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Habitual physical activity is associated with the maintenance of neutrophil migratory dynamics in healthy older adults

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1. Introduction

Innate immunity is the first line of defence against invading microorganisms. With advancing age there is a chronic deterioration of immune function, termed immunosenescence, leading to increased susceptibility to infection (Gavazzi and Krause, 2002; Butcher et al., 2003). Neutrophils are the most abundant leukocyte in blood and the first immune cell to arrive at sites of bacterial...
infection, making them critical intermediaries in the effective resolution of infection. Neutrophil dysfunction is evident in healthy older adults and is characterised by reduced directional migration, phagocytosis, oxidative burst and neutrophil extracellular trap (NET) production (Butcher et al., 2001; Sapey et al., 2013; Hazeldine et al., 2014; Shaw et al., 2010). Reduced chemotaxis may be particularly detrimental as it will delay recruitment of neutrophils to sites of infection, but it may also contribute to heightened inflammation during infection and pro-inflammatory insults in older adults. This is because neutrophils release proteases such as neutrophil elastase to aid migration through tissue (Cepinskas et al., 1999), thus damaging healthy tissue and inducing inflammation (Sapey et al., 2013). Inefficient chemotaxis will increase this unintentional damage and inflammation. It remains unclear why neutrophil dysfunction occurs with ageing but it is likely that defects are intrinsic to the cellular maturation process as neutrophils exit the bone marrow as mature non-proliferating cells (Shaw et al., 2010; Ward et al., 2011; Fortin et al., 2008). Our own recent work has shown that signalling pathways central to chemotaxis, namely phosphoinositide 3 kinase (PI3K), is dysregulated in neutrophils from older adults and reduced chemotaxis can be corrected by selective inhibition of this pathway (Sapey et al., 2013). Identifying ways to prevent or reverse neutrophil immunosenescence is critical to improving immunity in older adults.

One of the features of ageing in humans is a reduced level of physical activity. Less than half of over 65 year olds in the UK meet national guidelines for habitual physical activity (House of Lords Select Committee, 2013). Importantly, a growing body of evidence suggests that regular participation in physical exercise can improve or maintain immune function in old age, potentially slowing the progression of immunosenescence (Walsh et al., 2011; Simpson et al., 2012). The question thus arises of whether a significant part of immunosenescence is the consequence of an inactive lifestyle, rather than being due to the intrinsic ageing process. Despite the extent of neutrophil dysfunction with age and its potential contribution to ill-health, there are relatively few studies assessing the effects of exercise on neutrophil function in the elderly. Yan and colleagues showed that phagocytosis was better maintained in physically active compared to sedentary older men (Yan et al., 2001). In overweight older women, 6-weeks of aerobic exercise training reduced circulating neutrophil numbers – a potentially beneficial anti-inflammatory effect (Michishita et al., 2010). In young participants, neutrophil chemotaxis and phagocytosis was improved following 2 months of moderate aerobic exercise training (Syu et al., 2012). These studies highlight the potential for regular exercise to positively modify neutrophil function and potentially lower the risk of infectious diseases in later life.

Understanding the effects of habitual physical activity on immune function in the elderly could lead to new models of exercise prescription for older adults. To date, no studies have assessed broad neutrophil functions in healthy older individuals with a range of physical activity levels. The aim of this study was thus to assess a comprehensive range of neutrophil functions known to be reduced with age and determine the association with habitual physical activity. We hypothesized that regular physical activity in later life would be associated with maintenance of neutrophil function. Specifically we assessed directional migration, cell receptor expression, phagocytosis of *Escherichia coli* and the oxidative burst response to *E. coli*.

