Social isolation of aged mice drives dramatic release of inflammatory lipoxygenase-derived oxylipins

Mareike Wichmann-Costaganna[1], Raphaëlle Petit[2], Julia Lindner[2,3], Madlen Haase[2], Vivien Bachmann[1], Robert Klaus Hofstetter[1], Markus Werner[1], Christiane Frahm[2,#], Oliver Werz[1,#], Patrick Schädel[1,#]

[1]Department of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich Schiller University Jena, Germany

[2]Department of Neurology, Jena University Hospital, Am Klinikum 1, 07747 Jena, Germany

[3]Department of Hematology, Cellular Therapy, Hemostaseology and Infectious Diseases, University Hospital Leipzig, University of Leipzig Medical Center and Comprehensive Cancer Center Central Germany (CCCG), Leipzig-Jena, Germany

[#] shared correspondence

Address for correspondence (Patrick Schädel):

Department of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich Schiller University Jena, Philosophenweg 14, 07743 Jena, Germany

email: patrick.schaedel@uni-jena.de; telephone number: +49 (0) 3641 9-49815

Address for correspondence (Oliver Werz):

Department of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich Schiller University Jena, Philosophenweg 14, 07743 Jena, Germany
email: oliver.werz@uni-jena.de; telephone number: +49 (0) 3641 9-49801

Address for correspondence (Christiane Frahm):
Hans Berger Department of Neurology, Jena University Hospital, Am Klinikum 1, 07747 Jena, Germany
email: christiane.frahm@med.uni-jena.de; telephone number: +49 (0) 3641 9-325909

ORCiD: Mareike Wichmann-Costaganna (0000-0002-8215-5799), Julia Lindner (0000-0002-9805-5995), Madlen Haase (0000-0001-8803-6347), Raphaëlle Petit (0000-0002-0405-3180), Vivien Bachmann (0009-0000-2246-1682), Robert K Hofstetter (0000-0002-1077-9703), Markus Werner (0000-0002-0298-5136), Christiane Frahm (0000-0003-2234-7390), Oliver Werz (0000-0002-5064-4379), Patrick Schädel (0000-0002-9586-6653)

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Abstract

Oxylipins, signalling molecules derived from polyunsaturated fatty acids, act as key mediators controlling inflammatory processes. Ageing fuels the disruption of this network, promoting inflammageing. Social isolation, a common feature of ageing, may contribute to the emergence of pro-inflammatory responses, further aggravating conditions like cognitive decline and frailty. Here, we studied how repeated social isolation impacts inflammation-related oxylipin profiles in seven different organs and serum of aged mice. Additionally, we explored physical exercise as a tool to ameliorate age- and isolation-associated inflammation. Our results show that social isolation induces significant increases in interleukin-1β levels and stimulates a dramatic production of lipoxygenase (LOX)-derived oxylipins in an organ-dependent manner, particularly pronounced in liver, lung, and spleen. Physical exercise failed to mitigate the pro-inflammatory effects induced by social isolation. These effects did not occur in the circulatory system as serum oxylipin levels remained relatively unchanged by isolation. The unexpected and striking elevation of oxylipins across the organs highlights the detrimental effect of social isolation and proposes key roles of oxylipins in stress-related inflammageing.

Key Words

Exercise / Inflammageing / Oxylipins / Social isolation / Stress

Introduction

Ageing is a complex, multifactorial biological and social process, accompanied by the accumulation of deleterious changes often resulting in decreased health, cognitive decline, and overall loss of function and fitness (Harman 2006; Khan, Singer, and Vaughan 2017).

Disruption of homeostasis, caused by impaired immune functions, immunosenescence (Franceschi et al. 2000), and subsequent increase in chronic low-grade inflammation, termed inflammageing (Furman et al. 2019; Fulop et al. 2023), is considered one of the proposed hallmarks of ageing (Lopez-Otin et al. 2023). Inflammatory processes are tightly regulated by a network of mediators, such as cytokines, chemokines, and oxylipins (Franceschi et al. 2018). During ageing, this complex network becomes dysregulated and aggravates inflammatory processes, as oxylipins play vital roles as lipid mediators in facilitating the initiation, perpetuation, and resolution of inflammation (Arnardottir et al. 2014; Chiurchiu, Leuti, and Maccarrone 2018). Oxylipins are produced from polyunsaturated fatty acids (PUFA) including omega-6 (e.g., arachidonic acid (AA, C20:4)) and omega-3 (e.g., docosahexaenoic acid (DHA, C22:6), eicosapentaenoic acid (EPA, C20:5) members. Their mode of action can be both pro- and anti-inflammatory: while pro-inflammatory AA-derived prostaglandins (PG) and leukotrienes (LT) are biosynthesised via the cyclooxygenase (COX) and 5-lipoxygenase (LOX) pathway, anti-inflammatory and pro-resolving oxylipins are mainly omega-3-PUFA-derived and are generated by 5-, 12-, and 15-LOX. The latter oxylipins encompass specialized pro-resolving mediators (SPM) such as protectins, lipoxins (LX), maresins (MaR), resolvins (Rv), as well as their mono-hydroxylated precursors (e.g., 15-hydroxy-eicosapentaenoic acid (15-HEPE), 17-hydroxy-docosahexaenoic acid (17-HDHA)) (Serhan 2014; Leuti et al. 2020). Aberrant oxylipin biosynthesis is a characteristic feature of several age-related diseases, such as cardiovascular disease (Nayeem 2018; Bäck and Hansson 2019), atherosclerosis

79 (Gleim et al. 2012), and neurodegeneration (Whittington, Planel, and Terrando 2017; Chiurchiu et
80 al. 2022). In recent studies, we revealed that oxylipin formation exhibits unique organ- and cell
81 type-specific signatures (Schädel et al. 2021; Schadel et al. 2023), underlining the necessity to
82 consider age as variable when investigating inflammation.

83 Furthermore, lifestyle crucially determines biological health and the severity of ageing (Larsson,
84 Kaluza, and Wolk 2017; Wang, Chen, et al. 2023). Social isolation and loneliness are directly
85 associated with poor health outcomes, increased inflammation, and cognitive decline in ageing
86 (Leigh-Hunt et al. 2017; Cardona and Andres 2023). A meta-analysis of 148 studies on social
87 relationships and mortality risks has found that individuals with stronger social relationships
88 exhibit a higher likelihood of survival (Holt-Lunstad, Smith, and Layton 2010). Moreover,
89 genome-wide analyses revealed that people experiencing a high degree of subjective social
90 isolation show increased leukocyte transcriptional activity, especially in pro-inflammatory
91 pathways, directly linking social isolation with inflammation (Cole et al. 2007). Maintaining social
92 connections in old age has proven to be particularly beneficial, as the negative impact of social
93 isolation is estimated to be higher than that of diabetes (Yang et al. 2016). Mouse studies on social
94 isolation show elevated corticosterone levels, which acts as the main active endogenous
95 glucocorticoid in mice, indicating a physiological stress response, along with increased
96 inflammation and impaired cognitive and immune function (Berry et al. 2012; Ederer et al. 2022;
97 Muta et al. 2023). Conversely, a positive social environment is associated with better immunity
98 and increased lifespan (Garrido et al. 2022), underscoring the critical role of social connections in
99 both ageing and inflammatory processes. But how social isolation affects oxylipin networks and
100 which roles oxylipins play in physiological consequences of social isolation is unknown.

Metabolic interventions such as voluntary exercise (e.g., for humans taking a walk, for mice voluntary wheel running) are well established as a tool that may prolong the life and health span of an individual (Lopez-Otin et al. 2016) and subsequently may mitigate the negative impact of other lifestyle factors like isolation. While regular exercise yields a multitude of positive outcomes, the exact molecular drivers behind these benefits are not yet fully understood. Exercise not only decreases the secretion of pro-inflammatory cytokines but also shifts the oxylipin pathways toward omega-3 substrates, leading to elevated levels of SPM and their precursors (Markworth, Maddipati, and Cameron-Smith 2016; Pena Calderin et al. 2025). Additionally, it was found that exercise reduces perceived loneliness (Pels and Kleinert 2016). Although previous studies have examined inflammageing, age-related social isolation, and late-life exercise interventions individually, the combined effects and interactions of these factors remain unexplored. Here, we elucidated the implications of age-associated social isolation and related stress on the inflammatory microenvironment in aged mice, focusing on oxylipins, as well as cognitive function, and whether accompanied exercise (i.e., voluntary wheel running) can mitigate the detrimental effects of social isolation. We accounted for differential plasticity in various organs by screening seven different organ/tissue types, as well as serum (systemic circulation), creating a fundamental framework linking inflammation, ageing, and age-associated isolation stress.

Results

The inflammatory status of 7 organs (brain, heart, fat, liver, lung, muscle, spleen) and serum of adult (5 months) and aged (20 months) C57BL6/J/UKJ mice was assessed by analysis of inflammation-related cytokine and oxylipin profiles. To investigate the effect of social isolation stress at a later stage in life, we recurrently separated mice at an age of 18 months into single cages for three individual nights per week over a period of 8 weeks. Moreover, we addressed exercise as an established, anti-inflammatory metabolic intervention by providing a running wheel to a subgroup of isolated aged mice. In this context, aged mice additionally underwent a Barnes maze test to assess intervention-related changes in cognition. The detailed experimental setup is depicted in Figure 1.

Organ-specific inflammatory cytokines are differentially affected by ageing but significantly induced by recurring social isolation

Ageing significantly increased ($p = 0.018$) the body weight of the mice. Old mice in group-housing (O-GH) had a mean weight of 35.2 ± 0.6 g while the mean weight of adult mice in group-housing (Ad-GH) was 32.1 ± 0.9 g. Social isolation stress in old mice due to repeated isolation into single cages (O-ISO) did not significantly alter the final body weight (35.9 ± 1.7 g, $p = 0.692$). However, the body weight of these isolated aged mice (O-ISO) showed a higher degree of variation versus old mice in group-housing (O-GH) (Fig. 2a).

To address the inflammatory status of the investigated organs, we examined the protein level of the pro-inflammatory cytokine interleukin (IL)-1β, and its counteractor IL-1 receptor antagonist (IL-1ra). We found that ageing did not markedly change IL-1β levels in fat, muscle, and spleen (Fig. 2b). However, IL-1β levels were significantly decreased due to ageing in brain (Ad-GH: 8.7 ± 0.2, O-GH: 6.5 ± 0.3 pg/50 mg), heart (Ad-GH: 34.5 ± 5.3, O-GH: 12.3 ± 1.5 pg/50 mg), and

142 liver (Ad-GH: 273.7 ± 31.7, O-GH: 117.5 ± 17.6 pg/50 mg). Only in the lung, IL-1β was
143 significantly elevated (Ad-GH: 143.5 ± 8.1, O-GH: 205.9 ± 14.6 pg/50 mg) in the aged compared
144 to the adult mice (Fig. 2b). When the aged mice underwent social isolation (O-ISO), however,
145 IL-1β significantly increased in all studied organs except in spleen with only minor elevation
146 versus the O-GH mice (Fig. 2b, 1.8-fold, $p = 0.234$). Overall, this translates to a distinct, organ-
147 specific ageing-related impact on IL-1β secretion while stress due to isolation seems to consistently
148 promote inflammation in most organs. A similar pattern was observed for the levels of IL-1ra,
149 being significantly increased following isolation of aged mice in fat, heart, liver, muscle, and
150 spleen (Fig. 2c).

151 ***Social isolation potently induces lipoxygenase-derived oxylipin formation***

152 Since oxylipins are crucial regulators of all stages of inflammation (Funk 2001; Levy et al. 2001;
153 Serhan 2014) we assessed the oxylipin signature profiles of the different organs using a targeted
154 lipidomic approach based on UPLC-MS/MS. To get an overview of age- and isolation-related
155 changes in the complex oxylipin profiles, we performed an unbiased principal component analysis.
156 This analysis revealed distinct age-related alterations in the oxylipin profiles for brain, fat, heart,
157 and spleen between both group-housed adult (Ad-GH) and aged mice (O-GH) (Fig. 2d). An even
158 more pronounced separation of the clusters for all organs was observed following isolation of aged
159 mice (O-ISO) and only in the heart, age had a stronger effect on oxylipin profiles than isolation
160 (Fig. 2d).

