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ORIGINAL ARTICLE

Effects of long-term whole-body cold exposures on plasma concentrations of ACTH, beta-endorphin, cortisol, catecholamines and cytokines in healthy females

J. LEPPÄLUOTO[1], T. WESTERLUND[2], P. HUTTUNEN[3], J. OKSA[4], J. SMOLANDER[5], B. DUGUÉ[6,7] and M. MIKKELSSON[2]

[1]*Department of Physiology, University of Oulu, Finland*; [2]*Rheumatism Foundation Hospital, Heinola, Finland*; [3]*Department of Forensic Medicine, University of Oulu, Finland*; [4]*Oulu Regional Institute of Occupational Health, Oulu, Finland*; [5]*ORTON Orthopaedic Hospital, Helsinki, Finland*; [6]*Minerva Foundation Institute, Helsinki, Finland*; [7]*Laboratory of Exercise-Induced Physiological Adaptations (EA3813), University of Poitiers, France*

Objective. Cold therapy is used to relieve pain and inflammatory symptoms. The present study was designed to determine the influence of long-term regular exposure to acute cold temperature. Two types of exposure were studied: winter swimming in ice-cold water and whole-body cryotherapy. The outcome was investigated on humoral factors that may account for pain alleviation related to the exposures. ***Material and methods***. During the course of 12 weeks, 3 times a week, a group of healthy females ($n=10$) was exposed to winter swimming (water 0–2°C) for 20 s and another group ($n=10$) to whole-body cryotherapy (air −110°C) for 2 min in a special chamber. Blood specimens were drawn in weeks 1, 2, 4, 8 and 12, on a day when no cold exposure occurred (control specimens) and on a day of cold exposures (cold specimens) before the exposures (0 min), and thereafter at 5 and 35 min. ***Results***. Plasma ACTH and cortisol in weeks 4–12 on time-points 35 min were significantly lower than in week 1, probably due to habituation, suggesting that neither winter swimming nor whole-body cryotherapy stimulated the pituitary-adrenal cortex axis. Plasma epinephrine was unchanged during both experiments, but norepinephrine showed significant 2-fold to 3-fold increases each time for 12 weeks after both cold exposures. Plasma IL-1-beta, IL-6 or TNFα did not show any changes after cold exposure. ***Conclusions***. The main finding was the sustained cold-induced stimulation of norepinephrine, which was remarkably similar between exposures. The frequent increase in norepinephrine might have a role in pain alleviation in whole-body cryotherapy and winter swimming.

Keywords: Epinephrine; norepinephrine; pain alleviation; winter swimming; whole-body cryotherapy

Introduction

Local cold therapy or cryotherapy has long been used to relieve pain and inflammatory symptoms. The effects of local cryotherapy have accounted for cold-induced analgesia and a decrease in joint temperature protecting cartilages from collagenases in rheumatic diseases [1,2]. Whole-body cryotherapy is extreme cold therapy developed in Japan in the 1970s to treat pain and inflammation in different rheumatic diseases [3]. The therapy, which lasts 1–3 min, is given in a cold room in which the air temperature is −110°C and is now used in several European countries mostly for medical reasons [4,5]. Winter swimming, a form of whole-body cryotherapy, has also been used as a treatment method in rheumatic diseases or as a recreational pastime in healthy subjects in countries where waters freeze during winter. For instance, there are about 80,000 people in Finland who go in for winter swimming. Both winter swimming and whole-body cryotherapy are highly efficient cooling methods. In healthy subjects, the mean skin temperature decreases after whole-body cryotherapy to 12–16°C [5], indicating a thermal loss of 12–20 kJ kg^{-1} body weight and resulting in local analgesia [2].

Whole-body cryotherapy has been recommended for patients suffering from arthritis, osteoarthritis, fibromyalgia or ankylosing spondylitis, and after the therapy session the results show that subjective and objective pain ratings decrease and joint movements improve for as long as 24 h [6–10]. In order to understand the mechanisms of the effects of whole-body cryotherapy, humoral responses related to pain, stress and inflammation have also been reported in these studies. After a single therapy session, pro-opiomelanocortin-related hormones (ACTH and beta-endorphin) and adrenal hormones (epinephrine and cortisol) remained unchanged or decreased [11,12]. However, plasma norepinephrine showed a

*Correspondence author. Benoit Dugué, UFR Sciences du Sport de l'Université de Poitiers et Laboratoire des Adaptations Physiologiques aux Activités Physiques (EA3813), 4 allée Jean Monnet, 86000, Poitiers, France. Tel: +33 54945 4040. Fax: +33 5494 53396. Email: benoit.dugue@univ-poitiers.fr

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clear increase after exposure. It is worth noting that there is no previous information about the long-term effects of whole-body cryotherapy on plasma ACTH, beta-endorphin, cortisol or catecholamines in healthy subjects.

