Tupaia belangeri (Wagner, 1841), a Northern Treeshrew is an Animal Model of Metabolic Healthy Obesity

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ABSTRACT

Obesity has become a harmful factor for life expectancy through a series of diseases, but research on obesity in the past few decades has obtained only limited results. The discovery of the metabolic healthy obesity phenomenon brings a new direction for research of obesity problem, but the lack of an animal of metabolic healthy obesity limits its study. *Tupaia belangeri* is a new type of experimental animal emerging in recent years and is extremely widely used in various disease models because of its evolutionary status and high affinity with primates. Here, in order to judge whether this new experimental animal can serve as special materials in obesity research, we constructed an obesity model in *T. belangeri* by using high-fat food, then studied animal insulin sensitivity, blood pressure, blood biochemistry, routine blood, inflammatory response, and liver fat accumulation, etc. We found that *T. belangeri* had no metabolic disorders such as insulin resistance, hypertension, or hyperlipidemia after obesity, and there were also no typical obesity complications such as diabetes, inflammation, or fatty liver. To sum up, we suggest that *T. belangeri* can be used as an animal model of metabolic healthy obesity, and the special model organism of metabolic healthy obesity will provide us with new opportunities to study obesity problems.

INTRODUCTION

Obesity is associated with chronic diseases such as insulin resistance (Kahn *et al*., 2006), hypertension (Gruber *et al*., 2021), hyperlipidemia, and diabete (Pillon *et al*., 2021). Obesity has also been linked to fatty liver (Xie *et al*., 2017), heart disease, and cancer (Kivimäki *et al*., 2017). With the increasing global prevalence of obesity, it has become an important factor endangering human health and reducing life expectancy, and its harm is expected to deepen in the future

(Afshin *et al*., 2017; Kelly *et al*., 2008). Humans have made various attempts to solve the obesity problem (Cefalu *et al*., 2015), such as fasting (Ulgherait *et al*., 2021), exercise, and the administration of anti-feeding factor leptin (Duquenne *et al*., 2021; Montague *et al*., 1997). However, in the end, it has been found that the majority of dieters regained weight over the long-term, obesity rebounded very quickly after exercise stopped (Van Baak and Mariman, 2019), and exogenous leptin administration rarely worked *in vivo* (Blüher and Mantzoros, 2009; Myers *et al*., 2010). Although we have been working on this for decades, this problem has not been effectively solved (Roberto *et al*., 2015).

The discovery of the metabolic healthy obesity phenomenon brings new direction for research of obesity problem. The concept of healthy obesity was first conceived in the 1980s, and the first case of metabolic healthy obesity was found in the same year (Andres, 1980). As the phenomenon of metabolic healthy obesity has been widely reported, the discussion about healthy obesity has become popular. In recent researches, healthy obesity is that an

individual still remains in a good health state despite being obese, which have no insulin resistance, hypertension, diabetes, dyslipidemia, fatty liver, etc. (Wildman, 2009; Bluher, 2012; Stefan *et al*., 2013). In addition, researches also showed that the healthy obesity phenomenon is fairly prevalent in the obese population (Lavie *et al*., 2018; Lin *et al*., 2017), so further research on healthy obesity also has broad application prospects. In short, these data all indicate that metabolic healthy obesity has great potential in the diagnosis, treatment, and research of obesity (Phillips, 2016; Stefan *et al*., 2018), but the lack of an animal model of metabolic healthy obesity limits its study.

Tupaia belangeri is a small mammal, originating from Borneo, which is widely distributed in the Oriental realm (Roberts *et al*., 2011; Sargis *et al*., 2014). *T. belangeri* belongs to the genus *Tupaia* of the family Tupaiidae of the order Scandentia in China. It has many characteristics, such as small body size, high brain-to-body mass ratio, and low feeding cost (Zhang *et al*., 2020). Compared with rodents, *T. belangeri* has a higher affinity with humans (Janečka Jan *et al*., 2007; Roberts *et al*., 2009). So, under the general trend of increasingly limited research use of primates, it shows great development and application prospects (Shi *et al*., 2020). Accordingly, it has been rapidly developed into a new type of experimental animal and used as a model organism to study basic biology and various medical disease models (Ruan *et al*., 2016; Che *et al*., 2021; Zhang *et al*., 2019), all of which suggest that *T. belangeri* has great potential for human disease research. *T. belangeri* might be potential as an animal model of healthy obese. In the present study, we induced the obesity model of *T. belangeri* using high-fat food. Then, we measured animal glucose tolerance, insulin tolerance, blood biochemical indexes, inflammatory markers, and liver fat accumulation, etc. We predicted that animals did not show metabolic disorders and typical obesity complications. Moreover, the masses of heart, liver, kidney, and other organs of the obese animals still remained normal.

MATERIALS AND METHODS

Samples

Twenty-eight male *T. belangeri* (2 years old) animals were purchased from the Institute of Medical Biology, Chinese Academy of Medical Sciences (Kunming, China). Animals were housed at $25 \pm 1°C$, 12L:12D (lights on at 7:00 am), 50-60% humidity, and 250 lx illumination, and were housed in single cages, food and water were provided *ad libitum*. All animals were adapted for 2 weeks before the start of the experiment, and then randomly divided into control (n=14) and experimental (n=14) groups. The control group was fed standard chow (fat 24%, protein 32%, carbohydrate 44%), and the experimental group was fed a high-fat diet (fat 57%, protein 21%, carbohydrate 22%).

Glucose tolerance test and insulin tolerance test

For the glucose tolerance test (GTT), animals were intraperitoneally injected with glucose at a dose of 2g/kg after 8 h of fasting. After injection, venous blood was taken at 0, 15, 30, 60, 90, and 120 min, and the blood glucose concentration was measured using a blood glucose meter (SinocareGA-3; Sinocare, Changsha, China). For insulin tolerance testing (ITT), animals were injected with insulin (Novolin R; Novo Nordisk, Bagsvaerd, Denmark) at a dose of 0.75 U/kg after 8 h of fasting, and the blood glucose concentration was measured at 0, 15, 30, 60, 90, and 120 min.

