

Effect of Cold Temperature and Food Restriction on Hematology in Male Striped Hamsters

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Abstract

Many physiological processes including hematological profiles are usually used to assess the health status of animals, which are influenced by environmental factors such as temperature and food availability. In order to test the hypothesis that cold temperature and food shortage would reduce these physiological function, 37 adult male striped hamsters (*Cricetulus barabensis*) were randomly classified into the fed and food restricted groups, which were further assigned into the warm ($23^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and cold ($5^{\circ}\text{C} \pm 1^{\circ}\text{C}$) groups, respectively. Cold temperature enhanced erythrocyte mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), but reduced blood platelet count (PLT) and plateletocrit (PCT), indicating the increase of oxygen carrying capacity and the decrease of blood coagulation ability under low temperature, respectively. However, food restriction had no effect on the hematological parameters including red blood cells (RBC), haemoglobin concentration (HGB), mean corpuscular hemoglobin (MCH), haematocrit (PCV), MCV, PLT and so on. These results indicated that food shortage did not affect the oxygen-carrying capacity and blood coagulation ability. In conclusion, cold temperature and food shortage exert different influences on hematological parameters.

Keywords

Hematological Parameters, Cold Temperature, Food Restriction, Striped Hamsters (*Cricetulus barabensis*)

1. Introduction

Many physiological processes such as hematology are influenced by environmen-

tal factors such as temperature and food availability in animals [1]. Hematological profiles are usually used to monitor and assess the health status of animals, which are influenced by cold temperature and food shortage [1] [2]. For example, red blood cells (RBC), hemoglobin (HGB) and hematocrit (PCV) are indicative of animal metabolism related to the environment via the circulatory system [2] [3]. Generally, cold temperature increases the numbers of RBC and PCV in human and animals [4]-[6]. Similarly, hematological parameters are also impacted by food shortage. For instance, food restriction enhanced red blood cells (RBC), hemoglobin (HGB) and hematocrit (PCV) in rats [7]. Different degrees of food restriction may exert different influences on hematological parameters. For example, mild food restriction increased, but moderate food restriction did not affect red blood cells (RBC), hemoglobin (HGB) and hematocrit (PCV) in Wistar rats compared with the fed controls [8].

The striped hamster (*Cricetulus barabensis*) primarily distributes in northern China, Russia, Mongolia, and Korea [9]. This species is granivorous, nocturnal, solitary and feeds mainly on stems and leaves of plants during summer and on foraging crop seeds in winter [10]. The climate is characterized by warm and dry summer (extreme maximum temperatures is 42.6°C) and cold winter (extreme minimum temperatures below -20°C) [9] [11]. Thus hamsters must experience great seasonal variations in photoperiod, temperature, and food availability [12]. We have previously found that cellular and humoral immunity showed seasonal changes and they were also influenced by photoperiod, food restriction and cold temperature [12]-[14]. In the current study, we want to know how hematology would respond to the changes of temperature and food resources. We expected that cold temperature and food restriction would decrease hematological profiles in hamsters.

2. Materials and Methods

2.1. Animals and Experimental Design

The present study abided by Chinese laws and the requests of the Animal Care and Use Committee of Qufu Normal University. Samples of male striped hamsters were collected from Jiuxian Mountain (35°46.275'N, 116°59.976'E) in Qufu City, Shangdong province, China. Animals were raised alone in plastic cages (30 cm × 15 cm × 20 cm), and the bedding material was sawdust, the photoperiod was set as 12 h:12 h light-dark cycle (12 L:12 D) and room temperature was 23°C ± 1°C. Hamsters had free access to the food (i.e., standard rat pellets chow, Beijing KeAo Feed Co., Beijing, China) and water during the whole study. The captured adult hamsters were raised in laboratory for about four weeks, and then 37 males were chosen and randomly classified into the fed and food restricted groups, which were further assigned into the warm (23°C ± 1°C) and cold (5°C ± 1°C) groups, respectively. Consequently, there were four groups: the warm and fed ad libitum group (WF, n = 9), warm and food restricted group (WR, n = 10), cold and fed ad libitum group (CF, n = 9), and cold and food restricted group (CR, n = 9). The

