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Blood and Serum Analyses of Cold-Exposed Chipmunks

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To understand the adaptational strategy of Korean chipmunks (*Tamias sibiricus*) to cold temperature, blood and serum properties, and thyroid gland activity of cold-exposed chipmunks were examined. The number of erythrocytes and hemoglobin concentration increased, but platelets decreased in cold-exposed chipmunks compared with warm chipmunks. Serum total protein levels increased at early phase of cold-exposure, and decreased thereafter. Plasma glucose levels showed a transitory increase in cold temperature. Although there was significant decrease in serum total thyroxine level in cold-exposed chipmunks, serum total triiodothyronine level changed little. Histological analysis of thyroid glands demonstrated decreased thyroid activity, suggesting that differences in the blood and serum properties between the warm and cold-exposed chipmunks may be due to the different metabolic strategy associated with cold temperature.

Hibernation is an adaptive strategy for some mammalian species to conserve energy in cold or unfavorable environments related to food availability. These mammals can reduce dramatically their basal metabolic, heart, and respiratory rates as well as their body temperature during hibernation (Magnus and Henderson, 1988a; Weekley, 1995. Tomasi et al., 1998).

Of mammalian hibernators, chipmunks are most commonly studied and serve useful animal model for hibernation research due to its small body size and distinct behavioral pattern in relation to hibernation. Its annual cycle consists of two phases, the summer season during which squirrels reproduce and accumulate fat, and the hibernating phase which is composed of alternating dormancy and arousal bouts (Magnus and Henderson, 1988a,b).

In deep hibernating squirrels, body temperature can drop from normothermic values 35-38°C to a level of temperature as low as 6-8°C (Wang, 1989). Hibernating squirrel also can reduce cellular energy expenditure in excess of 88% that of maintaining euthermic activity, and heart rate decreases to less than 3% of normal. Oxygen consumption drops to 1% of that found in euthermia (Wang and Pehowich, 1985; Churchill et al., 1996).

It has been proposed that hibernating animals are good for investigating the mammalian metabolism at hypothermia to provide information for more applied aspect of low temperature science, such as organ preservation for transplantation, leading to a possible disclosure of hibernation mechanism for human welfare in the future (Kondo and Kondo, 1992; Churchill et al., 1996; Kondo, 1999).

Korean chipmunks are known to hibernate during winter months in the wild but their hibernation ecology as wel as physiology has rarely been studied. The present study describes some physiological adaptation of Korean chipmunks when they are exposed to cold temperature in the controlled laboratory condition.

Materials and Methods

Animals

36 Korean chipmunks (or known as Siberian chipmunk, *Tamias sibiricus*) which were all male collected during the spring from Kangwon Province of Korea were used for this study. The chipmunks were individually housed in the standard laboratory mouse cage and fed an ad libitum diet of hamster serial, sunflower seeds and apples. For acclimation in the laboratory, chipmunks were placed for 3 weeks under the controlled conditions of 23±2°C and 12:12 light-dark cycle.

While half of the acclimated chipmunks remained in the constant warm condition, the others were transferred

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to a cold room where temperature was gradually decreased from 20°C to 6°C over 3 weeks in constant dark and finally maintained at 6°C throughout the experiments. These chipmunks were treated as three period-groups, i.e. 3, 5 and 9 months cold-exposure. Hibernation state was confirmed by dormancy of the cold animals from daily observation.

Blood and serum analyses

Blood samples were taken into heparinized syringe by cardiac puncture under ether anesthesia. Numbers or levels of erythrocytes, platelets and hemoglobins were quantified by an automatic blood cell counter (Horiba). For the serum analysis, the blood was immediately centrifuged at 3,000 rpm for 5 min and the plasma was stored at -20°C for later analysis. Levels of serum glucose were determined by the glucose trinder (Sigma), following the procedure, including calibration and calculation, provided by the manufacturer.

