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Metabolic homeostasis of tissue macrophages across the lifespan

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Macrophage, Tissue residency, Metabolism, Aging, Organ homeostasis.

Highlights

The metabolic plasticity of tissue-resident macrophages (TMFs) largely diverges from the paradigms established in cultured monocyte-derived macrophages.

TMFs engage a diverging cellular metabolism across organs during homeostasis that supports their tissue-associated functions.

The clearance of specific organ waste products (phagocytosis) by TMFs often leads to organ-specific metabolic adaptions and vulnerabilities of TMFs.

Aging deregulates the tissue-specific balance of function and metabolism of TMFs, which contribute to inflammaging and tissue deterioration.

Abstract

Macrophages are present in virtually all organs. Apart from being immune sentinels, tissue-resident macrophages (TMFs) have organ-specific functions to

maintain homeostasis that require a specialized cellular metabolism. In addition, organ-dependent metabolic adaptations of TMFs appear to be fundamentally distinct in homeostasis and in response to a challenge, such as infection or injury. Moreover, TMF function becomes aberrant with advancing age, contributing to inflammaging and organ deterioration, and a metabolic imbalance may underlie TMF immunosenescence. Here, we outline the current knowledge on the particular metabolic states of TMFs across organs and the relevance for their function. Moreover, we discuss the concomitant decline of metabolic plasticity and functions of TMFs in the elderly highlighting potential novel therapeutic avenues to promote healthy aging.

36 **Functionally specialized macrophages permanently reside in tissues**
37 Known for their role as immune sentinels and for triggering immune responses,
38 macrophages are essential for life of higher order organisms. Infection or tissue
39 injury result in the recruitment of circulating monocytes that locally differentiate
40 into macrophages. Those tissue insult-associated macrophages induce and/or
41 resolve inflammation, clear pathogens and restore organ homeostasis [1,2].
42 Rewiring of the cellular metabolism by these recruited macrophages is a driving
43 force behind their distinct polarization states or activities [3] (**Box 1 and 2**).

44 Macrophages reside in distinct tissue milieus tailored towards the particular
45 function of the organ [4]. Yet, practically every organ harbors different types of
46 **tissue-resident macrophages TMFs** (see Glossary) in the steady-state (**Figure**
47 **1**). TMFs can rapidly detect changes in their environment, such as alterations in
48 metabolite levels, osmolarity, and pH [1–3], and adapt to the eventual
49 environmental constraints to maintain tissue health [1,2]. These adaptations
50 impose tissue-specific metabolic states and vulnerabilities on TMFs [5,6]. TMFs
51 colonize their homing organs early during development from the yolk sac (**Table**
52 **1**) and, once the hematopoiesis moves to the bone marrow, from circulating
53 monocytes [7]. These cells are functionally distinct from inflammation/resolution-
54 associated macrophages. TMFs tightly crosstalk with their neighboring
55 parenchymal or stromal cells, adopting tissue-specific activities that are vital for
56 organ health and function [1,2,6,8–11]. One of their main homeostatic functions
57 is the removal of tissue-specific waste or by-products, including dying or dead
58 cells, extracellular matrix, tissue-specific cell secretions (e.g. surfactant in the
59 lung or myelin in the brain), and subcellular particles such as exophers in the
60 heart or adipose tissue [12,13]. TMFs do so via multiple mechanisms including
61 **phagocytosis** and **efferocytosis** [14]. The clearance of cargos of distinct
62 composition via those different uptake processes requires TMFs to adopt
63 dedicated mechanisms for cargo degradation and recycling of the salvaged
64 nutrients and biological building blocks to fuel their own bioenergetics and
65 anabolism [14]. Thus, TMFs homing different organ niches engage an intricately
66 specialized cellular metabolism machinery (**Box 1**) to survive in their homing
67 environment and facilitate their tissue-specific functions [5,14]. In that sense,
68 TMFs face unique metabolic challenges compared with other cells: the

69 degradation and recycling of their unique ingested cargo, coupled with fueling
70 their own bioenergetics and anabolism.

71 However, the microenvironments, cellular compositions and physiology of tissues
72 are dynamically changing as a consequence of accumulating stresses over a
73 lifetime [15,16]. Aging also changes macrophage biology and functions,
74 decreasing their phagocytic/efferocytic as well as immune-resolving activities,
75 which contributes to their limited regenerative capacity. Instead, aged
76 macrophages become more pro-inflammatory, acting as major drivers of
77 “inflammaging” (chronic low-level inflammation) [17,18]. Those functional
78 changes of macrophages with advancing age are intertwined with intrinsic
79 metabolic alterations, such as mitochondrial dysfunction, **nicotinamide adenine**
80 **dinucleotide (NAD)** deficiency, ER stress, and deregulated nutrient sensing [19–
81 21]. Thus, the metabolic adjustments or dysfunctions of aged TMFs seem to
82 differ, much as the homeostatic metabolism of TMFs varies depending on their
83 homing organ.

84 Here, we highlight recent advances in our understanding of the connections
85 between tissue-specific cellular metabolism and the activities of the main TMF
86 populations across organs. Furthermore, we explore how aging affects the
87 cellular metabolism of TMFs and how, in turn, metabolic alterations contribute to
88 the abnormal functionality of TMFs in the elderly. Of note, most available
89 literature, which is discussed here, is based on studies in 2-4 months old (young)
90 and >12 months old (aged) mice. Data on human TMFs are explicitly highlighted.

91 The metabolic state of tissue-resident macrophages depends on their 92 homing organ

93 Analyses of macrophages in differing organ environments highlight their
94 metabolic plasticity and its link to their functions. First comparative analyses of
95 the physiological transcriptomes of mouse TMFs already found differential
96 expression of genes involved in metabolic processes [10,22,23]. TMFs across
97 different organs differentially express genes encoding solute carrier (SLC) and
98 ATP-binding cassette (ABC) transporters for metabolites, nutrients, ions and
99 other substrates [22]. These early studies identified tissue-specific gene
100 signatures of TMFs that are either specifically induced by certain metabolites,

such as peritoneal TMF-specific genes by vitamin A/retinoic acid [24], or include metabolic processes, such as lipoxin metabolism in heart TMFs and cholesterol/phospholipid handling in liver TMFs [10]. An unbiased transcriptomic analysis of metabolic diversity shows that all mouse TMFs highly express modules containing lipid handling-related genes and low levels of glycolysis, folate, serine and nucleotide metabolism genes compared with other mononuclear cells [25]. This study additionally distinguishes several TMF clusters based on specific lipid (species) metabolism modules [25]. Notably, mouse alveolar macrophages (AMs) accumulate higher amounts of lipids than splenic red pulp macrophages (RPMs), microglia or liver Kupffer cells (KCs) [26]. A targeted analysis of the expression of key metabolic proteins by flow cytometry further resolves the distinct metabolic state of mouse TMFs [27]. TMFs from the liver, spleen, small and large intestine are defined by similar clusters, while brain, lung and peritoneal TMFs appear to be metabolically unique [27]. This is in line with earlier reports showing that the transfer of macrophage progenitors/monocytes or TMFs from one organ to another induces the expression of organ-specific gene signatures by the transferred cells. For example, lipid metabolism-related genes, such as *Pparg,* are induced in monocyte-derived or peritoneal macrophages upon homing into the lung [23,28]. Peritoneal, spleen and liver TMFs show higher expression of GLUT1, PKM and G6PD than other TMFs, indicating an active glucose uptake, glycolysis and pentose phosphate pathway (PPP) [27]. Indeed, peritoneal TMFs more efficiently take up and metabolize glucose than lung AMs [29]. CD38 (NAD metabolism) and CD98 (amino acid transporter) expression is also comparatively higher in spleen and liver TMFs [27]. Lung, spleen, liver and peritoneal TMFs express CD36 that mediates lipid uptake, while peritoneal TMFs show the highest CPT1A (fatty acid [FA] oxidation [FAO]) and ACC1 (lipid biosynthesis) expression. Microglia only express notable amounts of GLUT1 [27]. Consistent with the notion of differential expression of metabolic genes by mouse TMFs across different organs [10,22–24], an unbiased analysis of mRNA expression identified oxidative phosphorylation (OXPHOS) and **electron transport chain (ETC)**-associated genes as the most differentially expressed gene signature across mouse and human TMFs from ten organs [6]. AMs, RPMs, large peritoneal macrophages (LPMs) and KCs expressed higher levels of OXPHOS-related genes than lean

135 WAT-MFs. Of note, those populations with the highest OXPHOS signature were
136 selectively lost upon genetic interference with OXPHOS in mice. Mechanistically,
137 a functional ETC is required by AMs, RPMs and LPMs to handle lipids and
138 cholesterol [6], in line with their high expression of CD36 and CPT1A proteins
139 [27]. OXPHOS dysfunction causes lipid/cholesterol accumulation, cellular stress,
140 cell cycle arrest and apoptosis in those TMF populations, which is rescued by
141 lowering the amount of lipids in the environment [6] in mice.

