Experimental Gerontology 108 (2018) 112–117

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Short report

Aging impairs mitochondrial respiratory capacity in classical monocytes

Brandt D. Pence[a,b,*], Johnathan R. Yarbro[a]

[a] School of Health Studies, University of Memphis, Memphis, TN, United States
[b] Center for Nutraceutical and Dietary Supplement Research, University of Memphis, Memphis, TN, United States

ARTICLE INFO

Keywords:
Innate immunity
Inflammation
Immunometabolism
Inflammaging
Metabolism

ABSTRACT

Aging is a critical healthcare concern, with age-related inflammation disposing individuals to a variety of diseases. Monocytes are affected by the aging process, with increased inflammation and impaired cellular functions such as phagocytosis. Mechanisms by which aging alters monocyte function are unknown, but recent research suggests that the balance of metabolic processes determine immune cell phenotype and function. Given the known association between aging and mitochondrial dysfunction in other tissues, we hypothesized that aging would impair mitochondrial function in monocytes. To test this, we isolated classical monocytes from young and older adults and tested mitochondrial function by a Seahorse assay. Aging reduced mitochondrial respiratory capacity and spare capacity in monocytes. Mitochondrial dysfunction is a potential mechanism by which aging alters monocyte phenotype and may impair inflammatory functions, especially in low-glucose environments where oxidative metabolism is necessary to meet energy demands.

1. Introduction

The proportion of U.S. adults over the age of 65 will reach nearly 20% by 2030 (Vincent and Velkoff 2010). This is likely to create a significant healthcare burden, as aging is highly associated with increased incidence of a variety of chronic disease (Kennedy et al. 2014). A state of chronic low-grade inflammation, popularly termed "inflammaging", underlies many these diseases (Franceschi and Campisi 2014). Although descriptively well-documented, the etiology of inflammation is not fully understood and may vary by immune cell type or individual. Age-related cellular damage is thought to trigger sterile inflammatory responses in phagocytes (Franceschi and Campisi 2014). Likewise, adaptive immunosenescence has been hypothesized to trigger compensatory innate immune activation (Franceschi et al. 2000), and age-related changes in the gut microbiota may also be involved in mediating inflammaging (Buford 2017). Inflammaging is therefore likely to be multifactorial, but a critical understanding of individual subcellular mechanisms governing the inflammaging process is nevertheless critical to the development of therapies to prevent or treat age- and inflammation-related chronic diseases.

Monocytes are circulating innate immune cells with functions including cytokine production, phagocytosis, and differentiation into macrophages and dendritic cells (Yang et al. 2014). Monocyte dysfunction affects the pathology of a variety of chronic and infectious diseases. For example, age-related monocyte dysfunction impairs the immune response to pneumococcal infection (Puchta et al. 2016). Monocyte dysfunction has also been described to associate with cancer (Mainwaring et al. 1999), renal diseases (Kato et al. 2008), hypercholesterolemia (Short et al. 2017), sepsis (Zhang et al. 2010), etc. However, the mechanisms regulating monocyte phenotype and function are not well-understood, especially in the aging context.

The field of immunometabolism has recently received considerable interest. Simplistically, rapid metabolic processes such as glycolysis contribute to increased inflammation, while slower metabolic processes such as fatty acid oxidation contribute to anti-inflammatory activities. A recent review covers these processes in comprehensive detail (O'Neill et al. 2016). Fatty acid oxidation occurs in the mitochondria, and thus dysfunction in mitochondria has the potential to suppress anti-inflammatory cellular activities and heighten inflammation. In aging, mitochondrial function is impaired in numerous cell types (Bratic and Larsson 2013) and thus represents a plausible underlying cause for inflammaging. However, mitochondrial function has not been widely investigated in this context. Recent research revealed age-associated mitochondrial dysfunction in macrophages (Stout-Delgado et al. 2016) and dendritic cells (Chougnet et al. 2015), but results from these studies are limited in their focus on specific disease states, and mitochondrial function has not been investigated in monocytes.

Therefore, we hypothesized that aging reduces mitochondrial function in monocytes. Given that this has not been evaluated previously, we conducted a small-scale study examining mitochondrial function in primary human classical monocytes as a proof-of-principle.

* Corresponding author at: 304 Elma Roane Fieldhouse, University of Memphis, Memphis, TN 38152.
E-mail address: bdpence@memphis.edu (B.D. Pence).

https://doi.org/10.1016/j.exger.2018.04.008
Received 12 February 2018; Received in revised form 6 April 2018; Accepted 11 April 2018
Available online 13 April 2018

2. Methods

2.1. Protocol availability

Detailed step-by-step protocols and required supplies for major assays described here (monocyte isolation, Seahorse assay, flow cytometry) are available on protocols.io (Pence 2017b, 2017c, 2017d).

2.2. Subjects

Subjects were recruited from the Greater Memphis metropolitan area community without regard to sex, race, or socioeconomic status. Subjects were excluded if they reported previous diagnosis of metabolic or inflammatory diseases or routinely took medications affecting metabolism or inflammation. A total of 18 subjects were recruited for the study and assigned based on age to aged (60–80 years old, $N = 9$) or young (18–35 year old, $N = 9$) groups. In the young group, one subject completed screening but did not return for additional data collection visits, therefore, data for the young group include only 8 subjects except where noted below. All subjects completed an informed consent document, and all protocols were approved by the Institutional Review Board at the University of Memphis (protocol #4361).

