Adaptation related to cytokines in man: effects of regular swimming in ice-cold water

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Summary

The cytokine response after thermal stress (sauna + swimming in ice-cold water) was investigated in subjectively healthy persons. Two groups were studied at the end of the winter season: habitual and inexperienced winter swimmers. Blood was collected at rest, after a sauna bath and after a short swim in ice-cold water. Conventional methods and ELISA kits were used to determined the blood picture, serum cortisol and dehydroepiandrosterone sulphate, plasma antidiuretic hormone (ADH) levels, and the levels of several cytokines in plasma and in the supernatants of blood cell cultures which were stimulated with lipopolysaccharide (LPS). In regular winter swimmers, the concentrations of plasma interleukin 6 (IL-6), leukocytes, and monocytes at rest were significantly higher than in inexperienced subjects. In experienced female winter swimmers, the plasma concentration of the soluble receptor for IL-6 was significantly lower than in inexperienced female swimmers. In both groups, granulocytosis, haemoconcentration and significant increases in the concentrations of ADH, cortisol and IL-6 were observed after the stimuli. However, the changes in the cortisol concentration were dramatically larger in habitual winter swimmers. A significant correlation was found between the delta values of cortisol and the basal concentrations of IL-6. In cell cultures, the LPS-induced release of IL-1β and IL-6 was higher at rest in the inexperienced winter swimmers. This release was dramatically suppressed after exposure to the stimuli in the inexperienced winter swimmers but tended to increase in the regular winter swimmers. These stresses appear to challenge both the neuro-endocrine and the immune systems and the results indicate that adaptive mechanisms occur in habitual winter swimmers.

Keywords: adaptive mechanism, ADH, blood picture, cortisol, interleukins, sauna, stress, winter swimming.

Introduction

Functional bi-directional communication exists between the endocrine and immune systems, and cytokines play a major role in these interactions. The cytokines are able to modulate the hypothalamic–pituitary–adrenocortical axis response in the hypothalamus, the pituitary gland and the adrenals. More than 30 different types of hormonal receptors have been demonstrated on lymphoid and accessory cells. In addition, immunocompetent cells secrete numerous hormones (Gaillard, 1994). The endocrine and immune systems seem to be so intimately linked with each other that they could be regarded as a single network rather than separate systems (Connor & Leonard, 1998). During exposure to stress (physical, psychological, chronic, acute), these systems (or networks) are challenged. However, information about mechanisms of adaptation to repeated physically stressful conditions in man is lacking. Most of the results of experiments in exercise/sport physiology have indicated a harmful effect of intensive physical training.

In Finland, swimming in ice-cold water is a fairly common sport or habit. The winter swimmers usually combine it with a hot sauna bath (Kauppinen & Urponen, 1988; Kauppinen *et al*., 1989). From a physiological point of view, strong changes in environmental temperature (almost 100°C) on a regular basis represents an obvious neuro-endocrine/cardiovascular stress which may lead to adaptive phenomena. Accordingly, this study was undertaken to investigate how thermal stress (sauna + swimming in ice-cold water) affects the endocrine and immune systems in two groups of subjectively healthy persons: habitual winter swimmers (HWS) and non-habitual winter swimmers (NHWS). We were especially interested in the pattern of cytokine and stress hormone responses after such stimuli. A preliminary report has been presented (Dugué *et al*., 1998).

Subjects and methods

Subjects

All specimens were taken from subjectively healthy persons who had given written informed consent to participate in the study. All reported the absence of chronic illness and no use of any medication (other than oral contraceptives) during the week before the experiment. They were 11 females (age 25–52 years, mean 38·7 years) and nine males (age 19–64 years, mean 44·0 years). Among them, five females and seven males were regular winter swimmers who practised this activity more than once a week during the winter. The average number of years of activity was 7·5 (range 1–15 years). The NHWS did not have any experience of winter swimming during the previous year. It should be pointed out that it was fairly difficult to recruit the NHWS.

