FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 45, Supp. 1, 2007
pp. 29-33

The influence of short-time period of an adaptation to decreased ambient temperature on interleukin-6 and corticosterone levels in female Wistar strain rats in the proestrous phase of the reproductive cycle

Arkadiusz Baran[1,2], Grzegorz Jakiel[1], Grażyna Wójcik[2]

[1]Department of Reproduction and Andrology, [2]Department of Human Physiology, Medical University of Lublin, Lublin, Poland

Abstract: To date, there has been little research examining whether short-time changes of external environmental conditions exert any effects on immune responses. The activation of metabolic changes, release of hormones responsive for immunomodulation and the action of interleukins play an important role in interaction with hormones of an anterior pituitary gland in the proestrous phase of the reproductive cycle. The aim of our study was to determine the effects of a short-time change of ambient temperature (30 minutes) on interleukin-6 (IL-6) and corticosterone plasma concentration of female rats in the proestrous phase of the reproductive cycle. The climatic chamber with automatically adjustable and monitored internal environmental parameters (temperature, oxygenation, humidity) was used during the experiment. The estimation of the vaginal lavage using a microscope was done to determine the estrous cycle. On the day of the experiment, animals were divided into 2 groups: the control group (ambient temperature 21°C ± 1°C; normoxia 21% O_2) and the test group (ambient temperature 10°C ± 1°C; normoxia 21% O_2) stayed in the climatic chamber for 30 minutes. The blood samples were collected before the experiment and after 30, 60, 90, 150 and 210 minutes from the beginning of the experiment. The concentrations of IL-6 and corticosterone were measured in blood plasma samples using ELISA method. There was a significant elevation of IL-6 levels after staying in 10°C during the first 150 minutes from the beginning of the experiment, with the highest value occuring after 60 minutes (426.6 pg/ml; SE - 146.1) with comparison to the value at first sampling (108.5 pg/ml; SE - 29.5; $p<0.05$) and with comparison to the control group at the same time from the beginning of the experiment (87.6 pg/ml; SE - 2.3; $p<0.05$). The changed level of corticosterone in the test group in comparison to control group was observed but the differences were insignificant. Our observations confirm the proposition, that even short-time changes of ambient conditions can activate adaptation mechanisms in the organism, which in part, is the activation of the immune system.

Key words: Temperature - Interleukin-6 - Acclimation - Climatic chamber - Rat

Introduction

The study was undertaken to determine the short-term effects of a cold environment on interleukin - 6 (IL-6) and corticosterone concentration in a rats' plasma. To date, there has been little research examining whether short-term changes in ambient temperature exert effects on the immune system. Cytokines, peptide hormones and neurotransmitters, as well as their receptors/ligands, are ubiquitous within the brain, endocrine and immune systems. These shared ligands and receptors are used as a common chemical language for communication within and between the immune and neuroendocrine systems. Such communication suggests an immunoregulatory role for the brain and sensory function for the immune system. Interplay between the immune, nervous and endocrine systems is most commonly associated with the pronounced effects of stress on immunity. The hypothalamic - pituitary - adrenal (HPA) axis is the key player in stress responses. It is well established that both external and internal stressors activate the HPA axis. Cytokines are chemical messengers that stimulate the HPA axis when the body is under stress or experiencing an infection [1]. These peptides contribute to a chemical signaling language that regulates development, tissue repair, haemopoiesis, inflammation and specific and nonspecific immune

