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Circulating soluble intercellular adhesion molecule-1 (sICAM-1) after exercise-induced muscular damage: does the use of whole-body cryostimulation influence its concentration in blood?

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**Abstract**

As soluble intercellular adhesion molecule-1 (sICAM-1) was recently hypothesized to be a key player in the mechanisms involved in exercise-induced muscular damage (EIMD), we investigated its circulating concentration changes in athletes before and after EIMD with and without the use of whole-body cryostimulation (WBC; 3 min at -110°C) at the exercise end **and repeated once a day during 4 days.**

We previously characterized plasma specimens from 11 endurance athletes who performed twice (randomized crossover design) strenuous running leading to EIMD, followed by passive recovery or WBC. Muscle soreness and inflammatory response were observed in both cases but the use of WBC induced a significant reduction in these responses (PlosOne 2011;6:e22748). We now found that sICAM-1 concentration slightly increased in both circumstances and remained elevated for 24 hours ( $p<0.01$ ). However, no significant WBC effect was observed concerning sICAM-1 changes indicating that this compound is not a major player both in EIMD and WBC physiological impacts.

Key words: Exercise-induced muscular damage (EIMD); Intercellular adhesion molecule-1 (ICAM-1); Soluble intercellular adhesion molecule-1 (sICAM-1); Whole-body cryostimulation; Whole-body cryotherapy; Recovery

57 Intercellular adhesion molecule-1 (ICAM-1) and its soluble form (sICAM-1) have  
58 recently been suspected to be key components in the mechanisms involved in  
59 exercise-induced muscular damage (EIMD) [4; 3]. It has been speculated that whole-  
60 body cryostimulation (WBC; 2 to 4 min at -110°C or less after exercise) which lowers  
61 EIMD also impacts the expression of ICAM-1 and the amount of circulating soluble  
62 ICAM-1 (sICAM-1) [4; 3].

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64 ICAM-1 is an adhesion protein present on endothelial cells and leukocyte surfaces.  
65 This factor mediates adhesion and transmigration of leukocytes through the  
66 endothelium. In addition, ICAM-1 directly contributes to inflammatory responses  
67 within the blood vessel wall by increasing endothelial cell activation. Surface  
68 expressed ICAM-1 can be shed from the cells and then circulates as soluble ICAM-1  
69 (sICAM-1) [3; 10; 7]. Initially thought to be a relevant marker of cardiovascular risk  
70 factor, sICAM-1 is nowadays more frequently considered as a biomarker of  
71 endothelial activation/dysfunction mimicking the amount of expressed ICAM on  
72 endothelial cells and leukocytes [7].

73  
74 We have recently presented that in the context of EIMD sarcomeres disrupt and then  
75 leukocytes (neutrophils, monocytes, and lymphocytes) transmigrate to the injured  
76 cells/tissue via ICAM-1 [4; 3]. Following this, pro-inflammatory cytokines and reactive  
77 oxygen species are produced in the muscle cells by leukocytes and amplify the initial  
78 muscle damage.

79  
80 Ferreira et al have suggested that a drop in core temperature induced by WBC would  
81 likely cause vasoconstriction of blood vessels and lower the amount of leukocytes  
82 arriving to the exercise-induced muscular inflammation site [4]. These authors  
83 propose a mechanism in which a decrease in the expression of ICAM-1 at the  
84 endothelium level would limit diapedesis through a reduction a: in the possibilities of  
85 activated leukocytes arrest, b: in a firm adherence on the endothelial cells, and c: in  
86 the initial steps of the trans-endothelial cell migration process. The level of this  
87 reduction in the expression of ICAM-1 could be followed by measuring the  
88 concentration of circulating sICAM-1.