Similar hormonal responses have been observed after winter swimming in ice-cold water in healthy subjects. Norepinephrine increases in response to winter swimming, but no changes can be seen in plasma epinephrine or cortisol [13,14]. There are some studies in which long-term effects of winter swimming have been documented. Norepinephrine resting levels and cold-induced responses become smaller, but beta-endorphin levels do not change after 3–8 months in regular winter swimming [13–15]. In laboratory conditions, repeated cold-water immersions have been observed leading to unchanged [16] or increased [17] norepinephrine responses.

Several studies have been conducted into the effects of cold exposures on the immune system. After 6 weeks of repeated cold-water immersions, the proportions of lymphocytes and monocytes and plasma tumour necrosis factor-alpha (TNFα) increased with no changes in plasma interleukin IL-6, IL-1-beta or C-reactive protein in young men [18]. In another study, resting levels of IL-6 and monocytes were higher in habitual than in inexperienced winter swimmers at the end of the winter season [19]. Immuno-stimulating effects of cold exposures have been reported in conjunction with heating or exercise [20]. It appears that no information is presently available about the long-term effects of extreme cold exposures on cytokines.

We hypothesized that the effects of long-term whole-body cold exposures could be associated with humoral factors. Stimulation of ACTH and cortisol has long been known to reduce inflammation processes. Spinal administrations of beta-endorphin [21], norepinephrine or an adrenoceptor agonist [22–24] have been shown to lead to pain alleviation. In some studies, cold exposures have been found to enhance the release of some cytokines to circulation [18–20]. We therefore studied the effects of long-term winter swimming and whole-body cryotherapy on plasma levels of pro-opiomelanocortin-related and adrenal hormones and proinflammatory cytokines to explore the involvement of humoral factors in these two forms of extreme cold exposure.

Methods

Subjects

Volunteers for the present study were recruited through an advertisement in a local newspaper. A total of 42 subjects, mostly women, applied. Those with high physical activity, on medication or recently practising winter swimming or whole-body cryotherapy were excluded. Twenty healthy women aged between 35 and 45 years were chosen (height 163 ± 8 cm (mean and SD), weight 67 ± 10 kg and body mass index 25 ± 3). Depending on body mass index, age and physical activity, the subjects were divided into 10 similar pairs randomized into 2 groups (10 subjects in each), one participating in winter swimming, the other in whole-body cryotherapy. In the group of winter swimmers, none used oral contraceptives, five had no menses, four used intrauterine devices and three subjects started winter swimming on the follicular phase of menses. In the group of extreme cold therapy, none used oral contraceptives, four had no menses, four used intrauterine devices and one subject started the experiment in the follicular phase. No information about the menstrual cycle was available in five subjects. The subjects have been described recently [25]. They gave their informed consent for the study and the experimental design was approved by the Ethics Committee of the Hospital District Päijät-Häme, Finland.

Experimental design

Subjects in the winter swimming group were exposed to swimming 3 times a week for 12 weeks in a pond in the area of the Rheumatism Foundation Hospital between 1500 h and 1715 h. They put on bathing suits at room temperature and immersed themselves in the pond water for 20 s with their heads out of the water. Water temperature was between 0 and 2°C. Four blood specimens were taken twice a week with the subjects in a sitting position in weeks 1, 2, 4, 8 and 12. The specimens were drawn at 0, 5 and 35 min in the week and day when no immersion occurred (control experiment). In the same week, the second series of specimens were drawn before the immersion (0 min) as well as at 5 and 35 min afterwards (cold experiment). In order to minimize any unpleasant experience from the first test immersion, the subjects were shown the immersion and blood specimens collection procedures and twice exposed to cold water for 1–3 s before the start of the experiments.

The whole-body cryotherapy group had three 2-min cold exposures (−110°C) per week for 12 weeks between 1500 and 1715 h. The subjects were exposed to cold in a specially built temperature-controlled unit (Zimmer, Elektromedizin, Germany) with three chambers at temperatures of −10, −60 and −110°C, respectively. During the exposures, the subjects wore bathing suits, masks, caps, gloves and socks. The first

two exposures were made only in the −10℃ or −60℃ chambers. Blood specimens for control and cold experiments were collected at 0, 5 and 35 min as above.