Correspondence: A.Baran, Dept. of Human Physiology, Medical University of Lublin, Radziwiłłowska Str. 11, 20-080 Lublin, Poland; tel.: (+4881) 5288434, e-mail: arkadiusz-baran@go2.pl

responses [2]. The immune system is regulated in part by the central nervous system (CNS), acting principally via the hypothalamic - pituitary - adrenal axis (HPA) and the sympathetic nervous system (SNS) [2]. In recent years, our understanding of the interactions between the HPA axis and immune-mediated inflammatory reactions has expanded enormously. Plasma corticosterone as an endogenous factor is a natural inhibitor of cytokine production [3]. Several cytokines are known to affect the release of anterior pituitary hormones by an action on the hypothalamus and/or the pituitary gland. The major cytokines involved are IL-1, IL-2, IL-6, TNF-α and interferon (IFN) [4]. The predominant effects of these cytokines are to stimulate the HPA axis and to suppress the hypothalamic - pituitary - thyroid (HPT) and gonadal axes and growth hormone (GH) release. The relative importance of systemically and locally produced cytokines in achieving these responses and their precise sites of action have not been fully established [1]. There is cumulating evidence that there are significant interactions between the immune and neuroendocrine systems which may explain, at least in part, some of the effects on growth, thyroid, adrenal and reproductive functions which occur in acute and chronic disease [5-7]. During stimulation of the immune system, peculiar alterations in hormone secretion occur (e.g. hypogonadism, hypercortisolism) [8-10]. A considerable amount of evidence has shown that physical and psychological stress elevates the plasma IL-6 levels [11,12]. Circulating cytokine concentrations are elevated in response to strenuous exercise and other forms of physical stress [13]. Although heat stress is known to accentuate exercise-associated immunomodulation, largely via augmented hormonal fluctuations [14,15], relatively little is known regarding the physiological modulation of the human immune system by cold exposure, either at rest or during sustained exercise [16]. Exposure to cold substantially augments hypothalamic - pituitary - adrenal axis and sympathetic nervous system activation, producing an enhanced secretion of cortisol and catecholamines, respectively [17]. Cold is known to affect leukocyte mobilization [18,19], and can suppress lymphocyte functional activities [18]. Limited evidence suggests that cold exposure may also initiate changes in cytokine expression associated with nonspecific acute phase reaction [16,20]. Because cytokines play a key role in the bidirectional communication between neuroendocrine and immune systems [21], it has been suggested that the interplay between hormones and cytokines during thermal stress may influence immune homeostasis in response to environmental challenge [16]. Adaptation of homeothermic organisms to change of environmental temperature results in the redistribution of the plastic and energetic potentials of the organism.

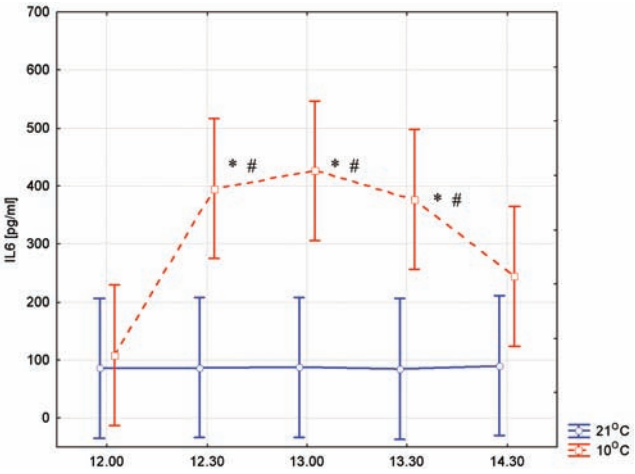

Fig. 1. Changes in the concentration of circulating interleukin - 6 (pg/ml) in the rat plasma following 30 minutes of acclimation to the novel environmental conditions (temperature: 21°C or 10°C) in the climatic chamber. Each data point represents mean value with a 95% confidence interval. Statistically significant difference compared to the control group (*$p<0.05$). Statistically significant difference compared to the value of samples collected before the experiment (#$p<0.05$).

The short length of the estrous cycle of rats makes it useful for investigation of changes occurring during the reproductive cycle. The estrous cycle lasts some days and is composed of: proestrus, estrus, metestrus and diestrus phases. The ovulation occurs from the beginning of proestrous to the end of estrus [22].