142 **Tissue-specific crosstalk of metabolism and function of tissue-resident**
143 **macrophages and its age-related changes**

144 *Large and small macrophages in the peritoneum*

145 LPMs (**Table 1**, **Figure 1**) specialize on clearance of dead cells/bacteria as well
146 as immune surveillance, while small peritoneal macrophages (SPMs, **Table 1**,
147 **Figure 1**) sense and regulate inflammation [1,2,5,30]. SPMs and LPMs have
148 active mitochondria and engage high levels of glutamine-driven OXPHOS that
149 aids their diverging responses upon danger sensing in mice [5,29–31]. De novo
150 NAD^+ synthesis via the kynurenine pathway-Sirt3 axis is required to sustain
151 mitochondrial metabolism in thioglycolate-elicited peritoneal MFs and dampens
152 their basal inflammatory state [32]. Yet, despite appearing more catabolic, the
153 bioenergetics of SPMs is less dependent on OXPHOS than that of LPMs [27],
154 the latter showing lipid accumulation and cellular stress upon OXPHOS
155 impairment [6]. Indeed, mouse LPM identity relies on transcription factors that
156 regulate lipid metabolism, such as C/EBPβ, GATA6 and RXRα/β [5]. They highly
157 express the efferocytic receptor TIM4 and numerous lipid-handling factors,
158 including the FA-synthesis enzyme ACC1 [27]. ACC1 inhibition dampens
159 efferocytosis by LPMs [27], in line with the need for lipid synthesis to expand
160 membranes for phagocytosis [33]. CD1d, a molecule involved in lipid antigen
161 presentation, regulates the expression of lipid handling genes and lipid import by
162 mouse LPMs via modulating the internalization of CD36 [34]. Moreover, upon IL-
163 4 exposure or as a late response to TLR4 stimulation, peritoneal macrophages
164 induce SREBP1, a transcription factor that controls lipogenesis, and FA and
165 sterol metabolism. The resulting remodeling of FA metabolism and *de novo*
166 synthesis contributes to anti-inflammatory functions of peritoneal macrophages
167 [35,36]. Accordingly, peritoneal macrophages accumulate triglyceride lipid

168 droplets upon LPS stimulation in mice via the inhibition of lipolysis by hypoxia-
169 inducible lipid droplet–associated (HILPDA) protein. HILPDA-deficient peritoneal
170 macrophages exhibit a lower lipid content and adopt anti-inflammatory features,
171 likely by shuttling triglycerides into the synthesis of prostaglandins [37]. Notably,
172 mouse SPMs exhibit increased expression of the rate-limiting enzyme of FAO,
173 CPT1A, whereas LPMs rely on ACC1 to fuel FAO, indicating preferred
174 bioenergetic pathways [27].

175 Resident LPMs are continuously replaced by monocytes in aging mice [7,38–40].
176 Metabolic alterations influence the decrease in phagocytosis/efferocytosis and
177 increased pro-inflammatory state of aged peritoneal macrophages [18,19].
178 Firstly, mouse $CD11b^+$ peritoneal macrophages display elevated signs of
179 oxidative stress (**reactive oxygen species [ROS]**), lower antioxidant protection
180 (glutathione and catalase activity) and a decreased phagocytic capacity [38].
181 Moreover, the metabolism of anti-inflammatory **glucocorticoids** is defective in
182 aged peritoneal macrophages. Glucocorticoid conversion and expression of the
183 immune-suppressive mediator glucocorticoid-induced leucine zipper (GILZ) are
184 impaired in LPMs from aged compared with young mice, in line with the
185 exacerbated inflammatory responses of aged LPMs to LPS stimulation [41].
186 Additionally, de novo NAD^+ synthesis is reduced in aged thioglycolate-elicited
187 peritoneal macrophages [32]. The drop in NAD^+ production correlates with
188 augmented expression of inflammatory proteins, decreased anti-inflammatory
189 factors as well as reduced OXPHOS and glycolysis in aged versus young human
190 **monocyte-derived macrophages (MDMs)** [32]. Finally, aged mouse **bone**
191 **marrow-derived macrophages (BMDMs)** also show defects in autophagy,
192 which in LPMs associate with metabolic alterations such as an increased
193 **extracellular acidification rate (ECAR)** [40].

194 *Alveolar macrophages in the lung*

195 AMs (**Table 1**, **Figure 1 and 2A**) recycle the lipid-rich pulmonary surfactant and
196 regulate inflammatory responses [1,2,6,29]. Hence, they are poised for lipid
197 metabolism, from cholesterol handling to catabolism and synthesis of FAs,
198 phospho- and other lipids. The homeostasis of AMs is controlled by the lipid
199 metabolism-associated transcriptions factors PPARγ, C/EBPβ, SREBP1, and
200 LXRα/β (cholesterol and oxysterol metabolism) [5,35,42,43]. Signaling via the

201 inhibitory C-type lectin-like receptor NKR-P1B also represses lipid uptake and
202 regulates lipid metabolism in AMs [44]. AMs have a high basal respiration that
203 sustains their lipid and cholesterol handling activity, whereas they poorly engage
204 in glycolysis [6,29,45]. A mutual activation between PPARγ and FABP5 mediating
205 macrophage pro-resolving programming was demonstrated in human AMs, being
206 FABP5 essential for BMDM mitochondrial OXPHOS [46]. Also, lysosomal protein
207 leakage hampers mitochondrial function in mouse AMs , resulting in deregulation
208 of lipid-metabolism genes and lipid accumulation in AMs [6,47]. Mouse and
209 human AMs, in contrast to BMDMs or MDMs (**Box 2**), do not rely on a glycolytic
210 switch upon LPS stimulation. The upregulation of pro-inflammatory mediators by
211 LPS-stimulated AMs requires OXPHOS [48–50] and an elevated mitochondrial
212 activity in AMs correlates with their exaggerated inflammatory responses to LPS
213 treatment in mice [26]. Apart from bioenergetic remodeling, human AMs enhance
214 their metabolism of polyamines, tryptophan and kynurenine upon LPS treatment
215 [51,52]. After exposure to *S. pneumonia*, mouse AMs upregulate the expression
216 of ETC complex I-V proteins and their **oxygen consumption rate (OCR)**, leading
217 to increased ATP concentrations and bacterial control [53]. In line, leptin receptor
218 deficiency of AM, which protects mice from *S. pneumonia*, causes reductions in
219 their ECAR as well as an elevated OCR, accumulation of TCA cycle metabolites
220 and lipids in an AMPK-dependent manner [26]. In contrast, mice harboring lactate
221 dehydrogenase (LDHA)-deficient myeloid cells (including AMs) are unable to
222 combat *M. tuberculosis*, which can block glycolysis in macrophages via NAD^+
223 depletion [54]. Consistently, forcing a glycolytic switch in AMs by metformin
224 treatment in vitro improves their capacity to control *M. tuberculosis* [45]. While
225 blocking glycolysis with 2-deoxyglucose in mice exacerbates the infection,
226 oxamate inhibition of LDH, which directs glucose metabolism through OXPHOS,
227 restricts *M. tuberculosis* replication in macrophages [47]. Importantly,
228 macrophages from the human respiratory tract actually display location-
229 dependent bioenergetic profiles [50]. Human bronchoalveolar lavage (BAL) MFs
230 from central airways, distal bronchus, and alveolar space as well as bronchial
231 fraction (BF) MFs display a predominant mitochondria-driven/OXPHOS
232 metabolism. Oxygen consumption of BAL- and BF-MFs is further increased upon
233 respiratory burst and decreases with exposure to a redox-active air pollutant. In
234 contrast, human induced sputum (IS) MFs (from large central airways) highly

235 engage glycolysis and OXPHOS, which remain unaltered upon stimulation. IS-
236 MFs are also more phagocytic and pro-inflammatory than BAL-MFs [50].

