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1 **Chronic exposure to a pollutant mixture at low doses led to tissue-specific metabolic**
2 **alterations in male mice fed standard and high-fat high-sucrose diet**

3

4 Danielle Naville, Guillain Gaillard, Benoit Julien, Nathalie Vega, Claudie Pinteur, Stéphanie
5 Chanon, Hubert Vidal, Brigitte Le Magueresse-Battistoni

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7

8

9 **Affiliations:**

10 Univ Lyon, CarMeN laboratory, INSERM U1060, INRA U1397, INSA Lyon, Université
11 Claude Bernard Lyon1, 69600 Oullins, France

12

13

14 **Corresponding author:**

15 Dr. B. Le Magueresse-Battistoni, PhD

16 CarMeN laboratory, INSERM U1060, Faculté de Médecine Lyon-Sud, Chemin du Grand
17 Revoyet, 69600 Oullins, France

18 *Phone* 33 (0)426235919, *Fax* 33 (0)426235916

19 E-mail : brigitte.lemagueresse@inserm.fr

20

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22 **Key words:** Mixture of pollutants, cocktail effect, endocrine disruptors, high-fat high-sucrose
23 diet, metabolic disturbances, obesity

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29 **Abstract**

30 Excessive consumption of industrialized food and beverages is a major etiologic factor in the
31 epidemics of obesity and associated metabolic diseases because these products are rich in fat
32 and sugar. In addition, they contain food contact materials and environmental pollutants
33 identified as metabolism disrupting chemicals. To evaluate the metabolic impact of these
34 dietary threats (individually or combined), we used a male mouse model of chronic exposure
35 to a mixture of low-dose archetypal food-contaminating chemicals that was added in standard
36 or high-fat, high-sucrose (HFHS) diet. Specifically, the mixture contained bisphenol A,
37 diethylhexylphthalate, 2,3,7,8-tetrachlorodibenzo-p-dioxine and polychlorinated biphenyl
38 153. Exposure lasted from 5 to 20 weeks of age. Metabolic exploration was conducted setting
39 the basis of candidate gene expression mRNA analyses in liver, jejunum and adipose tissue
40 depots from 20 week-old mice. Strong metabolic deleterious effects of the HFHS diet were
41 demonstrated in line with obesity-associated metabolic features and insulin resistance.
42 Pollutant exposure resulted in significant changes on plasma triglyceride levels and on the
43 expression levels of genes mainly encoding xenobiotic processing in jejunum; estrogen
44 receptors, regulators of lipoprotein lipase and inflammatory markers in jejunum and adipose
45 tissues as well as adipogenesis markers. Importantly, the impact of pollutants was principally
46 evidenced under standard diet. In addition, depending on nutritional conditions and on the
47 metabolic tissue considered, the impact of pollutants could mimic or oppose the HFHS
48 effects. Collectively, the present study extends the cocktail effect concept of a low-dosed
49 pollutant mixture and originally points to tissue-specificity responsiveness especially in
50 jejunum and adipose tissues.

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54 **Highlights:**

55 Low-dosed pollutants trigger metabolic disturbances in postnatally exposed male mice

56 The mixture of pollutants induced metabolic alterations in a tissue-specific manner

57 Jejunum is highly sensitive with regards to xenobiotic receptors and lipolysis

58 Pollutants have a greatest metabolic impact in standard nutritional conditions

59 Differences in the response to the pollutant mixture were seen between the 2 fat pads

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64 **1. Introduction**

65 According to WHO (World Health Organization), obesity is an “abnormal or
66 excessive fat accumulation that presents a risk to health”. This accumulation of fat is
67 deleterious because it could lead to a large spectrum of metabolic diseases (type 2 diabetes,
68 cardiovascular diseases...) which are among the first causes of mortality with 10 million
69 deaths in 2016. For example, more than 1.9 billion of adults in 2016, 18 years and older, were
70 overweight, of those over 650 million were obese (WHO, 2018a, b). Diabetes also exploded
71 these last decades with an average of 400 million worldwide in 2014 (WHO, 2017). In
72 addition, metabolic disorders constitute a heavy cost for society. For example, worldwide cost
73 of diabetes caring exceeded \$673 billion in 2015 (WHO, 2016).

74 Metabolic disorders are multifactorial diseases. Apart from genetic susceptibility and
75 low physical activity, environmental concern relates to excessive consumption of
76 industrialized food and beverages for their content in fat and sugar but also food contact
77 materials and environmental pollutants. Food contact materials may include phthalates and
78 bisphenols which can leach from packaging to cause contamination (Startin et al., 1987;
79 Vandenberg et al., 2007; Koch and Calafat, 2009; Muncke, 2009). Environmental pollutants
80 include persistent pollutants such as dioxins or polychlorobiphenyls (PCBs) which are
81 lipophilic and bioaccumulate through the food chain being virtually present in all animal
82 products (Schafer and Kegley, 2002).

83 Importantly, risk assessment evaluation bodies e.g., the European Food Safety
84 Authority (EFSA) or the U.S. Food and Drug administration (FDA) focused primarily on
85 single chemicals with the definition of tolerable daily intake (TDI) doses based on the no-
86 observed-adverse effect levels (NoAELs) or the lowest-adverse-effect levels in animal studies
87 (Dorne, 2010). Consistently, the mean exposures for the general population were found to be
88 below the current levels determined to be safe for chemicals (Schafer and Kegley, 2002; Koch

89 and Calafat, 2009). However, a recent concern arose with chemicals possibly acting at doses
90 lower than the NoAEL doses identified in animal studies and for some chemicals in the range
91 of the TDI doses (Vandenberg et al., 2007; Vandenberg et al., 2012), and interfering with
92 hormonal action, the so-called endocrine disruptors (Zoeller et al., 2014).

93 There are more than 900 molecules present in our environment that could be
94 considered as endocrine disruptors (EDs) (WHO/UNEP, 2013) and to which we are non-
95 voluntary but chronically exposed. Individually, their concentration may be too low to exert
96 an effect but through acting on identical biological outcomes, the resulting effects of mixtures
97 may grow as significant, an event now identified as the cocktail effect (Kortenkamp, 2014;
98 Svingen and Vinggaard, 2016; Le Magueresse-Battistoni et al., 2017; Le Magueresse-
99 Battistoni et al., 2018a). It is therefore of striking importance to further implement the
100 metabolic impact of mixtures of pollutants at dosage lower than their NoAEL determined in
101 animal studies.

102 Interestingly, we recently developed a mouse model of exposure to four pollutants
103 (Naville et al., 2013; Naville et al., 2015; Labaronne et al., 2017; Julien et al., 2018) selected
104 among archetypal EDs. Specifically, the mixture is made of two persistent pollutants, 2,3,7,8-
105 tetrachlorodibenzo-pdioxine (TCDD) and Polychlorinated Biphenyl 153 (PCB153) and two
106 non-persistent pollutants, Bisphenol A (BPA) and diethylhexylphthalate (DEHP). These
107 pollutants were chosen as an attempt to mimic a real-world exposure based on persistence
108 with two short-lived and two persistent molecules, on their large occurrence in a Western-
109 diet, on the broad range of signaling pathways activated not to mention the many cross-talks
110 occurring between them (Casals-Casas and Desvergne, 2011; Thayer et al., 2012; Vandenberg
111 et al., 2012). In addition, they are considered as metabolism disrupting chemicals (Casals-
112 Casas and Desvergne, 2011; Heindel et al., 2017). Notably, BPA whom worldwide
113 production reached millions of tons yearly and which is mainly used in the manufacture of

114 plastics and resins, has been well identified as triggering metabolic disturbances acting among
115 others through estrogeno-mimetic activities (Alonso-Magdalena et al., 2011; Vandenberg et
116 al., 2012; Vom Saal et al., 2012; Beausoleil et al., 2018; Le Magueresse-Battistoni et al.,
117 2018b). Phthalates are massively produced to soften plastics. They have been described as
118 anti-androgenic compounds although not acting through binding the Androgen Receptor (AR)
119 (Schug et al., 2011). PCB153 and TCDD (also known as the Seveso Dioxin) are persistent
120 with a very long half-life and they bioaccumulate in the fat (Casals-Casas and Desvergne,
121 2011). Dioxins act through binding to the Aryl hydrocarbon Receptor (AhR) and PCB153,
122 which could bind the Pregnane X and Constitutive Androstane Receptors (PXR and CAR,
123 respectively) but not AhR, may interfere with thyroid but also estrogen signaling (Diamanti-
124 Kandarakis et al., 2009; Casals-Casas and Desvergne, 2011; Pavek, 2016) to mediate their
125 metabolism disrupting effects (Angrish et al., 2012; Wahlang et al., 2013; Heindel et al.,
126 2017). Noteworthy, diet is the major route of exposure for pollutants including those present
127 in the mixture used in the present study (Diamanti-Kandarakis et al., 2009; Casals-Casas and
128 Desvergne, 2011; Thayer et al., 2012; Vandenberg et al., 2012).

129 With this model, we demonstrated that chronic exposure to such a mixture at dosage
130 lower than the NoAELs for each chemical of the mixture, could trigger metabolic
131 disturbances in the progeny of diet-induced obese mice in a sex-dependent way (Le
132 Magueresse-Battistoni et al., 2018c). Importantly, male mice developed alterations in
133 cholesterol metabolism whereas females exhibited altered glucose tolerance, probably linked
134 to reduced estrogen signaling in the liver, all occurring without additional obesogenic effects
135 (Naville et al., 2013). As a step further, the present study aims to evaluate the metabolic
136 impact of two challenging stressors, *i.e.* the high-fat high-sucrose (HFHS) diet and the
137 mixture of pollutants, independently and combined, focusing on males in a one-generation
138 study. Consistently, we analyzed the liver and the intestine, specifically the jejunum, because

139 these two organs play pivotal roles in detoxification processes and in glucose and lipid
140 metabolisms. In addition, the jejunum is a predominant organ for dietary fat and sugar
141 absorption. Adipose tissues were also surveyed as they play a major role in the development
142 of insulin resistance.

143 **2. Materials and Methods**

144 *2.1. Diet and animals*

145 Mouse studies were performed with the approval of the Regional Committee of Ethics for
146 Animal Experiments. Thirty-two C57Bl/6J male mice were purchased from Envigo (Gannat,
147 France) at the age of 4 weeks (wks) and were housed two per cage at 21°C with a normal
148 light-dark cycle and free access to water. Cages and bottles of water were made of
149 polypropylene to avoid BPA leaching from polycarbonate equipment. After 1 wk-
150 acclimatization, mice were divided into four groups depending on the diet: ST0 (standard diet
151 from Genestil, Royaucourt, France; without the mixture of pollutants), STp (standard diet
152 with the mixture of pollutants), HF0 (HFHS diet from Envigo, without the mixture of
153 pollutants) and HFp (HFHS diet with the mixture of pollutants). Pollutants (TCDD from
154 LGC-Promochem, Molsheim, France; PCB153, BPA and DEHP from Sigma-Aldrich, Lyon,
155 France) were each used at doses in the range of their tolerable daily intake (TDI) reference
156 dose and at least 10 times lower than their NoAEL dose (van Leeuwen et al., 2000; WHO,
157 2003; European Food Safety Authority Panel on Food Additives, 2005) (Supplementary Table
158 1). They were diluted in DMSO, solubilized in corn oil and mixed into the diet. All animals
159 received the same amount of DMSO and corn oil. To make sure mice received the adequate
160 quantity of pollutants, calculations for doses were made for 1 g of food for 17 g of body
161 weight/day and extra food devoid of pollutants but containing vehicle was given *ad libitum*.
162 Diet composition is detailed elsewhere (Labaronne et al., 2017). Mice were weighed weekly

163 and food changed three times a week. Exposure to pollutants lasted 15 wks after which
164 animals were fasted for 6 h and weighed. Blood was collected and mice euthanized by
165 cervical dislocation. The organs: liver, jejunum, perigonadal and inguinal subcutaneous
166 adipose tissues (pgAT and scAT, respectively) were rapidly removed, weighed, frozen in
167 liquid nitrogen and conserved at -80°C until experiments.