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This view may be correct if the rate of shedding (splicing) remains constant. It seems accepted that sICAM-1 is an accurate marker of inflammation reflecting the amount of ICAM-1 expressed on membranes. During inflammation and infection, membrane ICAM-1 expression on leukocytes and endothelial cells tremendously increases as well as the concentration of circulating sICAM-1. In this context sICAM-1 is a relevant biomarker which correctly reflects the expression of ICAM-1 on cell membranes. However, this might not always be the case. If the shedding of ICAM-1 is regulated, one can expect changes in the concentration of sICAM-1 in blood without changes in the expression of ICAM-1. The regulation of sICAM-1 in blood is definitely not clear [3]. It has previously been shown that an increase in the shedding of the ICAM-1 can be due to increased secretases activity, stimulated by catecholamines and other factors. One study showed that exercise may induce an increase in circulating sICAM-1 and such change could be blocked by the infusion of propranolol [9]. Moreover, ~~we have shown that~~ noradrenaline is produced and released in circulation after cold stimulation [7; 1] and can theoretically induce an extra release of sICAM-1 with or without changes in the expression of ICAM-1 [2]. In this case, the release of the soluble part of the ICAM-1 will leave a truncated form of the ICAM-1 at the membrane site which will not be able to play its role as an adhesion molecule. Therefore, the possibilities of leukocyte transmigration and the subsequent muscle inflammation will be reduced. This can be seen as a paradox, as in this case, an extra release of sICAM-1 may induce a decrease in muscle inflammation.

Nevertheless, there is for the time being no information available on the changes of sICAM-1 due to the combine effects of EIMD and whole-body cryostimulation. ~~A few years ago, we~~ We have previously studied the effects of EIMD and whole-body cryostimulation in high level athletes on perceived muscle soreness, inflammation markers and pro and anti-inflammatory cytokines. We were able to show that the heavy exercise our athletes had to perform led to muscle soreness accompanied by pro-inflammatory response. The use of whole-body cryostimulation (-110°C, 3min of exposure) after the exercise ~~and repeated once a day during 4 days~~ induced in our athletes both a reduction in muscle soreness and in the concentration of circulating

121 markers of inflammation (lower blood concentration in C-reactive protein and  
122 interleukin-1beta) and an increase in the concentration of anti-inflammatory  
123 circulating markers (increase in the concentration in blood interleukin-1 receptor  
124 antagonist) [8].

125  
126 The aim of the present study was to investigate ~~whether sICAM-1 is an important~~  
127 ~~regulating substance after EIMD and cryostimulation.~~ whether sICAM-1 is an  
128 important regulating substance in formation of EIMD and whether cryostimulation  
129 can modulate its concentration in blood (vs. passive recovery). To conduct this  
130 analysis, we used well preserved plasma specimens [5] collected from a previous  
131 experiment [8] where we investigated the effect of two different recovery modalities  
132 (passive recovery and WBC) after a simulated trail running race that created a EIMD  
133 in our athletes.

134  
135 The subjects and the methods have previously been described [8]. In summary, 11  
136 well-trained male runners (age  $31.8 \pm 2.0$  years;  $VO_{2max}$   $62.0 \pm 1.2$  ml.min<sup>-1</sup>.kg<sup>-1</sup>)  
137 participated in the study. All of them gave their informed consent and the study was  
138 approved by the local Ethics Committee (Île-de-France XI, France; Ref. 200978). The  
139 study had a crossover design. The subjects completed two simulated runs at a one-  
140 month interval on a treadmill followed in a random order by a passive recovery or  
141 with a recovery where WBC was used. The runs were organized on the same  
142 treadmill and were designed to generate fatigue. Blood specimens were collected  
143 before and after the simulated run, after the first recovery session, and before the  
144 recovery sessions after 24 h, 48 h, 72 h, 96 h following the end of the run.  
145 Hydration/nutrition before and during each session was standardized. Between trials,  
146 low intensity training was ensured.

147  
148 One week before the first simulated run, subjects were familiarized with the test  
149 scheme and preliminary tests were performed to determine maximal oxygen uptake,  
150 the first and the second ventilatory thresholds, and the maximal aerobic speed as  
151 previously described [8]. Concerning the proper test, the race lasted 48 minutes and  
152 was divided into 5 stages. The first stage consisted of a 6 minute run on treadmill on

153 a flat surface (intensity comprised between the subject first and the second  
154 ventilatory thresholds), followed by 3 minute uphill (+10% gradient; ≈80% of the  
155 subject maximal aerobic velocity) and 3 minute downhill (-15% gradient; first  
156 ventilatory threshold). Stages 2–5 consisted of 3 min at 0°, followed by 3 min uphill  
157 and 3 min downhill at the gradients and velocities as previously described [8]. After  
158 the run, subjects were randomly assigned to WBC or passive recovery. WBC sessions  
159 were administered in a cryogenic chamber (Zimmer MedizinSysteme GmbH, Ulm,  
160 Germany) and the subjects were exposed to -110°C for 3 min. **The exposures**  
161 **occurred at the end of the exercise and repeated once a day during 4 days at the**  
162 **same time of the day.** Before the exposure, subjects were instructed to dry any  
163 sweat, and clothe themselves in swimming trunks, a surgical mask, ear bands, gloves,  
164 dry socks and sabots. After **each** WBC session, subjects spent 10 min comfortably  
165 seated in an environment at 24°C wearing a bath robe. The control recovery was a  
166 passive recovery during which each subject was comfortably seated in an armchair  
167 for 30 min at 24°C. The subjects were not allowed to speak to anyone during the  
168 experiment.