237 The contribution of monocyte-derived cells to the AM pool increases with age to
238 up to 70% in mice [7]. Concomitantly, total numbers of AMs drop with age, they
239 have lower viability in culture and exhibit profound transcriptional changes.
240 Functionally, aged AMs decrease their phagocytic, efferocytic and bacterial
241 clearance activity while adopting a pro-inflammatory state, with an elevated
242 abundance of a pro-inflammatory MARCO^{+} CCL6-secreting AM population, both
243 in mice and humans [53,55–57]. Notably, cellular metabolism and bioenergetics
244 are deregulated in aged AMs, as evidenced by the altered expression of genes
245 involved in the mitochondrial ETC, PPAR signaling, ABC transporters as well as
246 metabolism and absorption of lipids/fat, cholesterol, amino acids and
247 carbohydrates in aged C57BL/6 mice [56,57]. Despite equal protein levels of ETC
248 complexes compared with their young counterparts, aged mouse AMs exhibit an
249 unstable mitochondrial membrane potential and enhanced oxidative stress,
250 failing to increase the expression of ETC complex proteins, their OCR and
251 intracellular ATP levels upon *S. pneumonia* exposure. This correlates with the
252 impaired clearance of *S. pneumonia* by aged versus young AMs, which is
253 ameliorated by pharmacologically reducing oxidative stress [53].

254 *Liver Kupffer cells*

255 KCs (**Table 1**, **Figure 1 and 2B**) balance inflammation versus tolerance, take up
256 pathogens as well as dysfunctional erythrocytes from the circulation and aid the
257 capacity of the liver to manage lipid excess [1,2,8,58]. Hence, they specialize on
258 iron metabolism and recycling and express high levels of the related transcription
259 factors Spi-C and NRF2. Moreover, KCs express PPARγ, LXRα and SREBP1 for
260 their active metabolism of lipids and cholesterol, which requires an active
261 mitochondrial metabolism [5,6,8]. For example, mouse KCs are an important
262 source of cholesteryl ester transfer protein, which is vital for HDL metabolism [59].
263 Interestingly, iron and lipid metabolism seem to be closely balanced by mouse
264 KCs, since their ability to clear LDL from the circulation to shuttle the recycled
265 cholesterol to hepatocytes is elevated upon systemic iron overload [60]. Notably,
266 two distinct KC populations were reported in mice: CD206^{lo} ESAM$^{–}$ CD36^{lo} KC1
267 and CD206^{hi} ESAM^{+} CD36^{hi} KC2. The minor KC2 population seems to have an

268 elevated metabolic activity, expresses high levels of lipid and carbohydrate
269 metabolism-related genes, and can modulate liver metabolism via the FA
270 transporter CD36 [9]. Overall, signatures for iron and lipid homeostasis are also
271 enriched in human resident KCs versus infiltrating pro-inflammatory liver
272 macrophages [61].

273 Healthy aging *per se* does not induce the contribution of monocytes to the KC
274 pool in mice [7], but the aged liver often accumulates lipids and iron [62–64].
275 Accordingly, livers from old animals harbor higher numbers of macrophages/KCs
276 with a pro-inflammatory phenotype [62–65]. Liver injury or inflammation causes
277 KC death and the repopulation of the pool by monocyte-derived cells [58].
278 Overall, these findings suggest the increased contribution of monocyte-derived
279 macrophages to the aged KC population in mice through amassing stresses over
280 a lifetime [62–64]. Aged KC exhibit reduced lysosomal activity and limited
281 clearance of erythrocytes [65]. Impaired efferocytosis and mitophagy is also
282 observed in aged BMDMs, leading to STING activation via ROS production or
283 mt-DNA leakage. Restoration of efferocytosis or STING blockade reduces the
284 extent of sterile inflammatory liver injury in aged mice [66,67]. Colloidal carbon
285 uptake and oxygen consumption are also reduced in aged KCs [68]. The NAD-
286 consuming enzyme CD38 is highly expressed on mouse KCs versus other TMFs
287 [27] and more pro-inflammatory $CD38^+$ KCs accumulate in aged livers. This
288 contributes to decreased NAD^+ levels in tissues of elderly individuals, which
289 associates with age-related diseases [69]. Finally, the reduced expression of
290 GILZ in aged KCs suggests a defective glucocorticoid metabolism [41].

291 *Splenic red pulp macrophages*

292 Beyond immune sentinels, spleen RPMs (**Table 1**, **Figure 1**) are vital for clearing
293 defective erythrocytes and platelets as well as salvaging iron [1,2]. Similar to KCs,
294 they rely on transcription factors to regulate active metabolism of iron (Spi-C and
295 NRF2) and lipids (PPARγ, LXRα and SREBP1) to maintain splenic and red blood
296 cell homeostasis [5,8]. Notably, PPARγ and Spi-C cooperate to maintain the
297 expression of many mouse RPM identity genes. Nevertheless, RPMs rely on
298 PPARγ from their neonatal development onward, but the population declines
299 upon the loss of Spi-C only several weeks after colonization of the spleen [70].
300 RPMs require a functional mitochondrial OXPHOS metabolism to maintain their

301 lipid handling capacity in homeostasis [6]. LPS treatment induced HILPDA
302 expression in mouse splenic macrophages, suggesting an accumulation of
303 triglycerides in lipid droplets to facilitate pro-inflammatory functions [37].

304 Monocytes gradually contribute to the RPM pool with aging [7] and their total
305 abundance also seems to be dynamic over a lifetime in mice [40,65,71]. Aged
306 RPMs express lower levels of the glucocorticoid metabolism regulator GILZ [41]
307 showing limited capacity for autophagy [40]. Notably, iron accumulates in the
308 spleen and RPMs of middle-aged (10-11 month-old) mice due to impaired iron
309 recycling activity and specific downregulation of the iron exporter ferroportin by
310 RPMs [65]. This elevated iron load in aged RPMs leads to increased oxidative
311 stress, mitochondrial dysfunction, compromised phagocytic capability and
312 ferroptosis, reducing the frequency of RPMs in spleens of middle-aged mice [65].
313 Nevertheless, other studies report a consistently elevated abundance of the RPM
314 population in older (20-24 month) versus young mice [40,71], which may suggest
315 a potential adaptation or other metabolic mechanisms that remain to be defined.

