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1 **The relationship between interleukin-6 in saliva, venous and capillary plasma, at**
2 **rest and in response to exercise**
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11 Highlights

12 - We measured IL-6 in saliva, venous and capillary plasma pre and post
13 exercise.
14 - Salivary IL-6 did not correlate to venous IL-6 at rest or post exercise.
15 - Capillary plasma IL-6 correlated to venous plasma in response to exercise.
16 - Capillary plasma responses may be reflective of systemic IL-6 responses to
17 exercise.
18

19 Abstract

20 IL-6 plays a mechanistic role in conditions such as metabolic syndrome, chronic
21 fatigue syndrome and clinical depression and also plays a major role in inflammatory
22 and immune responses to exercise. The purpose of this study was to investigate the
23 levels of resting and post exercise IL-6 when measured in venous plasma, saliva and
24 capillary plasma. Five male and five females completed 2 separate exercise trials,
25 both of which involved standardized exercise sessions on a cycle ergometer. Venous
26 blood and saliva samples were taken immediately before and after Trial A, venous
27 and capillary blood samples were taken immediately before and after Trial B. IL-6
28 values were obtained using a high-sensitivity enzyme-linked immunosorbent assay
29 (ELISA). In Trial A venous plasma IL-6 increased significantly from 0.4±0.14 pg/ml
30 to 0.99±0.29 pg/ml ($P<0.01$) while there was no increase in salivary IL-6. Venous
31 plasma and salivary IL-6 responses were not correlated at rest, post exercise or when
32 expressed as an exercise induced change. In Trial B venous and capillary plasma IL-6
33 increased significantly (venous: 0.22±0.18 to 0.74±0.28 pg/ml ($P=<0.01$); capillary:

0.37±0.22 to 1.08±0.30 pg/ml (P<0.01). Venous and capillary plasma responses did not correlate at rest (r=0.59, P=0.07) but did correlate post exercise (r=0.79, P=>0.001) and when expressed as an exercise induced change (r=0.71, P=0.02). Saliva does not appear to reflect systemic IL-6 responses, either at rest or in response to exercise. Conversely, capillary plasma responses are reflective of systemic IL-6 responses to exercise.

Keywords Interluekin-6, cytokines, exercise, venous, capillary, saliva

1. Introduction

Interleukin-6 (IL-6) is an important biomarker in metabolic syndrome [1], and is also reported to play a mechanistic role in the development of many disorders, including chronic fatigue syndrome [2] and clinical depression [3]. The discovery that IL-6 is released directly from exercising muscle [4] has led to an increase in the volume of research investigating the impact of exercise on plasma concentrations of IL-6. IL-6 is also considered to play a major role in a number of signaling pathways that govern metabolic, inflammatory and immune responses to exercise stimuli [1,5,6].

IL-6 is typically measured in the serum or plasma from whole blood samples, which are obtained using standard venipuncture techniques or, in the case of repeated samples, via venous catheter. Whole blood is generally considered the body fluid that most accurately represents the systemic response to a given stimulus. Importantly, however, venous blood collection is invasive and can prove impractical in situations where access is difficult [7].

The potential to use saliva or capillary blood samples in the place of traditional venous blood samples could be advantageous in settings where venous blood sampling is practically difficult. Some salivary proteins (such as cortisol and testosterone) are directly derived from the blood and therefore are considered to similarly reflect systemic responses. Thus, salivary levels are often used as surrogates for blood-borne levels of these analytes [8]. Capillary blood sampling is regularly utilized to measure health-related biomarkers (e.g. blood glucose, cholesterol, triglyceride levels), and is also used to measure exercise-related biomarkers (e.g. blood lactate, glucose, haematocrit, haemoglobin) in research and applied sports science environments. However, research into the validity of such measures has demonstrated the importance of evaluating the relationship between measurements