Biochemical analyses

Blood specimens were collected in cooled EDTA tubes for ACTH, beta-endorphin, IL-1-beta, IL-6 and TNFα in EDTA-sodium metabisulphite tubes for catecholamine measurements. Plasma specimens were separated within 120 min and stored at −70℃ before assays. For ACTH and beta-endorphin measurements, 2 mL plasma samples were extracted with Sep-pak C18 cartridges and assayed in radioimmunoassays as described previously [26,27]. Plasma cortisol was measured using a radioimmunoassay kit (Orion Diagnostica, Turku, Finland). Cytokines IL-1-beta, IL-6 and TNFα were determined using enzyme-immunoassay kits (R&D, Minneapolis, Minn., USA). Plasma catecholamines were purified by aluminium oxide extraction and assayed using an HPLC method using electrochemical detection [28].

The sensitivities of the ACTH and BE assays were 0.8 and 2 pg/tube, while the intra-assay coefficient of variation was below 10 % in both assays. The intra-assay coefficient of variation was 4.4 % and 4.8 % for NA and A, respectively. The sensitivities of the cytokine assays were 0.125, 0.156 and 0.12 pg/mL and the intra-assay coefficients of variation 6.9, 5.9 and 5.9 %, respectively.

Statistical analyses

Results are expressed as means ± SE. The changes in each humoral parameter during the 35 min experiments were analysed by repeated measures one-way analysis of variance with time as a factor separately in control and cold experiments. We used logarithm transformation for the analytes when the distribution was not Gaussian. Repeated measures two-way analysis of variance was used to analyse changes between cold and control experiments with time and treatments (cold versus control) as factors. The changes between winter swimming and whole-body cryotherapy experiments were analysed in two-way analysis of variance with time and treatments (winter swimming versus whole-body cryotherapy) as factors. Multiple comparisons between respective time-points were made using the Newman-Keul's test and between the zero time-point and other time-points with the Dunnett method. The results were considered statistically significant when $p < 0.05$.

Results

The results for ACTH, beta-endorphin and cortisol in winter swimming and whole-body cryotherapy are presented in Table I. Generally, no significant changes in the resting levels of the hormones (at 0 min) were seen during the experiments. Plasma ACTH showed a non-significant increase especially after winter swimming. This was due to elevated ACTH levels in two subjects, who evidently experienced the experimental situation as harmful during the first sessions. This resulted in significantly low plasma ACTH levels in week 12 at 15 and 35 min when compared to the 0 min value. After the whole-body cryotherapy, plasma ACTH in week 4 at 35 min was significantly lower than in week 1.

Plasma beta-endorphin did not show any significant response to winter swimming or extreme cold therapy: Plasma cortisol exhibited a non-significant increase after the first winter swimming, as did ACTH. In weeks 4, 8 and 12, plasma cortisol at 15 and 35 min was lower than in week 1 ($p < 0.01$) in winter swimmers. During the whole-body cryotherapy, plasma cortisol at 15 min was significantly lower in week 4 than in week 1. In week 12, plasma cortisol at 15 and 35 min was lower than at 0 min ($p < 0.05$).

Plasma epinephrine and norepinephrine: No significant changes were seen in the resting levels of epinephrine or norepinephrine during the experiments (Figures 1, 2). Plasma epinephrine showed no significant response either to winter swimming or to whole-body cryotherapy. However, plasma norepinephrine demonstrated a 2-fold to 3-fold increase ($p < 0.01$) immediately after the cold exposure in both winter swimming and whole-body cryotherapy, and the response remained at the same level throughout the 12-week experiment. Note that in week 4 the norepinephrine response remained at a significant level for 35 min after whole-body cryotherapy.

Plasma cytokines: The resting levels of IL-1-beta, IL-6 and TNFα were low and partly undetectable. The cytokines were assayed only from the samples at 0 and 35 min due to their slow responses (Table II). No significant changes were seen in weeks 1, 4 and 12 in the samples taken at 0 min and 35 min.

There were no statistically significant differences between the respective time-points of the winter swimming and whole-body cryotherapy sessions in any hormone or cytokine concentration.

Discussion

Of the humoral responses to long-term winter swimming and to whole-body cryotherapy, those of norepinephrine were the most striking. We observed a significant 2-fold to 3-fold increase in plasma

Table I. Plasma ACTH, cortisol and beta-endorphin (presented as mean and SE in parentheses) in female subjects participating in winter swimming or extreme cold therapy. The concentrations at rest, after 5 min and 35 min of exposure (and the corresponding time for the control session) at weeks 1, 4, 8 and 12 are presented. The number of subjects was 10 in each session. § denotes $p<0.05$ from the 0 min values and §§ $p<0.01$. An asterisk denotes $p<0.05$ from the respective 1st week value.

