

Urinary thrombomodulin and catecholamine levels are interrelated in healthy volunteers immersed in cold and warm water

Lasse Pakanen^{a,b}, Tiina Pääkkönen^{c,d}, Tiina M Ikaheimo^{b,e}, Hannu Rintamäki^{f,g}, Juhani Leppäluoto^g, Helena Kaija^{a,b}, Marja-Leena Kortelainen^{a,b}, Arja Rautio^h, and Katja Porvari^{a,b}

^aDepartment of Forensic Medicine; University of Oulu; Oulu, Finland; ^bMedical Research Center Oulu, Oulu University Hospital and University of Oulu; Oulu, Finland; ^cFaculty of Science; University of Oulu; Oulu, Finland; ^dFaculty of Biochemistry and Molecular Medicine; University of Oulu; Oulu, Finland; ^eCenter for Environmental and Respiratory Health Research; University of Oulu; Oulu, Finland; ^fFinnish Institute of Occupational Health; Oulu, Finland; ^gInstitute of Biomedicine; University of Oulu; Oulu, Finland; ^hCentre for Arctic Medicine; Thule Institute; University of Oulu; Oulu, Finland

ABSTRACT

Severe hypothermia has been shown to influence the levels of catecholamines and thrombomodulin, an endothelial protein essentially involved in the regulation of haemostasis and inflammation. A link between thrombomodulin and catecholamines during cold exposure has also been previously suggested. The aim of this study was to assess the influence of short-term cold exposure without hypothermia on catecholamines and the circulating and urinary thrombomodulin levels. Seven healthy male subjects were immersed in cold water (+10°C) for 10 minutes followed by a 20-minute immersion in +28°C water. Warm water immersion was performed separately for each subject (+30°C for 30 minutes). Thrombomodulin and catecholamine concentrations were measured from pre- and post-immersion (up to 23 hours) samples. In urine, the thrombomodulin level correlated strongly with adrenaline ($\rho = 0.806$) and noradrenaline ($\rho = 0.760$) levels. There were no significant differences in thrombomodulin levels between immersion temperatures. Post-immersion urinary thrombomodulin levels were significantly lower than the pre-immersion level at both immersion temperatures. Median concentrations of plasma noradrenaline and urinary adrenaline were higher after exposure to +10°C than to +30°C. Thus, further evidence of the association between thrombomodulin and catecholamines was gained in a physiologically relevant setting in humans. Additionally, it is evident that a short-term cold exposure was not able to elicit changes in the thrombomodulin levels in a follow-up period of up to 23 hours. These findings provide further understanding of the physiological responses to cold during immersion, and of the potential influence of stress on haemostatic and inflammatory responses.

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Introduction

Thrombomodulin (TM) is a transmembrane protein expressed in the endothelial cells of most blood vessels.¹ TM functions essentially as anticoagulant and anti-inflammatory regulator by acting as cofactor for thrombin.² The TM-thrombin complex elicits these effects mainly by activating protein C.³ However, the complex also inhibits fibrinolysis through the activation of thrombin-activatable fibrinolysis inhibitor.⁴ TM is found in the circulation and urine as soluble fragments which retain these functionalities.⁵

Recently, we have shown that rats exposed to severe hypothermia had low TM levels in circulation and myocardium while the myocardial TM transcript level was increased.⁶ In autopsy material, lethal hypothermia was associated with low myocardial TM transcript and TM protein levels as well as low urinary TM level compared with other causes of death.⁷ Furthermore, the circulating TM levels have been shown to increase in patients with induced milder hypothermia compared with normothermic patients after approximately a 2-hour period.⁸ However, no changes were

CONTACT Katja Porvari  katja.porvari@oulu.fi

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seen in healthy subjects immediately after a 10-minute dry cold exposure.⁹

Cold exposure is also known to increase the levels of catecholamines, adrenaline and noradrenaline.¹⁰ High urinary adrenaline and adrenaline-to-noradrenaline levels have been suggested as indicators of lethal hypothermia.¹¹⁻¹³ Furthermore, high circulating adrenaline level was associated with lowered myocardial *TM* expression in rats, and positive correlation was found between thrombomodulin and adrenaline concentrations in plasma.⁶ Significant correlations between *TM* and catecholamine levels were also seen in autopsy material.⁷ These findings suggested that there could be a regulatory link between catecholamines and *TM* during severe cold exposure, possibly via protein kinase A signaling pathway.

