

Yasar, Zerbu, Elliott, Bradley T., Kyriakidou, Yvoni, Nwokoma, Chiazor T., Postlethwaite, Ruth D., Gaffney, Christopher J., Dewhurst, Susan and Hayes, Lawrence (2021) Sprint interval training (SIT) reduces serum epidermal growth factor (EGF), but not other inflammatory cytokines in trained older men. *European Journal of Applied Physiology* .

Downloaded from: <http://insight.cumbria.ac.uk/id/eprint/6006/>

Usage of any items from the University of Cumbria's institutional repository 'Insight' must conform to the following fair usage guidelines.

Any item and its associated metadata held in the University of Cumbria's institutional repository Insight (unless stated otherwise on the metadata record) may be copied, displayed or performed, and stored in line with the JISC fair dealing guidelines (available [here](#)) for educational and not-for-profit activities

provided that

- the authors, title and full bibliographic details of the item are cited clearly when any part of the work is referred to verbally or in the written form
 - a hyperlink/URL to the original Insight record of that item is included in any citations of the work
- the content is not changed in any way
- all files required for usage of the item are kept together with the main item file.

You may not

- sell any part of an item
- refer to any part of an item without citation
- amend any item or contextualise it in a way that will impugn the creator's reputation
- remove or alter the copyright statement on an item.

The full policy can be found [here](#).

Alternatively contact the University of Cumbria Repository Editor by emailing insight@cumbria.ac.uk.

1 **Sprint interval training (SIT) reduces serum epidermal growth factor (EGF), but not other inflammatory**
2 **cytokines in trained older men**

3

4 Zerbu Yasar¹, Bradley T Elliott^{2,*}, Yvoni Kyriakidou², Chiazor T Nwokoma², Ruth D Postlethwaite^{1,3},
5 Christopher J Gaffney⁴, Susan Dewhurst⁵, and Lawrence D Hayes^{1,6}

6

7 *¹Active Ageing Research Group, Institute of Health, University of Cumbria, Lancaster, UK; ²Translational*
8 *Physiology Research Group, School of Life Sciences, University of Westminster, London, UK; ³Faculty of*
9 *Health and Life Sciences, Coventry University, Coventry, UK; ⁴Lancaster Medical School, Faculty of Health*
10 *and Medicine, Lancaster University, Lancaster, UK; ⁵Department of Rehabilitation and Sport Sciences,*
11 *Bournemouth University, Bournemouth, UK; ⁶School of Health and Life Sciences, University of the West of*
12 *Scotland, Glasgow, UK*

13

14 ORCID IDs: ZY0000-0001-8838-7286; BTE: 0000-0003-4295-3785; YK: 0000-0002-8883-2228; CTN: 0000-
15 0002-2931-5992; RDP: 0000-0003-3888-9338; CJG: 0000-0001-7990-2792; SD: 0000-0003-2747-9122; LDH:
16 0000-0002-6654-0072

17

18 * Corresponding author

19 B.T. Elliott

20 Translational Physiology Research Group,

21 School of Life Sciences, College of Liberal Arts & Sciences,

22 University of Westminster,

23 115 New Cavendish St,

24 London W1W 6UW

25 b.elliott@westminster.ac.uk

26

27

28

29

30

This article is formatted in British English

31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Acknowledgments

ZY received a PhD scholarship from the University of Cumbria. The cytokine arrays used within this work were funded by the University of Cumbria and the University of Westminster.

Abbreviations

ANOVA: analysis of variance
BLa: blood lactate
BMI: body mass index
EGF: epidermal growth factor
HIIT: high-intensity interval training
IFN γ : interferon gamma
IL: interleukin
MCP-1: monocyte chemoattractant protein-1
mRNA: messenger ribonucleic acid
N $_2$: nitrogen
O $_2$: oxygen
PPO: peak power output
RER: respiratory exchange ratio
RPE: rating of perceived exertion
SD: standard deviation
SIT: sprint interval training
TNF α : tumour necrosis factor alpha
VEGF: vascular endothelial growth factor
VO $_2$: oxygen uptake
VO $_{2peak}$: peak oxygen uptake

61 ABSTRACT

62 **Purpose:** The present study aimed to investigate the effect of age on circulating pro- and anti-inflammatory
63 cytokines and growth factors. A secondary aim was to investigate whether a novel sprint interval training (SIT)
64 intervention (3 x 20 s ‘all out’ static sprints, twice a week for 8 weeks) would affect inflammatory markers in
65 older men.

66 **Methods:** Nine older men (68 [1] years) and eleven younger men (28 [2] years) comprised the younger group.
67 Aerobic fitness and inflammatory markers were taken at baseline for both groups and following the SIT
68 intervention for the older group.

69 **Results:** Interleukin (IL)-8, vascular endothelial growth factor (VEGF), and monocyte chemoattractant protein-
70 1 (MCP-1) were unchanged for the older and younger groups at baseline (IL-8, $p = 0.819$; MCP-1, $p = 0.248$;
71 VEGF, $p = 0.264$). Epidermal growth factor (EGF) was greater in the older group compared to the younger
72 group at baseline (142 [20] pg.mL^{-1} and 60 [12] pg.mL^{-1} respectively, $p = 0.001$, Cohen's $d = 1.64$). Following
73 SIT, older men decreased EGF to 100 (12) pg.mL^{-1} which was similar to that of young men who did not
74 undergo training ($p = 0.113$, Cohen's $d = 1.07$).

75 **Conclusion:** Older aerobically trained men have greater serum EGF than younger aerobically trained men. A
76 novel SIT intervention in older men can shift circulating EGF towards trained younger concentrations. As lower
77 EGF has previously been associated with longevity in *C. elegans*, the manipulative effect of SIT on EGF in
78 healthy ageing in the human may be of further interest.

79

80

81 KEYWORDS

82 Ageing · Cytokines · Exercise · Growth factors · HIIT · Inflammation

83

84

85

86

87

88

89

90

91 INTRODUCTION

92 Human ageing involves a loss of function of multiple physiological systems, including the cardiovascular
93 system, respiratory system, musculoskeletal system, and immuno-senescence (Rebello-Marques et al. 2018).
94 Circulating cytokine dysregulation is well recognised as a consequence of biological ageing (Alvarez-Rodriguez
95 et al., 2012). The 'inflamm-ageing' hypothesis suggests that chronic ageing is associated with increased reactive
96 oxygen species and increased basal pro-inflammatory state (Franceschi et al. 2007). Indeed, tumour necrosis
97 factor alpha (TNF α) is greater in 80-year-olds relative to younger individuals and greater again in centenarians.
98 Similarly, interleukin (IL)-6 is elevated with increasing age (Bruunsgaard et al. 1999; Baylis et al. 2013;
99 Kanikowska et al. 2014) while intracellular pro-inflammatory cytokines (including interferon gamma [IFN γ]
100 and TNF α) are seen to be elevated in T cells of older vs young participants (Zanni et al. 2003).

101

102 The deleterious effects of ageing on immune function are linked to dysregulation of cytokines which are
103 responsible for the promotion of the pro-ageing senescence-associated secretory phenotype (Coppé et al. 2010).
104 It has been reported the senescence-associated secretory phenotype is promoted by excess body fat associated
105 with increased pro-inflammatory adipokines and cytokines, such as IL-6 and IL-8, alongside cytokines such as
106 monocyte chemoattractant protein-1 (MCP-1), IFN γ , and TNF α (Christiansen et al. 2005; Monzillo et al. 2012;
107 Sharabiani et al. 2011; Vieira et al. 2009). This is further compounded by decreased anti-inflammatory myokine
108 expression, which disrupts inflammatory balance, facilitating pathological developments including insulin
109 resistance, cardiovascular disease, sarcopenia, chronic kidney disease, neurodegenerative disease, and increased
110 inflamm-ageing of all organs (Muller et al. 2019). Moreover, growth factors, such as vascular endothelial
111 growth factor (VEGF) and epidermal growth factor (EGF), when overexpressed, facilitate increased
112 autoimmune diseases activity and tumorigenesis (Dasthangirisaheb et al. 2013; Kasza 2013). Concerning EGF
113 specifically, Meybosch et al. (2019) noted significant inverse correlations between EGF (normalised for body
114 surface area) and age, and EGF and body height. There was a notable and dramatic decrease in EGF post-
115 puberty, causing authors to emphasise the importance of EGF in maturation and growth during the early years of
116 life. What is unknown however, is the influence of physical fitness, physical activity levels, and exercise
117 training on EGF.

118

119 Interestingly, whilst the ageing process is omnipresent in humans, physical activity can meaningfully attenuate
120 the development of senescence-associated secretory phenotype (Garatachea et al. 2015). Masters athletes

121 possess superior muscle and cardiovascular function relative to untrained age-matched individuals, but still
122 show decreases in physiological function with increased age, suggesting lifelong exercise can delay, but not
123 prevent, ageing related changes to physiological systems, including inflammatory cytokine concentrations
124 (Campbell et al. 2019; Duggal et al. 2018; Elliott et al. 2017; Ganse et al. 2018; Pollock et al. 2015).