Our specific aims were to study the effect of 30 minutes of acclimation to novel ambient conditions on rat plasma levels of IL-6 and corticosterone to determine if cold stress is associated with alterations of profile of circulating IL-6 and corticosterone and to examine whether changes in these mediators are associated with each other.

Materials and methods

Female Wistar rats (*Rattus norvergicus*), three months old, weighing 200 to 300 g were used. The animals were housed in standard cages, six per cage, in a controlled temperature room (21°C ± 1°C), with a 12 h light: 12 h dark cycle, lights were turned on at 6:00 a.m. Standard laboratory chow and tap water were available *ad libitum*. The experimental protocol was approved by the Ethical Committee on Human and Animal Experimentation of The Medical University of Lublin.

One month prior to the experiment, every morning between 8:00 and 9:00 a.m. each animal cage was carried to the experimental room. The estimation of the vaginal lavage using a microscope was done to determine the phase of the estrous cycle. Unstained material was observed under a light microscope, without the use of the condenser lens, with 10 and 40 × objective lenses. Three types of cells could be recognized: round and nucleated ones were epithelial cells; irregular ones without nucleus were the cornified cells; and little round ones were the leukocytes. The proportion among them was used for the determination of the estrous cycle phases [22,23].

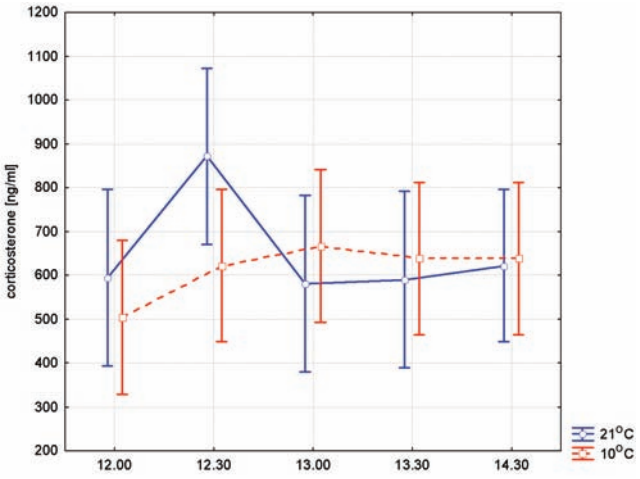

Fig. 2. Changes in the concentration of circulating corticosterone (ng/ml) in the rat plasma following 30 minutes of acclimation to the novel environmental conditions (temperature: 21°C or 10°C) in the climatic chamber. Each data point represents mean value with a 95% confidence interval.

On the day of the experiment, animals (in proestrus phase of estrous cycle) were divided into 2 groups: A. Control group - (CG - 21°C) (n = 6), acclimation in climatic chamber in normal environmental conditions (ambient temperature - 21°C ± 1°C; normoxia - 21% O_2; and relative humidity - 60%) during 30 minutes. B. Test group - (TG - 10°C) (n = 6), acclimation in climatic chamber in low temperature conditions (ambient temperature - 10°C ± 1°C; normoxia - 21% O_2; and relative humidity - 60%) during 30 minutes. The animals were tested once at a time in the climatic chamber. The experiment started at 12 a.m. The climatic chamber (Multiserv model KBI - 100, Lublin, Poland) is a box with plexiglas walls of dimensions 50×40×40 cm. Internal environmental parameters (oxygenation, temperature, humidity) are automatically adjustable and monitored. The blood samples were collected in tubes containing EDTA (using catheter implanted to the external jugular vein before the experiment [24], before and 30, 60, 90, 150 and 210 minutes from the beginning of the experiment. The collected samples were centrifuged and the plasma was stored at -20°C until assayed for IL-6 and corticosterone. Concentration of IL-6 and corticosterone in blood plasma were determined using commercially available ELISA kits (R&D Systems, Minneapolis; IBL Hamburg). The ELISAs were performed according to the instructions of the manufacturer. Statistic data obtained in this study are expressed as mean values with a 95% confidence interval. Data was analyzed by ANOVA with Tukey post-hoc analysis to determine differences. The level of significance was set at 0.05.