Blood pressure, heart rate, and temperature measurement

We used the tail-cuff method (Erken *et al*., 2013) to measure blood pressure and heart rate (BP-2000 Blood Pressure Analysis System; Visitech Systems, Apex, North Carolina). Animals were trained to adapt to the procedure several times before the measurement. During formal measurement, animals were allowed to adapt to the test room for 30 min, then the sensor was sleeved onto the tail of the animals. The blood flow signal was monitored by inflating and deflating the tail artery to measure the blood pressure and heart rate, and the average value was taken for three consecutive measurements. In addition, temperature is also the core index reflecting the physiological state. To determine whether the surface temperature distribution and nuclear temperature change after obesity, we imaged animals with an infrared imager (WIC640-SUW; Workswell, Roznov, Czech) to obtain the body surface temperature map (shooting distance: 1m) (Tattersall and Cadena, 2010). We measured the core temperature with a digital thermometer (inserted the probe into the anus approximately 2 cm and remained for 1 min to read).

Blood biochemistry and routine blood analysis

Blood was collected from the heart after fasting for 12h. 1/3 of the blood was anticoagulated with EDTA-K2, while 2/3 was placed at room temperature for 30 min, then centrifuged at 4000g, 4°C for 10 min to obtain serum. An automatic biochemical analyzer (Cobas 8000 C702; Roche, Barthel, Switzerland) measured serum total cholesterol, triglyceride, high density lipoprotein cholesterol, low density lipoprotein cholesterol, glucose, fructose amine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, cholinesterase, glutaminase aminotransferase, total protein, albumin, globulin, total bilirubin, total bile acid, creatine kinase,

lactate dehydrogenase, uric acid, urea nitrogen, and creatinine. Glycosylated hemoglobin level were measured using an automatic glycosylated hemoglobin analyzer (HLC-723GX; TOSOH, Yamaguchi, Japan) (Lahousen *et al*., 2002), and insulin and fasting C-peptide were detected using an automatic electrochemiluminescence analyzer (Cobas E601; Roche, Barthel, Switzerland). Routine blood tests were performed using an automatic blood cell counter (MEK-5105; NIHON KOHDEN, Tokyo, Japan), and the detection indexes included red blood cell count, white blood cell count, hemoglobin, and platelet count.

Enzyme-linked immunosorbent assay (ELISA)

Serum was obtained as described above, then the concentration level of CRP in the serum was measured using an ELISA kit (RayBiotech, Peachtree Corners, Georgia, USA), and α1-antitrypsin concentration level was detected with α1-antitrypsin kit (RayBiotech, Peachtree Corners, Georgia, USA).

Measurement of anatomical organs

To evaluate whether obesity affects the internal organs of *T. belangeri*, we used the cervical dislocation method and dissected the animals immediately. Then, the weights of the heart, kidney, large intestine, liver, lung, small intestine, spleen, stomach, testis, thymus, adrenal gland, pancreas, thyroid, and prostate were determined with an analytical balance.

Liver fat analysis

The liver was obtained after dissection. Liver fat accumulation was preliminarily assessed by weighing the liver and observing the adhesion of adipose tissue on its surface. Then, the triglyceride content in the liver was accurately determined using a triglyceride kit (Triglyceride Reagent T2449; Sigma-Aldrich, St. Louis, Missouri, USA). Specifically, 100 mg of tissue was homogenized in 500μl PBS, mixed with chloroform-methanol (2:1 (v/v)), transferred to the organic phase, air-dried overnight, resuspended in 1% Triton X-100 in absolute ethanol, and accurately quantified using a triglyceride assay kit.

Statistical analysis

All data were statistically analyzed using R language (version 4.1.1), and all data were tested for normality and variance homogeneity before analysis. Specifically, the Shapiro-Wilks test was used to evaluate the normality of data, and the F test was used to evaluate the homogeneity of the square difference of data. Repeated measurement variance analysis was used for the GTT and ITT test data, and the double-tailed t-test was used for other data. Statistical significance was set at $P < 0.05$.
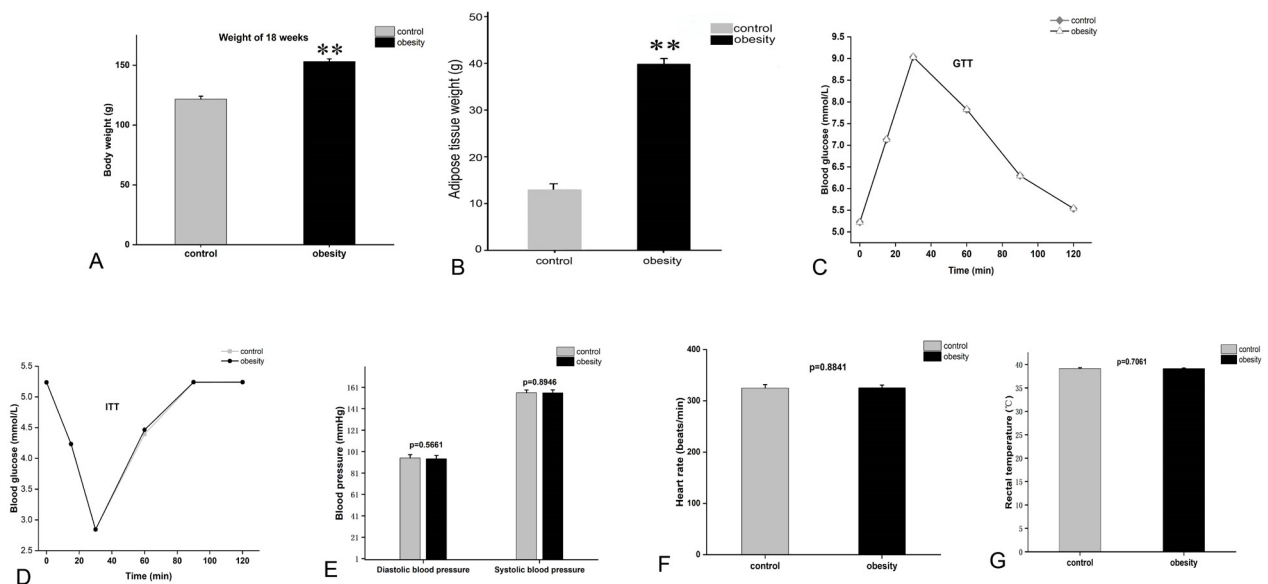