Design of the experiment and specimen collection

The experiments took place during late afternoon in and near a sauna situated 50 m from a lake as recently described (Dugué *et al*., 1999). The volunteers sat naked for 15 min in a sauna heated to 95°C with a relative humidity of 30–50%. Then they were subjected to cold air for about 1 min at a temperature between –15 and –5°C (this experiment was organized on four different occasions during late winter as it was not possible to monitor more than six individuals per session) while walking to the lake, followed by a 0·5 min swim in ice-cold water, and again a 1 min walk at the outdoor temperature when returning to the sauna building. This sequence of events is typical for winter swimming. Three blood specimen collections were organized in a small room at the sauna building, one at rest before the sauna bath after a period of 15 min of sitting according to the Scandinavian recommendation on specimen collection (Alström *et al*., 1993), a second collection directly after the sauna bath (the volunteers were not allowed showering/cooling options), and the last one after exposure to both cold air and water. Blood was collected from the cubital vein using minimum tourniquet, Venoject® needles and evacuated blood-collecting tubes for serum, EDTA-plasma and sterile adenine–citrate–dextrose (ACD)-plasma (Terumo Co.) and a Vacutainer CPT™ for cell separation (Becton Dick- inson). Blood for cytokine determination in plasma was collected in ice-chilled tubes and kept on ice until further processing. This study was approved by the ethical committee of our institute.

Isolation and stimulation of peripheral blood mononuclear cells

Venous blood was collected in tubes containing a sodium citrate gel and a density gradient medium (Vacutainer CPT™ for cell separation, Becton Dickinson). Specimens were then centrifuged at 1500 *g* for 20 min and the buffy coats transferred to sterile tubes, then washed twice with sterile phosphate buffer, and seeded at a concentration of 2×10^6 cells/ml in RPMI-1640 culture medium (Biological Industries) supplemented with 2 mmol l^{-1} glutamine, 100 U ml^{-1} penicillin, 100 mg ml^{-1} streptomycin and 10% heat-inactivated calf serum with or without lipopolysaccharide (LPS, *E. coli* 0111:B4, Sigma; at 1 ng ml^{-1}) at 37°C for 14 h in a humidified 5% CO_2 atmosphere. Supernatants were aspirated, freed of cells by centrifugation, and stored frozen at –20°C until analysis.

Stimulation of whole-blood culture

Specimens were collected in sterile ACD tubes (Terumo) and cultivated in dishes for cell culture (Costar) in the presence of 100 U ml^{-1} penicillin,

100 μg/ml streptomycin with or without LPS (10 ng ml^{-1}) at 37°C for 14 h in a humidified 5% CO_2 atmosphere. Samples were aspirated, centrifuged (800 g for 15 min), and the supernatants stored frozen at –20°C until analysis.

Blood picture and differential counts

Blood smears were drawn immediately after the specimen collection. The cell population was estimated after May–Grünwald–Giemsa coloration. The specimens for the blood picture determination were stored overnight at 4°C and analysed the following morning in a Sysmex-1000 machine at the clinical reference laboratory of Medix-Diacor Co, Espoo, Finland. The following data were recorded: leukocyte, erythrocyte and platelet counts, haemoglobin concentration, haematocrit, mean cell volume (MCV), mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet concentration.

