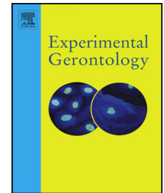
Experimental Gerontology 108 (2018) 112–117

Contents lists available at ScienceDirect

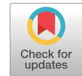
Experimental Gerontology

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
0531-5565/ © 2018 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/BY/4.0/).

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.

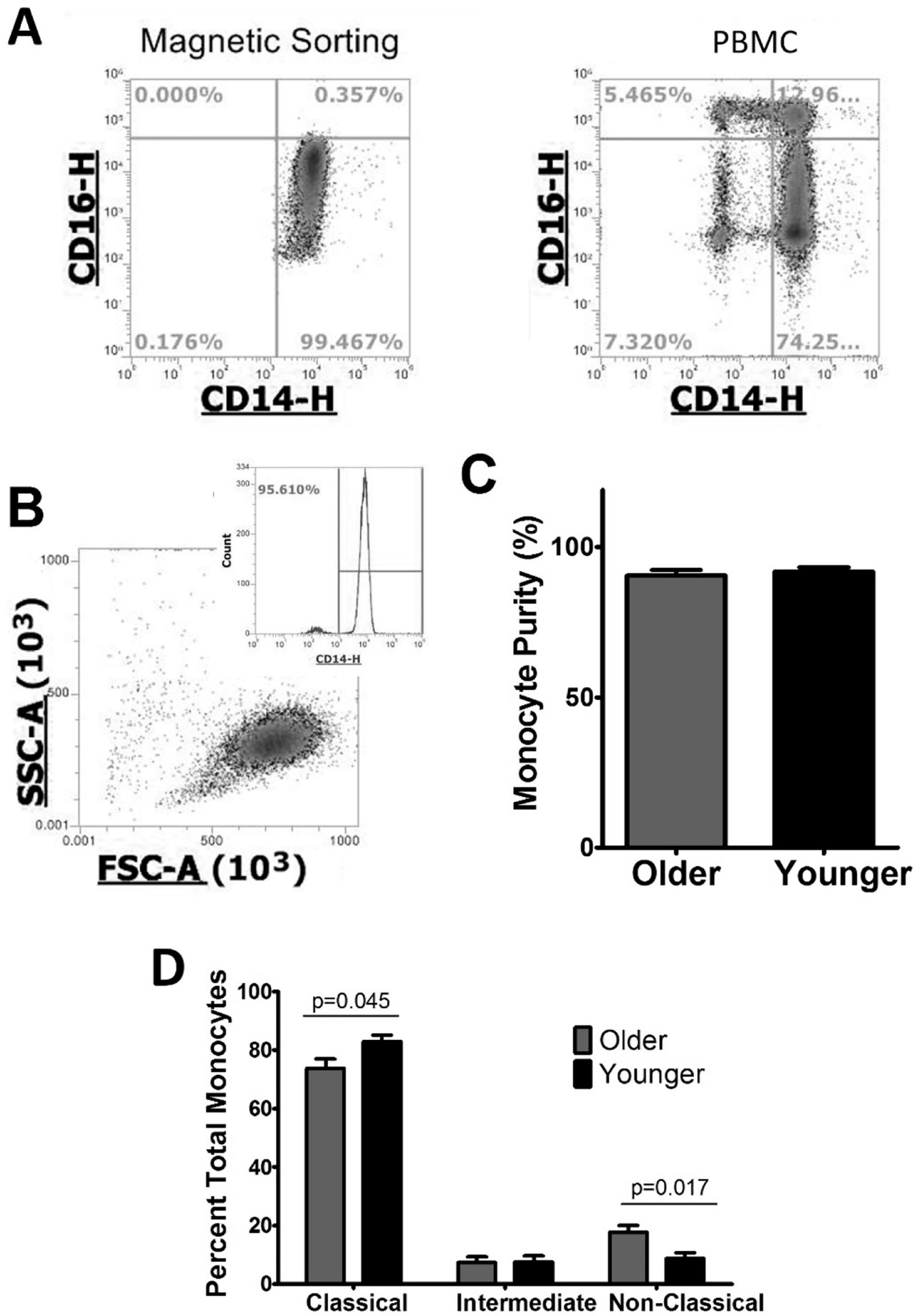
2.6. Data analysis

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).



