Trends Journal of Sciences Research, 2018, Volume 3, No 1, 1-9
http://www.tjsr.org/journal/index.php/tjsr
DOI: 10.31586/Biology.0301.01

Effect of Gradually Decreasing Photoperiod on Immune Function in Siberian Hamsters

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OPEN ACCESS Research Article

How to cite this article: Tian, Y.F., Zhao, H.S., Xu, D.L., Zhao, M.F., Zhang, Q.Q., Zhao, Q.Z., Zhang, Y.Q., Zhang, Q.L., Hu, X.K., Li, Z.Y. and Wang, Y. (2018). Effect of Gradually Decreasing Photoperiod on Immune Function in Siberian Hamsters. *Trends Journal of Sciences Research*, 3(1), 1-9. https://doi.org/10.31586/Biology.0301.01

Received: April 30, 2018

Accepted: June 25, 2018

Published: June 26, 2018

Abstract Animals usually use photoperiod as an important environmental cue to time the year. In terms of the winter immunocompetence enhancement hypothesis, animals in the non-tropical zone would actively enhance their immune function to decrease the negative influence of stressors such as low temperature and food shortage in winter. In the present study, we mimicked the transition from summer to winter by decreasing photoperiod gradually and examined the variations of immune repsonses in Siberian hamsters (Phodopus sungorus) to test this hypothesis. Twenty two female adult hamsters were randomly divided into the control (12h light: 12h dark, Control, n=11) and the gradually decreasing photoperiod group (Experiment, n=11). In the experiment group, day length was decreased from 12 h: 12 h light-dark cycle to 8 h: 16 h light-dark cycle at the pace of half an hour per week. We found that gradually decreasing photoperiod had no effect on body composition (wet carcass mass, subcutaneous, retroperitoneal, mesenteric and total body fat mass) and the masses of the organs detected such as brain, heart, liver and so on in hamsters. Similarly, immunological parameters including immune organs (thymus and spleen), white blood cells and serum bacteria killing capacity indicative of innate immunity were also not influenced by gradually decreasing photoperiod, which did not support the winter immunocompetence enhancement hypothesis. However, gradually decreasing photoperiod increased phytohaemagglutinin response post-24h of PHA challenge, which supported this hypothesis. There was no correlation between cellular, innate immunity and body fat mass, suggesting that body fat was not the reasons of the changes of cellular immunity. In summary, distinct components of immune system respond to gradually decreasing photoperiod differently in Siberian hamsters.

Keywords: *Phytohaemagglutinin response, Bacteria killing capacity, Body fat, Photoperiod, Siberian hamsters (Phodopus sungorus)*

Introduction

Photoperiod is an important environmental cue which is usually used by animals to time the year [1]. Therefore immune function in birds and mammals often demonstrates seasonal changes [1,2]. The winter immunocompetence enhancement hypothesis holds that animals in the temperate area would actively enhance immune responses against the negative effect of stressful conditions in winter (i.e., low temperature and decreased food availability). This hypothesis was supported by some field studies [3,4,5,6], but was against by other field studies [7,8,9]. In general, this hypothesis was supported in the laboratory studies in which immune enhancement can be induced by short days [1,10,11,12]. However, some other researches obtained opposite results. For example, the transition from long to short photoperiods reduced T-cell dependent antibody production in Siberian hamsters [13,14]. Therefore, more studies were required to clarify theses discrepancies.

Innate immunity, which is one arm of immune system, is usually assessed by serum bacterial killing capacity [15,16,17]. Phytohaemagglutinin (PHA) response, which indicates cellular immunity, involves a subcutaneous injection of PHA that induces local T-cell stimulation and proliferation resulting in swelling [18,19]. It belongs to adaptive immune system which is responsible for controlling intracellular pathogens [20]. Immune organs (i.e., thymus and spleen) are indirect immunological parameters [21,22]. White blood cells are also used to assess the overall health [20]. Moreover, adipose tissues have been regarded as important endocrine and immune organs besides its action as energy reserves [23,24,25].

Siberian hamsters (*Phodopus sungorus*), which are small seasonal breeding and granivorous rodents, live mainly in northern China [26,27]. Previous researches have shown that immune responses changed in response to 'winter-like' conditions [13,28]. In the present study, we mimicked the transition from summer to winter more naturally by decreasing photoperiod gradually and examined the changes of immune function in hamsters to test the winter immunocompetence enhancement hypothesis. We predicted that both innate and cellular immunity would increase in response to gradually decreasing photoperiod.