161 Looking at the changes of the individual screened oxylipins due to ageing, we found distinct organ-
162 specific ageing signatures with liver, muscle, and spleen exhibiting an overall decrease in
163 oxylipins, whereas brain, fat, and lung showed divergent oxylipin profiles with age (Fig. 2e, left).
164 In the heart, ageing led to increased amounts of oxylipins, particularly pronounced for PGs (Fig.

165 2e, left). Social stress in aged mice due to isolation, however, drives a remarkable increase of a
166 broad spectrum of oxylipins, especially derived from 5-, 12-, and 15-LOX across various organ
167 systems (Fig. 2e, right). Interestingly, in lung and spleen, the inflammation-resolving SPMs RvD5
168 and PDX produced by12/15-LOX were elevated along with pro-inflammatory LTs derived from
169 5-LOX. In the brain and heart, the oxylipin levels were mostly unaffected by isolation of the aged
170 mice (Fig. 2e, right).

171 In order to further characterise the age- and isolation-related effects on the oxylipin formation, we
172 classified the oxylipins according to their rate-limiting biosynthetic enzymes, i.e., COX, 5-LOX,
173 12-LOX, and 15-LOX. In detail, for COX products**,** age-related increases were most pronounced
174 in the heart (3.71-fold) with minor changes in other organs. Isolation did not affect COX products
175 in most organs (fat, heart, lung, and muscle), except for brain (0.71-fold), liver (2.79-fold) and
176 spleen (1.41-fold; Fig. 3a). Taken together, the effect size and its statistical significance indicate
177 that age and isolation only have a moderate impact on COX-derived oxylipins in most organs.

178 5-LOX products were much more affected by age and isolation. Thus, 5-LOX product levels
179 strongly decreased in fat, liver, muscle, and spleen of aged mice compared to adult controls (Fig.
180 3b). Conversely, ageing markedly increase 5-LOX products in the heart, which is surprisingly not
181 further aggravated by isolation (Fig. 3b). For all other organs – except muscle where 5-LOX
182 products were not detectable – isolation led to a marked increase of 5-LOX-derived oxylipins (Fig.
183 3b, brain: 2.0-fold, fat: 2.1-fold, liver: 18.2-fold, lung: 8.8-fold, spleen: 17.1-fold). The same trend
184 was observed for oxylipins produced by 12- and 15-LOX (Fig. 3c,d). Thus, ageing caused only
185 moderate changes in the levels of 12-LOX and 15-LOX products, while social isolation caused a
186 dramatic increase for both 12-LOX (brain: 5.7-fold, fat: 7.1-fold, liver: 30.1-fold, lung: 4.5-fold,
187 muscle: 22.2-fold, spleen: 4.3-fold) and 15-LOX products (brain: 1.6-fold, fat: 7.5-fold, liver:

188 33.4-fold, lung: 7.9-fold, muscle: 2.8-fold, spleen: 15.6-fold) in all organs except for the heart (no
189 elevation). In more detail, the radar charts showing individual representative oxylipins for each
190 enzyme class (COX: PGE_2; 5-LOX: LTB_4 and 5-HETE; 12-LOX: 12-HEPE, 12-HETE and 14-
191 HDHA; 15-LOX: PDX, 15-HEPE, 15-HETE and 17-HDHA) confirm the massive elevation of
192 LOX-derived oxylipins in all organs caused by social stress due to isolation, except for heart, with
193 minor or even opposite impact on COX products (e.g., PGE_2). Together, social isolation stress
194 consistently upregulates LOX-derived oxylipins in organs of aged mice while only the heart and,
195 to a lesser extent, the brain exhibit a lower susceptibility to social stress-related oxylipin alterations
196 (Fig. 3e). These increases in LOX-derived oxylipins were evident for conversion of all PUFAs as
197 substrates, i.e. AA, EPA and DHA.

198 ***Late-life exercise cannot rescue isolation effects on inflammatory state***

199 Since voluntary exercise yields a multitude of positive outcomes prolonging the life and health
200 span (Lopez-Otin et al. 2016) with impact on cytokines and oxylipins (Markworth, Maddipati, and
201 Cameron-Smith 2016; Pena Calderin et al. 2025), we further investigated whether exercise can
202 mitigate the effects on the inflammatory mediators induced by social isolation stress. We employed
203 voluntary wheel running as a late-life exercise intervention in socially isolated aged mice (runners:
204 O-EX, non-runners: O-ISO). After the intervention period, the group of O-ISO mice (non-runners)
205 showed a mean weight of 35.9 ± 1.7 g, whereas the group of O-EX mice (runners) showed a slight
206 decrease in body weight (33.9 ± 1.1 g), yet this difference was not significant (Fig. 4a, $p = 0.357$).
207 However, the weight loss seems to be connected to the individual running performance of the mice,
208 as the mouse with the lowest running performance across the 10-week-long experimental period
209 showed the highest weight and vice versa (Fig. 4b).

210 We then investigated the levels of IL-1β and IL-1ra and assessed the oxylipin signature profiles of
211 organs from aged mice during isolation that were either allowed to exercise (O-EX) or not (O-ISO).
212 Only in the lung, exercise led to a significant decrease in IL-1β levels (0.72-fold) (Fig. 4c) and a
213 small decrease by trend was observed in heart (0.72-fold, $p = 0.241$) and spleen (Fig. 4c, 0.62-fold,
214 $p = 0.151$). For all other organs, the IL-1β levels were essentially unchanged. Similarly, IL-1ra
215 levels remained essentially unaltered between O-EX and O-ISO, without significant impact of
216 exercise in any organ (Fig. 4d). Principal component analysis of the overall oxylipin profiles
217 showed no marked differences (no separation of clusters) for all organs comparing O-ISO versus
218 O-EX, with both the position of mean and 95% confidence intervals being similar (Fig. 4e). Yet,
219 when looking at individual oxylipins in detail (Fig. 4f), oxylipin levels in fat (increased) and liver
220 (decreased) were most affected by exercise, particularly pronounced for monohydroxylated fatty
221 acids like 5-HETE, 4-HDHA, 12-HETE, 12-HEPE etc. (Fig. 4f). Interestingly, in the lung, exercise
222 decreased PG and RvD5 formation but did not affect other oxylipins (Fig. 4f). Analysis of oxylipin
223 groups according to biosynthetic enzymes (i.e., COX, 5-, 12-, 15-LOX) reveals no significant
224 alterations due to exercise in any organ (Fig. S2a-d), and only in fat and liver a tendency for
225 changes was observed. Thus, the most notable change by trend upon exercise is the increase of 5-
226 and 15-LOX-derived products in fat tissue (5-LOX: 2.02-fold, $p = 0.131$; 15-LOX: 1.52-fold,
227 $p = 0.082$) while COX- and 12-LOX-derived products remain at a similar level to sedentary mice
228 (Fig. 4g). Furthermore, exercise led to a small decrease in LOX-derived products (5-LOX: 0.72-
229 fold, $p = 0.214$; 12-LOX: 0.52-fold, $p = 0.140$; 15-LOX: 0.67-fold, $p = 0.188$) in the liver, while
230 the level of COX-derived products was unaltered (Fig. 4h). Together, for aged mice undergoing
231 social isolation stress, the variable of voluntary exercise has only a minor impact on the massive
232 alterations of oxylipins and cytokine levels caused by isolation.

233 ***Cognitive function does not benefit from voluntary exercise in isolation***

234 In a previous study (Ederer et al. 2022), we addressed the relation between age and cognition in
235 male, group-housed mice by subjecting them to a Barnes maze test, revealing a cognitive decline
236 that occurs between the ages of 15 and 24 months. It was also shown that for aged mice, constant
237 social isolation further impairs cognitive function. To evaluate whether voluntary wheel running
238 can mitigate cognitive decline in ageing and social stress through repeated isolation and
239 resocialisation, we tested isolated aged mice in the Barnes maze to assess spatial learning, as well
240 as short- and long-term memory. The timeline of the implementation of the Barnes maze test is
241 shown in the schematic in Fig. 5a. During the training period for the Barnes maze test, both cohorts
242 with recurring social stress (O-ISO and O-EX) showed a similar, improved performance over time.
243 No strong exercise-mediated improvements were observed during training (Fig. 5b). The probe
244 trial (short-term memory) and the retention test (long-term memory) did not reveal cognitive
245 improvement following exercise (Fig. 5c,d). During the reversal test (cognitive flexibility), the
246 O-EX mice showed a shorter primary latency and an increased cognitive score compared to O-ISO
247 mice, suggesting a potential facilitation in task adaptation (Fig. 5e). Overall, exercise intervention
248 in late life does not seem to improve spatial learning, short and long-term memory in aged mice
249 experiencing social isolation stress, but O-EX showed an improved cognitive flexibility.

250

251 ***Reduced age-associated inflammatory repertoire of oxylipins and proteins in serum is***
252 ***maintained after isolation and exercise***

253 To determine whether the observed organ-specific effects due to ageing and isolation as well as
254 exercise are reflected in the systemic circulation, we characterised the inflammatory secretome
255 (oxylipins and proteins) in the serum of the mice cohorts from above. Based on both, the principal

256 component analysis of the oxylipin profiles and the detailed analysis of the individual oxylipins,
257 age but not isolation or exercise is the main driver for the observed alterations (Fig. 6a,b). While
258 in the PCA all aged cohorts (O-GH, O-ISO, O-EX) cluster tightly together, the oxylipin profile of
259 the adult cohort (Ad-GH) shows a distinct shift (Fig. 6a). The majority of the screened oxylipins
260 in serum are decreased due to age (O-GH/Ad-GH, Fig. 6b) with significant changes for 5- and 15-
261 LOX-derived products (5-LOX: 0.55-fold, 15-LOX: 0.54-fold, Fig. 6c). To our surprise and in
262 contrast to organs (see above), isolation only had a minor impact on oxylipin levels in serum,
263 reflected by the lack of changes for COX and 12-LOX products, and only moderate increases in
264 5- and 15-LOX-derived products in serum from O-ISO versus O-GH mice (5-LOX: 1.63-fold,
265 $p = 0.014$; 15-LOX: 1.44-fold, $p = 0.174$, Fig. 6c). These serum oxylipin levels of O-ISO did not
266 markedly change due to exercise, apart from a 4-fold increase in 5-LOX-derived products (O-ISO:
267 mean = 822.2 ± 124.0 pg/mL, O-EX: mean = 3341.3 ± 1661.3 pg/mL; $p = 0.211$) which was,
268 however, not significant and attributed to only 2 out of the 6 biological replicates (Fig. 6c).

