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


1Cardiff School of Sport, Cardiff Metropolitan University, Cardiff CF23 6XD, UK.
2Cardiff School of Health Sciences, Cardiff Metropolitan University, Cardiff CF5 2YB, UK.
*Corresponding author. E-mail address tcullen@cardiffmet.ac.uk

Highlights
• We measured IL-6 in saliva, venous and capillary plasma pre and post exercise.
• Salivary IL-6 did not correlate to venous IL-6 at rest or post exercise.
• Capillary plasma IL-6 correlated to venous plasma in response to exercise.
• Capillary plasma responses may be reflective of systemic IL-6 responses to exercise.

Abstract
IL-6 plays a mechanistic role in conditions such as metabolic syndrome, chronic fatigue syndrome and clinical depression and also plays a major role in inflammatory and immune responses to exercise. The purpose of this study was to investigate the levels of resting and post exercise IL-6 when measured in venous plasma, saliva and capillary plasma. Five male and five females completed 2 separate exercise trials, both of which involved standardized exercise sessions on a cycle ergometer. Venous blood and saliva samples were taken immediately before and after Trial A, venous and capillary blood samples were taken immediately before and after Trial B. IL-6 values were obtained using a high-sensitivity enzyme-linked immunosorbent assay (ELISA). In Trial A venous plasma IL-6 increased significantly from 0.4±0.14 pg/ml to 0.99±0.29 pg/ml (P<0.01) while there was no increase in salivary IL-6. Venous plasma and salivary IL-6 responses were not correlated at rest, post exercise or when expressed as an exercise induced change. In Trial B venous and capillary plasma IL-6 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 Interleukin-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 [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
obtained from venous versus capillary blood samples, and hence the standardization of the procedures involved in both sampling and analysis [9,10].

Several studies have investigated the potential for saliva to be used as a surrogate of blood to measure IL-6. Sjögren et al. (2005) found that while serum and salivary IL-6 levels were not correlated with each other, they were both negatively related to psychosocial resources and positively related to psychosocial risk factors [11]. Minetto et al. (2005) demonstrated that there was no correlation between salivary and plasma IL-6 concentrations at rest or in response to exercise [12]; however the same group later showed [13] that the sample collection method used in the earlier experiment may in part have accounted for this lack of correlation. Cox et al. (2008) measured plasma and salivary IL-6 in a cohort of 45 athletes; while they found no correlation at rest, the authors suggested it was possible that the pattern of regulation could be related in response to exercise [14].

Importantly, to our knowledge, no study has compared the cytokine responses to exercise in capillary and venous blood. IL-6 remains a regularly measured biomarker in numerous fields of research, and a more practical and convenient method of sampling would facilitate less invasive and time-consuming procedures. Therefore, this study aimed to investigate the relationship between IL-6 levels as measured in venous plasma, saliva and capillary plasma, both at rest and in response to exercise.

2. Materials and Methods

2.1 Participants

Ten healthy active individuals (5 male, 5 female) aged 25.5 ± 7.8 yr (mean ± SD) gave informed consent to participate in the study. Subjects completed health/physical activity questionnaires to ensure normal dietary and exercise habits before each test. All participants were free of injury and illness prior to and throughout the study. Ethical approval was obtained via ethics committee and conformed to the declaration of Helsinki.

2.2 Experimental Procedure
Participants were tested for maximal aerobic capacity using an incremental exercise test on an electromagnetically-braked cycle ergometer (Lode Excalibur, Groningen, Netherlands). Expired gases were measured using an online gas analyser (OxyconPro, Erich Jaeger GMBH & Co., Hoechberg, Germany), and heart rate was measured continuously via short-range telemetry (RS400, Polar Electro, Finland). Each stage of the incremental exercise lasted 3 minutes, with required power output being increased by 30W at each stage until volitional exhaustion. Males began the test at a required power output of 100W while females began at 50W. $\text{VO}_2\text{peak}$ was recorded as the highest 30-s period of oxygen consumption. Oxygen consumption values obtained during the incremental test were used to plot a linear regression of power output versus oxygen consumption. This allowed the calculation of individual power outputs for the subsequent exercise session.

