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A Comparison of Blood Buffering Capacity in Hibernators (*Mesocricetus auratus*) and Non-hibernators (*Mus musculus*) Acclimated to a Cold and Short Photoperiod

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Differences in blood buffering capacity and in blood glucose level homeostasis were examined in hibernators (golden hamsters) and non-hibernators (house mice) acclimated to a cold environment and short photoperiod. First, the pH and glucose levels were investigated in experimental animals maintained at a temperature of 22°C and a 12:12 light-dark cycle (control group) and then after a two-week period of adaptation to low ambient temperature and short photoperiod (temperature of 10°C and a 8:16 light-dark cycle) and additionally after 10 μl of 10% lactic acid was added to 450 μl of freshly collected blood samples. The pH of blood in cold acclimated animals decreased in comparison with control ones. In non-hibernators blood pH was significantly lower than in hibernators. The addition of lactic acid to blood samples results in a sharper drop of blood pH in non-hibernators than in hibernators. In control animals glucose levels didn't differ significantly between non-hibernators and hibernators, whereas after the cold acclimation period glucose levels in non-hibernators and hibernators stabilized at virtually the same but lower level. The results of blood glucose level measurements clearly indicated that this level – 130 mg/dl is the regulated level of glucose in the blood of both groups of animals. An improved buffering capacity of the blood revealed in hibernators could be considered as an adaptation to alleviate potentially negative effects of both respiratory acidosis and metabolic acidosis which accompany entrance into hibernation and arousal from hibernation. In conclusion the differences in buffering ability of blood to the resistance of changes of pH due to the addition of acid, revealed for non-hibernators and hibernators, indicate better adaptation of hibernators subjected to cold to their life in this environment.

Key words: pH, lactic acid, glucose, acid-base state, golden hamster, house mouse.

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Low ambient temperature in winter and limited access to food represent a challenge for homeothermic mammals, especially for small ones of less than one kilogram. These small-sized animals are exposed to the risk of a large loss of energy due to a high metabolic rate and a large, poorly isolated relative body surface. Thus, among these small-sized animals as many as six orders of mammals with the greatest number of representatives are able to hibernate and are also equipped with the most functionally efficient brown adipose tissue (BAT) *capable of vigorous thermogenic activity*. These two features are strongly associated with each other as revealed by the method of induction of maximal thermogenesis in BAT by intraperitoneal injection of noradrenaline (TĘGOWSKA & NARĘBSKI 1980). At thermoneutral temperature this induction results in an increase in body temperature reaching 37-38°C in non-hibernating mammals, whereas in hibernating mammals it yields to hyperthermia exceeding 42°C, i.e. life-threatening hyperthermia. However, even in the case of non-hibernators acclimated to cold, the metabolic rate in BAT is so fast that blood leaving this tissue exhibits a lack of oxygen, but is loaded with carbon dioxide instead (FOSTER & FRYD-

MAN 1978). This means that non-hibernators equipped with BAT are exposed to the risk of acidosis, for example when they are subjected to low ambient temperature. In the case of hibernators there is an additional and stronger risk of disturbance of the acid-base balance, especially during periods of entrance into hibernation and arousal from hibernation. Three phases of changes can be observed: (1) during entrance into hibernation – a period of decreased breathing rate – while carbon dioxide load results in blood acidosis – respiratory acidosis, (2) during arousal from hibernation – at the beginning of the phase a period of intense hyperventilation leading to a rapid respiratory alkalinization, (3) after alkalinization a period of metabolic acidification due to lactate production in shivering muscles; this phase is associated with shivering or non-shivering thermogenesis and a reduction of metabolic rate is observed along with stabilization of breathing during the phase and then progressively euthermic acid-base conditions are restored – the organism's pH homeostasis returns. Therefore it seems that animals subjected to such intense and multiple ups and downs of blood pH, undergoing acidosis as a physiological, transitory state during hibernation (MALAN *et al.* 1973), should be equipped with extremely effective blood buffering mechanisms, similar to those observed in burrowing mammals (HOLLOWAY & HEATH 1984). This is especially the case because hibernation is not a continuous process but is interrupted every 2-14 days (an average length of hibernation bout) by a series of energetically expensive, spontaneous arousals. During the arousals body temperature, lowered during hibernation, returns to a euthermic level, i.e. every 2-14 days animals risk blood pH fluctuations.

Although in the case of humans any disturbance of acidic-alkaline balance is always a pathological state, hibernating mammals benefit by acidosis in a way: for example when it contributes to depression of metabolic processes during entrance into hibernation (SNAPP & HELLER 1981). Thus immediate buffering of the pH would not be advantageous in certain cases. In this paper differences in buffering abilities of the blood between a hibernator (golden hamster) and a non-hibernator (house mouse) are assessed.