### 2. Materials and methods

#### 2.1. Participants and assessment of habitual physical activity

An assessment of physical activity and a broad range of physiological function were completed in 211 healthy older participants, 100 men (67 ± 5 years) and 111 women (66 ± 5 years), at the Wellcome Trust Clinical Research Facility (WT CRF), Birmingham, UK. For assessment of habitual activity levels participants wore a GT3X accelerometer (Actigraph, FL, USA) around the waist above the left leg for 7 days, except for during water based activities. Total steps per day were calculated using ActiLife Software v5 (Actigraph, FL, USA). Following 7 continuous days wearing an accelerometer the 20 most active (MA) and 20 least active (LA) participants were identified and asked to return to the WTCRF for an additional blood sampling. All participants were grouped (quintiles) by physical activity and then stratified for age and gender. The top ten MA males were then matched for age (±2 years) with ten LA males and the same was done for women. Upon recruitment to the study, participants again wore the accelerometer to ensure physical activity levels were similar to their first visit and completed the International Physical Activity Questionnaire (IPAQ). Details of participant age, physical activity levels, clinical and physiological characteristics are described in Table 1. Ten healthy young individuals (23 ± 4 years) were also recruited in order to compare neutrophil functions and serological status with age.

All participants were asked to abstain from physical exercise for 48 h before arriving at the clinic. Exclusion criteria was specified to ensure relatively good health and included: no general medical history in the previous 3 years: dementia, Parkinson’s, stroke, transient ischaemic attacks, liver disease, cancer, myocardial infarction, cardiomyopathy, valvular disease, angina pectoris, sudden chest pains, blood pressure >190/120 or tendencies to faint, asthma, hypertension, diabetes, use of Statins (Y/N) 1/19 5/15, use of blood pressure medication (Y/N) 5/19 14/15, current smoker (Y/N) 0/20 2/18, resting heart rate (bpm) 65 ± 9 64 ± 7, body fat (%) 26.2 ± 7.9 31.5 ± 6.7, body mass index (BMI) 23.5 ± 3.1 26.7 ± 3.9**,

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the forty older participants assessed.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Elderly activity group</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>10/10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.4 ± 5.0</td>
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<tr>
<td>Physical Activity</td>
<td>10370 ± 3221</td>
</tr>
<tr>
<td>Steps (per day)</td>
<td>29.4 ± 16.9</td>
</tr>
<tr>
<td>IPQ (AU)</td>
<td>55.2 ± 1.4</td>
</tr>
<tr>
<td>Physical Functioning</td>
<td>Timed-up-and-go (seconds)</td>
</tr>
<tr>
<td>Grip Strength (kg)</td>
<td>30 ± 9</td>
</tr>
<tr>
<td>Berg Balance Score (AU)</td>
<td>127 ± 13</td>
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<tr>
<td>Diastolic (mmHg)</td>
<td>77 ± 6</td>
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<tr>
<td>Resting Heart Rate (bpm)</td>
<td>65 ± 9</td>
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<tr>
<td>Current Smoker (Y/N)</td>
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</tr>
<tr>
<td>Use of Statins (Y/N)</td>
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</tr>
<tr>
<td>Physiological</td>
<td>2.5/19</td>
</tr>
<tr>
<td>White Blood Cells</td>
<td>3.08 ± 0.8</td>
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<tr>
<td>Neutrophils (10^9/L)</td>
<td>0.27 ± 0.08</td>
</tr>
<tr>
<td>Monocytes (10^9/L)</td>
<td>1.28 ± 0.28</td>
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<tr>
<td>Lymphocytes (10^9/L)</td>
<td>5.04 ± 0.4</td>
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<tr>
<td>Fasting Glucose (mmol/L)</td>
<td>12.3 ± 4.1</td>
</tr>
<tr>
<td>Fasting Insulin (μU/ml)</td>
<td>2.1 ± 1.1</td>
</tr>
<tr>
<td>HOMA-IR (AU)</td>
<td>1.14 ± 0.56</td>
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<tr>
<td>Triglycerides (mmol/L)</td>
<td>5.80</td>
</tr>
<tr>
<td>Cholesterol [statin* (mmol/L)]</td>
<td>5.75 ± 0.97</td>
</tr>
<tr>
<td>Cholesterol [statin* (mmol/L)]</td>
<td>9.9 ± 7.8</td>
</tr>
<tr>
<td>Leptin (mg/ml)</td>
<td>4.2 ± 2.0</td>
</tr>
<tr>
<td>Leptin: Adiponectin Ratio</td>
<td>2.5 ± 1.9</td>
</tr>
</tbody>
</table>