Fig. 1. **A,** Body weight (BW) of control and obesity group *T. belangeri* in 18th week. **B,** Adipose tissue weight of control and obesity group *T. belangeri* in 18th week. **C,** Glycemic response during GTT. **D,** Glycemic response during ITT. **E,** Blood pressure of control and obesity group *T. belangeri*. **F,** Heart rate of control and obesity group *T. belangeri*. **G,** Rectal temperature of control and obesity group *T. belangeri*; N= 14 per group; statistical significance is indicated: *$P < 0.05$, **$P < 0.01$.

RESULTS

T. belangeri *did not show impaired glucose tolerance or insulin resistance after obesity*

At the end of the experiment, the body weight and adipose tissue weight between the control group and experimental obesity group reached extremely significant level (Fig. 1A, B), which showed that the construction of obesity model is successful. Unexpectedly, *T. belangeri* did not suffer from impaired glucose tolerance, and also maintained good insulin sensitivity (Fig. 1C, D). Moreover, the blood glucose of the two groups reached its peak 30 min after glucose injection, and the blood glucose value basically returned to the normal level after 120 minutes (Fig. 1C). Thirty minutes after insulin injection, the blood glucose levels of the two groups reached their lowest levels and returned to normal values 90 min later (Fig. 1D). In addition, through repeated measurement analysis of variance, a significant difference was observed within the intra-group over time ($P < 0.05$), but there was

no significant difference between the groups ($P > 0.05$), indicating that both the control and obese groups responded to GTT and ITT and showed the same trend of change. In conclusion, through GTT and ITT, we confirmed that there was no impaired glucose tolerance or insulin resistance in *T. belangeri* after obesity.

Animal's blood pressure, heart rate, and temperature were not abnormal

The results showed that there was no increase in blood pressure in the obese group (Fig. 1E), and no statistical difference was found in the heart rate analysis between the two groups (Fig. 1F). Therefore, obesity does not lead to hypertension or abnormal heart rate. In addition, body temperature analysis showed that not only did the core temperature not change (Fig. 1G), but the size and distribution gradient of body surface temperature did not change significantly (Supplementary Fig. S1). In general, by analyzing the representative physiological indicators, we found that animal can still maintain their normal state.
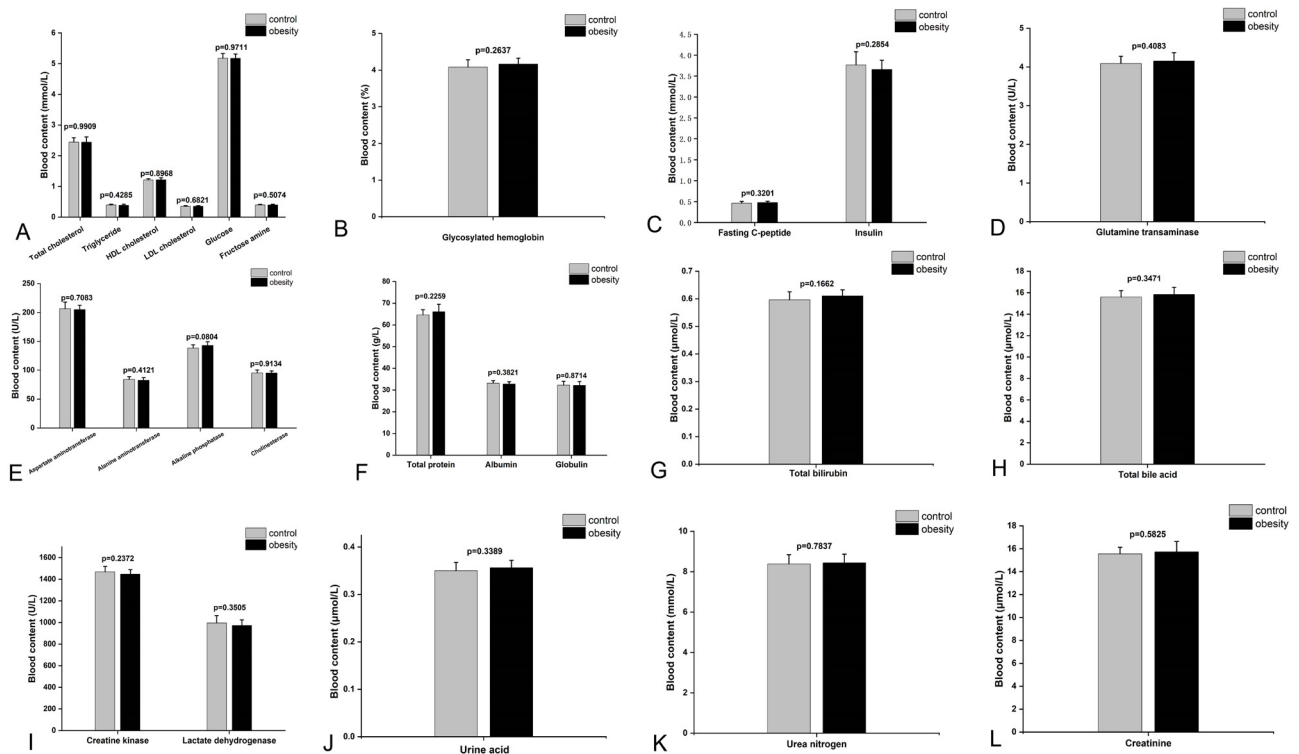