Results

The figures represent changes in concentrations of circulating interleukin - 6 (pg/ml) (Fig.1) and corticosterone (ng/ml) (Fig. 2) in the rat blood plasma following 30 minutes of acclimation to the novel environmental conditions (temperature: 21°C or 10°C) in the climatic chamber. Each data point represents a mean value with 95% confidence interval. There weren't significant elevations in IL-6 or corticosterone levels during the first 210 minutes from the beginning of the experiment in the control group (CG - 21°C). There were significant elevations in IL-6 levels after staying in 10°C (TG - 10°C) during the first 150 minutes from the beginning of the experiment, with the highest value occurring after 60 minutes (426.6 pg/ml; SE - 146.1) in comparison to the value before the beginning of the experiment (108.5 pg/ml; SE - 29.5; $p<0.05$) and in comparison to the control group (CG) at the same time from the beginning of the experiment (87,6 pg/ml; SE - 2.3; $p<0.05$). The changed levels of corticosterone in the test group (TG - 10°C) in comparison to the control group were observed but the differences were insignificant.

Discussion

Several studies have established that different types of stress can alter immune functions, cytokine levels and hormone levels [25] but only a few investigations have focused on the impact of heat or cold exposure on immunological changes. We have shown that exposure to cold environmental conditions modulates cytokine expression. Our findings provide evidence that changes in sympathoadrenal activation are linked to exertional- and cold-induced modification of this cytokine production profile. Unfortunately, our study measure only integrated accumulation of secreted cytokines, reflecting the net outcome of produced, absorbed, and degraded molecules within biological fluids. The numerous sources of cytokines have been identified in vitro and only a few studies have attempted to identify the origin of cytokines in vivo [26,27]. Some studies demonstrated that blood monocytes can be a source of circulating inflammatory cytokine production during exercise [15]. Blood monocytes are a first line of defense against invading pathogens and a major source of immuno-inflammatory mediators [28]. When activated by various noninfectious and infectious agents, such as bacteria-derived lipopolysaccharide (LPS), monocytes sequentially release a cascade of cytokines, including TNF-α, followed by IL-1β and IL-6 [28]. The prolonged cold exposure substantially magnified the extent of monocytosis [15]. Such cold-enhanced recruitment of monocytes has been previously documented in humans [16,19] and is presumably mediated by pronounced SNS activation accompanying prolonged cold stress. This activation may influence cell mobilization through indirect adjustments in hemodynamics or via direct receptor - mediated alterations in cellular adhesive properties, thereby affecting cell mobilization [29]. Exposure of humans, rats and mice to cold ambient temperature results in elevated blood pressure and heart rate. It appears that the tachycardia and hypertension are indirect results of SNS activation of thermoregulatory mechanisms, because elevated plasma norepinephrine levels corre-