67 obtained from venous versus capillary blood samples, and hence the standardization
68 of the procedures involved in both sampling and analysis [9,10].
69 Several studies have investigated the potential for saliva to be used as a surrogate of
70 blood to measure IL-6. Sjögren et al. (2005) found that while serum and salivary IL-6
71 levels were not correlated with each other, they were both negatively related to
72 psychosocial resources and positively related to psychosocial risk factors [11].
73 Minetto et al. (2005) demonstrated that there was no correlation between salivary and
74 plasma IL-6 concentrations at rest or in response to exercise [12]; however the same
75 group later showed [13] that the sample collection method used in the earlier
76 experiment may in part have accounted for this lack of correlation. Cox et al. (2008)
77 measured plasma and salivary IL-6 in a cohort of 45 athletes; while they found no
78 correlation at rest, the authors suggested it was possible that the pattern of regulation
79 could be related in response to exercise [14].
80 Importantly, to our knowledge, no study has compared the cytokine responses to
81 exercise in capillary and venous blood. IL-6 remains a regularly measured biomarker
82 in numerous fields of research, and a more practical and convenient method of
83 sampling would facilitate less invasive and time-consuming procedures.
84 Therefore, this study aimed to investigate the relationship between IL-6 levels as
85 measured in venous plasma, saliva and capillary plasma, both at rest and in response
86 to exercise.
87
88 ## 2. Materials and Methods
89
90 2.1 Participants
91
92 Ten healthy active individuals (5 male, 5 female) aged 25.5 ± 7.8 yr (mean ± SD)
93 gave informed consent to participate in the study. Subjects completed health/physical
94 activity questionnaires to ensure normal dietary and exercise habits before each test.
95 All participants were free of injury and illness prior to and throughout the study.
96 Ethical approval was obtained via ethics committee and conformed to the declaration
97 of Helsinki.
98
99 2.2 Experimental Procedure

100

101 Participants were tested for maximal aerobic capacity using an incremental exercise
102 test on an electromagnetically-braked cycle ergometer (Lode Excalibur, Groningen,
103 Netherlands). Expired gases were measured using an online gas analyser (OxyconPro,
104 Erich Jaeger GMBH & Co., Hoechberg, Germany), and heart rate was measured
105 continuously via short-range telemetry (RS400, Polar Electro, Finland). Each stage of
106 the incremental exercise lasted 3 minutes, with required power output being increased
107 by 30W at each stage until volitional exhaustion. Males began the test at a required
108 power output of 100W while females began at 50W. $\dot{V}O_{2\,peak}$ was recorded as the
109 highest 30-s period of oxygen consumption. Oxygen consumption values obtained
110 during the incremental test were used to plot a linear regression of power output
111 versus oxygen consumption. This allowed the calculation of individual power outputs
112 for the subsequent exercise session.
113 In both trials (A and B) participants completed the same standardized bout of high
114 intensity interval exercise on a cycle ergometer within 1 week of incremental exercise
115 testing: 5 x 4 minute intervals at 80% $\dot{V}O_{2\,peak}$ interspersed with 3-minute intervals at
116 50% $\dot{V}O_{2\,peak}$. During Trial A venous blood and saliva samples were collected
117 immediately pre- and post-exercise. During Trial B venous and capillary blood
118 samples were taken immediately pre- and post-exercise. Each participant performed
119 both exercise trials at the same time of day and with a minimum of 2 days rest
120 prior to each trial. Expired gases and heart rate were measured continuously
121 throughout each exercise session.

122

123 2.3 Sample collection/handling

124

125 Venous blood samples were collected into K_3EDTA tubes (Greiner Bio-one;
126 Frickenhausen, Germany). Whole mixed unstimulated saliva samples (approx. 1ml)
127 were collected by passively drooling though a straw [13]. Saliva samples were
128 aliquoted and stored at -80°C until further analysis. When thawed, saliva samples
129 were briefly centrifuged to avoid particulate matter and diluted 1:4 based upon prior
130 linearity and recovery experiments (data not shown). Prior to capillary blood
131 collection, participants briefly submerged their hand in warm water to aid blood flow
132 to the fingertip, whereupon 600μl of whole blood was obtained using 3 heparinized