168 *2.2. Metabolic explorations*

169 Metabolic tests were conducted on all animals with a one wk interval. After 12 wks of diet,
170 mice were fasted for 16 h and received an intragastric bolus of lipids (250µl of Isio4™ oil,
171 Lesieur). Blood samples were collected from the tail vein before gavage and at 1, 2, 3 and 4 h
172 after gavage for triglyceride measurement. This test will reflect the intestinal fat clearance.
173 After 13 wks of diet, mice were fasted 6 h and were orally administered a solution of 10% D-
174 xylose (1mg of D-xylose/g of body weight). Blood samples were collected at 20, 40, 60 and
175 120 min after gavage for xylose measurement. This exploration of the intestinal uptake of
176 ingested D-xylose will mirror a post-prandial sugar uptake. After 14 wks of diet, glucose
177 tolerance test (GTT) was performed on 6 h fasted mice as previously described (Gout et al.,
178 2010).

179 *2.3. Tissue and plasma dosages*

180 Blood glucose concentrations were determined using the Accu-Chek Performa glucometer
181 (Roche Diabetes Care France, Meylan). Insulin (Mouse ultrasensitive ELISA, Eurobio,
182 Courtaboeuf, France), triglycerides (Biolabo, Maizy, France), free fatty acid, total cholesterol
183 and cholesteryl esters, Alanine Aminotransferase and Aspartate aminotransferase activities
184 (all kits from Sigma-Aldrich), leptin and adiponectin (ELISA kits from Crystal Chem Europe,
185 Zaandam, Netherlands), were also assayed in plasma. Xylose plasma levels were determined
186 as described elsewhere (Conarello et al., 2007). Triglycerides and total cholesterol were also

187 measured in liver samples after extraction of the lipids using lipid extraction kit chloroform
188 free (Clinisciences, Nanterre, France).

189 2.4. Real time PCR analyses

190 Total RNA was extracted from liver, jejunum and adipose tissue samples. After reverse
191 transcription using the Prime Script RT Reagent kit (Takara Bio Europe SAS, Saint-Germain-
192 en-Laye, France), cDNAs were analyzed by real time PCR using the SYBR Premix Ex Taq™
193 (Takara) in the presence of specific primers specifically designed to encompass an intron
194 (Supplementary Table 2) as previously described (Naville et al., 2011). Data were normalized
195 using housekeeper genes which differ according to the tissues. They were chosen because
196 consistently expressed in all groups in a particular tissue and they encode TATA-Box Binding
197 Protein (*Tbp*) for scAT and pgAT, Beta-Glucuronidase (*Gusb*), for the liver, and
198 Hypoxanthine Phosphoribosyltransferase (*Hprt*) for the jejunum.

199 2.5. Histological analyses

200 The two adipose tissue depots were fixed with 4 % formaldehyde solution during 24 h and
201 then paraffin embedded. Serial sections of the paraffin-embedded blocks (4 µm thick) were
202 obtained and immunolabeled using the rabbit anti-laminin antibody (1:100, Sigma, L9393)
203 followed by alexafluor secondary antibody (Invitrogen, Villebon-sur-Yvette, France;
204 A11012). Measurement of adipocyte area was performed from the immunofluorescence
205 acquisition using DP74 camera (Olympus, France). At least 150 adipocytes for each sample
206 were considered. Image analyses were performed with ImageJ software.

207 2.6. Statistical analysis

208 Results are presented as means ± SEM. Groups were compared using one-way ANOVA
209 followed by Bonferroni multiple comparison post-hoc test. Two-way ANOVA for non-

210 repeated measures was also used to determine the overall effects of the diet (Standard
211 ST0/STp vs HFHS HF0/HFp), the mixture (non-exposure ST0/HF0 vs exposure STp/HFp)
212 and their interaction (diet x mixture). When significant effects without interaction were
213 obtained, differences were tested using Student's t-test between each pollutant-exposed group
214 and corresponding non-exposed group. GraphPad Prism 5.0 software was used for all
215 statistical analysis and differences were considered significant at P - values < 0.05.

216 **3. Results**

217 *3. 1. Metabolic characterization of males fed the HFHS versus the ST diet, containing or not* 218 *the mixture of pollutants*

219 Feeding 5-wk old male mice a HFHS diet for 15 wks resulted in a time-dependent increase of
220 body weight with first significant effects observed at 8 wks of age i.e. wk 3 of feeding
221 (Supplementary Fig. 1A). At the end of the 15-wk HFHS feeding, strong significant diet-
222 induced obesogenic effects were reported on body weight (+38%) and fat mass (+250% for
223 pgAT weight and +380% for scAT weight) (Fig. 1A and B). This was associated with
224 significant increases of hepatic and plasma triglycerides (TG), plasma cholesterol and blood
225 glucose levels (Fig. 1D, F and Supplementary Fig. 1D). An overall effect of the diet was also
226 noticed on plasma insulin level (Fig. 1D) resulting in a 4.5-fold significant enhancement of
227 the HOMA index of insulin resistance (HOMA-IR; Supplementary Fig. 1E). Moreover, the
228 ratio of adiponectin to leptin, a marker of insulin resistance (Mirza et al., 2011; Lopez-
229 Jaramillo et al., 2014), was strongly decreased in HFHS-fed mice (Fig.1E and Supplementary
230 Fig. 1F). These data are consistent with the metabolic tests performed on fasting animals
231 showing a significant decrease in blood lipid clearance (increased Area Under Curve, AUC,
232 after oral lipid load) and increased glucose absorption (estimated using the xylose absorption

233 test) as well as glucose intolerance in HFHS diet-induced obese mice (Fig. 1C and
234 Supplementary Fig. 1B and C).

235 Pollutant exposure did not result in body weight changes regardless of the nutritional context.
236 Interestingly, slight but significant effects ($P < 0.05$) of the mixture of pollutants could be
237 observed in STp-fed mice on several features including an increase of the intestinal fat
238 clearance after oral lipid load, a significant reduction in plasma levels of TG with a tendency
239 ($P=0.05$) to a reduction of plasma FFA levels and a significant increased ratio of adiponectin
240 to leptin, all seemingly and unexpectedly converging to some alleviation of metabolic
241 conditions (Fig. 1C, 1E, 1F and Supplementary Fig. 1F). On the opposite, mice exposed to the
242 mixture of pollutants and fed a HFHS diet exhibited a significant enhancement of the weight
243 of pgAT as compared to HF0 mice (Fig. 1B), in line with the observed trend ($P=0.08$) for
244 enhanced leptin plasma levels (Supplementary Fig. 1F).

245

246 3. 2. Gene expression analysis of proteins involved in lipid metabolism in the liver

247 As an attempt to better comprehend the metabolic features, we first focused on liver, a central
248 regulator of lipid metabolism and detoxification. Potential toxicity linked to pollutant
249 exposure was first excluded through measuring plasma transaminases. Indeed, both AST and
250 ALT levels were unchanged regardless of treatment (Supplementary Fig. 2). To understand
251 bases for the alleviation of the lipid profile in pollutant-exposed STp mice compared with ST0
252 mice (Fig. 1C and 1F), measurement of the expression levels of genes involved in the uptake
253 of circulating fatty acids, *de novo* lipogenesis, fatty acid breakdown, lipoprotein metabolism
254 and lipolysis as well as cholesterol homeostasis was conducted in liver. Specifically, studied
255 genes included *Cd36* encoding cluster of differentiation 36 and *Fabp1* encoding fatty acid
256 binding protein 1 involved in the uptake of FA, the lipid sensor *Nr1h3* encoding Liver X

257 receptor (LXR) α , *SrebF1* encoding Sterol Regulatory Element-Binding Protein 1
258 (SREBP1c), a master regulator of *de novo* lipogenesis, *Scd1* and *Acaca* encoding stearoyl-
259 CoA desaturase and acetyl-CoA carboxylase (ACC1), respectively. Lipid oxidation was
260 assessed measuring mRNA levels of *Nr1c1* (encoding peroxisome proliferator-activated
261 receptor α , PPAR α), its target genes *Cpt1a* (encoding carnitine palmitoyltransferase 1 α) and
262 *Vnn1* (encoding Vanin-1). Expression levels of *Apoa5* encoding Apolipoprotein A-V (APOA-
263 V) a lipoprotein lipase (LPL) activator and *Apoc3*, encoding an inhibitor of LPL (APO-C III)
264 were as well studied so were several key genes involved in cholesterol metabolism: *Cyp7a1*,
265 *Hmgcr*, *Cyp51*, *Abcg5*, *Abcg8*, *Abca1* encoding Cytochrome P450 Family 7 Subfamily A
266 Member 1, 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCoAR), P450 sterol 14 α -
267 demethylase and ATP-binding cassette (ABC) transporters, respectively.

268 On this panel of genes, effects observed in the liver were essentially due to the HFHS diet
269 (comparison HF0 vs ST0) with significant increases of *Cd36*, *SrebF1*, *Scd1* (Fig. 2A, C),
270 *Nr1c1*, *Vnn1* (Fig. 2D) and *Cyp7a1* (Fig.2E) expressions. As well, the fat-enriched diet
271 induced a decrease in the expression of *Apoa5* (Fig. 2B), *Hmgcr*, *Cyp51* (Fig. 2E) and *Abcg5*,
272 but not of *Abcg8*, *Abca*, *Nr1h3* and *Fabp1* (data not shown). It is interesting to note that some
273 alterations of gene expression induced by the fat-enriched diet were only observed in the
274 presence of pollutants: decreased expression of *Acaca* and increased expression of *Apoc3*. As
275 well, pollutants in a standard diet context significantly-increased the mRNA expression of
276 *Cyp7a1* (Fig. 2E). However, no effect of the pollutant mixture was observed on the other
277 investigated genes of the cholesterol field area including *Hmgcr*, *Cyp51* (Fig. 2E), *Nr1h3* as
278 well as *Abcg5*, *Abcg8* and *Abca1* (data not shown).

279 To discriminate between hepatic steatosis and steatohepatitis, a more severe liver disease than
280 fatty liver characterized by a chronic inflammatory state with high levels of cytokines among
281 which IL6 and tumor necrosis factor (TNF) α (Tilg and Moschen, 2008; Chen et al., 2017),

282 expression levels of *Il6* and *Tnfa* were surveyed but no impact was observed regardless of
283 treatments (Fig. 2F). Nonetheless, *Ccl2* encoding the chemokine monocyte chemoattractant
284 protein 1 (MCP1/CCL2) which levels could be increased from simple steatosis (Greco et al.,
285 2008) were found significantly enhanced by the HFHS diet (Fig. 2F).

286

287 3. 3. Gene expression analysis of proteins involved in lipid absorption and metabolism in 288 jejunum

289 In the present study, dietary fat and glucose absorption were shown impacted by the
290 nutritional stressors tested e.g., HFHS diet and/or the pollutant mixture (Fig. 1 and
291 Supplemental Fig. 1). Inasmuch as the jejunum is a predominant site for these functions, we
292 analyzed the expression levels of jejunal genes involved in the absorption of fatty acids
293 including *Got2* (encoding Plasma membrane Fatty Acid Binding Protein, FABPpm), *Cd36*
294 and *Slc27a4* (encoding Fatty Acid Transport Protein 4, FATP4). Expression of genes involved
295 in the cholesterol metabolism: *Npc1ll* gene (encoding Niemann Pick C1 Like-1, NPC1L1)
296 and the lipoprotein biosynthesis: *Sar1b* (encoding Secreted associated Ras related GTPase 1B,
297 SAR1B) and *Mttp* (encoding Microsomal Triglyceral Transfert Protein, MTP) were also
298 investigated, together with the anti-inflammatory marker *Il10* encoding the interleukin IL10.

