



**Reproducibility of measurement techniques used for creatine kinase, interleukin-6 and high-sensitivity c-reactive protein determination over a 48 h period in males and females**

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Reproducibility of measurement techniques for blood biomarker determination

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2 high-sensitivity c-reactive protein determination over a 48 h period in males and  
3 females**

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For Peer Review Only

## Reproducibility of measurement techniques for blood biomarker determination

**Abstract**

To examine the reproducibility of three measurement techniques used to determine creatine kinase, interleukin-6, and high-sensitivity C-reactive protein, fifty participants had blood samples taken on two occasions. Fingertip plasma samples were analysed using the Reflotron for CK determination. Venous blood samples collected into serum separator tubes were used for IL-6 and hs-CRP analyses. IL-6 was measured using an enzyme linked immune assay development kit. The hs-CRP was measured by an in-house ELISA method. Dependent t-tests showed no systematic bias between samples. The interdian CV was 20.0% for CK, 15.3% for IL-6 and 44.2% for hs-CRP. The intraclass correlation coefficient was 0.90 for CK, 0.98 for IL-6 and 0.70 for hs-CRP. The 95% limits of agreement were -69.7 to 63.5 IU/L for CK, -1.48 to 1.80 pg/ml for IL-6 and -1.10 to 0.91 µg/L for hs-CRP. The results demonstrate low absolute reproducibility, which may obscure a true experimental effect.

**Key words:** Exercise induced muscle damage; Reliability; Biomarkers; Cytokines; Inflammation

Reproducibility of measurement techniques for blood biomarker determination

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3 **53 Introduction**  
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7 55 Circulating blood biomarkers of muscle damage [e.g. creatine kinase (CK)] and inflammation  
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9 56 [e.g. interleukin 6 (IL-6)] are ubiquitous in exercise induced muscle damage (EIMD) research  
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11 57 (Clifford, Bell, West, Howatson, & Stevenson, 2016; McLeay, Stannard, Mundel, Foskett, &  
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13 58 Barnes, 2016; Tseng et al., 2016; Vieira et al., 2016), prolonged endurance research (Gill et  
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15 59 al., 2015; Nielsen, Oktedalen, Opstad, & Lyberg, 2016; Niemela, Kangastupa, Niemela,  
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17 60 Bloigu, & Juvonen, 2016; Scherr et al., 2011), and recovery related research within team  
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19 61 sports (Coelho, Morandi, de Melo, & Silami-Garcia, 2011; Fullagar, Skorski, Duffield, &  
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21 62 Meyer, 2016; Harper et al., 2016; Romagnoli et al., 2016). Such markers are frequently  
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23 63 employed to determine the magnitude of EIMD, and monitor readiness to train, despite their  
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25 64 inherent limitations. For example, there is large inter-individual variability of circulating  
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27 65 cytokines (Paulsen, Mikkelsen, Raastad, & Peake, 2012), and CK (Kraemer et al., 2013)  
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29 66 following EIMD. Furthermore, basal levels of these markers may also depend on a number of  
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31 67 individual factors such as age (Horska, Fishbein, Fleg, & Spencer, 2000), body composition  
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33 68 (Salvadori, Fanari, Ruga, Brunani, & Longhini, 1992) and training status (Vincent & Vincent,  
34  
35 69 1997), although these findings are equivocal. Nevertheless, when used in combination with  
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37 70 other measurement tools (e.g. maximum voluntary contraction, perceived soreness), they may  
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39 71 provide researchers and practitioners with important information regarding the muscle  
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41 72 damage and inflammatory response to various exercise paradigms, in addition to the recovery  
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43 73 profile across many sports. Therefore, if these blood markers are to be successfully  
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45 74 employed within a research context, and utilised by practitioners, it is essential that research  
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47 75 investigates the intra-individual variability (i.e. reproducibility) of the measurement  
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49 76 techniques utilised for their determination. This information is integral to interpreting a  
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51 77 change from baseline that is attributed to muscle damage and/or inflammation, and not  
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## Reproducibility of measurement techniques for blood biomarker determination

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3 78 simply due to random variability. For example, if the magnitude of change from baseline was  
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5 79 within the test-retest error, then the measurement techniques employed to determine these  
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7 80 biomarkers would not be considered appropriate.  
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11 82 Recent research measuring baseline CK and IL-6 in male soccer players across a 7 d period  
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13 83 reported a CV of 18.5% for both markers (Harper et al., 2016). Whilst such data is useful for  
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15 84 researchers and practitioners employing these measurements, in practice, such measures may  
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17 85 be collected on a more frequent basis. For example, in football a period of 72 h is considered  
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19 86 sufficient to achieve recovery to pre-match performance values, although in reality players  
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21 87 have much less time between fixtures and training (Owen et al., 2015). CK values typically  
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23 88 peak 24 – 48 h post exercise (Brancaccio, Maffulli, & Limongelli, 2007; Meister, Aus der  
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25 89 Funten, & Meyer, 2014; Young, Hepner, & Robbins, 2012), and therefore, measurements of  
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27 90 these markers within a 24 – 72 h period are likely to be employed (Howatson et al., 2010;  
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29 91 Niemela et al., 2016). Therefore, the aim of the present study was to examine the  
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31 92 reproducibility of three measurement tools used to detect the circulating blood biomarkers  
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33 93 (CK, IL-6 and hs-CRP) in healthy males and females, over a 48 h period. For CK fingertip  
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35 94 blood samples were obtained and analysed using the Reflotron. Venous blood samples were  
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37 95 collected for IL-6 and hs-CRP analyses using serum separator vacuette tubes. A R&D DuoSet  
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39 96 (DY206) enzyme linked immune assay (ELISA) development kit (R&D Systems, Abingdon,  
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41 97 UK) was used for IL-6 determination, and for hs-CRP an in-house ELISA method using anti-  
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43 98 human CRP antibodies, calibrators and controls from Abcam (Abcam®, Cambridge, UK)  
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45 99 was employed.  
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52 101 **Methods**

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## Reproducibility of measurement techniques for blood biomarker determination