133 200µl microvette capillary blood collection tubes (Sarstedt, Germany) – it should be
134 noted that this process took up to 10 minutes to complete. Nevertheless, for all
135 sample-types, samples were collected within a maximum of 15 minutes post-exercise.
136 Whole blood samples were fractionated by centrifugation (10min; 3,000xG), and the
137 resulting plasma was aliquoted and stored at -80°C until analysis.
138

139 2.4 Enzyme-linked immunosorbent assays
140

141 Plasma (both venous and capillary-derived) and salivary IL-6 concentrations were
142 analysed in duplicate using high sensitivity enzyme-linked immunosorbent assays
143 (ELISA) (Quantikine HS; R&D Systems Ltd., Abingdon, UK). The IL-6 assay has a
144 detection limit of 0.039 pg/ml and an inter/intra-assay coefficient of variation (CV) of
145 <10% across the range 0.15–10 pg/ml. Protein concentrations were determined in
146 relation to a four-parameter standard curve (GraphPad Prism, San Diego California,
147 USA)
148

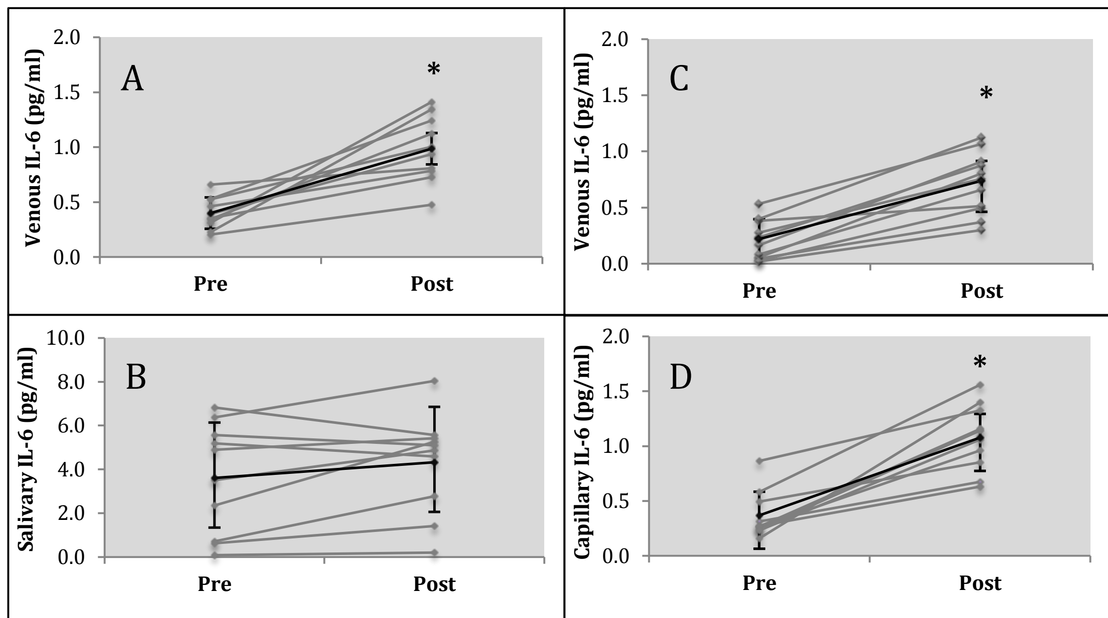
149 2.5 Statistical analysis
150

151 All data are presented as mean±standard deviation (SD) unless otherwise stated.
152 Results were evaluated using a linear mixed-model ANOVA with the factors ‘time’
153 and ‘sample-type’ included as factors. Student–Newman–Keuls (SNK) post-hoc tests
154 were performed where appropriate. Pearson’s correlation analyses were used to
155 investigate the relationships between IL-6 concentrations measured in venous,
156 salivary and capillary samples. Ninety-percent confidence intervals (90% CI) were
157 used to indicate the precision of estimates. SPSS 20.0 was used for all statistical
158 analysis.
159

