

1 ***Slc7a8* deletion is protective against diet-induced obesity and attenuates lipid accumulation** 2 **in multiple organs**

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11

12 **Abstract**

13 Adipogenesis, through adipocyte hyperplasia and/or hypertrophy, leads to increased adiposity,
14 giving rise to obesity. A genome-wide transcriptome analysis of adipogenesis in human adipose
15 derived stromal/stem cells identified SLC7A8 (Solute Carrier Family 7 Member 8) as a potential novel
16 mediator. This study has investigated the role of SLC7A8 in adipose tissue biology using a mouse
17 model of diet-induced obesity. *slc7a8* knockout (KO) and wildtype (WT) C57BL/6J mice were fed
18 either a control diet (CD) or a high-fat diet (HFD) for 14 weeks. On HFD, both WT and KO (WTHFD
19 and KOHFD) gained significantly more weight than their CD counterparts. However, KOHFD gained
20 significantly less weight than WT HFD. KOHFD significantly reduced the level of glucose intolerance
21 observed in WTHFD. KOHFD significantly reduced both adipocyte mass and hypertrophy in inguinal,
22 mesenteric, perigonadal and brown adipose depots, with a corresponding decrease in macrophage
23 infiltration. Additionally, KOHFD decreased lipid accumulation in the liver, heart, gastrocnemius
24 muscle, lung, and kidney. This study demonstrates that targeting *SLC7A8* protects against diet-
25 induced obesity by reducing lipid accumulation in multiple organs, and thereby has the potential to
26 mitigate the development of obesity-associated comorbidities.

27 **Author summary**

28 The development of obesity can be attributed to adipocyte hypertrophy or hyperplasia leading to
29 increased adiposity. The C57BL/6 mouse is an excellent model used to study metabolic syndromes
30 often associated with obesity development. Mice fed on a high-fat diet are susceptible to weight
31 gain leading to the development of obesity and its associated metabolic syndrome. Here, we report
32 findings from targeting a novel human adipogenic gene (*SLC7A8*) in condition of obesity
33 development using a mouse model of diet-induced obesity (DIO). The results indicate that deleting
34 *slc7a8* in mice significantly protects against DIO and improves glucose metabolism. Also, deficiency

35 in *slc7a8* was observed to significantly attenuates adipocyte hypertrophy in white and brown
36 adipose tissues, and reduced lipid accumulation in many organs. Furthermore, inflammation was
37 significantly reduced in adipose tissues and liver of *slc7a8* deficient mice in condition of DIO. Overall,
38 results from this study shows that *slc7a8* is an important molecular regulator of obesity
39 development and mediates its function by reducing lipid accumulation in multiple organs. Hence,
40 SLC7A8 could serve as a potential therapeutic target to combat the development of obesity and
41 other pathophysiological conditions associated with excess lipid accumulation in organs.

42 **Introduction**

43 Obesity is characterised by an excess accumulation of adipose tissue when energy intake exceeds
44 energy expenditure. The expansion of adipose tissue in obesity occurs either through adipocyte
45 hyperplasia or hypertrophy. The result is dysfunctional adipose tissue mainly due to adipocyte
46 hypertrophy, which leads to adverse metabolic consequences and chronic inflammation[1]. The
47 distribution of adipose tissue in obesity plays an important role in the development of obesity-
48 associated comorbidities. Accumulation of fat in the intra-abdominal depots (visceral depots) gives
49 rise to insulin resistance and is also associated with an increased risk of cardiovascular diseases[2].
50 Subcutaneous white adipose tissue (WAT) is the most common adipose tissue in healthy lean
51 individuals and serves as a metabolic sink for excess lipid storage[3]. Brown adipose tissue takes up
52 fatty acid in circulation to generate heat, which helps to clear plasma triglycerides thereby reducing
53 the accumulation of lipid at visceral depots[4]. In obesity, where the storage capacity of adipose
54 tissue is exceeded either due to an inability to produce new adipocytes (limited hyperplasia) or to
55 expand further (limited hypertrophy), excess fat is redistributed to peripheral organs such as the
56 liver and skeletal muscle which increases the risk of metabolic syndromes such as hyperglycaemia,
57 hyperinsulinemia, atherosclerosis, dyslipidemia and systemic inflammation[3, 5]. Hypertrophy in
58 brown adipose tissue (BAT) may impair its function in acting as a sink for excess blood glucose and
59 clearance of free fatty acids from circulation, thereby contributing to the development of insulin
60 resistance and hyperlipidemia in obesity[3]. Therefore, mitigating adipocyte hypertrophy in both
61 WAT and BAT depots is paramount to improving metabolic health.

62 Inflammation is a key consequence of adipose tissue expansion that occurs during weight gain and
63 contributes to the development of chronic low-grade systemic inflammation seen in obesity. This
64 expansion of adipose tissue is characterized by increased infiltration of immune cells, with a
65 predominance (around 60%) of macrophages, in response to chemokines that are produced by

66 hypertrophic adipocytes[6]. The majority are derived from circulating monocytes with a small
67 proportion coming from the proliferation of adipose tissue resident macrophages[7]. Tissue resident
68 macrophages present in normal or lean adipose tissue are of the M2 anti-inflammatory macrophage
69 phenotype that express markers such as mannose receptor (CD206), and are thought to be
70 responsible for maintaining tissue homeostasis[8]. Macrophage infiltration in adipose tissue
71 appears as crown-like clusters which is believed to signify an immune response to dying or dead
72 adipocytes[9]. These infiltrating macrophages undergo a phenotypic switch to a M1 pro-
73 inflammatory phenotype[10].

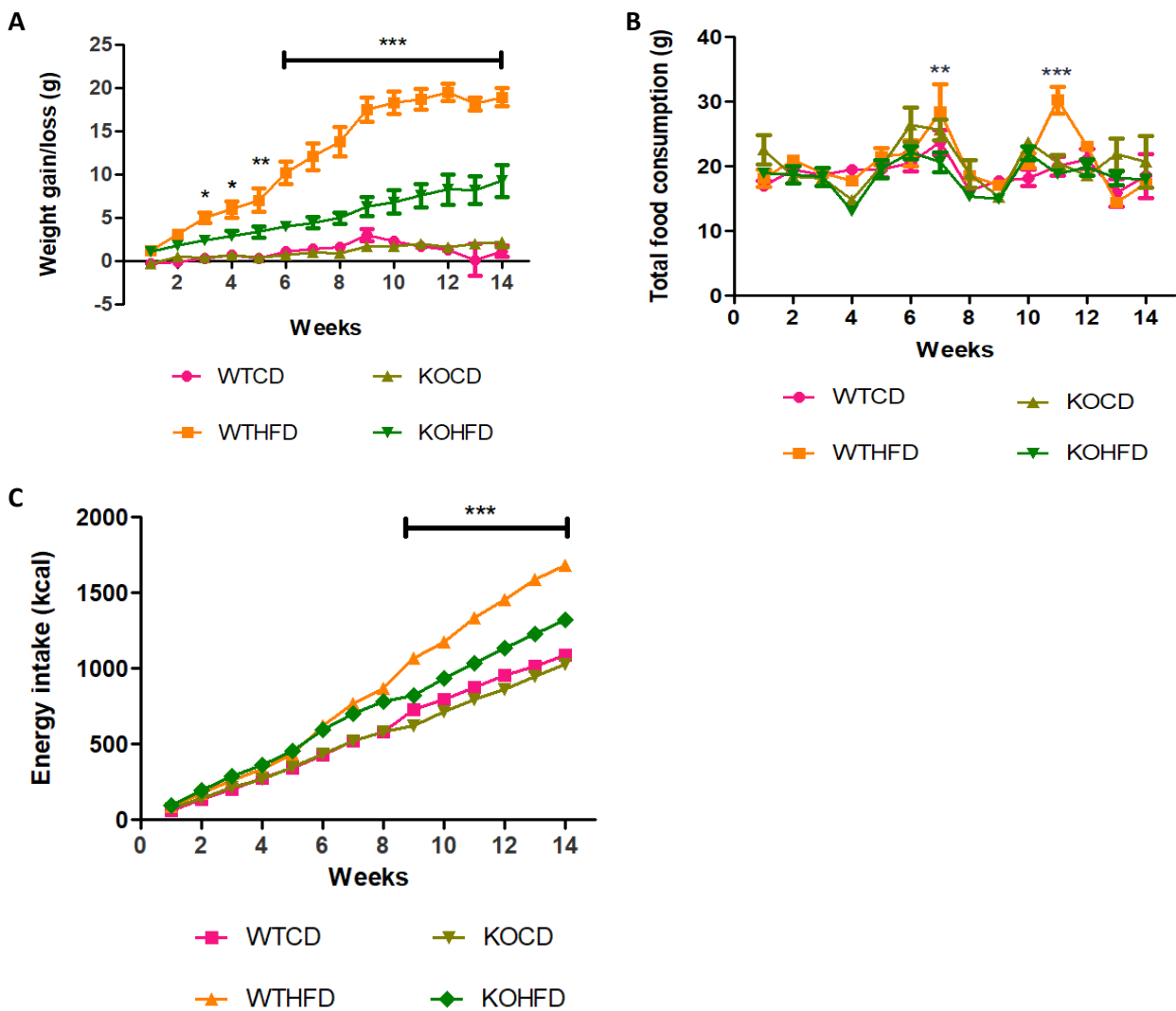
74 In addressing obesity, several studies have suggested exploiting the process of fat cell formation
75 (adipogenesis) to combat obesity development. These have led to several molecular determinants
76 being described to play important role in adipogenesis[11]. Except for PPAR γ [12, 13], molecular
77 determinants of adipogenesis have proven to be of limited clinical utility. Therefore, more research
78 is needed to identify new molecular determinants of adipogenesis which could play a role in obesity
79 development and serve potential therapeutic targets. We have previously undertaken an unbiased
80 exploratory comprehensive transcriptomic analysis of human adipose-derived stromal/stem cells
81 undergoing adipogenesis and identified several novel genes and transcription factors with possible
82 role in this process [14, 15]. One of the novel genes identified was *SLC7A8* (Solute Carrier Family 7
83 Member 8), not previously described in the context of adipogenesis and/or obesity, that was
84 significantly upregulated in the early phase of adipogenesis and declined significantly as the process
85 progressed [14]. This could suggest a role for this gene in the early stages of adipogenesis as a
86 potential driver of adiposity and consequently obesity. The aim of this study was to therefore
87 investigate the functional role of the *SLC7A8* in weight gain/obesity development and lipid
88 accumulation in various organs/tissues using a mouse model of diet induced obesity, as well as the
89 macrophage infiltration profile in some of these tissues.

90 **Results**

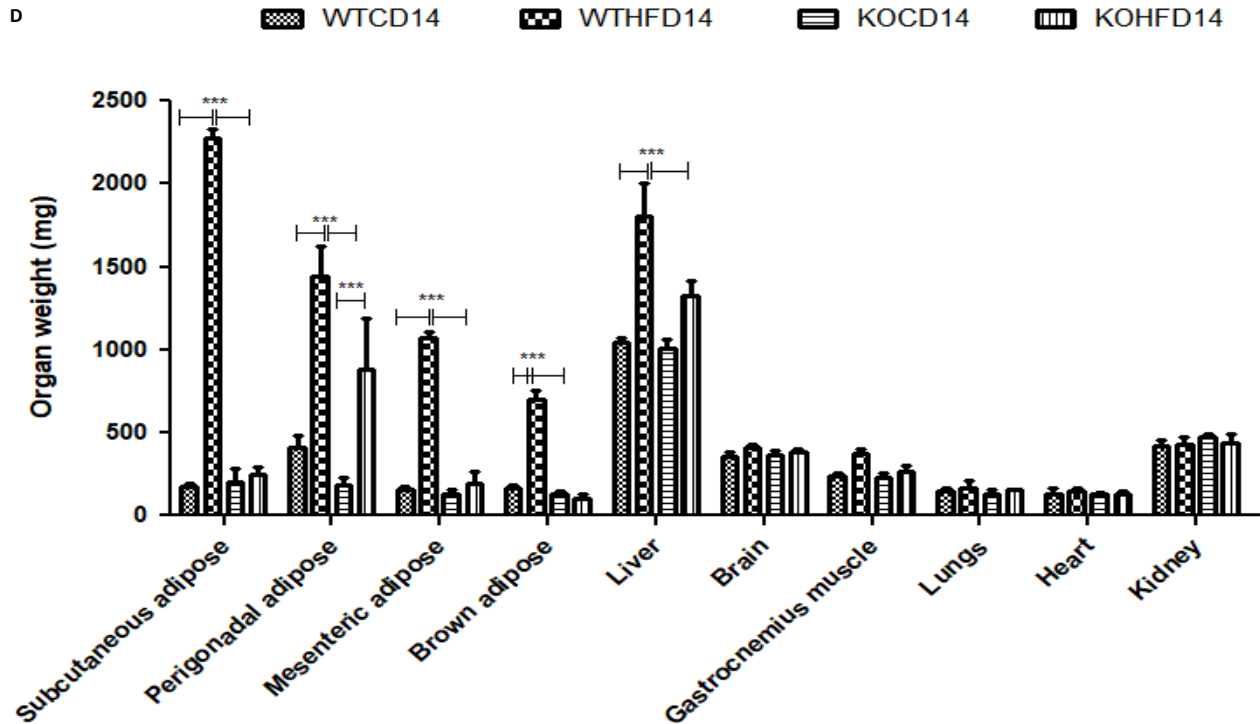
91 Deficiency of *slc7a8* protects against diet-induced obesity

92 WT and KO mice significantly gained weight at 14 weeks on HFD compared to WTCD ($p < 0.05$ to
93 $p < 0.001$) and KOCD ($p < 0.05$ to $p < 0.001$) (Figure 1A). No significant differences were observed
94 between WT and KO on CD. Interestingly, KOHFD gained significantly ($p < 0.05$ to $p < 0.001$) less weight
95 than WTHFD, which was evident from week 3 (Figure 1A). Significant weight gain in WTHFD was

96 associated with significantly larger ($p < 0.001$) iWAT, pWAT, mWAT, BAT and liver compared to WTCD
97 and KOHFD. Only the pWAT of KOHFD was significantly larger than in KOCD14 (Figure 1D). WTHFD
98 and KOHFD mice appear visibly larger in size when compared to their respective lean counterparts
99 (Figure 1 supplementary). Food consumption was similar across the four groups under study, except
100 that WTHFD had significantly greater food intake at 7 weeks when compared to KOHFD ($p < 0.01$)
101 and at 11 weeks when compared to WTCD ($p < 0.01$) and KOHFD ($p < 0.001$) (Figure 1B). Energy intake
102 increased significantly between WTCD and WTHFD ($p < 0.01$ at week 5 and $p < 0.001$ from week 6 to
103 week 14) and between KOCD and KOHFD ($p < 0.05$ at week 3, $p < 0.01$ at week 4, and $p < 0.001$ from
104 week 5 to 14) (Figure 1C). A significant difference ($p < 0.001$) in cumulative caloric intake was
105 observed between WTHFD and KOHFD from week 9 to week 14. No significant differences in caloric
106 intake were seen between KOCD and WTCD.