103 Participants

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105 The 50 (14 female), apparently healthy participants who volunteered for this study had the  
106 following characteristics: median (min - max) age = 26 (18 – 49) y; mean (SD) height = 176  
107 (9) cm and mean (SD) mass = 70.7 (11.8) kg. Participants were free from musculoskeletal  
108 injury, non-smokers, and engaged in regular physical activity (> 30 min, three times a week  
109 for at least 6 months). There was no control for the use of any oral or transdermal  
110 contraceptive medication. However, all females were eumenorrheic and completed the testing  
111 in the luteal phase of menstruation. Participants provided written informed consent, and were  
112 asked to adhere to written pre-measurement procedures for the duration of the study. These  
113 pre-measurement procedures stipulated that participants did not engage in any exercise for 7  
114 d prior to commencing the study, that no large meals or stimulants were consumed within 4 h  
115 of each measurement, that the participants followed and replicated the same diet during the  
116 testing period, and that at least 500 ml of fluid was consumed 2 h prior to each measurement.  
117 There was no exercise allowed in between the trials. Adherence to these procedures was  
118 monitored using a pre-measurement procedure checklist, which participants completed and  
119 signed prior to the commencement of each measurement. The apparent adherence was 100%  
120 in all instances. Additionally, a pre-test health and medical questionnaire was completed and  
121 checked prior to each trial, in order to monitor the participants sleep, diet, and any health  
122 conditions (e.g. the common cold). There were no reported cases of changes in health (i.e. the  
123 common cold) or diet during the testing period. Participants were free to leave the study at  
124 any point without reason, and anonymity, and confidentiality was ensured. Ethical approval  
125 was granted by the University of Hull, Department of Sport & Exercise Science Ethics  
126 Committee.

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Reproducibility of measurement techniques for blood biomarker determination

128 Experimental approach to the problem

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130 Participants visited the laboratory on two separate occasions with each visit separated by 48

131 h. Visit times were held constant within individuals ( $\pm 0.5$  h) to negate any effects of

132 circadian variation (Drust, Waterhouse, Atkinson, Edwards, & Reilly, 2005). Blood samples

133 were collected as described below.

134

135 Blood collection and analyses

136

137 *Collection and storage of blood samples*

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139 Prior to any blood collection, participants were asked to lay in a supine position for 15 min.

140 Fingertip capillary blood samples were collected using standard techniques. Firstly, the finger

141 was cleaned using a sterile alcohol wipe and an Accucheck softclicks lancet device (Roche

142 Diagnostics, Mannheim, Germany) was used to puncture the area. The initial drop of blood

143 was wiped away and the second drop used for analysis.

144

145 Venous blood samples were drawn from a superficial vein in the antecubital fossa of the

146 forearm using standard venepuncture techniques. Samples were collected into serum

147 separator vacuette tubes (Greiner Bio-one, Kremsmunster, Austria) and left to clot for 30 min

148 at room temperature before being centrifuged in a Heraeus Labofuge 400R (Kendro Labatory

149 products, Bishops Stortford, UK) at 1509 g and 19°C for 15 min. Subsequently, serum was

150 pipetted and stored in several 1.0 ml CryoPure tubes (SARSTEDT Ltd., Beaumont Leys,

151 Leicestershire, UK) in a -80°C freezer until transportation to the specialist assay laboratory at

152 Manchester Royal Infirmary.

## Reproducibility of measurement techniques for blood biomarker determination

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154 Haemoglobin (Hb) and haematocrit (Hct) concentrations were obtained to measure changes  
155 in plasma volume according to the Dill and Costill method (Dill & Costill, 1974). Blood  
156 measures were subsequently adjusted to account for shifts in plasma volume. The percentage  
157 change in plasma volume was either added or subtracted from the concentration of the blood  
158 biomarker as required.

159

160 Blood analyses

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162 *Creatine kinase*

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164 Plasma CK was determined from a 32  $\mu$ l fingertip capillary blood sample obtained using  
165 standard techniques while participants were semi-recumbent on a treatment couch. The  
166 sample of whole blood was immediately pipetted to a test strip and analysed for CK using a  
167 colorimetric assay procedure (Reflotron, Boehringer Mannheim, Germany). Measurements  
168 were obtained and analysed in triplicate and the average used. The Reflotron was used due to  
169 ecological validity as several studies employ this measurement tool (Coelho et al., 2011;  
170 Howatson, Goodall, & van Someren, 2009; Howatson et al., 2012; McLellan, Lovell, & Gass,  
171 2010; Owen et al., 2015). This system uses a plasma separation principle which is  
172 incorporated in the reagent carrier on the test strip. Briefly, following application of the  
173 whole blood to the test strip the sample flows into the reaction zone where erythrocytes are  
174 separated from the plasma. Subsequently, the formation of dye is measured kinetically at 642  
175 nm and at 37°C. According to manufacturer guidelines, the 'normal' range of CK activity is  
176 24 – 195 IU/L and 24 – 170 IU/L for males and females respectively. The intra-assay CV was  
177 9.0%.



## Reproducibility of measurement techniques for blood biomarker determination

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179 The analysis of IL-6 and hs-CRP was performed using serum in accordance with previously  
180 published methods (Pemberton, Aboutwerat, Smith, & Warnes, 2006). Paired samples were  
181 always analysed on the same plate with the same assay kit. If an absorbance value for a  
182 sample was greater than the highest standard (15µg/L for hs-CRP, 100pg/ml for IL-6) then  
183 the analysis was repeated using sample at a higher dilution.

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185 *Interleukin-6 (IL-6)*

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187 IL-6 was measured using a R&D DuoSet (DY206) enzyme linked immune assay (ELISA)  
188 development kit (R&D Systems, Abingdon, UK). The range of the assay is up to 100 pg/ml,  
189 and the minimum detection limit calculated from the mean plus two standard deviations of 12  
190 single replicate analyses of reagent blank was found to be 0.6 pg/ml. Intra and inter-assay CV  
191 was 5.9% and 17.2%, respectively.

192

193 *High-sensitivity C-reactive protein (hs-CRP)*

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195 The hs-CRP was measured by an in-house ELISA method using anti-human CRP antibodies,  
196 calibrators and controls from Abcam (Abcam®, Cambridge, UK). The ELISA technique is  
197 based on the antibody sandwich principle. First, the capture antibody (rabbit anti-human CRP  
198 antibody) was bound to a microtitre plate to create a solid phase. A blocking buffer  
199 containing BSA (Sigma-Aldrich®, Poole, UK) was then added. Following a wash, samples,  
200 standards and controls were incubated with the solid phase antibody that captures the CRP.  
201 After further washing, the conjugated detection antibody (HRP-labelled goat anti-human  
202 CRP antibody) was added. This detection antibody binds to a different epitope of CRP thus

## Reproducibility of measurement techniques for blood biomarker determination

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3 203 completing the sandwich. Subsequently the samples were washed to remove unbound  
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5 204 detection antibody, the substrate O-phenylenediamine (Sigma-Aldrich®, Poole, UK) was  
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7 205 added and colour developed in proportion to the amount of bound HRP. Colour development  
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9 206 was stopped by addition of strong acid and the intensity of colour then measured at  $\lambda = 490$   
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11 207 nm. The content of each sample was then calculated from the standard curve. The range of  
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13 208 the assay is up to 15  $\mu\text{g/L}$  and the minimum detection limit, calculated from the mean plus  
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15 209 two standard deviations of 8 replicate analyses of reagent blank, was 0.1  $\mu\text{g/L}$ . Intra and  
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17 210 inter-assay CV was 4.7% and 5.3%, respectively.  
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21 212 Statistical analyses