(caption on next page)

Fig. 1. Characterization of monocyte isolations and subpopulations. (**A**) Magnetic sorting (left panel) isolates classical monocytes and depletes intermediate and non-classical monocytes, as shown by representative comparison to monocytes isolated through peripheral blood mononuclear cell isolation (right panel). (**B**) Monocyte isolations by immunomagnetic negative sorting results in a highly-pure cellular population, as demonstrated by a representative CD14 stain and flow cytometry. (**C**) Monocyte purity does not differ between older and younger subjects. (**D**) Percentage of total monocytes in classical, intermediate, and non-classical subtypes. Indicated *p*-values are significantly different between groups. $N = 7–8$/group.

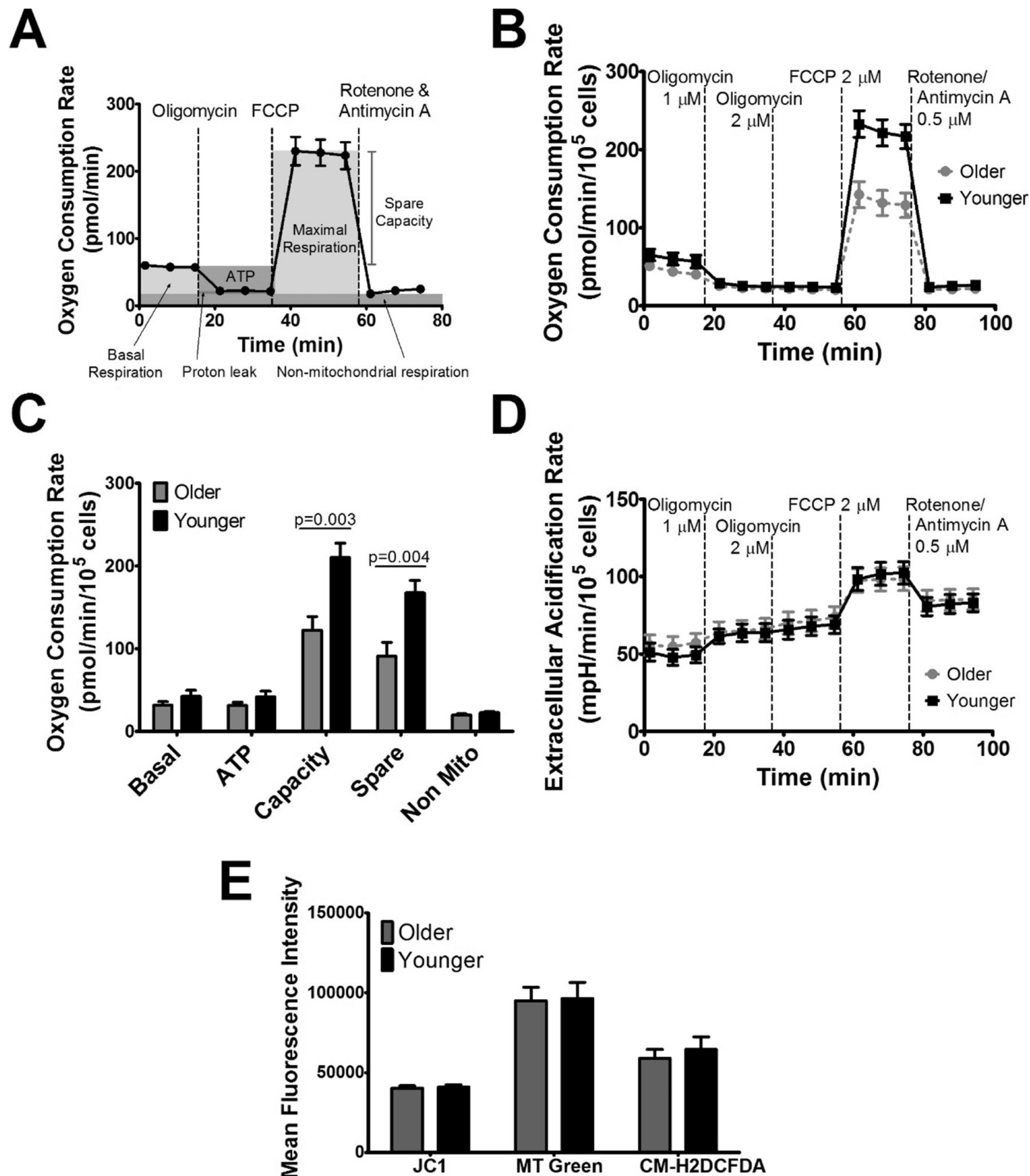


Fig. 2. Cell Mito Stress Test in classical monocytes. (**A**) Representative Seahorse Cell Mito Stress Test assay showing calculable parameters. (**B**) Oxygen consumption rate in older and younger subjects with sequential injections. (**C**) Calculated values for respiratory parameters. Basal: basal respiration. ATP: ATP-linked respiration. Capacity: maximal respiratory capacity. Spare: spare respiratory capacity. Non Mito: non-mitochondrial respiration. Indicated *p*-values are significantly different between groups. (**D**) Extracellular acidification rate in older and younger subjects with sequential injections. (**E**) Mitochondrial function markers by flow cytometry in classical monocytes. MT Green: MitoTracker Green FM. $N = 8$/group.

3.2. Subject characteristics

Of the 9 recruited subjects in the young group, 2 identified as Black/African American, 1 as Hispanic/Latino, and 6 as White/Caucasian. Five subjects were female and 4 subjects were male. Of the 9 recruited subjects in the older group, 4 identified as Black/African American and 5 identified as White/Caucasian. Four subjects were female and 5 subjects were male. The groups did not differ in distribution of race ($\chi^2 = 1.7576$, $p = 0.4153$) or sex ($\chi^2 = 0.2222$, $p = 0.6374$). Additionally, groups did not differ in height ($t = 1.4501$, $p = 0.1663$), weight ($t = 0.2396$, $p = 0.8137$), or body mass index ($t = -0.4087$, $p = 0.6881$).

3.3. Flow cytometry

We found increased non-classical (CD14^{dim}CD16^{+}) proportion in our aged subjects (Fig. 1D), in agreement with previous research (Hearps et al. 2012), although intermediate (CD14^{+}CD16^{+}) monocyte proportion did not differ.

3.4. Mitochondrial function

Aging impaired mitochondrial respiratory capacity and spare capacity (Fig. 2B-2C) in classical monocytes. Basal, ATP-linked, and non-mitochondrial respiration did not differ between older and young adults (Fig. 2C), although both basal OCR and ATP-linked OCR were non-significantly reduced in older subjects. Extracellular acidification rate (Fig. 2D) did not differ between groups during any step of the injection sequence.