IPAQ [International Physical Activity Questionnaire]; AU ( Arbitrary Units); BMI (Body Mass Index); HOMA-IR (Homeostasis Model Assessment for Insulin Resistance); Statin* (Prescribed statins); Statin (Not prescribed statins). Data are mean ± SD unless indicated otherwise. *p < 0.05, **p < 0.01, ***p < 0.001 compared to MA.
chronic obstructive pulmonary disease, rheumatoid arthritis or osteoarthritis, use of corticosteroids, type 1 or 2 diabetes, leg pain during exertion, and consultation with GP for feeling unwell in the previous 10-days. Ethical approval was granted by the Black Country Research Ethics committee (10/H1202/77) and all participants gave written informed consent.

2.2. Clinical assessment

Clinical assessments were taken by trained research nurses in the WTCRF. Briefly height and weight were recorded and body composition was measured using dual-energy X-ray absorptiometry (GE Lunar iDXA, GE Healthcare, Madison, WI) using Encore Software v13.6, as previously described (Schousboe et al., 2013). Blood pressure (BP) and heart rate were taken after 15 min sitting, using an Omron M5-1 automated BP machine (Omron Healthcare, Lake Forest, USA). An average of 3 BP readings taken over 5 min was taken as clinical BP (Omboni et al., 2007).

2.3. Functional testing

Each participant completed a short battery of physical functioning tests which are indicative of health and progression towards frailty in older people (Thrane et al., 2007; Nordin et al., 2008; Muir et al., 2008, 2010): Hand grip strength was determined by measuring both left and right hands using a hand held dynamometer (Takei TKK-5401, Takei Scientific Instruments, Japan) and the best scores used. Participants also completed the 6-metre timed-up and go (TUG) test, briefly, participants were timed to stand from sitting, walk 3-metres at their regular walking speed, turn around, walk back and sit down. The Berg Balance Scale (BBS), a measure of balance and postural stability, was also carried out in which participants were asked to complete 14 balance tasks and scores given. Scoring gives a risk of falling, with 41–56 a ‘low risk of falling’.

2.4. Blood sampling

Participants arrived at the clinic at 8am having fasted overnight and peripheral venous blood samples were taken into vacutainers containing either heparin, EDTA or a clotting agent for serum. Samples were then processed immediately for serum, plasma and immune cell isolation. Serum and plasma were separated from blood by centrifugation (3000g for 10 min), snap frozen in liquid nitrogen and stored at −80°C until analysis.

2.5. Neutrophil isolation

Neutrophils were isolated from heparinised blood following dextran induced sedimentation and separation on a discontinuous Percoll gradient as previously described (Butcher et al., 2001). Neutrophil purity and viability was determined by Giemsa staining (Diff-Quik; Gentaur Europe, Brussels, Belgium) and trypan blue (Sigma Aldrich, Dorset, UK) exclusion, respectively. Neutrophil purity and viability were consistently ≥96%. Neutrophils were then resuspended at 1 × 10⁶ ml⁻¹ in RPMI 1640 medium (Sigma–Aldrich) containing 0.15% bovine serum albumin (Sigma–Aldrich).

2.6. Neutrophil migration

Neutrophil migratory dynamics were assessed using an Insall chamber (Weber Scientific International Ltd., Teddington, UK) as previously described (Sapey et al., 2013; Muinonen-Martin et al., 2010). Briefly, coverslips were coated with 7.5% culture tested bovine serum albumin (Sigma–Aldrich) and neutrophils adhered to this surface for 20 min at room temperature. The use of this bovine serum albumin has previously been shown to mimic the ligand for CD11b and CD18, ICAM-1 (Zhu et al., 2000). The coverslip was then inverted on the Insall chamber before addition of buffer (RPMI-1640) alone as a control or buffer containing 100 nM of interleukin (IL)-8 (R&D Systems; Abingdon, UK).