			1st week			2nd Week			4th Week			8th Week			12th Week		
Analytes			Basal Value	After 5 min	After 35 min	Basal Value	After 5 min	After 35 min	Basal Value	After 5 min	After 35 min	Basal Value	After 5 min	After 35 min	Basal Value	After 5 min	After 35 min
ACTH (pmol/L)	Winter Swimming	Control Day	1.8	2.1	1.6	1.7	1.7	2.7	2.4	2.2	1.4	1.7	2.1	2.0	2.7	2.6	2.3
			(0.3)	(0.3)	(0.2)	(0.3)	(0.3)	(0.7)	(0.5)	(0.6)	(0.3)	(0.2)	(0.3)	(0.3)	(0.6)	(0.5)	(0.2)
		Exposure Day	3.2	5.1	2.7	2.6	2.5	2.1	2.2	2.7	1.8	1.9	1.8	2.5	2.7	2.3	1.2
			(1.3)	(2.3)	(1.1)	(0.7)	(0.5)	(0.3)	(0.4)	(0.4)	(0.4)	(0.4)	(0.3)	(0.4)	(0.3)	(0.2)	(0.2)
	Cryotherapy	Control Day	2.7	3.3	3.0	3.1	3.0	3.1	3.5	3.3	2.6	3.5	3.5	3.1	4.0	3.4	3.4
			(0.3)	(0.3)	(0.6)	(0.3)	(0.2)	(0.2)	(0.3)	(0.4)	(0.4)	(0.5)	(0.3)	(0.4)	(0.6)	(0.3)	(0.3)
		Exposure Day	2.9	3.5	3.2	3.1	3.8	3.3	2.8	3.0	2.1	3.0	3.0	3.0	3.4	3.8	3.3
			(0.3)	(0.5)	(0.2)	(0.3)	(0.4)	(0.2)	(0.4)	(0.3)	(0.2)	(0.3)	(0.2)	(0.3)	(0.2)	(0.4)	(0.4)
Cortisol (nmol/L)	Winter Swimming	Control Day	400	570	431	496	431	465	313	432	385	560	472	368	430	370	306
			(70)	(65)	(78)	(70)	(87)	(118)	(60)	(81)	(94)	(114)	(104)	(97)	(89)	(68)	(73)
		Exposure Day	619	638	941	537	551	506	346	323	341**	323	349	322**	399	400	335**
			(138)	(144)	(174)	(128)	(123)	(75)	(64)	(71)	(44)	(66)	(85)	(38)	(59)	(53)	(58)
	Cryotherapy	Control Day	160	206	180	224	160	142	208	163	141	204	194	197	244	221	180
			(14)	(34)	(39)	(43)	(19)	(13)	(30)	(26)	(28)	(39)	(21)	(39)	(44)	(32)	(34)
		Exposure Day	201	267	216	166	211	223	193	183	131	249	259	191	167	144	128§
			(36)	(62)	(41)	(30)	(25)	(35)	(28)	(29)	(30)	(58)	(64)	(34)	(9)	(11)	(14)
Beta- Endorphin (pmol/L)	Winter Swimming	Control Day	11.8	11.4	11.4	11.2	10.6	12.2	12.9	12.3	10.0	10.5	10.4	10.2	11.9	10.7	9.8
			(1.5)	(1.1)	(1.2)	(1.0)	(1.2)	(0.9)	(1.5)	(1.2)	(1.0)	(1.0)	(0.9)	(0.9)	(0.8)	(0.8)	(0.8)
		Exposure Day	12.0	13.4	13.2	13.0	12.0	12.3	13.0	11.3	11.4	9.1	9.2	11.1	11.0	9.2	11.1
			(1.2)	(1.1)	(1.2)	(1.0)	(1.0)	(1.2)	(1.3)	(0.8)	(1.1)	(0.7)	(0.8)	(1.1)	(0.9)	(0.8)	(1.1)
	Cryotherapy	Control Day	8.0	9.3	9.3	10.2	9.6	10.3	10.4	10.6	10.7	9.7	10.7	10.9	11.9	11.2	11.5
			(1.3)	(0.9)	(1.1)	(1.2)	(1.3)	(1.0)	(1.0)	(1.0)	(1.4)	(0.6)	(1.3)	(1.2)	(1.0)	(1.1)	(1.2)
		Exposure Day	9.7	11.1	10.9	10.4	10.4	10.1	9.1	9.6	9.2	9.8	8.7	9.9	11.0	11.2	10.3
			(1.1)	(1.0)	(1.3)	(0.6)	(0.9)	(1.0)	(1.2)	(0.8)	(0.5)	(0.8)	(1.0)	(0.8)	(0.8)	(0.7)	(0.9)