Although an association between *TM* and catecholamines has been described in hypothermia-related settings, it is unclear whether this connection also exists in physiological conditions. Furthermore, the influence of short-term cold stress on the circulating and urinary *TM* levels in a prolonged follow-up is unresolved. More detailed knowledge of *TM* secretion during and after cold exposure, as well as its possible regulatory pathways would give valuable information about the effects of cooling on haemostasis and other essential functions. Thus, the aim of this study was to test the hypotheses that *TM* and catecholamine levels are related in non-hypothermic conditions, and that the correlations might differ in cold and warm water exposures. We also hypothesized that short-term cold exposure could cause changes in *TM* levels during a follow-up period of up to 23 hours.

Material and methods

Subjects

Following approval of the experimental protocol by the University of Oulu and Northern Ostrobothnia Hospital research ethics committee and obtaining written informed consent, 7 healthy, non-smoking male participants volunteered for the study. Only males were recruited to reduce the dispersion of the results in a small study population. The characteristics (mean \pm standard deviation) of the participants were: age, 26 ± 2 years; height, 178 ± 4 cm; weight, 75 ± 7 kg; body mass index, 23.7 ± 1.6 kg/m². Maximal exercise tests were conducted to assess their physical condition. The mean maximal oxygen consumption

was 52.3 ± 5.2 ml/kg/min. The subjects were informed of the nature and risks of the experiment.

Experimental protocol

The experiment was carried out in the laboratory of physiology at the Finnish Institute of Occupational Health, Oulu, Finland (65°N 25°E). Any potential effects of cold acclimatisation were controlled by carrying out the experiments in late spring and early summer (between 6th May and 27th June). The subjects participated in the tests at least 2 hours after a light breakfast and were instructed not to use coffee or alcohol the evening before the measurements (>12 hours). They were instructed to avoid strenuous exercise and to sleep normally (e.g. 8 hours) during the preceding night. Two immersions of different exposure temperatures were conducted in random order for each subject. The immersions started at 9:00 a.m. (UTC +2).

The immersions were accomplished with head-out immersion in upright posture in a pool of water with a temperature of either +10°C or +30°C. The duration of the +10°C exposure was 10 minutes followed by an immersion in a pool with water temperature of +28°C for 20 minutes. This was done so as to limit the severity of the exposure, and also to allow further heat loss in a slightly below thermoneutral temperature. In the +30°C exposure, the same water temperature was used for the corresponding time (10 + 20 minutes). The total immersion time was thus 30 minutes. Blood and urine samples were taken before and after the immersions according to the schedule presented in Table 1.

Temperature measurements

Skin temperatures were measured from 10 sites: forehead, upper back, chest, abdomen, upper arm, lower

Table 1. The schedule of immersions and sampling.

Cumulative time from the start of immersion (h:min)	Samples taken	
	Blood	Urine
-1:00	x	x
0:00 (start of immersion)	x	
0:30 (end of immersion)	x	
1:00	x	x
2:00	x	
4:00	x	x
6:00	x	x
23:00	x	x