160 **3. Results**

161 In Trial A plasma IL-6 increased significantly in response to exercise from 0.4±0.14
162 pg/ml to 0.99±0.29 pg/ml ($P<0.01$) (Fig. 1A). There was no significant change in
163 salivary IL-6 pre to post exercise ($P=0.12$) (Fig. 1B). There was no correlation
164 between plasma and salivary IL-6, whether expressed as an exercise-induced change
165 ($r=-0.07$, $P=0.85$, 90% CI= -0.56 to 0.50) or the discrete pre-exercise ($r=-0.38$,

166 P=0.27, 90% CI= -0.77 to 0.21,) and post-exercise (r=0.49, P=0.15, 90% CI= -0.09 to
167 0.82) measurements.
168 In Trial B both venous and capillary plasma IL-6 increased significantly (venous:
169 0.22±0.18 to 0.74±0.28 pg/ml (P=<0.01); capillary: 0.37±0.22 to 1.08±0.30 pg/ml
170 (P<0.01) (Figs. 1C/D)). Venous and capillary plasma IL-6 did not correlate at rest
171 (r=0.59, P=0.07, 90% CI 0.06 to 0.86), but exhibited significant correlations both
172 post-exercise (r=0.79, P=>0.001, 90% CI= 0.42 to 0.93) and when expressed as an
173 exercise-induced change (r=0.71, P=0.02, 90% CI=0.26 to 0.91).



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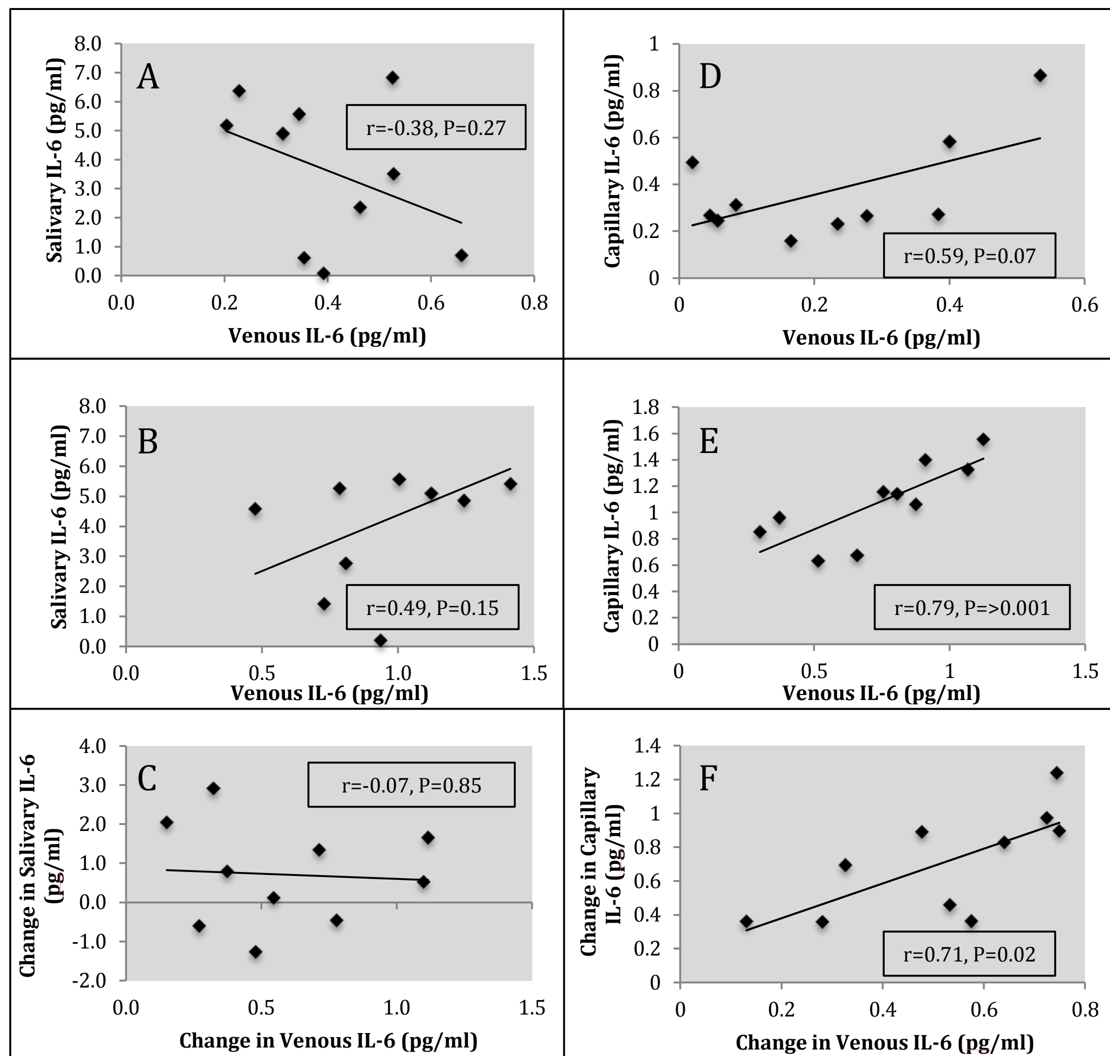
175 **Figure 1.** IL-6 responses to standardised bouts of high intensity intermittent exercise
176 in 10 matched individuals (venous (**A**) and salivary (**B)** IL-6 values [trial A], and
177 venous (**C**) and capillary (**D**) IL-6 values [trial B]). Grey lines represent individual
178 responses; black lines represent cohort-means (*=P>0.05).



