

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

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10
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:

34 0.37±0.22 to 1.08±0.30 pg/ml (P<0.01). Venous and capillary plasma responses did
35 not correlate at rest (r=0.59, P=0.07) but did correlate post exercise (r=0.79,
36 P=>0.001) and when expressed as an exercise induced change (r=0.71, P=0.02).
37 Saliva does not appear to reflect systemic IL-6 responses, either at rest or in response
38 to exercise. Conversely, capillary plasma responses are reflective of systemic IL-6
39 responses to exercise.

40 **Keywords** Interleukin-6, cytokines, exercise, venous, capillary, saliva

41

42 **1. Introduction**

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

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

56 The potential to use saliva or capillary blood samples in the place of traditional
57 venous blood samples could be advantageous in settings where venous blood
58 sampling is practically difficult. Some salivary proteins (such as cortisol and
59 testosterone) are directly derived from the blood and therefore are considered to
60 similarly reflect systemic responses. Thus, salivary levels are often used as surrogates
61 for blood-borne levels of these analytes [8]. Capillary blood sampling is regularly
62 utilized to measure health-related biomarkers (e.g. blood glucose, cholesterol,
63 triglyceride levels), and is also used to measure exercise-related biomarkers (e.g.
64 blood lactate, glucose, haematocrit, haemoglobin) in research and applied sports
65 science environments. However, research into the validity of such measures has
66 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 \pm 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. $\dot{V}O_{2\text{ peak}}$ 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% $\dot{V}O_{2\text{ peak}}$ interspersed with 3-minute intervals at
116 50% $\dot{V}O_{2\text{ peak}}$. 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 K₃EDTA tubes (Greiner Bio-one;
126 Frickenhausen, Germany). Whole mixed unstimulated saliva samples (approx. 1ml)
127 were collected by passively drooling through 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.