Flow cytometric parameters for mitochondrial function in classical monocytes did not differ between aged and young (Fig. 2E).

4. Discussion

In this study, we report for the first time that aging impairs mitochondrial respiration in classical monocytes. Aging is well-known to reduce mitochondrial function in other tissues (Bratic and Larsson 2013), therefore this result was in some ways unsurprising. However, the impact of aging on metabolism in immune cells is underexplored, and impairment in monocyte mitochondrial function is potentially problematic for several reasons as discussed below. Therefore, the results of this study have important implications for aging and immune function. Monocytes have been examined using Seahorse assays in a handful of previous studies (Dominguez-Andres et al. 2017; Duroux-Richard et al. 2016; Kramer et al. 2015), and the responses to the serial injections in our study are consistent with those studies. Additionally, our data supported previous reports that aging increases the proportion of non-classical monocytes in the circulation (Hearps et al. 2012).

Monocytes take on several phenotypes, traditionally broken down into classical (CD14^{+}CD16^{-}), intermediate (CD14^{+}CD16^{+}), and non-classical (CD14^{dim}CD16^{+}) (Ziegler-Heitbrock et al. 2010). In aging, the proportions of intermediate and non-classical monocytes are increased (Hearps et al. 2012), and this is known to be associated with higher rates of diseases such as Crohn's disease (Grip et al. 2007), cardiovascular disease (Shantsila et al. 2011), rheumatoid arthritis (Tsukamoto et al. 2017), and peripheral artery disease (Wildgruber et al. 2016). Both intermediate and non-classical monocytes display increased inflammatory features (Patel et al. 2017; Puchta et al. 2016; Strauss-Ayali et al. 2007), thus age-associated alterations in monocyte subset proportions may play a major role in inflammaging. A recent transcriptomics study identified gene expression patterns related to cellular respiration as being downregulated in the non-classical subtype (Metcalf et al. 2017). Our monocyte isolation strategy included CD16 depletion and thus only isolated classical monocytes, we expect that intermediate and non-classical monocytes may have even greater impairments in mitochondrial function in older adults. This is an acknowledged limitation of our study, but was done consciously to avoid the potential for bias in our mitochondrial respiratory outcome measure, given that we expected monocyte subset proportions to differ. Intermediate and non-classical monocytes have been linked with a wide variety of chronic diseases (Grip et al. 2007; Shantsila et al. 2011; Tsukamoto et al. 2017; Wildgruber et al. 2016), thus dysregulated mitochondrial function is a potential mechanism by which aging alters monocyte phenotype and predisposes individuals to disease. This hypothesis deserves further investigation.