sample size is adequate for statistical analysis based on the 3R principle of experimental animals. For animal welfare, males underwent a 10% food restriction for 19 days in the current experiment because of their sensitivity to food scarcity [15] [16]. Striped hamsters housed at room temperature began to die after 20 days of 10% food restriction (i.e. fed 90% of baseline food intake when hamsters ate ad libitum) (Professor Zhao Zhijun, Wenzhou University, personal communication). Therefore, we decided that hamsters would be subjected to 10% food restriction for 19 days in the present study, given their sensitivity to food shortage [15] [16]. Baseline food intake (g/day) of hamsters used for food restriction was measured for 6 days (once every other day). Average food consumption per day was calculated for each individual, and the restricted food amount was 90% of the baseline food intake. Baseline food intake (g/day) of each hamster was examined for 3 times (once every other day), and then average food intake per day for each animal was determined. The degree of food restriction was 90% of the baseline food intake. It was noteworthy that the experimental design and animals used were the same with our previously published paper [14].

2.2. Hematological Parameters Assays

At the end of the experiment, animals were killed and trunk blood was collected, and then 20 μ L whole blood was diluted immediately in 4 mL diluent, and after about 1 h later haematological parameters were counted in the Hematology Analyzer (Auto Counter 910EO+). Red blood cells (RBC), haemoglobin (Hb) concentration (HGB), mean corpuscular hemoglobin (MCH), haematocrit or packed cell volume (PCV), erythrocyte mean corpuscular volume (MCV), Mean corpuscular Hb concentration (MCHC), red blood cell distribution width (RDW) are indicative of oxygen-transport capacity [17]. Blood platelet count (PLT), plateletocrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) are reflective of coagulation ability [18]. The results of white blood cells (WBC), lymphocytes (LYMP), lymphocyte percent (LYMP%), intermediate granulocytes (MID) including eosinophil and basophil granulocytes, intermediate granulocytes percent (MID%), neutrophilic granulocytes (GRAN), neutrophil granulocytes percent (GRAN%) have been published in our previous paper [14].

2.3. Statistical Analysis

Data were analyzed using SPSS 27.0 software (SPSS Inc., Chicago, IL, USA). Prior to all statistical analyses, data were examined for normality and homogeneity of variance, using Kolmogorov-Smirnov and Levene tests, respectively. Group differences in hematological parameters were analyzed by a two-way ANOVA followed by Bonferroni *post hoc* tests. Significant group differences were further evaluated by one-way ANOVA followed by Turkey's *post hoc* tests. Results are presented as mean \pm SE, and $P < 0.05$ was considered to be statistically significant.

3. Results

Cold temperature increased MCV (Figure 1(c)), MCH (Figure 1(f)), while decreased PLT (Figure 1(h)), PCT (Figure 1(i)), and had no effect on RBC, PCV, RDW, HGB, MCHC, MPV and PDW (Figure 1, Table 1). All hematological parameters including RBC, PCV, MCV, RDW, HGB, MCH, MCHC, PLT, PCT, MPV and PDW were not influenced by food restriction or the interaction of temperature \times food restriction except that MCHC was affected by their interaction (Figure 1, Table 1).