Fig. 2. **A,** Total cholesterol, triglyceride, high density lipoprotein cholesterol, low density lipoprotein cholesterol, glucose, fructose amine levels in blood. **B,** Glycosylated hemoglobin levels in blood. **C,** Fasting c-peptide, insulin levels in blood. **D,** Glutamine transaminase levels in blood. **E,** Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, cholinesterase levels in blood. **F,** Total protein, albumin, globulin levels in blood. **G,** Total bilirubin levels in blood. **H,** Total bile acid levels in blood. **I,** Creatine kinase, lactate dehydrogenase levels in blood. **J,** Urine acid levels in blood. **K,** Urea nitrogen levels in blood. **L,** Creatinine levels in blood. Data are presented as mean±SEM; N= 14 per group; statistical significance is indicated: *$P < 0.05$, ** $P < 0.01$.

Blood biochemistry and routine blood indicators were normal

The blood lipid and blood glucose state were not significantly different (Fig. 2A, C). Testing and statistical analysis showed that aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, cholinesterase, glutamine aminotransferase, total protein, albumin, globulin, total bilirubin and total bile acids, which reflect liver health, did not differ statistically between groups (Fig. 2D, H). There were also no significant differences in creatine kinase and lactate dehydrogenase, indicators of heart health (Fig. 2I). In addition, there were no significant differences in uric acid, urea nitrogen and creatinine. Nitrogen, or creatinine, an important indicator of renal function, was also not significantly different (Fig. 2J, L). Similarly, there were no significant differences in red blood cell count, white blood cell count, haemoglobin or platelet count in the obese group compared to the control group (Fig. 3A, D).

Obesity animal does not show inflammation

The results showed that the CRP level did not increase in the obese group compared to the control group (Fig. 3E). Notably, consistent with the above results, there was no statistical difference in the levels of α1 - antitrypsin between the two groups (Fig. 3F).

Despite obesity, there was no impact on internal organs weight

In order to determine whether obesity has an impact on the internal organs of animals, we dissected the animals immediately after execution and accurately weighed the animal organs with an analytical balance. The analysis revealed that not only the heart, kidney, large intestine, liver, lung, small intestine, spleen, and stomach, but also the testis, an organ reflecting reproductive function, showed no statistical difference between the two groups (Fig. 4A). Interestingly, we also found that the weight of the thymus, an immune organ, did not difference (Fig. 4B), which is consistent with the above conclusion that obesity does not cause inflammation. In addition, we confirmed that the weights of the adrenal gland, pancreas, and thyroid were not altered in obese animals (Fig. 4D, E, H). Finally, we also confirmed that the prostate, an organ associated with urination and reproduction, was unchanged (Fig. 4F). In conclusion, the above results demonstrate that, although the animals become obese, this does not affect the internal organs weight.
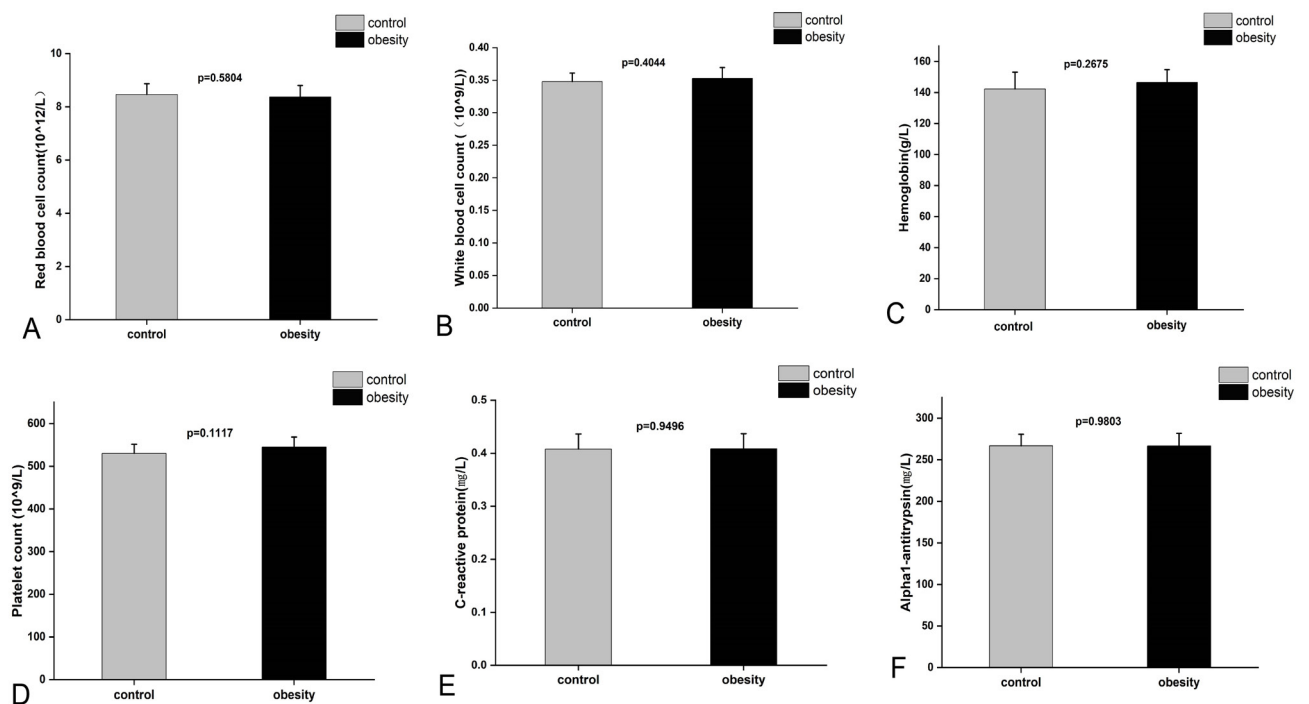