Here, we demonstrate that the primary aging impairment in monocyte mitochondrial function comes during maximal respiration, as basal respiration did not differ between young and old, and parameters of mitochondrial function as measured by flow cytometry in quiescent monocytes were also not changed. This suggests that monocytes from older individuals are likely to demonstrate the greatest dysfunction during high oxidative energy demand. Monocytes upregulate metabolism to meet energy demands during inflammation. Traditionally, this demand was thought to be primarily glycolytic, and thus mitochondrial function in monocytes has not been considered to be important to cellular function during inflammation. However, it was recently established that monocytes upregulate fatty acid oxidation during inflammatory events in low-glucose environments (Raulien et al. 2017). This compensatory upregulation of oxygen consumption in the absence of glucose appears to preserve the majority of monocyte effector functions (Raulien et al. 2017), and impairments in this is likely to dispose monocytes to functional dysregulation. Low-glucose environments include central nervous system sites of infection (Chow and Troy 2014) and atherosclerotic plaques (Ekstrand et al. 2017) and additionally result from conditions including sarcoidosis (Sarva et al. 2011) and meningitis (Kim 1980), so monocyte dysfunction in these conditions has the potential to severely impact the immune response to disease. Mitochondrial dysfunction therefore may impair monocyte metabolic flexibility, reducing their ability to mediate effector functions. Further, stimulation with some toll-like receptor agonists such as Pam3CSK4 increase both oxidative and glycolytic metabolism (Lachmandas et al. 2016). This is in directly contrast to LPS, which upregulates only glycolytic metabolism (Lachmandas et al. 2016) and was the historical basis for belief that monocytes are primarily glycolytic during inflammation (Cramer et al. 2003; Guida and Stewart 1998; Lang et al. 1985; Orlinska and Newton 1993). Given this new information that oxidative metabolism is increased with certain inflammatory stimuli, it is reasonable to speculate that mitochondrial dysfunction in monocytes may impair their ability to mediate immune responses to some pathogens. More research in this area is warranted.

Monocytes are precursor cells to macrophages and dendritic cells. These cells mediate important functions in peripheral tissues, including phagocytosis, antigen presentation, cytokine and chemokine production, and regulation and resolution of inflammation (Yang et al. 2014). It has been demonstrated that mitochondrial biogenesis is upregulated in monocytes undergoing differentiation to dendritic cells (Zaccagnino et al. 2012) and to macrophages (Daigneault et al. 2010). Aging is known to impair various functions in both cell types (Chougnet et al. 2015; Guo et al. 2014; Jiang et al. 2012), and mitochondrial dysfunction in the monocyte precursor cells is a potential mechanism by which this occurs.

The underlying mechanism for decreased mitochondrial respiration is unknown and is not evaluated here, and this finding could be explained by impaired fatty acid transport or oxidation, electron transport chain dysfunction, or other phenomena. Therefore, additional investigation is needed to delineate the mechanism(s) behind this observation. However, given the above discussion, the importance of this finding is clear in that age-related changes in monocyte mitochondrial metabolism have a clear potential to alter monocyte phenotype and/or function in a variety of ways relevant to human disease.

4.1. Conclusions

In summary, we demonstrated that aging impairs mitochondrial respiration in classical monocytes. The mechanisms by which this occurs are currently unknown, but mitochondrial dysfunction may skew monocytes toward inflammatory phenotypes and impair anti-pathogen and other effector functions. Future research to link mitochondrial function to monocyte phenotype, to confirm functional impairments, and to identify mechanisms by which aging impairs monocyte mitochondrial function is imperative, as monocytes mediate a variety of age-related chronic diseases.

Declarations of interest

None.