For follow-up flow cytometry studies (see below), we attempted to recruit the same participants as in the initial study. Six aged and 3 young participants from the previous groups were available to participate. Two additional aged subjects and 5 additional young subjects were therefore recruited to fill out subject numbers for the follow-up study. The groups did not appreciably differ in demographics or anthropometrics between the first study and the follow-up.

2.3. Monocyte isolation

Subjects reported to the laboratory following an overnight fast and had forearm vein blood collected into a 10 ml K_2EDTA vacutainer tube. Classical monocytes were isolated from whole blood by column-free immunomagnetic negative selection using a commercially-available kit (EasySep Direct Human Monocyte Isolation Kit, StemCell Technologies, Vancouver, CAN) and associated magnet system (EasyEights EasySep Magnet, StemCell Technologies) and counted using a Scepter 2.0 automated cell counter (EMD Millipore, Billerica, MA) with 40 µm sensor. The kit included CD16 depletion and thus isolated only classical monocytes (Fig. 1A). For data analysis of assays involving monocyte isolation, data from one aged subject were excluded on the basis of low monocyte purity (72%), bringing the total subject number in that group to 8 for those assays. Inclusion of this subject did not affect the results. Monocyte purity was established by percent of cells positive for CD14 (anti-CD14-PE, BD Biosciences, San Diego, CA) with analysis on an Attune NxT flow cytometer (Thermo Fisher Scientific, Grand Island, NY) (Fig. 1B). Purity of isolated monocytes was tested on all but one young subject at least once during the study and did not differ between aged and young subjects ($p = 0.609$, Fig. 1C). All downstream assays were immediately performed on freshly-isolated monocytes.

We additionally isolated peripheral blood mononuclear cells (PBMCs) and assessed monocyte subtypes by CD14 and CD16 (anti-CD16-BV421, BD Biosciences) for comparison purposes to demonstrate that the magnetic sorting kit removed intermediate and non-classical monocytes (Fig. 1A). PBMCs were isolated using SepMate-50 conical tubes and Lymphoprep (StemCell Technologies) according to manufacturer's instructions.

2.4. Extracellular flux assay

A Cell Mito Stress Test (Agilent, Santa Clara, CA) was performed based on manufacturer's instruction on a Seahorse XFp extracellular flux analyzer (Agilent). Monocytes were plated on a sterile XFp plate in duplicate at 1.5×10^5 total cells per well and serially stimulated in the following sequence: (A) 1 µM oligomycin; (B) 1 µM oligomycin (2 µM final concentration); (C) 2 µM carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP); (D) 0.5 µM rotenone/antimycin A. Respiratory parameters calculated included basal oxygen consumption rate (OCR), OCR for ATP production, total respiratory capacity, spare respiratory capacity, and non-mitochondrial respiration. A visualization of respiratory parameter calculations is shown in Fig. 2A. The dual oligomycin injection used in this study is non-standard. It was performed on the recommendation of technical support to overcome a slow monocyte response to oligomycin seen in our optimization studies. This slow response did not occur during our actual experiments and thus was likely an aberration specific to the sample used for optimization. However, to maintain consistency, we maintained the dual injection scheme throughout the study. A single oligomycin injection of 2 µM final concentration is sufficient for monocytes in the majority of cases and would give equivalent results.

2.5. Flow cytometry

Monocyte phenotype was determined by flow cytometry using a 2-laser Attune NxT equipped with 405 nm and 488 nm lasers and 10 detectors. Whole blood (100 µl) was blocked with 10 µg human IgG (Sigma-Aldrich, St. Louis, MO) and stained with anti-CD14-PE and anti-CD16-BV421 (BD Biosciences). After red blood cell lysis, cells were washed twice and analyzed. Monocytes were gated based on forward and side-scatter, and classical, intermediate, and non-classical monocyte subpopulations were determined based on CD14 and CD16 expression. One subject in the young group did not complete the blood draw for flow cytometry testing.

For follow-up studies testing mitochondrial function by flow cytometry, PBMCs were isolated with lymphoprep and SepMate-50 tubes as described above, and 5×10^5 PBMCs were stained with anti-CD14-PE and anti-CD16-BV421 antibodies for 1 h at 4 °C. Following washing, cells were stained for 30 min with JC-1 (MitoScreen, BD Biosciences, according to manufacturer's instructions) for determination of mitochondrial membrane potential, 200 nM MitoTracker Green FM (Cell Signaling Technology, Danvers, MA) for determination of mitochondrial mass, or 1 µM CM-H2DCFDA (Thermo Fisher Scientific) for determination of reactive oxygen species production. After staining, cells were washed with PBS and analyzed. Classical monocytes were determined as $CD14^+CD16^-$ cells, and mitochondrial stains were assessed by mean fluorescence intensity in the classical monocyte population.

2.6. Data analysis

Categorical demographic data (race, sex) were analyzed by Pearson's chi-square test. Normally-distributed continuous variables (height, weight, body mass index, classical monocyte proportion, non-classical monocyte proportion, monocyte purity, and all Seahorse data) were analyzed by independent-samples *t*-test. Non-normal (by Shapiro-Wilk test) continuous variables (intermediate monocyte proportion and mitochondrial content) were analyzed by Mann-Whitney *U* test. The significance level was $p < 0.05$. Reported results are mean ± SEM. All data were analyzed with R v. 3.3.3 (R Foundation for Statistical Computing, Vienna, AUT).

3. Results

3.1. Raw data

Raw data and analytical scripts are available at figshare.com (Pence 2017a).