late with elevated blood pressure in the cold [30,31]. There is a highly sensitive and linear effect of raising or lowering ambient temperature within the range 18-30°C on cardiovascular function [32]. Short duration, moderate cold-air exposure (2 h, 5°C) in a climatic chamber elicits significant plasma elevations of IL-6 in resting subjects [16]. Others have found that short-term exposure to cold air (1 h, 11°C) or cold water (1 h, 14°C) has no effect on systemic IL-1β or IL-6 release [33,34]. The different mechanisms of cytokine induction may be operative in moderate vs. severe cold exposure. The mechanism underlying the abovementioned differences is not clear. The cold-associated modulation of cytokine production may be related to induction of systemic endotoxemia, provoked by alterations in central hemodynamics and stress hormone release associated with enhanced thermoregulatory demands. The moderate cold exposure leads to a sharp reduction in splanchnic blood flow and ischemia [35] that promotes translocation of LPS into the systemic circulation [36]. Swoap et al. demonstrated that even a modest change in ambient temperature can influence the autonomic nervous system in such a way as to significantly influence arterial blood pressure, heart rate and metabolic rate [32]. Some investigators speculate that noncirculating cells, including vascular endothelium, hepatocytes and fibroblasts, may be chiefly responsible for the enhanced secretion of these cytokines with exercise [37,38]. There is an important role for reciprocal interactions between neuroendocrine and immune systems in the maintenance of homeostatic balance between pro- and anti-inflammatory cytokine responses [21,39]. Exercise and cold-induced catecholamine secretion is closely related to systemic IL-6 release [16]. IL-6 activates the HPA axis and induces the upregulation of cortisol and IL-1ra that in turn suppresses the synthesis of monocyte IL-1β, TNF-α and IL-6 thereby controlling the extent of local and systemic inflammatory responses [39]. The administration of IL-6 activates the HPA axis by increased activity of corticotrophin releasing hormone (CRH), elevated plasma ACTH and corticosterone levels [40,41]. Cytokine production may be differentially regulated by circulating catecholamines during exercise and cold exposure. Rhind et al. demonstrated that cold exposure differentially modulate cytokine production, upregulating the expression of IL-6 and IL-1ra but downregulating that of IL-1β and TNF-α. Secretion of sympathoadrenal hormones was significantly associated with changes in both circulating and intracellular cytokine profiles [15]. The molecular signaling pathways involved in exercise and/or thermal stress-induced cytokine alterations remain largely unknown. A change in ambient temperature by only a few degrees Celsius is enough to significantly impact metabolic rate, heart rate, and the mean blood pressure in mice and rats. Our findings are consistent with studies indicating that adrenergic and nonadrenergic mechanisms are involved in the regulation of cytokine production under various forms of physical stress [42]. We observed the insignificant changes of levels of corticosterone. Studies in rats, mice and humans have shown that the acute exposure to stressors is characterized by an increase in corticotrophin releasing hormone (CRH), adrenocorticotropin (ACTH) and corticosterone in rodents and cortisol in humans [43]. Chronic stress causes a decrease in hypothalamic CRH content, an increase in plasma levels of ACTH and glucocorticoids [43]. The final evidence for such cold-evoked, HPA axis associated modulation of cytokine expression must await future studies that interdict specific steps in the signaling pathways leading to cytokine induction.

Acknowledgements: This study was supported by grant from Medical University of Lublin (PW 224/06).

