

ORIGINAL ARTICLE

## Acute and long-term effects of winter swimming and whole-body cryotherapy on plasma antioxidative capacity in healthy women

B. DUGUÉ<sup>1,2</sup>, J. SMOLANDER<sup>3</sup>, T. WESTERLUND<sup>4</sup>, J. OKSA<sup>5</sup>,  
R. NIEMINEN<sup>6</sup>, E. MOILANEN<sup>6</sup> & M. MIKKELSSON<sup>4</sup>

<sup>1</sup>Laboratory of Exercise-Induced Physiological Adaptations (EA 3813), University of Poitiers, Poitiers, France, <sup>2</sup>Minerva Foundation Institute for Medical Research, BioMedicum, Helsinki, Finland, <sup>3</sup>Orton Orthopaedic Hospital and Orton Research Institute, Helsinki, Finland, <sup>4</sup>Rheumatism Foundation Hospital, Heinola, Finland, <sup>5</sup>Oulu Regional Institute of Occupational Health, Oulu, Finland, and <sup>6</sup>Immunopharmacological Research Group, University of Tampere Medical School and Research Unit Tampere University Hospital, Tampere, Finland

### Abstract

The effects of severe cold stress on total peroxy radical trapping antioxidant capacity of plasma (TRAP) were studied in two groups of healthy women: a whole-body cryotherapy group (WBC,  $n=10$ ) and a winter swimming group (WS,  $n=10$ ). The biovariability of TRAP values was also analysed. The WBC group was exposed to  $-110^{\circ}\text{C}$  for 2 min, whereas the exposure for the WS group lasted 20 s in ice-cold water. Sessions were organized three times per week for 12 weeks. Blood specimens were collected at 2, 4, 8 and 12 weeks at rest, 2 and 35 min after the cold exposures and at the corresponding times without cold exposure on a separate day. Conventional methods were used to determine TRAP values. The between-subject variation was 13.6% and the within-subject variation 6.4%. The index of individuality was 0.46, and the index of heterogeneity was 0.079. These results indicate a marked heterogeneity among subjects. During the first 4 weeks, the mean TRAP value significantly increased at 2 min after cold exposure in the WBC group, returning to baseline 35 min after the exposure. Similar changes were observed in the WS group. However, all changes due to cold were relatively mild ( $<5\%$ ). After 4 weeks no changes in TRAP values after the cold exposures were noticed and no long-term changes in basal TRAP values were observed. In the main, regular WBC and WS do not seem to be harmful as far as plasma antioxidative capacity is concerned.

**Key Words:** *Antioxidative capacity, biological variation, cold, cryotherapy, oxidative stress, TRAP, winter swimming*

### Introduction

In whole-body cryotherapy (WBC) patients, wearing minimal clothing, are exposed to very cold air ( $-110^{\circ}\text{C}$ ) in a special unit for 1 to 3 min. This procedure is mainly used to alleviate

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Correspondence: Benoit Dugué, PhD, Laboratory of Exercise-Induced Physiological Adaptations (EA 3813), 4 Allée Jean Monnet, FR-86 000 Poitiers, France. Tel: +33 5 49 45 4040. Fax: +33 5 49 45 3396. E-mail: benoit.dugue@univ-poitiers.fr

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inflammation and pain in, for example, arthritis [1], osteoarthritis [2] and fibromyalgia [3]. Winter swimming (WS), i.e. swimming or immersion in ice-cold water, is practised in northern countries, where seas, lakes and rivers freeze during the wintertime. Both WBC and WS represent intense but short-term cold exposure. The reported reasons for WS include improved general well-being, and self-treatment or body hardening against respiratory tract infections and musculoskeletal pains [4].

However, there are no well-controlled data to support these claims. Adaptation to repeated cold stress has been postulated as a mechanism resulting in increased resistance to diseases, but, again, there is little evidence to support this view [5]. Only a few works concerning the activation of the immune system and improved antioxidant protection in winter swimmers have been published. Dugué & Leppänen [6] have reported an increase in the resting concentrations of leucocytes, monocytes and plasma interleukin-6 in regular winter swimmers compared with those in inexperienced subjects. Moreover, after exposure to cold water, the capacity of blood mononuclear cells to produce interleukin-1-beta and interleukin-6 was significantly suppressed in inexperienced subjects, but tended to increase in regular winter swimmers. Siems et al. [7–9] have shown that acute WS in ice-cold water from 1 to 5 min leads to oxidative stress in experienced winter swimmers. However, the winter swimmers had significantly higher baseline values of reducing enzymes in erythrocytes (superoxide-dismutase, glutathione peroxidase and catalase) compared with control subjects [8]. They postulated that this improved antioxidant protection was the result of repeated exposure to non-damaging, mild, oxidative stress. The common limitation of these studies, however, is their cross-sectional design.