Cortisol, ADH, DHEA-SO_4 and cytokine measurements

ELISA kits for determination of cortisol were purchased from Orion Diagnostica; for the dehydroepiandrosterone sulphate (DHEA-SO_4) and prolactin, the kits was purchased from Diagnostic Products Corporation, and for IL-1β, IL-1Ra, IL-6 and its receptor the kits were purchased from R & D Systems Europe. Anti-diuretic hormone (ADH) was analysed with a radioimmunoassay developed at our institute (Fyhrquist *et al*., 1976). All the samples were analysed in the same series. The intra-assay variations were 5·0 and 3·1% for cortisol concentrations of 72·0 and 1241 nmol l^{-1}, respectively; <5·0% for prolactin concentrations of 5·0 and 100 μg l^{-1}; 6·9 and 6·4% for IL-1β concentrations of 1·5 and 4·6 ng l^{-1}; 6·2 and 5·3% for IL-1Ra concentrations of 150 and 880 ng l^{-1}; 11·4 and 5·9% for IL-6 concentrations of 0·36 and 2·73 ng l^{-1}; 8·6 and 2·6% for IL-6 receptor concentrations of 134 and 644 ng l^{-1}; 10·5 and 11·5% for ADH concentrations of 2·8 and 11·5 ng l^{-1}; and 7·2 and 6·0% for DHEA-SO_4 concentrations of 43 and 568 μg dl^{-1}. The detection limits for the determination of IL-1β, IL-1Ra, IL-6 and IL-6sR were 0·1, 14, 0·08 and 140 ng · e^{-1}, respectively.

Table 1 Blood picture and blood components before and after thermal stress (sauna + swimming in ice-cold water) in all of our participants.

	Mean value (range)	
Analytes	**Before**	**After**
Leukocytes (10^9/l)	6·6 (4·8–9·2)	7·7 (5·1–11·0)***
Neutrophils (10^9 l^{-1})	3·69 (1·99–5·29)	4·01 (1·91–5·78)*
Lymphocytes (10^9 l^{-1})	2·26 (1·48–3·37)	3·00 (1·86–4·82)***
Monocytes (10^9 l^{-1})	0·42 (0·06–0·78)	0·49 (0·05–0·94)
Eosinophils (10^9 l^{-1})	0·22 (0·00–0·51)	0·22 (0·0–0·77)
Erythrocytes (10^{12} l^{-1})	4·5 (4·0–5·4)	4·7 (4·2–5·5)***
Haemoglobin (g l^{-1})	137 (119–159)	144 (126–164)***
Haematocrit (vol. fraction)	40 (34–45)	42 (36–47)***
MCH (pg)	31 (29–33)	31 (29–33)
Cortisol (nmol l^{-1})	292 (130–480)	471 (148–805)***
DHEA-SO_4 (mg l^{-1})	2·3 (0·9–5·1)	2·4 (1·0–5·1)
ADH (ng l^{-1})	7·94 (2·54–14·3)	9·8 (5·6–16·2)**
Prolactin (ng l^{-1})	10·5 (2·5–44·0)	18·9 (4·0–44·2)**
IL-1β (ng l^{-1})	0·74 (ND–8·5)	0·68 (ND–7·5)
IL-1Ra (ng l^{-1})	198 (100–480)	189 (105–255)
IL-6 (ng l^{-1})	1·51 (0·55–3·2)	1·89 (0·72–4·4)***
IL-6 sR (μg l^{-1})	30 (20–38)	31 (21–39)*

Abbreviations: MCH, mean corpuscular haemoglobin; IL, interleukin; sR, soluble receptor; Ra, receptor antagonist; ADH, anti-diuretic hormone; ND, non-detectable; *P<0·05; **P<0·01; ***P<0·001 compared to the basal level.

Data processing and statistical analysis

The data were analysed using non-parametric statistical methods: Wilcoxon signed rank test for paired data and Mann–Whitney test for group comparison. The Friedman test was used to compare three groups. However, the interactions of different factors (i.e. age, gender, training) were studied using two-factor repeated-measures ANOVA.

Results

The changes in the concentrations of haematological analytes, pro-inflammatory cytokines and stress hormones are summarized in Tables 1 and 2.

At rest, the plasma IL-6-values observed in habitual winter swimmers (HWS) were significantly higher than the concentrations seen in the inexperienced winter swimmers (mean HWS 1·7 ng l^{-1}, SD 0·6, $n = 12$ versus mean NHWS 1·0 ng l^{-1}, SD 0·5, $n = 8$; P<0·05). In females HWS, plasma IL-6sR was significantly lower (mean 7 μg l^{-1}, SD 4, $n = 5$) than in female NHWS (mean 34 μg l^{-1}, SD 4, $n = 6$;

Table 2 Concentrations of serum and plasma components in the thermal test, values of habitual winter swimmers (HWS) and non habitual winter swimmers (NHWS) calculated separately.

