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Hibernation Pattern and Importance of Superoxide Dismutase for the Turkish Hamster, *Mesocricetus brandti* (Mammalia: Rodentia)

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Abstract: Specimens of *Mesocricetus brandti* were observed in order to assess the onset of hibernation and the inhibition rate of superoxide dismutase (SOD). The first individual began to hibernate on November 2 and the last animal awoke on May 5. Individual hibernation bouts varied from 8 to 63 days, and the longest uninterrupted hibernation was 4 days. In the hibernation period, the animals lost an average of 27.1 g, ranging from 2.7% to 38% of total body weight. In hibernating animals, the average inhibition rate of SOD was 82.3% while it was 77.5% in active animals. The inhibition rate of SOD in the hibernating animals was 6.3% higher on average than in active animals ($P < 0.05$).

Key Words: *Mesocricetus brandti*, hibernation, superoxide dismutase, Turkey

Türk Hamsteri'nin "*Mesocricetus brandti* (Mammalia: Rodentia)" Hibernasyon Özellikleri ve Süperoksit Dismutaz'ın Önemi

Özet: *Mesocricetus brandti*'nin hibernasyon özellikleri ve süperoksit dismutaz (SOD)'ın inhibisyon oranı belirlendi. Örneklerin ilk hibernasyona giriş tarihi 2 Kasım ve son örneğin hibernasyondan çıkış tarihi ise 5 Mayıs olarak belirlendi. Bireysel hibernasyon süreleri 8 ile 63 gün arasında değişmektetir ve en uzun kesintisiz hibernasyon süresi 4 gün olarak saptanmıştır. Hibernasyon periyodunda, hayvanlar yüzde 2,7' den yüzde 38'e kadar değişecek şekilde ortalama 27,1 g ağırlık kaybetmişlerdir. Hibernasyondaki hayvanlarda SOD'un ortalama inhibisyon oranı % 82,3, aktif hayvanlarda ise % 77,5 olarak saptandı. Hibernasyondaki hayvanlardaki SOD'un inhibisyon oranının aktif hayvanlardan % 6,3 oranında yüksek olduğu belirlendi ($P < 0,05$).

Anahtar Sözcükler: *Mesocricetus brandti*, hibernasyon, superoksit dismutaz, Türkiye

Introduction

Species of the genus *Mesocricetus* across the Palearctic region with 4-5 taxa are considered doubtful as valid species (Wilson and Reeder, 1993). Of these species, *Mesocricetus auratus* and *Mesocricetus brandti* are distributed throughout Turkey with *M. brandti* known as the Turkish hamster. Its distribution ranges from the West Anatolia steppes to the Caucasus Mountains and Iran (Yiğit et al., 2000a). Kilduff et al. (1993) recognized mammalian hibernation as an adaptation for energy conservation, indicating that sleep and hibernation might be homologous. Mammalian hibernators can be broadly grouped into 2 classes: facultative and obligate hibernators. Hibernation in facultative hibernators can be induced by environmental cues such as low temperatures, short photoperiods, and restricted food availability (Kilduff et al., 1993). The hamsters (*Mesocricetus* spp.) are included in this group (Kilduff et al., 1993). According to Darrow et al. (1986), Turkish hamsters hibernate when they are exposed to a short-day (10L:14D) and cold environment (6 °C). Darrow et al. (1986) also stated that during the 4 to 5 month hibernation season, Turkish hamsters are known to display 4 to 8 day bouts of torpor (where the body temperature is between 7 and 9 °C), alternating with 1 to 3 day intervals of euthermia. The Turkish hamster is also commonly used in laboratory experiments; studies on the reproductive cycle, growth, hibernation, laboratory

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care, behavior, and karyological analysis were conducted on this animal (Lyman and O'Brien, 1977; Lyman et al., 1981; Lyman et al., 1983; Pohl, 1987).