299 The HFHS diet (HF0 vs ST0) altered the expression of *Got2* and *Cd36* (increased expression,
300 Fig. 3A) as well as *Npc1ll* (decreased expression, Fig. 3B). The pollutant mixture had no
301 significant effect in a context of a standard diet but it has to be noted that *Slc27a4* and *Mttp*
302 expressions were only significantly modified by the HFHS diet in a context of pollutant
303 exposure (Fig. 3A and 3C).

304 The expression of genes encoding inhibitors of the lipoprotein lipase (LPL) enzyme
305 responsible for blood chylomicron clearance: *Angptl4* and *Apoc3* encoding Angiopoietin-Like
306 4 (ANGPTL4) and Apolipoprotein-CIII (APO-CIII), respectively, were also studied. Feeding
307 HFHS diet resulted in a significant enhancement of *Apoc3* and *Angptl4* mRNA levels both in
308 the absence or presence of pollutants (Fig. 3D). Of note, exposure to pollutants resulted in a
309 significant down-regulation of *Apoc3* mRNA and a downward trend for *Angptl4* ($P = 0.09$) in
310 ST fed mice only (Fig. 3D). Interestingly pollutants induced a significant decrease in the *Ill10*
311 expression at the mRNA level (Fig. 3E) in a context of standard diet and an inverse effect in
312 the context of HFHS diet (Fig. 3E).

313 Eventually, we found that genes involved in intestinal glucose transport and sensing such as
314 *Slc2a2* and *Slc5a1* (encoding the proteins Glucose Transporter 2, GLUT2 and sodium-
315 dependent glucose cotransporter SGLT1, respectively) (Roder et al., 2014) were expressed at
316 similar levels between groups (data not shown).

317 3. 4. Gene expression analysis of proteins involved in adipocyte differentiation

318 As a next step, we analyzed genes related to adipogenesis including *Nr1c3* encoding
319 peroxisome proliferator activated receptor (PPAR) γ , a master regulator of adipocyte
320 differentiation; *Fabp4* encoding Fatty Acid Binding Protein (FABP4), and *Cidec* encoding
321 Cell Death Inducing DFFA Like Effector C (CIDEC). Importantly, the two different ATs
322 studied herein responded differently to nutritional stressors. While in scAT, *Fabp4* and *Cidec*
323 mRNA levels were significantly enhanced in response to HFHS diet, they remained
324 unchanged in pgAT (Fig. 4A and 5A). Conversely, *Nr1c3* mRNA levels remained unchanged
325 in scAT from HF0 vs ST0 mice (Fig. 4A) but were significantly reduced in pgAT (Fig. 4A).
326 These distinct features were also observed for genes involved in TG metabolism including
327 *Pnpla2* encoding Adipose Triglyceride Lipase (ATGL); *Lipe* encoding Hormone-Sensitive

328 Lipase (HSL); and *Lpl* encoding Lipoprotein Lipase (LPL). While *Pnpla2* mRNA levels
329 remained unchanged in scAT, they were strongly decreased after HF feeding in pgAT (Fig.
330 4B and 5B). On the contrary, an enhanced expression of *Lipe* and *Lpl* was induced by the
331 HFHS diet in scAT while no change was observed in pgAT (Fig. 4B and 5B).

332 Pollutants also targeted scAT differently than pgAT. There was a significantly enhanced
333 expression of *Cidec* and *Lipe* and a trend to an enhanced expression of *Nr1c3*, *Fabp4*
334 ($P=0.07$) and *Lpl* ($P=0.06$) in scAT from STp mice as compared to ST0 mice (Fig.4A and 4B)
335 but no significant effect was observed in a context of HFHS diet. Conversely, the pollutant
336 effects in pgAT were observed in a context of HFHS diet with a significant decrease of *Nr1c3*
337 mRNA levels in HFp versus HF0 samples (Fig. 5A).

338 To complete the study, histological analyses were performed on both subcutaneous and
339 perigonadal ATs. Consistent with enhanced fat pads and body weight (Fig. 1), adipocytes
340 were of larger size in response to the HFHS diet. However, there was no major difference in
341 the mean diameter of adipocytes of both fat pads in response to exposure to the mixture of
342 pollutants (Supplementary Fig. 3).

343

344 3. 5. Gene expression analysis of inflammatory markers in adipocyte tissues

345 It is well described that AT inflammation conveys insulin-resistance (Ouchi et al., 2011).
346 Pollutants have also been described as pro-inflammatory in certain conditions (Rebourcet et
347 al., 2010; Kim et al., 2012; Magre et al., 2012; La Merrill et al., 2013). We therefore
348 examined the expression levels of genes including *Tnfa*, *Il-1b* and *Ccl2* encoding major pro-
349 inflammatory markers TNF α , interleukin 1 (IL1) β , and MCP1, respectively, as well as *Il10*
350 encoding an anti-inflammatory cytokine, interleukin 10. Apart from *Il10* which expression
351 levels did not change in response to HFHS diet alone (Fig. 4C and 5C), most pro-

352 inflammatory cytokines had their expression significantly enhanced in both AT samples from
353 HFHS-fed mice consistent with the phenotype of insulin resistance evidenced in this HF0
354 group as compared to ST0 group. *Il-1 β* expression was the exception with a down-regulation
355 of its mRNA levels in pgAT samples of HFHS-fed mice (Fig. 5C).

356 Interestingly, as well as for adipogenesis and lipolysis markers, pollutants had differential
357 impact depending on AT depots. In a standard diet context, the global impact was anti-
358 inflammatory in scAT samples with significant enhancement of *Il10* mRNA expression (Fig.
359 4C) and no impact on pro-inflammatory cytokines (Fig 4C). In HFHS-fed mice, the
360 inflammatory state based on the investigated studies remained unchanged by pollutant
361 exposure in scAT (Fig 5C). Conversely, pollutants tended to reduce the pro-inflammatory
362 state in pgAT, although *Il10* expression remained unchanged, with a significant and strong
363 down-regulation of *Il1b* mRNA levels in response to pollutant exposure in ST-fed mice.
364 Contrary to the observed decrease in *Il1b* expression after exposure to HFHS diet in the
365 absence of pollutant, the exposure to the mixture in this HFHS context induced an increased
366 expression of this gene (Fig. 5C).

367 3.6. Gene expression analysis of xenobiotic receptors

368 One way pollutants trigger metabolic disruption is through their direct interaction with
369 xenobiotic receptors and downstream activation of xenobiotic processing genes, among which
370 cytochromes P450 (CYPs) (Aleksunes and Klaassen, 2012). Therefore, a gene expression
371 analysis of the xenobiotic receptors was undertaken. The expression of *Ahr* encoding the
372 dioxin receptor AHR, could be detected in all investigated tissues consistent with its
373 ubiquitous expression (Barouki et al., 2012a). A HFHS effect on *Ahr* expression was observed
374 in jejunum, liver and scAT but not in pgAT with a significant down-regulation of the *Ahr*
375 mRNA levels (Fig. 6A). The AHR target gene *Cyp1a2* was also reduced at the mRNA levels
376 in liver (Fig. 6A) but could not be quantifiable in the jejunum and adipose tissues (not

377 shown). Expression of *Nr1i2* encoding Pregnane X Receptor (PXR) and *Nr1i3* encoding
378 Constitutive Androstane Receptor (CAR) was detected in jejunum and liver (Fig. 6B-C) but
379 not in ATs (not shown). Neither *Nr1i2* nor *Nr1i3* had their expression levels modified by
380 nutritional stressors in liver. In contrast, the expression of *Nr1i2* was significantly decreased
381 in the jejunum samples from HFHS-fed mice while *Nr1i3* expression tended to increase
382 (P=0.06) as compared to ST0-fed mice (Fig. 6C).

383 Interestingly, a trend (P = 0.06) for enhanced expression of *Nr1i3* was also detected in the
384 jejunum of STp as compared to ST0 mice, which is consistent with the significant
385 augmentation of the expression of *Cyp3a11*, a PXR target gene (Fu et al., 2016). In contrast,
386 the HFHS diet induced a significant and robust decrease of the expression levels of *Cyp3a11*
387 in jejunum and liver (Fig. 6B-C). Results in liver are consistent with previous reports
388 (Fromenty, 2013) although discrepancies still exist regarding the regulation of *Cyp3a11*
389 expression in the liver of high-fat fed mice (Ning and Jeong, 2017). Intriguingly, expression
390 levels of *Cyp2b10*, a CAR target gene (Aleksunes and Klaassen, 2012), was significantly
391 down-regulated in jejunum and up-regulated in liver from HFHS-fed mice. Noteworthy, a
392 significant down-regulation of the expression of *Cyp2b10* was observed in STp versus ST0
393 liver samples (Fig. 6B-C). No alteration of the expression of *Ugt1a1*, also a CAR target gene,
394 was noted between groups in jejunum and liver (data not shown).

395

396 3. 7. Gene expression analysis of estrogen and androgen receptors

397 To complete the study, we surveyed receptors for estrogen and androgen because steroid
398 hormones regulate energy metabolism (Mauvais-Jarvis, 2011) and that the pollutants of the
399 mixture have been endowed with estrogeno-mimetic activities by interacting either with
400 steroid signaling or its signaling machinery (Casals-Casas and Desvergne, 2011; Thayer et al.,
401 2012). ER α , encoded by the gene *Esr1*, is the main receptor in rodent liver (Gao et al., 2008).

402 The liver is also expressing the Androgen Receptor (AR, encoded by *Nr3c4*) (Lin et al.,
403 2008). While the expression of *Esr1* and *Nr3c4* was detected in the liver, these genes were not
404 found expressed in the jejunum (data not shown) contrary to *Gper1* encoding the G Protein-
405 Coupled Estrogen Receptor 30 (GPR30), a membrane estrogen receptor (Sharma et al., 2018).
406 These 3 genes were expressed in both scAT and pgAT (Fig. 7C and 7D). In response to the
407 HFHS diet, expression levels of *Nr3c4* were significantly reduced in ATs but augmented in
408 liver. *Esr1* expression levels were also altered by the HFHS diet in liver and ATs, but only
409 under pollutant exposure, with a significant increase in liver (Fig. 7A) and a significant
410 decrease in ATs (Figure 7C and 7D). Strongest effects were observed on *Gper1* mRNA levels
411 in ATs with 2.2 and 1.6-fold increases in scAT and pgAT, respectively, while there was a
412 significant decrease of 20% in jejunum (Fig. 7B-D). Pollutant exposure in a standard diet
413 context resulted in a 1.5-fold increase of *Esr1* mRNA levels ($P < 0.01$) in scAT but not in
414 pgAT or liver (Fig. 7). In jejunum, pollutants also induced a downward trend ($P=0.07$) in
415 *Gper1* expression, only in HFHS-fed mice (Fig. 7B). Importantly, the ratio *Esr1/Nr3c4* was
416 significantly up-regulated by pollutant exposure in the scAT of ST-fed mice but remained
417 unchanged in pgAT samples. A significant increase was induced by the HFHS diet in scAT
418 (Fig. 7C). In the liver, this ratio was significantly reduced in response to the HFHS diet (HF0
419 vs ST0) and a tendency to decrease ($P=0.05$) was observed under pollutant exposure in a
420 standard diet context (Fig. 7A).