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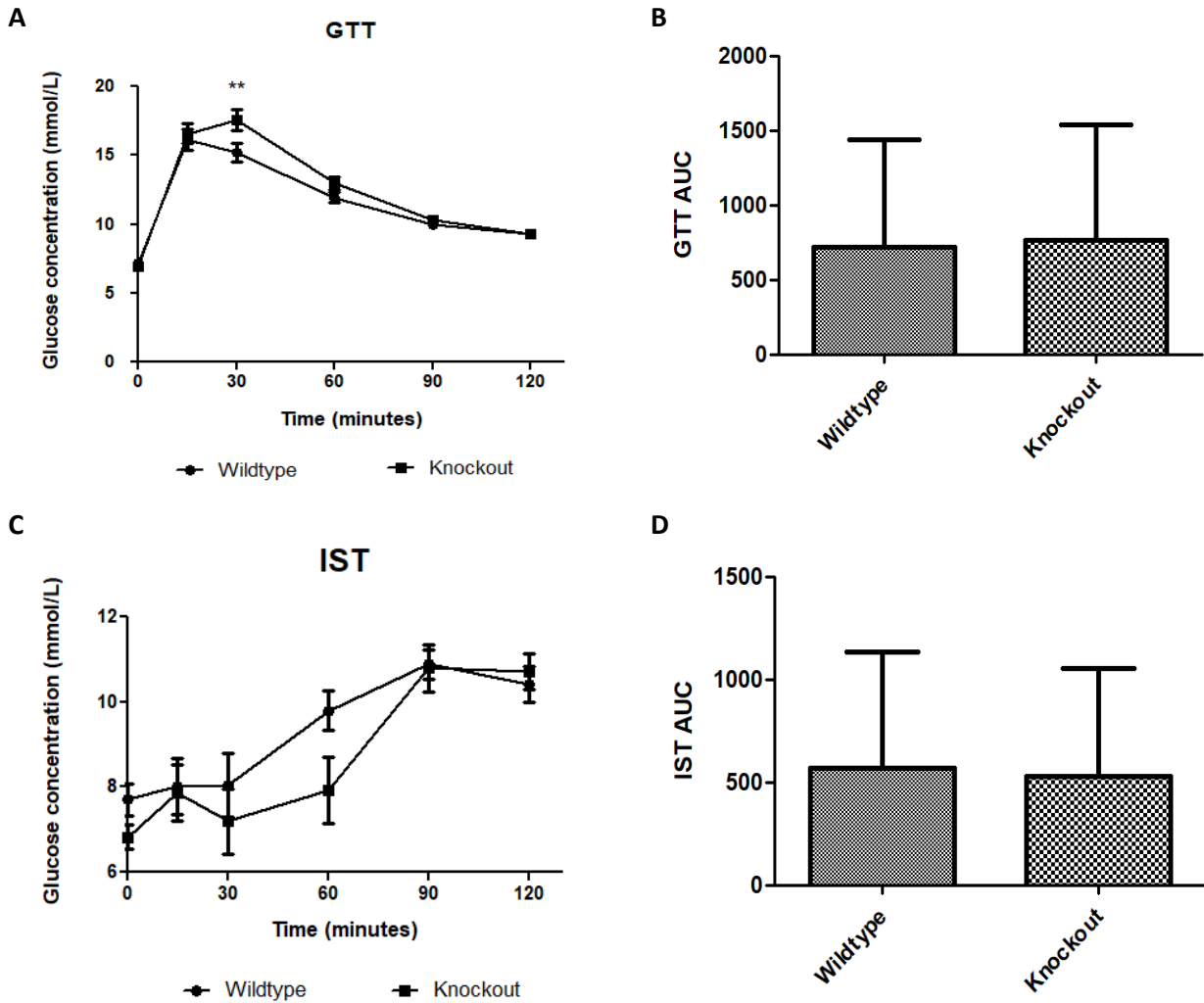
109 Figure 1: Effect of *slc7a8* deletion on body weight and caloric intake. WTHFD significantly gained weight
110 throughout the 14-week period starting from week 2 when compared to WTCD ($p < 0.05$ to $p < 0.001$). KOHFD
111 significantly gained weight in comparison to KOCD ($p < 0.05$). The difference in weight gain between WTHFD
112 and KOHFD was significant starting week 3, with the p-value increasing gradually from $p < 0.05$ to $p < 0.001$.
113 The WTCD and KOCD showed no differences in weight, A. Total cumulative food consumption was similar
114 across the four groups, except that WTHFD had significantly greater food intake at week 11 when compared
115 to WTCD and KOHFD, B. Energy intake increased significantly from $p < 0.01$ at week 2 to $p < 0.001$ from week
116 3 to week 14 between WTCD and WTHFD. A significant difference ($p < 0.001$) in caloric intake was observed
117 between WTHFD and KOHFD from week 11 to week 14. No significant differences in caloric intake were seen
118 between KOCD and WTCD, C. WTHFD showed significantly larger ($p < 0.001$) iWAT, pWAT, mWAT, BAT and
119 liver compared to WTCD and KOHFD, D. A-C: Week 1-5: N=18 for WTCD, WTHFD, KOHFD and N=17 for
120 KOCD; Week 6-8: N=12 for WTCD, WTHFD, KOHFD and N=11 for KOCD; Week 9-12: N=6 for WTCD,
121 WTHFD, KOHFD and N=5 for KOCD; Week 13-14: N=5 for WTCD, WTHFD, KOCD and N=6 for KOHFD.

122

123 Deficiency in *slc7a8* had no effect on glucose and insulin metabolism but significantly improved
124 glucose tolerance on HFD.

125 WT and KO mice showed no difference in metabolism of exogenous glucose (Figure 2A and B) and
126 insulin (Figure 2C and D) prior to introducing them on the experimental diets. However, significantly
127 elevated glucose levels ($p < 0.01$) were observed for the KO mice at 30 minutes. After 5 weeks on
128 experimental diets, no significant difference was observed in glucose metabolism between WT and
129 KO on either the CD or HFD (Figure 3A and B). Conversely, at 14 weeks, WTHFD had significantly
130 higher glucose levels compared to KOHFD and WTCD starting from 30 minutes (Figure 3C). Although
131 WTHFD had a larger AUC compared to KOHFD and WTCD, this was not statistically significant (Figure
132 3D). No significant differences were observed between the AUC of WTCD5, WTHFD5, KOCD5,

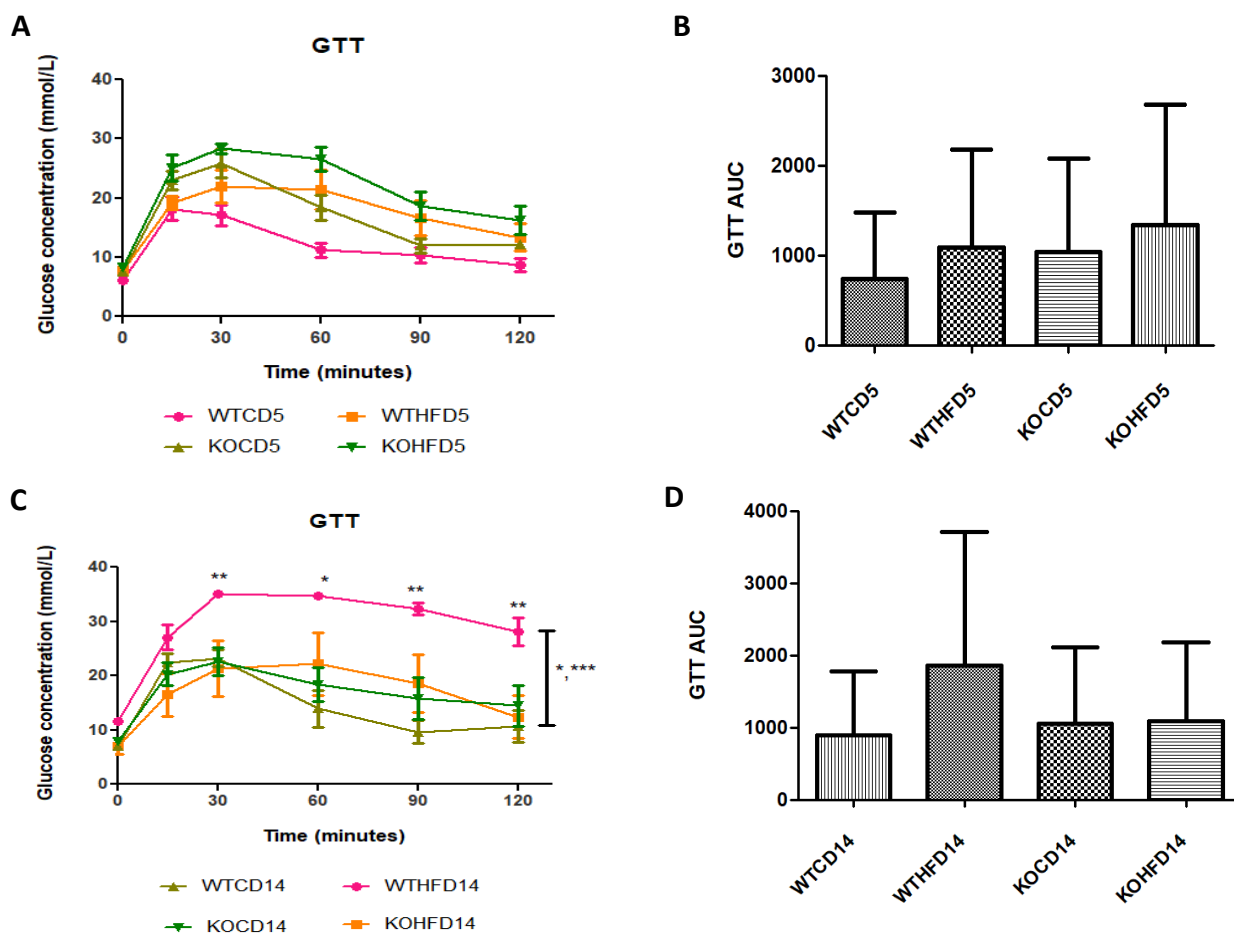
133 KOHFD5 when compared to their 14-week counterparts (WTCD14, WTHFD14, KOCD14 and
134 KOHFD14 (Figure 3B and D).



135

136

137 Figure 2: Effect of genotype on glucose tolerance and insulin sensitivity tests. GTT and IST were conducted
138 before introducing the C57BL/6J wildtype and *Slc7a8* knockout mice to CD and HFD. No significant differences
139 were observed in GTT and IST between the WT and KO except that significantly higher glucose levels ($p < 0.01$)
140 were observed for the KO mice at 30 minutes of the GTT, A. GTT: N=47 for WT and N=48 for KO; IST: N=47
141 for WT and N=44 for KO.



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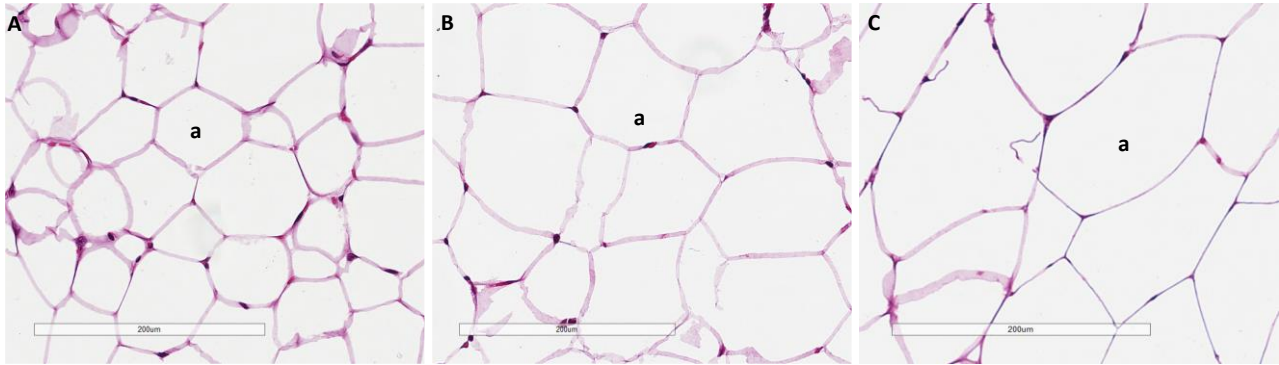
143 Figure 3: Glucose tolerance and insulin sensitivity tests of animals on experimental diet. No significant
 144 differences were observed after experimental feeding between WT and KO on either CD and HFD at 5 weeks,
 145 A and B. The WTHFD showed significantly higher glucose levels compared to KOHFD ($p < 0.05$, 0.01) and
 146 WTCD ($p < 0.05$, 0.001) at 14 weeks. No significant differences were observed between WTCD5, WTHFD5,
 147 KOCD5, KOHFD5 and their respective 14-week counterparts, B and D. $N = 6$ for WTCD5, WTHFD5, KOCD5,
 148 KOHFD5, WTCD14, WTHFD14, KOCD14, and $N = 5$ for KOHFD14

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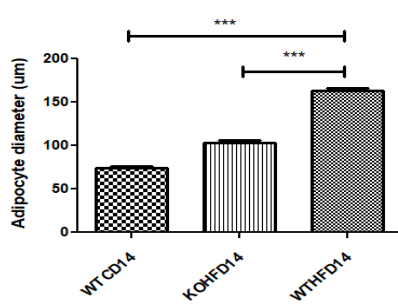
150 *Slc7a8* deletion attenuates adipocyte hypertrophy in white and brown adipose depots

151 The pWAT from WTCD (Figure 4A) and KOHFD (Figure 4B) had significantly smaller ($p < 0.001$)
 152 adipocyte sizes compared to WTHFD (Figure 4C) as indicated in the column graph (Figure 4D). The
 153 number of adipocytes per field was significantly higher in WTCD ($p < 0.001$) and KOHFD ($p < 0.05$)
 154 compared to WTHFD (Figure 4E). The iWAT in WTHFD (Figure 4H) had a significant increase
 155 ($p < 0.001$) (Figure 4I) in adipocyte hypertrophy compared to KOHFD (Figure 4G) and WTCD (Figure
 156 4F). Similarly, a significant increase ($p < 0.001$) was observed in adipocyte size of mWAT WTHFD
 157 (Figure 4M) in comparison to KOHFD (Figure 4L) and WTCD (Figure 4K), Figure 4N. The number of
 158 adipocytes per field was significantly lower in mWAT ($p < 0.01$) (Figure 3J) and iWAT ($p < 0.001$) (Figure
 159 3O) of WTHFD compared to WTCD, as well as in mWAT ($p < 0.01$) and iWAT ($p < 0.001$) of WTHFD

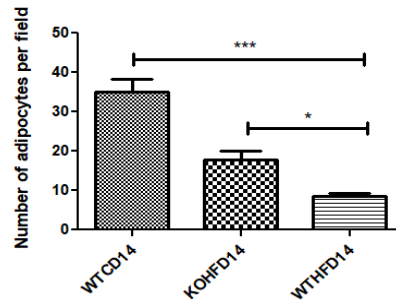
160 compared to KOHFD. Lipid droplet accumulation was greater in BAT of WTHFD (Figure 4R) compared
161 to WTCD (Figure 4P) and KOHFD (Figure 4Q). Additionally, as early as 5 weeks on experimental diet,
162 adipocyte hypertrophy was greater in WTHFD compared to KOHFD and WTCD in pWAT, mWAT,
163 iWAT, and larger lipid droplets were observed in BAT of WTHFD (Figure 2 supplementary).
164