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26 214 The number of participants required for this study was determined *a priori* with an alpha  
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28 215 level of 0.05 using a 2 tailed t-test for the main outcome measures for a follow up study (data  
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30 216 not shown) using Power Analysis and Sample Size Software (PASS) version 13.0 (NCSS,  
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32 217 LLC, Utah, USA). A sample size of  $n = 50$  achieved 99% and 94% power to detect the  
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34 218 required minimum worthwhile effects. Analyses were completed using the statistical software  
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36 219 package IBM SPSS Statistics version 19.0 (SPSS Inc, Chicago, IL, USA) and graphs created  
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38 220 using SigmaPlot version 12.3 (Systat Software Inc, CA, USA). Standard graphical methods  
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40 221 were preferred over null hypothesis significance testing to check statistical assumptions  
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42 222 (Grafen & Hails, 2002). For descriptive purposes the mean and standard deviation have been  
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44 223 used to report the central tendency and dispersion of the observed data. Combinations of  
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46 224 statistical methods were chosen in order to compare reliability between different measures  
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48 225 and different studies.  
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## Reproducibility of measurement techniques for blood biomarker determination

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3 227 Systematic bias was tested using two-tailed dependent t-tests, and also examined graphically  
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5 228 with Bland-Altman plots. Absolute measurement error was determined using repeated  
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7 229 measures CV and 95% limits of agreement (LoA). The CV (expressed as a percentage) was  
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9 230 calculated by dividing the standard deviation of the differences by the square root of two and  
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11 231 dividing the answer by the grand mean (Hopkins, 2000). The intra assay CV for CK, IL-6 and  
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13 232 hs-CRP was measured in duplicate for all participants (n = 50). The % CV for each sample  
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15 233 was calculated by finding the standard deviation of results 1 and 2, dividing that by the  
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17 234 duplicate mean and multiplying by 100. The average of the individual CV's was reported as  
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19 235 the intra-assay CV. Inter-assay CV for IL-6 and hs-CRP were measured in duplicate for both  
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21 236 high and low controls on ten different plates. The plate means for high and low were  
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23 237 calculated and used to obtain the overall mean, standard deviation and % CV. Overall % CV  
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25 238 was calculated by dividing the standard deviation of the plate means by the mean of the plate  
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27 239 means and multiplying this by 100. The average of the high and low % CV is reported as the  
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29 240 inter-assay CV. Relative reliability was determined using a two-way random model intraclass  
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31 241 correlation coefficient (ICC), which is a measure of the ratio of between-subject variance to  
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33 242 within-subject variance. A combination of reproducibility statistics were employed in order to  
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35 243 allow comparison with published research. Additionally, data were analysed separately by  
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37 244 sex. There were no significant differences between males and females ( $p \geq 0.23$ ) and  
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39 245 therefore, the data set was collapsed for the final analyses to increase statistical power and  
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41 246 satisfy the principle of parsimony. The two-tailed alpha level for significance testing was set  
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43 247 as  $p < 0.05$ .

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249 **Results**

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## Reproducibility of measurement techniques for blood biomarker determination

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3 251 The mean (SD) values of the circulating blood biomarkers obtained from measurement one  
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5 252 and two are shown in Table 1. Dependent t-tests indicated there was no systematic bias ( $p \geq$   
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7 253 0.24) for any of the variables (Table 1).  
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11 255 \*\*\*Insert Table 1 here \*\*\*  
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15 257 The CV, ICC and 95% LoA for CK, IL-6 and hs-CRP are reported in Table 2. The individual  
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17 258 mean circulating blood biomarker results plotted against their individual differences (Bland-  
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19 259 Altman plots) are shown in Figure 1.  
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22  
23 261 \*\*\*Insert Table 2 here \*\*\*  
24

25 262 \*\*\*Insert Figure 1 here \*\*\*  
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27 263

## 28 264 **Discussion**

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31 266 The aim of the present study was to examine the reproducibility of three measurement tools  
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33 267 used to assess CK, IL-6 and hs-CRP. The main findings in the present study were that  
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35 268 although there was no systemic bias between trials for CK, IL-6 and hs-CRP (Table 1), the  
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37 269 CV results show that all the blood biomarkers demonstrated high variability (i.e. low  
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39 270 reproducibility) (Table 2).  
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44 272 In support of the findings in the present study, CV's of 19% (Nicholson, Morgan, Meerkin,  
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46 273 Strauss, & McLeod, 1985) and 18.5% (Harper et al., 2016) for CK have been previously  
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48 274 reported. Conversely, lower CV's of 10.5% (Chen, Lin, Chen, Lin, & Nosaka, 2011) and  
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50 275 3.4% (Horder et al., 1991) for CK have also been shown. Discrepancy between these findings  
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## Reproducibility of measurement techniques for blood biomarker determination

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3 276 may be due to random biological variation, technical error, differences in timings between  
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5 277 measurements, and measurement technique. For example, Chen and colleagues used  
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7 278 spectrophotometry to measure CK compared to the Reflotron used in the present study. The  
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9 279 intra-assay CV for the analysis of CK in the present study was 9%, supporting the premise  
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11 280 that some of the variability resides within the measurement technique itself (i.e. the  
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13 281 Reflotron). Nevertheless, the CV in the present study was comparable to Harper and  
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15 282 colleagues, despite the different timings between measurements (i.e. 48 h compared to 7 d),  
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17 283 and measurement technique employed (i.e. Reflotron versus Cobas 8000). A Reflotron was  
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19 284 employed in the present study as this is typically utilised in research, and by practitioners  
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21 285 (Coelho et al., 2011; Howatson et al., 2012). However, both the Howatson and Horder  
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23 286 studies utilised a Reflotron and reported similar and more reliable CV's; < 3% and 3.4%  
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25 287 respectively. Therefore, differences in the interdian CV between the Howatson study and the  
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27 288 present study may be due to the population employed, as opposed to the measurement  
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29 289 technique. Howatson and colleagues used only male participants in their study (n = 12) who  
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31 290 were trained in the competitive national league for football (homogenous population),  
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33 291 compared to recreationally active males and females (heterogeneous population) in the  
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35 292 present study. We acknowledge that despite no significant differences between males and  
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37 293 females in the present study, the effects of menstruation, oral and transdermal contraceptives  
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39 294 were not measured. Subsequently, this could explain the higher interdian variability observed.  
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41 295 However, Harper and colleagues also used a homogenous population (i.e. males, n = 7) and  
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43 296 reported a similar interdian CV to the present study (i.e. 18.5%). Previous research has  
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45 297 suggested that high variability in baseline CK values could be due to minor injury, genetic  
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47 298 factors, physical activity status, and medication (Prelle et al., 2002). Subsequently, strict pre-  
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49 299 test procedures were employed in the present study with apparent 100% adherence. Despite  
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51 300 this, low reproducibility between testes was still observed. Therefore, researchers and  
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## Reproducibility of measurement techniques for blood biomarker determination