		Results[a]			Statistical analysis[b]	
Analyte	**Group**	**Basal level (1)**	**After sauna (2)**	**After cold stress (3)**	**At rest (*P*<)[c]**	**Delta values (*P*<)**
Monocyte total count (10^9 cells l^{-1})	HWS	0·48 (0·20–0·78)	0·40 (0·17–0·80)	0·44 (0·05–0·80)	0·05	0·08 for '2' versus '1' 0·007 for '3' versus '1'
	NHWS	0·32 (0·06–0·54)	0·45 (0·15–1·01)	0·58 (0·32–0·93)		0·35 for '3' versus '2'
Cortisol (nmol l^{-1})	HWS	296 (130–480)	459 (145–800)	544 (148–805)	0.78	0·009 for '2' versus '1' 0·03 for '3' versus '1'
	NHWS	285 (185–480)	258 (175–400)	359 (190–570)		0·56 for '3' versus '2'

[a]Mean values (extreme values); [b]Mann–Whitney test; [c]HWS versus NHWS.

P<0·03). Leukocyte counts were higher in female HWS (mean $7{\cdot}9 \times 10^9$ cells l^{-1}, SD 0·8, $n = 5$) than in female NHWS (mean $6{\cdot}3 \times 10^9$ cells l^{-1}, SD 0·9, $n = 6$; *P*<0·05). The same applies to total monocyte counts (mean HWS for the whole group $0{\cdot}48 \times 10^9$ cells l^{-1}, SD 0·18, $n = 12$ versus mean NHWS $0{\cdot}32 \times 10^9$ cells l^{-1}, SD 0·16, $n = 8$; *P*<0·05) and neutrophil counts (mean female HWS $4{\cdot}53 \times 10^9$ cells l^{-1}, SD 0·50, $n = 5$ versus mean female NHWS $3{\cdot}64 \times 10^9$ cells l^{-1}, SD 0·44, $n = 6$; *P*<0·05).

After the thermal stress (sauna + swimming in ice-cold water), the total leukocyte (*P*<0·001), neutrophil (*P*<0·05), lymphocyte (*P*<0·001) and erythrocyte counts (*P*<0·001), the haemoglobin concentration (*P*<0·001) and the haematocrit values (*P*<0·001) were significantly higher than in the controls. The concentrations of IL-6 (*P*<0·001) and its receptor (*P*<0·05), prolactin (*P*<0·01) and ADH (*P*<0·01) increased significantly after the stress, whereas the concentrations of IL-1β, IL-1Ra, DHEA-SO_4, and the MCV, MCH, MCHC values and platelet counts did not change significantly. When the results were corrected for haemoconcentration (according to the changes in the haematocrit values), the pattern of the changes was rather similar.

Interestingly, habitual winter swimming significantly influenced the changes in the monocyte counts (*P*<0·004) and the cortisol concentration (*P*<0·05). For these analytes, we also report the results obtained from specimens collected after the sauna session (Table 2). After these stimuli, the number of monocytes dropped in the HWS (*P*<0·05) whereas it rose in NHWS (*P*<0·02). The concentration of cortisol rose after the sauna in HWS (*P*<0·01) whereas a significant increase was seen in NHWS only after swimming in ice-cold water (*P*<0·05). When the results were corrected for haemoconcentration, a significant decrease was obtained for the NHWS after the sauna session (*P*<0·05). We also observed a significant and positive correlation between the changes in the concentration of cortisol after sauna and the level of plasma IL-6 at rest ($r = 0{\cdot}48$, $n = 20$, *P*<0·036) (Fig. 1).