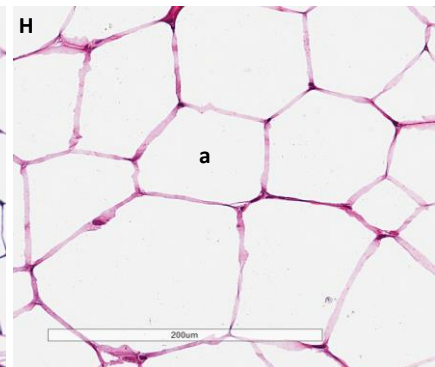
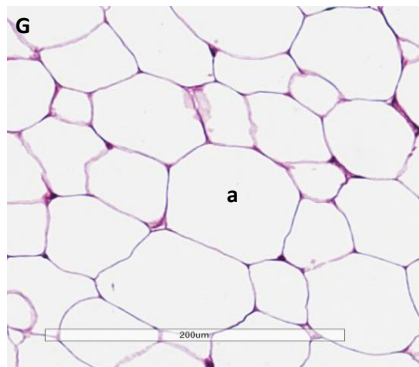
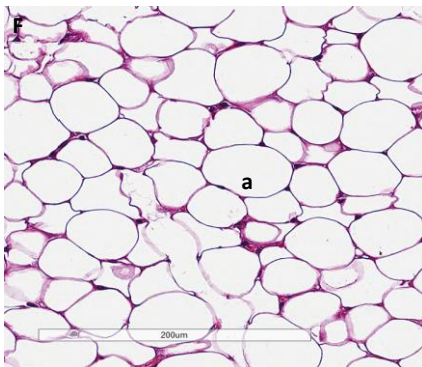
D 14 weeks perigonadal adipose



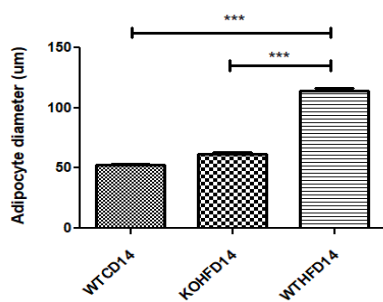
E 14 weeks perigonadal adipose



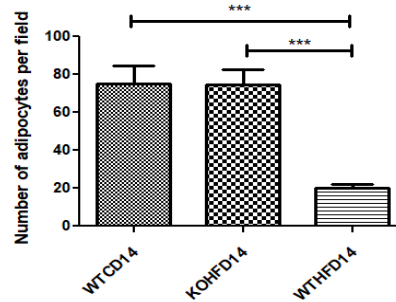
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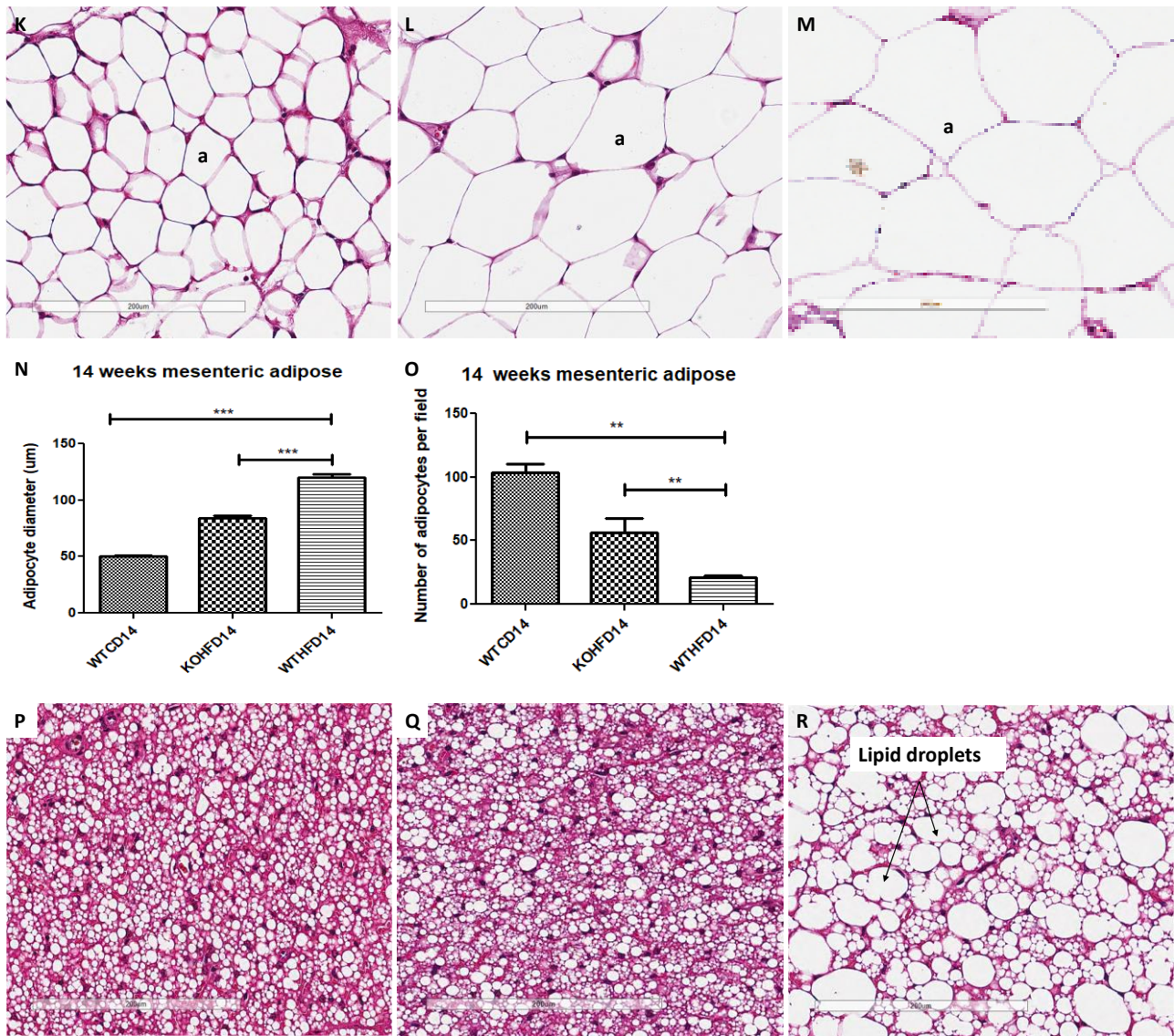
I 14 weeks inguinal subcutaneous adipose



J 14 weeks inguinal subcutaneous adipose



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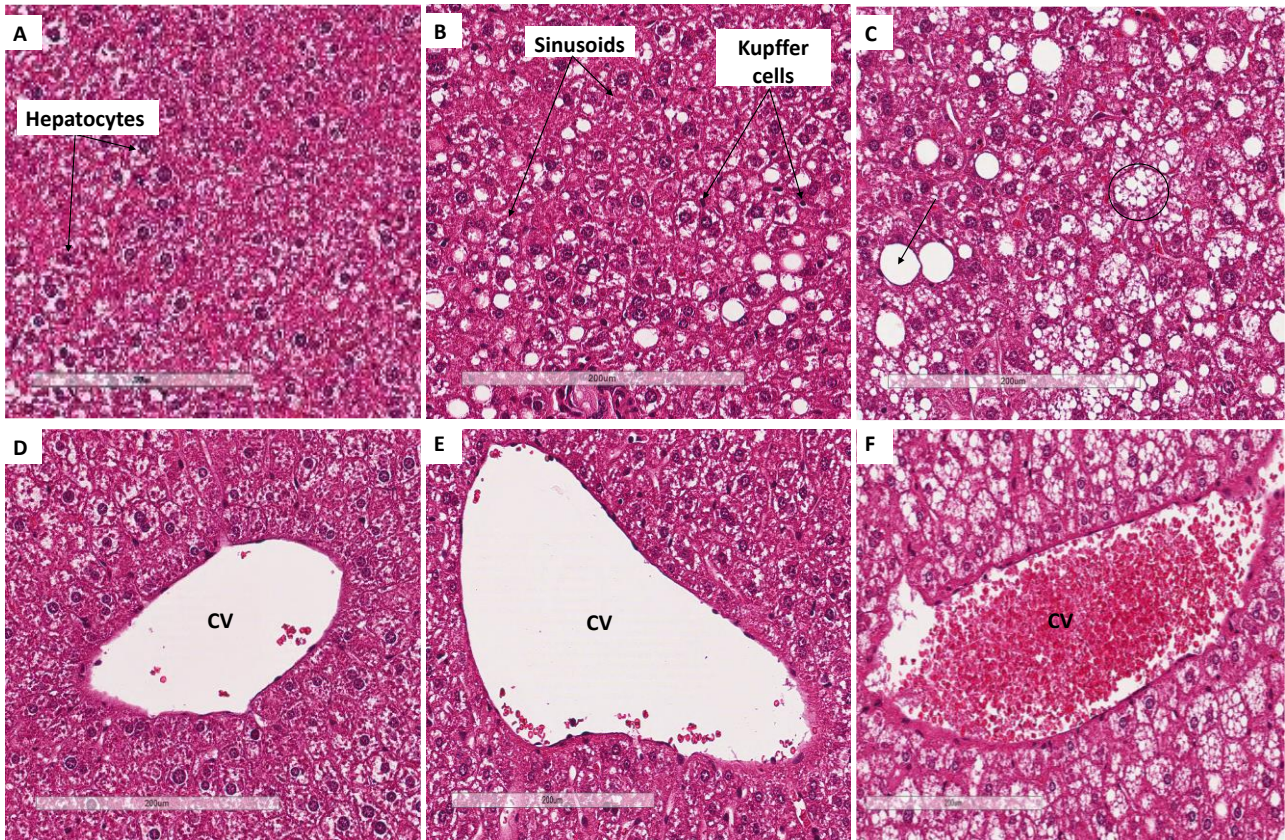
169 Figure 4: Adipocyte size distribution across the various adipose tissue depots. H&E-stained sections of
 170 perigonadal WAT (pWAT) revealed that the WTHFD, C, had significantly larger ($p < 0.001$), D, adipocytes than
 171 WTCD, A and KOHFD, B. The number of adipocytes per field was significantly smaller in WTHFD than KOHFD
 172 ($p < 0.05$) and WTCD ($p < 0.001$), E. Similarly, adipocyte diameter of WTHFD, H, of inguinal subcutaneous WAT
 173 (iWAT) was significantly greater ($p < 0.001$), I, than that of WTCD, F, and KOHFD, G. Conversely, the number
 174 of adipocytes per view was significantly lower ($p < 0.001$) in WTHFD compared to WTCD and KOHFD, J.
 175 Significant ($p < 0.001$), N, adipocyte hypertrophy was also observed in mWAT of WTHFD, M, compared to
 176 WTCD, K and KOHFD, L. Additionally, significantly ($p < 0.01$) fewer adipocytes were viewed per field in WTHFD
 177 compared to WTCD and KOHFD, O. Sections of the BAT revealed that WTCD, P, and KOHFD, Q, had smaller
 178 lipid droplets compared to those observed in WTHFD, R. Magnification= 20X, Scale bar= 200 μm. Key: a=
 179 adipocyte. N =120 adipocytes

180

181 Deletion of *slc7a8* reduces liver steatosis in diet induced obese mice

182 Liver sections from WTHFD (Figure 5C) showed lipid accumulation which can be categorised as
 183 microvesicular (circled, Figure 5C) and macrovesicular (indicated in arrow, Figure 5C) steatosis. This
 184 phenomenon was absent in liver sections of WTCD (Figure 5A). While macrovesicular steatosis was
 185 observed in KOHFD (Figure 5B), the lipid droplets were visibly smaller when compared to those

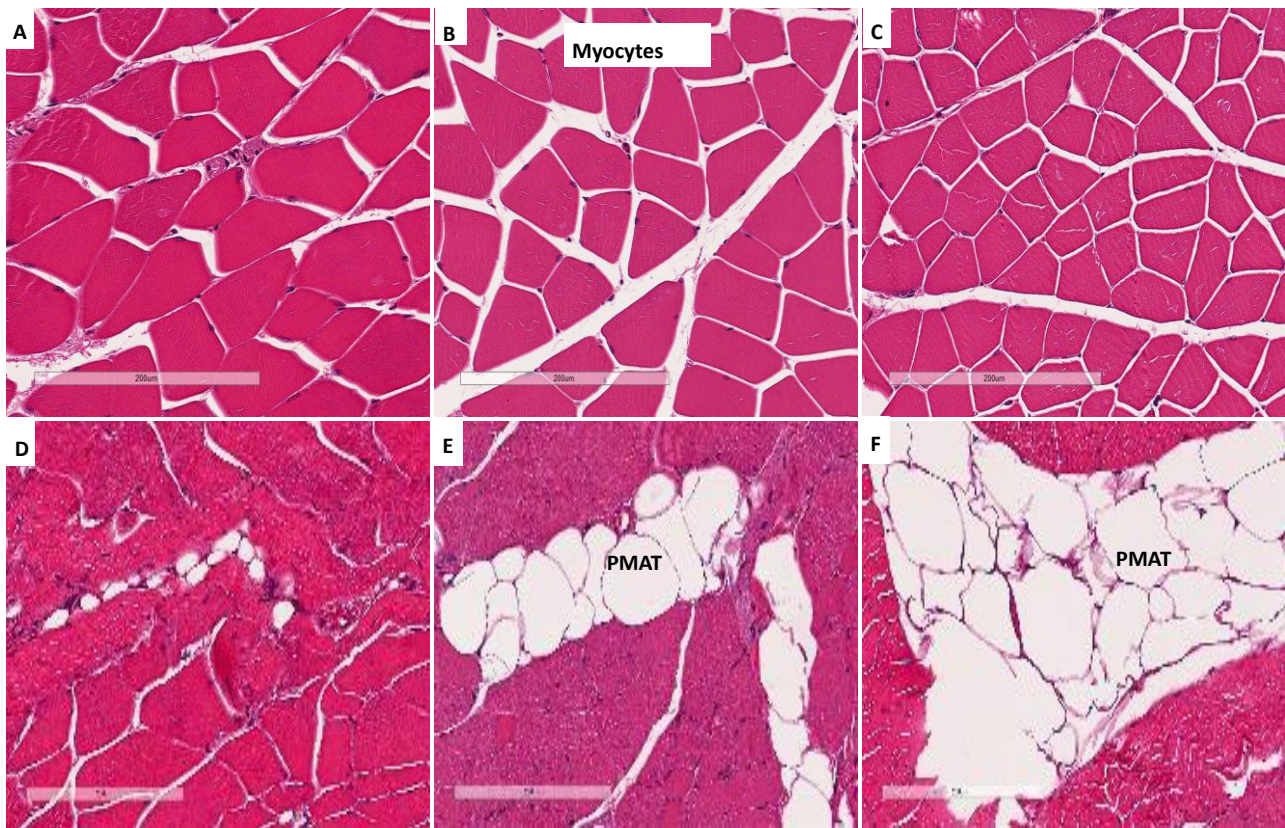
186 observed in WTHFD. Congestion of the central vein was observed in WTHFD (Figure 5F) but not in
187 WTCD (Figure 5D) and KOHFD (Figure 5E). No visible changes in sinusoid dilation and Kupffer cell
188 morphology were observed between WTCD, KOHFD and WTHFD. Additionally, lipid droplets in the
189 form of micro- and macrovesicular steatosis were observed as early as 5 weeks in WTHFD, while
190 macrovesicular steatosis was also seen in the KOHFD but was visibly smaller comparison to WTHFD
191 (Figure 3 supplementary).