Materials and methods

Animals and experimental design

The experiment was carried out in agreement with the animal procedures of the Animal Care and Use Committee of Qu Normal University. Adult female Siberian hamsters used in this study were the offspring of hamsters in our laboratory colony. Hamsters were housed individually after weaning in plastic cages (30cm×15cm×20cm) with sawdust as bedding under a constant photoperiod of 12 L:12 D (12 h:12 h light-dark cycle) and temperature of 23 ± 1 ℃. They had free access to the food and water throughout the experiment. The food was commercial standard rat pellets (Beijing KeAo Feed Co.) and its macronutrients were 6.2% crude fat, 18% crude protein, 23.1% neutral fiber, 5% crude fiber, 12.5% acid detergent fiber, and 10.0% ash, and the caloric value was 17.5 kJ/g. After body mass stabilized, 22 female hamsters (age: 10 to 12 months) were randomly assigned into the control

(Control, n=11) and the gradually decreasing photoperiod group (Experiment, n=11). The control group was maintained under 12 h: 12 h light-dark cycle throughout the experiment. Day length in the experimental group was decreased from 12 h: 12 h light-dark cycle to 8 h: 16 h light-dark cycle by decreasing half an hour per week and the experiment lasted for 8 weeks. At the end of the experiment, the photoperiod was 8 h: 16 h light-dark cycle.

Organs

Visceral organs were dissected as described previously [19]. In brief, the visceral organs, including heart, thymus, lungs, liver, spleen, kidneys, testes, epididymis, seminal vesicals and the digestive organs with contents (i.e., stomach, small intestine, caecum and colon) were dissected and weighed (± 1mg). The stomach, small intestine, caecum and colon were rinsed with saline to eliminate all the gut contents, before being weighed. All visceral organs were removed to obtain the mass of carcass mass.

Body composition

Mesenteric fat, retroperitoneal fat and subcutaneous fat were also dissected carefully and weighted. The mass of the three fat pads is considered as total body fat, and the fat content was calculated as mesenteric fat, retroperitoneal fat, subcutaneous fat and total fat mass divided by wet carcass mass [29].

Cellular immunity assays

PHA response (i.e., cellular immunity) was measured according to the previous description [18,19]. Briefly, we measured the footpad thickness of their left hind feet with a micrometer (Tesa Shopcal, Swiss) to ± 0.01 mm. Hamsters were then injected subcutaneously 0.1 mg of PHA dissolved in 0.03 ml sterile saline in the middle of the footpad. After 6 h, 12 h, 24 h, 48 h, 72 h injection, we measured footpad thickness. The PHA response was calculated as the difference between pre- and post- injection measurements divided by initial footpad thickness (PHA response = (post PHA – pre PHA) / pre PHA). Each measurement of PHA response was replicated six times on the same hamster.

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 (10^9cells/l) [29].

Innate immunity

Serum bacterial killing capacity indicative of innate immunity was carried out in a sterile laminar flow cabinet to evaluate the functional response by the animal's innate immune system against a

relevant pathogen, *Escherichia coli* [15,16,17]. 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.

Statistical analysis

Data were analyzed using SPSS 13.0 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 including PHA response were subjected to arcsine transformation. The differences of body mass between the control and experimental groups at any time point were analyzed by independent-samples t-test. Group differences in wet organ mass with body mass as the covariate were analyzed by General Linear Model multivariate analysis followed by Bonferroni *post hoc* tests. Group differences in other parameters (body compositions, PHA response, WBC) were analyzed by independent-samples t-test. Results were expressed as mean ± SE, and $P<0.05$ was considered to be statistically significant.

Results

Body composition

All composition including wet carcass mass, subcutaneous, retroperitoneal, mesenteric and total body fat mass were not affected by gradually decreasing photoperiod in hamsters (Table 1).

Table 1. Effect of gradually decreasing photoperiod on body composition in Siberian hamsters

Parameters	Groups		Statistical summary	
	Control	Experiment	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	31.7±2.7	-0.591	0.561
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.