arm, back of the hand, anterior thigh, dorsal side of the foot and calf (NTC DC 95, Digi Key, USA). The thermistors were attached to the skin with adhesive tape. Rectal temperature (T_{rect}) was measured 10 cm beyond the anal sphincter with an YSI 401 probe (Yellow Springs Instrument Co., Yellow Springs, USA). Skin and rectal temperature values were recorded at 30-s intervals with a datalogger (SmartReader 8+, ACR Systems, Canada) throughout the cold and warm exposure. Mean skin temperature (T_{sk}) was calculated as an area-weighted average according to the following formula: $0.07 \cdot (T_{\text{forehead}}) + 0.35 \cdot \text{mean}(T_{\text{chest}}, T_{\text{scapula}}, T_{\text{abdomen}}) + 0.14 \cdot \text{mean}(T_{\text{upper arm}}, T_{\text{lower arm}}) + 0.05 \cdot (T_{\text{dorsal hand}}) + 0.19 \cdot (T_{\text{thigh}}) + 0.13 \cdot (T_{\text{calf}}) + 0.07 \cdot (T_{\text{dorsal side of foot}})$.¹⁴ Mean body temperature (T_{b}) was calculated by the equation: $T_{\text{b}} = 0.65 \cdot T_{\text{rect}} + 0.35 \cdot T_{\text{sk}}$.¹⁵

TM and catecholamine analyses

TM and catecholamine (adrenaline and noradrenaline) concentrations in blood and urine samples were measured with enzyme-linked immunosorbent assay (TM: Quantikine Human Thrombomodulin Immunoassay kit, R&D Systems, Inc., Minneapolis, MN, USA; catecholamines: Cat Combi kit, DRG, Marburg, Germany) according to the instructions given by the manufacturers. According to the manufacturer, the mean minimum detectable dose of TM with the assay was 7.82 pg/ml. Coefficients of variance for intra/inter-assay precisions were <4% and <8%, respectively. The analytical sensitivities for adrenaline and noradrenaline were 0.011 ng/ml and 0.044 ng/ml, respectively. Coefficients of variance for intra-assay precisions were 15.0% for adrenaline and 16.1% for noradrenaline at 2.5 ng/ml and 24.4 ng/ml levels, respectively. The analyses of TM and catecholamines were done in the laboratory of the Department of Forensic Medicine, University of Oulu. Osmolality of the urine samples were measured in the laboratory of Oulu University Hospital (NordLab, Oulu). Haematocrit was analyzed with a Coulter Hematology Analyzer (Beckman Coulter Inc., Brea, CA, USA).

Statistical analyses

Statistical analyses were done with IBM SPSS Statistics 21 (IBM, Armonk, NY, USA). Values from different time points were analyzed as pre- and post-immersion sample groups. Differences between median values of TM, adrenaline and noradrenaline

were analyzed with Mann-Whitney non-parametric test, and the Benjamini-Hochberg corrections procedure was used to control false discovery rate in multiple comparisons. Correlations between TM and catecholamines were analyzed with Spearman's correlation coefficient. Data is given as medians and range unless stated otherwise.

Results

Changes in temperature

The +10°C immersion decreased the mean T_{sk} (Fig. 1A) and T_{b} (Fig. 1C) maximally by 11.1°C and 3.8°C, respectively. There were no obvious effects on