Antioxidant enzymes are of vital importance in an organism's defense against oxidative stress. The most important ones are superoxide dismutase, catalase, and glutathione peroxidase (Bolann and Ulvik, 1991; Kurata et al., 1993; Andersen et al., 1997). The free radicals such as superoxide, hydroxyl, and hydrogen peroxide damage all major macromolecules including nucleic acids, proteins, free amino acids, lipids, and carbohydrates (Kurata et al., 1993). On the other hand, heterothermic mammals are known to tolerate severe hypoxia. Additionally, many hibernating species in steady-state torpor are not hypoxic, despite the decrease in respiratory rates, but some heterothermic species may become hypoxic during torpor due to long periods of apnea (Drew et al., 2004). Reactive oxygen species (ROS) risk damage to macromolecules under hypoxic condition but have a way to protect themselves from ROS effects by eliminating free radicals via antioxidant enzymes (Storey, 1996). Thus we aimed to record the hibernation pattern and interpret differences in inhibition rate of SOD between hibernating and non-hibernating *M. brandti* individuals.

Materials and Methods

This study was carried out in 2004 and 2005 on 11 live specimens (6 females, 5 males) captured from different localities in central Turkey. The specimens were kept singly in cages to examine their hibernation behavior. They were provided with nesting materials, food (wheat seeds, carrot, and fresh grass), and water. The animals were kept under laboratory conditions designed to mimic their natural climatic environment (temperature, photoperiod, and humidity) in Ankara, and the maximum and minimum ambient temperature was recorded daily (°C) during the inspection period. Hibernation was monitored by means of the sawdust technique and the animals were visited daily to see whether there were active or hibernating. They were weighed during the periodic arousals, and the periodic arousals and the durations of individual bouts of hibernation were assessed. Three animals (no. 4, 7, and 11) were sacrificed under anesthesia during the hibernation and 3 after the hibernation period. Blood was taken from these animals into a citrated tube from a cardiac incision in order to measure the inhibition rate of superoxide dismutase (EC 1.15.1.1). The inhibition rate of superoxide dismutase was performed according to the method described by Sun et al. (1988). Test and blank tubes were monitored at 560 nm in a spectrophotometer (Shimadzu UV-1201V). The inhibition rate of superoxide radicals was calculated using the formula: Inhibition % = (absorbance value of blank tube - absorbance value of test tube × 100) / value of blank absorbance. Statistical analyses of the absorbance values obtained from both hibernating and non-hibernating animals were performed using one-way ANOVA.

Results

Entering Hibernation and Changes in Body Weights

Hibernation was first observed in one animal (no. 11) on November 2. This was followed by another animal (no. 1) on November 18. The last animal (no. 2) entered hibernation on March 11, hibernating for a total of only 8 days (see Table 1). The animals entered hibernation in a typical posture, coiled up in the nest material with their back up. The ambient temperature of the laboratory varied from 11 to 19 °C between December 2004 and May 2005.

The body weight of animals ranged from 108 g to 175 g with an average of 134.3 g before the animals began to hibernate. The body weights of animals decreased regularly prior to hibernation. No fluctuations in body weights were observed during the hibernation period with one exception (no. 2), which gained weight during this period. During the hibernation period, the rates of weight loss were found to vary from 6.8% to 38% with an average of 24.1%. The weight of the hamster that gained weight changed from 122 g to 131 g, increasing 6.8%. The changes in body weight of the sacrificed animals were not included in the results. However, the animals that were sacrificed also gained weight during the heterothermic period. A positive correlation was found between body weight and the onset of hibernation, with heavier animals entering hibernation earlier. No correlation was found between body weight and absolute weight loss. However, there was a correlation between body weight on entering and terminating hibernating (Table 1). The body weights of 7

Table 1. Hibernation behavior of 11 specimens of *M. brandti*. FHE = First hibernation entrance, BWH = Body weight on entering hibernation, LHA = Last hibernation arousal, BWA = Body weight on last hibernation arousal, RWC = Rate of weight change (%), NDS = Number of days spent in torpor, UHB = Uninterrupted hibernation bout (days).