The purpose of the present study was to compare the acute and long-term changes in plasma antioxidant capacity in women who attended WBC regularly with changes in women who started regular WS. Total peroxy radical trapping antioxidant capacity of plasma (TRAP) reflects the global combined antioxidant capacity of all individual antioxidants in blood and was chosen as the analyte to follow in our volunteers. The large number of baseline specimens we collected allowed us to analyse the biovariability (within- and between-subject variations) in TRAP values in healthy women.

## **Material and methods**

Twenty healthy women (aged 35–45 years) with normal body-weight (body mass index (BMI) < 28) and without hormonal substitution were chosen for the study from 42 volunteers, reached through an announcement in a local newspaper. The subjects were matched pairwise, having similar BMI, age, physical activity and use of hormonal contraception. The pairs were then randomly assigned either to the WBC group ( $n=10$ ) or the WS group ( $n=10$ ). None of the subjects had practised WBC or WS regularly before the study or had tried these activities during that winter. The subjects were moderately physically active, and no outdoor workers were included. They were asked to maintain their physical activity habits during the study period. The characteristics of the subjects are presented in Table I.

Before the study, all subjects underwent a medical check-up including a resting ECG. The protocol and procedures were done according to the Helsinki Declaration and were approved by the Ethics Committee of the Hospital District.

The study was carried out during the spring of 2002. The WBC group had three 2-min exposures per week for 3 months at  $-110^{\circ}\text{C}$  in a specially built, temperature-controlled unit (Zimmer, Elektromedizin). The unit has three chambers, where the subject passes

Table I. Physical characteristics of subjects in the winter swimming group (WS) and in the whole-body cryotherapy (WBC) group. Results are expressed as means and standard deviation (SD), in parentheses.

Variable	Winter swimming ( <i>n</i> =10)	Cryotherapy ( <i>n</i> =10)
Age, years	38 (3)	39 (2)
Height, cm	167 (7)	166 (6)
Weight, kg	68 (14)	67 (9)
Body mass index	24 (3)	24 (2)
Sum of four skinfolds, mm	62 (23)	56 (16)

through the first chamber ( $-10^{\circ}\text{C}$ ) and the second chamber ( $-60^{\circ}\text{C}$ ) before coming into the therapy chamber. During WBC, the subjects wore bathing suits, surgical masks, caps, gloves, socks and shoes. In the therapy chamber, the subjects were instructed to slightly move their fingers and legs.

The WS group had three exposures per week for 3 months (February, March, April) in a small pond in the hospital area. A mechanical pump in the pond stirred the water, by which the hole in the ice was kept open all the time. By the pier, there was a sauna building with a dressing-room at normal room temperature, where the subjects put on their bathing suits. The WS was done without a sauna bath. The subjects were instructed to stay in the water for about 20 s immersed to the neck (“head-out immersion”). In order to do this, they were asked to count slowly from 1 to 20, while immersed in the water. The personnel of the hospital’s WBC unit organized the exposures for the WBC group, but the WS group carried out the exposures by themselves according to the instructions.

When humans are exposed suddenly to severe cold stress, especially water, they may experience a “cold shock”, which is reduced rapidly with repeated cold stress [10]. To avoid this, the first 2-min exposure in the WBC group was at  $-10^{\circ}\text{C}$ , and the second at  $-60^{\circ}\text{C}$ . Similarly, the WS group was asked just to take a dip in the water during the first and second visits.

Blood specimens were taken at the beginning of the study, after 2 weeks, after 1, 2 and 3 months, so that the test protocol was carried out first without cold exposure and then on the next or next few days with the cold exposure. The blood specimens were taken at the beginning, after 2 and 35 min. The test protocol was carried out thus: the subjects lay down for 10 min and then sat up for 5 min. After that, the first blood specimen was taken. The second specimen was taken after 2 min or, on the day of the cold exposure, after the cold exposure (which was about 2 to 3 min after the first specimen), while the subject was sitting. After that, all subjects lay down, but the winter swimmers changed their clothes quickly before doing so. At 30 min the subjects started to sit up again and the last sample was taken after 5 min of sitting, 35 min after the start.