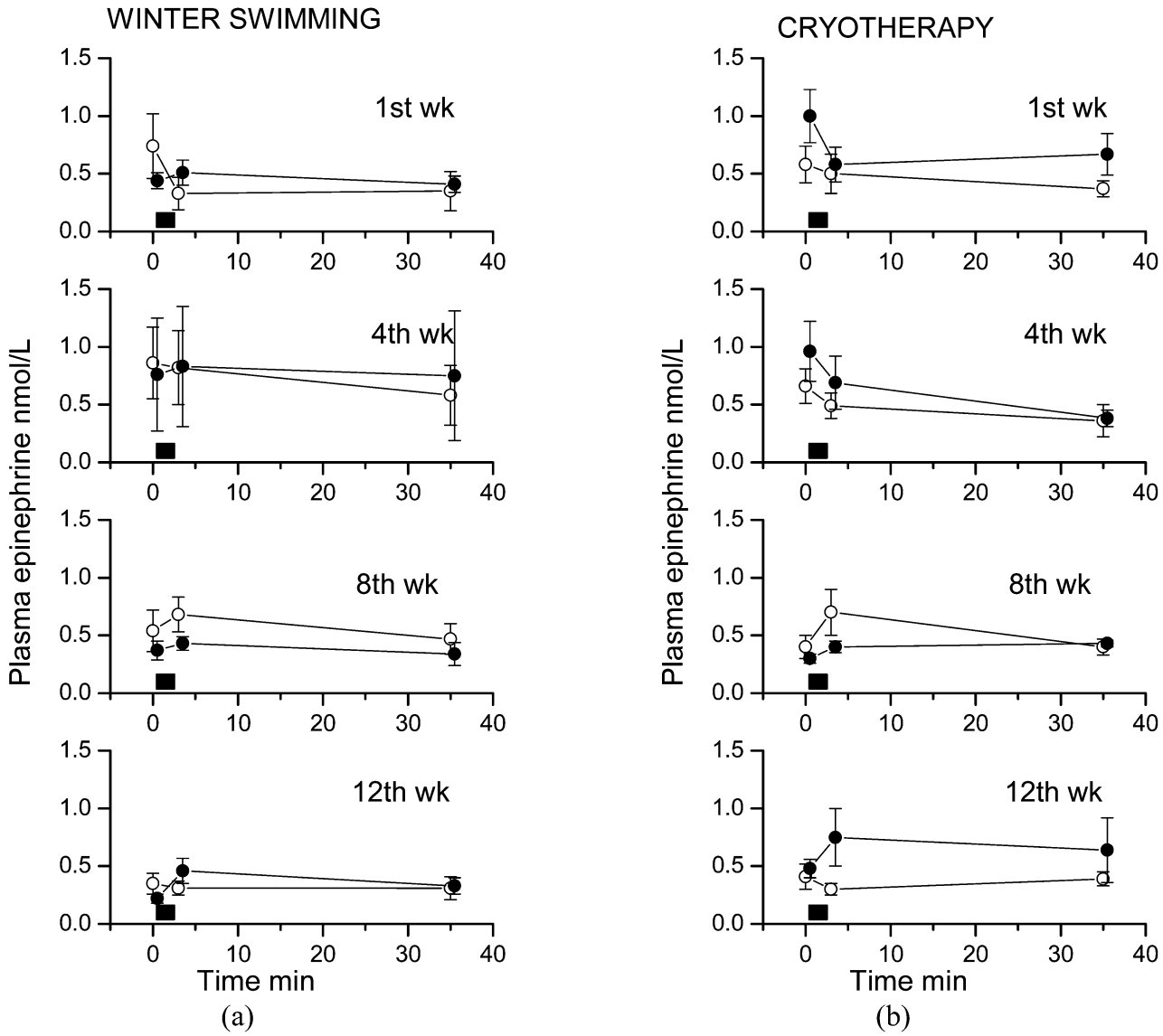

Figure 1. Plasma epinephrine in female subjects participating in winter swimming (a) or in extreme cold therapy (b) sessions at weeks 1, 4, 8 and 12. There were 10 subjects in each session. A black square on the x-axis denotes the cold or control session. Open circles denote control and black circles cold exposure. § denotes $p<0.05$ from the 0 min values and §§ $p<0.01$. An asterisk denotes $p<0.05$ from the respective 1st week value.