316 *Microglia in the central nervous system*

317 Beyond immune functions and efferocytosis, microglia (**Table 1**, **Figure 1 and**
318 **2C**) contribute to the generation of neurons and the formation of neuronal
319 synapses [1,2]. Microglia are characterized by a remarkable metabolic plasticity
320 [72–75]. Their preferred fuel is glucose, which can drive glycolysis or the TCA
321 cycle and OXPHOS, but mouse microglia can also utilize glutamine, FAs, ketone
322 bodies, lactate and other substrates for ATP production in a context-dependent
323 manner [27,72–78]. Interestingly, hexokinase 2 (HK2, rate limiting enzyme in
324 glycolysis) regulates microglia glycolysis and mitochondrial OXPHOS, and HK2
325 loss diminishes microglia OCR, ECAR and migratory behavior in mice [77].
326 Generally, mouse and human resting microglia engage a foremost mitochondrial
327 OXPHOS metabolism and undergo a species-specific glycolytic switch and TCA
328 cycle deregulation upon activation to facilitate their pro-inflammatory activities
329 [79,80]. Mitochondrial function and mitophagy of defective mitochondria notably
330 influence the functions of resting mouse microglia. Mitochondrial OXPHOS
331 metabolism also sustains their phagocytic function [81] and microglial
332 overexpression of PGC-1α, a master regulator of mitochondrial biogenesis,
333 ameliorates their inflammatory responses to ischemic injury and promotes

334 protective auto- and mitophagy [82]. Dysfunctional mitophagy impairs the
335 phagocytic capacity and prompts activation of microglia [83,84]. Upon
336 stimulation, the bioenergetic remodeling and inflammation-promoting activities of
337 microglia are largely independent from mitochondrial dynamics [85] and
338 regulated by the mTOR-HIF-1α pathway [76,86] as well as a balance of
339 mitochondrial translocator protein with HK2 [81]. However, HK2 is upregulated in
340 LPS-stimulated microglia, and its deletion potentiates their inflammatory
341 responses by decreasing mitochondrial membrane potential and increasing ROS
342 production [77]. Yet, in mouse experimental autoimmune encephalomyelitis,
343 microglia undergo a different type of metabolic remodeling and display enhanced
344 ETC complex I activity. This causes reverse electron transport and also leads to
345 ROS production by microglia, which results in neurotoxic damage [87].

346 The postnatal brain is enriched in metabolically active microglia, while different
347 inflammatory microglia subpopulations accumulate in the brain of aged mice
348 [88,89]. Of note, $CD8^+$ T cells in the white matter induce IFN-responsive microglia
349 associated to neurodegenerative disease and aging [89,90] without an overt
350 contribution of monocytes [7]. With aging, microglia often exhibit pro-
351 inflammatory, dysfunctional, and more active states, as well as dysregulated
352 cellular bioenergetics. Glycolysis and mitochondrial OXPHOS metabolism are
353 impaired in aged mouse microglia, shuttling glucose into glycogen synthesis. This
354 bioenergetic constraint of microglia in the aged brain is driven by a prostaglandin
355 E2/EP2 signaling axis, triggering their pro-inflammatory state and subsequent
356 cognitive decline [91]. Mitochondrial dysfunction and ATP-deficiency in aged
357 mouse microglia is also driven by gut microbiota due to elevated intestinal
358 permeability [92]. However, several studies suggest a bioenergetic
359 reprogramming of aged mouse microglia with an increase of glycolysis [93].
360 Moreover, mTOR signaling is upregulated in aged mouse and human microglia,
361 aggravating their inflammatory responses by controlling translation [94]. In line,
362 microglia expressing the human APOE4 allele accumulate in aged mice and
363 exhibit a dysfunctional TCA cycle, altered lipid handling and a HIF1α-mediated
364 switch to glycolysis in concert with adopting pro-inflammatory features [95].
365 Indeed, aged mouse (and human) microglia become lipid-laden and amass lipid
366 droplets and lysosomes/lysosomal storage bodies that contribute to impaired

367 phagocytosis, high levels of ROS and pro-inflammatory cytokines, and the
368 exacerbated reactions of aged microglia to ischemia [96–98]. For example, the
369 disrupted cholesterol handling capacity of aged microglia, which is controlled by
370 expression of the lipid receptor TREM2, limits their ability to clear cholesterol-rich
371 myelin upon (age-related) damage, causing its accumulation in lysosomes and
372 inflammasome activation [11,99]. Interestingly, TREM2 expression is also
373 required to maintain ATP levels in BMDMs and mTOR signaling in microglia in a
374 mouse model of Alzheimer's disease [100]. Of note, a subset of microglia that is
375 specialized on the clearance of myelin emerges in the white matter of aged mice.
376 These cells also depend on TREM2 and express higher levels of glycolytic,
377 lysosomal and cholesterol metabolism-related genes than pro-inflammatory
378 microglia [101].

379 *Cardiac macrophages*

380 Resident cardiac macrophages (RCMs, **Table 1**, **Figure 1**) diversify into several
381 sub-populations [102] that mediate immunity and regulate the functional and
382 metabolic homeostasis of the heart. They aid conduction and are involved in the
383 elimination, via the efferocytic receptor MerTK, of dying cardiomyocytes or
384 damaged mitochondria that were excreted by cardiomyocytes in exophers
385 [5,12,103–105]. LPS-induced cardiac inflammation [106] and myocardial
386 infarction (MI) decreases MerTK expression on mouse cardiac macrophages
387 through specific cleavage in a HIF-1α-dependent fashion, which hampers tissue
388 repair [103,107]. In concert, HIF-2α is upregulated and limits anti-inflammatory
389 features in those cells, likely by suppressing mitochondrial metabolism via
390 HILPDA upregulation and reduced lipolysis [107]. Indeed, mouse CCR2-
391 dependent (monocyte-derived) cardiac macrophages undergo a dynamic
392 metabolic reprogramming after MI with a rapid early increase in their glycolytic
393 rate and in the PPP as well as a decrease in OXPHOS, which however is largely
394 normalized after about a week [108]. In fact, the uptake of dying cells by mouse
395 cardiac macrophages after MI increases their intracellular FA levels, driving
396 mitochondrial OXPHOS. FAO and OXPHOS increase the transcription of the anti-
397 inflammatory cytokine IL-10 by cardiac macrophages through the NAD^+-SIRT1-
398 Pbx1 signaling axis [109]. Of note, monocyte-derived cells are rapidly recruited
399 to the heart and notably contribute to the cardiac macrophage pool throughout MI

400 in mice [104] and their MI-mediated metabolic alterations do not seem to be
401 intrinsic to RCMs [108].

402 Similarly, with increasing age, the self-renewal capacity of mouse RCMs declines
403 [102], although their numbers are unaltered [106]. However, the presence of
404 CCR2^{+} (monocyte-derived) pro-inflammatory macrophages increases in the
405 heart of aged mice, which are recruited by cardiac mesenchymal stromal cells
406 [110]. Nevertheless, the abundance of MerTK-expressing cardiac macrophages
407 also seems to increase with age, although the hearts of aged mice contain higher
408 frequencies of inflammatory cells than their young counterparts. Moreover, aged
409 mice are more susceptible to LPS-induced cardiac inflammation and electrical
410 dysfunction [106], which is likely caused by malfunctioning RCMs [105].
411 Interestingly, expression of the mitochondrial Ca^{2+} uniporter is decreasing in the
412 heart over a lifetime, which could contribute to an inflammatory activation of aged
413 cardiac macrophages [21]. Notably, treatment with an LXR agonist ameliorated
414 cardiac electric function of the heart by elevating the numbers of total and MerTK-
415 expressing RCMs in aged LPS-stimulated mice [106]. More details on RCM
416 immunometabolism can be found in a recent review [111].

417 *Kidney-resident macrophages*

418 Renal macrophages scavenge circulating immune complexes and contribute to
419 vascular and bud development in the kidney. They comprise a large population
420 of kidney-resident macrophages (KRMs) and numerically less bone marrow-
421 derived kidney macrophages (BMKMs, **Table 1**, **Figure 1**) [2,5,112]. Resting
422 renal macrophages appear metabolically quiescent, although glucose uptake,
423 glycolysis and mitochondrial respiration rates are higher in mouse KRMs than
424 BMKMs [112]. Indeed, kidney macrophages are associated with a comparatively
425 high OXPHOS signature but are less affected by impairment of OXPHOS than
426 other TMFs [6]. Macrophages from kidneys of a mouse model of lupus nephritis
427 upregulate gene signatures of glycolysis and OXPHOS and downregulate FA-
428 metabolism genes compared with controls [113]. In line, upon treatment with
429 immune complexes, both renal macrophage populations enhance their glycolytic
430 flux that is required for TNFα production, although KRMs outperform BMKMs
431 [112]. Inhibition of glucose uptake indeed reduces kidney inflammation upon
432 immune complex or nephrotoxic serum administration to mice [113]. In turn,

433 mitochondrial and iron homeostasis is hampered in mouse (and human) renal
434 macrophages during kidney fibrosis [114,115]. PINK1/Parkin-mediated
435 mitophagy is impaired in KRMs and they accumulate abnormal mitochondria.
436 Loss of PINK1 or the mitochondrial dynamic regulator mitofusin 2 further
437 aggravates inflammation in mouse models of kidney fibrosis [114]. Macrophages
438 also upregulate transferrin receptor 1 and are iron-deficient in fibrotic mouse
439 kidneys, which compromises their antioxidant response, promotes pro-
440 inflammatory activation and drives disease pathology [115].