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3 301 practitioners must account for this error when utilising CK values to determine the presence  
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5 302 of muscle damage, as changes in CK values could be used to prescribe recovery techniques  
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7 303 (e.g. pharmacological aids such as ibuprofen) and/or modify the training load, otherwise  
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9 304 incorrect application of both recovery techniques and changes to training load could be  
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11 305 performed. Additionally, this highlights the importance of not utilising just one measurement  
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13 306 (e.g. CK) when making such decisions.  
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18 308 Basal levels of CK are considered to be 24 – 195 IU/L, which is in agreement with the  
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20 309 findings in the present study (Table 1). Similarly, Clarkson and colleagues reported average  
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22 310 baseline CK values of 118 IU/L (range 33 – 481 IU/L) in 203 participants, although no  
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24 311 reproducibility statistics were reported in their study (Clarkson, Kearns, Rouzier, Rubin, &  
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26 312 Thompson, 2006). Post exercise concentrations of CK in athletes are thought to range  
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28 313 between 300 – 500 IU/L (Mougios, 2007). Furthermore, Coelho, Morandi, de Melo, &  
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30 314 Silami-Garcia (2011) observed average CK values (measured using a Reflotron) of 786 IU/L  
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32 315 (min – max; 96 – 1580 IU/L), 388 IU/L (38 – 749 IU/L), 299 IU/L (31 – 595 IU/L) and 317  
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34 316 IU/L (197 – 654 IU/L) 12 – 20 h, 36 – 48 h, 60 – 65 h and 90 – 110 h respectively following  
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36 317 a soccer game. Similarly, Howatson et al. (2012) observed a 3-4 fold increase in CK, which  
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38 318 peaked at 24 h post eccentric exercise. Subsequently, it appears that on average the  
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40 319 magnitude of changes in CK following exercise, is typically outside the CV reported in the  
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42 320 present study (i.e. 20%). Similarly, the magnitude of change in CK far exceeds the 95% LoA  
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44 321 reported in the present study (Figure 1), suggesting the test-retest error of this measurement  
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46 322 technique is low enough not to obscure a true experimental effect. However, Clarkson and  
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48 323 colleagues also measured CK 4, 7 and 10 days following a bout of eccentric exercise, and the  
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50 324 authors reported an increase in CK to 7713 IU/L (range 55 – 80, 550 IU/L), 2603 IU/L (range  
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52 325 49 – 21, 675 IU/L) and 486 IU (45 – 7034 IU/L) respectively, highlighting high variability in  
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## Reproducibility of measurement techniques for blood biomarker determination

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3 326 the CK response. The average percentage increase in CK was 6420, 2100 and 311% across  
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5 327 the 4, 7 and 10 d respectively. Subsequently, the minimum change in CK reported in the  
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7 328 aforementioned studies means that some individuals would fall within the test-retest error  
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9 329 value and 95% LoA reported in the present study (Figure 1). However, as the authors did not  
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11 330 conduct their own reproducibility statistics, it cannot be assumed that the test-retest error  
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13 331 would be comparable to the present study. In order for practitioners and researchers to  
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15 332 accurately distinguish between a 'true' experimental effect and test-retest error, it is vital that  
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17 333 the inter-dian and intra assay CV are reported, and compared to the experimental effect.  
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22 335 Previous research has reported a CV of 27% for IL-6 (Knudsen et al., 2008), which is slightly  
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24 336 higher than that found in the present study (15.3%). The average IL-6 values reported in the  
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26 337 present study (Table 1) appear to be higher than those reported in previous research  
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28 338 (Abdelmalek et al., 2013; Conceicao et al., 2012; Nieman et al., 2005; Scherr et al., 2011),  
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30 339 whilst the hs-CRP values are comparable to previous studies (Peake, Nosaka, Muthalib, &  
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32 340 Suzuki, 2006; Robson-Ansley et al., 2009; Scherr et al., 2011). The reasons behind the higher  
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34 341 baseline values for IL-6 in the present study are not clear. Factors such as upper respiratory  
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36 342 tract infections (Martin, Pence, & Woods, 2009), training status (Fischer, 2006), and time of  
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38 343 day (Abdelmalek et al., 2013) are known to affect IL-6 values. Time of day was  
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40 344 standardised within participants in the present study, however, between participant testing  
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42 345 time was not constant. Moreover, although all participants met the inclusion criteria regarding  
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44 346 regular physical activity, the population sample was heterogeneous. Therefore, the  
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46 347 combination of between participant differences in time of day, and the heterogeneity of  
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48 348 training status may have contributed to the variability in IL-6, and could explain the high  
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50 349 values observed (See Table 2, and Figure 1) compared to previous research. Furthermore, the  
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52 350 intra and inter-assay CV for IL-6 and hs-CRP were 5.9% and 17.2%, and 4.7% and 5.3%  
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## Reproducibility of measurement techniques for blood biomarker determination

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3 351 respectively. This suggests that the technical error component is somewhat large for the inter-  
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5 352 assay CV for IL-6 (17.2%). Conceicao et al. (2011) reported intra and inter assay variability  
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7 353 of 7.8% and 7.2% respectively for IL-6. Similarly, Peake, Nosaka, Muthalib, & Suzuki  
8  
9 354 (2005) reported an inter assay CV of 5.4% for IL-6. Furthermore, Robson-Ansley et al.  
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11 355 (2009) reported intra and inter assay CV's of < 4% and, 5% for hs-CRP respectively, which  
12  
13 356 is similar to the intra and inter-assay CV in the present study (4.7% and 5.3%). The high  
14  
15 357 inter-assay CV for IL-6 in the present study may account for the higher baseline values of IL-  
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17 358 6, which could have been caused by technical error in the measurement technique. However,  
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19 359 the five participants who had a baseline level of IL-6 > 10 pg/ml in trial 1 also had an almost  
20  
21 360 identical value for trial 2. Therefore, it is more plausible that these five participants had  
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23 361 higher IL-6 values due to the time of day, and training status, as previously discussed.  
24  
25 362 Nevertheless, the large interdian CV (Table 2), and low reproducibility reported in the  
26  
27 363 present study for IL-6 is clearly a combination of both technical and biological variation. The  
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29 364 high interdian CV for hs-CRP in the present study (Table 2), appears to support previous  
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31 365 findings demonstrating considerable intra-individual variability in males (n = 100) with either  
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33 366 previous history of coronary artery disease or no history (Bogaty et al., 2013), and healthy  
34  
35 367 older men (n = 50) measured over a four year period (Platz et al., 2010). Moreover, in a  
36  
37 368 mixed sex (n = 541) study the interdian CV for hs-CRP measured on average 18.9 d apart  
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39 369 was 46.2% (Bower, Lazo, Juraschek, & Selvin, 2012), which is almost identical to the 44.2%  
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41 370 reported in the present study, despite the fact that we utilised an in house assay for hs-CRP.  
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43 371 However, even when employing the same assay, reproducibility may still differ as it is  
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45 372 dependent on many factors (i.e. laboratory techniques, sample population, etc.).  
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47 373 Subsequently, it is important that researchers and practitioners conduct their own  
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49 374 reproducibility using their own measurement tools, and replicate this within populations, to  
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51 375 distinguish between the true test-retest error and experimental effect. Overall, these factors  
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## Reproducibility of measurement techniques for blood biomarker determination