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171 Blood specimens were collected from a superficial forearm vein using standard  
172 venipuncture techniques and EDTA tubes (Greiner Bio-one; Frickenhausen,  
173 Germany). Plasma aliquots were prepared and stored at -80°C. ELISA kits for the  
174 determination of sICAM-1 were purchased from R&D Systems Europe. The intra-  
175 assay imprecision (CVA) was 4.6% and 4.8% for sICAM-1 concentrations of 126 and  
176 476 mg/L, respectively. All specimens were analyzed in duplicate using Dynex MRXe  
177 spectrophotometer (Magellan Biosciences, Chelmsford, MA, USA).

178

179 Results are expressed in Figure 1 as means ± SD. Two-way analysis of variance for  
180 repeated measurements was used to analyze the changes between cold and control  
181 experiments with time and treatments (WBC versus control) as factors. We used  
182 logarithm transformation as the data distribution was not Gaussian. Post hoc  
183 multiple comparisons were made by PLSD test when appropriate to single out  
184 statistical significance. Statistical significance was set at  $p < 0.05$ .

185

186 We observed a significant increase (data not shown) in the blood concentration of  
187 sICAM-1 and leukocytes after exhausting exercise in athletes, who experienced a  
188 significant post exercise inflammatory response, with or without the use of a 3-min  
189 cryostimulation exposure as a recovery procedure at the end of the exercise. The  
190 concentrations of sICAM-1 in blood obtained 1 hour and 24 hours after the end of the  
191 exercise were significantly more elevated than those obtained at rest ( $p < 0.01$ ),  
192 whereas the sICAM-1 concentration obtained at the time point of 48, 72 and 96h  
193 were not significantly different than those obtained at rest (Fig 1). However, no  
194 significant interaction concerning the effect of cryostimulation was observed  
195 concerning circulating sICAM-1.

196

197 This work confirmed that the concentration of sICAM-1 in blood increases after a  
198 high intensity aerobic exercise [6]. In our previous analysis with the same subjects  
199 and blood specimens, we reported a reduction both in muscle soreness and in the  
200 concentration of circulating markers of inflammation (lower blood concentration in  
201 C-reactive protein and interleukin-1beta) and an increase in the concentration of  
202 anti-inflammatory circulating markers (increase in the concentration in blood  
203 interleukin-1 receptor antagonist) [8] when athletes experienced cryostimulation  
204 after exhausting exercise. Such significant physiological changes did not seem to alter  
205 the concentration of sICAM-1 in blood, though a minor reduction was observed  
206 compared with control group.

207

208 One limit of our study is the rather low number of participants ( $n=11$ ) which lowers  
209 the statistical power. Therefore, such trend might favor the hypothesis of a certain  
210 reduction in the expression of ICAM-1 on cell membranes (endothelial cells and  
211 leukocytes), a reduction in the secretase activity, or a combined effect at the  
212 expression level of the protein and in the activity of secretases.

213

214 Trans-endothelial migration is divided into at least four different steps: the  
215 attachment of circulating cells on to the endothelium; the activation of integrins on  
216 the leukocyte cell surface; the firm adhesion of the leukocyte and transmigration of



217 the cell through the endothelial cell barrier into the sub-endothelial space [7]. The  
218 role of ICAM-1 is to increase endothelial cell activation, to facilitate the firm arrest of  
219 leukocytes and to initiate the leukocytes' transmigratory process [7]. However, there  
220 are many other proteins (e.g. selectins, chemokines, integrins, IgSF) involved in  
221 diapedesis, and cryostimulation may influence the expression of those proteins  
222 involved in the transmigration process. If each of these proteins as well as ICAM-1  
223 were slightly less expressed after cryostimulation, a global significant impact might  
224 appear and reduces the exercise induced inflammation. Further studies dealing with  
225 the impact of cryostimulation and the changes of proteins involved in trans-  
226 endothelial migration would still be necessary. Also, it would be of interest to  
227 investigate downstream signaling using muscle and endothelial cells in order to get a  
228 deeper insight into exercise-induced muscle damage mechanisms and to rule out  
229 sICAM-1 and ICAM-1's roles.