Concentration of serum total proteins were measured by a protein assay kit (Bio-Rad). Serum total triiodothyronine (T_3) and total thyroxine (T_4) were quantified using the T_3 and T_4 microwell EIA (Biomerica). Levels of serum T_3 and T_4 were calculated by dose response curve on a logit-log graph paper.

Histological analysis for thyroid

To assess thyroid function by histological methods, the thyroid gland was obtained from the control and hibernating chipmunks and fixed in 10% neutral buffered formalin for 24 hours. The tissues were dehydrated in a graded ethanol series and embedded in paraffin. Serial 5 μm thick sections were prepared. For histological analysis, hematoxylin-eosin stain and periodic acid Schiffs (PAS) reaction were used. The height of follicular cell and diameter of colloid of the thyroid gland were measured.

Statistical analysis

Calculations of means, standard errors and Student's *t*-test were performed with a SigmaPlot version 6.0 (SPSS).

Results

Induction of hibernation

Most chipmunks maintained their activity for up to 3 months in the cold room before they became motionless, suggesting that they entered into hibernation state.

Blood analysis

Blood contents in both the control and hibernating chipmunks are presented in Figs. 1 and 2. The number

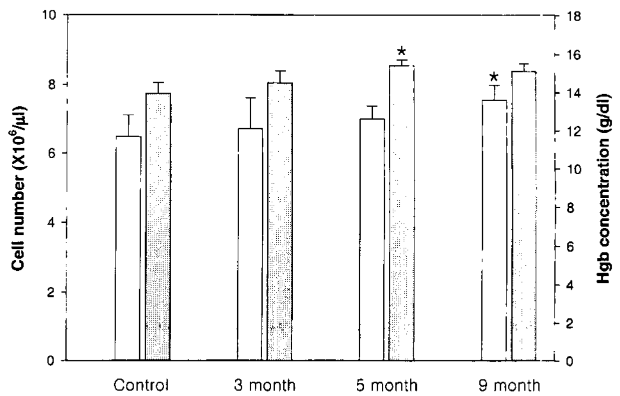

Fig. 1. The number of erythrocytes (white bar and the left scale) and hemoglobin concentration (gray bar and the right scale) in the control and cold-exposed chipmunks. Values are means±SEM of six to eight animals. *, Significantly different from control (P<0.05).

of erythrocytes and hemoglobin concentration increased steadily as time progressed in the cold room. Significant increase in erythrocytes counts and hemoglobin concentration were observed at 9 and 5 months of cold exposure, respectively. In contrast, platelets counts were significantly decreased at 5 and 9 month of cold-exposure.

Serum analysis

The changes in blood metabolic parameters, glucose and total protein and thyroid hormone levels are shown in Figs. 3 and 4. There was a 134% increase of total protein at 3 month of cold-exposed chipmunks, but thereafter the level decreased close to the control group at 5 month onwards of cold-exposure. Plasma glucose level remained unchanged until 3 month of cold-exposure followed by a significant increase at 5 month from 148.7±8.6 mg/dl to 209.7±26.4×10^3 mg/dl, but dropped to the control level at 9 month.

Although there was a tendency to increase of serum

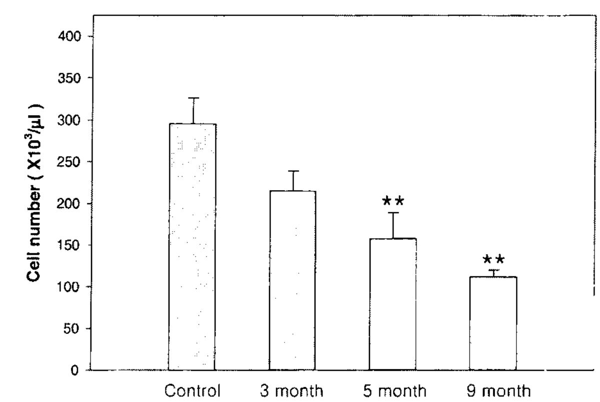

Fig. 2. Platelet counts of the control and cold-exposed chipmunks. Values are means±SEM of six to eight animals.
**, Significantly different from control (P<0.005).