125

126 Formalised physical activity, such as aerobic training and resistance training, have been widely researched for
127 health promoting benefits in older populations (Chodzko-Zajko et al. 2009; Hayes et al. 2015; Hayes and Elliott
128 2019; Sellami et al. 2019; 2020). Previous reviews have found both aerobic and resistance training to be
129 effective in attenuating senescence-associated secretory phenotype development (Muller et al. 2019; Sellami et
130 al. 2018). Further, a review by Muller and colleagues (2019) suggests high intensity interval training (HIIT) also
131 attenuates the senescence-associated secretory phenotype. Previously described by MacInnis and Gibala (2016),
132 HIIT utilises periods of high intensity exercise interspersed by lower intensity phases of recovery. Generally,
133 even with lower training volumes, HIIT produces similar health benefits when compared to classical forms of
134 aerobic training, and has been deemed time-efficient and enjoyable in various populations (Gibala et al. 2012;
135 Gillen and Gibala 2014; Hayes et al., 2020; Herbert et al. 2017; Hurst et al., 2018; Ramos et al. 2015; Weston et
136 al. 2014). Although HIIT is effective in improving physiological function, it has been suggested the perceived
137 difficulty of performing HIIT coupled with complex prescription may dissuade individuals from adopting HIIT
138 (Biddle and Batterham 2015; Buchheit and Laursen 2013). Yet, a distinct derivative of HIIT, sprint interval
139 training (SIT) offers an easier to prescribe exercise format (i.e. 'all-out'). SIT has been described as enjoyable,
140 tolerable, and easier to prescribe than HIIT, whilst still promoting positive physiological adaptations (MacInnis
141 and Gibala 2016; Olney et al. 2018; Stork et al. 2018; Thum et al. 2017; Vollard et al. 2017; Vollard and
142 Metcalfe 2017). Therefore, it is of interest to the field of exercise science and gerontology to investigate the
143 effects of SIT on immune-modulating cytokines and growth factors (Hwang et al. 2020).

144

145 To separate the effect of ageing from any effect of lifelong inactivity on circulating pro-inflammatory cytokines,
146 anti-inflammatory cytokines, and growth factors, we aimed to first establish the effect of age on circulating
147 inflammatory markers and growth factors in well trained young and older men, by comparing these biomarkers
148 in a cohort of young men, and a cohort of older men who were all aerobically trained. A secondary aim was to
149 examine the effect of a novel SIT stimuli on older aerobically trained men. It was hypothesised that older men

150 would show elevated pro-inflammatory cytokines relative to a young cohort, and SIT would reduce pro-
151 inflammatory cytokine concentrations.

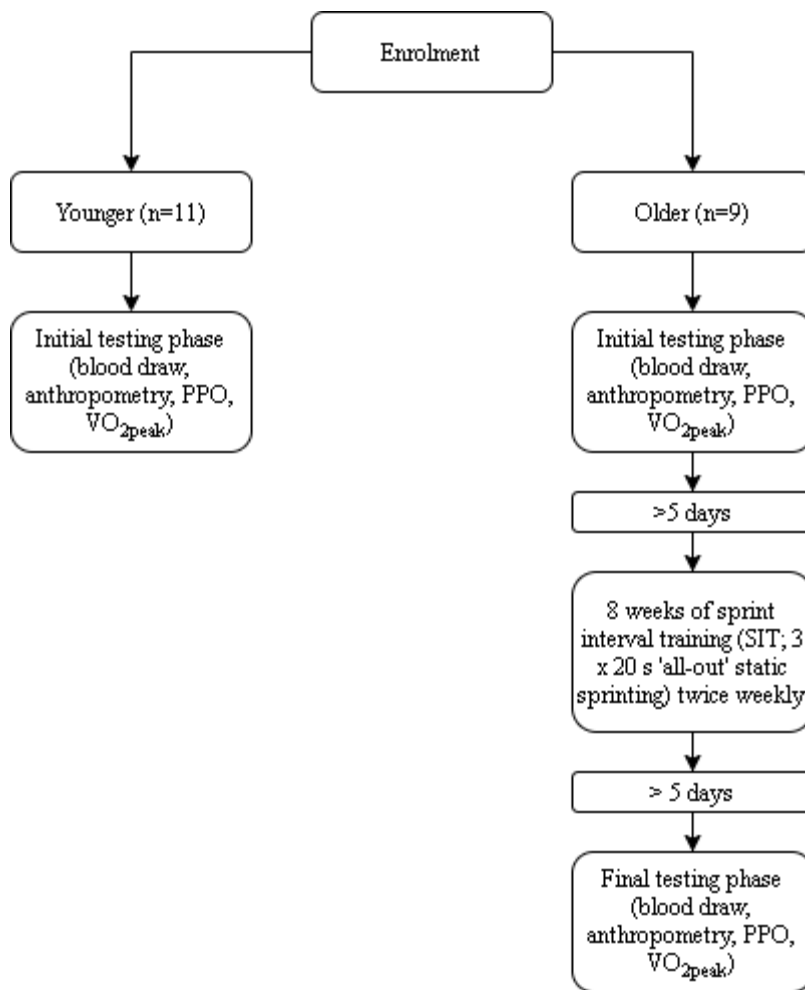
152

153 METHODS

154 *Participants*

155 Two cohorts were recruited for this study, younger (n = 11; 21-34 years of age) and older (n = 9; 63-73 years of
156 age) men, who regularly participated in a weekly minimum of 150 min.wk⁻¹ of moderate or high intensity
157 exercise for at least 6 months prior to participating in the study and continued habitual physical activity for the
158 duration of the study. Participants were free of exercise contraindicating disease or injury as determined by a
159 Physical Activity Readiness Questionnaire and American College of Sports Medicine pre-exercise participation
160 screening (Riebe et al. 2015). This study was carried out in accordance with the Declaration of Helsinki and
161 approved by the University of Cumbria Research Ethics Committee. Written informed consent was obtained
162 from all participants prior to study commencement and subjects were excluded if they presented with atrial
163 fibrillation. Descriptive statistics for participants are shown in Table 1: Participant anthropometric and
164 performance parameters at baseline (young and older pre-training) and following sprint interval training (SIT;
165 older post-training). Values given as mean (SD), and further described in the results section. Participants
166 attended all sessions with exercise suitable clothing and footwear. The younger cohort attended a single test
167 session whilst the older cohort attended two separate testing sessions five days prior to, and five days after, the
168 final SIT session of the intervention, which was 8 weeks in duration (Fig 1).

169



170

171 **Fig 1** Schematic representation of the methodological flow. PPO = peak power output. VO_{2peak} = peak oxygen
 172 uptake

173

174 *Blood draws and analysis*

175 Participants arrived at the exercise physiology laboratory between 08.00–11.00 h, following an overnight fast
 176 and having abstained from strenuous physical activity for a minimum of 48 h. Participants were reminded to
 177 maintain standardised conditions prior to each assessment point which included arriving in a hydrated state
 178 having abstained from caffeine and alcohol consumption for 24 h. Following 20 min supine rest, blood was
 179 sampled from the antecubital vein using standard venepuncture method into sterile serum separator vacutainer
 180 tubes (Becton Dickinson, Rutherford, NJ) that were kept at room temperature in the dark, for 30 min, to allow
 181 for clotting, after which samples were centrifuged at 1100 g for 15 min. Serum was then extracted, aliquoted,
 182 and stored at –80°C until subsequent analysis. Blood samples were collected at the same time of day for each

183 participant to control for biological variation and minimise inter-participant variation. Blood draws were
184 completed prior to any exercise testing.

185

186 *Anthropometry*

187 Height was measured to the nearest 0.1 cm, and mass to the nearest 0.01 kg using a Seca 286 measuring station
188 (Birmingham, UK), from which body mass index (BMI) was derived by dividing mass by the square of height
189 (kg/m^2).

190

191 *Peak power output (PPO)*

192 PPO was established using the 6 s Herbert test (Herbert et al. 2015b) on an air-braked cycle ergometer
193 (Wattbike Ltd., Nottingham, UK), which consisted of a maximal 6 s sprint from a standing start.