441 Inflammation-promoting monocyte-derived CCR2$^+$ macrophages are recruited to
442 kidneys of aged mice by mesenchymal stromal cells, despite comparable
443 numbers of F4/80$^+$ CCR2$^-$ KRMs in young mice [116]. However, eventual
444 metabolic alterations in aged renal macrophages remain to be investigated.

445 *White adipose tissue-resident macrophages*

446 Macrophages that reside in lean WAT-MFs (**Table 1**, **Figure 1**) promote lipid
447 storage as well as fat homeostasis and are generally metabolically quiescent,
448 showing low dependency on OXPHOS [2,5]. Interestingly, the transfer of
449 damaged mitochondria from adipocytes to WAT-MFs maintains WAT
450 homeostasis, but it is compromised upon obesity in mice [13]. Moreover, in
451 contrast to pro-inflammatory BMDMs (**Box 2**), inflammatory macrophages that
452 infiltrate obese WAT rely on mitochondrial metabolism to handle the lipid overload
453 due to overnutrition [6]. Indeed, mouse WAT-MFs undergo a dramatic remodeling
454 of their subset composition, functions and cellular metabolism towards a pro-
455 inflammatory state upon WAT hypertrophy and obesity [5,17]. A similar, but not
456 equal, PPARγ-dependent pro-inflammatory activation of WAT-MFs and
457 development of obesity is also associated with advanced aging [69,117]. Despite
458 similar total macrophage content, the relative contribution of CD38$^+$ WAT-
459 resident macrophages is increased with age in mice. Similar to the liver, pro-
460 inflammatory factors, locally released by senescent cells, induce CD38
461 expression by WAT-MFs, which depletes NAD$^+$ in aged WAT [69]. The
462 expression of genes regulating **catecholamine** degradation is also increased in
463 WAT-MFs from old versus young mice in an inflammasome-dependent manner
464 [118]. Hence, the use of triglycerides from adipocytes for energy production is
465 impaired in the elderly and associates with WAT accumulation and systemic

466 metabolic dysfunction. Aged NLRP3-deficient mice have an improved lipolysis
467 capacity compared with wild type controls, which correlates with normalized
468 expression of genes involved in the bioamine degradation pathway in WAT-MFs
469 [118]. However, subsequent analyses of the WAT of elderly human individuals
470 revealed an upregulation of catecholamine-degrading enzymes in adipocytes
471 rather than in WAT-MFs, highlighting species-specific mechanisms that
472 contribute to defective WAT lipolysis with aging [119].

473 *Langerhans cells*

474 In the elderly, wound healing is often impaired and associates with aberrant
475 inflammation [120]. Langerhans cells (LCs, **Table 1**, **Figure 1**) colonize the skin,
476 cornea and mucosal tissues, where they maintain barrier immunity [2]. Notably,
477 LCs are less abundant and poorly proliferate in healthy and wounded skin of aged
478 versus young mice. During wound healing, aged LCs upregulate inflammatory
479 pathways and downregulate metabolic processes, foremost mitochondrial
480 OXPHOS [120]. In concert, genetic interference of mitochondrial respiration in
481 LCs causes a decline of the population in young mice, which associates with a
482 compromised lipid metabolism in other TMFs [6]. In line, impairing FA synthesis
483 and lipid handling alters LC function and is associated with psoriasis [121]. LCs
484 use autophagy to recycle lipids and defects in this process, for example upon
485 excessive efferocytosis of dead cells in psoriatic skin, lead to lipid accumulation
486 [121]. Of note, autophagy is often compromised in aged macrophages and
487 causes aberrant metabolism and function [40]. Moreover, in elderly humans,
488 keratinocytes express lower levels of CXCL14, which limits monocyte infiltration
489 [122]. All these factors may contribute to reducing LC numbers in the skin of aging
490 individuals [120,122].

491 *Intestinal macrophages*

492 Intestinal macrophages (IMs, **Table 1**, **Figure 1**) comprise diverse subsets and
493 are important regulators of barrier integrity and gut homeostasis [1,123]. They
494 can ameliorate colitis and support the proliferation of colonic epithelial cells by
495 providing polyamines, which IMs synthesize in an mTORC1-dependent manner
496 [124]. Generally, in the human colon, lamina propria-associated macrophages
497 (LAMs) express lower levels of FAO-related genes compared with epithelial-
498 associated macrophages [125]. LAMs from patients with inflammatory bowel

499 disease further downregulate genes controlling fatty acid metabolism and
500 mitochondrial OXPHOS, while upregulating glycolytic genes in comparison with
501 LAMs from healthy donors [125]. Likely, the particular anatomical niche or
502 location of different IMs may impact their immunometabolism. Accordingly, the
503 metabolic states of IMs are controlled by the composition of ingested diet and the
504 gut microbiota, a topic recently reviewed [126]. For example, the dietary sugar
505 mannose reduces the pro-inflammatory activity of colonic macrophages during
506 colitis in mice. Mannose exposure elevated the abundance of glycolytic
507 metabolites and reduced the ECAR of LPS-stimulated macrophages in concert
508 with a GAPDH-mediated repression of the translation of TNFα [127]. Moreover,
509 ATP derived from microbes acts on myeloid cells to induce CSF2 production by
510 innate lymphoid cells type 3 that are located in solitary isolated lymphoid tissues
511 (SILT) in the mouse intestine. This drives the development of a specific SILT-
512 associated macrophage population, which exhibits a higher mitochondrial
513 membrane potential, enhanced ROS production and elevated expression of
514 OXPHOS- and phagocytosis-related genes than LAMs [123]. Commensal
515 microbes also secrete immunomodulatory metabolites such as short-chain fatty
516 acids (SCFAs, e.g. butyrate). Oral butyrate supplementation increases FAO and
517 OXPHOS in mouse IMs and ex vivo butyrate exposure reduces LPS-mediated
518 pro-inflammatory activation of IMs, which suggests that SCFAs mediate
519 hyporesponsiveness against commensal microbes [128]. However, eventual
520 alterations of the immunometabolism of IMs over the lifespan largely remain to
521 be investigated.

522 **Concluding remarks**

523 TMFs exist in a delicate metabolic balance with their homing tissue to maintain
524 homeostasis. The sensing of danger or pathogens and the removal of
525 extracellular components via phagocytosis or dead cells through efferocytosis are
526 common activities of the majority of TMFs. However, the metabolic remodeling of
527 TMFs upon pathogen recognition appears to be tissue- and context-dependent.
528 Moreover, the particular ingested cargos are tissue-specific and require a
529 dedicated handling or recycling machinery that differs largely. Hence, TMFs tailor
530 their cellular metabolism for their vital tissue-specific homeostatic activities. This
531 functional specialization may cause tissue-dependent metabolic vulnerabilities of

532 TMFs to alterations in their environment. This becomes particularly evident in
533 aged tissues. Generally, those cells adopt a more pro-inflammatory profile with
534 often reduced phagocytic capacities over the lifespan. Aged TMFs change along
535 with their environment, often exhibiting tissue-specific aberrant functions that are
536 driven by adjustments in their intrinsic metabolism. The extrinsic causes to the
537 age-related dysfunctions of TMFs remain poorly understood. Potential
538 contributors are the age-related impairment of the gut barrier function, which
539 induces systemic low-grade inflammation, and other local tissue perturbations,
540 such as age-related metabolic alterations of parenchymal cells [15,16,19,92].
541 Possibly, this altered functionality and metabolism could also be influenced by a
542 larger contribution of monocyte-derived cells to the aged TMF pool, which
543 remains to be further investigated. In turn, dysfunctional TMFs directly contribute
544 to organ deterioration with advanced age, locally and systemically, by losing their
545 homeostatic activities, inducing a pro-inflammatory phenotype and other
546 (metabolism-related) mechanisms [11,41,55,56,66,69].