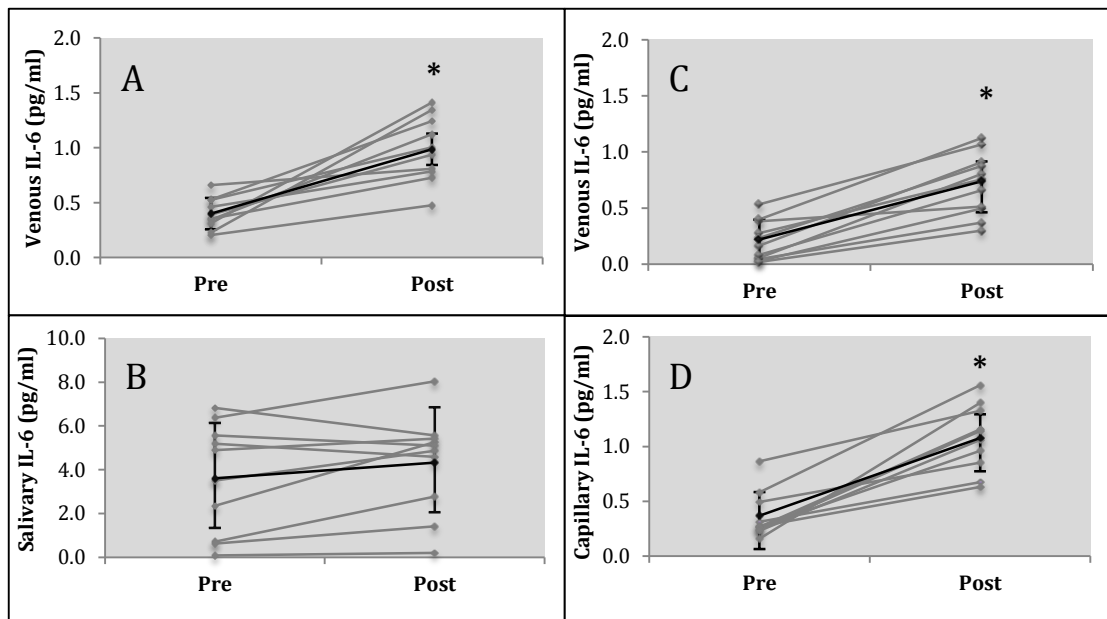
159

160 **3. Results**

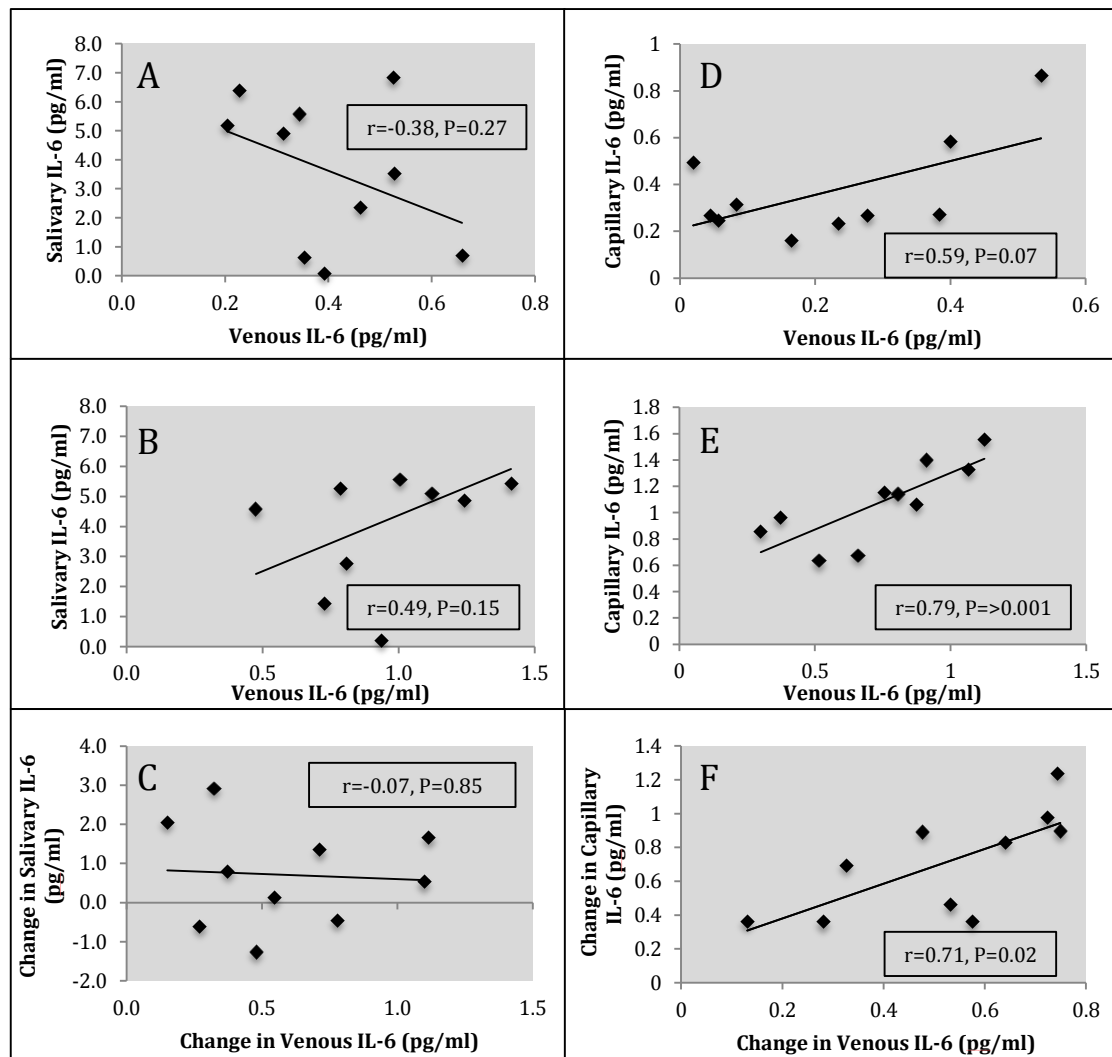
161 In Trial A plasma IL-6 increased significantly in response to exercise from 0.4±0.14
162 pg/ml to 0.99±0.29 pg/ml (P<0.01) (Fig. 1A). There was no significant change in
163 salivary IL-6 pre to post exercise (P=0.12) (Fig. 1B). There was no correlation
164 between plasma and salivary IL-6, whether 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).



174
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

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

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

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

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