Neutrophil migration was monitored using a Zeiss Axiocco 100 inverted microscope fitted with a Fast Mono 12-bit QICAM digital camera. Time-lapse recordings and calculation of neutrophil migratory dynamics were performed as previously described (Sapey et al., 2013). Briefly, the Insall chamber allows the formation of stable chemoattractant gradients, with defined, consistent direction in the y direction for each experiment (Muinonen-Martin et al., 2010). Only distance travelled in the y direction over time was included in calculations of chemotaxis. Migration was assessed using 3 parameters: Average cell speed (µm/min) of movement towards a chemokine (e.g. IL-8, termed chemokinesis), average velocity (µm/min) of cells (termed chemotaxis) and accuracy of movement (termed chemotactic index). Chemotactic index is expressed in a comparative scale (CS) ranging from −1 to +1. Movement directly towards the chemoattractant is +1 whilst directly away is −1.

Recordings lasted 20 min per experiment, with 20 slides captured using Improvision OpenLab software. The Java software ImageJ (Wayne Rasband, NIH, Bethesda, MD) was used to analyse cell tracks. All analysis was carried out by a single analyst, blinded to subject group and cell conditions.

2.7. Neutrophil phagocytosis and oxidative burst

Phagocytosis and oxidative burst were assessed in whole blood using commercially available kits and manufacturers’ guidelines (BD Biosciences, Oxford, UK). Briefly, phagocytosis was assessed in heparin treated whole blood and incubated at 4°C (control) or 37°C (test) with opsonised FITC-labelled E. coli. Phagocytosis was halted by the addition of cold phosphate buffered saline (PBS) whilst cell surface bound FITC was quenched by addition of Trypan Blue solution. Unbound free bacteria were removed by washing in PBS and erythrocytes lysed and leukocytes fixed using 1% fix/lyse solution provided in the kit. Cell DNA was counterstained by addition of propidium iodide (PI) before flow cytometry analysis was performed.

Oxidative burst was assessed in heparin treated whole blood which was incubated at 37°C with opsonised E. coli (test) or PBS (control). Solution containing dihydrodoramine-123, which is converted to fluorescent rodamine-123 in the presence of reactive oxidants, was included for 10 min at 37°C. Oxidative burst was halted by the addition of erythrocyte lysis/leukocyte fixation buffer before leukocyte DNA was stained and flow cytometry analysis performed.

Phagocytosis and oxidative burst quantitation was performed on a CyArd™ 430 flow cytometer (Beckman-Coulter, High Wycombe, UK) equipped with 3 solid-state lasers. FITC and R-123 were detected in FL1 whilst PI was detected in FL2 using the Argon (405 nm) laser. 10,000 neutrophils were acquired for analysis. Following compensation of FL1 v FL2 phagocytic and oxidative burst were determined by the relative increase in percentage and median fluorescence intensity (MFI) in FL1 compared to negative controls. Data were analysed using Summit v4.3 (DAKO, Cambridgeshire, UK).

2.8. Measurement of surface receptor expression

Freshly isolated neutrophils (1 × 10⁸ ml⁻¹) were stained with anti-CXCR1-FITC (eBioscience, Hatfield, UK, clone 8F1-1-4), anti-CXCR2-PE (eBioscience, clone 5E8-C7-F10), anti-CD16-APC (eBioscience, clone CB16), anti-CD16-FITC (BD Bioscience, clone 3G8), anti-CD11b-APC (BD Bioscience, clone ICRF44),
2.9. Serological and metabolic analysis

All measurements were made in duplicate using commercially available kits and manufacturers guidelines. Serum IL-1β, IL-4, IL-6, IL-8, IL-10, IL-17, GMC-SF, TNFα and MCP-1 were simultaneously measured by multiplex luminometry (Bio-Rad, Hemel Hempstead, UK). Samples were analysed using a Bio-Plex Luminx™ platform equipped with a 635 nm red and 532 nm green laser using Bio-Plex Manager™ software. Detection of C-reactive protein (CRP) was by high sensitivity ELISA using a commercial kit (IBL International, Hamburg, Germany). Plasma adiponectin and leptin were assessed separately by solid phase sandwich ELISA (R&D Systems, Abingdon, UK). Plasma insulin levels were determined by sandwich ELISA (Life Technologies, Paisley, UK). Plasma glucose, cholesterol and triglycerides (Instrumentation Laboratories, Warrington, UK) were determined photometrically on an automated chemistry analyser (iLab 650, Instrumentation Laboratories, Warrington, UK).