norepinephrine after the exposures for 12 weeks, but no response in plasma epinephrine at all. Two different kinds of stress may lead to similar physiological reaction [29]. Similar findings, cold-induced increases in plasma norepinephrine without any changes in plasma adrenaline, have earlier been seen after a single winter swimming [13,14] or extreme cold therapy session [4] and also in other types of cold exposure [30,31]. Since norepinephrine mostly originates from the sympathetic nerve endings and epinephrine from the adrenal medulla, we can conclude that both cold exposures activate the sympathetic nerve system.

It is interesting that the plasma norepinephrine response remained at the same levels in weeks 2, 4, 8 and 12 as in week 1 in both our winter swimming and whole-body cryotherapy sessions. There is no previous information about the long-term hormonal responses to whole-body cryotherapy, but in a winter swimming study plasma norepinephrine resting levels fell after 8 months [15], and the response to cold water decreased after 3 months [14]. In those experiments, however, a decrease in the basal norepinephrine level was seen in the subjects exposed to cold stimuli and in their controls. This phenomenon seemed to be related to seasonal chronobiology

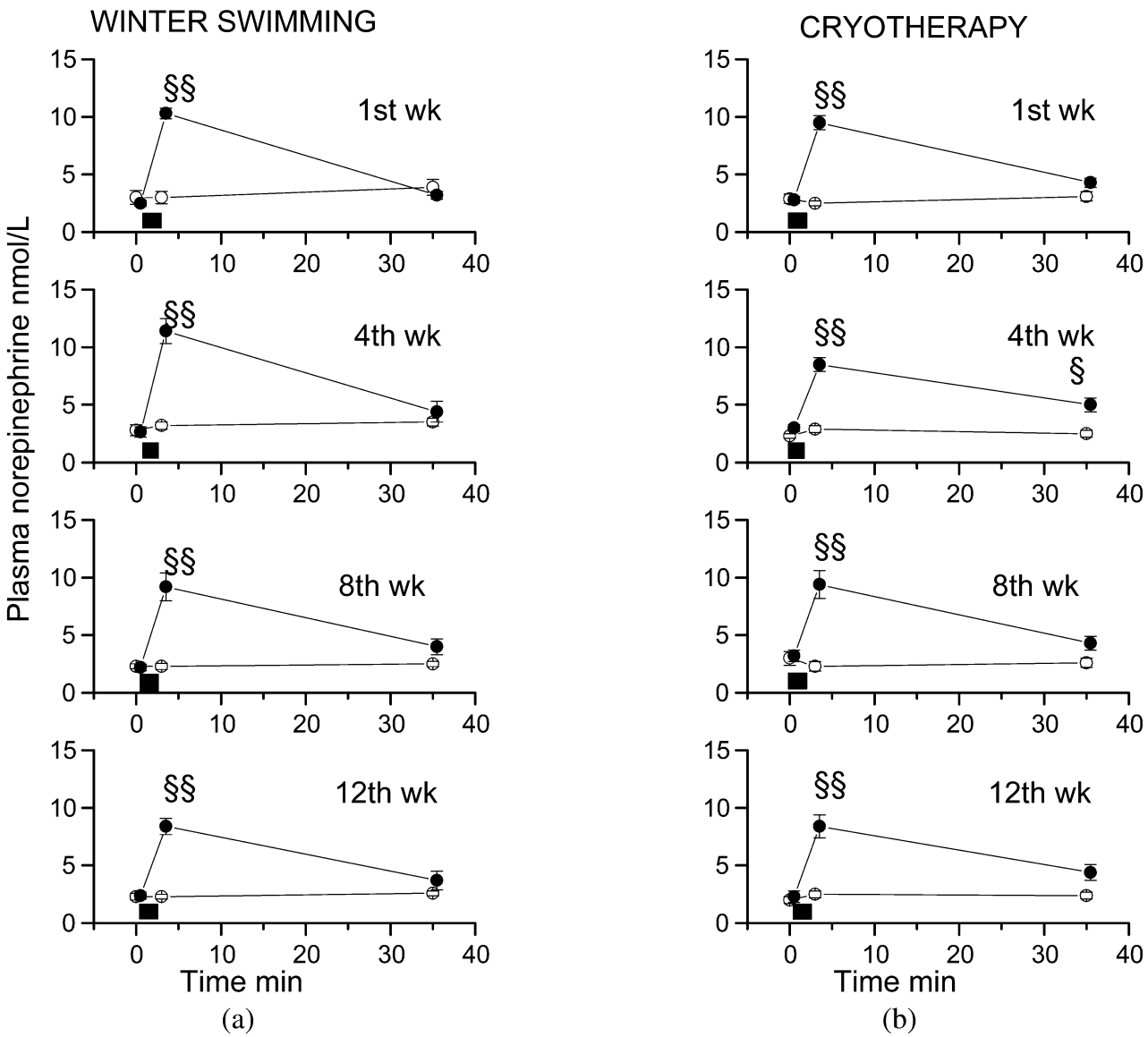

Figure 2. Plasma norepinephrine in female subjects participating in winter swimming (a) or in extreme cold therapy (b) sessions at weeks 1, 4, 8 and 12. There were 10 subjects in each session. A black square on the x-axis denotes the cold or control session. Open circles denote control and black circles cold exposure. § denotes $p<0.05$ from the 0 min values and §§ $p<0.01$. An asterisk denotes $p<0.05$ from the respective 1st week value.

during late autumn and the beginning of winter, at the time the experiments were carried out. Habituated (decreased) norepinephrine responses have been observed in studies utilizing repeated cold air exposures in laboratory conditions [32,33]. On the contrary, repeated cold-water immersions have been found to lead to increased [17] or unchanged [16] norepinephrine responses after acclimation. In our experimental setting, there was no habituation and the norepinephrine response remained unchanged during the 12-week period. This may be due to the short durations