421

422 **Discussion**

423 The results of the present study indicate that exposure to a mixture of low-dose pollutants for
424 15 weeks triggers metabolic effects in male mice challenged from 5 wks onwards in the
425 absence of general toxicity and that the resulting effects depended on the nutritional status,

426 i.e., ST or HFHS diet, thus extending previous data of the laboratory (Navelle et al., 2013;
427 Navelle et al., 2015; Labaronne et al., 2017). In addition, this study indicates, in an original
428 way, that low-dosed pollutant cocktail exposure elicited tissue-specific responses at least at
429 the gene expression mRNA level. Specifically, we herein demonstrated that jejunum and
430 adipose tissues were major targets of xenobiotics in this one-generation experimental study
431 and that scAT responded differently to the pollutant mixture than pgAT.

432 The protocol used herein was adapted from the previously described model (Navelle et al.,
433 2013; Navelle et al., 2015; Labaronne et al., 2017) in that mice were either fed a standard or a
434 HFHS diet containing the mixture of pollutants with treatments starting from the age of 5
435 weeks for a total duration of 15 weeks. As reviewed (Le Magueresse-Battistoni et al., 2017),
436 pollutants comprising the used mixture were selected to approach a realistic scenario with
437 both persistent and non-persistent chemicals, all bearing endocrine-disrupting activities and
438 all of high concern for human health for their suspected links with metabolic diseases
439 (Alonso-Magdalena et al., 2011; Casals-Casas and Desvergne, 2011; Gore et al., 2015). Thus,
440 this one-generation study allowed investigating in males (females were not explored in this
441 report) the metabolic impact of the pollutant mixture in a standard and in a HFHS diet context
442 to better understand the individual and combined contribution of both threats to the nowadays
443 epidemics of metabolic diseases. In addition to a thorough metabolic exploration of male mice
444 fed the high-fat high-sucrose diet, we investigated the regulation of target gene expression in
445 the liver, adipose tissues, and the jejunum. Both liver and jejunum play major detoxifying role
446 and express the repertoire of xenobiotic processing genes including xenobiotic receptors and
447 related transcription factors as well as phase I and II metabolizing enzymes and transporters
448 (Fu et al., 2016). In addition, the small intestine is the first organ directly in contact with the
449 dietary nutrients and the contaminating chemicals.

450 Within this study, HFHS diet recapitulated metabolic disorders linked to obesity and insulin
451 resistance including dyslipidemia, hyperglycemia, elevated plasma levels of insulin and
452 leptin. Interestingly, the metabolic impact was found more deleterious herein than in the two-
453 generation study with regards to insulin resistance (Homa-IR) and leptin levels (Navelle et al.,
454 2013). There was also impaired lipid homeostasis as evidenced through the oral lipid load
455 test and the findings of enhanced expression of several genes encoding proteins involved in
456 lipid transport and/or lipoprotein synthesis in the jejunum, in fat uptake and lipogenesis
457 favoring steatosis in the liver. In addition, fat mass was largely increased with an overall
458 inflammatory profile in both ATs (apart from a decreased *Il1b* expression in pgAT) and
459 augmented adipogenesis in scAT. Curiously, jejunum has so far not been extensively
460 investigated in response to HFHS diet and several features, indicative of alterations in
461 jejunum homeostasis, could be originally described in the present study. The anti-
462 inflammatory cytokine *Il10* gene expression was severely down-regulated concurrently with
463 the decreased expression of genes encoding the estrogen receptor *Gper*, the xenobiotic
464 receptors *Ahr* and *Nr1i2* as well as processing genes *Cyp3a11* and *Cyp2b10*. Additional work
465 would help to resolve why *Nr1i2* and *Nr1i3* mRNA levels did not change the same way in
466 liver and jejunum in response to HFHS diet along with their target genes, *Cyp3a11* and
467 *Cyp2b10*. Namely, CYP3A11 is an important phase I enzyme which catalyzes detoxification
468 of a wide range of substrates thus, in the long run, an inhibition of this enzyme, as observed in
469 both jejunum and liver, would be harmful consistent with reduced metabolization of
470 pharmacological drugs in obese patients (Fromenty, 2013). Whether increased *Cyp2b10*
471 mRNA levels will compensate for the decreased *Cyp3a11* expression in liver warrants further
472 study.

473 Interestingly, mice fed the HFHS diet in this experimental study retained some metabolic
474 adaptability as exemplified with the mild hepatic phenotype (accumulation of TG with limited

475 inflammation) and unchanged FFA levels restricting lipotoxicity. The upregulation of genes
476 encoding inhibitors of lipoprotein activity in liver and jejunum and the down-regulation of a
477 LPL activator in liver may have also contributed to this phenotype. In addition, gene
478 expression studies suggested restrained hypercholesterolemia through a reduced expression of
479 *Npc1l1* which encoding protein transports cholesterol in jejunum and of genes encoding
480 protein involved in liver cholesterol synthesis (*Hmgcr*, *Cyp51*) coupled to its enhanced
481 metabolism (*Cyp7a1*) in biliary acids. Importantly, AT depots responded differently to the
482 HFHS diet with regards to inflammation, adipocyte differentiation, *Ahr* expression as well as
483 estrogen and androgen receptors. Because it is known that estrogens favor subcutaneous over
484 visceral fat (Mauvais-Jarvis, 2011), it would be of interest to determine if the relative
485 limitation of the metabolic disorders observed in male mice fed the HFHS diet, particularly
486 concerning the fasting plasma glucose, could not be linked to the enhanced ratio of
487 *Esr1/Nr3c4* found in the scAT.

488 Analysis of the metabolic impact of a chronic exposure to the mixture of pollutants revealed
489 that effects were more marked in ST than in HFHS-fed mice. A major difference with the
490 HFHS threat consisted in the lack of any effect of pollutants on body weight which is
491 consistent with our previous data using the same mixture of pollutants (Naville et al., 2013;
492 Naville et al., 2015; Labaronne et al., 2017). In addition and contrasting with previous
493 published data using the chemicals present in the mixture but tested individually and at higher
494 doses (Wahlang et al., 2013; Wei et al., 2014; Duval et al., 2017; Heindel et al., 2017), the
495 pollutant cocktail did not trigger hepatic TG accumulation nor was the HFHS-induced TG
496 accumulation exacerbated in the pollutant-exposed mice. Discrepancies may be due to a
497 different diet composition, differences in the experimental chemical doses, the strain species
498 and the period of exposure to the pollutants which excluded in the present study the highly
499 vulnerable maternal period (Barouki et al., 2012b). However, one remarkable effect was the

500 alteration of the post-prandial plasma TG (in response to an oral lipid load) in ST-fed
501 pollutant-exposed males while glucose absorption was not affected. Post-prandial TG levels
502 result from intestinal TG secretion and clearance which depend on expression levels of
503 several binding proteins and apolipoproteins (Drover et al., 2005; Petit et al., 2007).
504 Interestingly, the significant increase of blood TG clearance in STp vs ST0 mice is consistent
505 with the decrease in the 6h-fasting plasma TG. In addition, the concomitant decrease in *Apoc3*
506 gene expression in the jejunum might suggest a higher efficiency of TG clearance by LPL
507 (Petit et al., 2007). The reduced plasma TG levels associated with the trend to decreased FFA
508 levels induced by the pollutants in a ST context were also indicative of changes in lipid
509 metabolism.

510 Regarding gene expression analysis of xenobiotic receptors, a trend for an elevation of *Nr1i3*
511 expression and a significant enhancement in *Cyp3a11* mRNA levels were shown in jejunum
512 of STp vs ST0 mice, but not in liver, again documenting that jejunum is highly responsive to
513 its environment. The meaning of the significant decrease of *Cyp2b10* expression in liver, an
514 enzyme which regulation is dependent on estrogens (Yamada et al., 2002) could be linked to
515 estrogen signaling impairment in response to pollutant exposure. Moreover, metabolic tissues
516 express estrogen and/or androgen receptors and there was a significant increase of the ratio of
517 *Esr1* to *Nr3c4* mRNA levels in scAT from STp vs ST0 mice, and a trend to a decrease of this
518 same ratio in liver, all indicative of changes in the estrogen signaling in these tissues.
519 Additionally, it is possible that the enhanced ratio of adiponectin to leptin that we observed in
520 the STp male mice and which suggests a better insulin sensitivity was linked to the estrogen
521 signaling changes in adipose tissues as adiponectin and leptin are synthesized by these tissues.
522 There were other features in addition to the effect of pollutants on plasma TG and on the ratio
523 of adiponectin to leptin which were the opposite of the effect induced by the HFHS diet, such

524 as the findings of reduced levels of *Il-1 β* mRNA levels in pgAT, *Apoc3* in jejunum, *Cyp2b10*
525 in liver as well as enhanced *Il10* expression in scAT and of *Cyp3a11* in jejunum, all from STp
526 vs ST0 mice. However, not all of the pollutant effects were opposite to those observed in
527 response to the HFHS diet. Interestingly, we observed effects mimicking HFHS-induced
528 metabolic disorders in the scAT. For example, there were enhanced expressions of genes
529 encoding markers of lipolysis and adipogenesis suggesting higher differentiation of
530 subcutaneous adipocytes. Collectively, and in line with a recently proposed hypothesis of an
531 estrogeno-mimetic effect of the pollutant mixture (Julien et al., 2018), our data could suggest
532 that the pollutant-induced changes of estrogen signaling may favor subcutaneous over visceral
533 fat as described in males fed the HFHS diet (this study) or injected with estradiol E2 (Clegg et
534 al., 2006), resulting in the “feminization” of the fat deposition. Whether, such “feminization”
535 will explain all of the changes described in STp fed mice will warrant further studies.

536 Another finding of the present study is that, according to the diet, the effects of the pollutants
537 were either to alleviate or to aggravate metabolic features. Indeed, exposure of HFHS-fed
538 mice to the pollutant mixture could aggravate some of the effects of the HFHS alone such as
539 the increased weight of the pgAT depot and the slight but not significant enhancement of
540 plasma leptin levels. There were also significant spotted impacts on gene expression in
541 jejunum and pgAT. Specifically, the increased (*Mttp*) or decreased (*Gper1* and *Slc27a4*)
542 expressions in jejunum were only observed in HFHS-fed pollutant exposed mice not in
543 absence of pollutant. As well, pollutants induced a strong significant increase of *Il10*
544 expression in jejunum in the HFHS context (the reverse effect being observed in ST-fed
545 pollutant exposed mice). In this HFHS context, pollutants were capable to decrease *Nr1c3* and
546 increase *Il1b* expression in pgAT only, thus amplifying the impact of the HFHS diet.

547 These data obtained from post-weaning studies induced metabolic changes somehow different
548 from the previous two-generation study in which male adult offspring showed reduced hepatic
549 cholesterol levels and alteration of cholesterol metabolism in response to the HFHS diet-
550 containing pollutants. If comparing the one- (this study) and two-generation (Naville et al.,
551 2013) studies on the various endpoints monitored, cholesterol metabolism does not appear to
552 be a major pollutant target in the present study apart from *Cyp7a1* enhancement.
553 Discrepancies do not seem to rely to the difference in pollutant dosage between the two
554 experimental protocols. Because we previously demonstrated that the low-dose pollutant
555 mixture could trigger metabolic disturbances leading to common and specific features as
556 compared to a HFHS diet (Labaronne et al., 2017), it may be considered that some effects of
557 the pollutants could be masked by the more pronounced effect of the HFHS diet in this study
558 regarding body weight and markers of insulin resistance (HOMA-IR, leptin levels) than in the
559 two-generation study (Naville et al., 2013). Thus, a more likely explanation for differences in
560 metabolic outcome in HFHS-fed pollutant exposed mice could be in line with the
561 Developmental Origin of Health and Disease (DOHaD) concept (Barker et al., 2002; Suzuki,
562 2018). This concept proposes that part of adult diseases finds their origins during fetal life
563 which is a highly vulnerable period of time specifically with regards to pollutant exposure
564 (Barker et al., 2002; Barouki et al., 2012b; Le Magueresse-Battistoni et al., 2016).