Acknowledgments

The authors would like to acknowledge the participants in this study.

Funding

This work was supported by startup funds to BP from the University of Memphis.

References

Bratic, A., Larsson, N.G., 2013. The role of mitochondria in aging. J. Clin. Invest. 123, 951–957.
Buford, T.W., 2017. (dis)trust your gut: the gut microbiome in age-related inflammation, health, and disease. Microbiome 5, 80.
Chougnet, C.A., Thacker, R.I., Shehata, H.M., Hennies, C.M., Lehn, M.A., Lages, C.S., Janssen, E.M., 2015. Loss of phagocytic and antigen cross-presenting capacity in aging dendritic cells is associated with mitochondrial dysfunction. J. Immunol. 195, 2624–2632.
Chow, E., Troy, S.B., 2014. The differential diagnosis of hypoglycorrhachia in adult patients. Am J Med Sci 348, 186–190.
Cramer, T., Yamanishi, Y., Clausen, B.E., Forster, I., Pawlinski, R., Mackman, N., Haase, V.H., Jaenisch, R., Corr, M., Nizet, V., Firestein, G.S., Gerber, H.P., Ferrara, N., Johnson, R.S., 2003. HIF-1alpha is essential for myeloid cell-mediated inflammation. Cell 112, 645–657.
Daigneault, M., Preston, J.A., Marriott, H.M., Whyte, M.K., Dockrell, D.H., 2010. The identification of markers of macrophage differentiation in PMA-stimulated THP-1 cells and monocyte-derived macrophages. PLoS One 5, e8668.
Dominguez-Andres, J., Arts, R.J.W., Ter Horst, R., Gresnigt, M.S., Smeekens, S.P., Ratter, J.M., Lachmandas, E., Boutens, L., van de Veerdonk, F.L., Joosten, L.A.B., Notebaart, R.A., Ardavin, C., Netea, M.G., 2017. Rewiring monocyte glucose metabolism via C-type lectin signaling protects against disseminated candidiasis. PLoS Pathog. 13, e1006632.
Duroux-Richard, I., Roubert, C., Ammari, M., Presumey, J., Grun, J.R., Haupl, T., Grutzkau, A., Lecellier, C.H., Boitez, V., Codogno, P., Escoubet, J., Pers, Y.M., Jorgensen, C., Apparailly, F., 2016. miR-125b controls monocyte adaptation to inflammation through mitochondrial metabolism and dynamics. Blood 128, 3125–3136.
Ekstrand, M., Widell, E., Hammar, A., Akyurek, L.M., Johansson, M., Fagerberg, B., Bergstrom, G., Levin, M.C., Fogelstrand, P., Boren, J., Levin, M., 2017. Depletion of ATP and glucose in advanced human atherosclerotic plaques. PLoS One 12, e0178877.
Franceschi, C., Campisi, J., 2014. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J. Gerontol. A Biol. Sci. Med. Sci. 69 (Suppl. 1), S4–9.
Franceschi, C., Bonafe, M., Valensin, S., 2000. Human immunosenescence: the prevailing of innate immunity, the failing of clonotypic immunity, and the filling of immunological space. Vaccine 18, 1717–1720.
Grip, O., Bredberg, A., Lindgren, S., Henriksson, G., 2007. Increased subpopulations of CD16(+) and CD56(+) blood monocytes in patients with active Crohn's disease. Inflamm. Bowel Dis. 13, 566–572.
Guida, E., Stewart, A., 1998. Influence of hypoxia and glucose deprivation on tumour necrosis factor-alpha and granulocyte-macrophage colony-stimulating factor expression in human cultured monocytes. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 8, 75–88.
Guo, Z., Tilburgs, T., Wong, B., Strominger, J.L., 2014. Dysfunction of dendritic cells in aged C57BL/6 mice leads to failure of natural killer cell activation and of tumor eradication. Proc. Natl. Acad. Sci. U. S. A. 111, 14199–14204.
Hearps, A.C., Martin, G.E., Angelovich, T.A., Cheng, W.J., Maisa, A., Landay, A.L., Jaworowski, A., Crowe, S.M., 2012. Aging is associated with chronic innate immune activation and dysregulation of monocyte phenotype and function. Aging Cell 11, 867–875.