In LPS-stimulated cultures, large amounts of IL-1β and IL-6 are produced (Table 3). To facilitate comparisons, the data were expressed as amount of cytokine produced per monocyte. The data obtained for IL-1β and IL-6 with the whole-blood cultures correlated well with the data obtained with the cultures of isolated peripheral blood mononuclear cells (*P*<0·05 in all cases). The basal LPS-induced releases of IL-1β and IL-6 were significantly higher in

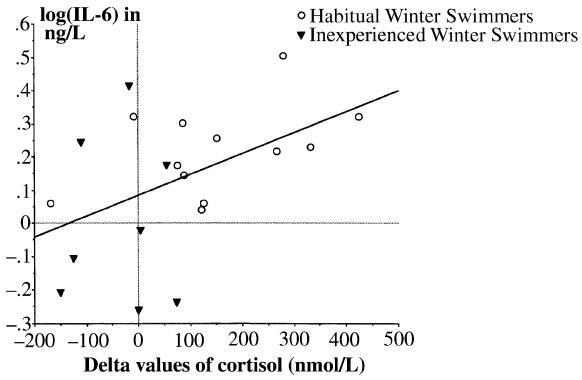

Figure 1 Correlation between the changes in cortisol and the basal concentration of interleukin-6. The delta values of cortisol (expressed in nmol l^{-1}) are the differences between the results obtained after the sauna session and those at rest. This correlation was found to be significant.

Table 3 LPS-induced release of cytokines before and after the thermal stress (sauna + swimming in ice-cold water).

	Non-habitual winter swimmers				Habitual winter swimmers				
	Whole blood		Mononuclear cells		Whole blood		Mononuclear cells		
Analytes	Before stress	After stress	Before stress	After stress	Before stress	After stress	Before stress	After stress	Interaction (due to training)
Interleukin-1β	7·0§ (2·8–13·1)	2·8* (1·6–4·8)	73·0‡ (33·0–151·8)	21·3* (9·7–38·2)	2·5§ (0·4–6·1)	5·3 (0·7–36·3)	18·7‡ (5·0–46·2)	29·6 (7·5–108·9)	$P<0·05$ with both methods
Interleukin-6	31·5§ (8·7–73·8)	9·3* (3·6–15·0)	175·0‡ (44·9–388·7)	37·4* (8·0–75·1)	8·9§ (0·5–22·5)	15·1 (2·5–57·2)	43·0‡ (24·5–103·5)	70·4 (28·3–220·9)	$P<0·05$ with both methods

The production of interleukin-1β and interleukin-6 (ng/10^6 monocytes) in LPS-stimulated isolated mononuclear cells and in whole-blood cultures. Both cultures were incubated for 14 h at 37°C. For each method, the interactions of the training factor was studied using two-factor repeated-measures ANOVA. §$P<0·05$ between groups with the whole-blood LPS-stimulated method, ‡$P<0·05$ between groups with the mononuclear cells LPS-stimulated method, *$P<0·05$ between situations. Due to strong vasoconstriction after the swimming in ice-cold water, we were not able to collect enough material for this series of experiment with three of the non-habitual winter swimmers.

the NHWS than in the HWS ($P<0·05$). Moreover, the NHWS showed a decrease in their capacity for producing IL-1β and IL-6 after the stimuli, whereas the HWS had their production slightly stimulated.

Discussion

Winter swimming is a form of self-care practice. It consists of taking a short swim in ice-cold water regularly throughout the winter season. Winter swimmers in Finland usually combine it with a hot sauna. Those practising it often strongly believe that regular swimming/bathing in ice-cold water is wholesome and that they fall ill less often than others. However, there are no well-controlled data to support this claim. Adaptation to repeated hot and cold stress has been postulated as a mechanism resulting in increased resistance to stress and diseases, but again little evidence exists. Surprisingly, there are only limited data available on the processes occurring during cold adaptation (e.g. Jansky *et al*., 1996).