Fig. 3. **A,** Red blood cell count levels in blood. **B,** White blood cell count levels in blood. **C,** Hemoglobin levels in blood. **D,** Platelet levels in blood. **E,** C-reactive protein concentration levels in blood. **F,** Alpha1-antitrypsin concentration levels in blood. Data are presented as mean±SEM; N= 14 per group; statistical significance is indicated: *$P < 0.05$, ** $P < 0.01$.
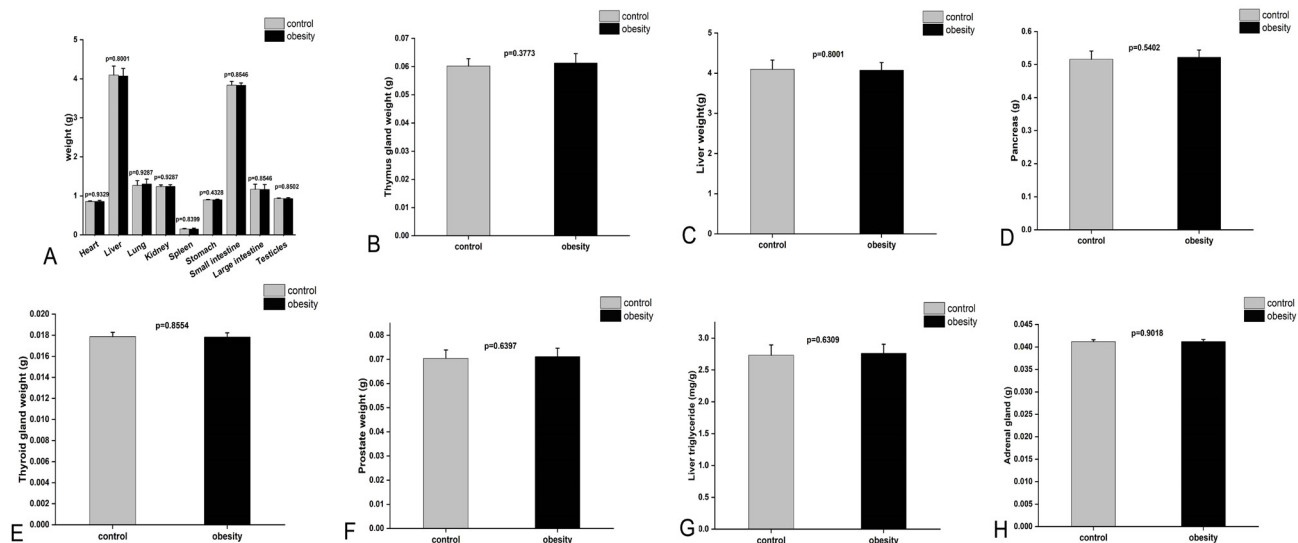
Fig. 4. **A,** Weight of heart, liver, lung, kidney, spleen, stomach, small intestine, large intestine, testis in control and obesity group. **B,** Weight of thymus gland in control and obesity group. **C,** Liver weight of control and obesity group *T. belangeri*, Vertical axis shows mass in grams for BW. **D,** Weight of pancreas in control and obesity group. **E,** Weight of thyroid gland in control and obesity group. **F,** Weight of prostate in control and obesity group. **G,** Liver triglyceride content in control and obesity group *T. belangeri*. **H,** Weight of adrenal gland in control and obesity group. Data are presented as mean ± SEM; N= 14 per group except H; statistical significance is indicated: *$P < 0.05$, **$P < 0.01$.

An obesity animal does not show liver fat accumulation

The results showed that the liver weight of obese animals did not increase (Fig. 4C), there was no fat attachment on the surface of the liver after obesity (Supplementary Fig. S2), and there was also no significant difference in triglyceride content in the liver between the two groups (Supplementary Fig. S2).

DISCUSSION

Because obesity is usually accompanied by impaired glucose tolerance and insulin resistance (Kornfeld *et al*., 2013), we performed GTT and ITT to determine whether these typical symptoms also occurred in obese *T. belangeri*. In the present study, *T. belangeri* did not suffer from impaired glucose tolerance, and also maintained good insulin sensitivity. Blood pressure, heart rate, and body temperature are important indicators to reflect physiological state of the body (Ayres, 2020). In our results, it showed that there was no increase in blood pressure in the obese group, heart rate analysis and body temperature. Therefore, obesity does not lead to hypertension or abnormal heart rate. Previous studies have shown that hyperlipidemia and hyperglycemia are typical complications of obesity (Pillon *et al*., 2021), and that these chronic metabolic diseases seriously affect health and reduce life expectancy (Lamharzi *et al*., 2004). We

found that the blood lipid and blood glucose state were not significantly different, which indicated that animals did not cause hyperlipidemia or hyperglycemia after obesity. Moreover, other blood biochemical and routine blood indicators showed no significant differences in red blood cell count, white blood cell count, haemoglobin or platelet count in the obese group compared to the control group, indicating that the animals remained healthy after obesity from the aspect of routine blood work.