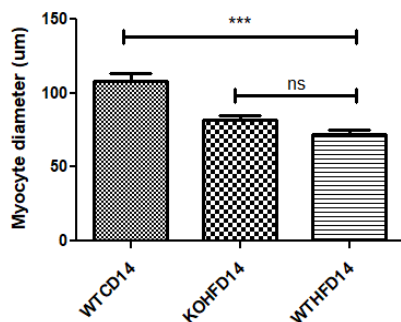
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193 Figure 5: Lipid accumulation in the liver. H&E-stained liver sections showed the presence of micro- and
194 macrovesicular steatosis in WTHFD, C which was not observed in WTCD, A and KOHFD, B. Venous
195 congestion in WTHFD, F was also observed but not in WTCD, D and KOHFD, E. Magnification = 20X, Scale
196 bar= 200 µm. Key: CV=central vein

197
198 Deficiency in *slc7a8* decrease lipid accumulation in gastrocnemius muscle

199 Myocyte atrophy was observed in WTHFD (Figure 6C) which had significantly smaller myocytes
200 ($p < 0.001$) (Figure 6G) than WTCD (Figure 6A). The deletion of *slc7a8* increases myocyte size in
201 KOHFD (Figure 6B) compared to WTHFD (Figure 6G). Accumulation of peri-muscular adipose tissue
202 (PMAT) (Figure 6F) was observed to be greater in WTHFD than in KOHFD (Figure 6E) and WTCD
203 (Figure 6D). At week 5, the KOHFD had significantly larger myocytes ($p < 0.001$) and less adipose
204 tissue accumulation than WTHFD (Figure 4 supplementary).



G 14 weeks gastrocnemius muscle



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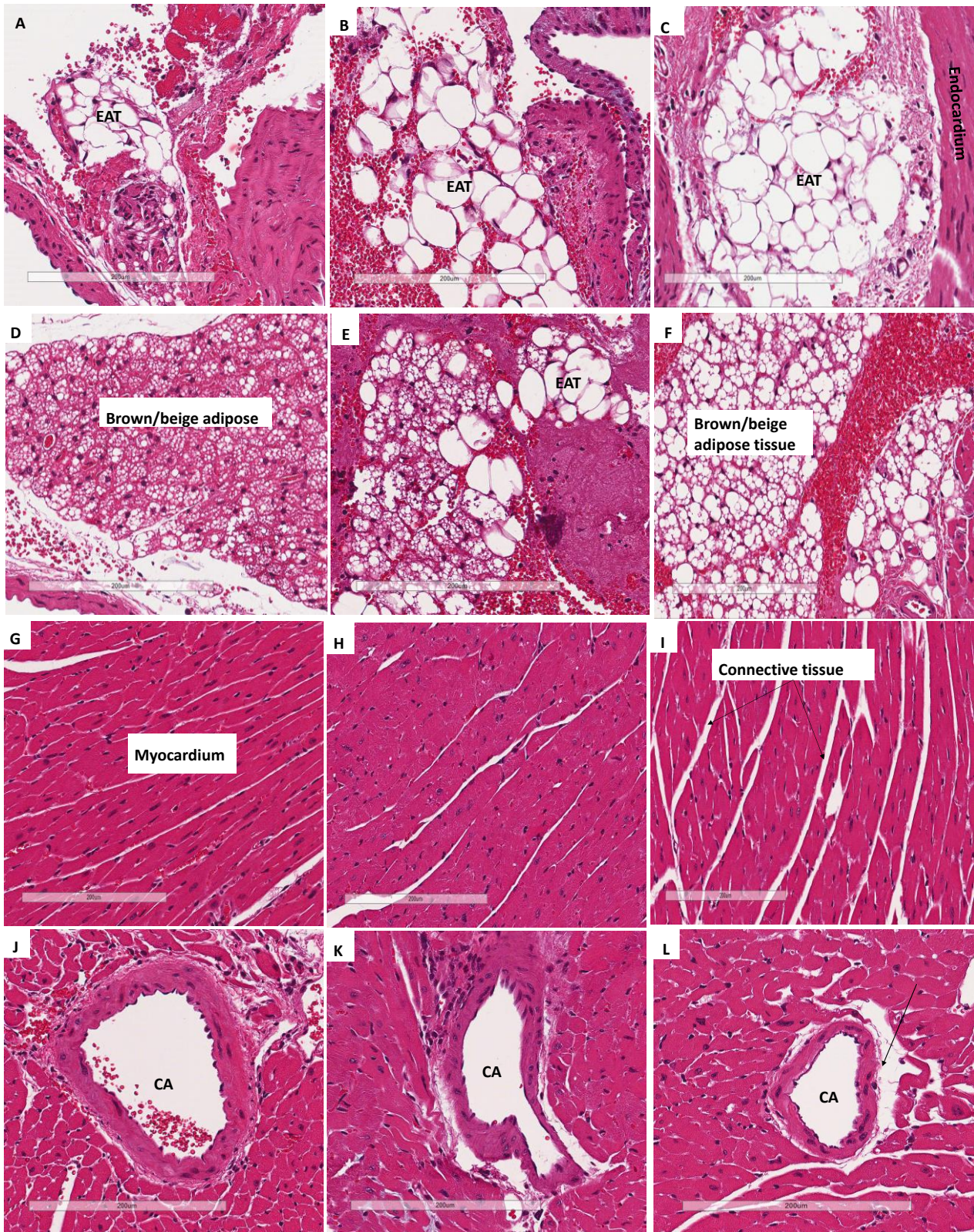
206 Figure 6: Effect of *slc7a8* deletion on adipose tissue accumulation and myocyte atrophy in gastrocnemius
207 muscle. KOHFD (Figure 6B) resulted in a protective effect against muscle atrophy when compared to WTHFD
208 (Figure 6C). WTHFD had significantly smaller ($p < 0.001$) (Figure 6G) myocytes than WTCD (Figure 6A).
209 Greater peri-muscular adipose tissue (PMAT) accumulation was seen in WTHFD (Figure 6F) when compared
210 to WTCD (Figure 6D) and KOHFD (Figure 6E). N= 120 myocytes

211

212 Deficiency in *slc7a8* reduces accumulation of epicardial adipose tissue

213 The increase in the accumulation of epicardial adipose tissue (EAT - white adipose tissue) observed
214 in WTHFD (Figure 7C) compared to WTCD (Figure 7A) was decreased following the deletion of
215 *slc7a8*, KOHFD (Figure 7B). Larger lipid droplets were observed in brown/beige adipose tissue (a
216 property of epicardial adipose tissue) in WTHFD (Figure 7F) when compared to WTCD (Figure 7D)
217 and KOHFD (Figure 7E). The connective tissue of the WTHFD (Figure 7I) was visibly thicker than that

218 of the WTCD (Figure 7G) and KOHFD (Figure 7H). Additionally, greater accumulation of adipose
219 tissue (black arrow) was observed surrounding the coronary artery (CA) of WTHFD (Figure 7L)
220 compared to KOHFD (Figure 7K) and WTCD (Figure 7J).



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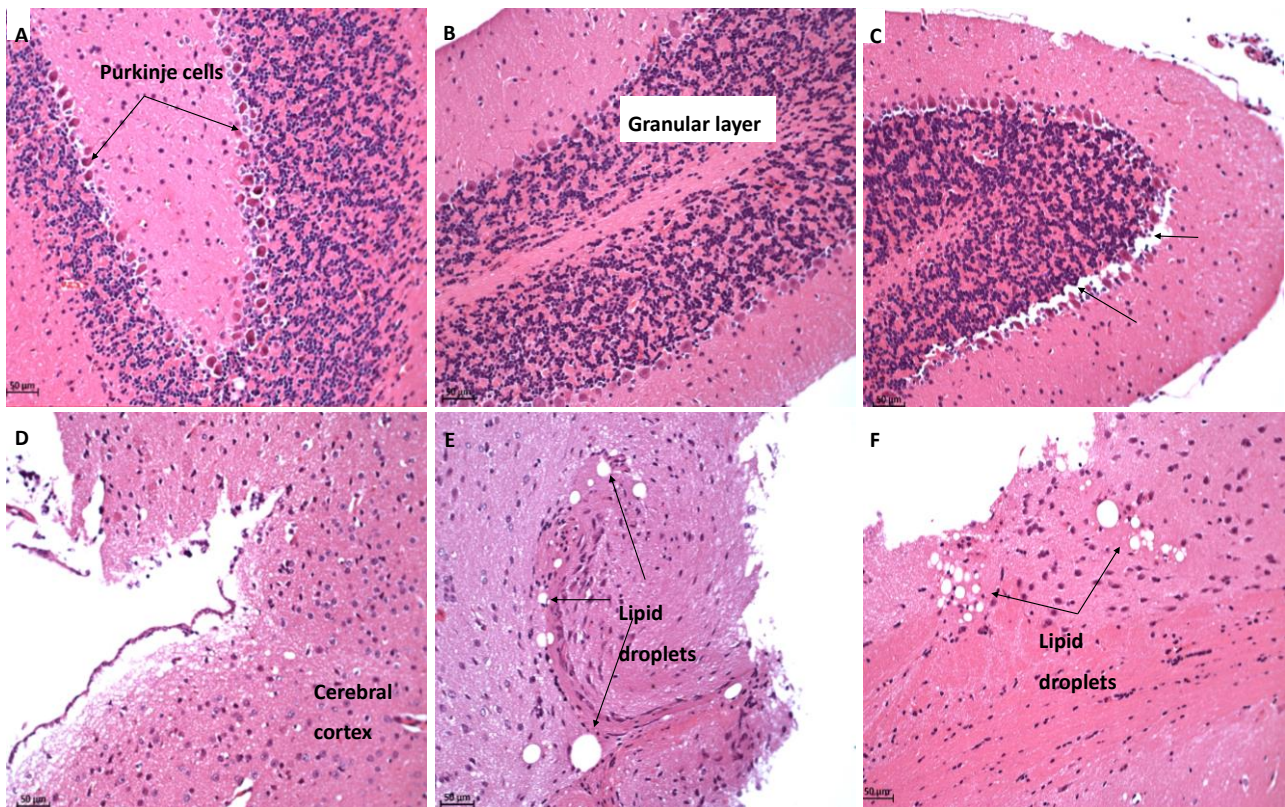
222 Figure 7: Effect of *slc7a8* on epicardial adipose tissue accumulation in the heart. H&E-stained heart sections
223 showed a greater accumulation of epicardial adipose tissue, seen as a brown/beige adipose depot, in the

224 WTHFD, C, compared to WTCD, A and KOHFD, B. The images demonstrate that the WTHFD, F, mice had
225 more connective tissue (cardiac muscle fibres) than WTCD, D and KOHFD, E. Additionally, the coronary artery
226 of the WTHFD, I, was surrounded by larger lipid deposits (black arrow) in comparison to WTCD, G and KOHFD,
227 H. Magnification = 20X, Scale bar= 200 μ m. Key: CA=coronary artery

228

229 Deficiency in *slc7a8* reduces lipid accumulation in the ganglion layer in diet induced obesity

230 Brain tissues of WTHFD (Figure 8C) showed vacuolation in the Purkinje cell layer (indicated by black
231 arrows) when compared to WTCD (Figure 8A). Deletion of *slc7a8* attenuated the vacuolation
232 observed in DIO, KOHFD (Figure 8B). In the cerebral cortex, lipid droplets were seen in KOHFD
233 (Figure 8E) and WTHFD (Figure 8F), but not in WTCD (Figure 8D). No visible morphological
234 differences were observed between Purkinje cells in WTCD (Figure 8A), KOHFD (Figure 8B) and
235 WTHFD (Figure 8C).



236

237 Figure 8: Effect of *slc7a8* deletion on lipid droplet accumulation in brain tissue. H&E-stained sections of brain
238 tissue showed vacuolation in the Purkinje cell layer of WTHFD, C, when compared to WTCD, A and KOHFD,
239 B. Lipid droplets were observed in the cerebral cortex of KOHFD, E and WTHFD, F, which was not seen in
240 WTCD, D. Magnification = 20X, Scale bar= 50 μ m

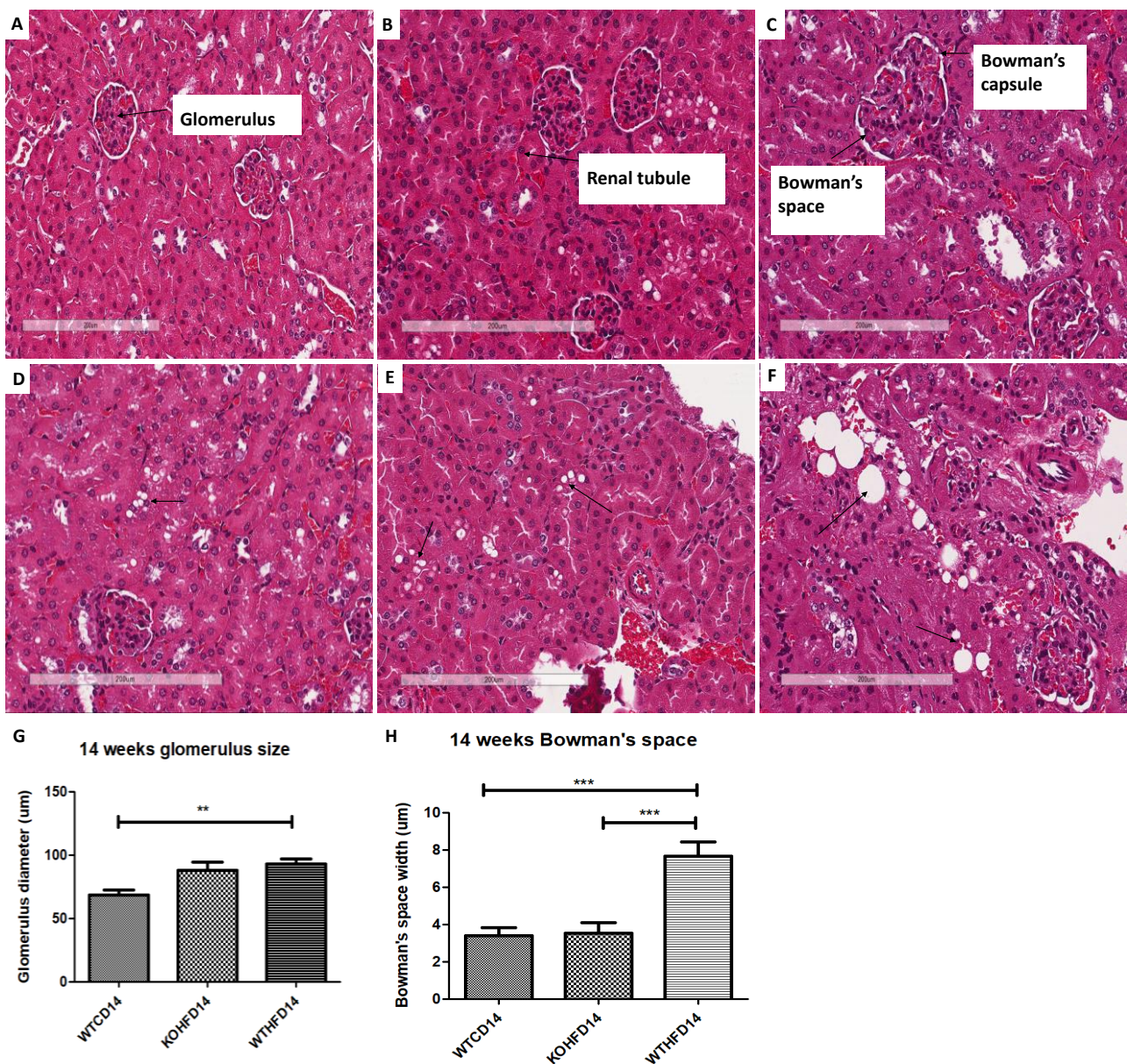
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244 Deficiency in *Slc7a8* reduces glomerulus size and lipid accumulation in the kidney

