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Effect of Shortening Day Length on Immune Function in Siberian Hamsters

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Abstract

In order to understand the effect of gradual changes in photoperiod on immune function, adult female Siberian hamsters (*Phodopus sungorus*) were randomly divided into the control group (12L:12D, Con, n = 11) and the shortening day length group (SD, n = 11), in which day length was reduced from 12:12 h to 8:16 h light-dark cycle at the pace of half an hour every week. Meanwhile the winter immunoenhancement hypothesis, which holds that animals' immune function would be enhanced in winter or winter-like conditions, was tested. Gradual shortening day length had no effect on body mass and body composition including wet carcass mass, the subcutaneous, retroperitoneal, mesenteric and total body fat masses in Siberian hamsters. The masses of liver and small intestine with contents were higher in the SD group than in the Con group, however other organ masses such as brain, heart, kidney and so on did not differ between the two groups. Phytohemagglutinin (PHA) response after 24 h of PHA injection was enhanced by the shortening photoperiod, which supported the winter immunoenhancement hypothesis. The masses of spleen and thymus, white blood cells, bacteria killing capacity indicative of innate immunity were not affected, which did not support this hypothesis. In summary, gradually decrease in day length increased cellular immunity, but had no effect on other immunological parameters in Siberian hamsters.

Keywords

Immune Function, Photoperiod, Siberian Hamsters (*Phodopus sungorus*)

1. Introduction

Photoperiod is an error free environmental cue and is generally used by animals

in non-tropical areas to estimate the time of the year. The winter immunoenhancement hypothesis postulates that animals' immune function would be enhanced in winter to offset the detrimental effects of stressors such as low temperature and food shortage in this season [1]. Short photoperiod is one of the prominent characteristics in winter. This hypothesis has been tested both in the field and laboratory researches. For example, immune function increases in some species [2] [3], while decreases in other animals [4] during winter compared with spring and summer. The laboratory studies also have shown that immune function can be boosted by short day length [1] [5], but immunity was reduced in other species [6] [7]. The discrepancies may be due to the differences in the species used and the experimental regime. Therefore further studies are required to clarify these discrepancies.

Siberian hamsters (*Phodopus sungorus*) mainly distribute in the northern part of Hebei Province and most parts of Inner Mongolia Autonomous Region. This species is confronted with seasonal changes in photoperiod in the natural environment [8]. Some researchers have investigated the effect of short photoperiod or winter on immune function in this species. For instance, delayed-type hypersensitivity (DTH) to 2,4-dinitrofluorobenzene (DNFB) and natural killer (NK) cell cytolytic activity indicative of cell mediated and innate immunity, respectively, increased in Siberian hamsters under short day length or in winter [5] [6]. However, lymphocyte proliferation indicative of cell mediated immunity [6], phagocytosis and fever both indicative of innate immunity [6] [9], and wound healing rate [10] all decreased under short photoperiod or in winter. These studies demonstrated that the different findings may be related to the parameters detected. In this study, the effect of shortening day length on immunological parameters were carried out to test the winter immunoenhancement hypothesis.

2. Materials and Methods

2.1. Animals and Experimental Design

All animal procedures were performed in accordance with the guidelines of the Animal Care and Use Committee of Qufu Normal University. Adult female Siberian hamsters used in this study were bought from Qufu Pet shop in Xiguan Market. The hamsters were housed individually in plastic cages (30 cm × 15 cm × 20 cm) with sawdust as bedding under a constant photoperiod of 12L:12D (12 h:12 h light-dark cycle) and temperature of 23˚C ± 1˚C. Standard rat pellets chow (Animal Feeding Center in Jining Medical University, Jining, China) and water were provided *ad libitum* throughout the experiment. After body mass stabilized, 22 female hamsters were selected and randomly assigned into the control group (12L:12D, Con, n = 11) and the shortening day length group (SD, n = 11), in which day length was reduced from 12:12 h light-dark cycle to 8:16 h light-dark cycle at the pace of half an hour per week. The experiment time lasted for 9 weeks.

2.2. Body Composition and Organs

Body composition and organs were measured as described in Xu and Wang (2010)

[11]. In brief, the visceral organs, including heart, thymus, lungs, liver, spleen, kidneys, paired adrenal glands, gonad (uterus and ovaries) and the digestive organs with contents (*i.e.*, stomach, small intestine, caecum, and colon) were dissected and weighed (±1 mg). The stomach, small intestine, caecum, and colon were rinsed with saline to eliminate all the gut contents, before being weighed. All the visceral organs were removed to obtain wet carcass. Moreover, subcutaneous fat, mesenteric fat and retroperitoneal fat were also dissected carefully and weighted. All the three parts of fat mass were regarded as total body fat mass. The percent content of subcutaneous, mesenteric, retroperitoneal, and total body fat were obtained by dividing the wet carcass mass, respectively [11] [12].