of our cold exposures and the very low temperatures we used (−110℃ for the air in the whole-body cryptherapy and 0–2℃ for the winter swimming). However, our experiment was non-hypothermic, which may be the main reason why we did not see habituation. Actually, similar results have been obtained previously on norepinephrine responses in subjects exposed to moderate cold water (14℃) [34–36]. One or two other factors, such as time of the season, age, gender and even traits of the volunteers, may also have interfered with the basal level of norepinephrine and its reactivity [15].

Previous studies have shown that local analgesia in cryotherapy requires skin temperature to be below 13.6℃, when nerve conduction and acetylcholine

Table II. Plasma interleukin (IL)-1beta and -6 and tumour necrosis factor (TNFα) in female subjects participating in the winter swimming or extreme cold therapy. The concentrations at rest and 35 min of exposure (and the corresponding time for the control session) at weeks 1, 4 and 12 are presented. No statistically significant differences between exposures (control versus exposure) or time (1, 4 or 12 weeks).

			1st week		4th week		12th week	
Analytes			Basal value	After 35 min	Basal value	After 35 min	Basal value	After 35 min
IL 1-beta (pg/mL)	Winter swimming	Control day	0.23 (0.11)	0.13 (0.01)	0.16 (0.01)	0.12 (0.01)	0.25 (0.13)	0.13 (0.01)
		Exposure day	0.13 (0.01)	0.13 (0.01)	0.21 (0.09)	0.13 (0.01)	0.13 (0.01)	0.13 (0.01)
	Cryotherapy	Control day	0.11 (0.03)	0.38 (0.31)	0.10 (0.01)	0.17 (0.04)	0.28 (0.19)	0.29 (0.19)
		Exposure day	0.11 (0.01)	0.16 (0.06)	0.13 (0.01)	0.61 (0.32)	0.08 (0.02)	0.16 (0.07)
IL-6 (pg/mL)	Winter swimming	Control day	0.9 (0.2)	0.9 (0.2)	1.0 (0.1)	0.7 (0.1)	0.7 (0.1)	0.6 (0.1)
		Exposure day	0.8 (0.1)	0.7 (0.1)	0.9 (0.2)	0.8 (0.1)	0.9 (0.1)	0.6 (0.2)
	Cryotherapy	Control day	1.1 (0.1)	1.1 (0.3)	1.2 (0.2)	1.4 (0.3)	1.1 (0.1)	1.2 (0.3)
		Exposure day	1.2 (0.3)	1.4 (0.2)	1.2 (0.2)	1.4 (0.3)	1.2 (0.2)	1.2 (0.2)
TNFα (pg/mL)	Winter swimming	Control day	1.1 (0.1)	1.2 (0.1)	1.2 (0.9)	1.2 (0.1)	1.1 (0.1)	1.1 (0.1)
		Exposure day	1.0 (0.1)	1.2 (0.1)	1.1 (0.1)	1.2 (0.1)	1.1 (0.1)	1.2 (0.1)
	Cryotherapy	Control day	1.6 (0.2)	1.8 (0.2)	1.3 (0.1)	1.4 (0.1)	1.4 (0.1)	1.4 (0.1)
		Exposure day	1.4 (0.1)	1.3 (0.1)	1.5 (0.3)	1.4 (0.1)	1.3 (0.19	1.4 (0.1)