245 No visible alterations were seen in the renal tubules of WTCD (Figure 9A), KOHFD (Figure 9B) and
246 WTHFD (Figure 9C). However, WTHFD had enlarged glomeruli which were significantly larger
247 ($p < 0.01$) than those of WTCD. Although glomerular sizes in KOHFD (Figure 9B) appeared to be
248 smaller than those of WTHFD, the difference was not statistically significant (Figure 9G). The
249 Bowman's space of WTHFD (Figure 9F) was significantly larger ($p < 0.05$) with the presence of many
250 larger lipid droplets than in WTCD (Figure 9D). Interestingly, the deletion of *slc7a8* significantly
251 reduced the Bowman's space enlargement and lipid droplet accumulation in DIO, KOHFD (Figure
252 9H) to a level observed in WTCD (Figure 9 D and H).



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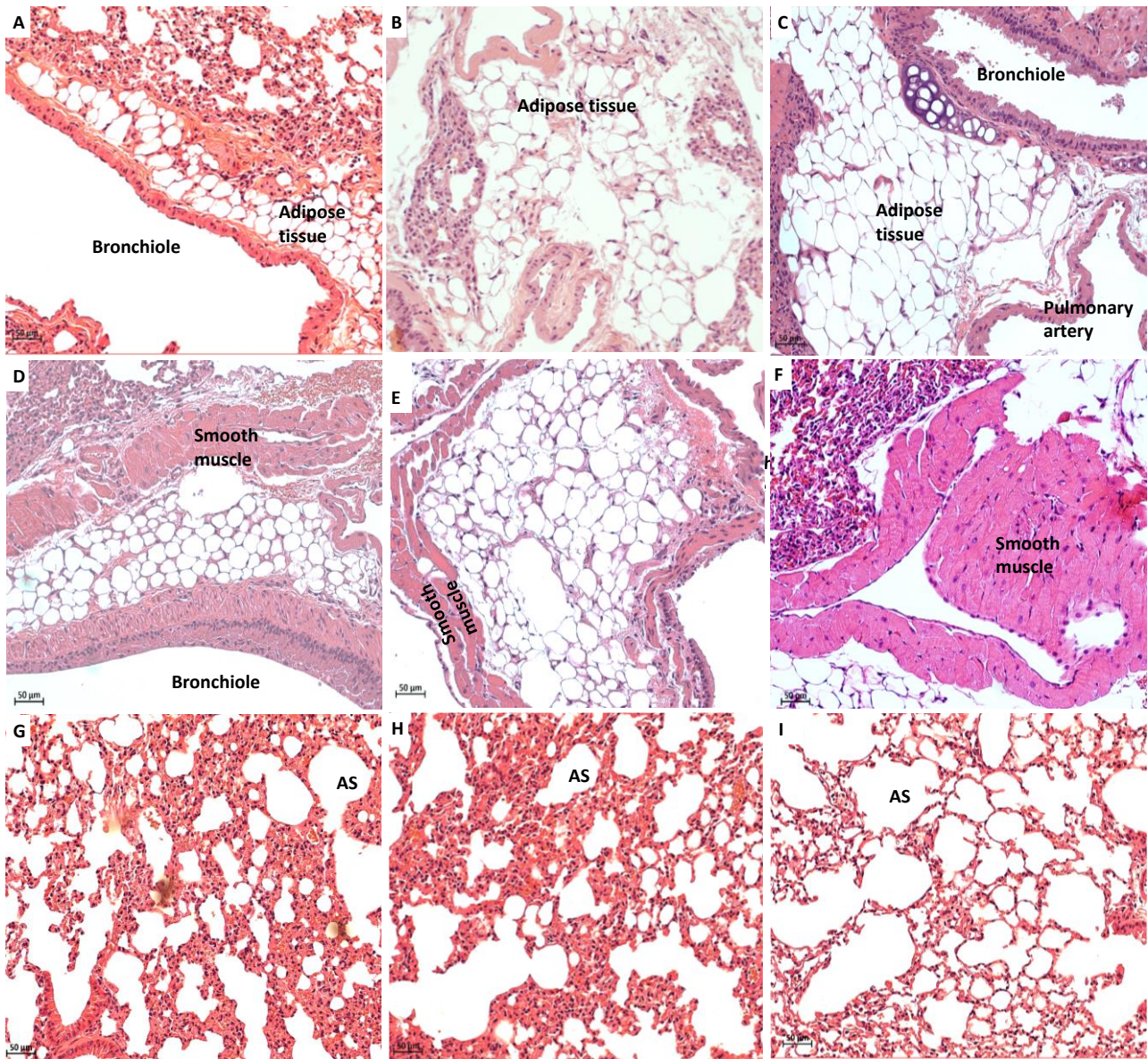
254 Figure 9: Effect of *slc7a8* deletion on lipid accumulation and glomerular size in the kidneys. H&E-stained
255 sections showed that WTHFD, C, had significantly enlarged glomeruli ($p < 0.01$), G, compared to WTCD, A; no

256 significant differences were observed between glomeruli of WTHFD and KOHFD, C. The width of the
257 Bowman's space was significantly larger ($p < 0.05$) in WTHFD when compared to WTCD and KOHFD, H.
258 Accumulation of lipid (black arrows) was greater in WTHFD, F, when compared to WTCD, D and KOHFD, F.
259 Magnification = 20X, Scale bar= 200 μm . N= 10 for glomeruli and bowman's space

260

261 Deficiency in *slc7a8* reduces adipose tissue accumulation in the lungs

262 Histological analysis of the lungs showed that adipose tissue tends to lie adjacent to bronchioles and
263 pulmonary arteries in WTCD (Figure 10A and D), KOHFD (Figure 10B) and WTHFD (Figure 10C). In
264 DIO, greater accumulation of adipose tissue was observed in WTHFD (Figure 10C) when compared
265 to WTCD (Figure 10A). The accumulation of adipose tissue in DIO appeared to reduce in KOHFD
266 (Figure 10B). The smooth muscle layer was enlarged in WTHFD (Figure 10F) in comparison to WTCD
267 (Figure 10D) and KOHFD (Figure 10E). Additionally, the alveolar walls around the alveolar sacs (AS)
268 of the WTHFD (Figure 10I) appeared thinner in comparison to WTCD (Figure 10G). Interestingly, the
269 deletion of *slc7a8* abated this effect that resulted from DIO, in KOHFD (Figure 10H). Lipid
270 accumulation in the lungs was observed as early as week 5 with more adipose tissue in WTHFD and
271 KOHFD compared to WTCD (Figure 5 supplementary).



272

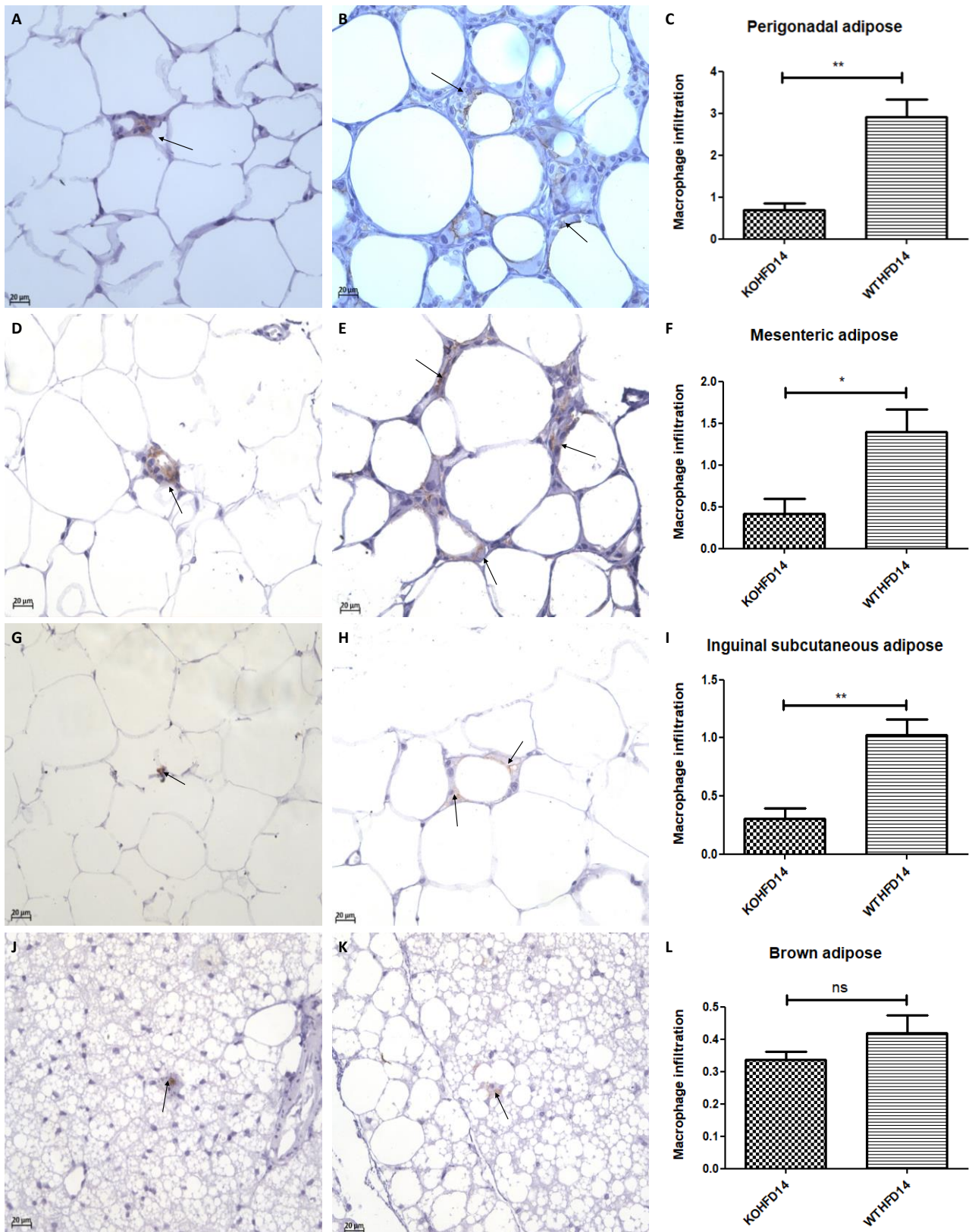
273 Figure 10: Effect of *slc7a8* deletion on lipid accumulation in the lungs. H&E-stained lung sections showed the
274 accumulation of adipose tissue around the bronchioles and pulmonary artery, which was greater in WTHFD,
275 C, compared to KOHFD, B, and WTCD14, A, D. An enlarged smooth muscle layer was observed in WTHFD,
276 F compared to KOHFD, E and WTCD, D. Additionally, thinner alveolar walls were observed in WTHFD, I,
277 in comparison to WTCD, G, and KOHFD, H. Magnification = 20X, Scale bar= 50 μm. Key: AS= Alveolar sacs

278

279 Deficiency in *slc7a8* reduces white adipose tissue inflammation in DIO

280 Immunohistochemical staining for F4/80, a mouse macrophage marker, was done to assess the
281 presence of macrophages in pWAT, mWAT, iWAT and brown adipose tissue. Deletion of *slc7a8*
282 significantly decreased macrophage infiltration (indicated by black arrows) in the pWAT (Figure 11C;
283 p<0.01), mWAT (Figure 11F; p<0.05) and iWAT (Figure 11I; p<0.01) of KOHFD (Figure 11A, D & G)
284 compared to WTHFD (Figure 11B, E & H). No significant difference was observed in the brown

285 adipose macrophage inflammation profile (Figure 11L) between WTHFD (Figure 11K) and KOHFD
286 (Figure 11J).



287

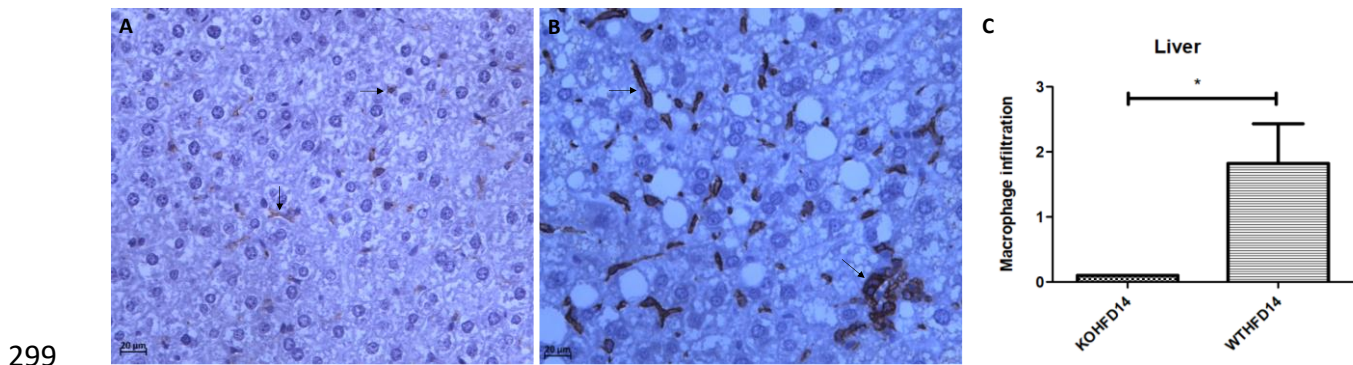
288 Figure 11: Effect of *slc7a8* deletion on macrophage infiltration in adipose tissues. KOHFD, A, showed a
289 significant decrease in macrophage infiltration (indicated by black arrows) in the pWAT ($p < 0.01$), C, compared
290 to WTHFD, B. A significant decrease in macrophage infiltration in mWAT ($p < 0.05$), F, was observed in KOHFD,

291 D, compared to WTHFD, E. A significantly decrease in macrophage infiltration ($p < 0.01$), I, in iWAT was seen
292 in KOHFD, G, when compared to WTHFD, H. No significant differences in macrophage infiltration in brown
293 adipose tissue, L, was observed between the KOHFD, J and WTHFD, K. Magnification = 40X, Scale bar= 20
294 μm . N= 5 fields

295

296 *Deficiency in slc7a8 reduces inflammation in the liver*

297 Deficiency in *slc7a8* resulted in a significant ($p < 0.05$) reduction in macrophages in the liver in DIO
298 (KOHFD), Figure 12A, compared to WTHFD (Figure 12B), Figure 12C.