Interestingly, we observed that at rest the concentrations of plasma IL-6, leukocytes and monocytes in regular winter swimmers are higher than in inexperienced winter swimmers. After taking a harsh sauna bath and a short swim in ice-cold water, the changes in the heart rate and blood pressure were similar to those previously reported (i.e. increase in heart rate and decrease in the diastolic blood pressure after the sauna bath, increase in systolic blood pressure after the sauna bath and the cold stress) (Kukkonen-Harjula *et al*., 1989; Zenner *et al*., 1980). Granulocytosis, haemoconcentration (probably due to sweating in the sauna) and a significant increase in the plasma concentrations of IL-6 and ADH occurred after these stressful stimuli. Similar findings have recently been reported in seven volunteers who first remained seated for 1 h in a water bath at 35 or 38°C and then for 2 h in a climatic chamber maintained at 5°C (Brenner *et al*., 1999). Interestingly, when studying the monocyte counts and the concentration of cortisol, we found a significant interaction of the changes, which were probably due to training. The monocyte count decreased after the sauna bath in HWS whereas it increased in NHWS. After the cold stress, the monocyte counts continued to rise in the NHWS and remained stable in HWS. The changes in the cortisol concentration were dramatically

greater in HWS than in NHWS after the stimuli. However, plasma cortisol decreased after the sauna in NHWS whereas it significantly increased in HWS. In both groups, after the ice-cold stress, the cortisol concentration was significantly higher than at rest. These observations can be explained in terms of adaptation. Recently, it has become clear that the release of cortisol in blood mainly happens after and not during the 15 min sauna exposure (Dugué *et al*., 1996a; Jezova *et al*., 1994; Kukkonen-Harjula *et al*., 1989) and that during this period the catabolism of cortisol may be increased (Jezova *et al*., 1994). This could explain the decrease in cortisol we observed in the NHWS. However, the secretory capacity of the adrenals of individuals who practise sport are enhanced (Kjaer, 1998). It could be hypothesized that the same happens in the cortisol synthesis/secretory system of regular winter swimmers, who did show an increase in plasma cortisol directly after sauna.

The increase in the concentrations of cortisol, ADH and prolactin show that the stimuli are able to activate the hypothalamic–pituitary–adrenal axis. It is known that IL-6 is a mediator of the interaction of the immune system with the hypothalamic–pituitary–adrenal axis, and it has recently been postulated that IL-6 may be a long-term regulator of the adrenal stress response (Päth *et al*., 1997; Salas *et al*., 1990). It has also recently been demonstrated that IL-6 stimulates the cortisol secretion from adult human adrenocortical cells (Weber *et al*., 1997). Moreover, it has been shown that depressed patients have higher plasma concentrations of IL-6 and cortisol (Dinan, 1994; Maes *et al*., 1997). The significant correlation we observed between the delta values of cortisol and the basal concentrations of IL-6 also suggests a chronic *in vivo* effect of IL-6 on the cortisol response.

We faced a methodological problem when investigating LPS-induced release of cytokines from blood cells. Nowadays, a number of authors prefer to study cytokine-stimulated release using cultures of whole blood rather than isolated peripheral blood mononuclear cells. Experiments with isolated cells have drawbacks, because the cells are removed from their physiological environment and artefacts may occur due to activation of the cells during their isolation (Dugué *et al*., 1996b). However, whole-blood cultures entail some inconveniences (Nerad *et al*., 1992). There are indications that the LPS-induced release of IL-1β and IL-6 is inhibited by cortisol (Arzt *et al*., 1994; Rock *et al*., 1992; Sauer *et al*., 1996; Waage *et al*., 1990). However, the whole-blood technique has been validated on specimens obtained from persons at rest. Therefore, we decided to investigate the LPS-induced release of cytokines using both methods. In these experiments, we used the lowest LPS concentration able to induce the production of cytokines by blood cells (i.e. 1 ng ml^{-1} for the peripheral blood mononuclear cell culture and 10 ng ml^{-1} for the whole-blood culture (Nerad *et al*., 1992)). Both techniques gave similar results for IL-1β and IL-6. Significant interaction due to previous training was observed when comparing the basal levels of the LPS-induced production of IL-1β and IL-6 and also the changes of LPS-stimulated release of these cytokines. The basal level of the LPS-induced release of IL-1β and IL-6 was significantly higher in the NHWS compared to the HWS. Moreover, the LPS-induced release of IL-1β and IL-6 was significantly suppressed after exposure to the stimuli in the NHWS but tended to increase in the HWS.