547 Notably, in many instances, the requirement of metabolic states to regulate
548 functional features of TMFs seems not to be generalizable, but dependent on
549 their particular activity in their homing organ and sub-tissular niche. We are only
550 beginning to uncover the mechanisms of how TMFs orchestrate their tissue-
551 specific functional and metabolic balance (see Outstanding Questions). While our
552 knowledge on the immunometabolism of microglia, peritoneal, lung and adipose
553 tissue macrophages is comparatively advanced across the lifespan, it remains
554 largely elusive for other TMF populations. Functional studies on spleen, liver,
555 heart, intestine, skin and kidney macrophages are limited, especially in aging.
556 Moreover, numerous other TMF populations were not covered here due to the
557 lack of available data. Moreover, most of our knowledge on TMF metabolism was
558 generated in mice. In some instances, species-specific variations were reported
559 in human TMFs [80,119]. Nevertheless, the main aspects of the metabolic
560 heterogeneity of mouse TMFs across organs were confirmed in humans, in
561 particular their tissue-specific functional dependence on mitochondrial OXPHOS
562 metabolism [6,20]. A combination of metabolic profiling (e.g. transcriptome,
563 proteome and metabolome) of human TMFs in concert with specific mouse
564 models for functional studies will aid filling those knowledge gaps. Understanding

565 the specific metabolic biology of TMFs holds a strong therapeutic potential to
566 control their activities in a location-specific manner [6,91], although further
567 research is required to reveal the mechanistic underpinnings. Revealing those, it
568 may be possible to extend healthy aging of TMF's homing organs by harnessing
569 their specific metabolism.

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590 ## Disclosure of interest

591 SKW serves as scientific adviser for ONA therapeutics. SF is a shareholder of
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Glossary

Bone marrow-derived macrophages (BMDM): cells differentiated from mouse bone marrow in culture under presence of macrophage-colony stimulating factor (M-CSF). Standard model for culturing primary macrophages, which can be polarized to M1/M2 states in response to different stimuli (see also **Box 2**).

Catecholamines: biogenic amine neurotransmitters released in response to stress. Sympathetic nerve-derived catecholamines induce the first step of triglyceride lipolysis in adipocytes to use free fatty acids for energy production. Macrophages sequester excess extracellular catecholamines to limit their activity.

Efferocytosis: immunologically silent uptake of apoptotic cells via specific surface receptors (e.g. TAM family receptors, TIM4, αV integrins) or soluble opsonins (e.g. Gas6, MFGE8, CD93), which are expressed by macrophages. It involves the formation of lamellipodia and membrane ruffles to engulf apoptotic bodies in efferosomes for elimination by acidification (similar to phagolysosome fusion).

Electron transport chain (ETC): core of oxidative phosphorylation (OXPHOS) for mitochondrial respiration, where all catabolic processes converge. The ETC contains Complex I to IV that accept (Complex I and II) and transport electrons derived from the oxidation of nutrients to generate a proton gradient across the inner mitochondrial membrane. These electrons are transferred to oxygen by Complex IV, while Complex V (ATP synthase) uses this membrane potential to produce ATP.

Extracellular acidification rate (ECAR): parameter calculated during extracellular flux analyses based on changes in pH. Indicator for glycolysis and other processes that acidify the milieu.

Glucocorticoids: steroid hormones. Macrophages locally convert glucocorticoids, such as cortisone, to their active anti-inflammatory form via the enzyme 11β-hydroxysteroid dehydrogenase type 1. Binding of active glucocorticoids to the transcription factor glucocorticoid receptor triggers the expression of glucocorticoid-induced leucine zipper (GILZ).

964 **Monocyte-derived macrophages (MDM)**: cells generated from ($CD14^+$) human
965 blood monocytes in vitro by treatment with M-CSF (pro-inflammatory) and/or
966 granulocyte-macrophage CSF (GM-CSF, anti-inflammatory) (see also **Box 2**).

967 **Nicotinamide adenine dinucleotide** (**NAD**): electron carrier involved in redox
968 reactions. Oxidized NAD^+ accepts electrons from metabolic reactions to convert
969 into its reduced form, NADH. NADH then donates electrons for chemical
970 reactions, for example to complex I of the ETC. NAD^+ is mainly located in
971 mitochondria and can be salvaged or newly synthesized from amino acids such
972 as tryptophan.

973 **Oxygen consumption rate (OCR)**: parameter calculated during extracellular flux
974 analyses based on oxygen concentration. Indicator for mitochondrial
975 respiration/OXPHOS.

976 **Phagocytosis:** specific receptor-mediated form of endocytosis aimed at clearing
977 large particles, cells or microbes. Those are recognized by professional
978 phagocytes like macrophages via non-opsonic (e.g. CD14, CD206, TLR4) and/or
979 opsonic receptors (e.g. Fc receptors) and internalized into phagosomes through
980 cytoskeleton rearrangements. Subsequent fusion with lysosomes results in
981 gradual acidification and phagolysosome formation for particle degradation.

982 **Reactive oxygen species (ROS)**: molecules containing at least one oxygen
983 atom with one or more unpaired electrons (free radicals) that are highly reactive,
984 for example superoxide radicals.

985 **Tissue-resident macrophages (TMF)**: cells that permanently reside in organs
986 (see also **Table 1**). They are either embryo-derived and proliferate locally to
987 maintain their population in tissues or differentiate from blood monocytes in their
988 respective organ. TMFs are highly adapted to their homing tissue and important
989 for organ homeostasis.

990 ## Text Boxes

991 **Box 1. Overview on cellular metabolic pathways engaged by macrophages**
992 Macrophages engage various metabolic pathways, closely connected with their
993 functional state and in adaptation to their environment. The metabolic toolbox of
994 macrophages comprises a complex network of interconnected metabolic circuits
995 integrating different nutrient sources, such as lipids for fatty acid oxidation (FAO),
996 simple sugars for glycolysis, and amino acids that can be metabolized through
997 various entry points (e.g. glutaminolysis or the urea cycle). In this network, the
998 amphibolic TCA cycle acts as the central mitochondrial metabolic hub for most
999 bioenergetic/catabolic and biosynthetic/anabolic processes. Firstly, metabolites
1000 may be fully oxidized to generate redox potentials in the form of NADH and
1001 $FADH_2$, supplying electrons to the ETC in the mitochondria for oxidative
1002 phosphorylation or generation of ROS [3]. NAD can be synthesized de novo from
1003 tryptophan through the kynurenine pathway, which is predominant in steady state
1004 macrophages, or recycled from NAM^+ via the NAD salvage pathway [20].
1005 Secondly, nutrient-derived metabolites can be used for the production of cellular
1006 building blocks. Thirdly, metabolites may be stored in intracellular deposits, for
1007 example, in the form of glycogen (metabolized from glucose) or lipid bodies
1008 (triglycerides surrounded by a phospholipid membrane containing cholesterol)
1009 [3]. A high capacity to handle distinct lipid species, such as triglycerides,
1010 phospholipids and sterol/cholesterol, is a particular characteristic of
1011 macrophages. While triglycerides and phospholipids can eventually be fully
1012 catabolized, macrophages actively efflux excess cholesterol forming extracellular
1013 HDL [5]. Finally, many TCA cycle intermediates produced by macrophages may
1014 function as signaling metabolites to other cells [3].