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3 376 highlight the importance of all studies reporting reproducibility statistics for the measurement  
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5 377 tools employed and ensuring adherence to pre-test procedures to control for any confounding  
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7 378 factors, which may affect baseline levels. Again, this highlights the potential problem of  
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9 379 using these bio-markers in isolation, and it is recommended that multiple measurement  
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11 380 techniques (e.g. wellness questionnaires, perceived exertion, maximal voluntary contraction)  
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13 381 are also obtained to ensure robust and reliable information is gathered.  
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18 383 The ICC's reported in the present study (Table 2) suggest 'high', 'high' and 'questionable'  
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20 384 reproducibility for CK, IL-6 and hs-CRP respectively. Therefore, if we had simply used the  
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22 385 ICC in isolation within our experimental design to determine whether these measurement  
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24 386 tools are reproducible, it would indeed suggest that CK and IL-6 are reproducible markers.  
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26 387 These findings appear to contradict the values observed for the CV's. High ranges observed  
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28 388 for the blood biomarkers (Figure 1) could have increased the size of the correlation.  
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30 389 Therefore, 'high' ICC's may be a reflection of more heterogeneous results rather than high  
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32 390 reproducibility. This highlights the potential problem when researchers purely employ a  
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34 391 correlation (e.g. Pearson's  $r$  or ICC) to determine the reproducibility of a measurement tool.  
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36 392 Consequently, it has been suggested that the CV may be a more accurate representation of the  
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38 393 reproducibility of a particular measure, and therefore, it is suggested that these results are  
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40 394 used to make recommendations in the present study. Nevertheless, previous research has  
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42 395 reported wide variation in ICC's for the blood biomarkers, ranging from questionable to  
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44 396 moderate. For example ICC'S of 0.48 (Navarro et al., 2012), 0.66 (Karakas et al., 2010) and  
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46 397 0.47 – 0.80 (Walshe et al., 2010) have all been reported for IL-6. Additionally, poor  
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48 398 reproducibility has been shown for hs-CRP with ICC'S of 0.62 (Navarro et al., 2012) and  
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50 399 0.66 (Karakas et al., 2010).  
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Reproducibility of measurement techniques for blood biomarker determination

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3 401 **Conclusion**

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7 403 Due to the high inter-dian CV of CK, IL-6 and hs-CRP, it could be recommended that these  
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9 404 variables are not used in isolation within research and by practitioners, as the high test-retest  
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11 405 error associated with these markers may obscure a true experimental effect. However, whilst  
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13 406 the CV values observed in the present study are outside the recommended threshold of 10%,  
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15 407 this does not necessarily imply that these biomarkers are not appropriate measurement tools.  
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17 408 To assess the usefulness of a measurement tool, it is critical to compare the CV with the  
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19 409 minimum worthwhile effect. As long as the CV is smaller than the minimum worthwhile  
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21 410 difference, then the measurement tool can be deemed appropriate.  
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26 412 Further research is required in order to investigate the biological variation and error of these  
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28 413 biomarkers over different periods of time. For example, it would be useful to quantify the  
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30 414 intra-individual variation and error over a more acute (1 – 12 h) and chronic (24 h – 7 d)  
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32 415 period as several blood samples are often obtained within research in order to investigate the  
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34 416 recovery pattern following different types of exercise. It is advised that researchers and  
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36 417 practitioners include a reproducibility trial within their research design in order to account for  
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38 418 potential error within their measurements, and allow for greater accuracy when reporting  
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40 419 changes in these biomarkers. Furthermore, future work should seek to perform repeated  
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42 420 reproducibility in the same population to provide an accurate profile of an individual's  
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44 421 variability in these markers.  
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Reproducibility of measurement techniques for blood biomarker determination

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428

#### 429 **Conflicts of interest**

430

431 The authors declare that this research was conducted in the absence of any commercial or  
432 financial relationships that could be construed as a potential conflict of interest.

433

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441

- 442 Abdelmalek, S., Chtourou, H., Aloui, A., Aouichaoui, C., Souissi, N., & Tabka, Z. (2013).  
443 Effect of time of day and partial sleep deprivation on plasma concentrations of IL-6  
444 during a short-term maximal performance. *European Journal of Applied Physiology*,  
445 *113*(1), 241-248. doi: 10.1007/s00421-012-2432-7
- 446 Bogaty, P., Dagenais, G. R., Joseph, L., Boyer, L., Leblanc, A., Bélisle, P., & Brophy, J. M.  
447 (2013). Time Variability of C-Reactive Protein: Implications for Clinical Risk  
448 Stratification. *Plos One*, *8*(4), e60759. doi: 10.1371/journal.pone.0060759
- 449 Bower, J. K., Lazo, M., Juraschek, S. P., & Selvin, E. (2012). Within-person variability in  
450 high-sensitivity C-reactive protein. *Arch Intern Med*, *172*(19), 1519-1521. doi:  
451 10.1001/archinternmed.2012.3712
- 452 Brancaccio, P., Maffulli, N., & Limongelli, F. M. (2007). Creatine kinase monitoring in sport  
453 medicine. *British Medical Bulletin*, *81-82*, 209-230. doi: 10.1093/bmb/ldm014
- 454 Chen, T. C., Lin, K. Y., Chen, H. L., Lin, M. J., & Nosaka, K. (2011). Comparison in  
455 eccentric exercise-induced muscle damage among four limb muscles. *European*  
456 *Journal of Applied Physiology*, *111*(2), 211-223. doi: 10.1007/s00421-010-1648-7
- 457 Clarkson, P. M., Kearns, A. K., Rouzier, P., Rubin, R., & Thompson, P. D. (2006). Serum  
458 creatine kinase levels and renal function measures in exertional muscle damage. *Med*  
459 *Sci Sports Exerc*, *38*(4), 623-627. doi: 10.1249/01.mss.0000210192.49210.fc
- 460 Clifford, T., Bell, O., West, D. J., Howatson, G., & Stevenson, E. J. (2016). The effects of  
461 beetroot juice supplementation on indices of muscle damage following eccentric  
462 exercise. *Eur J Appl Physiol*, *116*(2), 353-362. doi: 10.1007/s00421-015-3290-x