2.3. Cellular Immunity Assays

PHA response was measured as described previously [11]. Specifically, hamsters in the Con and SD groups on day 56 were caught, and then we measured their footpad thickness of the left hind foot with a micrometer (Digimatic Indicator ID-C Mitutoyo Absolute cod. 547-301, Japan) to ±0.01 mm. Immediately thereafter, hamsters in the two groups were injected subcutaneously 0.1 mg of PHA (PHA-P, Sigma L-8754) dissolved in 0.03 mL of sterile saline (pH 7.4) in the middle of the footpad. After 6 h, 12 h, 24 h, 48 h and 72 h injection, we measured the footpad thickness. The PHA response (*i.e.*, cellular immunity) was calculated as the difference between pre- and post-injection measurements divided by the initial footpad thickness (PHA response = (post PHA − pre PHA)/pre PHA). Six measures of footpad thickness were taken to obtain the value of each hamster [11].

2.4. White Blood Cells Assays

At the end of the experiment, after collecting trunk blood, 20 μL whole blood was diluted immediately in 0.38 mL solution containing 1.5% glacial acetic acid, 1% crystal violet (Sigma) and the leukocytes were counted in an improved Neubauer chamber using microscope. The total number of WBC was determined by counting all leucocytes in the four corner large-squares of the Neubauer chamber, and multiplying the raw data by 5×10^7 to obtain the final values (10^9 cells/L) [11] [13].

2.5. Innate Immunity

Serum bacterial killing capacity indicative of innate immunity was performed in a sterile laminar flow cabinet to assess the functional response by the animal's innate immune system against a relevant pathogen, *Escherichia coli* [12] [14]. Briefly, serum samples were diluted 1:20 in CO_2-independent medium (Gibco no. 18045, Carlsbad, GA, USA). A standard number of colony-forming units (CFUs) of *E. coli* (ATCC no. 8739, Microbial Culture Collection Center of Guangdong Institute of Microbiology, China) was added to each sample in a ratio of 1:10, and the mixture (*i.e.*, a number of *E. coli* dissolved in CO_2-independent medium, serum samples and CO_2-independent medium) was allowed to incubate at 37˚C for

30 min to induce bacterial killing. After incubation, 50 μl of each sample was added to tryptic soy agar plates in duplicate. All plates were covered and left to incubate upside down at 37˚C for 24 h, and then total CFUs were counted and bactericidal capacity was calculated as 100% minus the mean number of CFUs for each sample divided by the mean number of CFUs for the positive controls (containing only medium and standard bacterial solution), *i.e.* the percentage of bacteria killed relative to the positive control.

2.6. Statistical Analysis

Data were analyzed using SPSS 18.0 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. The ratio values of PHA response were subjected to arcsine transformation. Group differences in body mass on each day, body composition, WBC and innate immunity were analyzed by the independent Sample T-test. Group differences in organ masses with body mass as the covariate were analyzed by General Linear Model multivariate analysis followed by Bonferroni *post hoc* tests. Group differences in PHA response were analyzed by the repeated measure of ANOVA by followed by Bonferroni post hoc tests. PHA response at each time point between the two groups was analyzed by the independent Sample T-test. Results are presented as mean ± s.e.m, and $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Body Mass

Body mass between the Con and SD groups did not differ from the beginning (t = 0.343, df = 20, $P = 0.735$) to the end of the experiment (t = −0.065, df = 20, $P = 0.949$) (Figure 1).

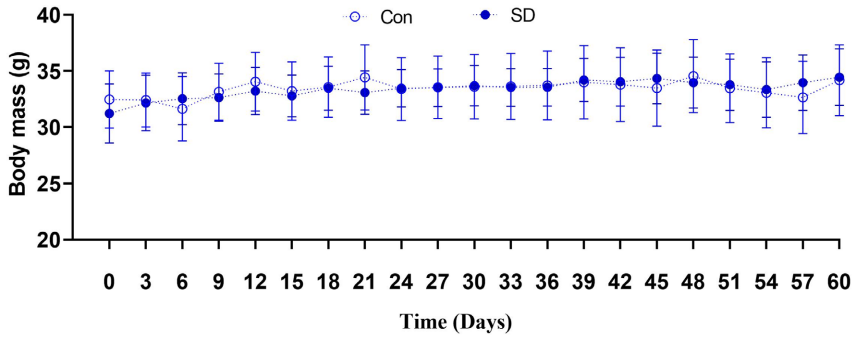

Figure 1. Effect of shortening day length on body mass in Siberian hamsters.

3.2. Body Composition

Shortening day length had no effect on body composition including wet carcass mass, subcutaneous, retroperitoneal, mesenteric, total body fat mass and their respective fat percent content in Siberian hamsters (Table 1).