269 We next employed an unbiased analysis of the repertoire of inflammation-related cytokines,
270 chemokines, enzymes, and growth factors in the serum of Ad-GH, O-GH, O-ISO, and O-EX mice,
271 using a commercially available Proteome Profiler, allowing us to screen for the relative abundance
272 of 111 candidates. In line with the reduced amounts of most of the oxylipins (Fig. 6b), ageing led
273 to an overall downregulation (82.0%) of the investigated proteins in serum (Fig. 6d). Isolation,
274 however, led to an increased expression of 82.9% of the proteins (Fig. 6d). Despite the seemingly
275 contradictory dynamics, the proportions of proteins being down-regulated with age and up-
276 regulated due to isolation are not congruent (Fig. S3). The exercise intervention (O-EX) led to less
277 pronounced and more balanced alterations for aged mice under isolation (O-ISO) with 59.5% up-
278 and 38.7% downregulated proteins (Fig. 6d). Acute phase proteins (APPs, e.g., complement factors

279 and pentraxins) and chemokines (particularly of the CCL and CXCL classes) were impaired due
280 to age versus adult mice (Fig. 6e,f). Such overarching class effects could not be observed for the
281 cohorts O-ISO and O-EX under isolation. Interestingly, the pro-inflammatory cytokine IL-6, the
282 chemokine CXCL1, and the LPS receptor cluster of differentiation (CD)14 were increased in
283 O-ISO versus O-GH mice (Fig. 6e,f). Exercise of aged mice under isolation, however, induced
284 higher levels of murine C-reactive protein (CRP), CCL11, CCL21, and vascular endothelial
285 growth factor (VEGF) versus O-ISO mice (Fig. 6e,f). These alterations in the abundance of
286 inflammation-related serum markers indicate a reduced immune competence with age, and distinct
287 pro-inflammatory responses due to isolation and exercise.

288

289 **Discussion**

290 Social isolation, a phenomenon commonly occurring in late life, is extensively described as a risk
291 factor for increased morbidity and mortality and is associated with higher levels of inflammation
292 and cognitive decline (Hodgson et al. 2020; Wang, Gao, et al. 2023). Considering inflammation
293 as a shared feature of ageing (Ferrucci and Fabbri 2018), isolation (Yang et al. 2013; Smith et al.
294 2020), and an inactive lifestyle (Fenton et al. 2018), we examined the inflammatory cytokine and
295 oxylipin secretome in mice undergoing recurring social isolation and resocialisation, and whether
296 voluntary physical exercise can ameliorate the inflammatory stress responses due to isolation in
297 single housing. Unexpectedly, we found dramatic elevation of inflammation-related LOX-derived
298 oxylipins due to social isolation in an organ-specific manner, especially affecting liver, lung, and
299 spleen. Surprisingly, physical exercise showed a neglectable impact on the organ-intrinsic oxylipin
300 secretome of isolated mice and was hardly evident in systemic circulation.

301 Previous studies have frequently investigated and described social isolation as a stressor, resulting
302 in a variety of adverse health outcomes (Grant, Hamer, and Steptoe 2009; Mumtaz et al. 2018).
303 The magnitude of these events, however, varies greatly depending on the type and duration of
304 social isolation (Shoji and Mizoguchi 2011; Hammig 2019). Studies on permanent or short- to
305 long-term social isolation reported mixed and sometimes contradictory results. Thus, a four-week
306 social isolation period of both male and female adult mice (3 months) affected female animals
307 more than males, but overall, the isolation only had minimal effects on serum levels of the stress
308 marker corticosterone, behaviour, and body weight (Smolensky et al. 2024). Conversely, shorter
309 periods of single housing of mice, such as 4 - 5 days only, tend to produce more pronounced
310 detrimental effects, including anxiety behaviour, elevated urinary stress hormones, and weight loss
311 (Muta et al. 2023). These findings suggest that, while isolation quickly triggers a strong stress

312 response, mice may adapt within a few weeks, resulting in lower or unchanged stress markers over
313 prolonged isolation. Nevertheless, despite seemingly modest stress responses, long-term isolation
314 of degus over a period of 20-24 months still promotes a pro-inflammatory state (Oliva et al. 2023).
315 Research on the effects of long-term isolation in human cohorts is limited. One study reported
316 elevated cortisol levels and heightened immune activation in men after an isolation period of
317 approximately 1.5 years (Yi et al. 2014). However, to allow comparability between studies on
318 isolation, further adjustments to the experimental conditions are needed. In our study, aged mice
319 underwent a social stress protocol of repeated isolation in single housing for three individual nights
320 per week and subsequent resocialisation in group housing over a total period of 8 weeks. Our
321 results confirm that such repeated social isolation acts as a potent and impactful stressor, elevating
322 the pro-inflammatory cytokine IL-1β. We showed before, that oxylipins are affected by ageing in
323 an organ-specific manner (Schädel et al. 2021), which we also confirm in this study. Additionally,
324 we here uncover a dramatic upregulation of LOX-derived oxylipin production in several organs
325 due to isolation, while COX products that are produced from the same PUFA substrates (Funk
326 2001) were hardly altered. Increased IL-1β has previously been linked to an isolation-associated
327 inflammatory phenotype (Magalhaes et al. 2024), but our findings demonstrate, for the first time
328 the tremendous effect of social isolation stress on oxylipins as responsible mediators of the
329 inflammatory response in several organs. Notably, these age- and isolation-related effects did not
330 occur systemically, reflected by the overall moderate changes of oxylipin levels in serum of mice.

331 The dramatic changes in oxylipin formation due to isolation may be attributed to a dysregulation
332 of the hypothalamic-pituitary-adrenal (HPA) axis in response to social isolation as a psychosocial
333 stressor, resulting in elevated glucocorticoid (GC) signalling. It is known that oxylipins,
334 particularly eicosanoids such as PGs, can be affected by stress-related GC (Umamaheswaran et al.

335 2018), and that GC may influence the expression of COX-1 and -2 (Goppelt-Struebe, Wolter, and
336 Resch 1989; Ehrchen et al. 2007). LOX expression has likewise been shown to be regulated
337 through exogenous GC, which can decrease the pro-inflammatory immune response along with
338 elevated 15-LOX-2 expression and elevated SPM levels (Riddick et al. 1997; Rao et al. 2023).
339 Although the murine paralog of 15-LOX-2, also called m8-LOX, is known to oxygenate free
340 substrates at the 8-position primarily, it has been demonstrated to oxygenate the 15-position in the
341 case of phospholipid-esterified fatty acid substrates (Bender et al. 2016). This could explain the
342 observed increase in 15-LOX-derived products. Our present results documenting elevated LOX
343 products in several organs and tissues of aged mice due to social isolation stress, predominantly in
344 immunological organs like spleen, liver, and lung, are in good agreement with the above-
345 mentioned impact of GC on oxylipin biosynthesis. Whether or not isolation of aged mice elevates
346 LOX expression and if GC are involved in this process remains to be explored in more detail.

347 Aberrations in inflammatory signalling caused by age and isolation may raise the risk of
348 dysregulated homeostasis, leading to chronic basal inflammation. Cognitive decline may also
349 result from age- and stress-related imbalances in oxylipins, as PG signalling and 5-LOX expression
350 have been associated with reduced cognitive function and depression-like symptoms (Luo et al.
351 2016; Minhas et al. 2021; Mrowetz et al. 2023). Furthermore, chronic social stress has been found
352 to significantly elevate levels of 12-LOX-derived metabolites 12-HETE and 12-HEPE in the brain
353 (Akiyama et al. 2022). In our study, isolation of aged mice elevated both, 5-LOX products with
354 mainly pro-inflammatory features (Haeggstrom 2018) but also 12/15-LOX-derived SPM like
355 RvD5, PDX, and MaR2 and their precursors 17-HDHA and 14-HDHA, which act as
356 immunoresolvents that terminate and resolve inflammation (Serhan 2014). Therefore, these
357 alterations in oxylipin profiles may be connected to a stress-induced activation of the innate

358 immune system, triggering the mobilisation of neutrophils, monocytes, and monocyte-derived
359 macrophages, as primary producers of oxylipins (McKim et al. 2018).

360 Lifestyle interventions such as diet or exercise are popular and well-characterised means of
361 mitigating or circumventing negative age-related health outcomes (Ngandu et al. 2015; Fitzgerald
362 et al. 2021). Exercise has been shown to exhibit anti-inflammatory features, which are, however,
363 highly dependent on type, duration, onset, and intensity (Gleeson et al. 2011). For example, Sun
364 et al. found that exercise indeed reduces inflammageing features like IL-1β secretion during long-
365 term exercise (12-month voluntary exercise) in young and aged mice exposed to infectious injury
366 (Sun et al. 2023). Thus, mice that underwent exercise were more protected from LPS challenge
367 than sedentary mice. However, this effect was less pronounced in mice that started the exercise
368 regimen at an older age (Sun et al. 2023), and is in line with results indicating that adaptive and
369 acute responses to physical activity decline with advancing age (Durham et al. 2010). This may
370 also explain why our results did not confirm previous findings showing that exercise stimulates
371 both pro- as well as anti-inflammatory pro-resolving lipid mediators (Calderin et al. 2022; Malan
372 et al. 2024; Pena Calderin et al. 2025). In contrast to these studies, our exercise protocol started in
373 late life (18 mo), in addition to the stress factor of repeated social isolation and resocialisation. We
374 suppose that the detrimental effects of social isolation override the benefits of physical exercise,
375 and thus, no ameliorating effect of exercise on pro-inflammatory signatures could be observed.

376 While our protocol of repeated isolation and resocialisation may better mimic isolation in the
377 elderly than current long-term isolation models, several limitations should be recognised. Past
378 studies have shown that the effects of social isolation occur in a sex-specific manner, with female
379 mice generally being more affected than males (Smolensky et al. 2024). To understand the origin
380 and implications of the proposed effects, future studies in female mice, ideally in comparison to

381 male counterparts, will be essential. Additionally, inclusion of an infection model, e.g., bacterial
382 pneumonia caused by *S. pneumoniae*, a common infection in the elderly (Gutierrez et al. 2006;
383 Ruiz et al. 2014), would be helpful to assess not only the basal inflammatory microenvironment
384 but also to evaluate immune competence and whether repeated social isolation stress may
385 significantly impair immunological resilience and resolution processes.

386 In future studies, the molecular signalling circuits that connect social isolation and inflammageing
387 need to be further explored. It is also important to recognise that the idea of gradual ageing is
388 increasingly challenged, with findings indicating that nonlinear ageing may be more common and
389 that abrupt changes might amplify ageing processes (Shen et al. 2024; Olecka et al. 2024; Olecka,
390 Morrison, and Hoffmann 2025). As such, social isolation stress in elderly may push individuals
391 past a critical threshold, triggering escalation of inflammation and accelerating ageing.

392 Our findings demonstrate that repeated cycles of social isolation and resocialisation may act as
393 stressors, increasing inflammation-related cytokine secretion and dramatically enhancing the
394 formation of LOX-derived oxylipins in defined organs, impacting inflammation and tissue
395 homeostasis. Notably and to the best of our knowledge, this is the first report revealing organ-
396 specific oxylipin modulation upon social isolation, offering a novel framework for future
397 interdisciplinary research at the intersection of inflammation, ageing, and social isolation. Under
398 our experimental conditions, voluntary physical exercise did not counteract the consequences of
399 social isolation stress on oxylipin production. Conclusively, the dramatic elevation of LOX-
400 derived oxylipins highlights the impact of social isolation stress, a common phenomenon in the
401 elderly, on inflammatory mediators within several specific organs and propose oxylipins as
402 determinants of stress-related inflammageing.

403 **Methods**

404 ***Animals and sample isolation***

405 Male C57BL/6J/UKJ mice were bred and housed at the Central Experimental Animal Facility at
406 Jena University Hospital, Jena, Germany. Mice were kept on a 14/10 h light/dark cycle and at 22
407 ± 2 °C with a relative humidity of 55 ± 10%. The mice had unrestricted access to water and food
408 (ssniff mouse V1534-300, ssniff Spezialdiäten GmbH, Soest, Germany). Four experimental
409 cohorts were established: (1) Ad-GH (*n* = 5), adult mice at 5 months of age that were kept in group-
410 housing throughout their lives; (2) O-GH (*n* = 6), 20-months-old mice that were kept in group-
411 housing throughout their lives; (3) O-ISO (*n* = 5 for oxylipin analysis, *n* = 18 for cognitive testing),
412 aged mice that were kept in group-housing until 18 months of age, then over a period of 8 weeks,
413 repeatedly separated for three individual nights per week (Sunday, Tuesday, Thursday) into single
414 cages before being returned to their littermates; and (4) O-EX (*n* = 6 for oxylipin analysis, *n* = 17
415 for cognitive testing), aged mice treated identically to O-ISO mice but provided with running
416 wheel during the isolation nights, allowing for voluntary wheel running.
417 After the experimental period, mice were euthanised by cervical dislocation. Whole blood was
418 collected immediately after death. To extract serum, blood was kept at room temperature for
419 around 60 min to ensure clotting. Samples were subsequently centrifuged for 30 min at 2,000 x*g*.
420 Serum was transferred into fresh reagent tubes and stored at -80 °C. Seven organs, namely brain,
421 fat (visceral WAT), heart, liver, lung, muscle (quadriceps and gastrocnemius), and spleen were
422 harvested, washed with PBS (pH 7.4, SERVA, Heidelberg, Germany; 47302.03), and stored
423 at -80 °C.