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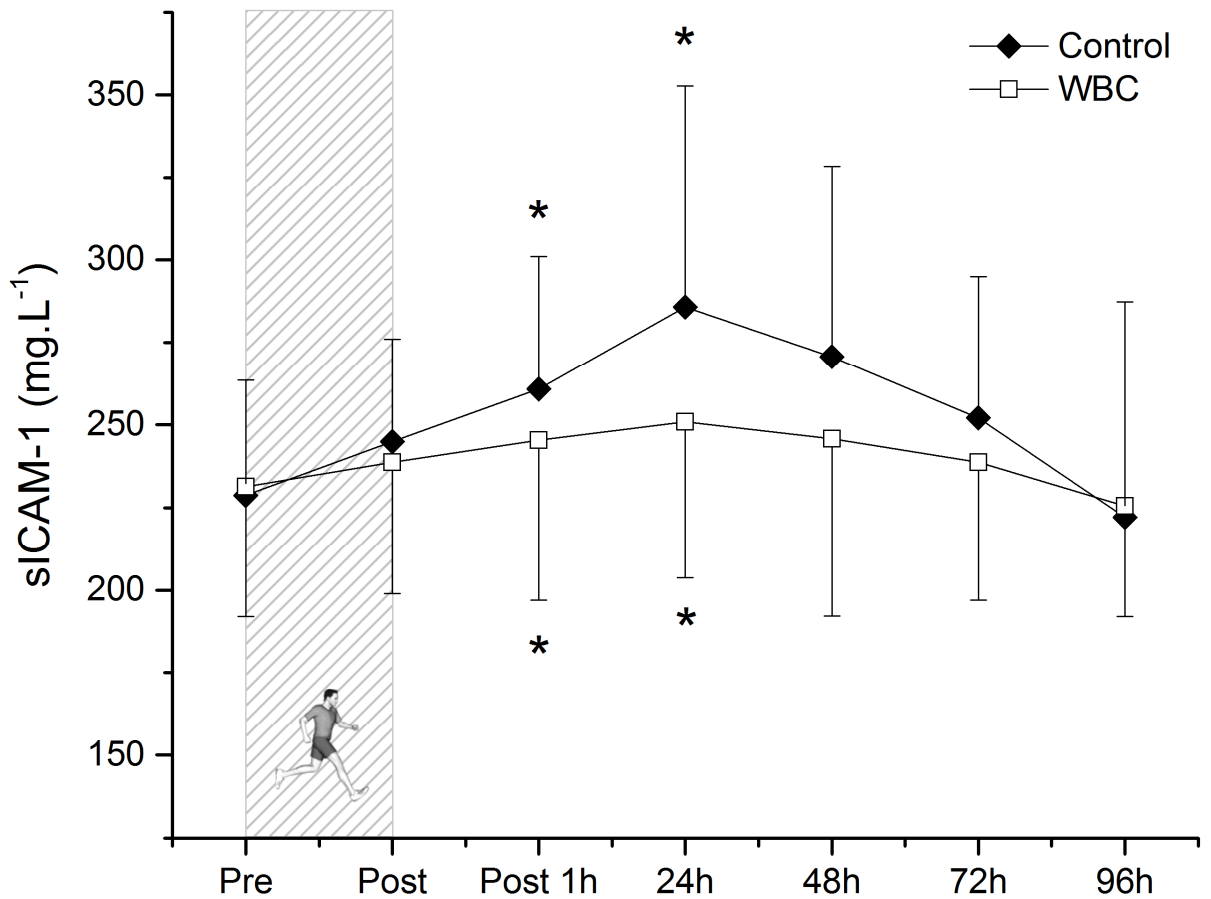
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Figure 1.

Changes in circulating sICAM-1 ( $\text{ng}\cdot\text{ml}^{-1}$ ) from basal concentration to recovery after exhausting physical exercise without (diamond) or with (square) using a 3min cryostimulation exposure in trained athletes. Asterisk indicates a significant increase ( $P<0.01$ ) in comparison to basal circulating concentrations.



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