2.10. Statistical analysis

All analyses were conducted using PASW version 18.0 (Chicago, IL, USA) and all data presented as mean ± standard deviation (SD) unless otherwise stated. Normality was assessed using Kolmogorov-Smirnov analysis; natural log transformation of distributed variables violating normality was completed. Analysis of covariance (ANCOVA) with Bonferroni correction and independent sample T-tests were used to determine differences between the groups. Covariates were included in the analyses if they were different between the most and least active elderly groups. Pearson correlation analysis was conducted to determine relationships between migratory dynamics and steps/day as well as metabolic variables. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as described previously using the HOMA2-IR online calculator (The Oxford Center for Diabetes, 2014; Wallace et al., 2004). As previously described, all experiments were preceded by studies of intra-subject and inter-subject variance to allow appropriate power calculations to be performed (Sapey et al., 2013). Statistical significance was accepted as p < 0.05.

3. Results

3.1. Participant characteristics

The physical and serological characteristics of the participants are shown in Table 1. Physical activity levels ranged from 1518 to 19,161 steps/day and as is typical with older people a low percentage, only 13%, completed >10,000 steps/day, causing a left-shift in distribution. There were no differences between the first and second wearing of the accelerometers and both were highly correlated, (r(38) > 0.784; p < 0.001 for all), therefore data presented here are from the second wear time only. The most active (MA) group was over twice as active for steps/day as the least active (LA) group (p < 0.001). These data highlighted that not only were the MA group walking more, but were less sedentary. Results from self-perceived activity reports (IPAQ) were similar to accelerometer data with approximately a twofold difference between the two groups (p = 0.026).

Although attempts were made to match the groups for as many potential confounding variables as possible, inevitably a number of differences were apparent. In particular, body mass index (BMI; p = 0.007) and body fat percentage (p = 0.029) were higher in the LA group which likely accounted for a number of metabolic differences. Fasting glucose (p = 0.010), insulin (p = 0.004) and leptin (p = 0.036) were all higher whilst adiponectin (p = 0.047) was lower, in the LA group. Importantly, none of these measures were clinically abnormal and other measures of health including blood pressure, resting heart rate, triglycerides, total cholesterol, grip strength, balance, walk speed, statin medication, and smoking were similar between groups (p > 0.05 for all).

There were also no differences in total immune cell counts for neutrophils, monocytes or lymphocytes between the two groups (Table 1). Taken together, these data show that our two populations were relatively healthy and we can be confident that any group differences are likely driven by differences in physical activity levels rather than morbidity.

3.2. Neutrophil migration and physical activity

Efficient neutrophil migration is a critical step in the resolution of infection and has recently been shown to be impaired with advancing age (Sapey et al., 2013). In agreement with published data, isolated neutrophils from young participants migrated towards the chemokine IL-8 with a greater directional speed [chemotaxis (p = 0.038)] and an overall greater chemotactic accuracy [chemotactic index (p = 0.020)], but not overall speed [chemokinesis (p = 0.098)], than older participants. When split into their respective activity groups, the LA group had significantly reduced neutrophil chemotaxis (Fig. 1A) compared to both the MA (p = 0.044) and young participants (p = 0.021) and there were still no differences between groups for chemokinesis (Fig. 1B). Chemotactic index for the LA group (Fig. 1C) was also reduced compared to the MA group (p = 0.002) and young participants (p = 0.001).

Correlation analysis revealed that increased steps/day were associated with increased neutrophil chemotactic index (r(38) = 0.689, p = 0.001) but not chemokinesis (both p > 0.05). These data suggest being physically active in later life could maintain neutrophil migratory dynamics, specifically accuracy and velocity, and that the amount of physical activity is related to the degree of migratory benefit.