Venous blood specimens (EDTA tubes, BD, USA) were obtained between 15.00 h and 17.15 h using a free-flowing blood-draw and a maximum 1-min tourniquet application. After the venipuncture, the specimens were placed in ice-water and protected from light and centrifuged within 2 h (1560g, 10 min at  $+8^{\circ}\text{C}$  (Heraeus Megafuge 1; OR Kendro Laboratory Products GmbH, Haunau, Germany). After centrifugation, the plasma was divided into aliquots and immediately deep-frozen at  $-70^{\circ}\text{C}$ .

Total antioxidant capacity (total TRAP) of plasma samples was measured by a chemiluminescence method, as described earlier [11]. In brief, peroxy radicals are produced at a constant rate by thermal decomposition of 2,2-azo-bis(2-aminopropane) hydrochloride (ABAP; Polysciences, Warrington, Pa., USA) in a test-tube and peroxy radical reactions are followed by luminol-enhanced chemiluminescence. The time at which the added

plasma sample extinguishes the reaction is directly proportional to the peroxy radical-trapping anti-oxidative capacity (TRAP) of the sample.

The reaction was initiated by mixing 450 µl of 100 mM phosphate buffer, pH 7.4 in saline, 50 µl of 400 mM ABAP and 50 µl of 10 mM luminol (5-amino-2,3-dihydro-1,4-phthalazinedione (Sigma Chemical Co., St. Louis, Mo., USA) in 100 mM borate buffer, pH 10. The cuvette was placed in a temperature-controlled sample carousel in a luminometer adjusted at 37°C, and the luminescence was measured at 35-s intervals. The reaction mixture was allowed to stabilize for 15 min after which the plasma sample (20 µl) was injected directly into the cuvette and the time for which it extinguished the chemiluminescence was determined. A water-soluble tocopherol, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Hoffman-La Roche Ltd., Basel, Switzerland) which is known to trap two peroxy radicals per molecule [12] was used as a standard and TRAP values of the unknown samples were calculated from the linear regression line for Trolox.

### Statistics

The possible changes in resting values of TRAP with time before the control day and experimental day were analysed separately for the WS and WBC groups by two-way analysis of variance with repeated measures on both factors (weeks, control/exposure). *Post-hoc* comparisons of TRAP values at different time-points against the resting values at the first exposure were done with the Duncan's test. At each point of time and test situation, the mean changes in TRAP values (with 95% CIs) were calculated from the corresponding resting TRAP values to values at 2 and 35 min after the control/exposure situation.

The biovariability was analysed as described by Fraser & Harris [13]. Two CVs were calculated, representing within- and between-subject variations (CVI and CVG, respectively). We also calculated the index of individuality (CVI/CVG) and the index of heterogeneity (i.e. the ratio of CVI to the theoretical CV, which is  $(2/(n-1))^{1/2}$ , where  $n$  is the number of specimens collected per subjects).

### Results

The results of the acute and long-term TRAP responses to WS and the WBC are presented in Tables II and III. No significant changes were observed in the resting values of TRAP during the 12 weeks of the study, either in the WBC group or in the WS group. During the control days when cold exposure was not applied and 3 blood specimens were collected during a period of 30 min, a trend towards a decrease in the TRAP values was observed. In the WBC group, when cold exposure was applied, a significant increase in the plasma anti-oxidative properties was observed 2 min after the cold stress in the first 4 weeks of the study. At weeks 8 and 12, no significant changes were observed 2 min after the stress. Thirty-five minutes after application of cold, the values of TRAP were not different from the baseline values at any time during the study. Concerning the results observed in the WS group, similar trends were noted in the control days and after application of the cold (i.e. the immediate increase in TRAP was significant,  $p < 0.05$ , at week 4, and also at week 12) However, the changes were milder than those observed in the WBC group.