References

[1] Haddad JJ, Harb HL. Cytokines and the regulation of hypoxia-inducible factor (HIF)-1alpha. *Int Immunopharmacol.* 2005;5:461-483.
[2] Safieh-Garabedian B, Poole S, Haddad JJ, et al. The role of the sympathetic efferents in endotoxin-induced localized inflammatory hyperalgesia and cytokine upregulation. *Neuropharmacology.* 2002;42:864-872.
[3] Leon LR, Blaha MD, DuBose DA. Time course of cytokine, corticosterone, and tissue injury responses in mice during heat strain recovery. *J Appl Physiol.* 2006;100:1400-1409.
[4] Bumiller A, Gotz F, Rohde W, Dorner G. Effects of repeated injections of interleukin 1beta or lipopolysaccharide on the HPA axis in the newborn rat. *Cytokine.* 1999;11:225-230.
[5] Turnbull AV, Prehar S, Kennedy AR, Little RA, Hopkins SJ. Interleukin-6 is an afferent signal to the hypothalamo-pituitary-adrenal axis during local inflammation in mice. *Endocrinology.* 2003;144:1894-1906.
[6] Wellby ML, Kennedy JA, Pile K, True BS, Barreau P. Serum interleukin-6 and thyroid hormones in rheumatoid arthritis. *Metabolism*. 2001;50:463-467.
[7] Bao X, Kennedy BP, Hopkins SR, Bogaard HJ, Wagner PD, Ziegler MG. Human autonomic activity and its response to acute oxygen supplement after high altitude acclimatization. *Auton Neurosci.* 2002;102:54-59.
[8] de Souza AL, Sztajnbok J, Marques SM, et al. Compartmentalization of interleukin-6 response in a patient with septic meningococcal peritonitis. *Clin Vaccine Immunol.* 2006;13: 1287-1290.
[9] Sweep CG, Van der Meer MJ, Hermus AR, et al. Chronic stimulation of the pituitary-adrenal axis in rats by interleukin-1 beta infusion: in vivo and in vitro studies. *Endocrinology.* 1992;130:1153-1164.
[10] Sutherland F, Cunningham H, Pontikes L, Parsons L, Klassen J. Elevated serum interleukin 6 levels in patients with acute intestinal ischemia. *Hepatogastroenterology*. 2003;50:419-421.
[11] Nukina H, Sudo N, Aiba Y, Oyama N, Koga Y, Kubo C. Restraint stress elevates the plasma interleukin-6 levels in germ-free mice. *J Neuroimmunol*. 2001;115:46-52.
[12] Besedovsky HO, del Rey A, Klusman I, Furukawa H, Monge AG, Kabiersch A. Cytokines as modulators of the hypothala-