194

195 *Peak oxygen uptake ($\text{VO}_{2\text{peak}}$)*

196 At least five min after PPO determination, $\text{VO}_{2\text{peak}}$ was determined using a Cortex II Metalyser 3B-R2 (Cortex,
197 Biophysik, Leipzig, Germany). Expiratory airflow was achieved using a volume transducer (Triple V® turbine,
198 digital) connected to an oxygen (O_2) analyser. Expired gases were analysed for O_2 with electrochemical cells
199 and for carbon dioxide CO_2 output with an infrared analyser. The Metalyser was calibrated according to
200 manufacturer's guidelines prior to each test. After a 60 min warm-up period, the O_2 and CO_2 sensors were
201 calibrated against environmental air in addition to reference gas of known composition (5% CO_2 , 15% O_2 , and
202 80% N_2) with volume calibrated by five inspiratory and expiratory strokes using a 3 L pump. Prior to
203 determination of $\text{VO}_{2\text{peak}}$, a chest strap heart rate monitor was attached to participants' chests, with heart rate
204 measured continuously throughout the test (Polar F1, Polar, Finland). The cycle ergometer (Wattbike Pro,
205 Wattbike, UK) was adjusted to manufacturer's guidance. Saddle height was adjusted relative to the crank
206 position and the foot was secured to a pedal with straps with participants' knee at almost full extension ($\sim 170^\circ$).
207 Participants mounted the cycle ergometer, and a rubber face mask was fitted (Hans Rudolph Inc, USA), which
208 was attached to the Cortex II Metalyser 3B-R2. VO_2 and VCO_2 were recorded continuously throughout the test.
209 Participants completed a 3 min warm-up at an intensity equivalent to $\sim 10\%$ of PPO. Subsequently, participants
210 cycled at increasing intensity with 25 W increments each min until they reached volitional exhaustion, with
211 rating of perceived exertion (RPE; 0-10 scale; Borg [1998]) recorded in the last 10 s of each stage. Immediately
212 following volitional exhaustion, participants had their index finger cleaned using a disinfectant wipe, and then a

213 lancet was used to lacerate the fingertip to obtain a blood sample for to measure blood lactate (Lactate Pro 2,
214 Arkray, Japan). $\text{VO}_{2\text{peak}}$ was confirmed when participants achieved a minimum of any four of the following
215 criteria; VO_2 plateau, $\text{RER} \geq 1.10$, peak heart rate within 10 beats of age predicted maximum and $[\text{BLa}] \geq 8$
216 $\text{mmol}\cdot\text{L}^{-1}$, final RPE of ≥ 9 .

217

218 *Cytokine array*

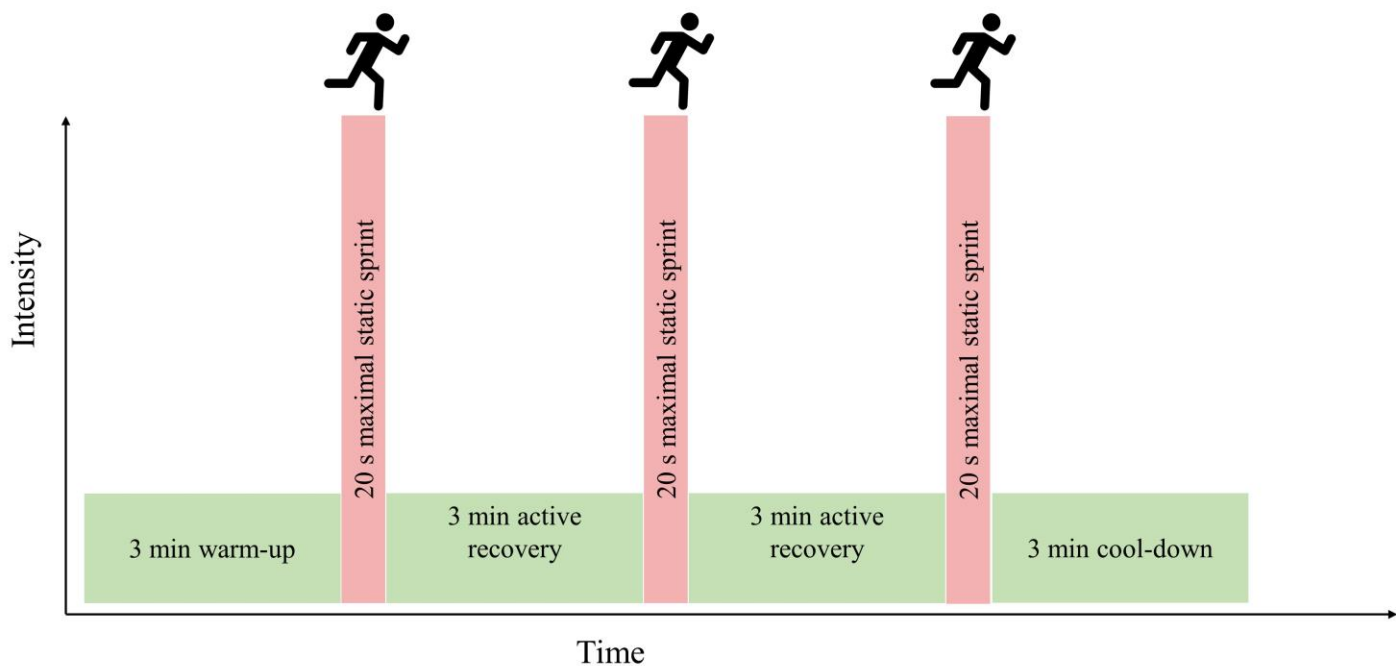
219 Cytokine concentrations were quantified in an aliquot of serum utilizing a chip array system (Cytokine array I,
220 Evidence Investigator, Affinity Biolabs, UK) with a sandwich chemiluminescent immunoassay technique for
221 epidermal growth factor (EGF), interleukins (IL-1a, -1b, -2, -4, -6, -8, -10), IFN- γ , MCP-1, TNF α , and VEGF.
222 Method precision and lower/upper limits of sensitivity have been previously reported (Karuppasamy et al.
223 2011), and quality controls were performed by the manufacturer using three known concentrations for each
224 cytokine.

225

226 *Exercise training*

227 Older participants attended two SIT sessions per week, 72 h apart, as our pilot work suggested older adults
228 would be suitably recovered from SIT in this timeframe (Yasar et al. 2019). Participants avoided strenuous
229 physical activity 24 h prior to SIT sessions whilst maintaining habitual physical activity according to self-
230 reporting. Participants warmed up for a period of 3 min at a self-paced intensity by performing static running.
231 Participants then performed three 20 s static sprints at an ‘all-out’ intensity, interspersed by 3 min self-paced
232 recovery phases. Following the final sprint, a 3 min self-paced cool down was performed (Fig 2). During all
233 sprints, participants were instructed to raise their feet to approximately knee height, with loud verbal
234 encouragement throughout each sprint.

235



236
 237 **Fig 2** Schematic representation of the sprint interval session. Participants performed this session twice weekly
 238 for eight weeks.

239

240 *Statistical Analysis*

241 Following confirmation of normality by a D'Agostino & Pearson normality test, cytokine data were examined
 242 by one-way analysis of variance (ANOVA) or Kruskal-Wallis test as appropriate, with post hoc interrogation by
 243 Dunnett's multiple comparison test (younger as comparison group). Descriptive statistics (younger vs older pre-
 244 training) and training effects (older group only) were examined by unpaired t-test or Mann Whitney test as
 245 appropriate. Fisher's exact test tested for dichotomous differences in whether a cytokine was above or below the
 246 minimum level of detection in the older and younger group. Relationships between variables were determined
 247 using Pearson's product-moment correlation coefficient. Effect size for paired comparisons is reported as
 248 Cohen's *d*, interpreted as trivial (<0.20), small ($\geq 0.20-0.49$), moderate ($\geq 0.50-0.79$), and large (≥ 0.80).
 249 Parametric data sets are summarised in text as mean and standard deviation (SD) whilst non-parametric are
 250 given as median (upper - lower quartile). Figures are presented as grouped dot plots, as recommended by
 251 Drummond and Vowler (2011). Alpha level was not set dichotomously as significant or non-significant as
 252 recommended by Hurlbert and colleagues (2019). All figures were generated in GraphPad (5.02, GraphPad

253 Software, USA) or R (version 3.6.1, [R Core Team (2019)]) utilizing the *Hmisc* [Harrell et al. 2020] and the
 254 *corrplot* [Wei et al. 2017] packages.

255

256 RESULTS

257 *Anthropometric and performance measures*

258 At baseline, older men did not differ from younger men in terms of body mass ($p = 0.635$, Cohen's $d = 0.13$),
 259 BMI ($p = 0.070$, Cohen's $d = 0.04$) resting heart rate BMI ($p = 0.517$, Cohen's $d = 0.30$), systolic blood pressure
 260 BMI ($p = 0.803$, Cohen's $d = 0.11$), diastolic blood pressure BMI ($p = 0.896$, Cohen's $d = 0.06$), or BMI ($p =$
 261 0.070 , Cohen's $d = 0.04$). However, older men did exhibit a lower VO_{2peak} ($p = 0.004$, Cohen's $d = 1.48$) and
 262 PPO ($p < 0.001$ Cohen's $d = 4.05$; Table 1). The SIT intervention produced a trivial increase in older
 263 participants' BMI ($p = 0.039$, Cohen's $d = 0.12$), a small increase in VO_{2peak} ($p = 0.268$, Cohen's $d = 0.23$), a
 264 small increase in PPO ($p = 0.072$, Cohen's $d = 0.35$), a small decrease in resting heart rate ($p = 0.263$, Cohen's d
 265 $= 0.40$) a trivial reduction in systolic blood pressure ($p = 0.701$, Cohen's $d = 0.13$), and a small decrease in
 266 diastolic blood pressure ($p = 0.347$, Cohen's $d = 0.33$).