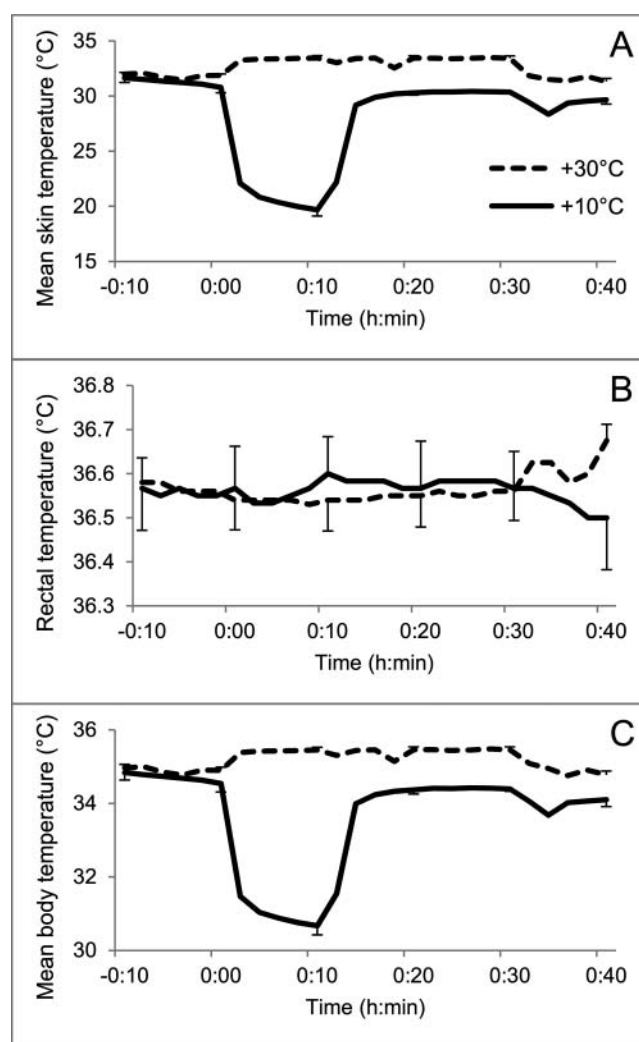


Figure 1. The temperature data during immersions. Mean skin (A) and body temperatures (C) decreased during immersion in +10°C water, but the rectal temperature (B) was unaffected. No obvious changes were seen in immersion to +30°C water. Error bars represent standard error.

T_{sk} and T_b at $+30^\circ\text{C}$. T_{rect} remained fairly constant in both exposure temperatures (Fig. 1B).

Haematocrit and urine osmolality

Median urinary osmolality was higher before immersion (658.0, 250.0–924.0 mosm $\text{kg}[\text{H}_2\text{O}]^{-1}$) than after exposure to $+10^\circ\text{C}$ (421.0, 140.0–822.0 mosm $\text{kg}[\text{H}_2\text{O}]^{-1}$) or $+30^\circ\text{C}$ (338.5, 143.0–955.0 mosm $\text{kg}[\text{H}_2\text{O}]^{-1}$). Mean (\pm SE) haematocrit was higher after exposure to $+10^\circ\text{C}$ ($46.6 \pm 0.4\%$) than to $+30^\circ\text{C}$ ($44.7 \pm 0.4\%$). The pre-immersion haematocrit was $46.1 \pm 0.5\%$.

TM concentrations

The pre-immersion median TM concentration was 4.54 (3.34–6.41) ng/ml in plasma and 15.97 (1.05–32.04) ng/ml in urine for both immersions. The post-immersion osmolality-corrected urinary TM levels were significantly lower in both exposure temperatures compared with the pre-immersion level (Fig. 2A). There were no significant differences between exposure to $+10^\circ\text{C}$ or $+30^\circ\text{C}$, or in the plasma TM levels. Proportioning the plasma TM levels with haematocrit did not cause significant changes to the results (data not shown).

Catecholamine concentrations

The median plasma noradrenaline concentration (Fig. 2C) was increased after immersion to $+10^\circ\text{C}$ (4.93, 1.55–12.29 ng/ml) compared with the pre-immersion level (4.03, 1.64–6.98 ng/ml; $p = 0.017$) or the exposure to $+30^\circ\text{C}$ (3.79, 1.97–7.24 ng/ml; $p = 0.021$). There were no significant differences between plasma adrenaline levels. Proportioning the plasma catecholamine levels with haematocrit did not cause significant changes to the results (data not shown). Urinary adrenaline level (Fig. 2B) was lower in $+30^\circ\text{C}$ exposure (4.04, 0.72–40.81 ng/ml) than the pre-immersion level (13.27, 1.66–39.20 ng/ml) or in the $+10^\circ\text{C}$ exposure (7.91, 0.94–40.31 ng/ml). The differences were significant after osmolality correction ($p = 0.003$ and $p = 0.011$ compared with pre-immersion level and $+10^\circ\text{C}$, respectively).