3.3. Neutrophil migration and a relationship with metabolic phenotype

In order to determine if the relationship between neutrophil migration and physical activity might be mediated by differences in metabolic parameters between the LA and MA groups, data was analysed to include BMI, body fat percentage, adiponectin, leptin, glucose and insulin concentrations as covariates. Inclusion of these variables did not statistically alter our findings for any
chemotactic index (Supplementary Table 1). Correlation analysis
individual migratory parameters, chemokinesis, chemotaxis or
chemotactic index (Supplementary Table 1). Correlation analysis
did however reveal that certain metabolic markers were associated
with the overall chemotactic index. Fig. 2A shows that adiponectin
(\(r(38) = 0.515, p = 0.05\)) and insulin (Fig. 2D; \(r(38) = -0.617, p = 0.01\)) were associated with chemotactic index, whilst leptin
(Fig. 2B; \(r(38) = -0.359, p = 0.22\)) and glucose (Fig. 2C; \(r(38) = -0.327, p = 0.263\)) were not. These data suggest that exposure
to raised adiponectin and reduced insulin may play a role in medi-
atating the effects of exercise on neutrophil migration.

3.4. Phagocytosis and oxidative burst and relation to physical activity

A primary mechanism of bacterial clearance by neutrophils is
phagocytosis through exposure to reactive oxygen species such as
superoxide anions. All neutrophils ingested bacteria and pro-
duced an oxidative burst. Phagocytosis of opsonised FITC-labelled
E. coli showed no differences between young, LA or MA groups
(Fig. 3A) and the oxidative burst also did not differ between groups
(Fig. 3B).

3.5. Neutrophil receptor expression

To understand whether the differences in migration were due to
the detection of the chemokine IL-8, or the adhesion to the cover-
slip chemokine and adhesion receptor expression was assessed on
isolated neutrophils (Table 2). All neutrophils expressed the recep-
tors measured. No differences were observed between groups for
the IL-8 receptors CXCR1 and CXCR2, or the adhesion molecule
CD11b. There was a small group effect for the adhesion molecule
CD11b \(F_{2, 39} = 3.64, p = 0.036, \eta^2 = 0.168\) which was due to
lower expression on the LA neutrophils compared to the MA
\(p = 0.048\) and young \(p = 0.041\) groups. CD11b was included as
a covariate in the analysis of Chemotactic Index but did not alter
our findings. Our data suggest that differences in neutrophil migra-
tion are not the result of chemokine receptor expression.

3.6. Systemic inflammation and neutrophil migratory dynamics

Exposure to inflammatory mediators is known to influence the
function of neutrophils including migration. In order to determine
whether systemic inflammation could have contributed to migratory
differences levels of pro-inflammatory cytokines, acute phase proteins and anti-inflammatory cytokines were assessed. Table 3
reveals the young subjects had lower levels of circulating IL-6
\(p = 0.011\), IL-8 \(p = 0.049\), MCP-1 \(p = 0.002\), CRP \(p = 0.039\),
IL-10 \(p = 0.004\) and IL-13 \(p = 0.033\) than the combined older
participants. No differences between activity groups for the LA
and MA groups were evident.

4. Discussion

With a growing body of evidence to suggest that physical activ-
ity in later life reduces the risk of age related diseases it is impre-
sive to understand which specific aspects of physiology benefit
from a healthy lifestyle (Brandt and Pedersen, 2010; Pedersen,
2011). The immune system characteristically becomes dysregu-
lated with age leading to a severe functional loss and exposing
older individuals to increased risk of infection and disease
(Pawelec et al., 2005; Panda et al., 2009). The beneficial effects
and impact of physical activity on the immune system, especially
adaptive immunity, have been extensively studied in young
healthy individuals (LaVoy et al., 2013; Simpson et al., 2010;
Spielmann et al., 2011). However few studies have specifically
focused on the neutrophil in older adults and the effects of habitual
physical activity in later life. This study is the first to compare neu-
rophil phagocytosis, superoxide production and detailed migratory
dynamics towards the chemokine IL-8 in habitually
physically active and less active healthy older individuals. We
show for the first time that a 2-fold difference in physical activity
is associated with better preserved neutrophil migratory dynamics
in healthy older people.