424 ***Cognitive testing with Barnes maze***

425 The Barnes maze is employed to assess spatial learning, short- and long-term memory, memory
426 retrieval, and cognitive flexibility by training animals to associate distal cues with a fixed escape
427 box (Ederer et al. 2022). Mice are placed on a brightly lit circular platform with 20 holes, one of
428 which provides access to an escape box. The protocol comprises one day of habituation, followed
429 by 6 training days (3 trials/day with an inter-trial interval of 60 min) to assess learning. The
430 following day, a probe trial, for which all holes are closed, evaluates short-term memory. After
431 three days without further training, a retention test assesses long-term memory retention with the
432 original hole with the escape box opened again. Lastly, one day later the position of the escape
433 box is placed 180° opposite to the original position. This reversal test measures cognitive
434 flexibility.

435 ***Organ homogenisation***

436 For fat, liver, lung, muscle and spleen, organ homogenates were prepared by weighing 20-40 mg
437 of organ tissue in tubes containing around 100 mg of lysing matrix D per 10 mg organ (M.P.
438 Biomedicals, Irvine, CA, USA; 116540434). The lysis buffer was added at a ratio of 20 µL per
439 milligram organ (1% (*v*/*v*) NP-40 (AppliChem, Darmstadt, Germany; A1694), 1 mM sodium
440 orthovanadate (AppliChem; A2196), 10 mM sodium fluoride (AppliChem; A3904), 5 mM sodium
441 pyrophosphate (Sigma Aldrich, St. Louis, MO, USA; S8282), 25 mM β-glycerophosphate (Sigma
442 Aldrich; G9422), 5 mM EDTA (AppliChem; A2937), 25 µM leupeptin (Sigma Aldrich; L2884),
443 3 mM soybean trypsin inhibitor (Sigma Aldrich; T9128) and 1 mM phenylmethanesulfonyl
444 fluoride (Sigma Aldrich; P7626)). For the homogenisation of brain and heart, one hemisphere and
445 the whole heart, respectively, were weighed into tubes containing lysing matrix D, and 5 µL of

446 lysis buffer per milligram organ was added. All organs were homogenised using a FastPrep-24™
447 5G bead beating homogeniser using established protocols (M.P. Biomedicals).

448 ***Cytokine quantification via ELISA***

449 For cytokine quantification, homogenates were centrifuged at 21,100 x*g*, 4 °C for 10 min. The
450 supernatant was transferred into fresh reagent tubes and stored at -20 °C until further analysis. The
451 final concentration of the homogenates was 50 mg/mL for fat, liver, lung, muscle, spleen, and
452 200 mg/mL for brain and heart.

453 Quantification of cytokines in organ homogenates was performed using commercially available
454 ELISA kits for murine IL-1β (DY401-05) and IL-1ra (DY406-15), manufactured by R&D Systems
455 (Minneapolis, MN, USA). The assays were completed per the manufacturer's instructions.
456 Quantification was based standard curves generated individually for each experiment.

457 ***Oxylipin quantification via UPLC-tandem mass spectrometry***

458 For oxylipin quantification, immediately after homogenisation, homogenates were mixed 1:1 with
459 ice-cold methanol (fisher chemical; 10653963). Samples were kept on ice for around 20 min, and
460 then centrifuged at 21,100 x*g*, 4 °C for 10 min. The amount of supernatant equivalent to 20-30 mg
461 organ was then transferred into glass vials containing 990 µL ice-cold methanol and 10 µL of
462 deuterium-labelled internal standard [200 nM d_8-5*S*-hydroxyeicosatetraenoic acid (HETE)
463 (Cayman Chemical; 334230), d_4-LTB_4 (Cayman Chemical; 320110), d_5-LXA_4 (Cayman
464 Chemical; 24936), d_5-RvD2 (Cayman Chemical; 11184), d_4-PGE_2 (Cayman Chemical; 10007273)
465 and 10 µM d_8-AA (Cayman Chemical; 390010). Finally, lysis buffer was added to yield a total
466 volume of 3 mL and stored overnight at -20 °C to ensure protein precipitation.

467 After centrifugation (1,200 x*g*, 4°C for 10 min), supernatants were transferred to clean glass vials
468 and acidified by addition of 9 mL of MilliQ water (pH 3.5). Solid phase extraction of organ
469 samples was performed as previously described (Werner et al. 2019). Purified samples were
470 evaporated under continuous N_2 flow, and the residue was resuspended in 200 µL of an equal
471 mixture of methanol and water (VWR Chemicals, 83645320). After final centrifugation at
472 21,100 x*g*, 4°C for 5 min, the supernatant was used for ultra-performance liquid chromatography
473 coupled with tandem mass spectrometry (UPLC-MS/MS) analysis. Analytes were separated on an
474 Acquity UPLC system (Waters, Milford, MA, USA) equipped with an Acquity UPLC BEH C18
475 column (1.7 µm, 2.1 mm × 100 mm; Waters, Eschborn, Germany) and a pre-column of identical
476 material with column temperature set to 50°C. The gradient was adjusted as listed in Table 1.
477 Analytes were detected using a QTRAP 5500 mass spectrometer (ABSciex, Darmstadt, Germany)
478 with electrospray ionisation, operated as previously described.(Werner et al. 2019)

479 **Table 1:** Composition of mobile phase and elution gradient for targeted lipidomics.

Time [min]	Flow rate [mL/min]	Eluent A [%] (H_2O/MeOH 90/10 + 0.01 % CH_3COOH)	Eluent B [%] (MeOH + 0.01 % CH_3COOH)
0	0.3	64.4	35.6
4.8 - 19.0	0.3	45.5	54.5
26.7	0.3	15.6	84.4
29.7	0.3	2.2	97.8
30.7	0.3	64.4	35.6

480

481 The QTRAP 5500 was operated in negative mode using scheduled multiple reaction monitoring

482 (MRM) with the MRM window set to 60 s. The retention time and constitutional stereochemistry
483 of analytes were confirmed by external standards (Cayman Chemical), and individual calibration
484 curves were obtained for quantification, using the ratios of the areas of external standards to their
485 corresponding internal standard (ES/IS). Additional information, such as the specific transitions,
486 detection and quantitation limits for each screened metabolite, is listed in Table S1. Representative
487 unsmoothed chromatograms from samples showing peaks of oxylipins relevant to this study are
488 provided in Fig. S4. For further analysis, the determined concentration was normalised to pg or ng
489 oxylipin per 50 mg organ. The normalised raw data is provided in Table S2.

490 ***Assessment of serum proteome (Proteome Profiler)***

491 Serum samples were pooled from 4-5 biological replicates to a final volume of 100 µL. The serum
492 samples were assessed for the relative abundance of 111 cytokines using the commercially
493 available Proteome Profiler Mouse XL Cytokine Array Kit (ARY028) by R&D Systems, following
494 the manufacturer's guidelines. After treatment with IRDye 800CW streptavidin (LI-COR, Bad
495 Homburg, Germany), and the membranes were analysed with an LI-COR Odyssey Infrared
496 Imaging System. Pictures of the raw blots are included in Fig. S1.

497 ***Data handling and statistical analysis***

498 Results are presented as mean ± standard error of mean (SEM) unless stated otherwise. For data
499 analysis and visualisation GraphPad Prism (Version 10.4.1), Origin Pro (Version 2023b), RStudio
500 (2024.12.0) and Adobe Illustrator (version 29.3) were used. For the analysis of the oxylipin data,
501 values below the determined limits of detection (LOD) were set to the corresponding LOD, and
502 values between LOD and the lower limit of quantification (LLOQ) were set to ½ LLOQ, unless
503 stated otherwise. For the principal component analysis, RStudio in combination with the R
504 packages FactoMineR (https://cran.r-project.org/web/packages/FactoMineR/index.html) and

505 factoextra (https://cran.r-project.org/web/packages/factoextra/index.html) was used, and
506 metabolites for which all values were below the LLOQ were excluded from analysis. The ROUT
507 outlier test was used to identify outliers within data sets, with a Q-value set to 0.5. Detected outliers
508 were excluded from further analysis. The distribution of datasets was checked by Shapiro-Wilk
509 test with α set to 0.05 and, if implied, data was log-transformed for statistical analysis. For group
510 comparisons, an unpaired, two-tailed Student's t-test was performed. Statistical significance was
511 assumed for comparisons with $p \leq 0.05$. Significance is indicated as: *$p \leq 0.05$, **$p \leq 0.01$,
512 ***$p \leq 0.001$, ****$p \leq 0.0001$, or # respectively, ns, not significant.

513

514 **List of abbreviations**

515 AA – arachidonic acid

516 Ad – adult

517 APP – acute-phase proteins

518 CD – cluster of differentiation

519 COX – cyclooxygenase

520 CRP – C-reactive protein

521 DHA – docosahexaenoic acid

522 EPA – eicosatetraenoic acid

523 EX – exercise

524 GC – glucocorticoid

525 GH – group-housed

526 HDHA – hydroxydocosahexaenoic acid

527 HETE – hydroxyeicosatetraenoic acid

528 HPA – hypothalamic-pituitary-adrenal

529 IL – Interleukin

530 IL-1ra – IL-1 receptor antagonist

531 ISO – isolated for 3 individual nights per week

532 LLOQ – limit of lower quantification

533 LOD – limit of detection

534 LOX – lipoxygenase

535 LT – leukotriene

536 LX – lipoxin

537 MaR – maresin

538 MRM – multiple reaction monitoring

539 O – aged

540 PG – prostaglandin

541 Rv – resolvin

542 SEM – standard error of mean

543 UPLC-MS/MS – ultra-performance liquid chromatography-tandem mass spectrometry

544 VEGF – vascular endothelial growth factor

545

Declarations

Ethical Statement

All studies were conducted in compliance with the recommendations of the European Commission for the protection of animals used for scientific purposes and with the approval of the local authorities Thüringer Landesamt für Verbraucherschutz, Germany license UKJ-19-014, and TWZ09-2022.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analysed during the current study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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CRediT author statement

Mareike Wichmann-Costaganna: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing- Original Draft, Writing- Review & Editing, Visualization,

Project Administration. **Raphaëlle Petit:** Methodology, Formal analysis, Investigation, Writing- Review & Editing. **Julia Lindner:** Methodology, Formal analysis, Investigation, Writing- Review & Editing. **Madlen Haase:** Methodology, Formal analysis, Investigation, Writing- Review & Editing. **Vivien Bachmann:** Investigation. **Robert Klaus Hofstetter:** Methodology, Validation, Writing- Review & Editing. **Markus Werner:** Methodology, Validation, Writing- Review & Editing. **Christiane Frahm:** Conceptualization, Supervision, Project administration, Resources, Writing- Review & Editing, Funding acquisition. **Oliver Werz:** Conceptualization, Resources, Writing- Original Draft, Writing- Review & Editing, Supervision, Project administration, Funding acquisition. **Patrick Schädel:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing- Original Draft, Writing- Review & Editing, Supervision, Project administration.