565 In conclusion, the present study underlines that the threat posed by a Western-style diet
566 relates to the fat and sugar content but that pollutants which contaminate food are also part of
567 the equation. In addition to evidences for metabolic deleterious effects of the HFHS diet in
568 line with obesity associated metabolic features and insulin resistance, we demonstrated, at
569 least at the mRNA expression level that metabolic tissues responded in a tissue-specific way
570 to the cocktail of pollutants and that the greatest metabolic impact of pollutants was evidenced
571 in standard nutritional conditions in this one-generation study. Importantly, jejunum was

572 highly sensitive with regards to xenobiotic receptors and lipolysis and AT depots responded
573 differently to both nutritional stressors. Moreover, the impact of pollutants was found to
574 mimic or oppose the HFHS effects in a manner depending on nutritional conditions and on
575 the metabolic tissue considered. Collectively and although pollutants in the mixture have not
576 been tested individually, we here reinforce previous observations (Le Magueresse-Battistoni
577 et al., 2017) that the resulting effect of a mixture of low dosed pollutants cannot be derived
578 from the expected effect of one chemical at a time based on its dosage and this is raising
579 important implications in terms of recommendations for food market security.

580

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588

589 **References**

- 590 WHO, 2018a. Obesity and overweight. <http://www.who.int/mediacentre/factsheets/fs311/en/>.
- 591 WHO, 2018b. The top 10 causes of death. [http://www.who.int/news-room/fact-sheets/detail/the-](http://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death)
- 592 [top-10-causes-of-death](http://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death).
- 593 WHO, 2017. Diabetes. <http://www.who.int/news-room/fact-sheets/detail/diabetes>.
- 594 WHO, 2016. Obesity and diabetes: the slow-motion disaster Keynote address at the 47th meeting of
- 595 the National Academy of Medicine. Chan M. [http://www.who.int/dg/speeches/2016/obesity-](http://www.who.int/dg/speeches/2016/obesity-diabetes-disaster/en/)
- 596 [diabetes-disaster/en/](http://www.who.int/dg/speeches/2016/obesity-diabetes-disaster/en/).
- 597 STARTIN, J.R., SHARMAN, M., ROSE, M.D., et al., 1987. Migration from plasticized films into foods. 1.
- 598 Migration of di-(2-ethylhexyl)adipate from PVC films during home-use and microwave cooking. Food
- 599 additives and contaminants 4, 385-398.
- 600 VANDENBERG, L.N., HAUSER, R., MARCUS, M., et al., 2007. Human exposure to bisphenol A (BPA).
- 601 *Reprod Toxicol* 24, 139-177.
- 602 KOCH, H.M., CALAFAT, A.M., 2009. Human body burdens of chemicals used in plastic manufacture.
- 603 *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 364, 2063-
- 604 2078.
- 605 MUNCKE, J., 2009. Exposure to endocrine disrupting compounds via the food chain: Is packaging a
- 606 relevant source? *The Science of the total environment* 407, 4549-4559.
- 607 SCHAFFER, K.S., KEGLEY, S.E., 2002. Persistent toxic chemicals in the US food supply. *Journal of*
- 608 *epidemiology and community health* 56, 813-817.
- 609 DORNE, J.L., 2010. Metabolism, variability and risk assessment. *Toxicology* 268, 156-164.
- 610 VANDENBERG, L.N., COLBORN, T., HAYES, T.B., et al., 2012. Hormones and endocrine-disrupting
- 611 chemicals: low-dose effects and nonmonotonic dose responses. *Endocrine reviews* 33, 378-455.
- 612 ZOELLER, R.T., BERGMAN, A., BECHER, G., et al., 2014. A path forward in the debate over health
- 613 impacts of endocrine disrupting chemicals. *Environ Health* 13, 118.
- 614 WHO/UNEP, 2013. State of the science of endocrine disrupting chemicals - 2012. An assessment of
- 615 the state of the science of endocrine disruptors prepared by a group of experts for the United
- 616 Nations Environment Programme (UNEP) and WHO. Åke Bergman, Jerrold J. Heindel, Susan Jobling,
- 617 Karen A. Kidd and R. Thomas Zoeller eds <http://www.who.int/ceh/publications/endocrine/en/>.
- 618 KORTENKAMP, A., 2014. Low dose mixture effects of endocrine disrupters and their implications for
- 619 regulatory thresholds in chemical risk assessment. *Current opinion in pharmacology* 19, 105-111.
- 620 SVINGEN, T., VINGGAARD, A.M., 2016. The risk of chemical cocktail effects and how to deal with the
- 621 issue. *Journal of epidemiology and community health* 70, 322-323.
- 622 LE MAGUERESSE-BATTISTONI, B., LABARONNE, E., VIDAL, H., et al., 2017. Endocrine disrupting
- 623 chemicals in mixture and obesity, diabetes and related metabolic disorders. *World J Biol Chem* 8,
- 624 108-119.
- 625 LE MAGUERESSE-BATTISTONI, B., VIDAL, H., NAVILLE, D., 2018a. Environmental Pollutants and
- 626 Metabolic Disorders: The Multi-Exposure Scenario of Life. *Frontiers in endocrinology* 9, 582.
- 627 NAVILLE, D., PINTEUR, C., VEGA, N., et al., 2013. Low-dose food contaminants trigger sex-specific,
- 628 hepatic metabolic changes in the progeny of obese mice. *FASEB journal : official publication of the*
- 629 *Federation of American Societies for Experimental Biology* 27, 3860-3870.
- 630 NAVILLE, D., LABARONNE, E., VEGA, N., et al., 2015. Metabolic outcome of female mice exposed to a
- 631 mixture of low-dose pollutants in a diet-induced obesity model. *PloS one* 10, e0124015.
- 632 LABARONNE, E., PINTEUR, C., VEGA, N., et al., 2017. Low-dose pollutant mixture triggers metabolic
- 633 disturbances in female mice leading to common and specific features as compared to a high-fat diet.
- 634 *The Journal of nutritional biochemistry* 45, 83-93.
- 635 JULIEN, B., PINTEUR, C., VEGA, N., et al., 2018. Evidence for estrogeno-mimetic effects of a mixture of
- 636 low-dose pollutants in a model of ovariectomized mice. *Environmental toxicology and pharmacology*
- 637 57, 34-40.
- 638 CASALS-CASAS, C., DESVERGNE, B., 2011. Endocrine disruptors: from endocrine to metabolic
- 639 disruption. *Annual review of physiology* 73, 135-162.

640 THAYER, K.A., HEINDEL, J.J., BUCHER, J.R., et al., 2012. Role of environmental chemicals in diabetes
641 and obesity: a National Toxicology Program workshop review. *Environmental health perspectives*
642 120, 779-789.

643 HEINDEL, J.J., BLUMBERG, B., CAVE, M., et al., 2017. Metabolism disrupting chemicals and metabolic
644 disorders. *Reprod Toxicol* 68, 3-33.

645 ALONSO-MAGDALENA, P., QUESADA, I., NADAL, A., 2011. Endocrine disruptors in the etiology of type
646 2 diabetes mellitus. *Nature reviews. Endocrinology* 7, 346-353.

647 VOM SAAL, F.S., NAGEL, S.C., COE, B.L., et al., 2012. The estrogenic endocrine disrupting chemical
648 bisphenol A (BPA) and obesity. *Molecular and cellular endocrinology* 354, 74-84.

649 BEAUSOLEIL, C., EMOND, C., CRAVEDI, J.P., et al., 2018. Regulatory identification of BPA as an
650 endocrine disruptor: Context and methodology. *Molecular and cellular endocrinology* 475, 4-9.

651 LE MAGUERESSE-BATTISTONI, B., MULTIGNER, L., BEAUSOLEIL, C., et al., 2018b. Effects of bisphenol
652 A on metabolism and evidences of a mode of action mediated through endocrine disruption.
653 *Molecular and cellular endocrinology* 475, 74-91.

654 SCHUG, T.T., JANESICK, A., BLUMBERG, B., et al., 2011. Endocrine disrupting chemicals and disease
655 susceptibility. *The Journal of steroid biochemistry and molecular biology* 127, 204-215.

656 DIAMANTI-KANDARAKIS, E., BOURGUIGNON, J.P., GIUDICE, L.C., et al., 2009. Endocrine-disrupting
657 chemicals: an Endocrine Society scientific statement. *Endocrine reviews* 30, 293-342.

658 PAVEK, P., 2016. Pregnane X Receptor (PXR)-Mediated Gene Repression and Cross-Talk of PXR with
659 Other Nuclear Receptors via Coactivator Interactions. *Frontiers in pharmacology* 7, 456.

660 ANGRISH, M.M., METS, B.D., JONES, A.D., et al., 2012. Dietary fat is a lipid source in 2,3,7,8-
661 tetrachlorodibenzo-rho-dioxin (TCDD)-elicited hepatic steatosis in C57BL/6 mice. *Toxicological*
662 *sciences : an official journal of the Society of Toxicology* 128, 377-386.

663 WAHLANG, B., FALKNER, K.C., GREGORY, B., et al., 2013. Polychlorinated biphenyl 153 is a diet-
664 dependent obesogen that worsens nonalcoholic fatty liver disease in male C57BL6/J mice. *The*
665 *Journal of nutritional biochemistry* 24, 1587-1595.

666 LE MAGUERESSE-BATTISTONI, B., VIDAL, H., NAVILLE, D., 2018c. Sex-specific metabolic alterations
667 induced by environmental pollutants. *Current Opinion in Toxicology* 8 1-7
668 <http://www.hal.inserm.fr/inserm-01743795>.

669 VAN LEEUWEN, F.X., FEELEY, M., SCHRENK, D., et al., 2000. Dioxins: WHO's tolerable daily intake
670 (TDI) revisited. *Chemosphere* 40, 1095-1101.

671 WHO, 2003. Polychlorinated biphenyls: human health aspects. *Concise International Chemical*
672 *Assesment Document 55*. World Health Organization, Geneva, Switzerland.

673 EUROPEAN FOOD SAFETY AUTHORITY PANEL ON FOOD ADDITIVES, F., PROCESSING AIDS AND
674 MATERIALS IN CONTACT WITH FOOD, 2005. Opinion of the scientific panel on food additives,
675 flavourings, processing aids and materials in contact with food (AFC) related to bis(2-
676 ethylhexyl)phthalate (DEHP) for use in food contact materials. Question N° EFSA-Q-2003-191. *EFSA J*
677 243, 1-20.

678 GOUT, J., SARAFIAN, D., MUTEL, E., et al., 2010. Metabolic and melanocortin gene expression
679 alterations in male offspring of obese mice. *Molecular and cellular endocrinology* 319, 99-108.

680 CONARELLO, S.L., JIANG, G., MU, J., et al., 2007. Glucagon receptor knockout mice are resistant to
681 diet-induced obesity and streptozotocin-mediated beta cell loss and hyperglycaemia. *Diabetologia*
682 50, 142-150.

683 NAVILLE, D., REBOURCET, D., CHAUVIN, M.A., et al., 2011. Direct and indirect impact of 2,3,7,8-
684 tetrachlorodibenzo-p-dioxin (TCDD) on adult mouse Leydig cells: an in vitro study. *Toxicology letters*
685 207, 251-257.