Jiang, M., Xiang, Y., Wang, D., Gao, J., Liu, D., Liu, Y., Liu, S., Zheng, D., 2012. Dysregulated expression of miR-146a contributes to age-related dysfunction of macrophages. Aging Cell 11, 29–40.
Kato, S., Chmielewski, M., Honda, H., Pecoits-Filho, R., Matsuo, S., Yuzawa, Y., Tranaeus, A., Stenvinkel, P., Lindholm, B., 2008. Aspects of immune dysfunction in end-stage renal disease. Clinical journal of the American Society of Nephrology : CJASN 3, 1526–1533.
Kennedy, B.K., Berger, S.L., Brunet, A., Campisi, J., Cuervo, A.M., Epel, E.S., Franceschi, C., Lithgow, G.J., Morimoto, R.I., Pessin, J.E., Rando, T.A., Richardson, A., Schadt, E.E., Wyss-Coray, T., Sierra, F., 2014. Geroscience: linking aging to chronic disease. Cell 159, 709–713.
Kim, R.C., 1980. Rheumatoid disease with encephalopathy. Ann. Neurol. 7, 86–91.
Kramer, P.A., Chacko, B.K., George, D.J., Zhi, D., Wei, C.C., Dell'Italia, L.J., Melby, S.J., George, J.F., Darley-Usmar, V.M., 2015. Decreased bioenergetic health index in monocytes isolated from the pericardial fluid and blood of post-operative cardiac surgery patients. Biosci. Rep. 35.
Lachmandas, E., Boutens, L., Ratter, J.M., Hijmans, A., Hooiveld, G.J., Joosten, L.A., Rodenburg, R.J., Fransen, J.A., Houtkooper, R.H., van Crevel, R., Netea, M.G., Stienstra, R., 2016. Microbial stimulation of different toll-like receptor signalling pathways induces diverse metabolic programmes in human monocytes. Nature microbiology 2, 16246.
Lang, C.H., Bagby, G.J., Spitzer, J.J., 1985. Glucose kinetics and body temperature after lethal and nonlethal doses of endotoxin. Am. J. Phys. 248, R471–8.
Mainwaring, C.J., Williams, M.A., Singer, C.R., Lush, R.J., Smith, J.G., Haynes, C.L., Kelsey, S.M., 1999. Monocyte dysfunction in patients with multiple myeloma and lymphoplasmacytic disorders is related to serum paraprotein levels. Br. J. Haematol. 105, 948–954.
Metcalf, T.U., Wilkinson, P.A., Cameron, M.J., Ghneim, K., Chiang, C., Wertheimer, A.M., Hiscott, J.B., Nikolich-Zugich, J., Haddad, E.K., 2017. Human monocyte subsets are transcriptionally and functionally altered in aging in response to pattern recognition receptor agonists. J. Immunol. 199, 1405–1417.
O'Neill, L.A., Kishton, R.J., Rathmell, J., 2016. A guide to immunometabolism for immunologists. Nat. Rev. Immunol. 16, 553–565.
Orlinska, U., Newton, R.C., 1993. Role of glucose in interleukin-1 beta production by lipopolysaccharide-activated human monocytes. J. Cell. Physiol. 157, 201–208.
Patel, V.K., Williams, H., Li, S.C.H., Fletcher, J.P., Medbury, H.J., 2017. Monocyte inflammatory profile is specific for individuals and associated with altered blood lipid levels. Atherosclerosis 263, 15–23.
Pence, B.D., 2017a. Aging Impairs Mitochondrial Respiratory Capacity in Classical Monocytes. FigShare. https://figshare.com/s/d05c1c037a09683ebf72, Accessed date: 6 April 2018.
Pence, B.D., 2017b. Cell Mito Stress Test for Monocyte Mitochondrial Function. http://dx.doi.org/http://dx.doi.org/10.17504/protocols.io.kw7cxhn. Retrieved February 12, 2018.
Pence, B.D., 2017c. Flow Cytometry for Monocyte Phenotype and Mitochondrial Mass. http://dx.doi.org/http://dx.doi.org/10.17504/protocols.io.kw8cxhw. Retrieved February 12, 2018.
Pence, B.D., 2017d. Isolation of Monocytes from Whole Blood With Magnetic Negative Sorting. http://dx.doi.org/http://dx.doi.org/10.17504/protocols.io.kwtcxen. Retrieved February 12, 2018.