Correlations between TM and catecholamines

Urinary adrenaline and noradrenaline levels correlated strongly with the urinary TM concentration (Table 2).

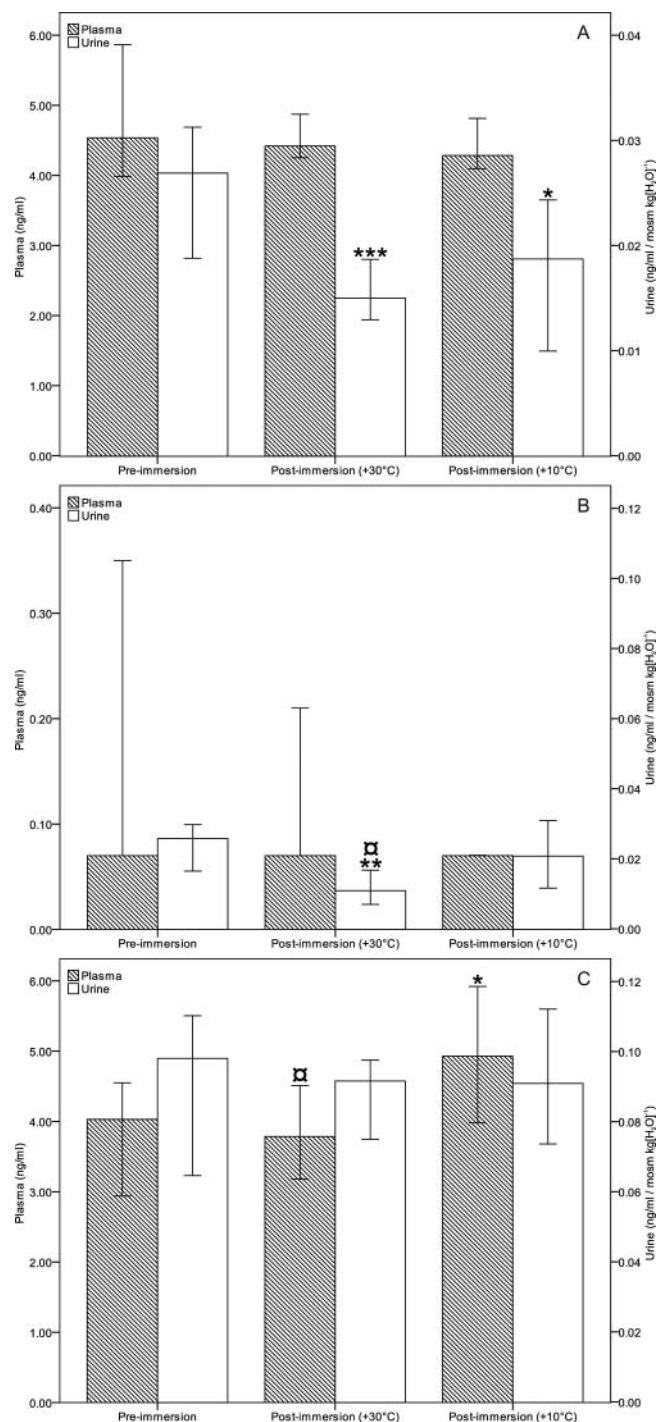


Figure 2. Median levels of thrombomodulin (A), adrenaline (B) and noradrenaline (C) in plasma and urine (corrected with osmolality). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with pre-immersion level; □ $P < 0.05$ compared with $+10^\circ\text{C}$ immersion. Error bars represent 95% confidence interval.

The correlations were observed in both pre- and post-immersion data, and the correlations were similar at both exposure temperatures. Plasma TM level exhibited a weak to moderate inverse relation to urinary adrenaline and plasma noradrenaline level.

Table 2. The correlations between thrombomodulin (TM) and catecholamine parameters.