mus-pituitary-adrenal axis. *J Steroid Biochem Mol Biol.* 1991; 40:613-618.
[13] Ostrowski K, Schjerling P, Pedersen BK. Physical activity and plasma interleukin-6 in humans - effect of intensity of exercise. *Eur J Appl Physiol.* 2000;83:512-515.
[14] Rhind SG, Gannon GA, Shephard RJ, Buguet A, Shek PN, Radomski MW. Cytokine induction during exertional hyperthermia is abolished by core temperature clamping: neuroendocrine regulatory mechanisms. *Int J Hyperthermia.* 2004;20: 503-516.
[15] Rhind SG, Castellani JW, Brenner IK, et al. Intracellular monocyte and serum cytokine expression is modulated by exhausting exercise and cold exposure. *Am J Physiol Regul Integr Comp Physiol.* 2001;281:R66-R75.
[16] Brenner IK, Castellani JW, Gabaree C, et al. Immune changes in humans during cold exposure: effects of prior heating and exercise. *J Appl Physiol.* 1999;87:699-710.
[17] Speziale G, Ferroni P, Ruvolo G, et al. Effect of normothermic versus hypothermic cardiopulmonary bypass on cytokine production and platelet function. *J Cardiovasc Surg (Torino).* 2000;41:819-827.
[18] Beilin B, Shavit Y, Razumovsky J, Wolloch Y, Zeidel A, Bessler H. Effects of mild perioperative hypothermia on cellular immune responses. *Anesthesiology.* 1998;89:1133-1140.
[19] Jansky L, Vybiral S. Thermal homeostasis in systemic inflammation: modulation of neuronal mechanisms. *Front Biosci.* 2004;9:3068-3084.
[20] Fairchild KD, Viscardi RM, Hester L, Singh IS, Hasday JD. Effects of hypothermia and hyperthermia on cytokine production by cultured human mononuclear phagocytes from adults and newborns. *J Interferon Cytokine Res.* 2000;20: 1049-1055.
[21] Tanner MA, Berk LS, Felten DL, Blidy AD, Bit SL, Ruff DW. Substantial changes in gene expression level due to the storage temperature and storage duration of human whole blood. *Clin Lab Haematol.* 2002;24:337-341.
[22] Mandl A.M. The phases of the oestrous cycle in the adult white rat. *J Exp Biol.* 1951;28:584-588.
[23] Marcondes FK, Miguel KJ, Melo LL, Spadari-Bratfisch RC. Estrous cycle influences the response of female rats in the elevated plus-maze test. *Physiol Behav.* 2001;74:435-440.
[24] Thrivikraman KV, Huot RL, Plotsky PM. Jugular vein catheterization for repeated blood sampling in the unrestrained conscious rat. *Brain Res Brain Res Protoc.* 2002;10: 84-94.
[25] Blanque R, Meakin C, Millet S, Gardner CR. Dual mechanisms of action of interferon-gamma in potentiating responses to LPS in mice: IL1, TNFalpha and IL6 production in serum and hypothermia. *Gen Pharmacol.* 1999;32:453-461.
[26] Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund PB. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J Physiol.* 2000;529 Pt 1:237-242.
[27] Pedersen BK, Steensberg A, Schjerling P. Exercise and interleukin-6. *Curr Opin Hematol.* 2001;8:137-141.
[28] van Furth R. Human monocytes and cytokines. *Res Immunol.* 1998;149:719-720.
[29] Dugue B, Leppanen E. Adaptation related to cytokines in man: effects of regular swimming in ice-cold water. *Clin Physiol.* 2000;20:114-121.
[30] Maes M, De Meyer F. Relationships of climatic data to immune and hematologic variables in normal human. *Neuroendocrinol Lett.* 2000;21:127-136.
[31] Demura S, Takahashi K, Kawahara N, Watanabe Y, Tomita K. Serum interleukin-6 response after spinal surgery: estimation of surgical magnitude. *J Orthop Sci.* 2006;11:241-247.
[32] Swoap SJ, Overton JM, Garber G. Effect of ambient temperature on cardiovascular parameters in rats and mice: a comparative approach. *Am J Physiol Regul Integr Comp Physiol.* 2004;287:391-396.
[33] Mercer JB, Osterud B, Tveita T. The effect of short-term cold exposure on risk factors for cardiovascular disease. *Thromb Res.* 1999;95:93-104.
[34] Jansky L, Pospisilova D, Honzova S, Ulicny B, Sramek P, Zeman V et al. Immune system of cold-exposed and cold-adapted humans. *Eur J Appl Physiol Occup Physiol.* 1996;72: 445-450.
[35] Gaffin SL, Gardner JW, Flinn SD. Cooling methods for heatstroke victims. *Ann Intern Med.* 2000;132: 678.
[36] Gercekoglu H, Tarim O, Agar I, et al. Effects of hypothermia on blood endogenous endotoxin levels during cardiopulmonary bypass. *J Card Surg.* 1997;12:223-227.
[37] Keller P, Keller C, Robinson LE, Pedersen BK. Epinephrine infusion increases adipose interleukin-6 gene expression and systemic levels in humans. *J Appl Physiol.* 2004;97:1309-1312.
[38] Penkowa M, Keller C, Keller P, Jauffred S, Pedersen BK. Immunohistochemical detection of interleukin-6 in human skeletal muscle fibers following exercise. *FASEB J.* 2003;17: 2166-2168.
[39] Dinarello CA. Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock. *Chest.* 1997;112:321S-329S.
[40] Lenczowski MJ, Bluthe RM, Roth J, et al. Central administration of rat IL-6 induces HPA activation and fever but not sickness behavior in rats. *Am J Physiol.* 1999;276:652-658.
[41] Wang X, Jiang W, Zhao G, et al. Mild hypothermia protects against sodium taurocholate (NaTc)-induced acute pancreatitis in rats with adverse effects on serum cytokines. *Pancreas.* 2005;30:e80-e86.
[42] Alkharfy KM, Kellum JA, Matzke GR. Unintended immunomodulation: part I. Effects of common clinical conditions on cytokine biosynthesis. *Shock* 2000; 13: 333-345.
[43] Retana-Marquez S, Salazar ED, Velazquez-Moctezuma J. Effect of acute and chronic stress on masculine sexual behavior in the rat. *Psychoneuroendocrinology* 1996; 21: 39-50.