Table 1. Effect of shortening day length on body composition in Siberian hamsters.

Parameters	Groups		Statistical summary	
	Con	SD	t	*P*
Sample size	11	11		
Initial body mass (g)	32.5 ± 2.55	31.3 ± 2.6	−0.343	0.735
Final body mass (g)	34.2 ± 3.2	34.5 ± 2.5	−0.065	0.949
Wet carcass mass (g)	24.5 ± 2.35	24.05 ± 1.65	−0.150	0.882
Subcutaneus fat (g)	1.766 ± 0.434	1.449 ± 0.254	−0.631	0.535
Subcutaneus fat content (%)	5.7 ± 0.9	5.2 ± 0.7	−0.368	0.717
Retroperitoneal fat (g)	0.233 ± 0.576	0.200 ± 0.298	−0.513	0.614
Retroperitoneal fat content (%)	0.8 ± 0.1	0.8 ± 0.1	−0.032	0.975
Mesenteric fat (g)	0.503 ± 0.750	0.426 ± 0.288	−0.956	0.350
Mesenteric fat content (%)	1.8 ± 0.1	1.6 ± 0.1	−1.113	0.279
Total body fat (g)	2.502 ± 0.544	2.074 ± 0.290	−0.693	0.496
Total body fat content (%)	8.2 ± 1.1	7.6 ± 0.8	−0.441	0.664

Values are means ± SE. Values for a specific parameter that shares different superscripts are significantly different at $P < 0.05$, determined by independent t-test analysis.

3.3. Organs

Decreasing photoperiod increased the masses of the liver and small intestine with contents, but did not affect other organ masses such as the heart, lungs, thymus, spleen, stomach and so on (Table 2).

Table 2. Effect of shortening day length on wet organ mass in Siberian hamsters.

Parameters	Groups		Statistical summary	
	Con	SD	$F_{1,19}$	*P*
Sample size	11	11		
IBAT (g)	0.106 ± 0.358	0.120 ± 0.033	1.635	0.216
Brain (g)	0.372 ± 0.019	0.392 ± 0.036	2.999	0.100
Heart (g)	0.195 ± 0.048	0.214 ± 0.034	1.524	0.232
Lungs (g)	0.272 ± 0.066	0.296 ± 0.060	0.781	0.388
Thymus (g)	0.017 ± 0.099	0.019 ± 0.008	0.248	0.624
Liver (g)	1.520 ± 0.360[b]	1.814 ± 0.629[a]	4.550	0.046
Spleen (g)	0.164 ± 0.079	0.275 ± 0.319	1.200	0.287
Kidneys (g)	0.368 ± 0.123	0.390 ± 0.104	0.650	0.430
Adrenal gland (g)	0.013 ± 0.006	0.013 ± 0.008	0.002	0.964
Gonads (Uterus and ovary) (g)	0.196 ± 0.214	0.155 ± 0.076	0.446	0.512
Stomach with contents (g)	1.272 ± 0.336	1.311 ± 0.521	0.057	0.815
Stomach (g)	0.322 ± 0.070	0.300 ± 0.085	0.861	0.365

Continued

Small intestine with contents (g)	1.384 ± 0.217[b]	1.593 ± 0.421[a]	4.875	0.040
Small intestine (g)	0.582 ± 0.209	0.516 ± 0.218	0.838	0.371
Small intestine length (cm)	27.136 ± 3.073	28.346 ± 3.778	1.425	0.247
Caecum with contents (g)	0.904 ± 0.159	1.030 ± 0.323	1.994	0.174
Caecum (g)	0.224 ± 0.064	0.244 ± 0.097	0.343	0.565
Caecum length (cm)	5.236 ± 1.419	4.982 ± 1.378	0.178	0.678
Colon with contents (g)	0.600 ± 0.217	0.695 ± 0.242	1.746	0.202
Colon (g)	0.267 ± 0.110	0.270 ± 0.078	0.001	0.971
Colon length (cm)	12.673 ± 2.425	14.118 ± 2.213	3.412	0.080
Total digestive tract (g)	1.073 ± 0.337	1.030 ± 0.324	0.215	0.648
Total digestive tract length (cm)	45.046 ± 4.345	47.446 ± 6.234	2.662	0.119

Data are mean ± SE. Values for a specific parameter that share different superscripts are significantly different at $P < 0.05$, determined by General Linear Model multivariate analysis with body mass as the covariate followed by Bonferroni post hoc tests.

3.4. White Blood Cells

WBC did not differ between the Con and SD groups (t = −0.509, df = 20, P = 0.616) (Figure 2).