1015 **Box 2. The metabolic paradigm of cultured M1/M2 polarized macrophages**
1016 M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophage polarization
1017 arises from cultured BMDMs or MDMs stimulated with LPS/IFN-γ or IL-4,
1018 respectively [3,5,19,20]. Classically activated M1 macrophages exhibit increased
1019 (aerobic) glycolysis, ROS generation, inducible nitric oxide synthase (iNOS)
1020 expression and a pro-inflammatory cytokine profile. Increased glucose uptake is
1021 accompanied by the expression of a highly active isoform of the rate-limiting

1022 enzyme of glycolysis, phosphofructosekinase 2 (uPFK2). Shunting of glycolytic
1023 intermediates into the oxidative branch of the pentose phosphate pathway (PPP)
1024 generates NADPH for antimicrobial ROS production by NADPH oxidases. Two
1025 breaks in the TCA cycle of M1 macrophages at isocitrate dehydrogenase 1
1026 (IDH1) and succinate dehydrogenase (SDH) decrease electron flow into the ETC.
1027 IDH1 converts isocitrate to α-ketoglutarate (αKG) and its downregulation causes
1028 citrate and cis-aconitate accumulation, which can serve for lipid synthesis and
1029 itaconate production, respectively. Itaconate inhibits SDH, thereby facilitating the
1030 second break in the TCA cycle. Succinate accumulation stabilizes the
1031 transcription factor HIF-1α, driving pro-inflammatory gene expression. Further, its
1032 rapid oxidation by SDH can lead to reverse electron transport due to the depletion
1033 of the electron carrier coenzyme Q, resulting in excessive mitochondrial ROS
1034 generation [3,5,19,20]. Moreover, M1 macrophages can export citrate into the
1035 cytoplasm to generate acetyl-CoA, which regulates histone acetylation of pro-
1036 inflammatory genes [129]. Yet, mitochondrial oxidative stress can reprogram
1037 macrophages towards aerobic glycolysis. This reduces mitochondrial ROS and
1038 acetyl-CoA production that are required for inflammasome activation and histone
1039 acetylation, respectively [130].
1040 Alternatively activated M2 macrophages display increased OXPHOS, arginase 1
1041 expression and an anti-inflammatory cytokine profile. M2 macrophages rely on
1042 increased FAO-derived acetyl-CoA, enabled by CD36 mediated uptake of fatty
1043 acids, and anaplerotic glutamine metabolism to fuel the TCA cycle.
1044 Glutaminolysis generates αKG, which is a TCA cycle intermediate and acts as
1045 cofactor for the histone demethylase JMJD3 to orchestrate anti-inflammatory
1046 gene expression [3,5,19,20]. To reduce ROS production by the oxidative PPP,
1047 M2 macrophages increase expression of the sedoheptulose kinase, CARKL, to
1048 drive the non-oxidative branch of the PPP and decrease PPP flux [14]. Lastly,
1049 conversion of arginine into polyamines by arginase 1 increases mitochondrial
1050 protein expression and supports cell proliferation [5].
1051 While the metabolic paradigms established in cultured M1/M2 macrophages
1052 formed the basis of macrophage immunometabolism and serve as easy-to-
1053 manipulate model system, caution is needed when extending these results to
1054 TMFs. Environmental conditions in vitro are not comparable to the specific organ
1055 and tissue milieus that are key for the metabolic adaptations of TFMs.

1056 ## Outstanding Questions Box

1057 What are the metabolic states of TMF populations in different organs, also
1058 according to their sub-tissular niches?

1059 Are metabolic adaptions and requirements of TMFs conserved between mice and
1060 humans in youth and age? Are there relevant sex-differences?

1061 How much are metabolic adaptions of TMFs solely required for a particular
1062 activity (e.g. cargo degradation)? To what extent are the surrounding milieu and
1063 nutrient availability involved?

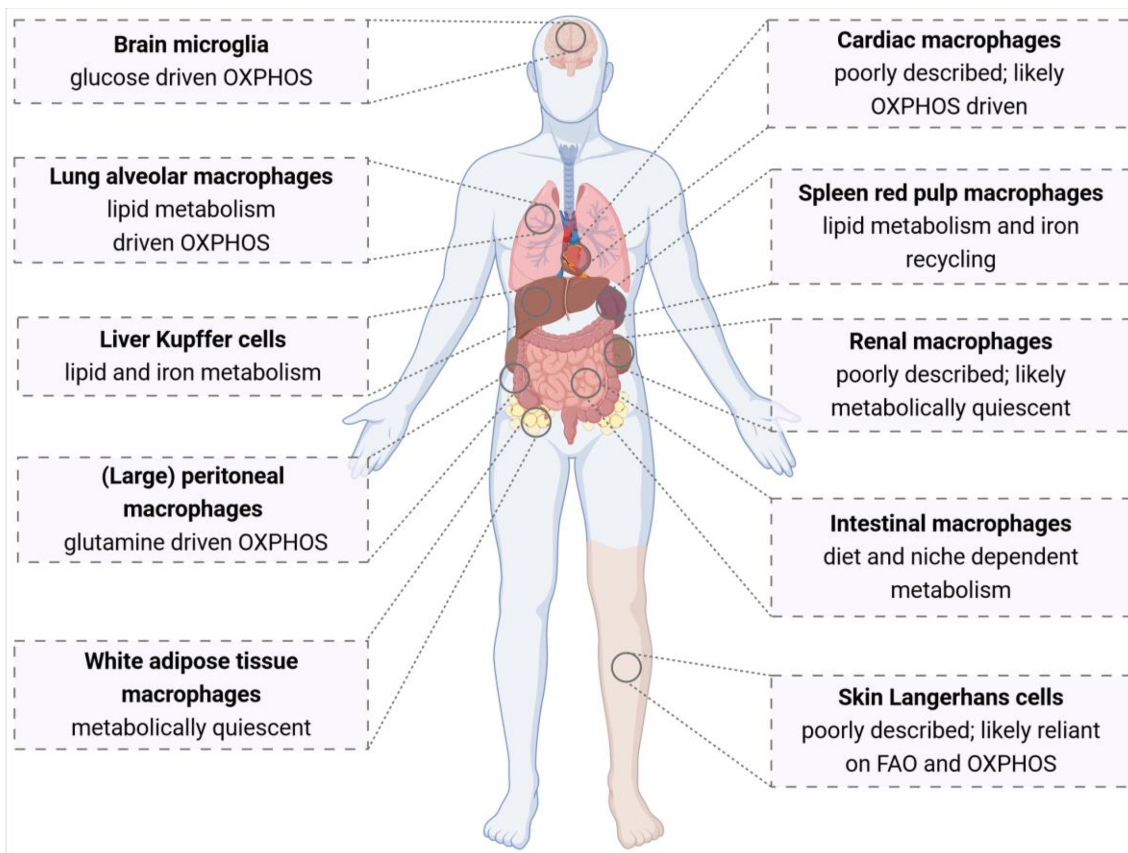
1064 How can infectious or non-infectious danger signals and changing environments
1065 in the tissue affect the metabolic and functional features of TMFs (in aging)? What
1066 are the precise local and systemic mechanisms?

1067 Conversely, how does metabolic-functional impairment of aged TMFs cause
1068 tissue dysfunction? What triggers and can break this vicious cycle?