Concerning the biological variation in the TRAP values, the descriptive statistics of our sample of basal values of TRAP in healthy young adult females showed a mean of 1115 µmol/l, a standard deviation of 150 µmol/l and the 25th–75th percentiles of

Table II. TRAP response (in  $\mu\text{mol/l}$ ) to whole-body cryotherapy during successive weeks at time 0 (pre-exposure), and after 2 and 35 min compared with measurements on control days. Results are expressed as means and 95% CIs, in parentheses.

Week	Control day			Whole-body cryotherapy		
	0	Change 2 min	Change 35 min	0	Change 2 min	Change 35 min
	Mean (95 % CI)	Mean (95 % CI)	Mean (95% CI)	Mean (95% CI)	Mean (95 % CI)	Mean (95 % CI)
0	1086.9 (998.7; 1175.1)	3.8 (-8.3; 15.9)	3.9 (-15.2; 23.0)	1108.2 (1026.5; 1189.9)	35.3 (14.8; 55.8)	15.3 (-5.6; 36.3)
2	1092.6 (960.9; 1224.3)	-8.5 (-29.5; 12.5)	1.0 (-26.9; 28.9)	1112.8 (993.9; 1231.7)	25.6 (6.8; 44.4)	-5.0 (-9.1; 9.1)
4	1102.0 (980.5; 1223.5)	8.0 (-9.7; 25.7)	-0.2 (-9.7; 9.3)	1103.4 (983.5; 1223.3)	21.8 (8.3; 35.2)	10.2 (-11.4; 31.8)
8	1118.6 (985.7; 1251.5)	-9.2 (-39.5; 21.1)	-19.7 (-52.4; 13.0)	1114.4 (997.2; 1231.6)	6.4 (-19.7; 32.5)	-17.6 (-36.7; 1.5)
12	1063.5 (939.5; 1187.5)	-3.6 (-16.2; 9.0)	-11.8 (-25.8; 2.2)	1081.6 (952.7; 1210.5)	20.7 (-2.7; 44.0)	2.0 (-10.8; 14.8)

Abbreviation: TRAP=total peroxy radical trapping antioxidant capacity of plasma.

Table III. TRAP response (in  $\mu\text{mol/l}$ ) to winter swimming during successive weeks at time 0 (pre-exposure), and after 2 and 35 min compared with measurements on control days. Results are expressed as means and 95% CIs, in parentheses.

Week	Control day			Winter swimming		
	0	Change 2 min	Change 35 min	0	Change 2 min	Change 35 min
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95 % CI)	Mean (95% CI)
0	1159.8 (1037.6; 1282.0)	-1.7 (-16.9; 13.5)	-5.9 (-20.5; 8.7)	1111.4 (988.0; 1234.9)	6.9 (-12.5; 26.2)	-2.7 (-18.3; 12.9)
2	1106.8 (1009.8; 1203.8)	-3.6 (-23.7; 16.5)	-12.0 (-23.9; -0.1)	1177.3 (1081.9; 1272.7)	1.8 (-18.2; 21.7)	-4.9 (-24.8; 15.0)
4	1210.8 (1100.9; 1320.7)	-12.0 (-34.0; 10.0)	-15.1 (-52.8; 22.6)	1173.7 (1050.2; 1297.1)	28.9 (15.3; 42.5)	-4.0 (-30.5; 22.5)
8	1087.6 (1005.8; 1169.4)	-11.6 (-21.7; -1.5)	-15.6 (-33.9; 2.7)	1115.8 (1006.7; 1224.9)	8.9 (-15.0; 32.8)	-20.9 (-48.8; 7.0)
12	1106.5 (1010.7; 1202.3)	-6.7 (-21.5; 8.1)	-3.8 (-26.1; 18.5)	1090.5 (994.4; 1186.6)	19.7 (2.1; 37.3)	-17.6 (-44.5; 9.3)

Abbreviations: TRAP=total peroxy radical trapping antioxidant capacity of plasma.

1017–1212  $\mu\text{mol/l}$ . The between-subject variation was 13.6% and was higher than the within subject variation, which was found to be 6.4%. The index of individuality was 0.46, and the index of heterogeneity was 0.079.

## Discussion

From a physiological point of view, experiencing acute cold temperature on a regular basis for a period of 12 weeks represents an obvious stress, which could lead to some adaptive mechanisms. This is one of the tentative explanations of hardening the body after cold treatment. It has been suspected that an adaptation to cold stimuli and the improvement in the body hardening could be related to an increase in the protection against oxidative stress [7–9]. Oxidative stress is now known to play a part in the development of several pathologies including cancer, Alzheimer's disease, arthritis, and others. Therefore, it is important to find a situation that naturally improves the protection of the body against oxidative stress and that could have some practical applications in the development of therapies for a large number of individuals.