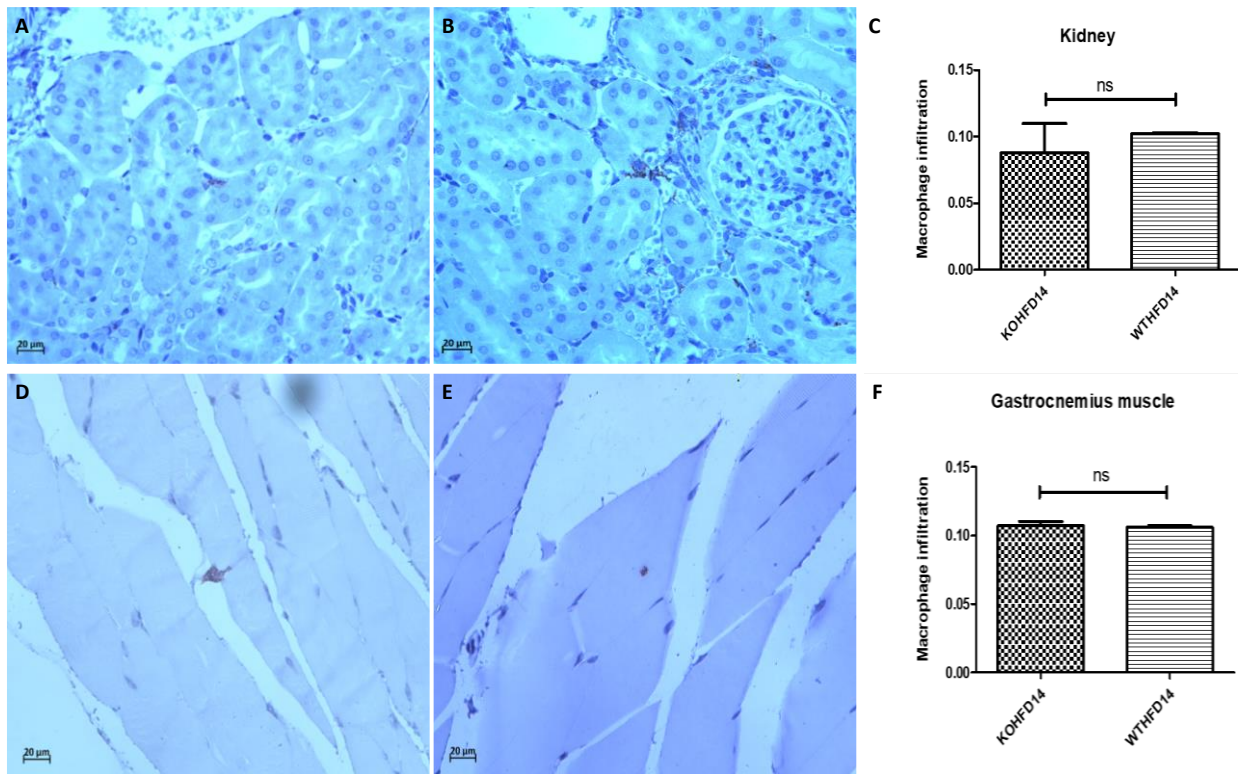


300 Figure 12: Effect of *slc7a8* deletion on the presence of macrophages in the liver. WTHFD, B, had a significantly
301 greater infiltration ($p < 0.05$) of macrophages compared to KOHFD, A, C. Magnification = 40X, Scale bar= 20
302 μm . N= 10 fields

303

304 Deficiency in *slc7a8* had no effect on the presence of macrophages in kidney and gastrocnemius
305 muscle in DIO

306 The presence of macrophages in the kidney of KOHFD (Figure 13A) and WTHFD (Figure 13B) was
307 similar (Figure 13C). This observation was the same for gastrocnemius muscle of KOHFD (Figure 13D)
308 and WTHFD (Figure 13E) with no statistical difference in macrophage profile between them (Figure
309 13F).



310

311 Figure 13: Effect of *slc7a8* on macrophage infiltration profile of kidney and gastrocnemius muscle. KOHFD, A,
312 had slightly fewer macrophages infiltrating into the kidney in comparison to WTHFD, C. However, no significant
313 differences were noted between KOHFD and WTHFD. No significant differences, F, were observed in
314 infiltration between the gastrocnemius muscles of KOHFD, D and WTHFD, E. Magnification = 40X, Scale bar=
315 20 μ m. N= 5 fields

316

317 Discussion

318 Obesity is characterized by excessive accumulation of adipose tissue, and it is associated with the
319 development of metabolic syndromes affecting many organs and tissues in the body. The search for
320 molecular factors that play a role in attenuating lipid accumulation in condition such as diet induced
321 obesity is paramount to identifying good candidates for therapeutic interventions that mitigate the
322 development of obesity associated comorbidities. Studies of adipogenesis in human derived
323 stromal/stem cells *in vitro* have served as an excellent model for identifying molecular factors with
324 a potential role in adipocyte formation and lipid accumulation/metabolism [11, 14]. This study
325 investigated the role of a previously identified novel human adipogenic gene, SLC7A8 [14] in diet-
326 induced obesity, and its effect on adipose tissue accumulation in different organs and tissues. To
327 achieve this, *slc7a8* knockout (KO) and wildtype (WT) C57BL/6 mice were fed either a HFD or
328 nutrient matched CD for 14 weeks followed by the analyses of different parameters.

329 Weight gain, food, and caloric intake between WTCD and KOCD were similar, indicating that *slc7a8*
330 deletion had no effect on food intake, caloric consumption, and weight gain on a normal diet.
331 WTHFD gained significantly more weight ($p < 0.001$) than WTCD starting from week 3 (Figure 1A) with
332 a significantly higher caloric intake ($p < 0.01$ to $p < 0.001$) than WTCD (Figure 1C). Total food
333 consumption was not significantly different during the 14-week period except at week 8 where food
334 consumption in WTHFD was significantly elevated ($p < 0.05$). This indicates that the occurrence of
335 diet-induced obesity was due to an increase in caloric intake when on HFD. Interestingly, the *slc7a8*
336 deficient genotype on HFD (KOHFD) gained significantly less weight ($p < 0.05$ to $p < 0.001$) compared
337 to the WTHFD starting from week 3 (Figure 1A). This suggests that *slc7a8* deletion is protective
338 against diet-induced obesity. The significant decrease in weight gain in KOHFD was accompanied by
339 significantly lower tissue mass of iWAT, mWAT, pWAT, BAT and liver compared to WTHFD (Figure
340 1D). Strikingly, it was observed that KOHFD gained significantly more weight ($p < 0.05$ to $p < 0.001$)
341 than KOCD from week 8, and this corresponded to a significantly larger pWAT in KOHFD than KOCD
342 (Figure 1D). This indicates that weight gain by KOHFD is due to pWAT expansion and suggests that
343 pWAT is the primary site of lipid accumulation in the KO phenotype.

344 BAT in WTHFD (Figure 4R) displayed enlarged lipid droplets compared to WTCD (Figure 4P). A recent
345 study showed that following 20 weeks of feeding mice on a HFD, lipid accumulation did not influence
346 the function of brown adipose tissue. However, the authors speculated that if the period of HFD
347 feeding was extended, a malfunction of BAT would be observed in obese mice[16]. We have
348 observed in this current study that KOHFD (Figure 4Q) attenuates adipocyte hypertrophy and lipid
349 accumulation in BAT. This suggests that *slc7a8* deletion could be protective against the long-term
350 effect of BAT hypertrophy and malfunctioning caused by DIO.

351 Furthermore, it was observed that WTHFD had a significantly greater caloric intake than KOHFD
352 (Figure 1C) while food consumption was similar except at week 11 where a significant difference
353 ($p < 0.05$) was observed. It is possible that the deletion of *slc7a8* regulates weight gain on HFD by
354 burning calories quicker than WTHFD since both KOHFD and WTHFD had similar caloric intake up to
355 week 8 (Figure 1C) but as early as week 5, adipocyte hypertrophy was already significantly greater
356 in WTHFD compared to KOHFD (Figure S2). Additionally, food and caloric intake was similar between
357 KO and WT on a normal diet, with differences only being observed on HFD; this could suggest satiety
358 in KOHFD as caloric intake significantly decreased after week 8 (Figure 1C).

359 Adipose tissue expansion in obesity is commonly associated with conditions such as hyperglycaemia,
360 impaired glucose tolerance and insulin resistance[17]. To investigate the effect of *slc7a8* deletion
361 on the metabolism of exogenous glucose and insulin, GTT and IST were performed on all animals
362 (KO and WT) prior to introducing them to an experimental diet (Figure 2A and 2B). Importantly,
363 there was no significant difference between the *slc7a8* KO and WT mice for both tests. This shows
364 that the deletion of *slc7a8* had no effect on their ability to metabolise glucose and insulin efficiently.
365 It was noted, however, that significantly higher levels of blood glucose were seen in KO mice at 30
366 minutes of the GTT (Figure 2A), which later return to normal without any change in the AUC between
367 KO and WT (Figure 2B). Both WTCD and KOCD at 5 and 14 weeks showed a similar trend in glucose
368 metabolism (Figure 3A and C) with no difference in the AUC (Figure 2B and D), suggesting glucose
369 metabolism is unaltered in *slc7a8* deficient mice on a normal diet. Under condition of DIO, WTHFD
370 showed significantly higher levels of glucose intolerance compared to WTCD, and this effect was
371 significantly improved in KOHFD with blood glucose levels returning to baseline levels at the end of
372 the GTT (Figure 3C). This demonstrates that *slc7a8* deletion significantly improves glucose
373 metabolism in DIO.

374 WTHFD showed significantly larger adipocytes in the pWAT, mWAT and iWAT (Figure 4) compared
375 to WTCD. The adipose tissue hypertrophy in WTHFD may increase susceptibility to hyperglycaemia.
376 In an obese phenotype, insulin signalling is usually impaired, which results in reduced glucose uptake
377 by muscles and thus increased glucose levels in the circulation[18]. pWAT is significantly larger
378 ($p < 0.001$) than iWAT and mWAT in WTHFD (Figure 6 supplementary), which may be suggestive of
379 pWAT being the main site of lipid accumulation in this group as was observed in the KO group.
380 Abdominal/visceral obesity is critical to the development of metabolic syndrome, and accumulation
381 of adipose tissue in the abdomen correlates with metabolic syndrome, compared to lipid
382 accumulation in the subcutaneous depot[19]. Larger pWAT in WTHFD may thus be responsible for
383 the glucose intolerance observed in these mice. Lipid accumulation in the liver presented as
384 microvesicular steatosis (characterised by small lipid droplets in the cytoplasm of hepatocytes) and
385 macrovesicular steatosis (large lipid droplets) (Figure 5), are both of which important in the
386 development of non-alcoholic fatty liver disease (NAFLD)[20, 21], and were observed in WTHFD but
387 not in WTCD. The presence of lipid droplets in WTHFD liver could be due to the redistribution of
388 excess lipid to peripheral organs such as the liver or muscles seen in the obese phenotype, when
389 the storage capacity of adipose tissue is exceeded[3, 5]. The liver has previously been reported to
390 be the major site for storage of free-fatty acids (FFA) released from white adipose tissue in an obese

391 phenotype[22]. Furthermore, the vast majority of hepatic triglycerides in obese individuals with
392 NAFLD are from FFA released from adipose tissue[23]. The observations made in our study indicate
393 that KOHFD attenuates both macrovesicular and microvascular steatosis seen in WTHFD, suggesting
394 that *slc7a8* deletion could be protective against NAFLD in DIO.

395 DIO is often associated with the recruitment and accumulation of macrophages in adipose depots.
396 The F4/80 antibody is a marker for macrophages in mouse tissues[10, 24] and was utilised in this
397 study. Adipose tissues from obese WTHFD mice showed significantly more macrophages, which
398 indicates increased inflammation when compared to KOHFD (Figure 11). Thus, *slc7a8* deletion
399 significantly improves the inflammatory profile of adipose tissues in DIO. The liver tissue sections in
400 WTHFD showed significantly elevated levels of macrophages and congestion of the central vein. The
401 observed histopathological changes in liver which occur due to DIO were improved by *slc7a8*
402 deletion in KOHFD (Figure 12).

403 Apart from metabolic syndromes that are associated with excess adipose tissue accumulation,
404 obese individuals are also prone to developing pulmonary disorders such as chronic obstructive
405 pulmonary disease (COPD) or asthma[25]. In DIO, the lungs of WTHFD showed an increase in adipose
406 tissues accumulation around the bronchioles and pulmonary arteries, which was reduced in KOHFD
407 (Figure 10). Additionally, the smooth muscle layer was visibly thicker, and alveolar walls thinner in
408 the WTHFD in comparison to KOHFD (Figure 10). A previous study showed that accumulation of
409 adipose tissue in the lungs increased with an individual's body mass index (BMI)[26]. Additionally,
410 an increase in adipose tissue affects the structure of the lungs, resulting in the blockage of airways
411 and causing inflammation which ultimately gives rise to pulmonary disease[25, 26]. We observed
412 that the deletion of *slc7a8* attenuates adipose tissue accumulation in DIO, and this could mitigate
413 the development of obesity associated lung pathologies.

414 DIO resulted in a significant reduction in gastrocnemius muscle myocyte size in WTHFD compared
415 to WTCD, and the deletion of *slc7a8* decreased this effect of DIO (KOHFD) on myocytes size (Figure
416 6G). Additionally, peri-muscular adipose tissue accumulation, which was observed to increase in
417 muscle of WTHFD, decreased in KOHFD (Figure 6E & F). Peri-muscular adipose tissue has previously
418 been shown to promote age and obesity related muscle atrophy by increasing muscle
419 senescence[27]. Hence, a decrease in lipid accumulation due to *slc7a8* deletion in our study suggests
420 an improvement in DIO associated muscular disease. Conversely, there was no significant difference
421 in the gastrocnemius muscle macrophage profile between WTHFD and KOHFD.

422 The development of cardiovascular diseases is associated with an increase in adiposity[28]. In DIO,
423 the heart of WTHFD showed greater accumulation of epicardial adipose tissue, which was found to
424 decrease in the absence of *slc7a8*, KOHFD (Figure 7). Epicardial adipose tissue is located between
425 the myocardium and epicardium and has properties of brown or beige adipose tissue. It is important
426 for maintaining energy homeostasis and thermoregulation of the heart[29]. However, accumulation
427 of epicardial adipose tissue is associated with increasing BMI and poses a risk for the development
428 of cardiovascular disease[28].