No.	FHE	BWH	LHA	BWA	RWC	NDS	UHB
1	November 18, 2004	175 g	April 17, 2005	146 g	16.5	28	4
2	March 11, 2005	122 g	April 16, 2005	131 g	6.8	8	2
3	February 8, 2005	135 g	April 17, 2005	110 g	18.5	9	3
4	November 29, 2004	133 g	January 28, 2005*	114 g*	14*	24*	4
5	January 5, 2005	120 g	April 13, 2005	95	20.8	31	3
6	January 3, 2005	117 g	April 18, 2005	97	17	43	3
7	January 12, 2005	108 g	January 28, 2005*	105*	2.7*	6*	2
8	December 18, 2004	128 g	May 5, 2005	92	28	60	4
9	November 27, 2004	134 g	May 4, 2005	83	38	60	3
10	November 27, 2004	147 g	May 4, 2005	103	29.9	63	4
11	November 2, 2004	158 g	January 28, 2005*	139 g*	12*	22*	4

*= Sacrificed to allow for analysis of superoxide dismutase

animals between entering and terminating hibernation periods were found to be statistically significant (ANOVA test, $P = 0.0099$, $P < 0.05$).

Hibernation Period

The heterothermic period lasted from early November to the first week of May (6 months and 3 days). The longest time spent in torpor in the heterothermic period by an individual was 63 days (no. 10), followed by 60 days in no. 8 and 9. The shortest torpor in this period was observed in no. 2 (8 days) (Figure). The bouts of hibernation lasted from 1 to 4 days. The maximum uninterrupted periods of hibernation were 4 days in 5 animals (no. 1, 4, 8, 10, and 11), followed by 3 days in 4 animals (no. 3, 5, 6, and 9), and 2 days in 1 animal (no. 2). It was also observed that the Turkish hamster ate during the periodic arousals of the heterothermic period but they were highly torpid between hibernation bouts.

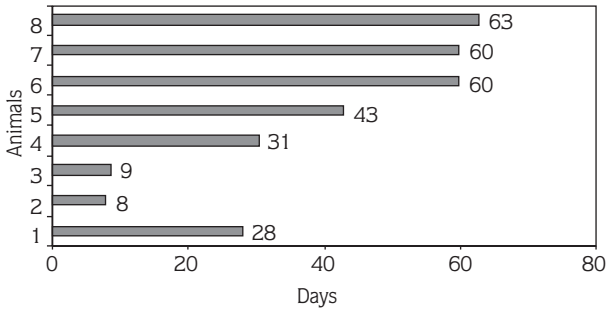

Figure. Horizontal bars indicate the number of days spent in hibernation for 8 specimens of *M. brandti.*

Inhibition rate of superoxide dismutase (SOD)

In total, 6 animals were used in the analysis of SOD. Of these, 3 (no. 4, 6, and 11) were in the heterothermic period and the remaining 3 (no. 6, 8 and 10) were out of the hibernation period. Of the 3 animals in the heterothermic period, 2 animals (no. 4 and 7) were in torpor while the third was in the arousal stage. According to the spectrophotometric analyses, the absorbance values at 560 nm were 0.012 for no. 4, 0.015 for no. 7, and 0.016 for no. 11. Therefore, the inhibition rates were 85.5%, 81.9%, and 80.7%, respectively. The mean value of animals in the heterothermic period was 82.7%. The lowest inhibition rate was calculated from no. 11, which was in the arousal stage in the heterothermic period.

The absorbance values of the animals that were out of the hibernation period were 0.017 for no. 10, 0.019 for no. 8, and 0.020 for no. 6. The inhibition rates in these animals varied from 79.5% to 75.9% with an average of 77.6%. According to these findings, the inhibition rates in the hibernation period were significantly higher (ANOVA test, $P = 0.0465$, $P < 0.05$) than in the active period, with a difference of 6.3%.

Discussion

In the laboratory study by Lyman and O'Brien (1977), Turkish hamsters were exposed to a constant temperature (5 ± 2 °C) and photoperiod regime. It was

reported that Turkish hamsters hibernated between November 15 and April 15 and spent 36% of the heterothermic period in hibernation. The duration of the heterothermic period was similar to the result found in this study but the percentage of hibernation within the heterothermic period ranged from 4% to 43%, with an average of 20.03%. These values are markedly below those reported by Lyman and O'Brien (1977). Darrow et al. (1986) reported that Turkish hamsters were known to display 4 to 8 day bouts of torpor, alternating with 1 to 3 day intervals of euthermia. In contrast to Darrow et al. (1986), the maximum observed torpor lasted 4 days.