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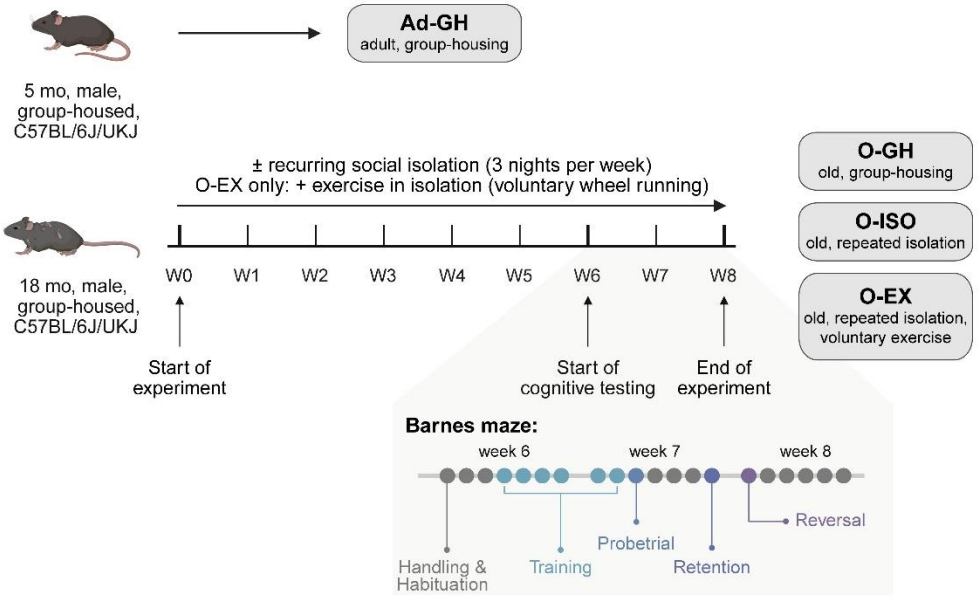
583 **Figures**

584 ***Figure 1: Schematic representation of the recurring social isolation and physical exercise***
585 ***intervention.***

586 5-month-old, adult male mice (Ad-GH, *n* = 5) were kept in group housing until sacrificed. For the
587 O-GH cohort, aged mice were maintained in group housing throughout their lives and underwent
588 cognitive testing in the last three weeks before sacrifice. To investigate the effect of recurring
589 social isolation in late-life, 18-month-old mice were repeatedly isolated into single cages for 3
590 individual nights per week, and resocialised with their corresponding littermates throughout a total
591 of 8 subsequent weeks. The single cages were equipped with (= O-EX) or without (= O-ISO)
592 running wheels. Aged mice underwent cognitive testing (*n* = 17-18) during the last weeks before
593 the end of the experiment. Organs and blood for serum investigation (*n* = 5-6) were collected
594 immediately after sacrifice.

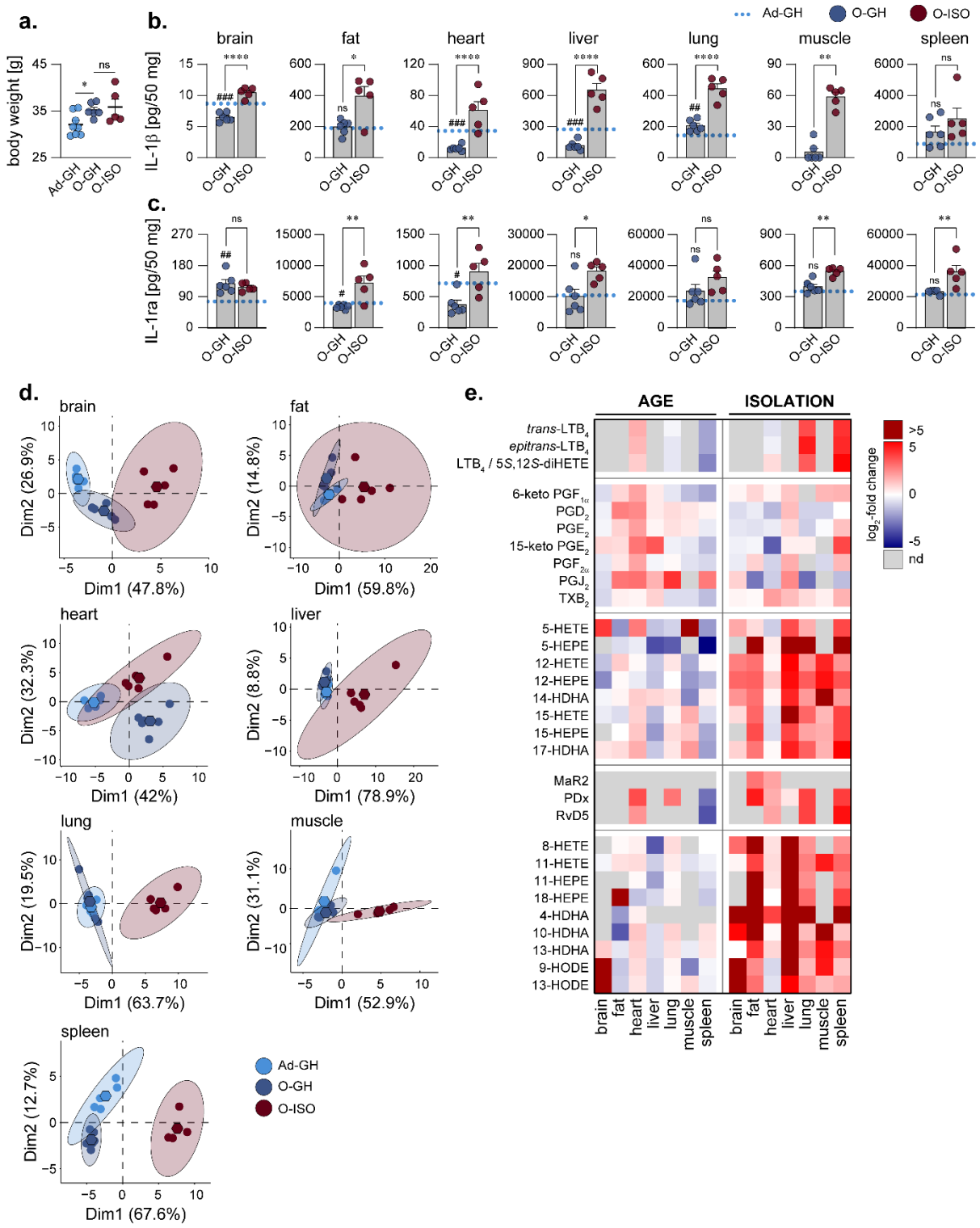

Ad-GH
adult, group-housing
5 mo, male, group-housed, C57BL/6J/UKJ
± recurring social isolation (3 nights per week)
O-EX only: + exercise in isolation (voluntary wheel running)
O-GH
old, group-housing
O-ISO
old, repeated isolation
O-EX
old, repeated isolation, voluntary exercise
18 mo, male, group-housed, C57BL/6J/UKJ
W0
W1
W2
W3
W4
W5
W6
W7
W8
Start of experiment
Start of cognitive testing
End of experiment
Barnes maze:
week 6
week 7
week 8
Handling & Habituation
Training
Probetrial
Retention
Reversal

595

596

597 ***Figure 2: Ageing leads to organ-specific inflammatory phenotypes that are aggravated by***
598 ***social isolation stress.***

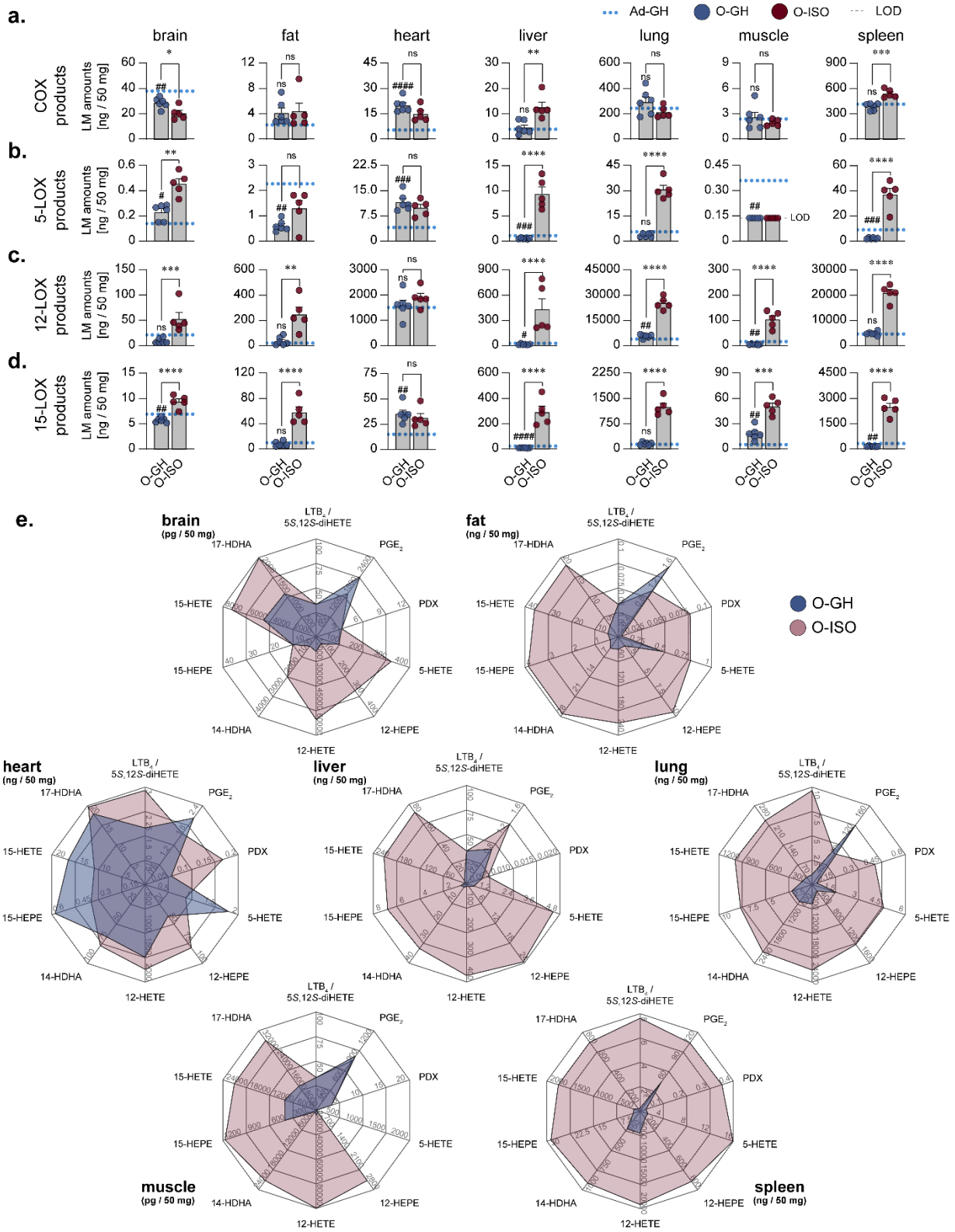
599 **(a)** Body weight of adult (5 months) and aged (20 months) mice after being kept in group-housing
600 (Ad-GH and O-GH, respectively) or repeatedly isolated (O-ISO). **(b,c)** Concentration of the
601 cytokines IL-1β and IL-1ra in pg per 50 mg organ. **(d)** Principal component analysis of organ-
602 specific oxylipin profiles. Hexagons indicate the mean PCA score of all screened replicates within
603 respective experimental groups. **(e)** Log_2-fold changes for the organ-specific concentrations of
604 individual oxylipins for the comparison of aged (O-GH) and adult (Ad-GH) mice kept in group-
605 housing (left panel), and the comparison of aged, repeatedly isolated (O-ISO) and aged, group-
606 housed (O-GH) mice (right panel). Fold-changes that could not be calculated due to missing values
607 (e.g., below limit of detection = nd) are depicted in grey. **Statistics**: Data are shown as (**a**-**c**) mean
608 ± SEM. The number of biological replicates is n = 5 for Ad-GH and O-ISO, n = 6 for O-GH.
609 Unpaired, two-tailed Student's t-tests with or without Welch-correction were performed for
610 indicated comparisons. #, comparison of O-GH versus Ad-GH.