## Reproducibility of measurement techniques for blood biomarker determination

- 1  
2  
3 463 Coelho, D. B., Morandi, R. F., de Melo, M. A. A., & Silami-Garcia, E. (2011). Creatine  
4 464 kinase kinetics in professional soccer players during a competitive season. *Brazilian*  
5 465 *Journal of Kineanthropometry and Human Performance*, 13(3), 189.
- 6 466 Conceicao, M. S., Libardi, C. A., Nogueira, F. R. D., Bonganha, V., Gaspari, A. F., Chacon-  
7 467 Mikahil, M. P. T., . . . Madruga, V. A. (2012). Effects of eccentric exercise on  
8 468 systemic concentrations of pro- and anti-inflammatory cytokines and prostaglandin  
9 469 (E2): comparison between young and postmenopausal women. *European Journal of*  
10 470 *Applied Physiology*, 112(9), 3205-3213. doi: 10.1007/s00421-011-2292-6.
- 11 471 Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood,  
12 472 plasma, and red-cells in dehydration. *Journal of Applied Physiology*, 37(2), 247-248.
- 13 473 Drust, B., Waterhouse, J., Atkinson, G., Edwards, B., & Reilly, T. (2005). Circadian rhythms  
14 474 in sports performance - An update. *Chronobiology International*, 22(1), 21-44. doi:  
15 475 10.1081/cbi-200041039
- 16 476 Fischer, C. P. (2006). Interleukin-6 in acute exercise and training: what is the biological  
17 477 relevance? *Exerc Immunol Rev*, 12, 6-33.
- 18 478 Fullagar, H., Skorski, S., Duffield, R., & Meyer, T. (2016). The effect of an acute sleep  
19 479 hygiene strategy following a late-night soccer match on recovery of players.  
20 480 *Chronobiol Int*, 33(5), 490-505. doi: 10.3109/07420528.2016.1149190
- 21 481 Gill, S. K., Hankey, J., Wright, A., Marczak, S., Hemming, K., Allerton, D. M., . . . Costa, R.  
22 482 J. (2015). The Impact of a 24-h Ultra-Marathon on Circulatory Endotoxin and  
23 483 Cytokine Profile. *Int J Sports Med*, 36(8), 688-695. doi: 10.1055/s-0034-1398535
- 24 484 Grafen, G., & Hails, R. (2002) *Modern Statistics for the Life Sciences* (pp. 153 - 184 ). New  
25 485 York, USA: Oxford University Press.
- 26 486 Harper, L. D., Hunter, R., Parker, P., Goodall, S., Thomas, K., Howatson, G., . . . Russell, M.  
27 487 (2016). Test-Retest Reliability of Physiological and Performance Responses to 120  
28 488 Minutes of Simulated Soccer Match Play. *J Strength Cond Res*, 30(11), 3178-3186.  
29 489 doi: 10.1519/jsc.0000000000001400
- 30 490 Hopkins, W. G. (2000). Measures of reliability in sports medicine and science. *Sports*  
31 491 *Medicine*, 30(1), 1-15. doi: 10.2165/00007256-200030010-00001
- 32 492 Horder, M., Jorgensen, P. J., Hafkenschied, J. C. M., Carstensen, C. A., Bachmann, C.,  
33 493 Bauer, K., . . . Vogt, W. (1991). Creatine kinase determination - a european evaluation  
34 494 of the creatine kinase determination in serum, plasma and whole blood with the  
35 495 Reflotron system *European Journal of Clinical Chemistry and Clinical Biochemistry*,  
36 496 29(10), 691-696.
- 37 497 Horska, A., Fishbein, K. W., Fleg, J. L., & Spencer, R. G. S. (2000). The relationship  
38 498 between creatine kinase kinetics and exercise intensity in human forearm is  
39 499 unchanged by age. *American Journal of Physiology-Endocrinology and Metabolism*,  
40 500 279(2), E333-E339.
- 41 501 Howatson, G., Goodall, S., & van Someren, K. A. (2009). The influence of cold water  
42 502 immersions on adaptation following a single bout of damaging exercise. *Eur J Appl*  
43 503 *Physiol*, 105(4), 615-621. doi: 10.1007/s00421-008-0941-1
- 44 504 Howatson, G., Hoad, M., Goodall, S., Tallent, J., Bell, P. G., & French, D. N. (2012).  
45 505 Exercise-induced muscle damage is reduced in resistance-trained males by branched  
46 506 chain amino acids: a randomised, double-blind, placebo controlled study. *Journal of*  
47 507 *International Society of Sports Nutrition*, 12(9), 20. doi: 10.1186/1550-2783-9-20
- 48 508 Howatson, G., McHugh, M. P., Hill, J. A., Brouner, J., Jewell, A. P., van Someren, K. A., . . .  
49 509 Howatson, S. A. (2010). Influence of tart cherry juice on indices of recovery  
50 510 following marathon running. *Scandinavian Journal of Medicine & Science in Sports*,  
51 511 20(6), 843-852. doi: 10.1111/j.1600-0838.2009.01005.x