267

268 **Table 1:** Participant anthropometric and performance parameters at baseline (young and older pre-training) and
 269 following sprint interval training (SIT; older post-training). Values given as mean (SD).

	Young (n = 11)	Older	
		Pre-SIT (n = 9)	Post-SIT (n = 9)
Age (years)	28 (5)	68 (3)*	-----
BMI (kg.m⁻²)	23 (2)	23 (3)	24 (3) †
VO_{2peak} (mL.kg.min⁻¹)	55 (11)	39 (6)*	41 (8)
PPO (W)	1149 (131)	696 (89)*	727 (76)
Resting heart rate (b·min⁻¹)	53 (10)	56 (7)	55 (7)
Systolic blood pressure (mmHg)	127 (10)	129 (16)	126 (14)
Diastolic blood pressure (mmHg)	77 (8)	77 (10)	77 (10)

270 SIT = sprint interval training, BMI = body mass index, VO_{2peak} = peak oxygen uptake, PPO = peak power
 271 output. * young different to older at the $p < 0.05$ level, †older pre-SIT different to older post-SIT at the $p < 0.05$
 272 level.

273

274 *Cytokines*

275 Of the 12 cytokines measured by chip array, IL-1a, IL-1b, IL-2, IL-4, IL-6, IL-10, IFN- γ and TNF α were
 276 frequently below the limit of detection of array methodology and thus concentrations are not further reported.
 277 For clarity, we report on cytokines whereby $> 75\%$ of samples returned with values above the lower limit of
 278 detection. Ordinal analysis of the data suggests that pro-inflammatory cytokines IL-1a, IL-1b, IL-6 were more
 279 frequently observed in the older cohort, whilst classically anti-inflammatory cytokines IL-2 and IL-10 were
 280 more often observed quantifiable in the younger cohort. However, Fisher's exact test revealed no differences
 281 between younger and older for the frequency of cytokines above or below the limit of detection (Table 2). Pro-
 282 inflammatory cytokines IL-8 and MCP-1, and growth factors VEGF and EGF were consistently detected and
 283 further described below.

284

285 **Table 2:** Cytokine marker state at baseline for young (n = 11) and older (n = 9). Markers were accepted if $>$
 286 75% of samples returned concentrations $>$ lower limit of detection. P values represent Fisher's exact test for
 287 whether the proportion of cytokine detected was different between the young and older group.

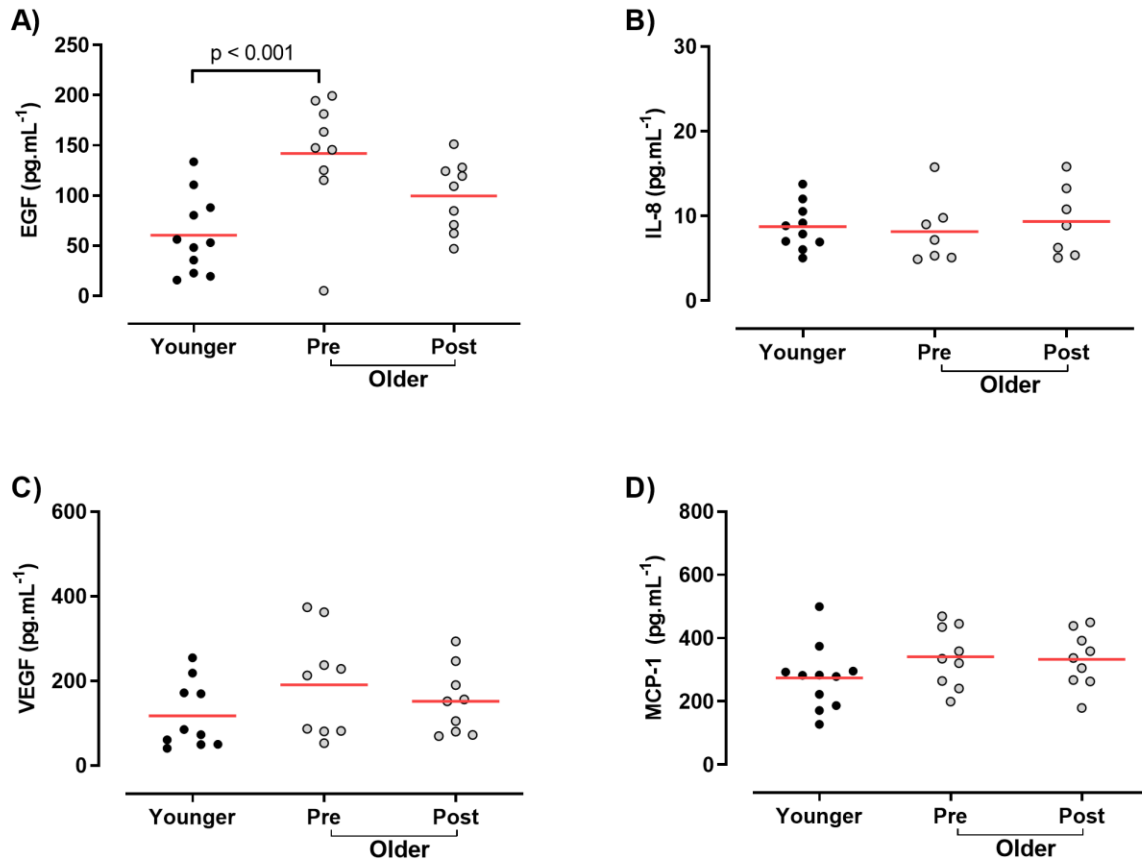
Cytokine	Young N = 11	Older N = 9	Lower limit of detection (pg.mL ⁻¹)	Accepted (y/n)	P value
EGF	11	9	2.9	Yes	1.000
IL-1a	4	5	0.8	No	0.653
IL-1b	3	4	1.6	No	0.642
IL-2	3	0	4.8	No	0.218
IL-4	0	0	6.6	No	1.000
IL-6	4	6	1.2	No	0.370
IL-8	10	7	4.9	Yes	0.569
IL-10	2	0	1.8	No	0.479
IFN- γ	0	0	3.5	No	1.000

MCP-1	11	9	13.2	Yes	1.000
TNFα	0	0	4.4	No	1.000
VEGF	10	9	14.6	Yes	1.000

288

289 The effect of age and SIT on EGF, IL-8, VEGF and MCP-1, was compared by one-way (condition [younger,
290 older pre-training, older post-training]) ANOVA. EGF showed an effect of condition ($p = 0.002$). The effect of
291 condition was examined post hoc by Dunnett's multiple comparison test, with the younger condition as the
292 comparison. Older pre-training EGF was higher compared to the younger group ($p = 0.001$, Cohen's $d = 1.64$;
293 Fig 3), whilst the older post-training values were the same as the younger group ($p = 0.113$, Cohen's $d = 1.07$;
294 younger 60 [12] pg.mL⁻¹, older pre-training 142 [20] pg.mL⁻¹, older post-training 100 [12] pg.mL⁻¹). There was
295 a large decrease in EGF in the older cohort as a result of SIT ($p = 0.101$, Cohen's $d = 0.87$). There was no effect
296 of group on remaining pro-inflammatory cytokines (IL-8, $p = 0.819$, Cohen's $d = 0.28$; younger 9 [3] pg.mL⁻¹,
297 older pre-training 8 [4] pg.mL⁻¹, older post-training 9 [4] pg.mL⁻¹; MCP-1, $p = 0.248$, Cohen's $d = 0.68$; younger
298 274 [102] pg.mL⁻¹, older pre-training 341 [95] pg.mL⁻¹, older post-training 333 [88] pg.mL⁻¹) or VEGF ($p =$
299 0.264 , Cohen's $d = 0.72$; younger 117 [79] pg.mL⁻¹, older pre-training 191 [123] pg.mL⁻¹, older post-training
300 152 [80] pg.mL⁻¹; Fig 3b-d). When examining the magnitude of effect of training in the older group, there was a
301 trivial effect of SIT on MCP-1 ($n = 9$; Cohen's $d = 0.09$), and a small increase in IL-8 ($n = 7$; Cohen's $d = 0.30$)
302 and a small decrease in VEGF ($n = 9$; Cohen's $d = 0.38$).