Organs

Gradually decreasing photoperiod had no influence on all wet organ mass detected including IBAT, brain, heart, lung, thymus, spleen, liver, kidneys, gonads, stomach, small intestine, caecum and colon in hamsters (Table 2).

Table 2. Effect of gradually decreasing photoperiod on wet organ mass in Siberian hamsters

Parameters	Groups		Statistical summary	
	Control	Experiment	$F_{1,19}$	*P*
Sample size	11	11		
IBAT (g)	0.106±0.358	0.120±0.033	2.886	0.106
Brain (g)	0.372±0.019	0.392±0.036	3.098	0.094
Heart (g)	0.195±0.048	0.214±0.034	2.034	0.170
Lungs (g)	0.272±0.066	0.296±0.060	0.969	0.337
Thymus (g)	0.017±0.099	0.019±0.008	0.172	0.683
Liver (g)	1.520±0.360	1.814±0.629	3.121	0.093
Spleen (g)	0.164±0.079	0.275±0.319	1.125	0.302
Kidneys(g)	0.368±0.123	0.390±0.104	0.884	0.359
Adrenal gland (g)	0.013±0.006	0.013±0.008	0.019	0.891
Gonads (Uterus and ovary) (g)	0.196±0.214	0.155±0.076	0.153	0.700
Stomach with contents (g)	1.272±0.336	1.311±0.521	0.323	0.576
Stomach(g)	0.322±0.070	0.300±0.085	0.102	0.752
Small intestine with contents (g)	1.384±0.217	1.593±0.421	4.205	0.054
Small intestine (g)	0.582±0.209	0.516±0.218	0.283	0.601
Small intestine length (cm)	27.136±3.073	28.346±3.778	1.536	0.230
Caecum with contents (g)	0.904±0.159	1.030±0.323	1.846	0.190
Caecum (g)	0.224±0.064	0.244±0.097	0.719	0.407
Caecum length (cm)	5.236±1.419	4.982±1.378	0.274	0.607
Colon with contents (g)	0.600±0.217	0.695±0.242	1.532	0.231
Colon (g)	0.267±0.110	0.270±0.078	0.221	0.643

Colon length (cm)	12.673±2.425	14.118±2.213	3.872	0.064
Total digestive tract (g)	1.073±0.337	1.030±0.324	< 0.001	0.986
Total digestive tract length (cm)	45.046±4.345	47.446±6.234	2.185	0.156

Values for a specific parameter that shares different superscripts are significantly different at $P<0.05$, determined by a multivariate analysis with body mass as the covariate.

White blood cells

Gradually decreasing photoperiod did not affect WBC in hamsters (t=-0.509, df=20, P=0.616) (Figure 1).

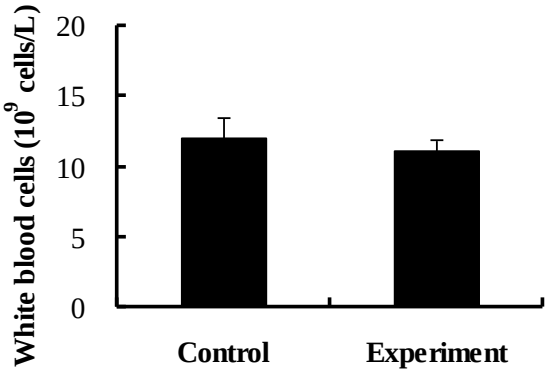

Figure 1. Effect of gradually decreasing photoperiod on white blood cells in Siberian hamsters. Different letters on the columns indicate statistical significance at $P < 0.05$, determined by independent t-test analysis.

Cellular immune response

PHA response decreased significantly with the time of PHA injection ($F_{4,80}$=59.366, P<0.001) and there was also significant interaction between PHA injection time and groups ($F_{4,80}$=3.018, P=0.023). Gradually decreasing photoperiod increased PHA response post-24 hours of PHA injection, but had no effect on PHA response after 6h (t=1.926, df=20, P=0.068), 12h (t=0.873, df=20, P=0.393), 48h (t=-0.602, df=20, P=0.554), 72h (t=-0.062, df=20, P=0.951) of PHA injection (Figure 2). No correlation was observed between PHA response after 24h immunochallenge and body mass (r=0.049, P=0.829), total body fat mass (r=-0.010, P=0.966).