## Reproducibility of measurement techniques for blood biomarker determination

- 1  
2  
3 512 Karakas, M., Baumert, J., Greven, S., Ruckerl, R., Peters, A., & Koenig, W. (2010).  
4 513 Reproducibility in Serial C-Reactive Protein and Interleukin-6 Measurements in Post-  
5 514 Myocardial Infarction Patients: Results from the AIRGENE Study. *Clinical*  
6 515 *Chemistry*, 56(5), 861-864. doi: 10.1373/clinchem.2010.143719  
7 516 Knudsen, L. S., Christensen, I. B. J., Lottenburger, T., Svendsen, M. N., Nielsen, H. J.,  
8 517 Nielsen, L., . . . Johansen, J. S. (2008). Pre-analytical and biological variability in  
9 518 circulating interleukin 6 in healthy subjects and patients with rheumatoid arthritis.  
10 519 *Biomarkers*, 13(1), 59-78. doi: 10.1080/13547500701615017  
11 520 Kraemer, W. J., Looney, D. P., Martin, G. J., Ratamess, N. A., Vingren, J. L., French, D.  
12 521 N., . . . Fleck, S. J. (2013). Changes in creatine kinase and cortisol in national  
13 522 collegiate athletic association division i american football players during a season.  
14 523 *Journal of Strength and Conditioning Research*, 27(2), 434-441. doi:  
15 524 10.1519/JSC.0b013e318281d1b0  
16 525 Martin, S. A., Pence, B. D., & Woods, J. A. (2009). Exercise and Respiratory Tract Viral  
17 526 Infections. *Exercise and sport sciences reviews*, 37(4), 157-164. doi:  
18 527 10.1097/JES.0b013e3181b7b57b  
19 528 McLeay, Y., Stannard, S. R., Mundel, T., Foskett, A., & Barnes, M. (2016). Effect of Alcohol  
20 529 Consumption on Recovery From Eccentric Exercise Induced Muscle Damage in  
21 530 Females. *Int J Sport Nutr Exerc Metab*, 1-20. doi: 10.1123/ijsnem.2016-0171  
22 531 McLellan, C. P., Lovell, D. I., & Gass, G. C. (2010). Creatine kinase and endocrine responses  
23 532 of elite players pre, during, and post rugby league match play. *J Strength Cond Res*,  
24 533 24(11), 2908-2919. doi: 10.1519/JSC.0b013e3181c1fcb1  
25 534 Meister, S., Aus der Funten, K., & Meyer, T. (2014). Repeated monitoring of blood  
26 535 parameters for evaluating strain and overload in elite football players: is it justified? *J*  
27 536 *Sports Sci*, 32(13), 1328-1331. doi: 10.1080/02640414.2014.927070  
28 537 Mougios, V. (2007). Reference intervals for serum creatine kinase in athletes. *British Journal*  
29 538 *of Sports Medicine*, 41(10), 674-678. doi: 10.1136/bjism.2006.034041  
30 539 Navarro, S. L., Brasky, T. M., Schwarz, Y., Song, X., Wang, C. Y., Kristal, A. R., . . .  
31 540 Lampe, J. W. (2012). Reliability of serum biomarkers of inflammation from repeated  
32 541 measures in healthy individuals. *Cancer epidemiology, biomarkers & prevention : a*  
33 542 *publication of the American Association for Cancer Research, cosponsored by the*  
34 543 *American Society of Preventive Oncology*, 21(7), 1167-1170. doi: 10.1158/1055-9965  
35 544 Nicholson, G. A., Morgan, G., Meerkink, M., Strauss, E., & McLeod, J. G. (1985). The  
36 545 creatine-kinase reference interval - an assessment of intraindividual and  
37 546 interindividual variation. *Journal of the Neurological Sciences*, 71(2-3), 225-231. doi:  
38 547 10.1016/0022-510x(85)90061-9  
39 548 Nielsen, H. G., Oktedalen, O., Opstad, P. K., & Lyberg, T. (2016). Plasma Cytokine Profiles  
40 549 in Long-Term Strenuous Exercise. *J Sports Med (Hindawi Publ Corp)*, 2016,  
41 550 7186137. doi: 10.1155/2016/7186137  
42 551 Nieman, D. C., Henson, D. A., Dumke, C. L., McAnulty, S. R., McAnulty, L. S., Gross, S. J.,  
43 552 & Lind, R. H. (2005). Muscle Damage Is Linked To Cytokine Changes Following A  
44 553 160-km Race. *Medicine and Science in Sports and Exercise*, 37, S336-S336. doi:  
45 554 10.1097/00005768-200505001-01740  
46 555 Niemela, M., Kangastupa, P., Niemela, O., Bloigu, R., & Juvonen, T. (2016). Acute Changes  
47 556 in Inflammatory Biomarker Levels in Recreational Runners Participating in a  
48 557 Marathon or Half-Marathon. *Sports Med Open*, 2(1), 21. doi: 10.1186/s40798-016-  
49 558 0045-0  
50 559 Owen, A., Dunlop, G., Rouissi, M., Chtara, M., Paul, D., Zouhal, H., & Wong del, P. (2015).  
51 560 The relationship between lower-limb strength and match-related muscle damage in