303

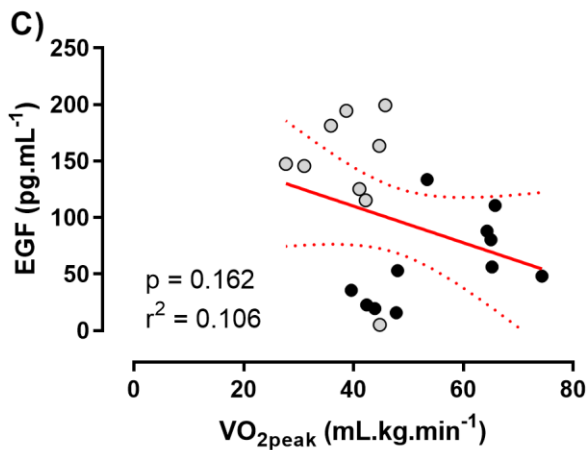
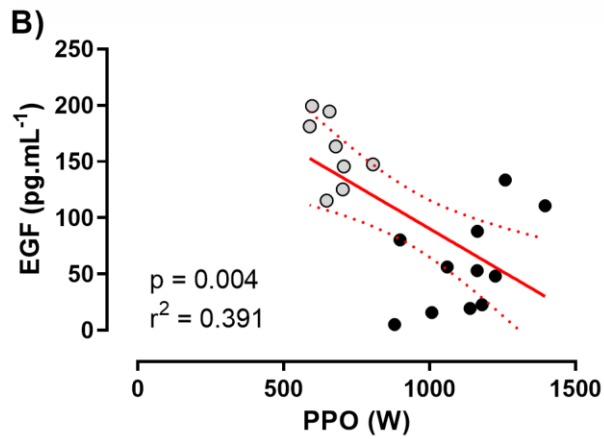
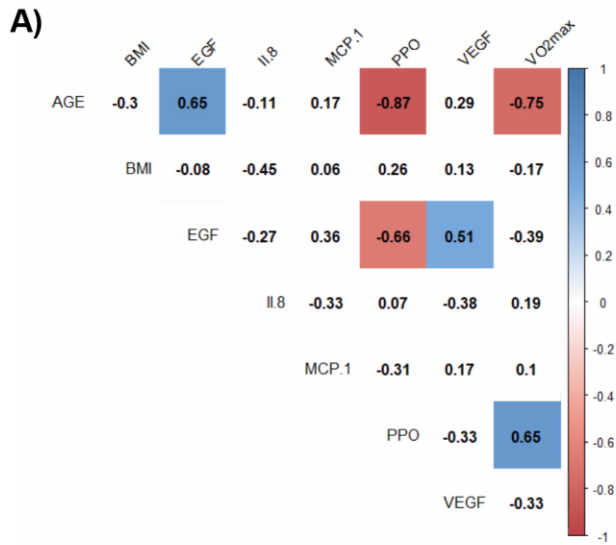


304

305 **Fig 3** Cytokine concentrations of young, older pre- and older post-sprint interval training. a) EGF, b) IL-8, c)
 306 VEGF and d) MCP-1. Young shown in black circles, older shown in grey. Red horizontal lines indicate group
 307 means

308

309 Relationships between baseline characteristics and circulating cytokines were examined by Pearson's correlation
 310 matrix (Fig 4a). Age was strongly and negatively correlated with PPO and VO_{2peak}, and moderately associated
 311 with EGF (Fig 4b). The EGF-PPO relationship was moderate (p = 0.004, r² = 0.391; Fig 3b), and the EGF-
 312 VO_{2peak} relationship was weak (p = 0.162, r² = 0.106; Fig 4c).



313

314 **Fig 4** Correlations between physiological and cytokine markers. a) Correlation matrix where values indicate r
 315 correlation coefficient and filled squares indicate where $p < 0.05$. Shading indicates strength of relationship

316 (blue = positive, red = negative correlation). b) EGF (pg.mL^{-1}) as a function of PPO (W), C) EGF (pg.mL^{-1}) as a

317 function of VO_{2peak} ($mL.kg.min^{-1}$). For both b) and c), linear correlation indicated by red line, 95% confidence
318 indicated by red dashed lines. Grey circles indicate older, black indicates younger

319

320 DISCUSSION

321 The primary findings from the present study were 1) baseline EGF was greater in trained older men compared to
322 younger participants, 2) there was no baseline differences in most (IL-1a, IL-1b, IL-2, IL-6, IL-8, IFN- γ , MCP-
323 1, and TNF α) pro-inflammatory cytokines between trained older men and trained younger men, and 3) we make
324 the novel observation that EGF was reduced to levels of younger men by a novel 8 week SIT intervention in
325 trained older men.

326

327 Of the cytokines measured in the present work, only EGF was different between younger and older at baseline.
328 EGF has a well understood action via the activation of the EGF receptor which is linked to inflammatory
329 responses in terms of wound healing in mouse model keratinocytes, cellular proliferation, chronic kidney
330 disease and tumorigenesis in humans, all of which are negative outcomes of ageing (Choi et al. 2018; Kasza
331 2013; Rayego-Mateos et al. 2018). However, data presented here should not be read as support of EGF as an
332 activity-independent marker of biological age, as the addition of a novel exercise stimulus reduced EGF
333 concentration in older participants. Indeed, it has been previously shown that overweight sedentary individuals
334 possess lower plasma EGF compared to normal weight controls (Accattato et al. 2017). What physiological
335 effect these alterations in EGF have on healthspan and lifespan can only be speculated at with the data presented
336 here, but it is interesting to observe that a gain-of-function mutation in the EGF receptor promotes longevity in
337 the model organism *C. elegans*, whilst loss-of-function mutations negatively affect longevity (Iwasa et al. 2010;
338 Rongo 2011; Siddiqui et al. 2012).

339

340 We demonstrated 8 weeks of SIT reduced EGF in SIT-naïve but aerobically trained older men. We are unaware
341 of other studies that investigate the effect of exercise training (i.e. >1 month) on EGF in older men. However,
342 Accattato et al. (2017) established a single bout of endurance exercise (20 min run at 70% VO_{2peak}) acutely
343 suppresses EGF in younger individuals, yet resistance training has been shown to acutely increase EGF in
344 healthy trained men (Diaz-Castro et al. 2020). Thus, it is clear the type of exercise (resistance vs endurance)
345 influences EGF response after a period of training as recent studies in C2C12 myotubes have shown that EGF

346 receptor inhibition promotes a slow twitch (oxidative) over a fast-twitch muscle phenotype (Ciano et al., 2019).
347 Thus, after resistance training, an increase in EGF would be associated with an increase in muscle protein
348 synthesis and hypertrophy whereas a decrease in EGF after endurance exercise is associated with oxidative
349 adaptation. The clinical significance of these changes in EGF following exercise training is unclear however.
350 Whilst greater EGF receptor prevalence is associated with multiple cancer types (Fisher et al., 2018; Gao et al.,
351 2016; Tokunaga et al., 1995), cardiovascular disease (Makki et al., 2013), and in vitro EGF has been shown to
352 influence cellular proliferation and differentiation rates (included in C2C12 myocytes [Ciano et al., 2019]), it is
353 difficult to speculate concerning the biological role that post-SIT EGF suppression exerts in older men here.

354

355 Ageing is associated with a fast-to-slow muscle fibre type shift (Brunner et al. 2007; Deschenes 2004), as is
356 chronic endurance training (Hawley et al. 2014), and this observation is maintained in lifelong endurance trained
357 older individuals (Dubé et al. 2016). In a cohort of both healthy controls and chronic obstructive pulmonary
358 disease patients, greater muscle EGF messenger ribonucleic acid (mRNA) expression was associated with fewer
359 slow twitch muscle fibres and lower VO_{2peak} (Ciano et al. 2019). Interestingly, our data suggest lifelong
360 endurance training into older age is associated with higher EGF expression than younger adults, yet a relatively
361 high VO_{2peak} . The reasonably expected large percentage of slow twitch fibre type expression in our trained older
362 participants may correlate with higher EGF expression, and the introduction of a 'fast twitch' promoting training
363 stimulus could thus be speculated to induce the witnessed depression in circulating EGF, yet muscle biopsies
364 would be required to confirm the fibre type shift.

365

366 Ageing is associated with an increased basal expression of circulating pro-inflammatory cytokines (Michaud et
367 al. 2013). A recent meta-analysis concluded that chronic (at least 4 weeks) aerobic exercise in middle aged and
368 older individuals decreased pro-inflammatory markers TNF α and IL-6 (Zheng et al. 2019). In addition, low
369 physical activity levels and high sitting time increase overall risk of death from inflammation-related chronic
370 disorders in people aged >60 years (Cabanas-Sanchez et al. 2018). In line with this, our results demonstrate that
371 aerobically trained older men possess low circulating concentrations of several pro-inflammatory cytokines. Our
372 data are thus in line with the hypothesis that basal inflammation seen in older individuals may be partly
373 inactivity-induced, and not a result of chronological ageing *per se*. This is supported by the fact that several of

374 the cytokines reported here were below assay limits of detection, our participants did not show the elevated
375 systemic inflammation typically seen in inactive older populations.