Neutrophil migration is a key component for the resolution of
infection and control of inflammation. Along with age-associated
reductions in bacterial clearance, migratory dysfunction can lead
to increased morbidity and mortality from bacterial infections
(Li and Mackowiak, 2007). Defective migration results in poor
resolution of inflammation and wound repair in aged mice
(Brubaker et al., 2013). Additionally, if the neutrophil does not

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migrate accurately between tissues, collateral damage is augmented by elevated proteinase release from the cell, enhancing the inflammatory milieu and potentially contributing to the increased infection associated frailty (Liou and Campbell, 1996; Pham, 2006; Fleming and Elliot, 2005). Recent work from our group showed that neutrophils from older adults migrated with a similar speed but reduced velocity and accuracy when compared to younger participants (Sapey et al., 2013). Our findings here show that velocity and accuracy were similarly reduced in our older cohort compared to young subjects, but we now add that the reduced habitual activity of our elders is associated with this loss of migratory accuracy.

A number of studies have highlighted the beneficial effects of physical exercise on immune function in the young and to a lesser extent the old. However, few studies have assessed whether neutrophil migration is associated with physical activity. Neutrophil migration was improved following six months of moderate exercise training in hypertensive older women (65 ± 5 years) to a similar level of migration as that in age-matched healthy women (Fuente et al., 2005). Furthermore, migration was also improved 2-fold in young sedentary individuals following a 2 month moderate intensity cycling exercise program (Syu et al., 2012). Interestingly, in both these studies following a period of no-training or de-training neutrophil migration reverted back to levels prior to commencing training. These data suggest that efficient neutrophil migration is not only improved by physical exercise but is highly plastic and fundamentally dependent on it. In support of a physical activity effect on neutrophil function, we show that just a 2-fold difference in habitual physical activity levels was sufficient to be associated with increased neutrophil chemotaxis and chemotactic index. Moreover, none of our participants were actively engaged in structured exercise training as stated in their IPAQ’s, thus higher habitual physical activity levels resulted in significantly better migration.

To investigate the potential mechanisms we assessed whether cell surface receptor expression related to migratory dynamics was altered with physical activity levels. The two main neutrophil receptors for IL-8 are the G-coupled protein receptors CXCR1 and CXCR2, which signal through a range of pathways, with the PI3K/AKT and Rac GTPase pathways central to chemotaxis and migratory accuracy (King and Insall, 2009). CXCR2 has been shown to increase in muscle fibres and vascular endothelium following acute exercise, likely stimulating angiogenesis in the working tissue (Frydelund-Larsen et al., 2007). However we found that physical...
activity levels did not correlate with CXC1R or CXC2R expression on neutrophils. Our group recently showed that neither CXC1R nor CXC2R were altered with age and instead migratory dysfunc-