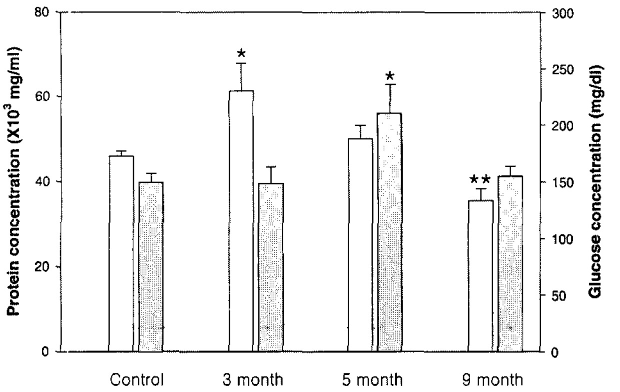

Fig. 3. The total protein (white bar and the left scale) and glucose concentration (gray bar and the right scale) in the control and cold-exposed chipmunks. Values are means±SEM of six to eight animals.
* and **, Significantly different from control at P<0.05 and P<0.005 levels, respectively.

T_3 level by cold treatment, especially at 5-month stage but no significant increase was observed. During the experiment, the T_3 level ranged from 1.5±0.2 μg/dl to 2.1±0.3 μg/dl. The T_4 level was about two times higher than T_3 and showed a sharp decrease from 2.8±0.2 μg /dl to 0.8±0.2 μg/dl by 3-month cold-exposure. The decreased level persisted to the 9-month period. The T_3 : T_4 ratio increased remarkably after cold exposure, reached its peak of 3.59±1.4 by 5 month and thereafter decreased by 9-month. The ratio was higher than the control level.

Histological change of thyroid gland

Thyroid glands of the control chipmunks had uniformly small follicle lined by high cuboidal cells and the cyst-like follicles of various sizes. As shown in Figs. 5 and 6, the height of follicular cells decreased in proportion to the

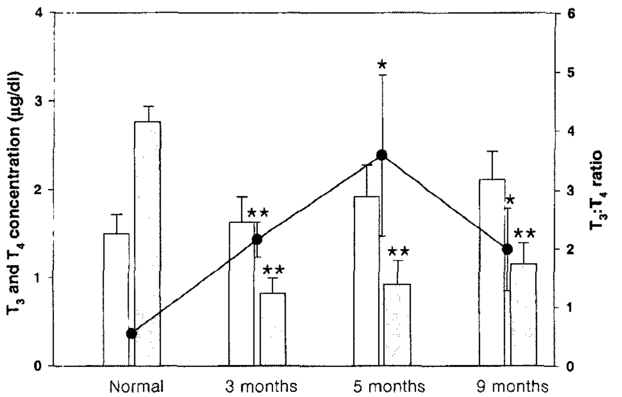

Fig. 4. The T3 levels (white bar and the left scale) and T4 levels (gray bar and the left scale) in the control and cold-exposed chipmunks. Dark circle represents the pattern of T3:T4 ratio (right scale). Values are means±SEM of six to eight animals.
* and **, Significantly different from control at P<0.05 and P<0.005 levels, respectively.

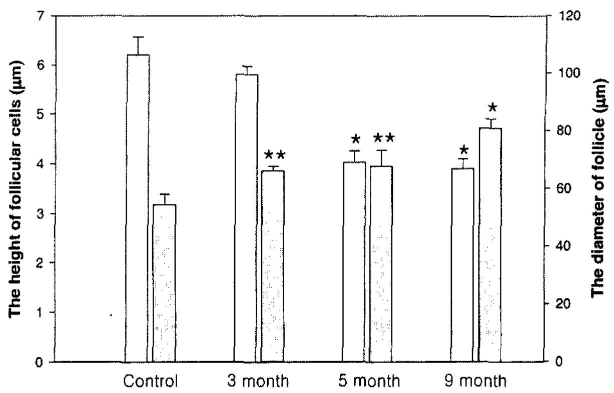

Fig. 5. Means of follicular cell height (white bar and the left scale) and diameter (gray bar and the right scale) of cross-sectional colloid in the control and cold-exposed chipmunks. Values are means±SEM of six to eight animals.
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time of cold exposure, especially in the 5 and 9 months of cold-exposed chipmunks. However, the diameter of follicles was opposite; it significantly increased at the end