According to Lyman and O'Brien (1977), the animals lost weight in the first 2 and 3 weeks; the younger ones could easily regain their original weight if kept in the cold for several months and the heavy animals lost more weight than the lighter ones. We performed the hibernation study on adult specimens and noted that they lost an average of 19.7% body weight, except for no. 2, which hibernated for only 8 days in the heterothermic period. Our findings show that weight loss was not correlated with the original weights of an animal but was related to duration of hibernation. Animals (no. 8, 9, and 10) that spent 60 days or more in hibernation lost more weight than any other animals.

Lyman et al. (1981) studied the relationship between the duration of hibernation and lifespan. They established 3 categories for animals due to their hibernation behavior: 1) poor hibernators, animals that hibernated 0% to 11% of their lives; 2) moderate hibernators, animals that hibernated 12% to 18% of their lives; 3) good hibernators, animals that hibernated 19% to 33% of their lives in the heterothermic period. We applied these categories to our animals, and concluded that 2 hamsters (no. 2 and 3) were poor hibernators, 2 (no. 1 and 5) were moderate hibernators, and 4 (no. 6, 8, 9, and 10) were good hibernators. Lyman et al. (1983) compared the hibernation tendencies of Turkish hamsters with different chromosome numbers (2n = 42 and 44). Animals were kept in a cold room (5 ± 2 °C) between November and April. Animals with 2n = 42 and 44 spent on average 12.1% and 17.1% of their lives in hibernation, respectively. This represents a significant difference in hibernation period ($P < 0.001$). According to a previous karyological report (Yiğit et al., 2000a) and our analyses, our study population of Turkish hamsters contained only those with 2n = 42. Pohl (1987) separated Turkish hamsters into groups and applied different photoperiod cycles at different temperatures. He reported that hamsters entered hibernation and also lost body weight when exposed to short photoperiod (8L:16D) and cold conditions (10 °C). Our results are consistent with this conclusion.

The hibernation biology of *Glis glis orientalis* and *Spermophilus xanthoprymnus* was studied by Çolak et al. (1998) and Yiğit et al. (2000b). Çolak et al. (1998) reported that animals entered hibernation on November 28 with an average body weight of 180 g at 18 °C. There were marked fluctuations in the body weight of dormice. Yiğit et al. (2000b) previously found that ground squirrels begin hibernation in late August and finish in the middle of February, and lose an average of 28% body weight. According to the hibernation data in Table 2 including data reported by Çolak et al. (1998) and Yiğit et al. (2000b), hibernation bouts of dormice and ground squirrels are longer than those of Turkish hamsters. Çolak et al. (1998) stated that *G. glis* entered hibernation at a high temperature (approximately 18 °C) because they are obligate hibernators. Dormice essentially hibernate because of their annual physiological rhythms, whereas hamsters are facultative hibernators. Therefore, hamsters require an ambient temperature lower than 18 °C for entering hibernation. Dormice and ground squirrels are clearly better hibernators than Turkish hamsters.

Mammalian hibernators are exposed to hypoxia because of long apneic periods during hibernation. Rice et al. (2002) performed a study to determine the level of

Table 2. Duration of heterothermic periods and torpor of 2 hibernator species compared to *M. brandti*.

	M. brandti (present study)	*G. g. orientalis* Çolak et al. (1998)	*S. xanthoprymnus* Yiğit et al. (2000b)
Minimum and maximum days spent in hibernation	8 – 63	21 – 100	5 – 181
Minimum and maximum days in torpor	1 – 4	1- 13	1 – 36

oxidative stress during hibernation by measuring plasma ascorbic acid in *Spermophilus parryii* and *Spermophilus tridecemlineatus*. They found plasma ascorbic acid levels 4 times higher for *S. parryii* and 3 times higher for *S. tridecemlineatus* during hibernation compared with the homothermic period. High levels of plasma ascorbic acid are regarded as an indicator of oxidative stress. During hypoxia, animals will raise the level of antioxidant enzymes to eliminate ROS effects (Fridovich, 1975; Andersen et al., 1997). Similarly, the inhibition rate of SOD in hamsters was found to be 6.3% higher for animals in the heterothermic period as compared with animals in the homothermic period. Therefore, this rate may be correlated with the hibernation ability of mammalian hibernators. Comparative studies on other hibernating rodents are necessary to elucidate the relationships between the duration of the heterothermic period and the level of oxidative stress enzymes.