179

180 **Figure 2.** The effect of sample type and procedure on IL-6 concentration.
181 Correlations between venous and salivary IL-6 at rest (**A**), post–exercise (**B**), and
182 when expressed as a pre to post exercise change (**C**). Venous and capillary IL-6
183 correlation at rest (**D**), post–exercise (**E**) and when expressed as a pre-versus-post
184 exercise change (**F**).

185

186 **4. Discussion**

187 In this study the exercise induced increase in IL-6 was observed in plasma and
188 capillary samples but not in saliva (Fig.1). This study confirms the modest increases
189 in plasma IL-6 seen with similar high intensity interval exercise protocols [15].
190 Capillary and venous plasma IL-6 correlated post-exercise and when expressed as an
191 exercise-induced change, but not at rest (Figs. 2 D-F). Capillary plasma IL-6 values
192 were consistently higher than those in venous plasma (mean differences: 0.15 and
193 0.34 pg/ml at rest and post-exercise, respectively). However there was no significant

difference between the exercise-induced increase in IL-6 between capillary and venous samples. The higher values from the capillary samples may be due to a small local inflammatory response to the action of the pinprick, and the fact that the blood is obtained from the capillaries surrounding the potential site of inflammation rather than from the circulation (as with a venous sample). While the correlation between resting capillary and venous plasma IL-6 approached significance (P=0.07), it should be noted that the study used a small homogenous (n=10) sample population with very low resting IL-6 levels, and it is possible that a larger and more diverse cohort of subjects may have led to a statistically significant correlation.

Salivary IL-6 did not correlate with venous IL-6 at rest, post-exercise or when expressed as an exercise-induced change (Figs. 2 A-C). This confirms the previous suggestions [12] that salivary IL-6 does not share similar patterns of regulation to venous IL-6 in response to exercise, and hence that saliva is not an appropriate surrogate measure of systemic IL-6. While the working muscle is primarily responsible for systemic increases in IL-6 in response to exercise [4], it has been hypothesized that salivary responses are due to local tissue macrophages and the acinar cells of the salivary glands [11]. Consequently, it is likely that salivary IL-6 is more reflective of a local inflammatory response than a systemic metabolic response to an exercise stimulus.

In conclusion, this study confirms previous observations that salivary IL-6 does not change in response to aerobic exercise [12], and should not be used as a surrogate for systemic IL-6 responses, either at rest or in response to exercise. Conversely, IL-6 measured from capillary blood appears to be sensitive to exercise, and apparently shares the same pattern of regulation as is seen in venous blood. However, caution should be applied when interpreting these preliminary findings and further investigation is required; in particular, the impact of exercise on capillary IL-6 should be investigated within different cohorts undergoing a range of different exercise protocols.