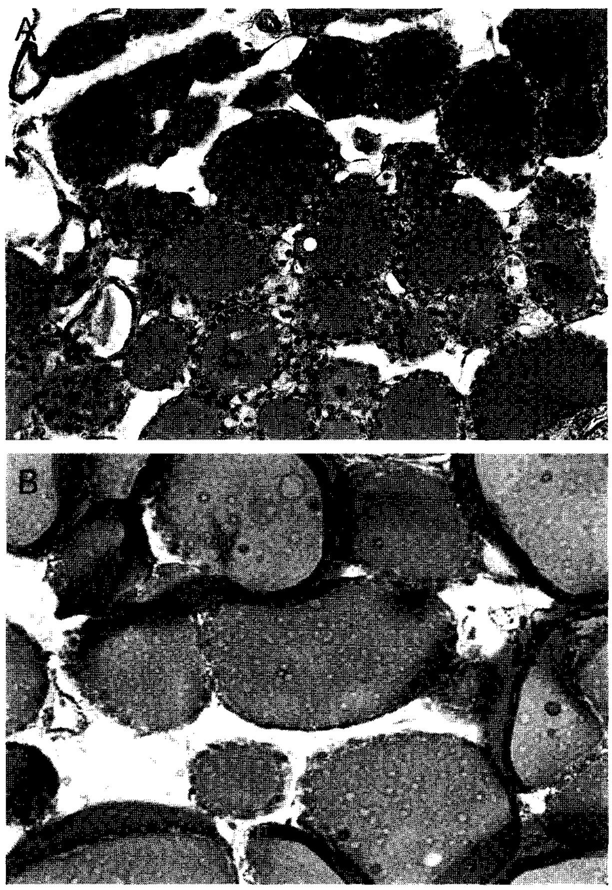

Fig. 6. Thyroid gland histology of control (A) and 9 months (B) of cold-exposed chipmunks. The low cuboidal follicular cell and lager follicle were detected in cold-exposed chipmunks compared with control one. Periodic acid Schiff's reaction. X 400.

of prolonged cold-exposure.

Discussion

Korean chipmunks were observed to enter into dormant or hibernation state within three months of cold-exposure at 6°C in the laboratory, although it is yet premature to say whether or not the chipmunks showed a similar pattern of hibernation as they were in the wild. However, some physiological changes measured during the cold-exposure periods indicate about their physiological adaptation to unfavorable situations such as food deprivation or cold temperature.

There are numerous conflicting reports about the alteration of erythrocytes number during hibernation, i.e. no changes (Reznik et al., 1975), a decrease (Spurrier and Dawe, 1973), and an increase (Novoselova et al., 2000). The increased level of erythrocytes counts with hemoglobin concentration shown in the present research during cold-exposure period may be explained by a decrease in the body size of torpid animal due to slower blood flow (Novoselova et al., 2000) or an increase in the average life span of the circulating erythrocytes (Deveci et al., 2001). Although it is not clear what mechanisms are activated in the slow blood flow of hibernating animals, a decrease in platelets counts in hibernating animals may prevent clot formation and maintain tissue perfusion under conditions of very slow flow result from low temperature (Deveci et al., 2001). Blood composition including erythrocytes and platelets counts in the hibernators may contribute to an innate tolerance to low temperature.