References

Andersen, H.R., Nielsen, J.B., Nielsen, F. and Grandjean, P. 1997. Antioxidative enzyme activities in human erythrocytes. Clinical Chemistry, 43: 562-568.

Bolann, B.J. and Ulvik, R.J. 1991. Improvement of a direct spectrophotometric assay for routine determination of superoxide dismutase activity. Clinical Chemistry, 37**:** 1993-1999.

Çolak, E., Yiğit, N., Sözen, M. and Özkurt, S. 1998. Hibernation and body weight in Dormice, *Glis glis orientalis* (Nehring, 1903) (Rodentia: Gliridae), maintained under uncontrolled conditions. Turk. J. Zool. 22: 1-7.

Darrow, J.M., Tamarkin, L., Duncan, M.J. and Goldman, B.D. 1986. Pineal melatonin rhythms in female Turkish Hamsters: Effects of photoperiod and hibernation. Biology of Reproduction, 35: 74-83.

Drew, K.L., Harris, M.B., Lamanna, J.C., Smith, M.A., Zhu, X.W. and Ma, Y.L. 2004. Hypoxia tolerance in Mammalian Heterotherms. Journal of Experimental Biology, 207: 3155-3162.

Fridovich, I. 1975. Superoxide dismutase. Annual Review of Biochemistry, 44: 147-159.

Kilduff, T.S., Krilowicz, B., Milsom, W.S., Trachsel, L. and Wang, L.C.H. 1993. Sleep and mammalian hibernation: Homologous adaptations and homologous processes. American Sleep Disorders Association and Sleep Research Society, 16: 372-386.

Kurata, M., Suzuki, M. and Agars, N. 1993. Antioxidant systems and erythrocyte life-span in mammals. Comparative Biochemistry and Physiology, *B* 106**:** 477-487.

Lyman, C.P. and O'Brien, R.C. 1977. Laboratory study of the Turkish Hamster *Mesocricetus brandti*. Breviora, 442**:** 1-27.

Lyman, C.P., O'Brien, R.C., Greene, G.C. and Papafrangos, E.D. 1981. Hibernation and longevity in the Turkish Hamster *Mesocricetus brandti*. Science, 212: 668-670.

Lyman, C.P., O'Brien, R.C., and Bossert, W.H. 1983. Differences in tendency to hibernate among groups of Turkish hamster (*Mesocricetus brandti)*. Journal of Thermal Biology, 8: 255-257.

Pohl, H. 1987. Control of annual rhythms of reproduction and hibernation by photoperiod and temperature in the Turkish hamster. Journal of Thermal Biology, 12**:** 119-123.

Rice, M.E., Forman, R.E., Chen, B.T., Avshalumov, M.V., Cragg, S.J. and Drew, K.L. 2002. Brain antioxidant regulation in mammals and anoxia-tolerant reptiles: balanced for neuroprotection and neuromodulation. Comparative Biochemistry and Physiology C, 133**:** 515-525.

Storey, K.B. 1996. Oxidative stress: animal adaptations in nature. Brazilian Journal of Medical and Biological Research 29: 1715-1733.

Sun, Y., Oberley, L.M. and Li, Y. 1988. A simple method for clinical assay of superoxide dismutase. Clinical Chemistry, 34: 497-500.

Wilson, E. and Reeder, M.D. 1993. Mammal Species of the World: A taxonomic and geographic reference. Second Ed. Smithsonian Institution Press, Washington and London, 1206 pp.

Yiğit, N., Çolak, E., Sözen, M., Özkurt, S. and Verimli, R. 2000a. The distribution, morphology, and karyology of the genus *Mesocricetus* (Mammalia: Rodentia) in Turkey. Folia Zoologica, 49: 167-174.

Yiğit, N., Çolak, E. and Sözen, M. 2000b. A study on the hibernation of *Spermophilus xanthoprymnus* (Bennet, 1835) (Mammalia: Rodentia) in Turkey. Turk. J. Zool. 24: 87-93.