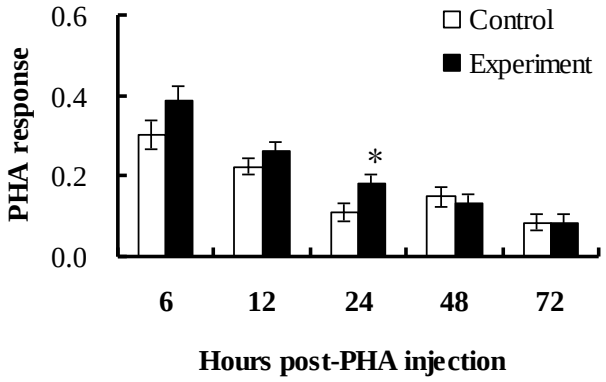

Figure 2. Effect of gradually decreasing photoperiod on PHA response in Siberian hamsters. Asterisk (*) indicates statistical significance at $P < 0.05$, determined by independent t-test analysis.

Innate immunity

Bacteria killing capacity indicative of innate immunity was not influenced by gradually decreasing photoperiod in hamsters （ t=1.538, df=19, *P*=0.140 ） , however it was 56.2% higher in the experimental group than in the control group (Figure 3). There was no correlation between innate immunity and body mass (r=-0.104, *P*=0.654), total body fat mass (r=0.044, *P*=0.850).

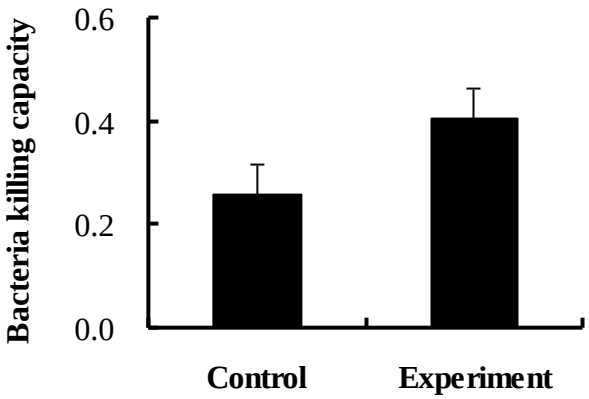

Figure 3. Effect of gradually decreasing photoperiod on innate immunity in Siberian hamsters. Different letters on the columns indicate statistical significance at $P < 0.05$, determined by independent t-test analysis.

Discussion

In the present study, we found that distinct components of immune system respond to gradually decreasing photoperiod differently. PHA response post-24 h of PHA injection increased upon decreasing photoperiod, which supported the 'winter immunoenhancement hypothesis' [1,5]. This result agreed with other researches in which the delayed-type hypersensitivity responses of Siberian hamsters increased upon short day length [30]. However, this finding was inconsistent with others in which cellular and humoral immunity were not responsive to the change of photoperiod in Aztec mouse (*Peromyscus aztecus hylocetes*) [31]. The reason might be the difference of species, which the former distributes mainly in the temperate zone and the latter mainly lives in the tropical area being insensitive to the changes of photoperiod. Previous research has shown that melatonin mediates photoperiod control of endocrine adaptations and humoral immunity in male Siberian hamsters [32]. The enhancing effect of decreasing photoperiod on cellular immunity in Siberian hamsters might be mediated by melatonin secretion [33]. The influence of melatonin on immune responses in female hamsters requires further researches.

Variation of immune responses is often related with the change of energy reserves such as body fat mass [34,35]. For example, cellular or humoral immunity may be suppressed due to excessive body mass or fat loss during starvation [19,34,36] or experimental reductions in body fat [35]. In our study, we found that subcutaneous, retroperitoneal, mesenteric and total fat mass were all not affected by gradually decreasing photoperiod. In addition, no correlation was observed between body fat mass and PHA response, innate immunity in hamsters, indicating that fat mass could not explain the change of cellular and innate immunity.

In summary, gradually decreasing photoperiod differently enhanced cellular immunity in Siberian hamsters, however it did not affect the masses of thymus and spleen, white blood cells and innate immunity. The actions of melatonin in immune responses require further study.

Acknowledgements

We are grateful for the support of the National Natural Science Foundation of China (31370427; 31370444) to DLX.

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