686 MIRZA, S., QU, H.Q., LI, Q., et al., 2011. Adiponectin/leptin ratio and metabolic syndrome in a
687 Mexican American population. *Clinical and investigative medicine. Medecine clinique et*
688 *experimentale* 34, E290.

689 LOPEZ-JARAMILLO, P., GOMEZ-ARBELAEZ, D., LOPEZ-LOPEZ, J., et al., 2014. The role of
690 leptin/adiponectin ratio in metabolic syndrome and diabetes. *Hormone molecular biology and*
691 *clinical investigation* 18, 37-45.

692 TILG, H., MOSCHEN, A.R., 2008. Insulin resistance, inflammation, and non-alcoholic fatty liver
693 disease. *Trends in endocrinology and metabolism: TEM* 19, 371-379.

694 CHEN, Z., YU, R., XIONG, Y., et al., 2017. A vicious circle between insulin resistance and inflammation
695 in nonalcoholic fatty liver disease. *Lipids in health and disease* 16, 203.

696 GRECO, D., KOTRONEN, A., WESTERBACKA, J., et al., 2008. Gene expression in human NAFLD.
697 *American journal of physiology. Gastrointestinal and liver physiology* 294, G1281-1287.

698 RODER, P.V., GEILLINGER, K.E., ZIETEK, T.S., et al., 2014. The role of SGLT1 and GLUT2 in intestinal
699 glucose transport and sensing. *PLoS one* 9, e89977.

700 OUCHI, N., PARKER, J.L., LUGUS, J.J., et al., 2011. Adipokines in inflammation and metabolic disease.
701 *Nature reviews. Immunology* 11, 85-97.

702 REBOURCET, D., ODET, F., VEROT, A., et al., 2010. The effects of an in utero exposure to 2,3,7,8-
703 tetrachloro-dibenzo-p-dioxin on male reproductive function: identification of Ccl5 as a potential
704 marker. *International journal of andrology* 33, 413-424.

705 KIM, M.J., PELLOUX, V., GUYOT, E., et al., 2012. Inflammatory pathway genes belong to major targets
706 of persistent organic pollutants in adipose cells. *Environmental health perspectives* 120, 508-514.

707 MAGRE, S., REBOURCET, D., ISHAQ, M., et al., 2012. Gender differences in transcriptional signature of
708 developing rat testes and ovaries following embryonic exposure to 2,3,7,8-TCDD. *PLoS one* 7, e40306.

709 LA MERRILL, M., EMOND, C., KIM, M.J., et al., 2013. Toxicological function of adipose tissue: focus on
710 persistent organic pollutants. *Environmental health perspectives* 121, 162-169.

711 ALEKSUNES, L.M., KLAASSEN, C.D., 2012. Coordinated regulation of hepatic phase I and II drug-
712 metabolizing genes and transporters using AhR-, CAR-, PXR-, PPARalpha-, and Nrf2-null mice. *Drug*
713 *metabolism and disposition: the biological fate of chemicals* 40, 1366-1379.

714 BAROUKI, R., AGGERBECK, M., AGGERBECK, L., et al., 2012a. The aryl hydrocarbon receptor system.
715 *Drug metabolism and drug interactions* 27, 3-8.

716 FU, Z.D., SELWYN, F.P., CUI, J.Y., et al., 2016. RNA Sequencing Quantification of Xenobiotic-Processing
717 Genes in Various Sections of the Intestine in Comparison to the Liver of Male Mice. *Drug metabolism*
718 *and disposition: the biological fate of chemicals* 44, 842-856.

719 FROMENTY, B., 2013. Drug-induced liver injury in obesity. *Journal of hepatology* 58, 824-826.

720 NING, M., JEONG, H., 2017. High-Fat Diet Feeding Alters Expression of Hepatic Drug-Metabolizing
721 Enzymes in Mice. *Drug metabolism and disposition: the biological fate of chemicals* 45, 707-711.

722 MAUVAIS-JARVIS, F., 2011. Estrogen and androgen receptors: regulators of fuel homeostasis and
723 emerging targets for diabetes and obesity. *Trends in endocrinology and metabolism: TEM* 22, 24-33.

724 GAO, H., FALT, S., SANDELIN, A., et al., 2008. Genome-wide identification of estrogen receptor alpha-
725 binding sites in mouse liver. *Molecular endocrinology (Baltimore, Md)* 22, 10-22.

726 LIN, H.Y., YU, I.C., WANG, R.S., et al., 2008. Increased hepatic steatosis and insulin resistance in mice
727 lacking hepatic androgen receptor. *Hepatology (Baltimore, Md)* 47, 1924-1935.

728 SHARMA, G., MAUVAIS-JARVIS, F., PROSSNITZ, E.R., 2018. Roles of G protein-coupled estrogen
729 receptor GPER in metabolic regulation. *The Journal of steroid biochemistry and molecular biology*
730 176, 31-37.

731 GORE, A.C., CHAPPELL, V.A., FENTON, S.E., et al., 2015. EDC-2: The Endocrine Society's Second
732 Scientific Statement on Endocrine-Disrupting Chemicals. *Endocrine reviews* 36, E1-E150.

733 WEI, J., SUN, X., CHEN, Y., et al., 2014. Perinatal exposure to bisphenol A exacerbates nonalcoholic
734 steatohepatitis-like phenotype in male rat offspring fed on a high-fat diet. *The Journal of*
735 *endocrinology* 222, 313-325.

736 DUVAL, C., TEIXEIRA-CLERC, F., LEBLANC, A.F., et al., 2017. Chronic Exposure to Low Doses of Dioxin
737 Promotes Liver Fibrosis Development in the C57BL/6J Diet-Induced Obesity Mouse Model.
738 *Environmental health perspectives* 125, 428-436.

739 BAROUKI, R., GLUCKMAN, P.D., GRANDJEAN, P., et al., 2012b. Developmental origins of non-
740 communicable disease: implications for research and public health. *Environ Health* 11, 42.

741 DROVER, V.A., AJMAL, M., NASSIR, F., et al., 2005. CD36 deficiency impairs intestinal lipid secretion
742 and clearance of chylomicrons from the blood. *The Journal of clinical investigation* 115, 1290-1297.

743 PETIT, V., ARNOULD, L., MARTIN, P., et al., 2007. Chronic high-fat diet affects intestinal fat absorption
744 and postprandial triglyceride levels in the mouse. *Journal of lipid research* 48, 278-287.
745 YAMADA, H., GOHYAMA, N., HONDA, S., et al., 2002. Estrogen-dependent regulation of the
746 expression of hepatic Cyp2b and 3a isoforms: assessment using aromatase-deficient mice. *Toxicology*
747 *and applied pharmacology* 180, 1-10.
748 CLEGG, D.J., BROWN, L.M., WOODS, S.C., et al., 2006. Gonadal hormones determine sensitivity to
749 central leptin and insulin. *Diabetes* 55, 978-987.
750 BARKER, D.J., ERIKSSON, J.G., FORSEN, T., et al., 2002. Fetal origins of adult disease: strength of
751 effects and biological basis. *International journal of epidemiology* 31, 1235-1239.
752 SUZUKI, K., 2018. The developing world of DOHaD. *Journal of developmental origins of health and*
753 *disease* 9, 266-269.
754 LE MAGUERESSE-BATTISTONI, B., VIDAL, H., NAVILLE, D., 2016. [Impact of in utero exposure to
755 pollutants on metabolic health in adults]. *Medecine sciences : M/S* 32, 51-56.

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776 **Figure legends**

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779 **Figure 1:** (A) Body weight measured at the end of the protocol (after 6 h of fasting). (B)
780 Weight of perigonadal and subcutaneous adipose tissues (pgAT and scAT) at the end of the
781 protocol. (C) Tests performed after 12 wks of diet (Oral lipid load expressed as differences of
782 triglyceride levels from time 0 and corresponding AUC) (D-F) Biochemical parameters:
783 Blood glucose and plasma insulin (D); adponectin/leptin ratio (E); plasma and hepatic
784 Triglycerides; plasma Free Fatty Acids (FFA) (F). Values are means \pm SEM with n=8.
785 ***P<0.001; ** P<0.01; *P<0.05

786 **Figure 2:** Effect of the HFHS diet and the mixture of pollutants on the hepatic expression of
787 genes related to Fat uptake (A), Lipoprotein lipase (B), lipogenesis (C), lipid oxidation (D),
788 cholesterol homeostasis (E) and inflammation (F). Values are means \pm SEM with n=8.
789 ***P<0.001; ** P<0.01; *P<0.05

790 **Figure 3:** Effect of the HFHS diet and the mixture of pollutants on the jejunum expression of
791 genes related to Fatty Acid absorption (A), cholesterol metabolism (B), lipoprotein
792 biosynthesis (C), and encoding LPL inhibitors (D) or inflammatory markers (E). Values are
793 means \pm SEM with n=8. ***P<0.001; ** P<0.01; *P<0.05

794 **Figure 4:** Effect of the HFHS diet and the mixture of pollutants on the scAT expression of
795 genes related to adipocyte differentiation (A), triglyceride metabolism (B) or encoding
796 inflammatory markers (C). Values are means \pm SEM with n=8. ***P<0.001; ** P<0.01;
797 *P<0.05

798 **Figure 5:** Effect of the HFHS diet and the mixture of pollutants on the pgAT expression of
799 genes related to adipocyte differentiation (A), triglyceride metabolism (B) or encoding

800 inflammatory markers (C). Values are means \pm SEM with n=8. ***P<0.001; ** P<0.01;
801 *P<0.05

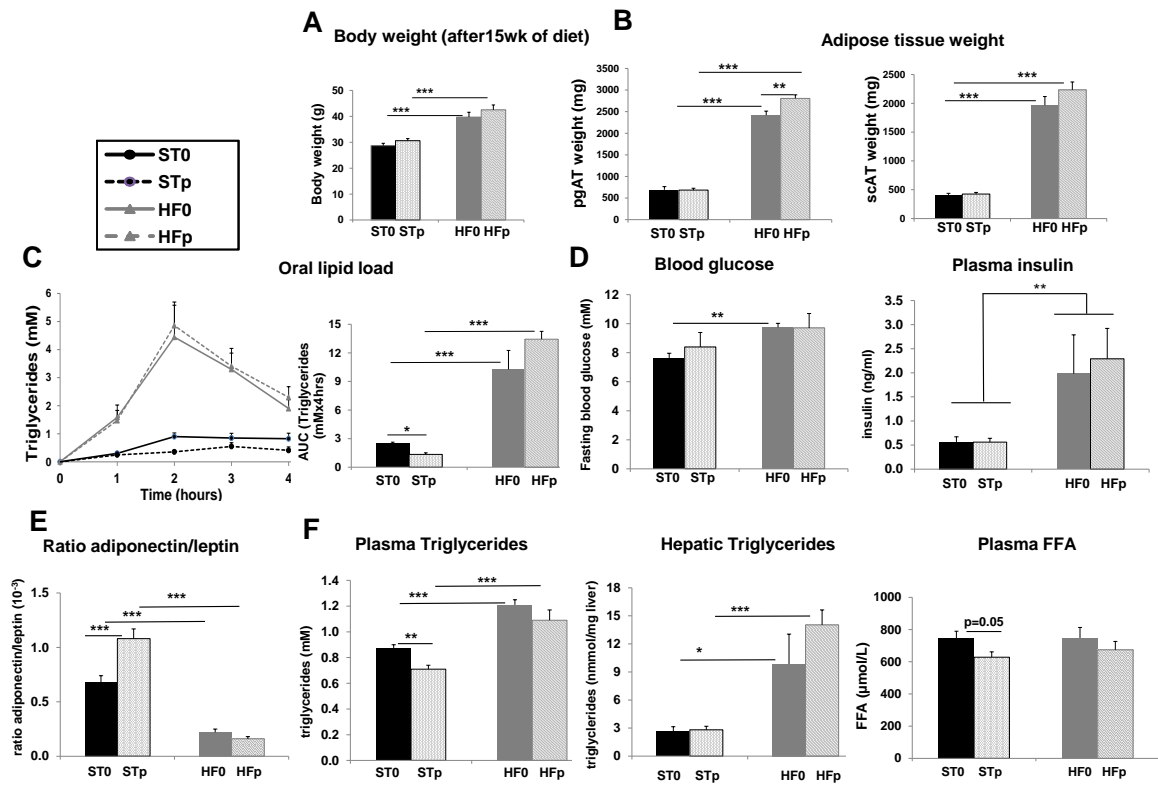
802 **Figure 6:** Effect of the HFHS diet and the mixture of pollutants on the expression of genes
803 encoding xenobiotic receptors. Ahr and Cyp1a2 mRNA expression in liver; Ahr mRNA
804 expression in jejunum, scAT and pgAT (A). Nr1i2 and Nr1i3 mRNA expression and their
805 respective target gene in liver (B) and jejunum (C). Values are means \pm SEM with n=8.
806 ***P<0.001; *P<0.05