In both trials (A and B) participants completed the same standardized bout of high intensity interval exercise on a cycle ergometer within 1 week of incremental exercise testing: 5 x 4 minute intervals at 80% $\text{VO}_2\text{peak}$ interspersed with 3-minute intervals at 50% $\text{VO}_2\text{peak}$. During Trial A venous blood and saliva samples were collected immediately pre- and post-exercise. During Trial B venous and capillary blood samples were taken immediately pre- and post-exercise. Each participant performed both exercise trials at the same time of day and with a minimum of 2 days rest prior to each trial. Expired gases and heart rate were measured continuously throughout each exercise session.

2.3 Sample collection/handling

Venous blood samples were collected into K$_3$EDTA tubes (Greiner Bio-one; Frickenhausen, Germany). Whole mixed unstimulated saliva samples (approx. 1ml) were collected by passively drooling though a straw [13]. Saliva samples were aliquoted and stored at -80°C until further analysis. When thawed, saliva samples were briefly centrifuged to avoid particulate matter and diluted 1:4 based upon prior linearity and recovery experiments (data not shown). Prior to capillary blood collection, participants briefly submerged their hand in warm water to aid blood flow to the fingertip, whereupon 600μl of whole blood was obtained using 3 heparinized
200 μl microvette capillary blood collection tubes (Sarstedt, Germany) – it should be noted that this process took up to 10 minutes to complete. Nevertheless, for all sample-types, samples were collected within a maximum of 15 minutes post-exercise. Whole blood samples were fractionated by centrifugation (10 min; 3,000xG), and the resulting plasma was aliquoted and stored at -80°C until analysis.

2.4 Enzyme-linked immunosorbent assays

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

2.5 Statistical analysis

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

3. Results

In Trial A plasma IL-6 increased significantly in response to exercise from 0.4±0.14 pg/ml to 0.99±0.29 pg/ml (P<0.01) (Fig. 1A). There was no significant change in salivary IL-6 pre to post exercise (P=0.12) (Fig. 1B). There was no correlation between plasma and salivary IL-6, whether expressed as an exercise-induced change (r=-0.07, P=0.85, 90% CI= -0.56 to 0.50) or the discrete pre-exercise (r=-0.38,
P=0.27, 90% CI= -0.77 to 0.21,) and post-exercise (r=0.49, P=0.15, 90% CI= -0.09 to 0.82) measurements.

In Trial B both venous and capillary plasma IL-6 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) (Figs. 1C/D)). Venous and capillary plasma IL-6 did not correlate at rest (r=0.59, P=0.07, 90% CI 0.06 to 0.86), but exhibited significant correlations both post-exercise (r=0.79, P=>0.001, 90% CI= 0.42 to 0.93) and when expressed as an exercise-induced change (r=0.71, P=0.02, 90% CI=0.26 to 0.91).

Figure 1. IL-6 responses to standardised bouts of high intensity intermittent exercise in 10 matched individuals (venous (A) and salivary (B) IL-6 values [trial A], and venous (C) and capillary (D) IL-6 values [trial B]). Grey lines represent individual responses; black lines represent cohort-means (*=P>0.05).
Figure 2. The effect of sample type and procedure on IL-6 concentration. Correlations between venous and salivary IL-6 at rest (A), post–exercise (B), and when expressed as a pre to post exercise change (C). Venous and capillary IL-6 correlation at rest (D), post–exercise (E) and when expressed as a pre-versus-post exercise change (F).

4. Discussion
In this study the exercise induced increase in IL-6 was observed in plasma and capillary samples but not in saliva (Fig.1). This study confirms the modest increases in plasma IL-6 seen with similar high intensity interval exercise protocols [15]. Capillary and venous plasma IL-6 correlated post-exercise and when expressed as an exercise-induced change, but not at rest (Figs. 2 D-F). Capillary plasma IL-6 values were consistently higher than those in venous plasma (mean differences: 0.15 and 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


12. Minetto M, Rainoldi A, Gazzoni M, Terzolo M, Borrione P, Termine A,