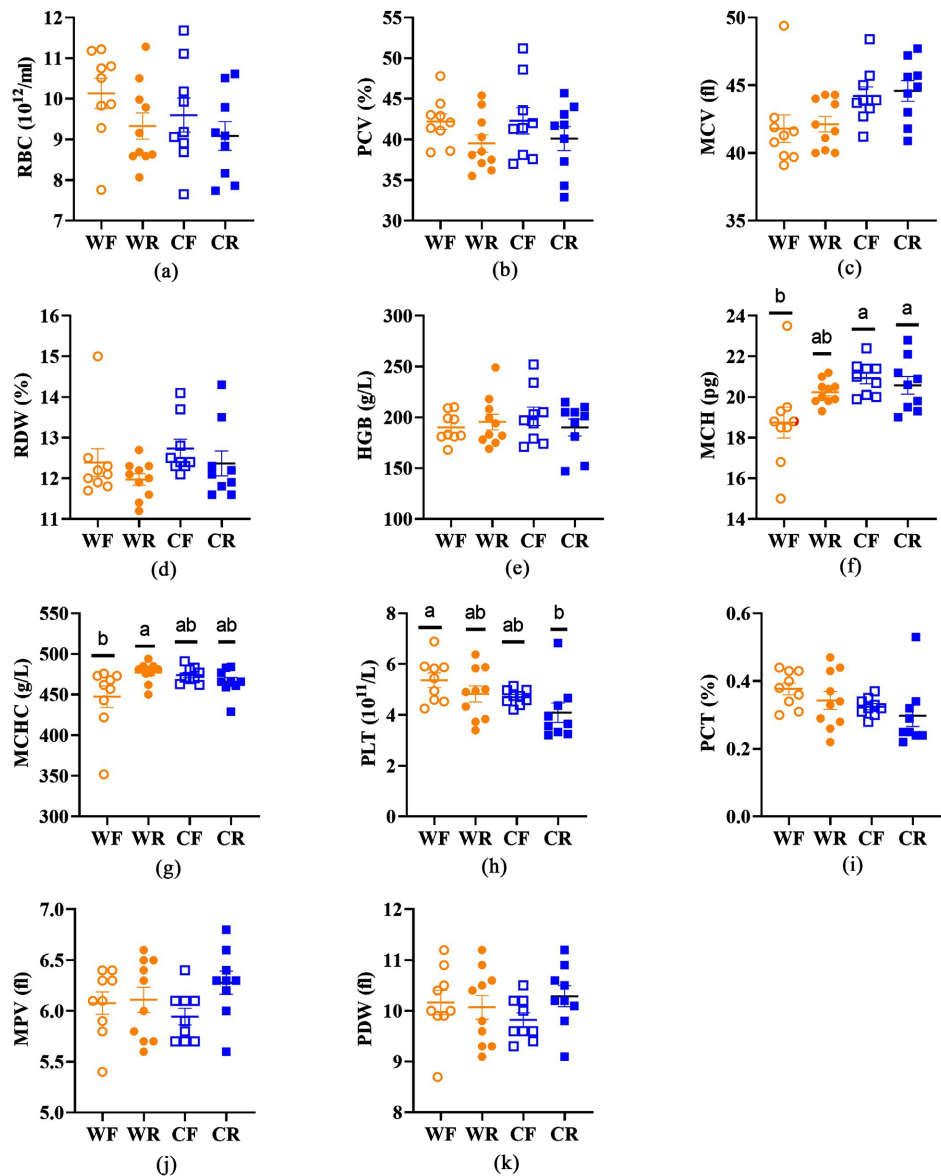


Figure 1. Effect of cold temperature and food restriction on hematological profiles in male striped hamsters. Different letters (a, b or ab) above the scattered dots indicate significant difference at $P < 0.05$ in each organ, determined by a two-way ANOVA and Bonferroni post-hoc tests. CR: the cold and food restricted group; CF: the cold and fed *ad libitum* group; WR: the warm and food restricted group; WF: the warm and fed *ad libitum* group.

Table 1. Effect of cold temperature and food restriction on the hematological parameters in striped hamsters.