In addition to insulin resistance, hypertension, hyperlipidemia, and hyperglycemia (Kahn *et al*., 2006; Gruber *et al*., 2021; Pillon *et al*., 2021), obesity is often associated with inflammatory reactions (Bapat *et al*., 2022), and CRP is a classic marker of vascular inflammation (Koenig *et al*., 1999). In addition, α1-antitrypsin is also an important inflammatory marker (Teckman *et al*., 1996). It showed that the CRP level did not increase in the obese group compared to the control group, which was consistent with the levels of α1-antitrypsin. Considering the typicality of hepatic fat accumulation in obesity (Xie *et al*., 2017), we analyzed fat accumulation in the liver. It showed that the liver weight of obese animals did not increase, there was no fat attachment on the surface and triglyceride content of the liver after obesity. These results indicate that animal does not accumulate fat in the liver after obesity. In conclusion, through the study of liver weight, fatty tissue adhesion on the liver surface, and triglyceride content in

the liver, we confirmed that the animals did not show liver fat accumulation after obesity.

Based on the above studies, we found that *T. belangeri* can maintain glucose tolerance, blood pressure, blood biochemical, inflammatory markers, organs weight, and liver fat content normal status after obesity. This shows that this animal can maintain metabolic and physiological health after obesity, so it has a great potential in the study of metabolic health obesity as an animal model. Besides, we found that humans (Lavie *et al.*, 2018; Lin *et al.*, 2017) and *Lasiopodomys brandtii* (Liu *et al.*, 2016), a rodent, also have a phenomenon of metabolic healthy obesity, and many other species that have not yet been studied may also maintain a healthy state after obesity. Although the phenomenon of metabolic health obesity exists in many species, whether the internal mechanism leading to this phenomenon is consistent across different species, whether it is all by the same key molecules and the same key pathways, is also a key question to be answered. All in all, healthy obesity may be an opportunity for understanding obesity problems, and it is worthy of further and extensive exploration in this field. In previous obesity research, we obtained relatively limited results when we focused on addressing obesity-related metabolic disorders and various disease consequences (Kivimäki *et al.*, 2017). Therefore, this special animal model provides us with a new direction to study obesity, and also provides us with a new idea to research the current severe obesity problems. With the worsening global prevalence of obesity, it is of practical significance to explore new direction, and this new experimental animal may play an important role in further research.

Although our study revealed those findings, it has some limitations. First, health has many aspects (López-Otín and Kroemer, 2021), and we have mainly measured the metabolic aspect of animals. Whether other aspects, such as tissue, cellular and molecular levels, can also remain healthy require further research. Therefore, metabolic health cannot be equated with complete and overall health. The overall health of the animals needs more comprehensive evaluation. Besides, our experiments are observational and descriptive, and we have not further researched the internal molecular mechanism of the metabolic healthy obesity phenomenon. However, the research on the molecular mechanism of metabolic healthy obesity is the next most important work. Finally, the animals used in this experiment were males, but whether there was consistency in females requires further investigation. In summary, this study demonstrates that *T. belangeri* has a great potential in the study of metabolic health obesity as an animal model. It provides new opportunities and ideas for re-understanding obesity problems.

IRB approval

All animal procedures were compliance with the Animal Care and Use Committee of School of Life Science, Yunnan Normal University.

Ethical statement

This study was approved by the Ethics Committee (No.13-0901-011).

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20230505090553

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Afshin, A., Sur, P., Estep, K., Lee, A., Marczak, L., Moradi-Lakeh, M., Naghavi, M. and Vos, T., 2017. Health effects of overweight and obesity in 195 countries over 25 years. *N. Eng. J. Med.*, **377**: 13-27. https://doi.org/10.1056/NEJMoa1614362

Andres, R., 1980. Effect of obesity on total mortality. *Int. J. Obes.*, **4**: 381-386.

Ayres, J.S., 2020. The biology of physiological health. *Cell*, **181**: 250-269. https://doi.org/10.1016/j.cell.2020.03.036

Bapat, S.P., Whitty, C., Mowery, C.T., Liang, Y., Yoo, A., Jiang, Z., Peters, M.C., Zhang, L.J., Vogel, I., Zhou, C., Nguyen, V.Q., Li, Z., Chang, C., Zhu, W.S., Hastie, A.T., He, H., Ren, X. and Qiu, W., 2022. Obesity alters pathology and treatment response in inflammatory disease. *Nature*, **604**: 337-342. https://doi.org/10.1038/s41586-022-04536-0

Bluher, M., 2012. Are there still healthy obese patients? *Curr. Opin. Endocrinol. Diabetes Obes.*, **19**: 341-346. https://doi.org/10.1097/MED.0b013e328357f0a3

Blüher, S. and Mantzoros, C.S., 2009. Leptin in humans: Lessons from translational research. *Am. J. clin. Nutr.*, **89**: 991S-997S. https://doi.org/10.3945/ajcn.2008.26788E

Cefalu, W.T., Bray, G.A., Home, P.D., Garvey, W.T., Klein, S., Pi-Sunyer, F.X., Hu, F.B., Raz, I., Van, Gaal. L., Wolfe, B.M. and Ryan, D.H., 2015. Advances in the science, treatment, and prevention of the disease of obesity: Reflections from a diabetes care editors' expert forum. *Diabetes Care*, **38**: 1567-1582. https://doi.org/10.2337/dc15-1081

Che, P., Wang, M., Larson-Casey, J.L., Hu, R.H., Cheng, Y.E., Hamdaoui, M., Zhao, X.K., Grytz, R., Brent, Carter. A. and Ding, Q., 2021. A novel tree shrew model of pulmonary fibrosis. *Lab. Investig.*, **101**: 116-124. https://doi.org/10.1038/s41374-020-00476-3

Duquenne, M., Folgueira, C., Bourouh, C., Millet, M., Silva, A., Clasadonte, J., Imbernon, M., Fernandois, D., Martinez-Corral, I., Kusumakshi, S., Caron, E., Rasika, S. and Deliglia, E., 2021. Leptin brain entry via a tanycytic LepR–EGFR shuttle controls lipid metabolism and pancreas function. *Nat. Metab.*, **3**: 1071-1090. https://doi.org/10.1038/s42255-021-00432-5

Erken, H.A., Erken, G. and Genç, O., 2013. Blood pressure measurement in freely moving rats by the tail cuff method. *Clin. exp. Hyperten.*, **35**: 11-15. https://doi.org/10.3109/10641963.2012.685534

Ezzati, M., Bentham, J. and Bilano, V., 2017. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: A pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet*, **390**: 2627-2642.