376

377 VEGF is a potent angiogenetic factor (Apte et al. 2019) and is essential for exercise-induced angiogenesis and
378 subsequent improvements in performance (Wagner et al. 2006). In younger adults, resting VEGF was not
379 changed following a HIIT intervention of 6 weeks (Żebrowska et al. 2019). VEGF positively associates with age
380 in adults (Ruggiero et al. 2011) and has previously been reported to be increased in sedentary older individuals
381 relative to lifelong exercisers, and further increased in sedentary individuals by 6 weeks of HIIT (Grace et al.
382 2015). We see no difference either in younger vs older trained individuals, or any pre-to-post training effect in
383 our older population. Thus, any effects of ageing on circulated VEGF may be negated by lifelong exercise
384 behaviour. In a similar manner MCP-1 positivity associates with age in mice and is elevated in older frail
385 individuals relative to non-frail age matched controls (Yousefzadeh et al. 2018). As MCP-1 was not elevated in
386 our cohort of trained older individuals relative to our younger population, this provides further support of the
387 use of MCP-1 and VEGF as a marker of biological age, however, the addition of an inactive ageing control
388 group to our model is needed to confirm this.

389

390 Some limitations to our study design should be acknowledged. We specifically sought to examine trained older
391 individuals, comparing them to trained younger adults to remove any effect of inactivity on ageing. However,
392 the addition of an inactive older group would have been a useful addition to confirm inactivity-associated ageing
393 changes in pro-inflammatory cytokines and growth factors that others have reported. Likewise, a young training
394 group would have provided insight as to whether they possess more plasticity with regards to serum cytokine
395 concentrations. Additionally, this study did not include women and therefore findings cannot be extrapolated to
396 women. Having multiple cytokine markers below useful limits of detection was a methodological weakness of
397 the approach that we have utilised here, and future studies will need to consider the use of high-sensitivity
398 biochip cytokine arrays, individual ELISA per marker, or the use of multiplex ELISA techniques, however,
399 these methodological approaches are associated with greater resource commitments. Additionally, the present
400 study did not verify objectively measured physical activity of participants during the study. Instead, the present
401 study relied on self-reporting, which is subject to self-reporting bias.

402

403 In conclusion, here we make novel observations on the state of circulating pro- and anti-inflammatory markers
404 in trained older individuals. EGF was greater in endurance trained older individuals compared to younger men,
405 however, the addition of a novel SIT intervention in older men can shift circulating EGF towards trained
406 younger concentrations. As EGF has previously been associated with longevity in *C. elegans*, the manipulative
407 effect of SIT on EGF in healthy ageing in the human may be of further interest.

408

409 **Declarations**

410 *Funding*

411 Funding was provided by institutions employing the authors.

412

413 *Conflict of interests*

414 We declare no conflict of interest or competing interests.

415

416 *Ethical approval*

417 Ethical approval was obtained for this study and all participants provided informed consent. All authors have
418 read the manuscript and consent for this work to be published. Data can be made available on request. Code
419 details are not applicable within this manuscript, but all software details are given.

420

421 **Authors' contributions are given according to the CRediT taxonomy:**

422 Conceptualization: Zerbu Yasar, Bradley T Elliott, Susan Dewhurst, Lawrence D Hayes; Methodology: Zerbu
423 Yasar, Bradley T Elliott, Susan Dewhurst, Lawrence D Hayes; Formal analysis and investigation: Zerbu Yasar,
424 Bradley T Elliott, Chiazor T Nwokoma, Lawrence D Hayes; Investigation: Zerbu Yasar, Bradley T Elliott, Ruth
425 D Postlethwaite, Christopher J Gaffney, Lawrence D Hayes; Resources: Zerbu Yasar, Bradley T Elliott, Ruth D
426 Postlethwaite, Christopher J Gaffney, Lawrence D Hayes, Affinity biomarker labs; Writing - original draft
427 preparation: Zerbu Yasar, Bradley T Elliott, Lawrence D Hayes; Writing - review and editing: Zerbu Yasar,
428 Bradley T Elliott, Yvoni Kyriakidou, Chiazor T Nwokoma, Ruth D Postlethwaite, Christopher J Gaffney, Susan
429 Dewhurst, and Lawrence D Hayes; Visualization: Bradley T Elliott; Supervision: Bradley T Elliott, Susan
430 Dewhurst, Lawrence D Hayes; Project administration: Zerbu Yasar, Bradley T Elliott, Lawrence D Hayes;
431 Funding acquisition: Bradley T Elliott, Susan Dewhurst, Lawrence D Hayes.

432

433

434

435 REFERENCES

- 436 Accattato F, Greco M, Pullano SA, Carè I, Fiorillo AS, Pujia A, Montalcini T, Foti DP, Brunetti A, Gulletta E
437 (2017) Effects of acute physical exercise on oxidative stress and inflammatory status in young, sedentary obese
438 subjects. PLoS One 12: e0178900. <https://doi.org/10.1371/journal.pone.0178900>
- 439 Álvarez-Rodríguez L, López-Hoyos M, Muñoz-Cacho P, Martínez-Taboada VM (2012) Aging is associated
440 with circulating cytokine dysregulation. Cell Immunol 273: 124-132.
441 <https://doi.org/10.1016/j.cellimm.2012.01.001>.
- 442 Apte RS, Chen DS, Ferrara N (2019) VEGF in signaling and disease: Beyond discovery and development. Cell
443 176:1248–1264. <https://doi.org/10.1016/j.cell.2019.01.021>
- 444 Drummond GB, Vowler SL (2011) Show the data, don't conceal them Br J Pharmacol 163:1392.
445 <https://doi.org/10.1111/j.1476-5381.2011.01251.x>
- 446 Baylis D, Bartlett DB, Patel HP, Roberts HC (2013) Understanding how we age: insights into inflammaging.
447 Longev Healthspan 2:8. <https://doi.org/10.1186/2046-2395-2-8>
- 448 Borg G (1998) Borg's perceived exertion and pain scales. Human Kinetics, Champaign, IL
- 449 Brunner F, Schmid A, Sheikhzadeh A, Nordin M, Yoon J, Frankel V (2007) Effects of aging on Type II muscle
450 fibers: A systematic review of the literature. J Aging Phys Act 15:336–348.
451 <https://doi.org/10.1123/japa.15.3.336>
- 452 Bruunsgaard H, Andersen-Ranberg K, Jeune B, Pedersen AN, Skinhøj P, Pedersen BK (1999) A high plasma
453 concentration of TNF-alpha is associated with dementia in centenarians. J Gerontol A Biol Sci Med Sci
454 54:M357-364. <https://doi.org/10.1093/gerona/54.7.M35>
- 455 Buchheit M, Laursen PB (2013) High-intensity interval training, solutions to the programming puzzle: Part I:
456 Cardiopulmonary emphasis. Sports Med 43:313–338. <https://doi.org/10.1007/s40279-013-0029-x>.
- 457 Cabanas-Sánchez V, Guallar-Castillón P, Higuera-Fresnillo S, García-Esquinas E, Rodríguez-Artalejo F,
458 Martínez-Gomez D (2018) Physical activity, sitting time, and mortality from inflammatory diseases in older
459 adults. Front Physiol 9:898. <https://doi.org/10.3389/fphys.2018.00898>

460 Campbell A, Grace F, Ritchie L, Beaumont A, Sculthorpe N (2019) Long-term aerobic exercise improves
461 vascular function into old age: A systematic review, meta-analysis and meta regression of observational and
462 interventional studies. *Front Physiol* 10:31. <https://doi.org/10.3389/fphys.2019.00031>

463 Choi SY, Lee YJ, Kim JM, Kang HJ, Cho SH, Chang SE (2018) Epidermal growth factor relieves inflammatory
464 signals in staphylococcus aureus-treated human epidermal keratinocytes and atopic dermatitis-like skin lesions
465 in Nc/Nga mice. *Biomed Res Int* 2018. <https://doi.org/10.1155/2018/9439182>

466 Christiansen T, Richelsen B, Bruun JM (2005) Monocyte chemoattractant protein-1 is produced in isolated
467 adipocytes, associated with adiposity and reduced after weight loss in morbid obese subjects. *Int J Obes* 29:146–
468 150. <https://doi.org/10.1038/sj.ijo.0802839>

469 Ciano M, Mantellato G, Connolly M, Paul-Clark M, Willis-Owen S, Moffatt MF, Cookson WOCM, Mitchell
470 JA, Polkey MI, Hughes SM, Kemp PR, Natanek SA (2019) EGF receptor (EGFR) inhibition promotes a slow-
471 twitch oxidative, over a fast-twitch, muscle phenotype. *Sci Rep* 9:9218. [https://doi.org/10.1038/s41598-019-](https://doi.org/10.1038/s41598-019-45567-4)
472 [45567-4](https://doi.org/10.1038/s41598-019-45567-4)

473 Coppé J-P, Desprez P-Y, Krtolica A, Campisi J (2010) The senescence-associated secretory phenotype: The
474 dark side of tumor suppression. *Annu Rev Pathol* 5:99–118. [https://doi.org/10.1146/annurev-pathol-121808-](https://doi.org/10.1146/annurev-pathol-121808-102144)
475 [102144](https://doi.org/10.1146/annurev-pathol-121808-102144)