807 **Figure 7:** Effect of the HFHS diet and the mixture of pollutants on the expression of genes
808 encoding estrogen and androgen receptors in liver (A), jejunum (B), scAT (C) and pg AT (D).
809 Values are means \pm SEM with n=8. ***P<0.001; ** P<0.01; *P<0.05

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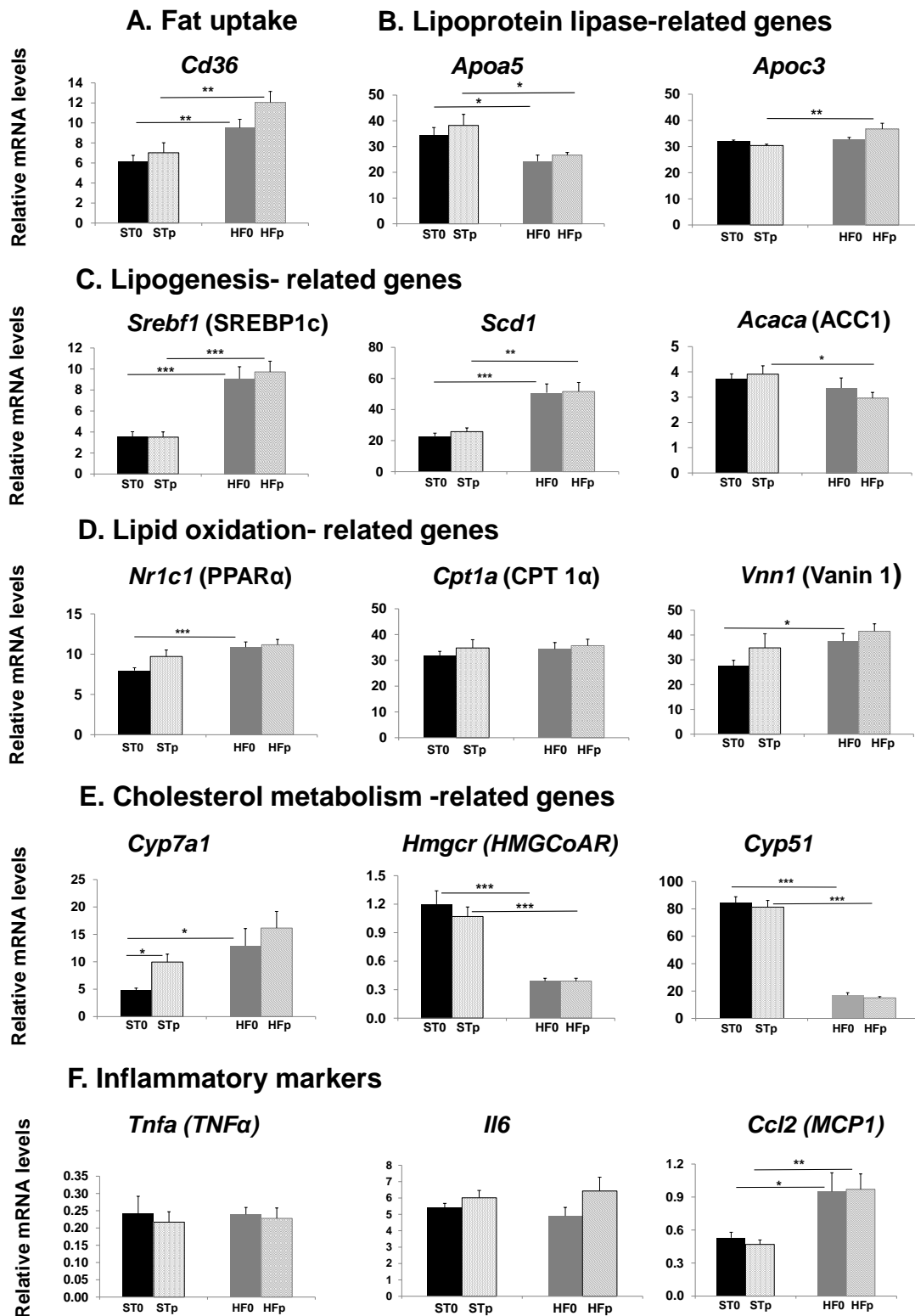
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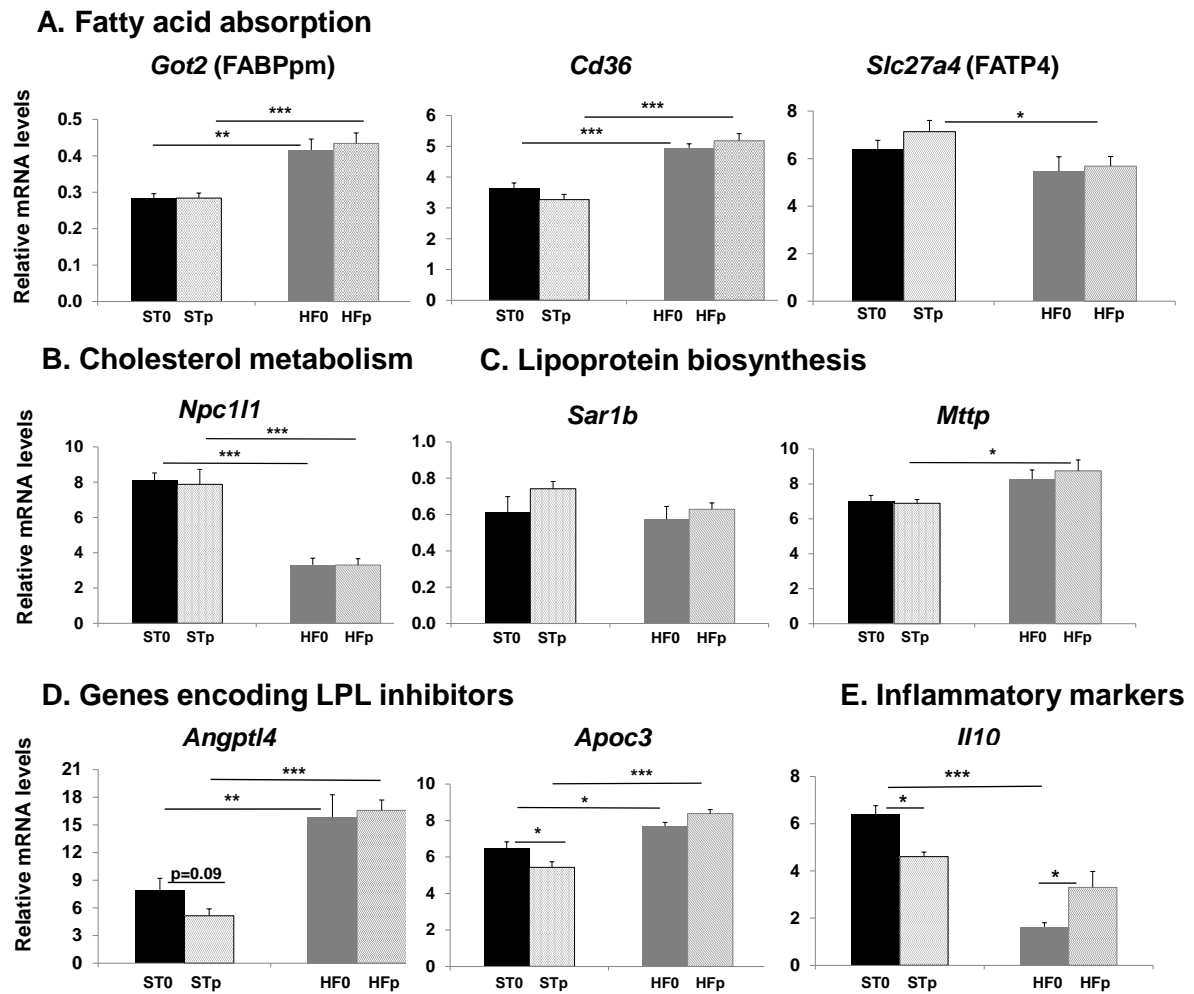