Gruber, T., Pan, C., Wiedemann, T., Lefort, S., Le, Thuc. O., Legutko, B., García-Villalón, A.L., Ussar, S., Pfluger, P., Woods, S.C., Ertürk, A. and García-Cáceres, C., 2021. Obesity-associated hyperleptinemia alters the gliovascular interface of the hypothalamus to promote hypertension. *Cell Metab.*, **33**: 1155-1170.e1110. https://doi.org/10.1016/j.cmet.2021.04.007

Janecka, J.E., Miller, W., Pringle, T.H., Wiens, F., Zitzmann, A., Helgen, K.M., Springer, M.S. and Murphy, W.J., 2007. Molecular and genomic data identify the closest living relative of primates. *Science*, **318**: 792-794. https://doi.org/10.1126/science.1147555

Kahn, S.E., Hull, R.L. and Utzschneider, K.M., 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*, **444**: 840-846. https://doi.org/10.1038/nature05482

Kelly, T., Yang, W. and Chen, C.S., 2008. Reynolds, K. and He, J. Global burden of obesity in 2005 and projections to 2030. *Int. J. Obes.*, **32**: 1431-1437. https://doi.org/10.1038/ijo.2008.102

Kivimäki, M., Kuosma, E., Luukkonen, R., Alfredsson, L., Fransson, E., Goldberg, M., Knutsson, A., Koskenvuo, M., Nordin, M., Oksanen, T., Pentti, J., Rugulies, R., Singh-Manoux, A., Steptoe, A., Theorell, T., Vahtera, J., Virtanen, M., Westerholm, P., Westerlund, H., Zins, M., Hamer, M. and Jokela, M., 2017. Overweight, obesity, and risk of cardiometabolic multimorbidity: Pooled analysis of individual-level data for 120 813 adults from 16 cohort studies from the USA and Europe. *Lancet Publ. Hlth.*, **2**: e277-e285. https://doi.org/10.1016/S2468-2667(17)30074-9

Koenig, W., Sund, M., Fröhlich, M., Fischer, H.G., Löwel, H., Döring, A., Hutchinson, W.L. and Pepys, M.B., 1999. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: Results from the MONICA (monitoring trends and determinants in cardiovascular disease) Augsburg cohort study, 1984 to 1992. *Circulation*, **99**: 237-242. https://doi.org/10.1161/01.CIR.99.2.237

Kornfeld, J.W., Baitzel, C., Könner, A.C., Nicholls, H.T., Vogt, M.C., Herrmanns, K., Scheja, L., Haumaitre, C., Wolf, A.M., Knippschild, U., Seibler, J., Cereghini, S., Heeren, J., Stoffel, M. and Brüning, J.C., 2013. Obesity-induced overexpression of miR-802 impairs glucose metabolism through silencing of Hnf1b. *Nature*, **494**: 111-115. https://doi.org/10.1038/nature11793

Lahousen, T., Roller, R.E., Lipp, R.W. and Schnedl, W.J., 2002. Silent haemoglobin variants and determination of HbA(1c) with the HPLC Bio-Rad Variant II. *J. Clin. Pathol.*, **55**: 699. https://doi.org/10.1136/jcp.55.9.699

Lamharzi, N., Renard, C.B., Kramer, F., Pennathur, S., Heinecke, J.W., Chait, A. and Bornfeldt, K.E., 2004. Hyperlipidemia in concert with hyperglycemia stimulates the proliferation of macrophages in *Atherosclerotic lesions*: potential role of glucose-oxidized LDL. *Diabetes*, **53**: 3217-3225. https://doi.org/10.2337/diabetes.53.12.3217

Lavie, C.J., Laddu, D. and Arena, R., 2018. Healthy weight and obesity prevention: JACC health

promotion series. *J. Am. College Cardiol.*, **72**: 1506-1531. https://doi.org/10.1016/j.jacc.2018.08.1037

Lin, H., Zhang, L., Zheng, R. and Zheng, Y., 2017. The prevalence, metabolic risk and effects of lifestyle intervention for metabolically healthy obesity: A systematic review and meta-analysis: A PRISMA-compliant article. *Medicine*, **96**: e8838. https://doi.org/10.1097/MD.0000000000008838

Liu, X.Y., Yang, D.B., Xu, Y.C., Gronning, M.O., Zhang, F., Wang, D.H. and Speakman, J.R., 2016. Photoperiod induced obesity in the Brandt's vole (*Lasiopodomys brandtii*): A model of healthy obesity? *Dis. Models Mech.*, **9**: 1357-1366. https://doi.org/10.1242/dmm.026070

López-Otín, C. and Kroemer, G., 2021. Hallmarks of health. *Cell*, **184**: 33-63. https://doi.org/10.1016/j.cell.2020.11.034

Montague, C.T., Farooqi, I.S., Whitehead, J.P., Soos, M.A., Rau, H., Wareham, N.J., Sewter, C.P., Digby, J.E., Mohammed, S.N., Hurst, J.A., Cheetham, C.H., Earley, A.R., Barnett, A.H., Prins, J.B. and O'Rahilly, S., 1997. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature*, **387**: 903-908. https://doi.org/10.1038/43185

Myers, M.G., Leibel, R.L., Seeley, R.J. and Schwartz, M.W., 2010. Obesity and leptin resistance: Distinguishing cause from effect. *Trends Endocrinol. Metab.*, **21**: 643-651. https://doi.org/10.1016/j.tem.2010.08.002

Phillips, C.M., 2016. Metabolically healthy obesity: Personalised and public health implications. *Trends Endocrinol. Metab.*, **27**: 189-191. https://doi.org/10.1016/j.tem.2016.02.001