Table 3

| Pro-Inflammatory | Old | Young | All | MA (n = 20) | LA (n = 20)
<table>
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<tbody>
<tr>
<td>IL-1β (pg/ml)*</td>
<td>0.21 ± 0.05</td>
<td>0.33 ± 0.04</td>
<td>0.31 ± 0.05</td>
<td>0.35 ± 0.07</td>
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</tr>
<tr>
<td>IL-6 (pg/ml)*</td>
<td>1.36 ± 0.92</td>
<td>5.11 ± 1.49*</td>
<td>5.71 ± 2.27</td>
<td>4.46 ± 1.99</td>
<td></td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>5.63 ± 0.58</td>
<td>6.79 ± 0.30*</td>
<td>6.49 ± 0.32</td>
<td>7.11 ± 0.51</td>
<td></td>
</tr>
<tr>
<td>IL-17 (pg/ml)*</td>
<td>10.09 ± 10.09</td>
<td>16.01 ± 6.35*</td>
<td>17.40 ± 11.33</td>
<td>14.48 ± 6.13</td>
<td></td>
</tr>
<tr>
<td>TNFα (pg/ml)*</td>
<td>0.23 ± 0.11</td>
<td>3.59 ± 2.47</td>
<td>3.36 ± 4.40</td>
<td>2.48 ± 1.96</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)*</td>
<td>1.30 ± 0.81</td>
<td>2.13 ± 0.45*</td>
<td>2.46 ± 0.81</td>
<td>1.77 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>GM-CSF (pg/ml)*</td>
<td>ND</td>
<td>21.3 ± 1.52</td>
<td>24.4 ± 2.44</td>
<td>1.79 ± 1.79</td>
<td></td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>27.63 ± 2.44</td>
<td>43.84 ± 2.57**</td>
<td>43.31 ± 3.84*</td>
<td>44.42 ± 3.44*</td>
<td></td>
</tr>
</tbody>
</table>
| Anti-Inflammatory | Old | Young | All | MA (n = 20) | LA (n = 20)
| IL-4 (pg/ml)*    | 0.11 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 |          |          |
| IL-10 (pg/ml)*   | 7.12 ± 3.12 | 19.34 ± 3.45** | 17.33 ± 4.67 | 21.56 ± 5.19* |          |
| IL-13 (pg/ml)    | 4.13 ± 1.20 | 7.15 ± 0.71* | 6.29 ± 0.87 | 8.10 ± 1.14 |          |

ND (not-detec-ta-ble); IL (Interleukin); TNFα (tumour necrosis factor-α); CRP (C-Reactiv-e Protein); GM-CSF (Granulocyte/Monocyte Colony Stimulating Factor); MCP-1 (Monocyte Chemoattractant Protein-1). *Not normally distributed and log-transformed. **p < 0.05 and ***p < 0.01 compared to young group.
nalling and has inherent anti-inflammatory effects. Here adiponectin levels were positively associated with chemotactic index. Our group showed adiponectin delayed spontaneous neutrophil apoptosis whilst others have shown reduced IL-8 production in obese individuals associated with reduced serum adiponectin (Rossi and Lord, 2013; Trellakis et al., 2012). Recently, adiponectin was associated with improved neutrophil migration towards acute kidney injury in mice (Jin et al., 2013). A recent study suggested that the addition of 20-weeks aerobic exercise to dieting in overweight postmenopausal women resulted in increased adiponectin concentrations (Wang et al., 2015). Together these findings suggest a positive role for adiponectin on neutrophil function and migration. Further work is required to understand metabolism in neutrophils and the role exercise plays.

Our study is not without limitations. We did not measure body composition, physical activity levels or physical functioning in our young cohort. Our data suggest that neutrophil dysfunction, specifically chemotactic index, may be a consequence of physical inactivity. This would have been further supported had the activity levels of the young cohort matched the most active elderly. Furthermore we did not consider dietary differences between groups. It is clear that different compositions (carbohydrate, protein, fats) of diet can have effects on metabolism and therefore potentially cell function. However, we measured a number of inflammatory and endocrine markers which are associated with poor diet and increased adiposity and determined they did not have a direct effect on neutrophil migration. Future studies assessing immunological differences between young and old individuals should consider physical activity and dietary differences. Finally we have previously observed that PI3K is constitutively activated in elderly individuals and blocking PI3K in these cells restores migratory dynamics. Future studies should assess PI3K expression associated with physical activity and determine where the signalling defect occurs.

In conclusion our study suggests impaired neutrophil migration, but not bactericidal function, in older adults may be in part the result of physical inactivity. It is unlikely that these effects are driven by systemic inflammation or reduced surface chemokine receptor expression but a positive correlation with adiponectin levels suggest an influence of this metabolic hormone. Increasing habitual physical activity levels in elderly individuals may therefore be beneficial for neutrophil mediated immunity.

Conflicts of Interest

All authors declare they have no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbbi.2016.02.024.

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