429 Renal injury and disease have been associated with obesity and studies in mice have documented
430 renal morphological changes due to HFD[30, 31]. An increase in glomerular size and accumulation
431 of lipid droplets in the kidneys was observed in DIO in WTHFD, suggesting an increase in body weight
432 could contribute to renal abnormalities. These changes associated with a greater risk of renal
433 disease was reduced in KOHFD (Figure 9), suggesting that *slc7a8* deletion may improve kidney health
434 in DIO.

435 This study demonstrates that deletion of *slc7a8* in mice is protective against DIO by significantly
436 reducing adipose tissue mass as well as lipid accumulation in multiple organs and tissues, resulting
437 in improve glucose tolerance in diet induced obesity. Furthermore, our histological findings reveal
438 that the negative effects of DIO on different organs and tissues were improved with *slc7a8* deletion,
439 suggesting a contributing role of this gene to the development of some obesity associated
440 comorbidities. Overall, the results from this study suggest that *slc7a8* could be an important
441 therapeutic target for controlling DIO, as well as for mitigating the development of some of the
442 pathophysiological conditions associated with obesity. Nevertheless, further studies will be required
443 to provide additional knowledge on how *slc7a8* regulates plasma parameters such hormones, lipids
444 and the cytokine inflammatory profile in DIO to reduce lipid accumulation at multiple organs and
445 tissues.

446 **Materials and methods**

447 **Animals**

448 This study was approved by the Research Ethics Committee, Faculty of Health Sciences and the
449 Animal Ethics Committee, University of Pretoria (Ref. No.: 474/2019). *Slc7a8* (*Slc7a8^{tm1Dgen}*)
450 heterozygous and wildtype C57BL/6J mating pairs obtained from Jackson Laboratory (*Bar Harbor,*
451 *Maine, United States of America*) were used to generate *Slc7a8* wildtype (WT) and knockout (KO)

452 genotypes. Genotypes were confirmed by PCR (supplementary methods S1). Both WT and KO mice
453 were fed either a high-fat diet (HFD; D12492) or control diet (CD; D12450J) from Research Diets, Inc.
454 (*New Brunswick, New Jersey, United States of America*) for a period of 14 weeks, with termination
455 time points at weeks 5 and 14. Weekly measurements of weight, food consumption and calorie
456 intake were done. Unless otherwise stated, the nomenclature used for the different genotypes on
457 either a CD or HFD for 14 weeks will be WTCD (wildtype mice on control CD), WTHFD (wildtype mice
458 on HFD), KOCD (*Slc7a8* Knockout mice on control CD) and KOHFD (*Slc7a8* knockout mice on HFD).

459 Glucose tolerance and insulin sensitivity tests

460 Glucose tolerance tests (GTT) and insulin sensitivity tests (IST) were performed in both KO and WT
461 mice prior to introducing them to either CD or HFD. Mice were fasted for 4 hours, and the baseline
462 glucose concentration measured. A 45% D-(+)- glucose solution (*Sigma-Aldrich, St. Louis, Missouri,
463 United States of America*) was then administered interperitoneally at 1.5 mg/g body weight and an
464 insulin solution (*Sigma-Aldrich, St. Louis, Missouri, United States of America*) at 0.8mU/g body
465 weight for GTT and IST, respectively. Blood from the tail vein was used to measure glucose
466 concentration at 15, 30, 60, 90 and 120 minutes using an Accu-Check Instant Blood Glucose Meter
467 (*Roche Diagnostics, Basel, Switzerland*).

468 Histology and immunohistochemistry of mouse tissues and organs

469 Mice on either CD or HFD were euthanised at week 5 and 14 followed by the collection of white
470 adipose tissue from the inguinal (iWAT), perigonadal (pWAT) and mesenteric (mWAT) depots;
471 interscapular brown adipose tissue (BAT); and liver, kidneys, heart, brain, lungs and gastrocnemius
472 muscle. 10% formalin fixed paraffin embedded (FFPE) tissue sections were processed for histological
473 analysis.

474 FFPE tissue sections were cut using a microtome and baked at 62°C for 20 minutes followed by
475 haematoxylin and eosin (H&E) staining using a Leica Autostainer XL (*Leica Microsystems, Wetzlar,
476 Germany*). Slides were mounted with DPX (distyrene, plasticiser, xylene) and imaged using an
477 Axiocam 305 color microscope camera (*ZEISS, Oberkochen, Germany*) and ZEN 2.6 blue edition
478 software (*ZEISS*).

479 Immunohistochemical analysis of macrophages was performed as previously described[31]. Briefly,
480 tissue sections were stained with F4/80 rat anti mouse antibody clone A3-1 (*Bio-Rad Laboratories,
481 Sandton, Johannesburg, South Africa*). FFPE sections were baked overnight at 54°C, followed by

482 dewaxing in xylene. The sections were then hydrated through a series of ethanol concentrations,
483 rinsed with distilled water and treated with 3% hydrogen peroxide for 5 minutes at 37°C. Heat-
484 induced epitope retrieval was performed in citrate buffer pH 6,1 (*Dako Target Retrieval Solution*
485 *S1699, Dako, Carpinteria, California, United States of America*) using a 2100 Retriever Unit (*Electron*
486 *Microscopy Sciences, Hatfield, Pennsylvania, United States of America*). The sections were rinsed in
487 PBS/Tween buffer and treated with 5% Normal Goat Serum (Dako X0907) for 30 minutes after which
488 they were incubated overnight at 4°C with a 1:25 dilution of F4/80 monoclonal rat anti-mouse
489 antibody BM8 (*ThermoFisher Scientific*) or 1:100 F4/80 rat anti mouse antibody clone A3-1 (*Bio-Rad*
490 *Laboratories, Sandton, Johannesburg, South Africa*). The sections were rinsed in PBS/Tween buffer
491 before incubating for 60 min in 1:200 goat anti-rat IgG (H+L) antibody conjugated to horseradish
492 peroxidase (HRP) (Invitrogen, ThermoFisher Scientific). The slides were then developed in 3,3'
493 diaminobenzidine (DAB) chromogen to visualise F4/80 protein staining. All images were taken and
494 analysed at 20x magnification.

495 Statistical and image analyses

496 Images from H&E and immunohistochemical staining were analysed using ImageJ Fiji
497 (<https://imagej.nih.gov/ij/download.html>) or Aperio ImageScope version 12.4.3.5008 software
498 (*Leica Biosystems, Wetzlar, Germany*). Morphometric analysis of the various tissue sections was
499 estimated by measuring the diameter of at least 120 cells distributed across the tissue. Semi-
500 quantitative analysis of F4/80 staining using ImageJ Fiji was done according to the protocol
501 described by Crowe and Jue, 2019[32] to quantify macrophages in the tissues.

502 Statistical analyses were conducted using GraphPad Prism 5 (*GraphPad Software, San Diego,*
503 *California*). Values are expressed as mean \pm SEM. One-way ANOVA followed by Bonferroni
504 corrections was used to compare means between three or more categories. When comparing two
505 means, a two-tailed unpaired Student's t-test was used. Two-way ANOVA with Bonferroni
506 corrections was used where necessary. Statistically significant results are indicated as *P<0.05,
507 **P<0.01, ***P<0.001.

508 **Acknowledgments**

509 We would like to thank Mr Muchavengwa Chovheya and Mrs Ilse van Rensburg at the Onderstepoort
510 Veterinary Animal Research Unit (OVARU) for assisting with animal care, housing, and experiments; to Mrs

511 Rene Sutherland at the Department of Oral Pathology and Oral Biology for assisting with tissue processing
512 and the Department of Anatomy for allowing us to use their facility including the light microscope.

513 **Author Contributions**

514 Conceptualization, M.A.A. and M.S.P.; methodology, M.A.A, R.R.P and M.B.vH.; formal analysis, R.R.P and
515 M.A.A.; investigation, R.R.P.; data curation, R.R.P and M.B.vH.; writing—original draft preparation, R.R.P.;
516 writing—review and editing, R.R.P. M.A.A, M.B.vH and M.S.P.; supervision, M.A.A and M.S.P.; project
517 administration, M.A.A.; funding acquisition, M.A.A and M.S.P All authors have read and agreed to the
518 published version of the manuscript.

519 **Funding:** This research was funded by the National Research Foundation grant no. 114044 and National
520 Health Laboratory Services grant no. 004 94683 (M.A.A.); the South African Medical Research Council
521 University Flagship Project (SAMRC-RFA-UFSP-01-2013/STEM CELLS), the SAMRC Extramural Unit for Stem
522 Cell Research and Therapy, the Institute for Cellular and Molecular Medicine of the University of Pretoria
523 (M.S.P.).

524 **Conflicts of Interest:** The authors have no conflicts of interest to declare.

525

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638

639 Supporting Information

640 Supplementary methods

641 S1: Genotyping of mice

642 Genomic DNA was extracted from tail biopsy of mouse pups using the KAPA Mouse Genotyping Kit
643 (*Wilmington, Massachusetts, United States of America*) and the KAPA Express Extract Protocol. The
644 extractions were performed in a volume of 100 μ l and was set up as follows: 88 μ l PCR-grade water,
645 10 μ l of 10X KAPA Extract Express buffer, 2 μ l of 1 U/ μ l KAPA Express Extract enzyme and
646 approximately 2 mm of mouse tail tissue. Enzymatic lysis was performed in the Applied Biosystems
647 9700 thermal cycler (*Foster City, California, United States of America*) at 75°C for 10 minutes and
648 enzyme inactivation at 95°C for 5 minutes. The DNA extracts were subsequently diluted 10-fold in
649 10 mM TRIS-HCL (pH 8.5).

650 To determine the wildtype, heterozygous and knockout *SLC7A8* genotypes, the following gene-
651 specific primer sequences were used: 5'-CAAATGCCAGCTGTCCTGACCTCAC-3' forward primer for
652 the wildtype allele, 5'-GGGTGGGATTAGATAAATGCCTGCTCT-3' forward primer for the knockout
653 allele and 5'-CAGACTTAGGGATGGTGACGCCTAG-3' for the common reverse primer. All
654 oligonucleotides used in the study were synthesised by Integrated DNA Technologies (*Coralville,*
655 *Iowa, United States of America*). The PCR reaction mixture consisted of 6.5 μ l of PCR-grade water,
656 12.5 μ l of the KAPA2G Fast Genotyping buffer, 1.25 μ l of both the 10 μ M wildtype forward primer
657 and 10 μ M knockout forward primer, 2.5 μ l of 10 μ M common reverse primer and 1 μ l of the diluted
658 DNA extract. The PCR amplifications were performed in a total volume of 25 μ l and cycled in the ABI
659 Applied Biosystems 9700 thermal cycler. The thermal cycling conditions used were as such: 95°C for
660 3 minutes followed by 95°C for 15 seconds, 60°C for 15 seconds, 72°C for 15 seconds and a final
661 extension for 2 minutes at 72°C. After amplification, 10 μ l of each amplicon was separated on a 2%
662 agarose gel alongside a Thermo Scientific FastRuler Low Range DNA ladder (*Waltham,*
663 *Massachusetts, United States of America*). Electrophoresis was performed in 1 x TAE (diluted from
664 UltraPure 10 x TAE buffer (*ThermoFischer Scientific, Waltham, Massachusetts, United States of*
665 *America*) at 120V for 40 minutes. The gel was stained with Ethidium Bromide Solution, Molecular
666 Grade (*Promega, Madison, Wisconsin, United States of America*) and viewed under UV light using
667 the Molecular Imager Gel Doc XR System (*Bio-Rad, Hercules, California, United States of America*).
668 The expected amplicon sizes were 206bp for the wildtype allele and 390bp for the knockout allele.
669 Only wildtype and knockout mice for the *SLC7A8* gene were used in the study.

670 Supplementary figures

WTCD14



WTHFD14



KOCD14

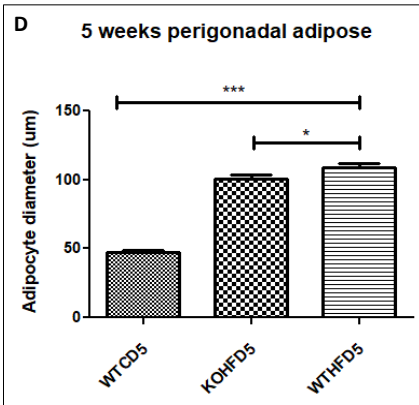
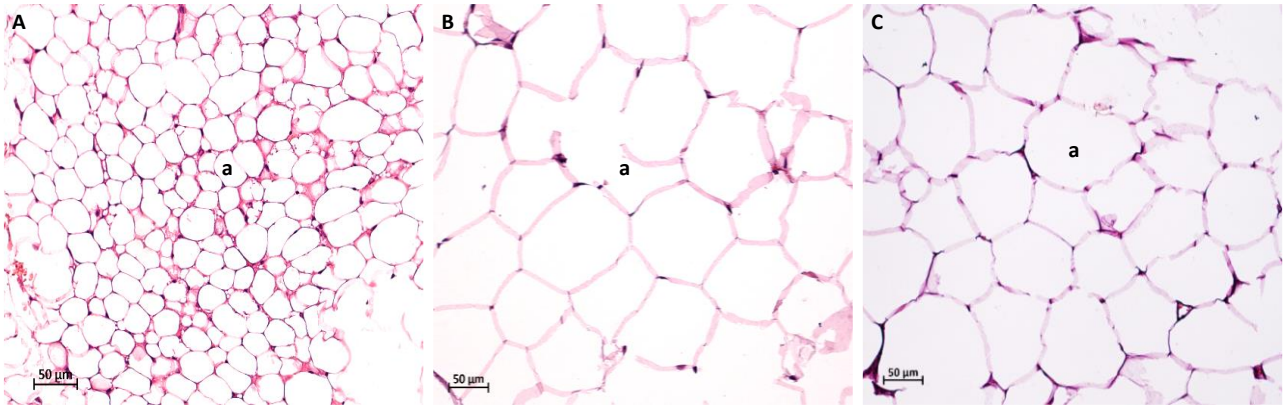


KOHFD14

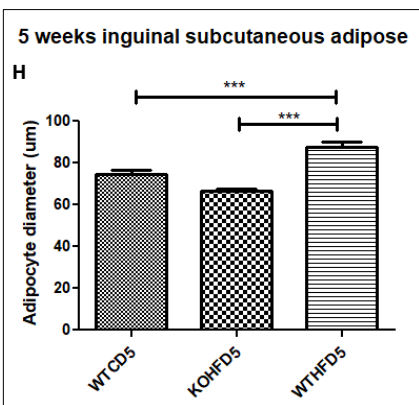
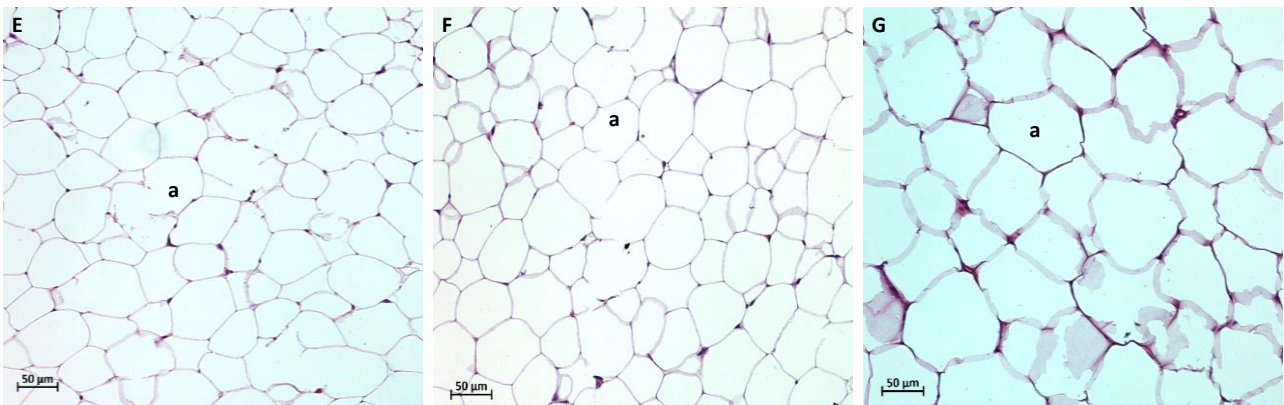


671

672 Figure 1 supplementary: Mice used in the study. The WTHFD14 and KOHFD14 mice look larger in size when
673 compared to WTCD14 and KOCD14, respectively.