The transitory increase in plasma glucose levels in 5-month cold-exposure group of Korean chipmunks was in contrast to the results of previous researchers done with other hibernating mammals (William and Peter, 1970; Magnus and Henderson, 1988a). Plasma glucose and glycogen in the liver of arctic squirrels decline steadily during hibernating periods (William et al., 1970), indicating that glucose consumption exceed gluconeogenesis, an imbalance between catabolism and synthesis of glucose, during hibernation. However, Nizielski et al. (1986) noted that glucose utilization is negligible at the time of deep torpor in ground squirrels, resulting in the increase in blood glucose level.

Serum total protein level showed an earlier peak at 3-month period than glucose, which was a significant increase compared with the level in active chipmunks. This increase may be considered as a process of cold acclimation by which the hibernating squirrels survive fasting period of several months as shown in hibernating black bear (Nelson, 1980). After acclimation period at early hibernation stage, the protein level of the chipmunks returned to similar level of warm animal at 5-month period and decreased further to a significantly lower level at 9-month period.

Although thyroid hormones have multiple functions, more important roles lie in the activities related to metabolism, growth and reproduction. Therefore, it is general understanding that the synthesis of thyroid hormones decreases due to the decreased metabolic activities during hibernation (McNabb, 1992). This postulation is supported by the present study clearly. Serum total T_4 significantly decreased in Korean chipmunks exposed to prolonged cold temperature, with little change or slight increase in total serum T_3 level. Some other mammalian hibernators, however, show different response to cold temperature. Both thyroid hormones increased during hibernation or dormant periods in ground squirrels, woodchuck, and black bear (Demeneix and Henderson, 1978; Young et al., 1979; Tomasi et al., 1998; Magnus and Henderson, 1988a,b; Nevretdinova, 1989). The reasons for the elevation in plasma T_3 and T_4 during hibernation are not clear, but it may serve as a storage form of T_4 and T_3 for use after the animals arouse from hibernation (Young, 1984). Alternatively, Nevretdinova (1992) postulated that thyroid also participated in the lipid metabolism in relation to membrane viscosity during hibernation as well as to energy metabolism.

The dynamics of thyroid hormones are not so simple to understand that several factors should be considered in interpretation of their metabolism. As we examined thyroid gland of Korean chipmunks to monitor the production activity of thyroid hormones based on histology of the gland (Fig. 5), the results showed smaller cuboidal follicular cells and larger colloid diameter during cold-exposure period, representing decreased activities of the thyroid gland. However, various mammals, which have an ability for adaptation to transient cold exposure, response with increased thyroid activities and basal metabolism, so thyroid follicular cell height was significantly greater (Olson et al., 1999; Buffenstein et al., 2001). The slightly increased T_3 level of the cold-exposed chipmunks in current report did not reflect increased thyroid gland activity but correlate with the increase of $T_3:T_4$ ratio, suggesting relatively more T_3 was induced by T_4 deiodination as response of cold acclimation.

In the present study, we observed significant changes in blood components, thyroid hormone levels, and thyroid morphology in the cold-exposed Korean chipmunks, although they were able to survive cold temperature with somewhat different adaptational strategy from other mammalian hibernators. Since this is the first attempt to simulate hibernation of Korean chipmunks under controlled condition, the obtained results would be considered as preliminary. Therefore, future research on this topic would be necessary to have a more thorough investigation in the hibernation mechanism of this little hibernator.

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References

Buffenstein R, Woodley R, Thomadakis C, Daly TJ, and Gray DA (2001) Cold-exposed changes in thyroid function in a poikilothermic mammal, the naked mole-rat. *Am J Physiol Regul Integr Comp Physiol* 280: 149-155.

Churchill TA, Simpkin S, Wang LC, Green CJ, Williams SR, Busza AL, and Fuller BJ (1996) Metabolic effects of cold storage on livers from euthermic and hibernating Columbian ground squirrels. *Cryobiology* 33: 34-40.

Demeneix BA and Henderson NE (1978) Serum T_4 and T_3 in active and torpid ground squirrels, *Spermophilus richardsoni. Gen Comp Endocrinol* 35: 77-85.

Deveci D, Stone PC and Egginton S (2001) Differential effect of cold acclimation on blood composition in rats and hamsters. *J Comp Physiol* 171: 135-143.