Siems et al. [7–9] have investigated the effects of acute cold stimuli (winter swimming) on a number of markers of oxidative stress and have shown that these stimuli induce a decrease in several major plasma antioxidants (i.e. ascorbic acid, uric acid), and an increase in the concentration of hydroxynonenal in plasma – a marker of lipid peroxydation. In addition, the erythrocytic level of oxidized glutathione and the ratio of oxidized glutathione/total glutathione also increased following cold exposure. However, these investigators also reported a higher enzymatic protection (i.e. in the activity of red blood cells catalase, glutathione peroxydase, superoxide dismutase) for those who regularly practise WS activities compared with control subjects. However, no information is available concerning the rapidity of changes in the enzymatic protection. We therefore studied the effects of two acute cold-stress situations: swimming in ice-cold water and whole-body cryotherapy, three times per week over a period of 12 weeks. Our outcome value was the measurement of TRAP, which represents the total antioxidant activity of the plasma and provides an insight into the delicate *in vivo* balance between oxidants and antioxidants. If improved protection against oxidative stress develops, the decrease in the values of TRAP supposed to be seen after the first exposures to cold stress should diminish during the development of the study.

Surprisingly, we observed a significant increase – and not a decrease, as expected – in the values of TRAP after acute cold stimuli in both groups at the beginning of the period of adaptation. The general response pattern of the WS group was similar to but milder than the pattern seen in the WBC group. The rather low number of volunteers investigated and the variable outdoor conditions, uncontrolled activity before WS, and duration of exposure (self-timing) may have had an influence on those results. However, we chose to use outdoor exposure instead of an indoor pool because that is the natural way people do practise their WS activities [14]. We also did not regard it as good practice to increase the number of volunteers simply to gain statistical significance. After 4 weeks of experiencing cold stress three times a week, almost no changes were observed after the cold stress compared with the pre-cold stress results. Furthermore, no significant changes were observed in basal TRAP values throughout the time of the study. These striking results are at variance with those of Siems et al. However, a change in the TRAP values after application of cold stimuli indicates that oxidative stress is involved when the body is submitted to cold stimuli. Similar kinds of changes have already been observed after heavy endurance physical

exercise [15–17]. These data may suggest that stimuli as acute exercise and cold stress activate antioxidant defences in the body. This activation can be viewed as an adaptive defensive mechanism to cope with increased oxidative stress.

In this study, we have determined 10 times the basal values of TRAP in strictly standardized conditions in 20 healthy female volunteers through a period of 12 weeks without noticing any significant changes. This allowed us to study the biological variation of TRAP values. We found a very low index of heterogeneity, which indicates that all subjects presented similar intrinsic variances. However, when comparing the between-subject (CVG) versus the within-subject variation (CVI), we found that the former was roughly twice as high as the latter. This led to a CVI/CVG ratio of 0.46. It has previously been shown when the CVI/CVG is  $< 1.4$ , population-based reference values are useful, but when the ratio is  $< 0.6$ , these values become unsuitable for assessing whether a change has occurred [13]. Moreover, it has to be remembered that our results stemmed from a group of female subjects who were 35–45 years old. It would be expected that if one considered a larger sample of subjects including males and females, persons of different age, of different BMI, and of different physical activity levels, the CVI/CVG ratio would become even lower. Therefore, comparing TRAP values between different groups of subjects may not be effective, especially when the number of subjects is limited. There are numerous studies published in the literature where groups of patients are compared with groups of healthy subjects. Our results stress that special attention should be given to confounding factors, and if at some point reference values are needed, then group-based reference values should be chosen in preference to the general health-related reference values. Strategies and recommendations on the collection of different kinds of reference values have recently been published [18–20].

In conclusion, TRAP values showed wide variations between subjects. However, changes in TRAP values after cold stimuli in both the WBC and the WS groups were rather mild. No long-term changes in TRAP values were observed. Nevertheless, our results do not exclude minor changes in single antioxidants. In the main, regular cryotherapy and winter swimming three times per week for 12 weeks do not appear to be harmful as far as antioxidative capacity is concerned.

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