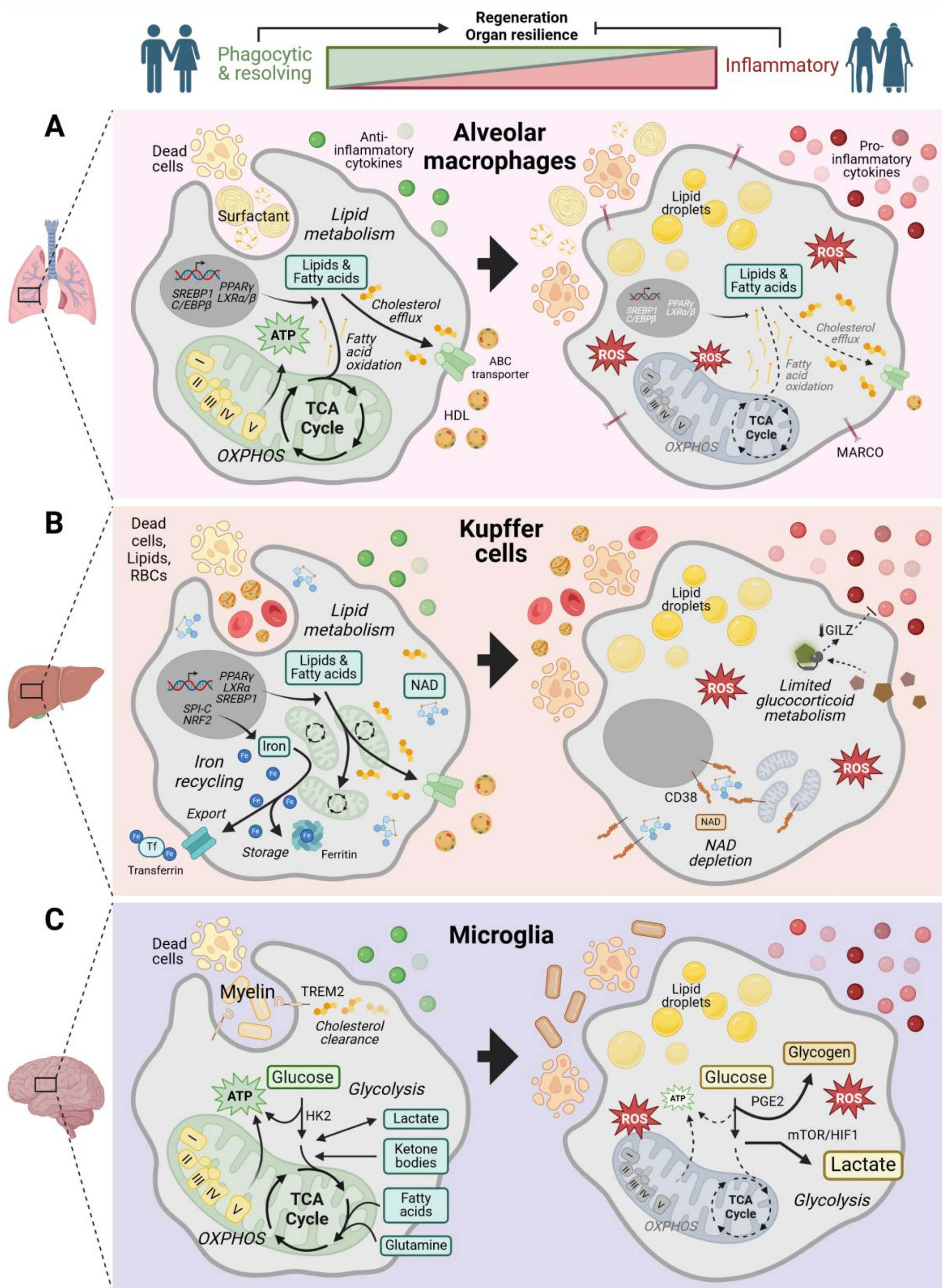
1069 How can metabolic states dictate the activity of particular TMFs? Can we
1070 modulate TMF metabolism to control their activities on a tissue-specific level and
1071 how?

1072 Can tissue-specific metabolic requirements or vulnerabilities of TMFs offer new
1073 therapeutic targets without undesired systemic effects to facilitate healthy aging?

1074 ## Figure legends



1075 **Figure 1. Overview on location and metabolic characteristics of main tissue**
1076 **macrophage populations**
1077 The immunometabolism and homing organs of the most studied TMF populations
1078 in the steady-state is illustrated. Of note, the depicted metabolic states of TMFs
1079 are largely based on findings in mice. Figure created with BioRender.com



1080 **Figure 2. Metabolic specialization by selected tissue macrophages and**
1081 **alterations with advancing age**
1082 The upper panel shows the general activities of TMFs and their changes over a
1083 lifetime.

1084 (A) Alveolar macrophages (lung) take up surfactant and dead cells, engage an
1085 active lipid metabolism program, and harbor actively respiring mitochondria in
1086 young mice (left). In aged mice (right), alveolar macrophages adopt a pro-
1087 inflammatory state, have a diminished capacity to handle lipids, accumulate
1088 dysfunctional mitochondria and produce ROS.
1089 (B) Kupffer cells (liver) from young mice remove red blood cells, excess lipids,
1090 and dead cells, mainly via scavenger receptors. Their metabolic state is tailored
1091 towards recycling iron and lipid metabolism (left). Upon aging (right), Kupffer cells
1092 become pro-inflammatory, produce ROS, and express NAD-depleting molecules
1093 as well as inhibitors of glucocorticoid activation.
1094 (C) Microglia (brain) clear myelin as well as dead cells and fuel their active
1095 glycolytic and mitochondrial bioenergetics with a context-dependent diversity of
1096 nutrients (foremost glucose) in young mice (left). In older mice (right),
1097 mitochondrial activity declines in microglia, they are more pro-inflammatory,
1098 accumulate ROS, produce glycogen and, in some cases, enhance glycolysis.
1099 Figure created with BioRender.com

Tables

Population name	Organ/tissue	Main markers (mouse)	Origin	Monocyte-contribution over a lifetime[a]
Large peritoneal macrophages (LPMs)	Peritoneum	F4/80^{hi} CD11b^{hi} MHCII^{lo}	Yolk sac EMPs, self-maintain	<5% --> 40%
Small peritoneal macrophages (SPMs)	Peritoneum	F4/80^{lo} CD11b^{lo} MHCII^{hi}	Adult monocytes	/
Alveolar macrophages (AMs)	Lung	CD11c^{+} SiglecF^{+} CXC3R1^{-}	Yolk sac EMPs[b], self-maintain	10% --> 70%
Kupffer cells (KCs)	Liver	F4/80^{+} Clec4F^{+} TIM4^{+}	Yolk sac EMPs[b], self-maintain	<5%
Red pulp macrophages (RPMs)	Spleen	F4/80^{+} VCAM1^{+} CD11b^{lo}	Yolk sac EMPs[b], self-maintain	<5% --> 50%
Microglia	CNS	F4/80^{+} CX3CR1^{+} CD11b^{+}	Yolk sac EMPs, self-maintain	0%
Resident cardiac macrophages (RCMs)	Heart	CD64^{+} CX3CR1^{+} TIM4^{+}	Yolk sac EMPs[b], self-maintain	Yes, % TBD
Kidney resident macrophages (KRM)	Kidney	F4/80^{hi} CX3CR1^{+} CD11b^{+}	Yolk sac EMPs[b], self-maintain	5% --> 40%
BM-derived kidney macrophages (BMKM)	Kidney	F4/80^{hi} CX3CR1^{+} CD11b^{+}	Adult monocytes	/
White adipose tissue macrophages (WAT-MF)	Fat (lean)	F4/80^{+} CD11b^{+} CD206^{+}	Yolk sac EMPs, self-maintain	Yes, % TBD
Langerhans cells (LCs)	Epidermis & others	F4/80^{+} EpCAM^{+} CD11b^{+}	Yolk sac EMPs[b], self-maintain	<5%
Intestinal macrophages (IMs)	Gut	F4/80^{+} CD11b^{+} (TIM4)$^{+}$	Not well defined[c]	/

Table 1. Characteristics of main tissue resident macrophage populations
Information is compiled from [1,2,5,7]. CNS, central nervous system; EMP, erythro-myeloid progenitor; ^{hi}, high; ^{lo}, low; TBD, to be determined. [a] from birth to about 36 weeks of age in mice. [b] some studies describe fetal liver monocytes as (additional) origin of those TMF populations. However, fetal liver monocytes are currently considered to derive from yolk sac EMPs themselves. [c] the TIM4^{+} sub-populations appears to be embryonically-derived.