Acidosis contributes to a profound reduction of metabolic rate which is to some extent associated with inhibition of the glycolysis process and an increase in activity of gluconeogenic processes. Additionally, during deep torpor blood glucose concentrations are elevated which subjects hibernators to different blood glucose levels many times as hibernation is composed of a series of torpor bouts and short arousals. Non hibernators do not experience this. Therefore the blood glucose level was compared in hibernators and non-hibernators acclimated to cold.

Material and Methods

Experimental animals, six golden hamsters (*Mesocricetus auratus*) and six house mice (*Mus musculus*) were housed in individual cages. Standard food for rodents and water were offered *ad libitum*. Since in hibernators and non-hibernators the dependence of physiological processes upon ambient conditions (such as ambient temperature and length of daylight phase) could be different, animals of both groups were subjected to a two-week period of acclimation, not only to short photoperiod (8:16 light-dark cycle) but also to low ambient temperature (10°C). Besides these twelve experimental animals, six golden hamsters and six house mice (control group) which were not acclimated to low temperatures and short photoperiod (temperature of 22°C and a 12:12 light-dark cycle) were also examined.

All the experimental animals were anaesthetised with ketamine at a dose of 100 mg/kg to collect blood from the cervical vein into eleven heparinised capillary tubes. The measurements of blood pH were carried out using a Sentron 501 pH PocketFET pH-meter before and after lactic acid treatment. Lactate ions are generated in high concentrations during intense muscle activity, e.g. during shivering thermogenesis. After examination of blood pH from the first capillary tube, the remaining blood – from 10 capillary tubes (450 μl), was transferred into a porcelain container and treated with a lactic acid solution (10 μl in 100 μl of water) added in the amount of 10 μl and then the pH of the blood and lactic acid mixture was measured.

The glucose level was determined in blood using a Precision Q·I·D TM glucometer, produced by MediSense UK Ltd.

The use and handling of animals for this experiment were approved by the Local Ethical Committee.

Statistical analysis

The results were processed statistically by the use of a parametric test of differences between averages in Statistica with the Student's *t*-test, except for non-normally distributed data, which were compared using the Mann-Whitney U test. The normal distribution of tested data was examined by means of the Shapiro-Wilk test. The differences were considered to be significant at $P<0.05$.

Table 1

A comparison of blood pH levels in golden hamsters and house mice immediately after blood collection and after the addition of lactic acid solution

No.	Blood pH – golden hamsters			Blood pH – house mice		
	Control group	After acclimation		Control group	After acclimation	
		native blood	added lactic acid		native blood	added lactic acid
1	7.4	7.5	6.4	7.4	7.1	4.8
2	7.6	7.6	6.3	7.3	7.2	5.5
3	7.6	7.3	6.4	7.3	7.2	5.6
4	7.5	7.5	6.7	7.2	7.1	5.7
5	7.4	7.4	6.4	7.4	7.3	5.9
6	7.5	7.4	5.9	7.2	7.1	5.4
x	7.50	7.45	6.35	7.30	7.17	5.48
SD	0.089	0.105A	0.259B	0.089	0.082A	0.376B

Means with factors marked with the same letter are significantly different (P<0.01)