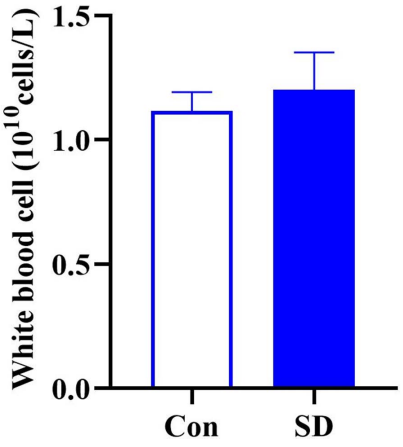

Figure 2. Effect of shortening day length on white blood cells in Siberian hamsters.

3.5. Cellular Immune Response

PHA response changed significantly with time ($F_{4,80}$ = 59.366, $P < 0.001$), and differed between the Con and SD groups ($F_{4,80}$ = 3.018, P = 0.023) (Figure 3). Specifically, the SD hamsters had higher PHA response after 24 h of PHA injection (t = 2.337, df = 20, P = 0.026), however PHA response after 6 h (t = 1.926, df = 20, P = 0.068) , 12 h (t = 0.873, df = 20, P = 0.393), 48 h (t = −0.602, df = 20, P = 0.554), 72 h (t = −0.062, df = 20, P = 0.951) of PHA injection did not differ between the two groups.

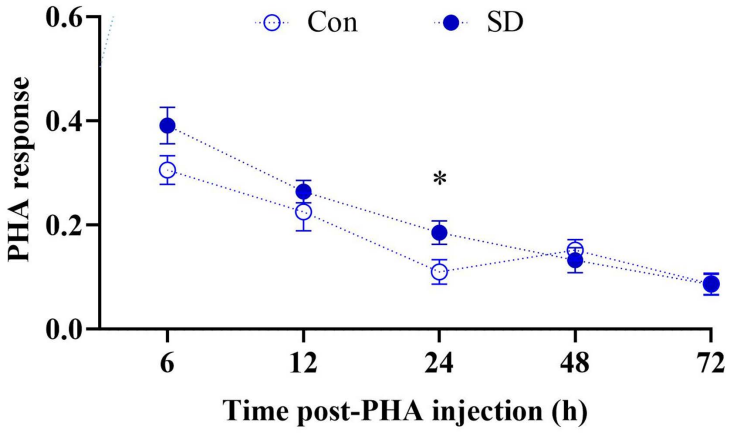

Figure 3. Effect of shortening day length on PHA response in Siberian hamsters. Asterisk (*) indicates significant group differences.

3.6. Innate Immunity

There was no group difference in bacterial killing capacity (BKA) indicative of innate immunit (t = −1.538, df = 19, P = 0.140) (Figure 4).

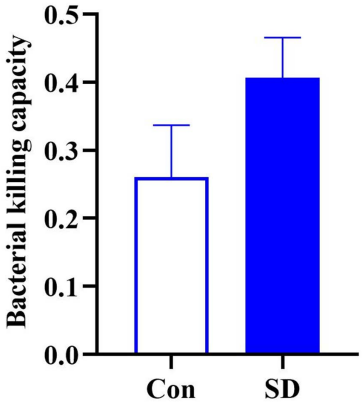

Figure 4. Effect of shortening day length on innate immunity in Siberian hamsters.

4. Discussion

Our data demonstrated that gradually decrease in photoperiod had no effect on body mass in Siberian hamsters. As expected, shortening day length increased PHA response post 24 hours of PHA injection, suggesting its enhancing effect on cellular immunity in Siberian hamsters, which supported the winter immunoenhancement hypothesis. This finding agreed with other researches in which short day increased cellular immunity compared with long day [1] [5] [15] [16]. The enhancement of cellular immunity under short day length may lie in that the increased secretion of melatonin which can boost immune responses [16]-[19]. Previous study has shown that melatonin mediates the effect of short photoperiod on humoral immunity in male Siberian hamsters [20], the effect of melatonin on cellular immunity in this species needs further research. The improvement of cellular

immunity in Siberian hamsters in the present study was inconsistent with our previous research in striped hamsters (*Cricetulus barabensis*) in which cellular immunity was suppressed under short day length compared with the long day length [21]. The reason may be the differences in the species used and the experimental regimes in which striped hamsters were acclimated directly under 8:16 h light-dark cycles for 54 days [21]. No significant impact of shortening day length on total body fat mass, thymus and spleen mass, WBC, bacteria killing capacity indicates that different elements of immune system responded to the decrease of day length distinctly in this species, which did not support the winter immunoenhancement hypothesis.

5. Conclusion

In summary, body mass, body composition such as total body fat mass, and most organ masses were not affected by the gradual decrease in day length in Siberian hamsters. In addition, short photoperiod had no influence on the wet masses of spleen and thymus, WBC and innate immunity, which did not support the winter immunoenhancement hypothesis. However, short photoperiod boosted cellular immunity, which supported this hypothesis. Therefore, further studies are needed to test this hypothesis in more species in future.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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