476 Deschenes MR (2004) Effects of aging on muscle fibre type and size. *Sports Med* 34:809–824.
477 <https://doi.org/10.2165/00007256-200434120-00002>

478 Diaz-Castro J, Moreno-Fernandez J, Chiroso I, Chiroso LJ, Guisado R, Ochoa JJ (2020) Beneficial effect of
479 ubiquinol on hematological and inflammatory signaling during exercise. *Nutrients* 12: 424.
480 <https://doi.org/10.3390/nu12020424>

481 Dubé JJ, Broskey NT, Despines AA, Stefanovic-Racic M, Toledo FGS, Goodpaster BH, Amati F (2016) Muscle
482 characteristics and substrate energetics in lifelong endurance athletes. *Med Sci Sports Exerc* 48:472–480.
483 <https://doi.org/10.1249/MSS.0000000000000789>

484 Duggal NA, Pollock RD, Lazarus NR, Harridge S, Lord JM (2018). Major features of immunosenescence,
485 including reduced thymic output, are ameliorated by high levels of physical activity in adulthood. *Aging Cell*
486 17: e12750. <https://doi.org/10.1111/ace1.12750>

487 Elliott BT, Herbert P, Sculthorpe N, Grace FM, Stratton D, Hayes LD (2018) Lifelong exercise, but not
488 short-term high-intensity interval training, increases GDF11, a marker of successful aging: a preliminary
489 investigation. *Physiol Rep* 5:e13343. <https://dx.doi.org/10.14814%2Fphy2.13343>

490 Fisher SA, Tam YT, Fokina A, Mahmoodi MM, Distefano MD, Schoichet MS (2018) Photo-immobilized EGF
491 chemical gradients differentially impact breast cancer cell invasion and drug response in defined 3D hydrogels.
492 *Biomaterials* 178:751-766. <https://doi.org/10.1016/j.biomaterials.2018.01.032>

493 Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panourgia MP, Invidia L, Celani L, Scurti M,
494 Cevenini E, Castellani GC, Salvioli S (2007) Inflammaging and anti-inflammaging: a systemic perspective on
495 aging and longevity emerged from studies in humans. *Mech Ageing Dev* 128:92–105.
496 <https://doi.org/10.1016/j.mad.2006.11.016>

497 Frank E Harrell Jr, with contributions from Charles Dupont and many others. (2020) Hmisc: Harrell
498 Miscellaneous. R package version 4.4-0. <https://CRAN.R-project.org/package=Hmisc>

499 Ganse B, Ganse U, Dahl J, Degens H (2018) Linear decrease in athletic performance during the human life
500 span. *Front Physiol* 9:1100. <https://doi.org/10.3389/fphys.2018.01100>

501 Gao L, Wang FQ, Li HM, Yang JG, Ren JG, He KF, Liu B, Zhang W, Zhao YF (2016) CCL2/EGF positive
502 feedback loop between cancer cells and macrophages promotes cell migration and invasion in head and neck
503 squamous cell carcinoma. *Oncotarget* 7:87037-87051. <https://doi.org/10.18632/oncotarget.13523>

504 Garatachea N, Pareja-Galeano H, Sanchis-Gomar F, Santos-Lozano A, Fiuza-Luces C, Morán, M, Emanuele E,
505 Joyner MJ, Lucia A (2015) Exercise attenuates the major hallmarks of aging. *Rejuvenation Res* 18:57–89.
506 <https://doi.org/10.1089/rej.2014.1623>

507 Gibala MJ, Little JP, Macdonald MJ, Hawley JA (2012) Physiological adaptations to low-volume, high-
508 intensity interval training in health and disease. *J Physiol* 590:1077–1084.
509 <https://doi.org/10.1113/jphysiol.2011.224725>

510 Gillen JB, Gibala MJ (2014) Is high-intensity interval training a time-efficient exercise strategy to improve
511 health and fitness? *Appl Physiol Nutr Metab* 39:409–412. <https://doi.org/10.1139/apnm-2013-0187>

512 Grace FM, Herbert P, Ratcliffe JW, New KJ, Baker JS, Sculthorpe NF (2015) Age related vascular endothelial
513 function following lifelong sedentariness: positive impact of cardiovascular conditioning without further
514 improvement following low frequency high intensity interval training. *Physiol Rep* 3:e12234.
515 <https://doi.org/10.14814/phy2.12234>

516 Hawley JA, Hargreaves M, Joyner MJ, Zierath JR (2014) Integrative biology of exercise. *Cell* 159:738–749.
517 <https://doi.org/10.1016/j.cell.2014.10.029>

518 Hayes LD, Herbert P, Sculthorpe N, Grace F (2020) High intensity interval training (HIIT) produces small
519 improvements in fasting glucose, insulin, and insulin resistance in sedentary older men but not masters athletes.
520 *Exp Gerontol.* 140:111074. <https://doi.org/10.1016/j.exger.2020.111074>

521 Hayes LD, Sculthorpe N, Herbert P, Baker JS, Spagna R, Grace FM (2015) Six weeks of conditioning exercise
522 increases total, but not free testosterone in lifelong sedentary aging men. *Aging Male* 18:195-200.
523 <https://doi.org/10.3109/13685538.2015.1046123>

524 Hayes LD, Elliott BT (2019) Short-term exercise training inconsistently influences basal testosterone in older
525 men: A systematic review and meta-analysis. *Front Physiol* 9:1878. <https://doi.org/10.3389/fphys.2018.01878>

526 Herbert P, Hayes LD, Sculthorpe NF, Grace FM (2017) HIIT produces increases in muscle power and free
527 testosterone in male masters athletes. *Endocr Conn* 6:430–436. <https://doi.org/10.1530/EC-17-0159>

528 Herbert P, Sculthorpe N, Baker JS, Grace FM (2015) Validation of a six second cycle test for the determination
529 of peak power output. *Res Sports Med* 23:115–125. <https://doi.org/10.1080/15438627.2015.1005294>

530 Hurlbert SH, Levine RA, Utts J (2019) Coup de grâce for a tough old bull: “statistically significant” expires. *Am*
531 *Stat* 73:352–357. <https://doi.org/10.1080/00031305.2018.1543616>

532 Hurst C, Weston KL, Weston M (2019) The effect of 12 weeks of combined upper- and lower-body high-
533 intensity interval training on muscular and cardiorespiratory fitness in older adults. *Aging Clin Exp Res* 31:
534 661–671. <https://doi.org/10.1007/s40520-018-1015-9>

535 Hwang JH, McGovern J, Minett GM, Della Gatta PA, Roberts L, Harris JM, Thompson EW, Parker TJ, Peake
536 JM, Neubauer O (2020) Mobilizing serum factors and immune cells through exercise to counteract age-related
537 changes in cancer risk. *Exerc Immunol Rev* 26:80-99.

538 Iwasa H, Yu S, Xue J, Driscoll M (2010) Novel EGF pathway regulators modulate *C. elegans* healthspan and
539 lifespan via EGF receptor, PLC-gamma, and IP3R activation. *Aging Cell* 9: 490–505.
540 <https://doi.org/10.1111/j.1474-9726.2010.00575.x>

541 Kanikowska D, Pyda M, Korybalska K, Grajek S, Lesiak M, Bręborowicz A, Witowski J (2014) Age-related
542 limitations of interleukin-6 in predicting early mortality in acute ST-elevation myocardial infarction. *Immun*
543 *Ageing* 11:23. <https://doi.org/10.1186/s12979-014-0023-7>

544 Karuppasamy P, Chaubey S, Dew T, Musto R, Sherwood R, Desai J, John L, Shah AM, Marber MS, Kunst G
545 (2011) Remote intermittent ischemia before coronary artery bypass graft surgery: a strategy to reduce injury and
546 inflammation? *Basic Res Cardiol* 106:511–519. <https://doi.org/10.1007/s00395-011-0185-9>

547 Kasza A (2013) IL-1 and EGF regulate expression of genes important in inflammation and cancer. *Cytokine*
548 62:22–33. [10.1016/j.cyto.2013.02.007](https://doi.org/10.1016/j.cyto.2013.02.007)

549 MacInnis MJ, Gibala MJ (2017) Physiological adaptations to interval training and the role of exercise intensity.
550 *J Physiol* 595:2915–2930. <https://doi.org/10.1113/JP273196>

551 Makki N, Thiel KW, Miller FJ (2013) The epidermal growth factor receptor and its ligands in cardiovascular
552 disease. *Int J Mol Sci* 14: 20597-20613. <https://doi.org/10.3390/ijms141020597>

553 Meybosch S, De Monie A, Anne C, Bruyndonckx L, Jurgens A, De Winter BY, Trouet D, Ledeganck KJ (2019)
554 Epidermal growth factor and its influencing variables in healthy children and adults. *PLoS One* 14: e0211212.
555 <https://doi.org/10.1371/journal.pone.0211212>

556 Michaud M, Balardy L, Moulis G, Gaudin C, Peyrot C, Vellas B, Cesari M, Nourhashemi F (2013)
557 Proinflammatory cytokines, aging, and age-related diseases. *J Am Med Dir Assoc* 14:877–882.
558 <https://doi.org/10.1016/j.jamda.2013.05.009>