Kondo N and Kondo J (1992) Identification of novel blood proteins specific for mammalian hibernation. *J Biol Chem* 267: 473-478.

Kondo N (1999) Hibernation control mechanism and possible applications to humans. *JBIS*. 52: 343-347.

McNabb FMA (1992) Thyroid Hormones. Prentice-Hall International, Englewood Cliffs, NJ. pp 283.

Magnus TH and Henderson NE (1988a) Thyroid hormone resistance in hibernating ground squirrels, *Spermophilus richardsoni*. I. Increased binding of triiodo-L-thyronine and L-thyroxine by serum proteins. *Gen Comp Endocrinol* 69: 352-360.

Magnus TH and Henderson NE (1988b) Thyroid hormone resistance in hibernating ground squirrels, *Spermophilus richardsoni*. II. Reduction of hepatic nuclear receptors. *Gen Comp Endocrinol* 69: 361-371.

Nelson RA (1980) Protein and fat metabolism in hibernating bears. *Fed Proc* 39: 2955-2958.

Nevretdinova ZG (1989) Seasonal rhythms in the functioning of the hypophyseo-thyroid system of the suslik *Citellus parryi. Zh Evol Biokhim Fiziol* 25: 310-317.

Nizielski SE, Morley JE, Bartness TJ, Seal US, and Levine AS (1986) Effects of manipulations of glucoregulation on feeding in the ground squirrel. *Physiol Behav* 36: 53-58.

Novoselova EG, Kolaeva SG, Makar VR, and Agaphonova TA (2000) Production of tumor necrosis factor in cells of hibernating ground squirrels Citellus undulatus during annual cycle. *Life Sci* 67: 1073-1080.

Olson JM, McNabb FM, Jablonski MS and Ferris DV (1999) Thyroid development in relation to the development of endothermy in the red-winged blackbird (*Agelaius phoeniceus*). *Gen Comp Endocrinol* 116: 204-212.

Reznik G, Reznik-Schuller H, Emminger A, and Mohr U (1975) Comparative studies of blood from hibernating and non-hibernating European hamsters (*Cricetus cricetus L*). *Lab Anim Sci* 25: 210-215.

Spurrier WA and Dawe AR (1973) Several blood and circulatory changes in the hibernation of the 13-lined ground squirrel, *Citellus tridecemlineatus. Comp Biochem Physiol* 44: 267-282.

Tomasi TE, Hellgren EC, and Tucker TJ (1988) Thyroid hormone concentrations in black bears (*Ursus americanus*): hibernation and pregnancy effects. *Gen Comp Endocrinol* 109: 192-199.

Wang LCH and Pehowich DJ (1985) Physiological and biochemical aspects of mammalian hibernation. In: Gilles R (ed), Circulation, respiration, and metabolism. Springer-Verlag, Berlin, pp 135-149.

Wang LCH (1989) Ecological, physiological, and biochemical aspects of torpor in mammals and birds. In: Wang LCH (ed), Advances in comparative and environmental physiology. Vol 4, Springer-Verlag, Berlin, pp 361-379.

Weekley LB (1995) Tissue and plasma peptidase activity is altered during hypothermic hibernation in the 13 lined ground squirrel (*Spermophilus tridecemlineatus*). *Physiol Behav.* 57: 595-598.

William AG and Peter M (1970) Cyclic changes in carbohydrate concentrations during hibernation in the arctic ground squirrel. *Am J Physiol.* 218: 1228-1232.

Young RA, Danforth E Jr, Vagenakis AG, Krupp PP, Frink R, and Sims EA (1979) Seasonal variation and the influence of body temperature on plasma concentrations and binding of thyroxine and triiodothyronine in the woodchuck. *Endocrinology* 104: 996-999.

Young RA (1984) Interrelationships between body weight, food consumption and plasma thyroid hormone concentration cycles in the woodchuck, *Marmota monax. Comp Biochem Physiol* 77: 533-536.

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