compared to before adaptation. Circulating CRE (at 08:00, $P < 0.01$; at 24:00, $P < 0.05$) and A/G (at 08:00, $P < 0.05$; at 24:00, $P < 0.05$) levels showed significant decreases after adaptation compared to before adaptation. There were no obvious changes in the levels of CK, LD, ALT, AST, BUN, and ALB at 08:00 and 24:00, or in GLU at 24:00 and TP and GLB at 08:00. *$P < 0.05$, **$P < 0.01$

at 08:00 and lower at the other two time points, and was significantly different from the values before adaptation. ACTH concentrations were significantly increased at 24:00 after adaptation compared to before. Overall, the two hormones showed a downward trend from 08:00 to 24:00 across the three time points.

To investigate the effect of handling training on biochemical parameters, we tested a total of 11 parameters: GLU, TP, GLB, CRE, CK, LDH, ALT, AST, BUN, ALB, and A/G. The results demonstrated that circulating GLU (at 08:00), TP (at 24:00), and GLB (at 24:00) levels showed significant upregulation after handling adaptation compared

to the values before adaptation. Circulating CRE (at 08:00 and 24:00) and A/G (at 08:00 and 24:00) levels showed significant declines after adaptation compared to before adaptation. In contrast, no significant changes were observed in the levels of other biochemical parameters: CK, LDH, ALT, AST, BUN, ALB (at 08:00 and 24:00), GLU (at 24:00) and TP, GLB (at 08:00) (Figure 2).

4 | DISCUSSION

Our results show, for the first time, that the *Mongolian gerbil* has a similar circadian rhythm to human beings, as demonstrated by the variation over 24 hours in the levels of the two hormones cortisol and ACTH. Secondly, the gerbil has the capacity to adapt to handling and blood collection after training for 1 week.

The circadian rhythm plays an important role in many diseases such as obesity,[23,24] diabetes,[25] cardiovascular disease,[26] etc. Thus, a diurnal animal would be a more suitable and reliable animal model in which to study these diseases. Use of such models may provide better confirmation of proposals for treatments.[27] Our current results testing the dynamics of two hormones showed that the *Mongolian gerbils*' circadian rhythm is much like that of human beings. Although the only significant differences in circulating cortisol and ACTH levels after adaptation were between cortisol levels at 08:00 and ACTH levels at 24:00, they both showed downward trends from 08:00 to 24:00 across the three time points, with the highest level at 08:00. The cortisol concentration was lower at 08:00 before adaptation to handling, which might be the result of stress. In human beings, circulating ACTH and cortisol levels show a circadian rhythm, with hormone levels low in the late evening, reaching a minimum after sleep, and rising to a maximum in the morning.[27,28] Therefore, the circadian rhythm of *Mongolian gerbils* is more similar to human beings than that of rats and mice. Although, the degree of diurnality of the *Mongolian gerbil* is not as marked as that of the antelope ground squirrel and the African grass rat, the gerbil has been domesticated by many laboratories and is easier to access, and thus it is a more suitable animal model for research into circadian rhythm in the future.

Our results showed that handling and blood collection is stressful to animals and a week of handling adaptation could minimize the influence of stress. Adaptation to training by handling significantly affects serum parameters in gerbils. Cortisol is secreted by adrenal glands via the hypothalamic-pituitary-adrenocortical axis system and has been shown in many studies to be a good biomarker of stress,[29] but it has not been tested as a stress marker in the *Mongolian gerbils* yet. We show here that stress of handling can also affect the serum levels of other compounds, increasing CRE and A/G, and decreasing GLU, TP, and GLB, while having almost no influence on CK, LDH, ALT, AST, BUN, and ALB serum levels. Our results therefore show that the influence of handling and blood collection stress on CK, LDH, ALT, AST, BUN, and ALB serum levels can be ignored, but more attention should be paid to the effects on the serum parameters which changed after adaptation in relevant animal experiments because they are indicators of many biochemical reactions. For instance, CRE is a metabolic parameter produced by muscle and is a biomarker of renal function and is freely filtered in kidneys.[30,31] A/G is a biomarker of diseases in which would be changed in serum, especially liver disease,[32] and TP and GLB are also involved in immunity and related diseases.[33] Serum GLU level is an indicator of metabolic diseases such as diabetes or obesity, which is regulated by hormones such as pineal hormone and insulin.[34] Besides the biochemical indicators we investigated, other hormones such as luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels have also been found to increase with stress from handling.[35] The serum parameters we tested are just some of blood indicators and further investigation of the influence of handling and blood collection on other serum parameters is needed.

In conclusion, researchers should consider the effects of stress, particularly in the measurement of blood parameters, and should be aware of the physiological changes caused by handling and how these may influence the results. Rodents such as *Mongolian gerbils* may have the ability to adapt to handling. We recommend that training such as adaptation to handling is a good way to minimize the influence of stress before animal experiments.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

All listed authors meet the requirements for authorship. XYD and ZWC conceived and designed the experiments. XCL, XZ and YHL performed the experiments and analyzed the data. XCL, XZ and XYD wrote the manuscript text. All authors read and approved the manuscript.

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