formation become suppressed [2,37]. During the whole-body cryotherapy of 2 min at −110℃ this temperature was reached in the back and in the extremities, excluding the hands and feet, which were covered by gloves and socks [5]. Surprisingly, pain has been found to be alleviated in covered hands and feet during whole-body cryotherapy [4], although their temperatures stay well above 13.6℃. This has been explained by a lessening of nerve transmission over a large area of the body [4]. It is also possible that humoral mechanisms may be responsible for alleviating symptoms after whole-body cryotherapies.

The persistent highly significant norepinephrine response we observed in this study during winter swimming and whole-body cryotherapy might be one possible explanation for the beneficial pain-alleviating effects of whole-body cryotherapy treatments observed previously (*vide supra*). Sympathetic stimulation releases norepinephrine both from peripheral nerve endings and brainstem nuclei [24]. In addition, spinal administration of norepinephrine in experimental animals and epidural injections of an adrenoceptor agonist in human subjects, respectively, alleviate pain [22,23,38]. A cold-induced increase in norepinephrine may therefore have a role in suppressing pain at the spinal level. Circulating norepinephrine reaches the spinal cord via the posterior spinal arteries supplying, for example, the substantia gelatinosa where pain afferents from skin terminate [39].

On the first cold exposure, plasma ACTH and cortisol showed a slight increase, but one that was not significant. It was due to high plasma ACTH and cortisol levels in two of the subjects participating in the winter swimming study, although all the subjects were accustomed to the cold exposures before the experiment. We then observed significantly decreased plasma ACTH and cortisol levels in weeks 4–12. The decreases in plasma ACTH and cortisol were probably related to a habituation response towards the whole experimental situation. Previously, unchanged plasma cortisol levels have also been observed in a long-term winter swimming study [14]. A more intensive whole-body cryotherapy (−160℃ every day for 2 weeks) has been shown to increase plasma ACTH, beta-endorphin and cortisol in arthritis patients [40], although non-specific stress effects cannot be excluded.

Similarly, we did not observe any significant changes in plasma beta-endorphin during the 12 weeks of winter swimming or whole-body cryotherapy. This finding concurs with a previous study in which plasma beta-endorphin did not change during an 8-month winter swimming period [15]. Plasma beta-endorphin accounts mostly for beta-lipotropin, which has slow kinetics, and hence minor changes in its levels may have remained unnoticed.

In one earlier study, long-term cold-water immersions of healthy males resulted in slight elevations in plasma TNFα and lymphocyte and monocyte counts, but no changes were seen in plasma IL-6 or IL-1-beta [18]. In another study, lymphocyte and monocyte cell counts and plasma IL-6 were higher in habitual than in inexperienced winter swimmers [19]. In our study, plasma levels of cytokines IL-1-beta, IL-6 or TNFα at 1, 4 or 12 weeks did not show any significant changes during winter swimming and extreme cold therapy and were fairly close to the detection limits of the assays. Though the previously cited studies used ultrasensitive methods (more sensitive that the one we used) for detecting cytokines, we can infer that long-term extreme cold exposures of short duration do not lead to clear activation of proinflammatory cytokines

IL-1-beta, Il-6 or TNFα. It has been suggested that cold and exercise-induced increases in plasma norepinephrine may account for changes in cell counts and a rise in plasma IL-6 [20]. Despite large increases in plasma norepinephrine after the cold exposures in the present study, no such association was found.

In summary, the humoral responses of long-term winter swimming or whole-body cryotherapy were remarkably similar. There was no stimulation in traditional stress hormones such as ACTH, beta-endorphin, adrenaline and cortisol or in proinflammatory cytokines. However, our present observations showed that both winter swimming and whole-body cryotherapy resulted in increased levels of circulating norepinephrine after exposures for 12 weeks. We point out that our study was performed in healthy female subjects; it is presently not known whether the humoral responses are similar also in patients. However, sustained norepinephrine stimulation during whole-body cryotherapy could have a role in pain alleviation.

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