a.
b.
c.
d.
e.
Ad-GH
O-GH
O-ISO
body weight [g]
IL-1β [pg/50 mg]
IL-1ra [pg/50 mg]
brain
fat
heart
liver
lung
muscle
spleen
Dim1 (47.8%)
Dim2 (26.9%)
Dim1 (59.8%)
Dim2 (14.8%)
Dim1 (42%)
Dim2 (32.3%)
Dim1 (78.9%)
Dim2 (8.8%)
Dim1 (63.7%)
Dim2 (19.5%)
Dim1 (52.9%)
Dim2 (31.1%)
Dim1 (67.6%)
Dim2 (12.7%)
AGE
ISOLATION
trans-LTB4
epitrans-LTB4
LTB4 / 5S,12S-diHETE
6-keto PGF1α
PGD2
PGE2
15-keto PGE2
PGF2α
PGJ2
TXB2
5-HETE
5-HEPE
12-HETE
12-HEPE
14-HDHA
15-HETE
15-HEPE
17-HDHA
MaR2
PDx
RvD5
8-HETE
11-HETE
11-HEPE
18-HEPE
4-HDHA
10-HDHA
13-HDHA
9-HODE
13-HODE
log2-fold change
nd

611

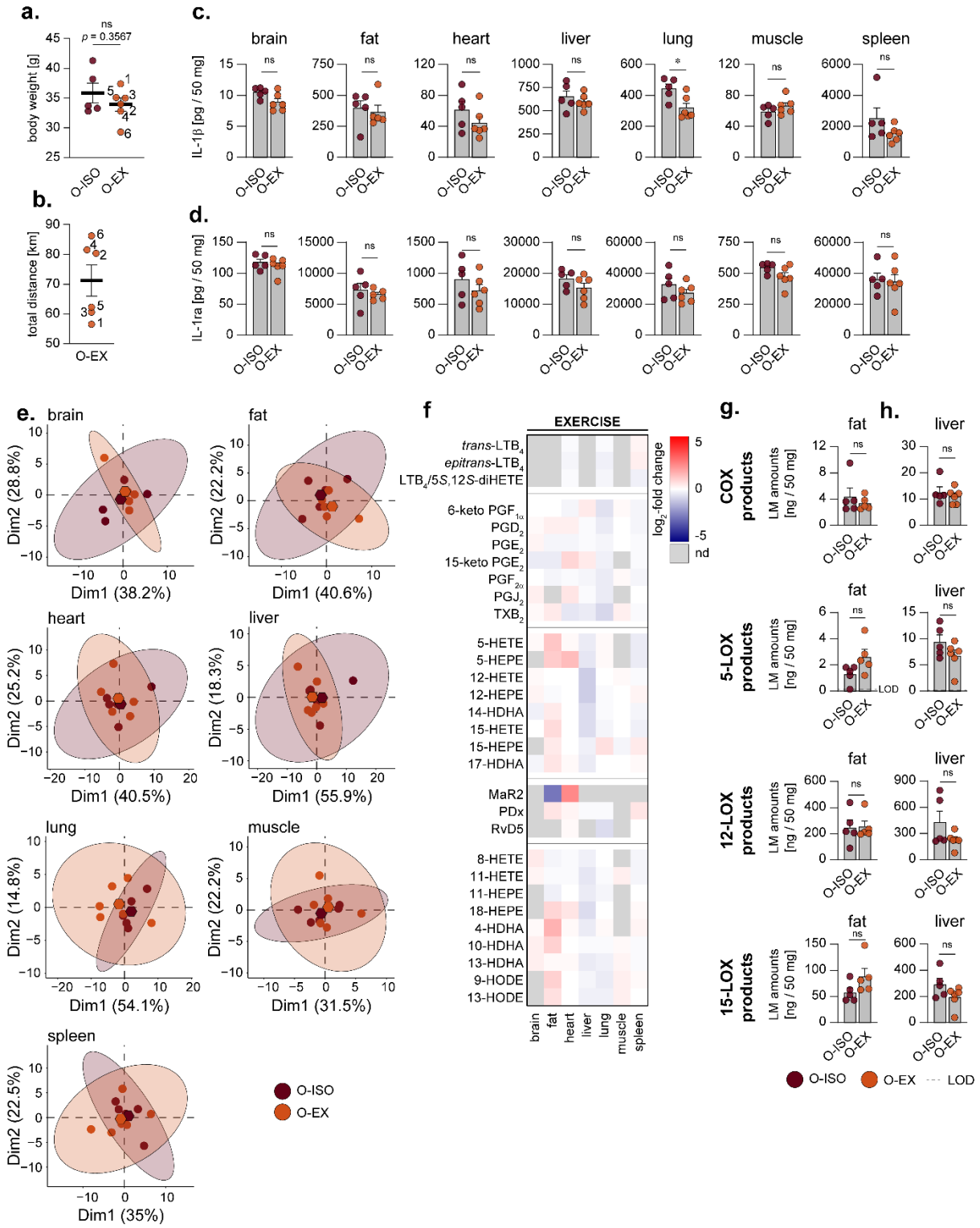
612 ***Figure 3: Social isolation stress leads to induction of LOX pathways.***

613 Total grouped amounts of oxylipins that are products of the COX **(a)** or LOX **(b-d)** pathways.
614 Metabolites were grouped as follows: *COX* – PGD_1, PGE_1, $PGF_{1\alpha}$, 6-keto $PGF_{1\alpha}$, PGD_2, PGE_2,
615 15-keto PGE_2, $PGF_{2\alpha}$, $PGF_{2ß}$, PGJ_2, PGD_3/PGE_3, $PGF_{3\alpha}$, TXB_2; *5-LOX* – RvE1, RvE2, *trans*-
616 LTB_4, *epitrans*-LTB_4, LTB_4/5*S*,12*S*-diHETE, 5*S*,6*R*-diHETE, 20-OH LTB_4, LTB_5, 5-HETE, 5-
617 HEPE, 7-HDHA; *12-LOX* – MaR1, MaR2, 12-HETE, 12-HEPE, 14-HDHA; *15-LOX* – PDx, PD1,
618 RvD1, RvD2, RvD3, RvD4, RvD5, RvE4, LXA_4, LXB_4, LXA_5, 5*S*,15*S*-diHETE, 15-HETE, 15-
619 HEPE, 17-HDHA. Values are given as ng per 50 mg organ. LOD of the oxylipins is indicated, if
620 applicable. **(e)** Radar charts of individual oxylipins in aged, group-housed mice (O-GH, blue) and
621 aged mice undergoing repeated isolation (O-ISO, red). **Statistics**: Data are shown as (a-d) mean ±
622 SEM. The number of biological replicates is n = 5 for Ad-GH and O-ISO, n = 6 for O-GH.
623 Unpaired, two-tailed Student's *t*-tests with or without Welch-correction were performed for
624 indicated comparisons. #, comparison of O-GH versus Ad-GH.


a.
b.
c.
d.
e.
Ad-GH
O-GH
O-ISO
LOD
brain
fat
heart
liver
lung
muscle
spleen
COX products
5-LOX products
12-LOX products
15-LOX products
LM amounts [ng / 50 mg]
O-GH
O-ISO
LTB4 / 5S,12S-diHETE
PGE2
PDX
5-HETE
12-HEPE
12-HETE
14-HDHA
15-HEPE
15-HETE
17-HDHA
(pg / 50 mg)
(ng / 50 mg)

626 ***Figure 4: Exercise cannot ameliorate increased oxylipin secretion in aged mice affected by***
627 ***social isolation stress.***

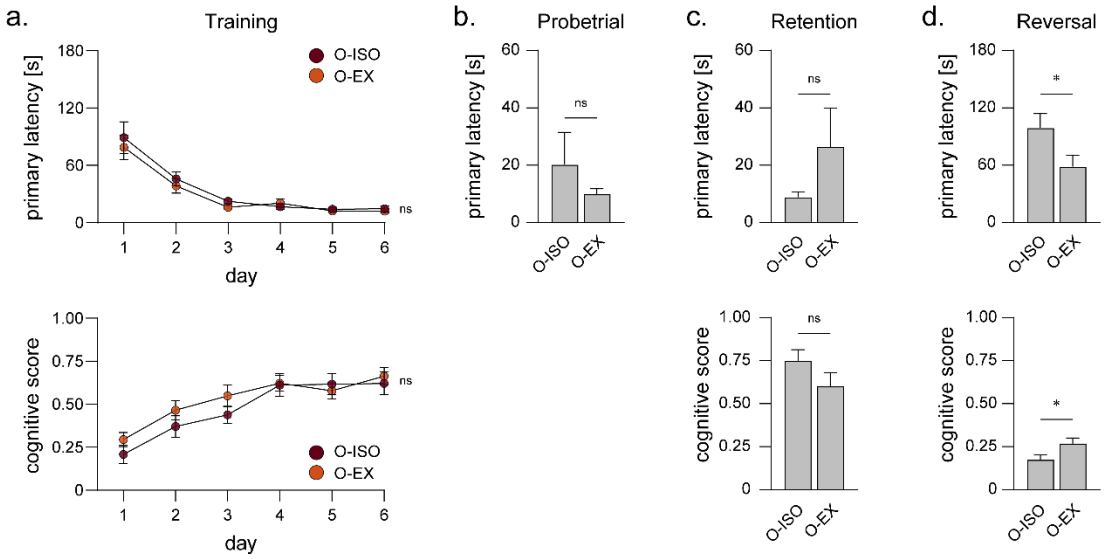
628 **(a)** Body weight of aged (20 month) mice after being repeatedly isolated into single cages with
629 (O-EX) or without running wheels (O-ISO) for 3 separate nights per week over a period of 8
630 weeks. **(b)** Total running performance of O-EX mice is summarised in km over the experimental
631 period of eight weeks. **(a,b)** Numbers indicate the individual animals in the O-EX cohort. **(c,d)**
632 Concentration of IL-1β and IL-1ra in pg per 50 mg organ from O-ISO and O-EX mice. **(e)** Principal
633 component analysis of organ-specific oxylipin profiles from O-ISO and O-EX mice. Hexagons
634 indicate the mean PCA score of all screened replicates within respective experimental groups. **(f)**
635 Log_2-fold changes for the organ-specific levels of individual oxylipins comparing the levels of O-
636 EX versus O-ISO mice. Fold-changes that could not be calculated due to missing values (e.g.,
637 below limit of detection = nd) are depicted in grey. **(g,h)** Total amounts of grouped oxylipin species
638 derived from the COX or LOX pathways in **(g)** fat and **(h)** liver. Oxylipins were grouped as
639 indicated in Fig. 3. Values are given as ng per 50 mg organ. Limit of detection (LOD) of oxylipins
640 is indicated, if applicable. **Statistics**: Data are shown as (**a-d**, **g-h**) mean ± SEM. The number of
641 biological replicates is n = 5 for O-ISO and n = 5-6 for O-EX. Unpaired, two-tailed Student's t-
642 tests with or without Welch-correction were performed for indicated comparisons.