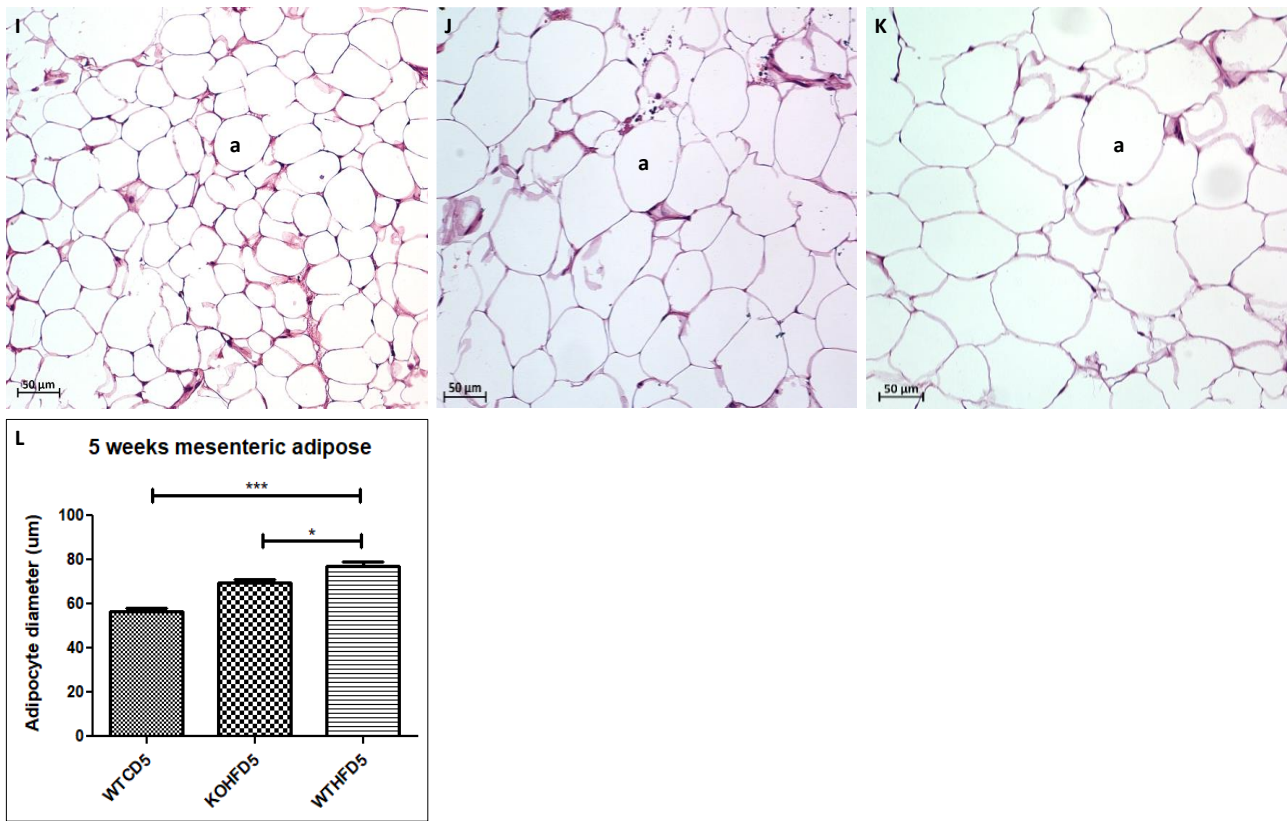
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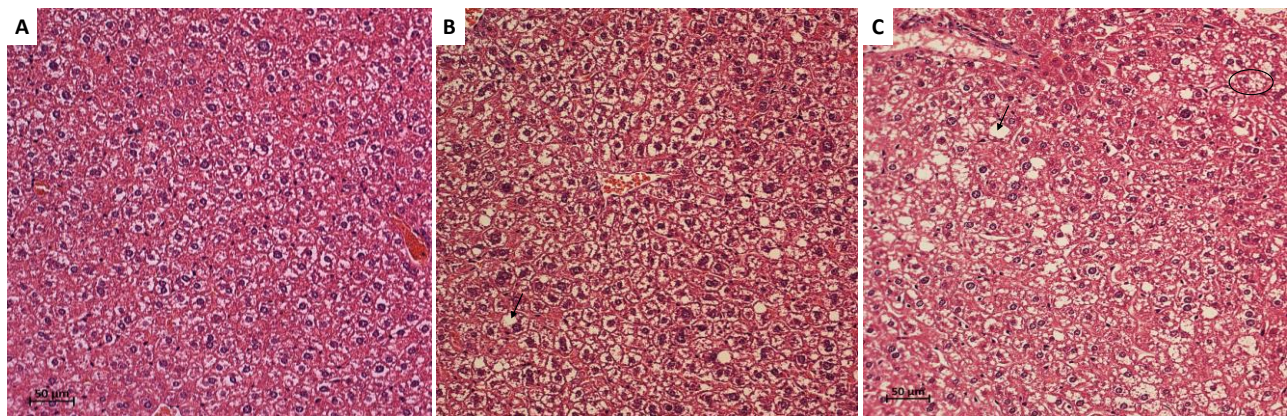
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679 Figure 2 supplementary: Adipocyte hypertrophy at 5 weeks. Adipocyte diameter of WTHFD, C in pWAT was
680 significantly larger than WTCD, A ($p < 0.001$) and KOHFD, B ($p < 0.05$). In iWAT, WTHFD, G adipocyte
681 hypertrophy was significantly greater ($p < 0.05$) than WTCD, E and KOHFD, F. WTHFD, K in mWAT showed
682 significantly larger adipocytes than WTCD, I ($p < 0.001$) and KOHFD, J ($p < 0.05$). Accumulation of enlarged lipid
683 droplets were observed in WTHFD, O than WTCD, M and KOHFD, N.

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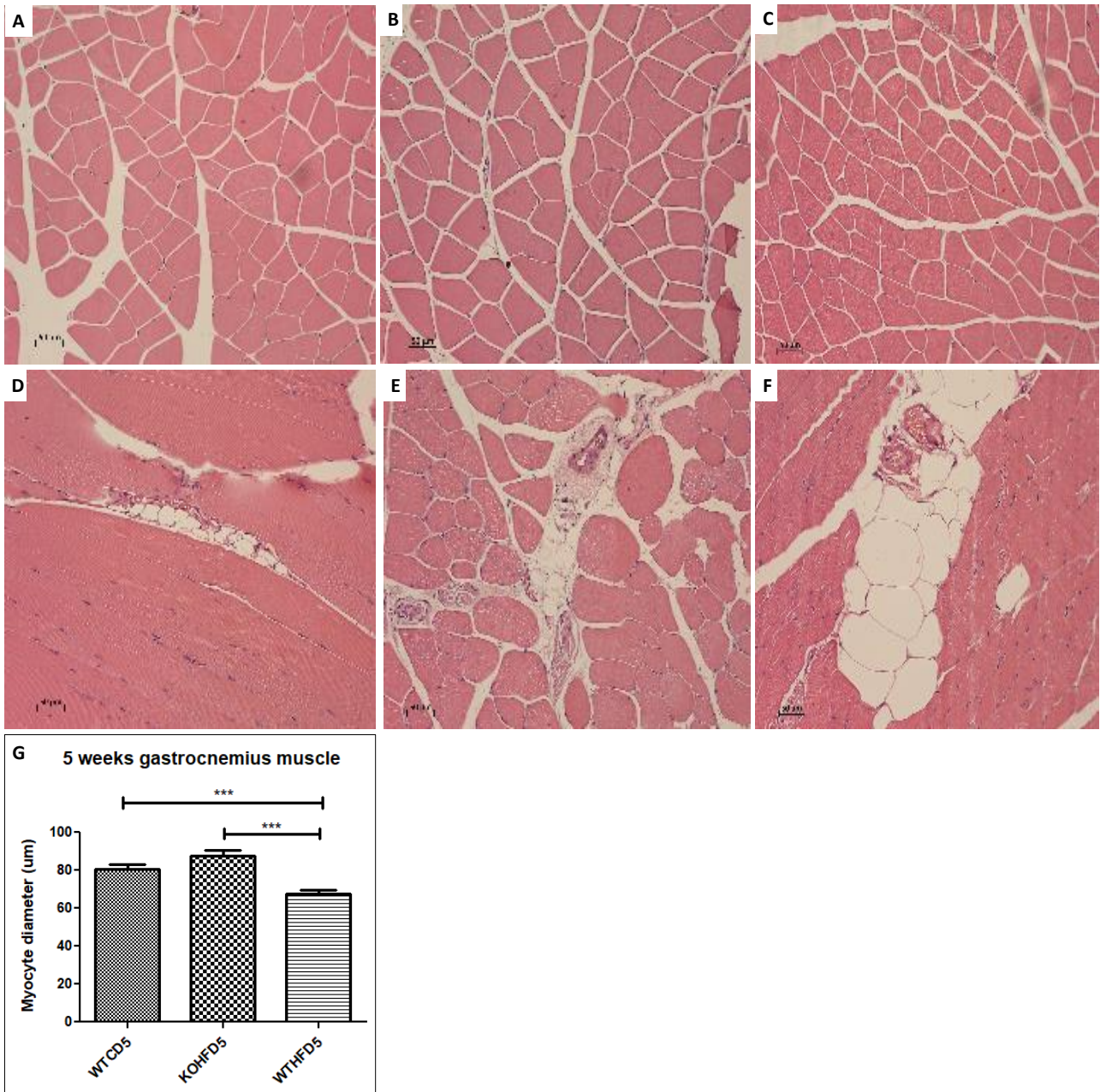


685

686 Figure 3 supplementary: Lipid droplets in the liver at 5 weeks. WTHFD, C and KOHFD, B had lipid droplets in
687 the tissue, while none were observed in WTCD, A.

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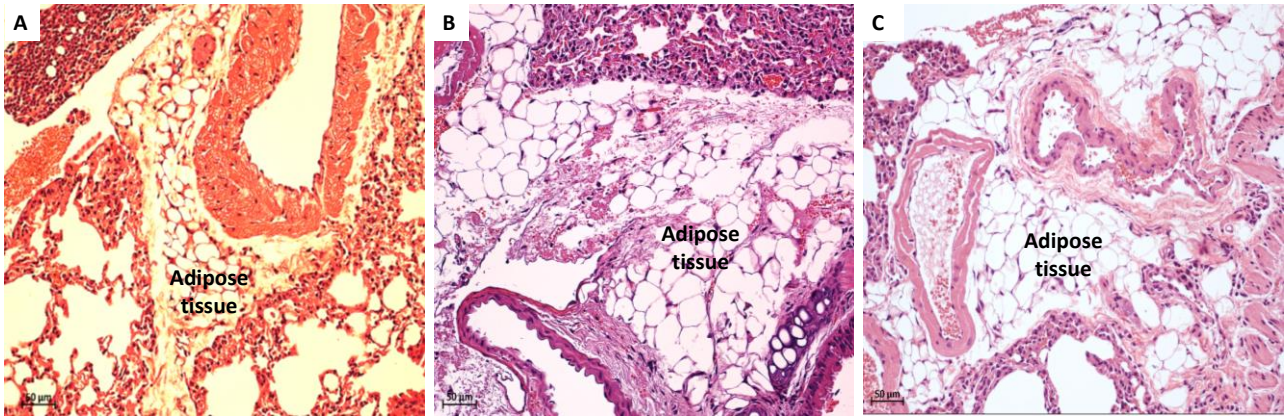
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691 Figure 4 supplementary: Significantly larger myocytes ($p < 0.001$), G, were observed in the WTCD, A and
692 KOHFD, B in comparison to those in the WTHFD, C group. The distribution of peri-muscular adipose tissue
693 shows that greater accumulation of the adipose was observed in WTHFD, F, compared to WTCD, D and
694 KOHFD, E.

695

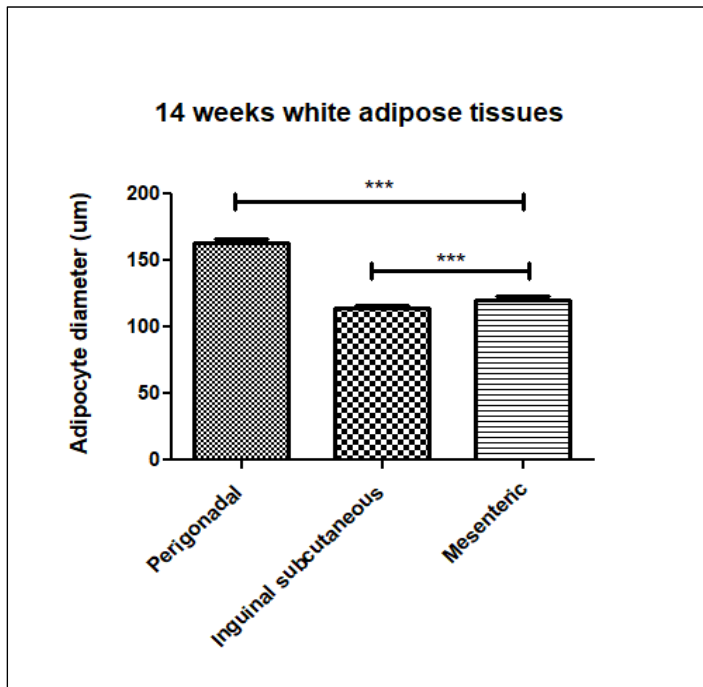
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699 Figure 5 supplementary: Accumulation of adipose tissue in the lungs. Greater accumulation was observed in
700 WTHFD, C and KOHFD, B in comparison to WTCD, A.

701



703 Figure 6 supplementary: Perigonadal adipose tissue in WTHFD is significantly larger ($p < 0.001$) than inguinal
704 and mesenteric adipose tissues.