Pillon, N.J., Loos, R.J.F., Marshall, S.M. and Zierath, J.R., 2021. Metabolic consequences of obesity and type 2 diabetes: Balancing genes and environment for personalized care. *Cell*, **184**: 1530-1544. https://doi.org/10.1016/j.cell.2021.02.012

Roberto, C.A., Swinburn, B., Hawkes, C., Huang, T.T., Costa, S.A., Ashe, M., Zwicker, L., Cawley, J.H. and Brownell, K.D., 2015. Patchy progress on obesity prevention: Emerging examples, entrenched barriers, and new thinking. *Lancet*, **385**: 2400-2409. https://doi.org/10.1016/S0140-6736(14)61744-X

Roberts, T.E., Lanier, H.C., Sargis, E.J. and Olson, L.E., 2011. Molecular phylogeny of treeshrews (Mammalia: Scandentia) and the timescale of diversification in Southeast Asia. *Mol. Phylogenet. Evol.*, **60**: 358-372. https://doi.org/10.1016/j.ympev.2011.04.021

Roberts, T.E., Sargis, E.J. and Olson, L.E., 2009. Networks, trees, and treeshrews: Assessing support and identifying conflict with multiple loci and a problematic root. *Syst. Biol.*, **58**: 257-270. https://doi.org/10.1093/sysbio/syp025

Ruan, G.P., Yao, X., Liu, J.F., He, J., Li, Z.A., Yang, J.Y., Pang, R.Q. and Pan, X.H., 2016. Establishing a tree shrew model of systemic lupus erythematosus and cell transplantation treatment. *Stem Cell Res. Ther.*, **7**: 121. https://doi.org/10.1186/s13287-016-0385-1

Sargis, E.J., Woodman, N., Morningstar, N.C., Reese, A.T. amd Olson, L.E., 2014. Island history affects faunal composition: The treeshrews (Mammalia: Scandentia: Tupaiidae) from the Mentawai and Batu Islands, Indonesia. *Biol. J. Linn. Soc.,* **111**: 290-304. https://doi.org/10.1111/bij.12195

Shi, Z., Xing, H., Qi, C., Fang, M. and Fu, J., 2020. Zhang X. Chinese tree shrews as a primate experimental animal eligible for the study of alcoholic liver disease: Characterization and confirmation by MRI. *Exp. Anim.*, **69**: 110-118. https://doi.org/10.1538/expanim.19-0073

Stefan, N., Häring, H.U. and Schulze, M.B., 2018. Metabolically healthy obesity: The low-hanging fruit in obesity treatment? The lancet. *Diabetes Endocrinol.*, **6**: 249-258. https://doi.org/10.1016/S2213-8587(17)30292-9

Stefan, N., Häring, H.U., Hu, F.B. and Schulze, M.B., 2013. Metabolically healthy obesity: Epidemiology, mechanisms, and clinical implications. *Lancet Diabetes Endocrinol.*, **1**: 152-162. https://doi.org/10.1016/S2213-8587(13)70062-7

Sumithran, P. and Proietto, J., 2013. The defence of body weight: A physiological basis for weight regain after weight loss. *Clin. Sci.*, **124**: 231-241. https://doi.org/10.1042/CS20120223

Tattersall, G.J. and Cadena, V., 2010. Insights into animal temperature adaptations revealed through thermal imaging. *Imag. Sci. J.*, **58**: 261-268. https://doi.org/10.1179/136821910X12695060594165

Teckman, J.H., Qu, D. and Perlmutter, D.H., 1996. Molecular pathogenesis of liver disease in alpha1-antitrypsin deficiency. *Hepatology* (Baltimore, Med.), **24**: 1504-1516. https://doi.org/10.1053/jhep.1996.v24.ajhep0241504

Ulgherait, M., Midoun, A.M., Park, S.J., Gatto, J.A., Tener, S.J., Siewert, J., Klickstein, N., Canman, J.C., Ja, W.W. and Shirasu-Hiza, M., 2021. Circadian autophagy drives iTRF-mediated longevity. *Nature*, **598**: 353-358. https://doi.org/10.1038/s41586-021-03934-0

van Baak, M.A. and Mariman, E.C.M., 2019.

Mechanisms of weight regain after weight loss-the role of adipose tissue. *Nat. Rev. Endocrinol.*, **15**: 274-287. https://doi.org/10.1038/s41574-018-0148-4

Wildman, R.P., 2009. Healthy obesity. *Curr. Opin. Clin. Nutr. Metab. Care*, **12**: 438-443. https://doi.org/10.1097/MCO.0b013e32832c6db7

Xie, C., Yagai, T., Luo, Y., Liang, X., Chen, T., Wang, Q., Sun, D., Zhao, J., Sun, L., Jiang, C., Xue, X., Tian, Y., Wu, Y. and Jiang, C., 2017. Activation of intestinal hypoxia-inducible factor 2α during obesity contributes to hepatic steatosis. *Nat. Med.*, **23**: 1298-1308. https://doi.org/10.1038/nm.4412

Zhang, J., Luo, R.C., Man, X.Y., Lv, L.B., Yao, Y.G. and Zheng, M., 2020. The anatomy of the skin of the Chinese tree shrew is very similar to that of human skin. *Zool. Res.*, **41**: 208-212. https://doi.org/10.24272/j.issn.2095-8137.2020.028

Zhang, L., Shen, Z.L., Feng, Y., Li, D.Q., Zhang, N.N., Deng, Y.Q., Qi, X.P., Sun, X.M., Dai, J.J., Yang, C.G., Yang, Z.F., Qin, C.F. and Xia, X.S., 2019. Infectivity of Zika virus on primary cells support tree shrew as animal model. *Emerg. Microbes Infect.*, **8**: 232-241. https://doi.org/10.1080/22221751.2018.1559707