## Reproducibility of measurement techniques for blood biomarker determination

- 1  
2  
3 561 elite level professional European soccer players. *J Sports Sci*, 33(20), 2100-2105. doi:  
4 562 10.1080/02640414.2015.1064155
- 5 563 Paulsen, G., Mikkelsen, U. R., Raastad, T., & Peake, J. M. (2012). Leucocytes, cytokines and  
6 564 satellite cells: what role do they play in muscle damage and regeneration following  
7 565 eccentric exercise? *Exercise Immunology Review*, 18, 42-97.
- 8 566 Peake, J. M., Nosaka, K., Muthalib, M., & Suzuki, K. (2006). Systemic inflammatory  
9 567 responses to maximal versus submaximal lengthening contractions of the elbow  
10 568 flexors. *Exercise Immunology Review*, 12, 72-85.
- 11 569 Pemberton, P. W., Aboutwerat, A., Smith, A., & Warnes, T. W. (2006). Ursodeoxycholic  
12 570 acid in primary biliary cirrhosis improves glutathione status but fails to reduce lipid  
13 571 peroxidation. *Redox Report*, 11(3), 117-123. doi: 10.1179/135100006x116600
- 14 572 Platz, E. A., Sutcliffe, S., De Marzo, A. M., Drake, C. G., Rifai, N., Hsing, A. W., . . . Kristal,  
15 573 A. R. (2010). Intra-individual variation in serum C-reactive protein over four years:  
16 574 An implication for epidemiologic studies. *Cancer Causes Control*, 21(6), 847-851.  
17 575 doi: 10.1007/s10552-010-9511-z
- 18 576 Prella, A., Tancredi, L., Sciacco, M., Chiveri, L., Comi, G. P., Battistel, A., . . . Moggio, M.  
19 577 (2002). Retrospective study of a large population of patients with asymptomatic or  
20 578 minimally symptomatic raised serum creatine kinase levels. *J Neurol*, 249(3), 305-  
21 579 311.
- 22 580 Robson-Ansley, P., Barwood, M., Canavan, J., Hack, S., Eglin, C., Davey, S., . . . Ansley, L.  
23 581 (2009). The effect of repeated endurance exercise on IL-6 and sIL-6R and their  
24 582 relationship with sensations of fatigue at rest. *Cytokine*, 45(2), 111-116. doi:  
25 583 10.1016/j.cyto.2008.11.006
- 26 584 Romagnoli, M., Sanchis-Gomar, F., Alis, R., Risso-Ballester, J., Bosio, A., Graziani, R. L., &  
27 585 Rampinini, E. (2016). Changes in muscle damage, inflammation, and fatigue-related  
28 586 parameters in young elite soccer players after a match. *J Sports Med Phys Fitness*,  
29 587 56(10), 1198-1205.
- 30 588 Salvadori, A., Fanari, P., Ruga, S., Brunani, A., & Longhini, E. (1992). Creatine-kinase and  
31 589 creatine kinase-mb isoenzyme during and after exercise testing in normal and obese  
32 590 young-people. *Chest*, 102(6), 1687-1689. doi: 10.1378/chest.102.6.1687
- 33 591 Scherr, J., Braun, S., Schuster, T., Hartmann, C., Moehlenkamp, S., Wolfarth, B., . . . Halle,  
34 592 M. (2011). 72-h Kinetics of High-Sensitive Troponin T and Inflammatory Markers  
35 593 after Marathon. *Medicine and Science in Sports and Exercise*, 43(10), 1819-1827. doi:  
36 594 10.1249/MSS.0b013e31821b12eb
- 37 595 Tseng, K. W., Tseng, W. C., Lin, M. J., Chen, H. L., Nosaka, K., & Chen, T. C. (2016).  
38 596 Protective effect by maximal isometric contractions against maximal eccentric  
39 597 exercise-induced muscle damage of the knee extensors. *Res Sports Med*, 24(3), 243-  
40 598 256. doi: 10.1080/15438627.2016.1202826
- 41 599 Vieira, A., Siqueira, A. F., Ferreira-Junior, J. B., do Carmo, J., Durigan, J. L., Blazeovich, A.,  
42 600 & Bottaro, M. (2016). The Effect of Water Temperature during Cold-Water  
43 601 Immersion on Recovery from Exercise-Induced Muscle Damage. *Int J Sports Med*,  
44 602 37(12), 937-943. doi: 10.1055/s-0042-111438
- 45 603 Vincent, H. K., & Vincent, K. R. (1997). The effect of training status on the serum creatine  
46 604 kinase response, soreness and muscle function following resistance exercise.  
47 605 *International Journal of Sports Medicine*, 18(6), 431-437.
- 48 606 Walshe, I., Robson-Ansley, P., Gibson, A. S. C., Lawrence, C., Thompson, K. G., & Ansley,  
49 607 L. (2010). The reliability of the IL-6, sIL-6R and sgp130 response to a preloaded time  
50 608 trial. *European Journal of Applied Physiology*, 110(3), 619-625. doi: 10.1007/s00421-  
51 609 010-1548-x

## Reproducibility of measurement techniques for blood biomarker determination

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2  
3 610 Young, W. B., Hepner, J., & Robbins, D. W. (2012). Movement demands in Australian rules  
4 611 football as indicators of muscle damage. *J Strength Cond Res*, 26(2), 492-496. doi:  
5 612 10.1519/JSC.0b013e318225a1c4  
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**Figure caption**

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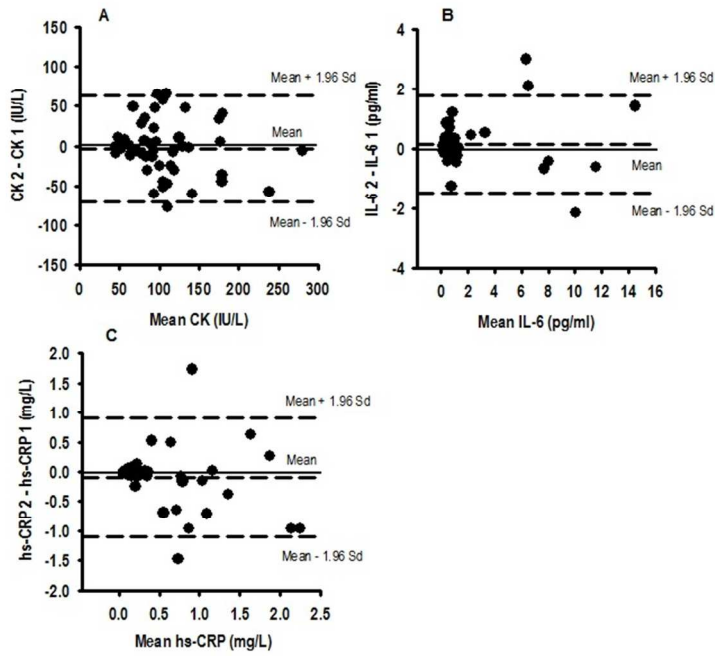
13 620 Figure 1. Bland-Altman plots showing individual differences between the absolute values  
14 621 plotted against their individual means (n = 50) for **A**) CK = creatine kinase, **B**) IL-6 =  
15 622 interleukin-6 and **C**) hs-CRP = high sensitivity C-reactive protein. Horizontal dashed lines =  
16 623 95% limits of agreement; solid line = zero reference line;  $S_d$  = within-subject standard  
17 624 deviation  
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Table 1. Circulating blood biomarker values obtained during trial 1 and trial 2 (n = 50)

Measure	Trial 1			Trial 2			Trial 1 – Trial 2 differences			
	Mean	SD	Range	Mean	SD	Range	Mean diff	95% CI	S <sub>d</sub>	p value
CK (IU/L)	109.8	40.4	42.6 – 283.0	108.7	42.7	41.3 – 210.0	-1.0	-7.9, 10.0	31.6	0.82
IL-6 (pg/ml)	3.96	6.60	0.02 – 33.02	4.18	7.25	0.10 – 31.80	0.21	-0.31, 0.74	1.24	0.41
hs-CRP (mg/L)	0.63	0.70	0.04 – 2.73	0.53	0.58	0.02 – 2.00	-0.10	-0.26, 0.07	0.51	0.24

Any discrepancies between the mean diff and means is due to rounding error; SD = between-subject standard deviation; mean diff = mean difference, 95% CI = lower and upper bounds of the 95% confidence interval for the mean difference, S<sub>d</sub> = within-subject standard deviation CK = creatine kinase; IL-6 = interleukin 6, hs-CRP = high sensitivity C reactive protein

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Table 2. Reproducibility statistics for the circulating blood biomarkers (n = 50)

<b>Measure</b>	<b>CV (%)</b>	<b>ICC</b>	<b>95% LoA</b>
CK (IU/L)	20.0	0.90	-69.7, 63.5
IL-6 (pg/ml)	15.3	0.98	-1.48, 1.80
hs-CRP (mg/L)	44.2	0.70	-1.10, 0.91

See footnotes of Table 1 for an explanation of the abbreviations; CV = coefficient of variation for repeated measures; ICC = intraclass correlation coefficient (two-way random model for a single rater); 95% LoA = 95% limits of agreement.

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