a.
ns
p = 0.3567
body weight [g]
O-ISO
O-EX
b.
total distance [km]
O-EX
c.
brain
fat
heart
liver
lung
muscle
spleen
IL-1β [pg / 50 mg]
d.
IL-1ra [pg / 50 mg]
e.
Dim1 (38.2%)
Dim2 (28.8%)
Dim1 (40.6%)
Dim2 (22.2%)
Dim1 (40.5%)
Dim2 (25.2%)
Dim1 (55.9%)
Dim2 (18.3%)
Dim1 (54.1%)
Dim2 (14.8%)
Dim1 (31.5%)
Dim2 (22.2%)
Dim1 (35%)
Dim2 (22.5%)
O-ISO
O-EX
f
EXERCISE
trans-LTB4
epitrans-LTB4
LTB4/5S,12S-diHETE
6-keto PGF1α
PGD2
PGE2
15-keto PGE2
PGF2α
PGJ2
TXB2
5-HETE
5-HEPE
12-HETE
12-HEPE
14-HDHA
15-HETE
15-HEPE
17-HDHA
MaR2
PDx
RvD5
8-HETE
11-HETE
11-HEPE
18-HEPE
4-HDHA
10-HDHA
13-HDHA
9-HODE
13-HODE
log2-fold change
nd
g.
COX products
5-LOX products
12-LOX products
15-LOX products
LM amounts [ng / 50 mg]
LOD
h.
O-ISO
O-EX
LOD

643

644 ***Figure 5: Cognitive function assessment of mice undergoing recurring social isolation with***
645 ***and without physical exercise.***

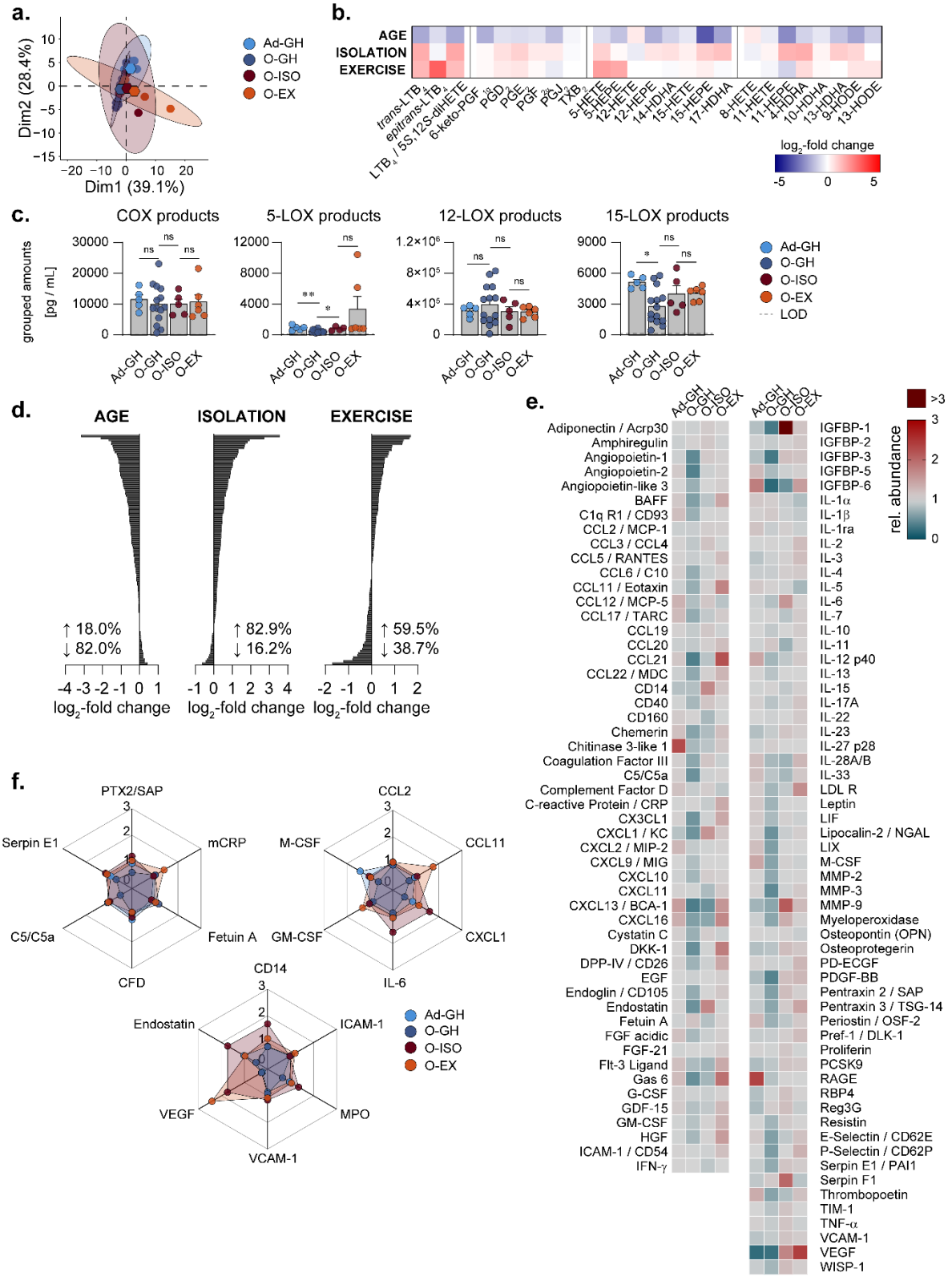
646 Results from the Barnes maze test, depicted as the primary latency period and as cognitive scores
647 for **(b)** training, **(c)** probe trial, **(d)** after a retention period, and **(e)** in a reversal test. **Statistics**:
648 Data are shown as mean ± SEM. The number of biological replicates is n = 18 for O-GH and n =
649 17 for O-EX. Unpaired, two-tailed Student's t-tests with or without Welch-correction were
650 performed for indicated comparisons.


a.
Training
O-ISO
O-EX
primary latency [s]
180
120
60
0
1
2
3
4
5
6
day
ns
cognitive score
1.00
0.75
0.50
0.25
0
b.
Probetrial
60
40
20
ns
c.
Retention
d.
Reversal
*

651

652 ***Figure 6: Impact of age and social isolation on inflammatory oxylipins and proteins in serum***

653 **(a)** Principal component analysis of oxylipin profile of serum. Hexagons indicate the mean PCA
654 score of all screened replicates within respective experimental groups. **(b)** Log_2-fold changes for
655 the concentrations of individual lipid mediators, detected in serum, comparing the levels of (AGE)
656 O-GH versus Ad-GH, (ISOLATION) O-ISO versus O-GH, and (EXERCISE) O-EX versus O-ISO
657 mice. **(c)** Total amounts of grouped LM species that are products of the COX or LOX pathways in
658 serum. Metabolites were grouped as indicated for Fig. 3. Values are given as pg per mL serum.
659 Limit of detection (LOD) of the metabolites is indicated, if applicable. **(d)** Log_2-fold changes of
660 111 screened, circulating proteins in pooled serum samples and percentages of regulated proteins
661 for the depicted comparisons. **(e)** Heatmap showing the relative abundance of the investigated
662 proteins in all experimental cohorts. **(f)** Radar charts displaying the relative abundance of selected
663 (top left) acute-phase proteins, (top right) classical cytokines and chemokines, and (bottom)
664 various surface markers and inflammatory proteins. **Statistics**: Data are shown as mean ± SEM
665 (**c**). The number of biological replicates is $n = 5$ for Ad-GH and O-ISO, $n = 14$ for O-GH and $n =$
666 6 for O-EX. (**d-f**) Samples were pooled from the serum of 4-5 biological replicates for all screened
667 cohorts. Unpaired, two-tailed Student's *t*-tests with or without Welch-correction were performed
668 for indicated comparisons.


a.
Dim2 (28.4%)
Dim1 (39.1%)
Ad-GH
O-GH
O-ISO
O-EX
b.
AGE
ISOLATION
EXERCISE
log2-fold change
c.
COX products
5-LOX products
12-LOX products
15-LOX products
grouped amounts [pg / mL]
LOD
d.
AGE
ISOLATION
EXERCISE
↑ 18.0%
↓ 82.0%
↑ 82.9%
↓ 16.2%
↑ 59.5%
↓ 38.7%
log2-fold change
e.
rel. abundance
f.
PTX2/SAP
Serpin E1
mCRP
C5/C5a
Fetuin A
CFD
CCL2
M-CSF
CCL11
GM-CSF
CXCL1
IL-6
CD14
Endostatin
ICAM-1
VEGF
MPO
VCAM-1

669

670 **Supplementary Information**

671 ***Figure S1: Raw pictures of proteome profiler membranes***

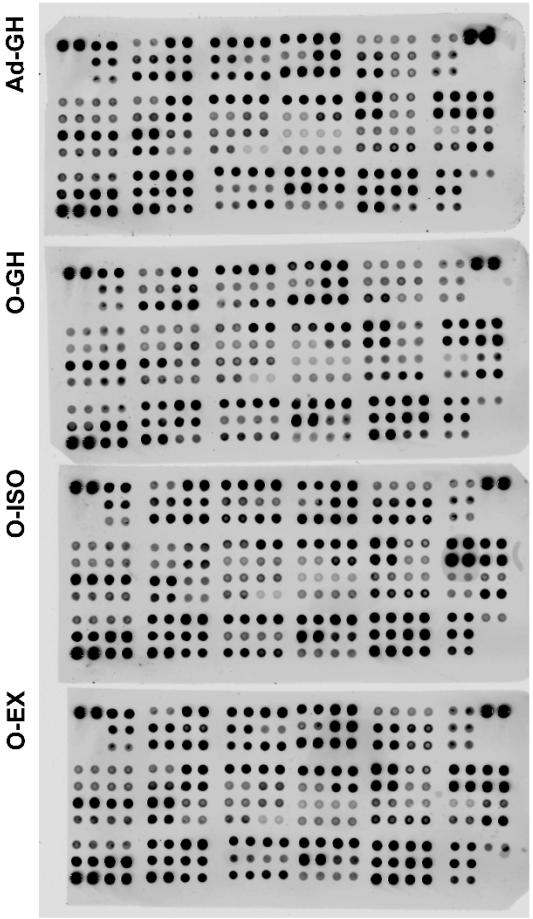
672 **(a)** Schematic layout of the proteome profiler overlay template used for the analysis of circulating
673 proteins in pooled serum samples. **(b)** List of the 111 screened proteins allotted to their
674 corresponding position on the membrane. **(c)** Pictures of the membranes used for the analysis of
675 circulating serum proteins in pooled serum samples.

a.

b.

spot	legend	spot	legend
A1-A2	reference spots	F1-F2	IGFBP-3
A3-A4	adiponectin	F3-F4	IGFBP-5
A5-A6	amphiregulin	F5-F6	IGFBP-6
A7-A8	angiopoietin-1	F7-F8	IL-1α
A9-A10	angiopoietin-2	F9-F10	IL-1β
A11-A12	angiopoietin-like 3	F11-F12	IL-1ra
A13-A14	CD257	F13-F14	IL-2
A15-A16	CD93	F15-F16	IL-3
A17-A18	MCP-1	F17-F18	IL-4
A19-A20	MIP-1α/β	F19-F20	IL-5
A21-A22	RANTES	F21-F22	IL-6
A23-A24	reference spots	F23-F24	IL-7
B3-B4	MRP-1	G1-G2	IL-10
B5-B6	eotaxin	G3-G4	IL-11
B7-B8	MCP-5	G5-G6	IL-12
B9-B10	TARC	G7-G8	IL-13
B11-B12	MIP-3β	G9-G10	IL-15
B13-B14	MIP-3α	G11-G12	IL-17A
B15-B16	SCYA21	G13-G14	IL-22
B17-B18	MDC	G15-G16	IL-23
B19-B20	CD14	G17-G18	IL-27
B21-B22	CD40	G19-G20	IL-28A/B
C3-C4	CD160	G21-G22	IL-33
C5-C6	chemerin	G23-G24	LDL R
C7-C8	chitinase 3-like 1	H1-H2	leptin
C9-C10	CD142	H3-H4	LIF
C11-C12	C5a	H5-H6	lipocalin-2
C13-C14	complement factor D	H7-H8	LIX
C15-C16	CRP	H9-H10	M-CSF
C17-C18	fractalkine	H11-H12	MMP-2
C19-C20	KC	H13-H14	MMP-3
C21-C22	MIP-2	H15-H16	MMP-9
D1-D2	MIG	H17-H18	myeloperoxidase
D3-D4	IP-10	H19-H20	osteopontin
D5-D6	I-TAC	H21-H22	osteoprotegerin
D7-D8	BCA-1	H23-H24	gliostatin
D9-D10	CXCL16	I1-I2	PDGF-BB
D11-D12	cystatin C	I3-I4	pentraxin 2
D13-D14	DKK-1	I5-I6	pentraxin 3
D15-D16	CD26	I7-I8	periostin
D17-D18	EGF	I9-I10	Pref-1
D19-D20	endoglin	I11-I12	proliferin
D21-D22	endostatin	I13-I14	PCSK9
D23-D24	fetuin A	I15-I16	RAGE
E1-E2	FGF acidic	I17-I18	RBP4
E3-E4	FGF-21	I19-I20	Reg3G
E5-E6	Flt-3 ligand	I21-I22	resistin
E7-E8	Gas 6	J1-J2	reference spots
E9-E10	G-CSF	J3-J4	E-selectin
E11-E12	GDF-15	J5-J6	P-selectin
E13-E14	GM-CSF	J7-J8	serpin E1
E15-E16	HGF	J9-J10	serpin F1
E17-E18	ICAM-1	J11-J12	thrombopoietin
E19-E20	IFN-γ	J13-J14	TIM-1
E21-E22	IGFBP-1	J15-J16	TNF-α
E23-E24	IGFBP-2	J17-J18	VCAM-1
		J19-J20	VEGF
		J21-J22	WISP-1
		J23-J24	negative control