Puchta, A., Naidoo, A., Verschoor, C.P., Loukov, D., Thevaranjan, N., Mandur, T.S., Nguyen, P.S., Jordana, M., Loeb, M., Xing, Z., Kobzik, L., Larche, M.J., Bowdish, D.M., 2016. TNF drives monocyte dysfunction with age and results in impaired anti-pneumococcal immunity. PLoS Pathog. 12, e1005368.
Raulien, N., Friedrich, K., Strobel, S., Rubner, S., Baumann, S., von Bergen, M., Korner, A., Krueger, M., Rossol, M., Wagner, U., 2017. Fatty acid oxidation compensates for lipopolysaccharide-induced Warburg effect in glucose-deprived monocytes. Front. Immunol. 8, 609.
Sarva, H., Chapman, R., Omoregie, E., Abrams, C., 2011. The challenge of profound hypoglycorrhachia: two cases of sarcoidosis and review of the literature. Clin. Rheumatol. 30, 1631–1639.
Shantsila, E., Wrigley, B., Tapp, L., Apostolakis, S., Montoro-Garcia, S., Drayson, M.T., Lip, G.Y., 2011. Immunophenotypic characterization of human monocyte subsets: possible implications for cardiovascular disease pathophysiology. Journal of thrombosis and haemostasis : JTH 9, 1056–1066.
Short, J.D., Tavakoli, S., Nguyen, H.N., Carrera, A., Farnen, C., Cox, L.A., Asmis, R., 2017. Dyslipidemic diet-induced monocyte "priming" and dysfunction in non-human Primates is triggered by elevated plasma cholesterol and accompanied by altered histone acetylation. Front. Immunol. 8, 958.
Stout-Delgado, H.W., Cho, S.J., Chu, S.G., Mitzel, D.N., Villalba, J., El-Chemaly, S., Ryter, S.W., Choi, A.M., Rosas, I.O., 2016. Age-dependent susceptibility to pulmonary fibrosis is associated with NLRP3 Inflammasome activation. Am. J. Respir. Cell Mol. Biol. 55, 252–263.
Strauss-Ayali, D., Conrad, S.M., Mosser, D.M., 2007. Monocyte subpopulations and their differentiation patterns during infection. J. Leukoc. Biol. 82, 244–252.
Tsukamoto, M., Seta, N., Yoshimoto, K., Suzuki, K., Yamaoka, K., Takeuchi, T., 2017. CD14brightCD16+ intermediate monocytes are induced by interleukin-10 and positively correlate with disease activity in rheumatoid arthritis. Arthritis research & therapy 19, 28.
Vincent, G.K., Velkoff, V.A., 2010. The next four decades, the older population in the United States. Current Population Reports P25–1138.
Wildgruber, M., Aschenbrenner, T., Wendorff, H., Czubba, M., Glinzer, A., Haller, B., Schiemann, M., Zimmermann, A., Berger, H., Eckstein, H.H., Meier, R., Wohlgemuth, W.A., Libby, P., Zernecke, A., 2016. The "intermediate" CD14++CD16+ monocyte subset increases in severe peripheral artery disease in humans. Sci. Rep. 6, 39483.
Yang, J., Zhang, L., Yu, C., Yang, X.F., Wang, H., 2014. Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. Biomarker research 2, 1.
Zaccagnino, P., Saltarella, M., Maiorano, S., Gaballo, A., Santoro, G., Nico, B., Lorusso, M., Del Prete, A., 2012. An active mitochondrial biogenesis occurs during dendritic cell differentiation. Int. J. Biochem. Cell Biol. 44, 1962–1969.
Zhang, Y., Zhou, Y., Lou, J., Li, J., Bo, L., Zhu, K., Wan, X., Deng, X., Cai, Z., 2010. PD-L1 blockade improves survival in experimental sepsis by inhibiting lymphocyte apoptosis and reversing monocyte dysfunction. Crit. Care 14, R220.
Ziegler-Heitbrock, L., Ancuta, P., Crowe, S., Dalod, M., Grau, V., Hart, D.N., Leenen, P.J., Liu, Y.J., MacPherson, G., Randolph, G.J., Scherberich, J., Schmitz, J., Shortman, K., Sozzani, S., Strobl, H., Zembala, M., Austyn, J.M., Lutz, M.B., 2010. Nomenclature of monocytes and dendritic cells in blood. Blood 116, e74–80.