559 Muller L, Di Benedetto S, Pawelec G (2019) The immune system and its dysregulation with aging. *Subcell*
560 *Biochem* 91:21-43. https://doi.org/10.1007/978-981-13-3681-2_2

561 Monzillo LU, Hamdy O, Horton ES, Ledbury S, Mullooly C, Jarema C, Porter S, Ovalle K, Moussa A,
562 Mantzoros CS (2003) Effect of lifestyle modification on adipokine levels in obese subjects with insulin
563 resistance. *Obes Res* 11:1048–1054. <https://doi.org/10.1038/oby.2003.144>

564 Pollock RD, Carter S, Velloso CP, Duggal NA, Lord JM, Lazarus NR, Harridge SDR (2015) An investigation
565 into the relationship between age and physiological function in highly active older adults. *J Physiol* 593:657–
566 680. <https://doi.org/10.1113/jphysiol.2014.282863>

567 R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical
568 Computing, Vienna, Austria. URL <https://www.R-project.org/>

569 Ramos JS, Dalleck LC, Tjonna AE, Beetham KS, Coombes JS (2015) The impact of high-intensity interval
570 training versus moderate-intensity continuous training on vascular function: A systematic review and meta-
571 analysis. *Sports Med* 45:679–692. <https://doi.org/10.1007/s40279-015-0321-z>

572 Rayego-Mateos S, Rodrigues-Diez R, Morgado-Pascual JL, Valentijn F, Valdivielso JM, Goldschmeding R,
573 Ruiz-Ortega M (2018) Role of epidermal growth factor receptor (EGFR) and its ligands in kidney inflammation
574 and damage. *Mediators Inflamm* 2018:8739473. <https://doi.org/10.1155/2018/8739473>

575 Rebelo-Marques A, De Sousa Lages A, Andrade R, Ribeiro CF, Mota-Pinto A, Carrilho, F, Espregueira-Mendes
576 J (2018) Aging hallmarks: The benefits of physical exercise. *Front Endocrinol* 9:258.
577 <https://doi.org/10.3389/fendo.2018.00258>

578 Riebe D, Franklin BA, Thompson PD, Garber CE, Whitfield GP, Magal M, Pescatello LS (2015) Updating
579 ACSM’s recommendations for exercise preparticipation health screening. *Med Sci Sports Exerc* 47:2473–2479.
580 <https://doi.org/10.1249/MSS.0000000000000664>

581 Rongo C (2011) Epidermal growth factor and aging: a signaling molecule reveals a new eye opening function.
582 *Aging* 3:896–905. <https://doi.org/10.18632/aging.100384>

583 Ruggiero D, Dalmaso C, Nutile T, Sorice R, Dionisi L, Aversano M, Bröet P, Leutenegger A-L, Bourgain C,
584 Ciullo M (2011) Genetics of VEGF serum variation in human isolated populations of cilento: Importance of
585 VEGF polymorphisms. *PLoS ONE* 6:e16982. <https://doi.org/10.1371/journal.pone.0016982>

586 Sellami M, Ben Abderrahmen A, Dhabi W, Hayes LD, Zouhal H (2020) Hemoglobin, hematocrit and plasma
587 volume variations following combined sprint and strength: Effect of advanced age. *Sci Sports Epub ahead of*
588 *print.* <https://doi.org/10.1016/j.scispo.2019.10.012>

589 Sellami M, Bragazzi NL, Slimani M Hayes LD, Jabbour G, De Giorgio A, Dugue B (2019) The effect of
590 exercise on glucoregulatory hormones: A countermeasure to human aging: Insights from a comprehensive
591 review of the literature. *Int J Environ Res Public Health* 16:1709. <https://dx.doi.org/10.3390%2Fijerph16101709>

592 Siddiqui S, Fang M, Ni B, Lu D, Martin B, Maudsley S (2012) Central role of the EGF receptor in
593 neurometabolic aging. *Int J Endocrinol* 2012:739428. <https://doi.org/10.1155/2012/739428>

594 Sellami M, Guasmi M, Denham J, Hayes LD, Stratton D, Padulo J, Bragazzi NL (2018) Effects of acute and
595 chronic exercise on immunological parameters in the elderly aged: Can physical activity counteract the effects
596 of aging? *Front Immunol* 9:2187. <https://doi.org/10.3389/fimmu.2018.02187>

597 Stork MJ, Gibala MJ, Martin Ginis KA (2018) Psychological and behavioral responses to interval and
598 continuous exercise. *Med Sci Sports Exerc* 50:2110–2121. <https://doi.org/10.1249/MSS.0000000000001671>

599 Taiyun Wei and Viliam Simko (2017). R package "corrplot": Visualization of a Correlation Matrix (Version
600 0.84). Available from <https://github.com/taiyun/corrplot>

601 Tokunaga A, Onda M, Okuda T, Teramoto T, Fujita I, Mizutani T, Kiyama T, Yoshiyuki T, Nishi K, Matsukura
602 N (1995) Clinical significance of epidermal growth factor (EGF), EGF receptor, and c-erbB-2 in human gastric
603 cancer. *Cancer* 75:1418-1425. [https://doi.org/10.1002/1097-0142\(19950315\)75:6+%3C1418::AID-](https://doi.org/10.1002/1097-0142(19950315)75:6+%3C1418::AID-)
604 [CNCR2820751505%3E3.0.CO;2-Y](https://doi.org/10.1002/1097-0142(19950315)75:6+%3C1418::AID-CNCR2820751505%3E3.0.CO;2-Y)

605 Thum JS, Parsons G, Whittle T, Astorino TA (2017) High-intensity interval training elicits higher enjoyment
606 than moderate intensity continuous exercise. *PLoS ONE* 12:e0166299.
607 <https://doi.org/10.1371/journal.pone.0166299>

608 Vollaard NBJ, Metcalfe RS (2017) Research into the health benefits of sprint interval training should focus on
609 protocols with fewer and shorter sprints. *Sports Med* 47:2443–2451. <https://doi.org/10.1007/s40279-017-0727-x>

610 Vollaard NBJ, Metcalfe RS, Williams S (2017) Effect of number of sprints in an SIT session on change in
611 VO_{2max} : A meta-analysis. *Med Sci Sports Exerc* 49:1147–1156.
612 <https://doi.org/10.1249/MSS.0000000000001204>

613 Wagner PD, Olfert IM, Tang K, Breen EC (2006) Muscle-targeted deletion of VEGF and exercise capacity in
614 mice. *Respir Physiol Neurobiol* 151:159–166. <https://doi.org/10.1016/j.resp.2005.09.007>

615 Weston KS, Wisløff U, Coombes JS (2014) High-intensity interval training in patients with lifestyle-induced
616 cardiometabolic disease: a systematic review and meta-analysis. *Br J Sports Med* 48:1227–1234.
617 <https://doi.org/10.1136/bjsports-2013-092576>

618 Yasar Z, Dewhurst S, Hayes LD (2019) Peak power output is similarly recovered after three- and five-days' rest
619 following sprint interval training in young and older adults. *Sports* 7:94. <https://doi.org/10.3390/sports7040094>

620 Yousefzadeh MJ, Schafer MJ, Noren Hooten N, Atkinson EJ, Evans MK, Baker DJ, Quarles EK, Robbins PD,
621 Ladiges WC, LeBrasseur NK, Niedernhofer LJ (2018) Circulating levels of monocyte chemoattractant protein-1
622 as a potential measure of biological age in mice and frailty in humans. *Aging Cell* 17.
623 <https://doi.org/10.1111/acer.12706>

624 Zanni F, Vescovini R, Biasini C, Fagnoni F, Zanlari L, Telera A, Di Pede P, Passeri G, Pedrazzoni M, Passeri
625 M, Francheschi C, Sansoni P (2003) Marked increase with age of type 1 cytokines within memory and
626 effector/cytotoxic CD8+ T cells in humans: a contribution to understand the relationship between inflammation
627 and immunosenescence. *Exp Gerontol* 38:981–987. [https://doi.org/10.1016/s0531-5565\(03\)00160-8](https://doi.org/10.1016/s0531-5565(03)00160-8)

628 Żebrowska A, Jastrzębski D, Sadowska-Krępa E, Sikora M, Di Giulio C (2019) Comparison of the effectiveness
629 of high-intensity interval training in hypoxia and normoxia in healthy male volunteers: A pilot study. *Biomed*
630 *Res Int* 2019. <https://doi.org/10.1155/2019/7315714>

631 Zheng G, Qiu P, Xia R, Lin H, Ye B, Tao J, Chen L (2019) Effect of aerobic exercise on inflammatory markers
632 in healthy middle-aged and older adults: A systematic review and meta-analysis of randomized controlled trials.
633 *Front Aging Neurosci* 11:98. <https://doi.org/10.3389/fnagi.2019.00098>

634

635