Some authors have reported that immune function was increased by light or moderate exercise but decreased by excessive physical work. Kvernmo *et al*. (1992) observed a significant decrease in LPS-induced TNF production in highly trained athletes at rest compared to sedentary controls. The same may have applied to our subjects in the case of LPS-induced release of IL-6 and IL-1β. Moreover, the same authors observed a decreased LPS-induced TNF production following exercise (Kvernmo *et al*., 1992). In the present study, we were also able to find a decrease in the LPS-induced release of cytokines but only in the NHWS. It is believed that exercise is followed by transient immunosuppression which is due to the release of glucocorticoids. Although the concentration of cortisol is dramatically increased in the HWS after the stress (even more than in the NHWS), the LPS-induced release of cytokines is not decreased and even tends to increase. This may be due to changes in the corticosteroid sensitivity of peripheral blood mononuclear cells (e.g. down-regulation of glucocorticoid receptors, changes in the affinities of these receptors). However, the changes in the concentration of blood cells after thermal stress may also be due to redistribution of cells. Redistributed cells may have different abilities to produce cytokines.

It is believed that stress suppresses immune function and increases susceptibility to infections and diseases, and impairs wound healing, etc. It seems paradoxical that an organism would suppress its immune system at a time when it is likely to need an active immune response. For instance, under conditions of stress, it may be injured or infected by a stressor agent such as an attacker (Sapolsky, 1993). However, stress is also thought to exacerbate inflammatory diseases such as psoriasis, asthma and arthritis (which may be ameliorated by suppression of the immune system) (Dhabhar & McEven, 1997). Also, stress is not a stringent concept – it is used as a term for various immune phenomena and other conditions (Dugué *et al*., 1992, 1993). As stated, our winter swimmers believed that regular ice-cold baths are wholesome and make them resistant to diseases. However, is the immune response involved? The basal levels of several analytes (leukocytes, monocytes, IL-6) involved in the immune response are higher than in controls, indicating that the immune system is slightly stimulated, leading to speculation that they are more prepared to react to an infection. However, in the same persons, some other immune aspects are impaired e.g. the mononuclear cells released a lower amount of IL-1β and IL-6. This could be interpreted as a counter-action breaking the development of an inflammatory process.

However, it should also be taken into account that it may be difficult to interpret our *in vivo* and *in vitro* results together, especially in the case of IL-6. In blood, the major IL-6-producing cell line is thought to be the monocytes, as the other cells (e.g. B and T cells and granulocytes) have been shown to produce only little (De Rijk *et al*., 1996). However, in the circulation, IL-6 may also be derived from other sources such as endothelial cells (De Rijk *et al*., 1997). Moreover, the results obtained after stimulation of the cells indicate their degree of priming to produce cytokines. They do not indicate the amount of cytokine that the cells are currently producing.

This study provides new information on the responses to hot and cold temperature and on the possible adaptation that subjects may develop if they experience such stress regularly. We showed that the neuroendocrine and immune systems are challenged after thermal stress. Moreover, at rest, the blood concentrations of several analytes involved in the immune system were significantly enhanced in the HWS. An adaptation to repeated hot and cold stress has previously been postulated as a mechanism for body sturdiness, resulting in an increased tolerance to stress and diseases. Exposure to repeated intensive short-term cold stimuli is often applied in hydrotherapy/physiotherapy to promote strength. Our results could contribute to the explanation of such phenomena.

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