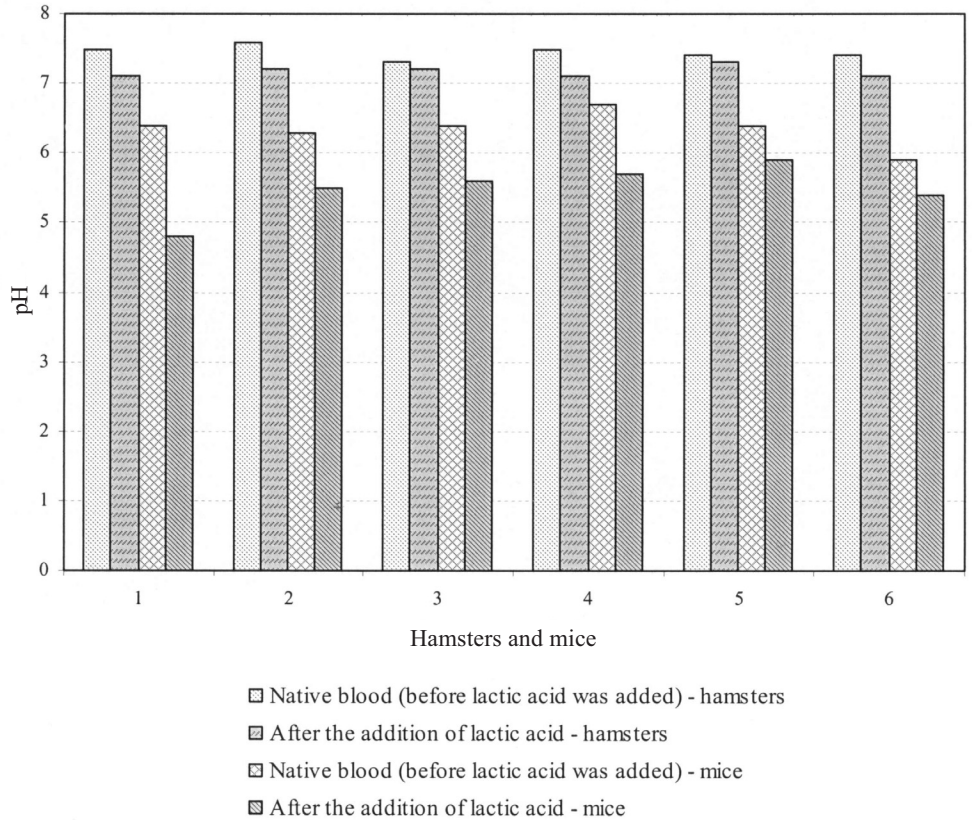

Fig. 1. A comparison of blood pH level in cold acclimated hibernators and non-hibernators immediately after blood collection and after the addition of lactic acid solution.

Results

As shown in Table 1, the pH of blood in the control group was 7.5 and 7.3 in golden hamster and house mouse, respectively. In cold acclimated animals blood pH was somewhat smaller: the pH change was of – 0.05 and of – 0.13 pH units compared to control values in golden hamster and house mouse, respectively, but these blood pH drops were not significant. Blood pH level in experimental animals acclimated to low temperature and short photoperiod was significantly higher in hibernators (golden hamsters) than in non-hibernators (house mice) at P<0.001. The addition of 10 μl of 10% lactic acid solution to blood samples resulted in a sharp drop of blood pH in both species of mammals. The pH drop was significantly higher (P<0.01) in house mice than in hamsters (1.69 vs 1.1).

Table 2

A comparison of blood glucose levels in hibernators and non-hibernators

No.	Glucose mg/dl			
	hamsters		mice	
	control group	after acclimation	control group	after acclimation
1	227	128	122	125
2	166	143	142	115
3	186	122	131	105
4	71	127	153	111
5	162	134	129	150
6	126	122	168	157
x	156.33	129.33	140.83	127.17
SD	53.29	8.04	17.20	21.53

Blood glucose levels in hibernators (golden hamsters) and non-hibernators (house mice) acclimated to low temperature and short photoperiod did not differ significantly from each other.

Figure 1 presents a comparison of blood pH levels in golden hamsters and house mice immediately after blood collection and after the addition of the lactic acid solution.

Blood glucose levels in hibernators (golden hamsters) and non-hibernators (house mice) acclimated to a low temperature and short photoperiod did not differ significantly from each other (Table 2).

Discussion

The differences in adaptation to lowered ambient temperature and short photoperiod in hibernators (golden hamsters) and non hibernators (house mice) were examined in this study; blood buffering capacities and homeostasis of glucose level in blood were the object of the study. These parameters were investigated in animals of a control group (T=22^{o}C and L:D 12:12) and then after a two-week period of adaptation to low temperature and short photoperiod (T=10^{o}C and L:D 8:16).

The blood pH level measured in hamsters acclimated to a low temperature was 7.45. This value is very close to pH values found during euthermia in other hibernators, but during the winter: *Citellus tridecemlineatus*: 7.38 (KENT & PEIRCE 1967), *Citellus tridecemlineatus*: 7.40 (MUSACCHIA & VOLKERT 1971), *Spermophilus tridecemlineatus*: 7.37 (FRERICHS *et al*. 1994), marmots: *Marmota monax* and *Marmota flaviventris*: 7.45 (GOODRICH & LYMAN 1971), European hamsters: *Cricetus cricetus*: 7.40 (MALAN *et al.* 1973). Unfortunately no data are available confirming that blood pH in these hibernators was (as in our experimental animals) higher before cold adaptation. Clearly it would be beneficial to know whether blood pH levels in these animals were higher or lower before adaptation to cold.

In cold acclimated house mice blood pH was 7.17. This suggests incomplete compensation of metabolic changes associated with cold stress, supported by the following observations: during the first few days after exposure to cold a few mice died. On the contrary, all hamsters survived. In the later stages of acclimation no incidents of death were noted among both mice and hamsters. Nevertheless the results indicated that cold stress was more harmful for mice than for hamsters.