	Urine TM		Plasma TM	
	ρ	p	ρ	p
Urine adrenaline	0.806	< 0.001	-0.471	0.003
Urine noradrenaline	0.760	< 0.001	-0.323	0.048
Plasma adrenaline	0.094	ns	0.121	ns
Plasma noradrenaline	-0.107	ns	-0.385	0.002

ns, not significant.

Discussion

The urinary TM and catecholamine levels correlated significantly in pre- and post-immersion samples. Similar correlations have been demonstrated in plasma and urine in both cold-related^{6,7} and non-cold-related¹⁶ settings. In rats, a high circulating adrenaline level correlated with low cellular *TM* transcript levels.⁶ Based on these findings, it is plausible that adrenaline — and possibly also noradrenaline — increase the release of TM from endothelium to circulation and inhibit the transcriptional activation of *TM*.

The short-term cold exposure with significant superficial cooling but without decrease in core temperature was not able to produce significant changes in the TM levels in plasma or urine in this study. In our previously conducted study with rats, mild hypothermia was associated with lower urinary and higher circulating TM levels compared with severe hypothermia.⁶ However, the urine of lethal hypothermia victims contained lower TM concentrations⁷ than the baseline values measured in the present study. These findings would suggest that the elimination of TM is diminished in the first phases of cold exposure, possibly reflecting an increased need in the circulation. In prolonged, severe exposure, the production mechanisms are probably slowed down or exhausted.

The TM levels in urine were significantly lower during the follow-up period of both exposure temperatures than before immersion. It is, thus, possible that the immersion itself might have influence on the results. There is no published data on the effects of water immersion on TM expression or secretion, but it is known to affect hemodynamic parameters. Immersion increases central plasma volume and cardiac output, and causes a relative hyperperfusion of peripheral tissues.¹⁷⁻¹⁹ Some changes in haemostatic potential may also occur during head-out immersion in thermoneutral water, demonstrated by a decrease

in prothrombin time and the activity of plasminogen activator inhibitor.²⁰ Thus, the influence of water immersion and the subsequent rise in external hydrostatic pressure seems to be the main contributing factor affecting the concentrations of circulating and urinary TM.

An increase in the plasma noradrenaline level was produced by exposure to +10°C, but not with the exposure to +30°C. Also, the post-immersion levels of urinary adrenaline were higher in +10°C than +30°C temperature. It is well-known that the circulating noradrenaline level reacts more sensitively to cold stress,¹⁰ and adrenomedullary activation generally requires a more severe stimulation.²¹ However, high levels of urinary adrenaline have been regarded more specific to severe hypothermia than noradrenaline.^{12,13}

The cold-induced increase in diuresis is a well-known phenomenon,²² associated with a concomitant decrease in urine osmolality.²³ To eliminate the influence of dilution on the results, the TM and catecholamine concentrations were proportioned to the respective osmolality of the samples. Thus, the pre- and post-immersion analyses can be regarded comparable. Urine creatinine has been proposed for the same purpose,²⁴ but osmolality could be less sensitive to changes in renal function. Proportioning the plasma levels of TM and catecholamines with haematocrit, in order to control for haemodilution or haemoconcentration, did not cause significant changes to the results.

In conclusion, further evidence of the association between TM and catecholamines was gained in healthy subjects, suggesting that these potential regulatory mechanisms are valid in a physiological setting. A short-term cold exposure causing superficial cooling and increase in catecholamine levels was not able to elicit changes in TM levels during a follow-up of up to 23 hours. These observations provide further understanding of the responses of the body to stress factors such as cold and immersion. The findings could be useful, for instance, in developing diagnostic methods for assessing cold stress, or therapeutic tools concerning haemostatic and inflammatory processes. However, future studies are needed to reveal the exact regulatory pathways of TM expression and secretion on cellular level.

Abbreviations

T_b mean body temperatureT_{rect} rectal temperature

T_{sk} mean skin temperature
 TM thrombomodulin

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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