	WF	WR	CF	CR	Temperature		Food		T × F	
	9	10	9	9	F _{1,33}	P	F _{1,33}	P	F _{1,33}	P
RBC (10 ¹² /L)	10.1 ± 0.4	9.3 ± 0.3	9.6 ± 0.4	9.1 ± 0.4	1.121	0.297	3.234	0.081	0.160	0.692
PCV (%)	42.2 ± 1.0	39.5 ± 1.1	42.3 ± 1.6	40.1 ± 1.5	0.077	0.783	3.543	0.069	0.034	0.856
MCV (fl)	41.8 ± 1.0	42.1 ± 0.6	44.2 ± 0.7	44.6 ± 0.8	9.960	0.003	0.206	0.653	0.001	0.970
RDW (%)	12.4 ± 0.3	12.0 ± 0.1	12.7 ± 0.2	12.4 ± 0.3	2.016	0.165	2.265	0.142	0.010	0.921
HGB (g/L)	190.1 ± 4.8	195.5 ± 7.7	201.0 ± 9.0	190.1 ± 8.3	0.129	0.722	0.129	0.722	1.130	0.295
MCH (pg)	18.7 ± 0.8 ^b	20.2 ± 0.2 ^{ab}	20.9 ± 0.3 ^a	20.6 ± 0.4 ^a	7.625	0.009	1.552	0.222	4.093	0.051
MCHC (g/L)	447.3 ± 13.2 ^b	477.1 ± 4.0 ^a	474.1 ± 3.2 ^{ab}	465.7 ± 5.5 ^{ab}	1.057	0.311	2.042	0.162	6.558	0.015
PLT (10 ⁹ /L)	536.3 ± 28.4 ^a	482.6 ± 31.7 ^{ab}	470.0 ± 10.0 ^{ab}	408.7 ± 38.2 ^b	5.743	0.022	3.865	0.058	0.017	0.897
PCT (%)	0.38 ± 0.02	0.34 ± 0.03	0.32 ± 0.01	0.30 ± 0.03	4.399	0.044	1.686	0.203	0.023	0.881
MPV (fl)	6.1 ± 0.1	6.1 ± 0.1	5.9 ± 0.1	6.3 ± 0.1	0.025	0.876	2.779	0.105	1.885	0.179
PDW (fl)	10.2 ± 0.2	10.1 ± 0.2	9.8 ± 0.1	10.3 ± 0.2	0.089	0.767	0.772	0.386	1.789	0.190

Data are mean ± s.e.m. Firstly, values were analyzed by a two-way ANOVA and Bonferroni *post-hoc* tests. Group differences were further examined by one-way ANOVA followed by Turkey's *post hoc* tests, in which a specific parameter that share different superscripts (a, b or ab) are significantly different at $P < 0.05$. WF: the warm and fed *ad libitum* group; WR: the warm and food restricted group; CF: the cold and fed *ad libitum* group; CR: the cold and food restricted group. T × F indicates the interaction of cold stress × food restriction. RBC: red blood cells, PCV or HCT: red blood cell specific volume (Packed cell volume or haematocrit), MCV: erythrocyte meancorpuscular volume, RDW: red blood cell distribution width, HGB: hemoglobin concentration, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular Hb concentration, PLT: blood platelet count, PCT: plateletocrit, MPV: mean platelet volume, PDW: platelet distribution width.

4. Discussion

In the present study, we found that cold treatment significantly increased MCV and MCH in male hamsters, implying the increased oxygen carrying capacity of the plasma upon low temperature exposure. The higher oxygen-transport capacity in cold-adapted hamsters might be compatible with the augment of food intake, metabolism and thermogenic capacity across many species including striped hamster during winter or under low temperature [19]-[22]. However, cold exposure decreased PLT and PCT, indicating reduction of blood coagulation ability under low temperature. Moreover, cold temperature had no effects on RBC, PCV, RDW, HGB, MCHC, MPV and PDW in male hamsters. These results were inconsistent with other researches in which cold exposure led to the increase of RBC and HGB in cold-adapted frogs [23], or RBC and PCV in birds [4]-[6], PCV in American bullfrogs (*Rana catesbeiana*) [24] and RBC increased in cold water swimmers [25]. All the hametological parameters detected in male hamsters were not affected by food restriction. These results were inconsistent with other research in which food restriction increased RBC, PCV and HGB in the albino rats [7], or food restriction had no negative effects on hematological parameters such as RBC, PCV and HGB in Wistar rats [8]. The distinct results in different studies may be due to the dif-

ferences in the species used, sex, age or the experimental protocols.

In summary, cold temperature enhanced MCV and MCH, but reduced PLT and PCT, indicating the increase of oxygen carrying capacity and the decrease of blood coagulation ability under low temperature, respectively. However, food restriction had no effect on the hematological parameters detected such as RBC, HGB, MCH, PCV, MCV, PLT and so on, implying that food shortage did not affect the oxygen-carrying capacity and blood coagulation ability. In conclusion, cold temperature and food shortage exert different influences on hematological parameters. One limitation in the present research was that only males were studied and future work that includes females is needed to test for sex-specific responses.

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Authors' Contributions

D. L. X. designed the study and supervised the analyses. X. Y. L. examined the hematological parameters and wrote the draft paper. X. K. H. performed the experiment. D. L. X. revised the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare that we have no competing interests.

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