Hibernation is characterized by dynamic changes of the acidic-alkaline balance (MALAN & MIOSKOWSKI 1988; MALAN *et al.* 1988) and a danger of hypoxia and temporary lactate load. Therefore it should be expected that hibernators (golden hamsters) have an elevated blood buffering capacity as compared to non-hibernators (house mice). This suggestion was supported by results obtained by GORDON and FOGELSON (1991). They showed that hibernating animals were more resistant to reduced oxygen content in environment and increased content of carbon dioxide. As mentioned before, these two factors could give rise to respiratory acidosis. However, there is no data currently available concerning possible differences in buffering abilities of blood to metabolic acidosis that is elicited not only during the period of exposure to cold (shivering thermogenesis in non-hibernators), but also during arousal from hibernation, when intense shivering thermogenesis yields the increased amount of lactate. Thus, lactic acid was used here and blood pH was measured before and after treatment. Addition of lactic acid to the blood was aimed to simulate natural lactate load in the blood and to determine to what extent experimental animals were able to buffer this acid.

Data presented in Table 1 clearly indicate that hibernators have a greater ability to compensate the excess of lactate as compared to non hibernators.

Both levels of pH: 6.35 vs 5.48 and magnitude of changes: 1.1 vs 1.69, respectively, in hamster and mouse prove this thesis.

It is worth mentioning that even if the pH of blood did not decreased, the decrease in buffering capacity (balanced acidosis) alone would be a severe disturbance of homeostasis since every additional effort could result in a sudden decrease in pH and induce inappropriate functioning of the organism (KOZŁOWSKI *et al.* 1970). Thus it would be extremely advantageous if animals were provided facilities for muscle effort (treadmill) in analogous experiments. Preliminary investigations (not presented in this paper) indicated a stronger decrease in pH level of blood after lactic acid treatment in such animals as compared with that in resting animals whose blood was treated with lactic acid as well. This suggests a decrease in buffering capacity. The decline in activity of animals while preparing for hibernation and entering it always during sleep are probably associated with the control of buffering capacity, which takes place in order to satisfy the demands of the arousal process.

The concentration of glucose in blood of examined rodents was also investigated. The experimental animals had constant access to food containing all necessary nutritional components. Thus, they were not subjected to hunger stress, which, as it was already reported, results in a decrease of glucose level in blood (KRILOWICZ 1985). Nevertheless a decrease of glucose level in blood was observed in the experimental animals.

A statistical comparison of glucose concentration in native blood of golden hamsters and house mice revealed an interesting relationships between the two species. Glucose levels in blood measured in animals of control group hibernators and non hibernators (156 vs 140 mg/dl), despite visible decreasing tendencies, did not differ significantly. The adaptation of animals to cold resulted in a decrease of blood glucose levels both in hibernators and non hibernators to similar values: 129 mg/dl and 127 mg/dl, respectively. They did not differ significantly from each other (Table 2). These results may suggest both different metabolic mechanisms responsible for cold stress compensation and indicate that glucose level is stabilised at the minimal physiological level even in the case of animals being in great danger of overcooling. This suggestion is supported by the well known fact that glucose homeostasis is maintained in the brain of hibernators (YEH *et al.* 1995), e.g. by release of glucose from glycogen. This phenomenon takes place when animals are exposed to severe cold and during starvation (KRILOWICZ 1985) as well. It was even suggested that acute cold (5°C) for 48 consecutive hours exerts an "insulin-like" effect on glucose uptake, i.e. glucose level increases in insulin-sensitive tissues such as skeletal and heart muscles, white and brown adipose tissues (VALLERAND *et al.* 1992). This effect (homeostasis of glucose level in tissues) could be responsible for a such significant decrease of blood glucose level in the experimental animals. A more drastic decrease of glucose level in hibernators could be associated with more efficient (and thus consuming glucose more efficiently and faster) BAT. In contrary to what was observed in the case of pH changes, where the pH levels in hamsters and mice varied much from each other, blood glucose levels stabilized at a similar level in both species. This suggests that the measured glucose levels, virtually the same in hibernators and non-hibernators, represent the minimal glucose levels necessary for the normal course of metabolic processes.

Both of the investigated parameters: changes of glucose level in blood and effectiveness of blood buffering capacities vary between hibernators and non hibernators. These differences suggest that hibernators exhibit better adaptation to life in low temperatures. However, more experimentation is required especially on torpid non hibernators to obtain an accurate conclusion.

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