References

1. **Pedersen BK & Febbraio MA.** Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nat. Rev. Endocrinol.* 2012 Apr 3;8(8):457-65.
2. **Arnold MC, Papanicolaou DA, O'Grady JA, Lotsikas A, Dale JK, Straus

227 **SE, Grafman J**. Using an interleukin-6 challenge to evaluate
228 neuropsychological performance in chronic fatigue syndrome. *Psychol Med.*
229 2002;32:1075–1089.
230 3. **Hiles SA, Baker AL, de Malmanche T, Attia J.** A meta-analysis of
231 differences in IL-6 and IL-10 between people with and without depression:
232 exploring the causes of heterogeneity. *Brain Behav Immun.* 2012
233 Oct;26(7):1180-8.
234 4. **Steensberg A, van HG, Osada T, Sacchetti M, Saltin B, Klarlund PB.**
235 Production of interleukin-6 in contracting human skeletal muscles can account
236 for the exercise-induced increase in plasma interleukin-6. *J Physiol.* 2000;529:
237 237–242.
238 5. **Fischer CP.** Interleukin-6 in acute exercise and training: what is the biological
239 relevance? *Exerc Immunol Rev.* 2006;12: 6–33.
240 6. **Pedersen BK.** Edward F. Adolph Distinguished Lecture: Muscle as an
241 endocrine organ: IL-6 and other myokines. *J Appl Physiol.* 2009;107:1006-
242 1014.
243 7. **Williamson S, Munro C, Pickler R, Grap MJ, Elswick RK Jr.** Comparison
244 of Biomarkers in Blood and Saliva in Healthy Adults. *Nurs Res Pract*. 2012.
245 8. **Papacosta E, Nassis GP**. Saliva as a tool for monitoring steroid, peptide and
246 immune markers in sport and exercise science. *J Sci Med Sport*. 2011
247 Sep;14(5):424-34.
248 9. **Ignell C, Berntorp K**. Evaluation of the relationship between capillary and
249 venous plasma glucose concentrations obtained by the HemoCue Glucose
250 201+ system during an oral glucose tolerance test. *Scand J Clin Lab Invest.*
251 2011 Dec;71(8):670-5.
252 10. **Foxdal P, Sjödin A, Ostman B, Sjödin B.** The effect of different blood
253 sampling sites and analyses on the relationship between exercise intensity and
254 4.0 mmol.l-1 blood lactate concentration. *Eur J Appl Physiol Occup Physiol.*
255 1991;63(1):52-4.
256 11. **Sjögren E, Leanderson P, Kristenson M, Ernerudh J.** Interleukin-6 levels
257 in relation to psychosocial factors: Studies on serum, saliva, and in vitro
258 production by blood mononuclear cells. *Brain Behav Immun*. 2006
259 May;20(3):270-8
260 12. **Minetto M, Rainoldi A, Gazzoni M, Terzolo M, Borrione P, Termine A,**

261 **Saba L, Dovio A, Angeli A, Paccotti P.** Differential responses of serum and
262 salivary interleukin-6 to acute strenuous exercise. *Eur J Appl Physiol*.
263 2005;93(5-6):679–686.
264 13. **Minetto MA. Gazzoni M. Lanfranco F. Baldi M. Saba L. Pedrola R. Komi**
265 **PK. Rainoldi A**. Influence of the sample collection method on salivary
266 interleukin-6 levels in resting and post-exercise conditions. *Eur J Appl Physiol*.
267 2007;101:249–256.
268 14. **Cox AJ, Pyne DB, Gleeson M, Callister R**. Resting plasma and salivary IL-6
269 concentrations are not correlated in distance runners. *Eur J Appl Physiol*.
270 2008;103:477–479.
271 15. **Zwetsloot KA, John CS, Lawrence MM, Battista RA, Shanely RA.** High-
272 intensity interval training induces a modest systemic inflammatory response in
273 active, young men. *J Inflamm Res.* 2014 Jan 9;7:9-17.
274