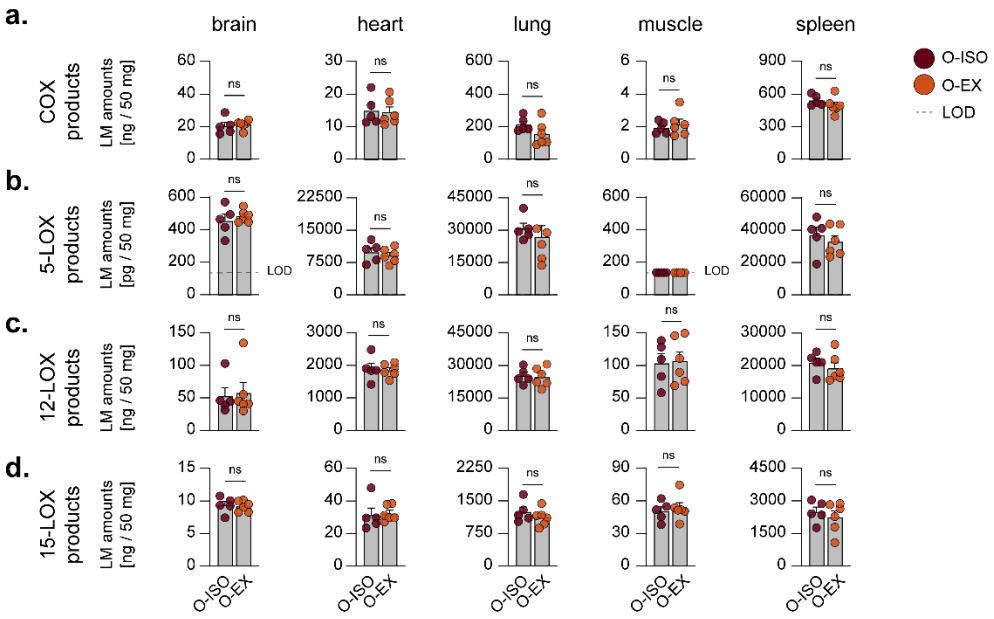
c.



676

677 ***Figure S2: Enzyme-derived oxylipin levels for isolated mice with and without exercise.***

678 **(a-d)** Total amounts of grouped LM species for isolated mice with (O-EX) and without exercise
679 (O-GH) that are products of the COX or LOX pathways in brain, heart, lung, muscle, spleen.
680 Metabolites were grouped as follows: **(a)** *COX* – PGD_1, PGE_1, $PGF_{1\alpha}$, 6-keto $PGF_{1\alpha}$, PGD_2, PGE_2,
681 15-keto PGE_2, $PGF_{2\alpha}$, $PGF_{2ß}$, PGJ_2, PGD_3/PGE_3, $PGF_{3\alpha}$, TXB_2; **(b)** *5-LOX* – RvE1, RvE2, *trans*-
682 LTB_4, *epitrans*-LTB_4, LTB_4/5*S*,12*S*-diHETE, 5*S*,6*R*-diHETE, 20-OH LTB_4, LTB_5, 5-HETE, 5-
683 HEPE, 7-HDHA; **(c)** *12-LOX* – MaR1, MaR2, 12-HETE, 12-HEPE, 14-HDHA; **(d)** *15-LOX* –
684 PDx, PD1, RvD1, RvD2, RvD3, RvD4, RvD5, RvE4, LXA4, LXB4, LXA5, 5*S*,15*S*-diHETE, 15-
685 HETE, 15-HEPE, 17-HDHA. Values are given as ng per 50 mg organ. LOD of the metabolites is
686 indicated, if applicable.


a.
b.
c.
d.
brain
heart
lung
muscle
spleen
COX products
5-LOX products
12-LOX products
15-LOX products
LM amounts [ng / 50 mg]
LM amounts [pg / 50 mg]
ns
LOD
O-ISO
O-EX

687

688 ***Figure S3: Circulatory proteome changes.***

689 **(a)** Log_2-fold changes of 111 screened, circulating proteins in pooled serum samples and
690 percentages of up- or downregulated proteins for the comparison of aged, isolated mice (O-ISO)
691 against adult, group-housed (Ad-GH) mice.

a.

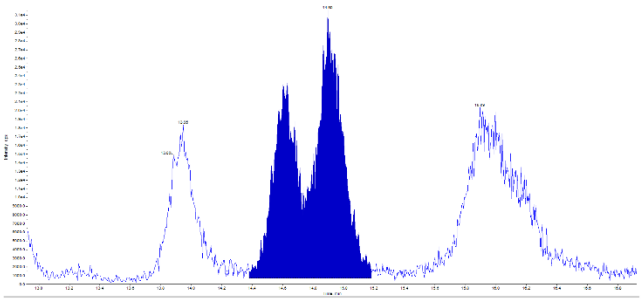
692

693

694 ***Figure S4: Representative chromatograms.***

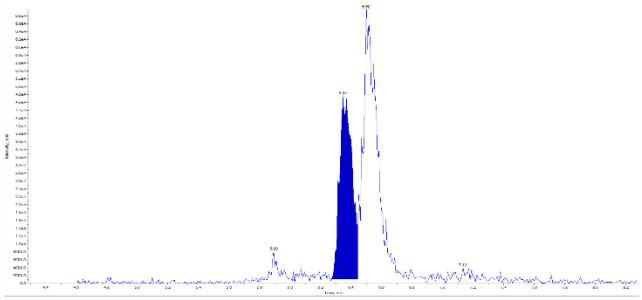
695 Representative. unsmoothed chromatograms including the integration of relevant metabolites **(a)**
696 LTB_4/5*S*,12*S*-diHETE, **(b)** PGE_2, **(c)** 15-HETE, **(d)** 15-HEPE, **(e)** 17-HDHA, and **(f)** PDx. Given
697 values represent the measured mass on column, the calculated mass in the sample, and the
698 normalized amount per 50 mg organ.

a.



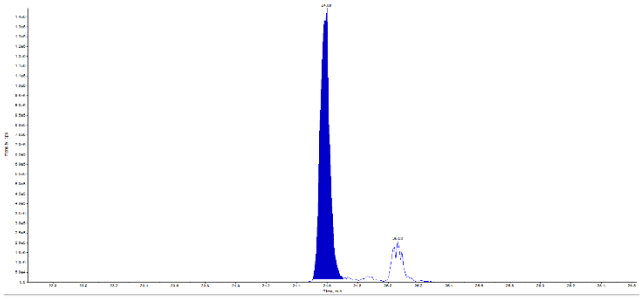
LTB_4/5*S*,12*S*-diHETE
lung of Ad-GH_4
= 15.18 pg measured mass
≙ 303.6 pg / sample
≙ 759.0 pg / 50 mg lung

b.



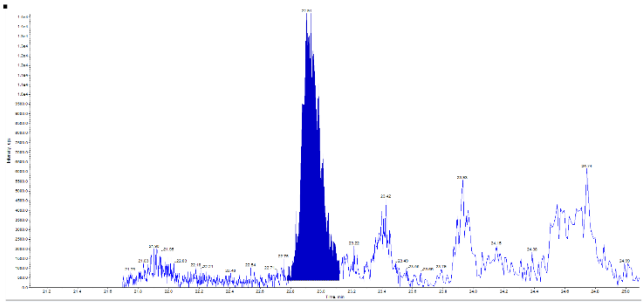
PGE_2
liver of O-GH_1
= 18.01 pg measured mass
≙ 360.3 pg / sample
≙ 900.7 pg / 50 mg lung

c.



15-HETE
lung of Ad-GH_2
= 2,401.61 pg measured mass
≙ 48,032.3 pg / sample
≙ 120,080.7 pg / 50 mg lung

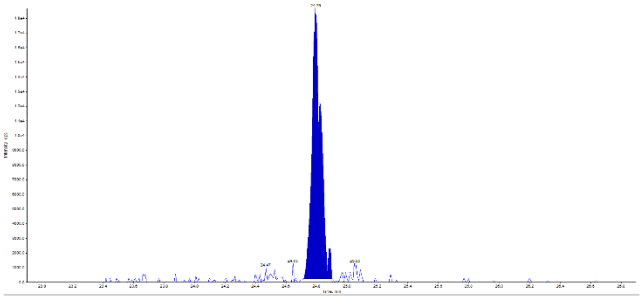
d.



15-HEPE
lung of O-GH_2
= 48.15 pg measured mass
≙ 963.0 pg / sample
≙ 2,407.6 pg / 50 mg lung

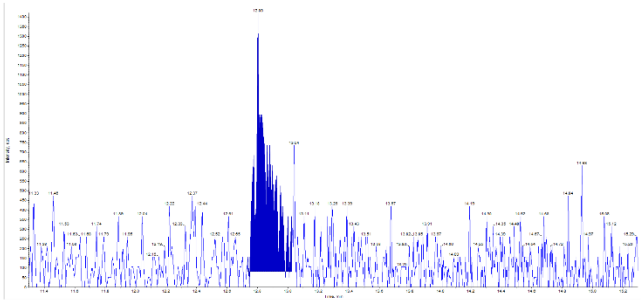
699

e.

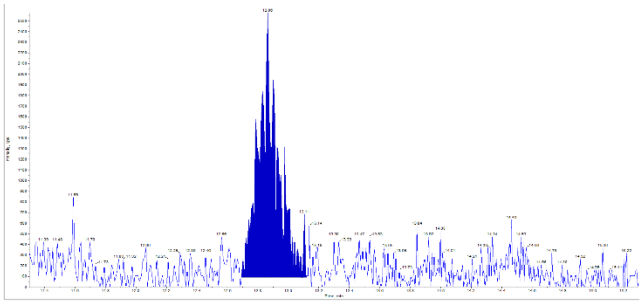


17-HDHA
muscle of O-ISO_5
= 756.82 pg measured mass
≙ 15,136.3 pg / sample
≙ 25,227.2 pg / 50 mg muscle

f.



PDx
fat of Ad-GH_4
< LOD



PDx
fat of O-ISO_2
= 2.23 pg measured mass
≙ 44.6 pg / sample
≙ 74.3 pg / 50 mg fat

700

701 ***Table S1: Screened metabolites, RT, transitions, LOD and LLOQ***

702 ***Table S2: Oxylipin raw data***

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