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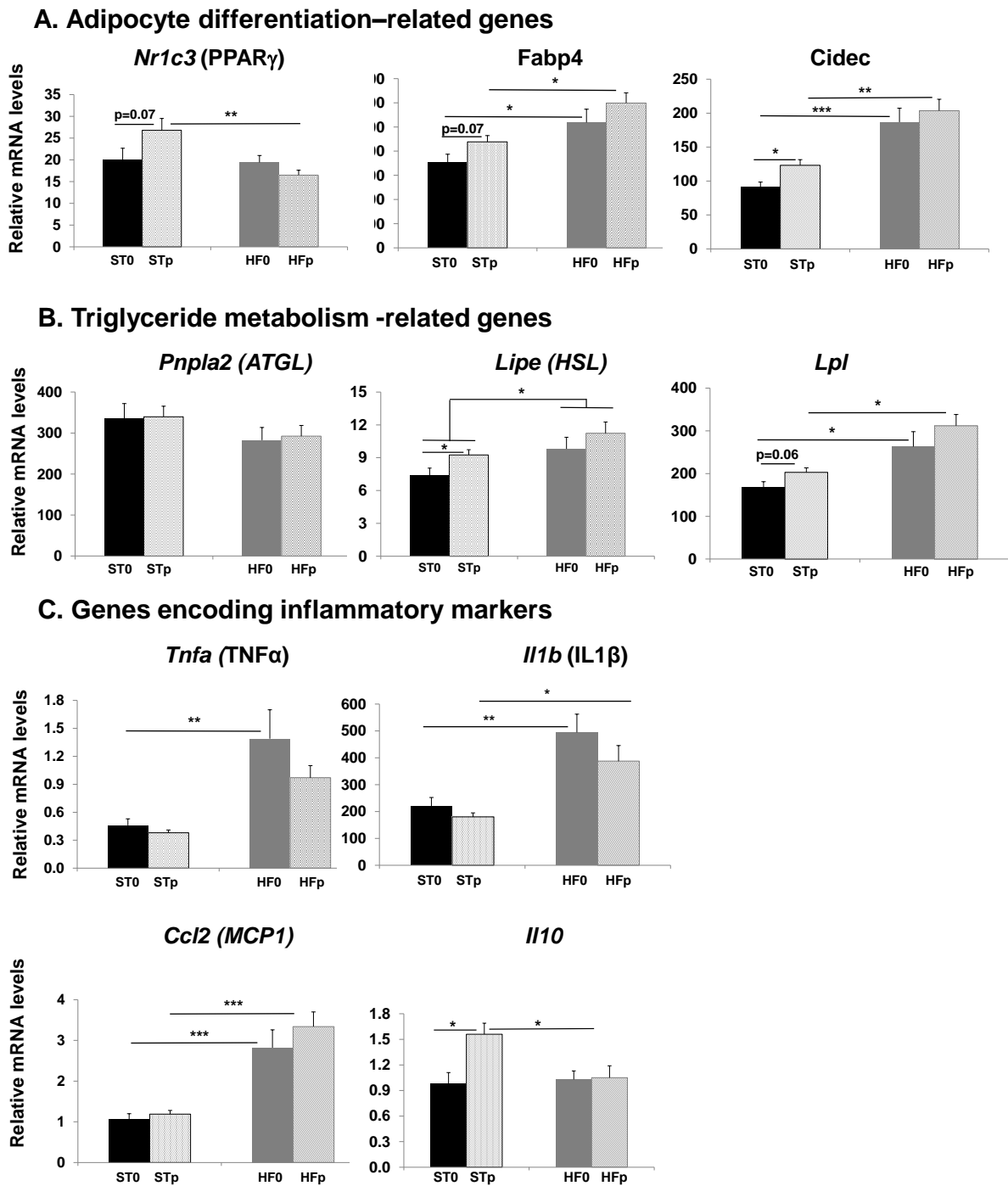
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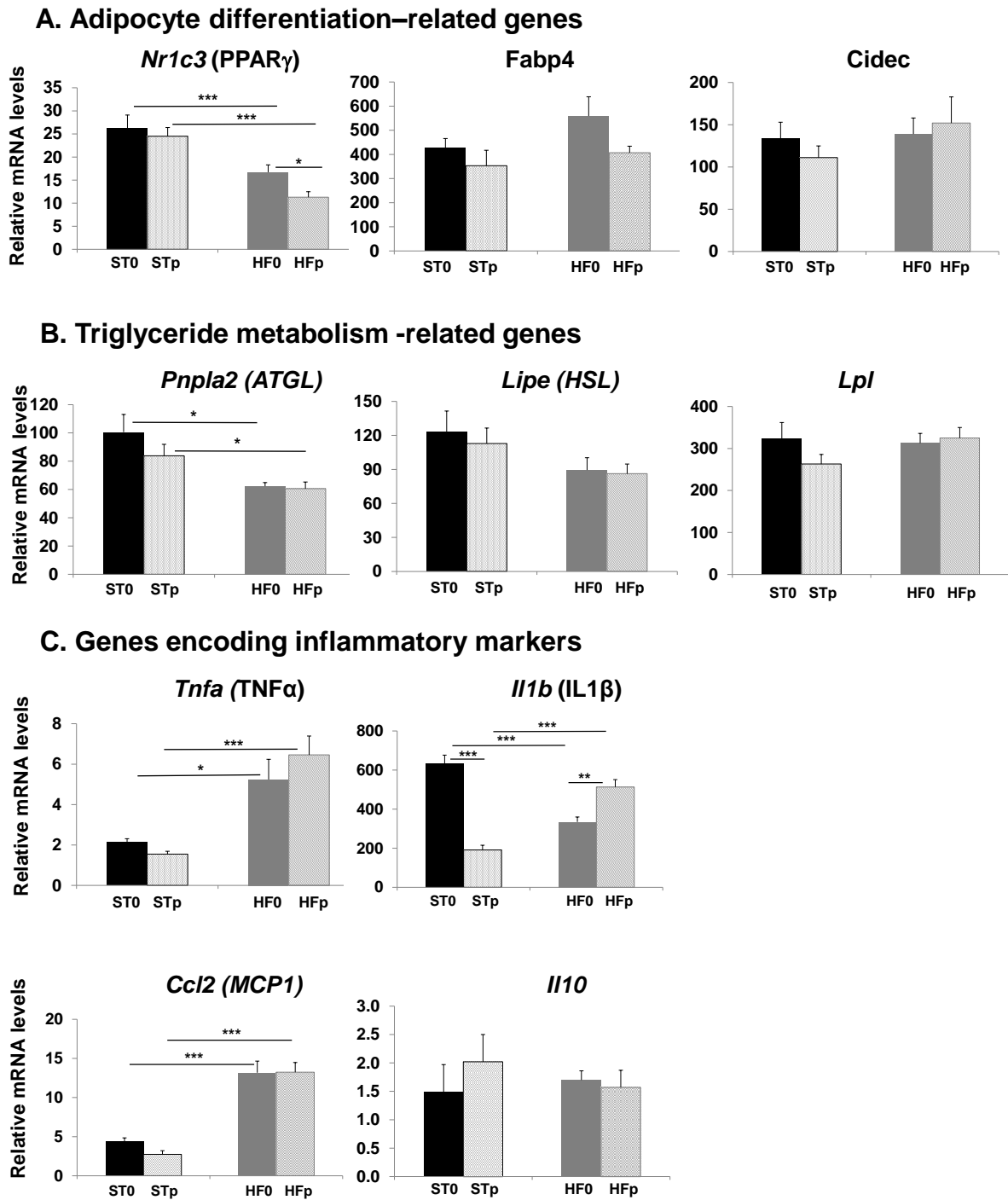
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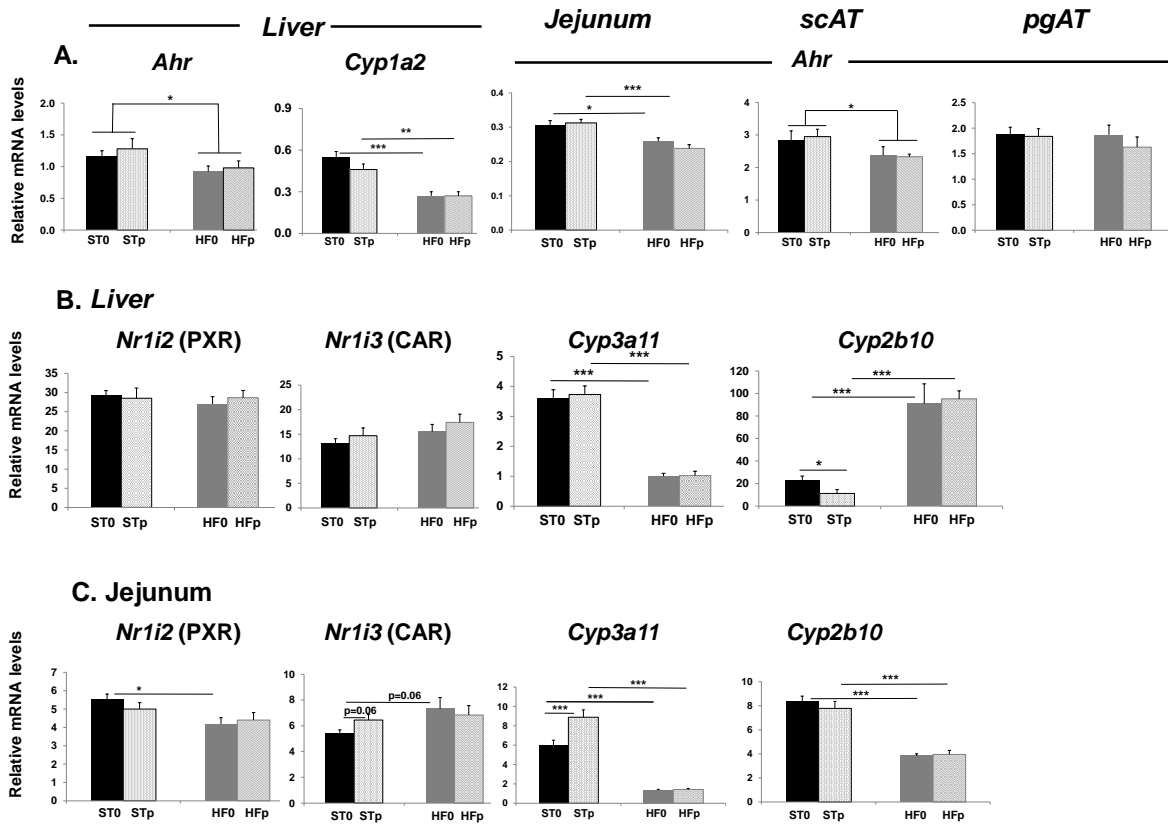
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861 Figure 6



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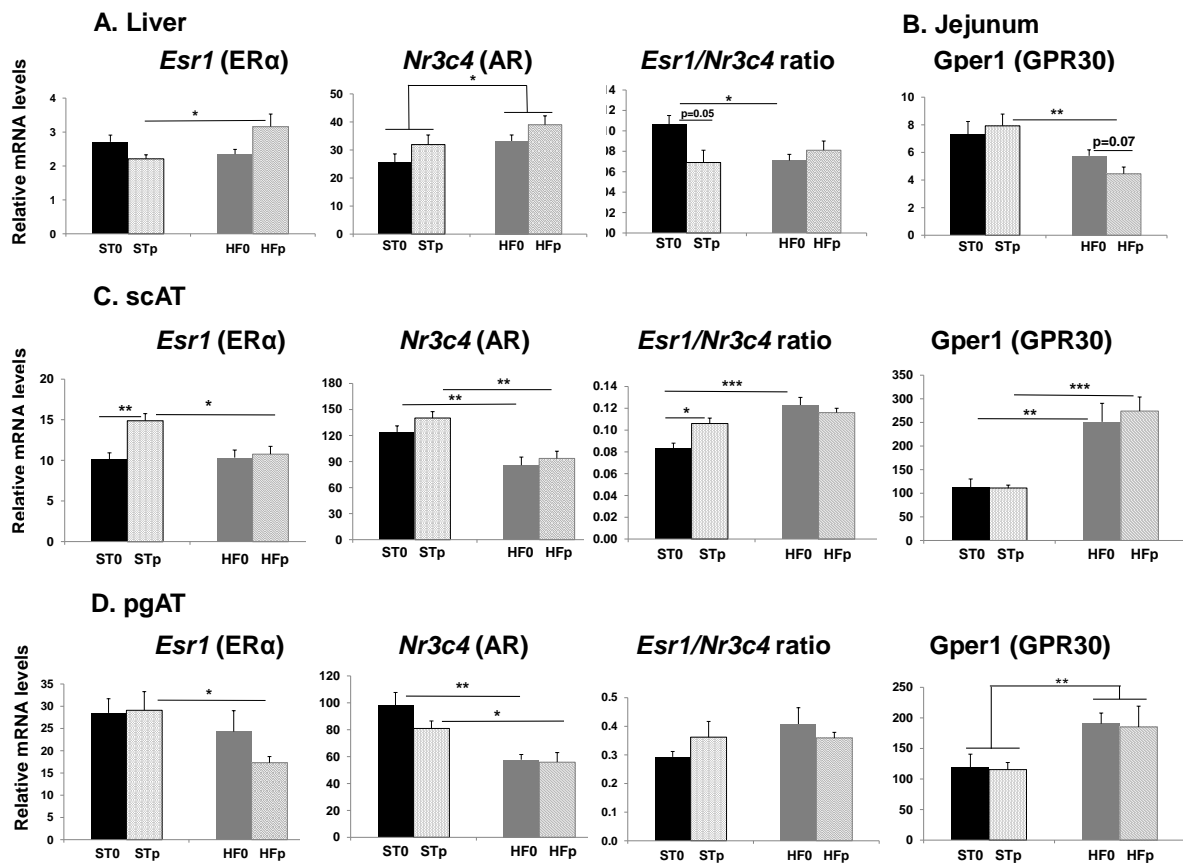
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878 **Supplementary Tables**

879 **Supplementary Table 1: References doses of the pollutants present in the**

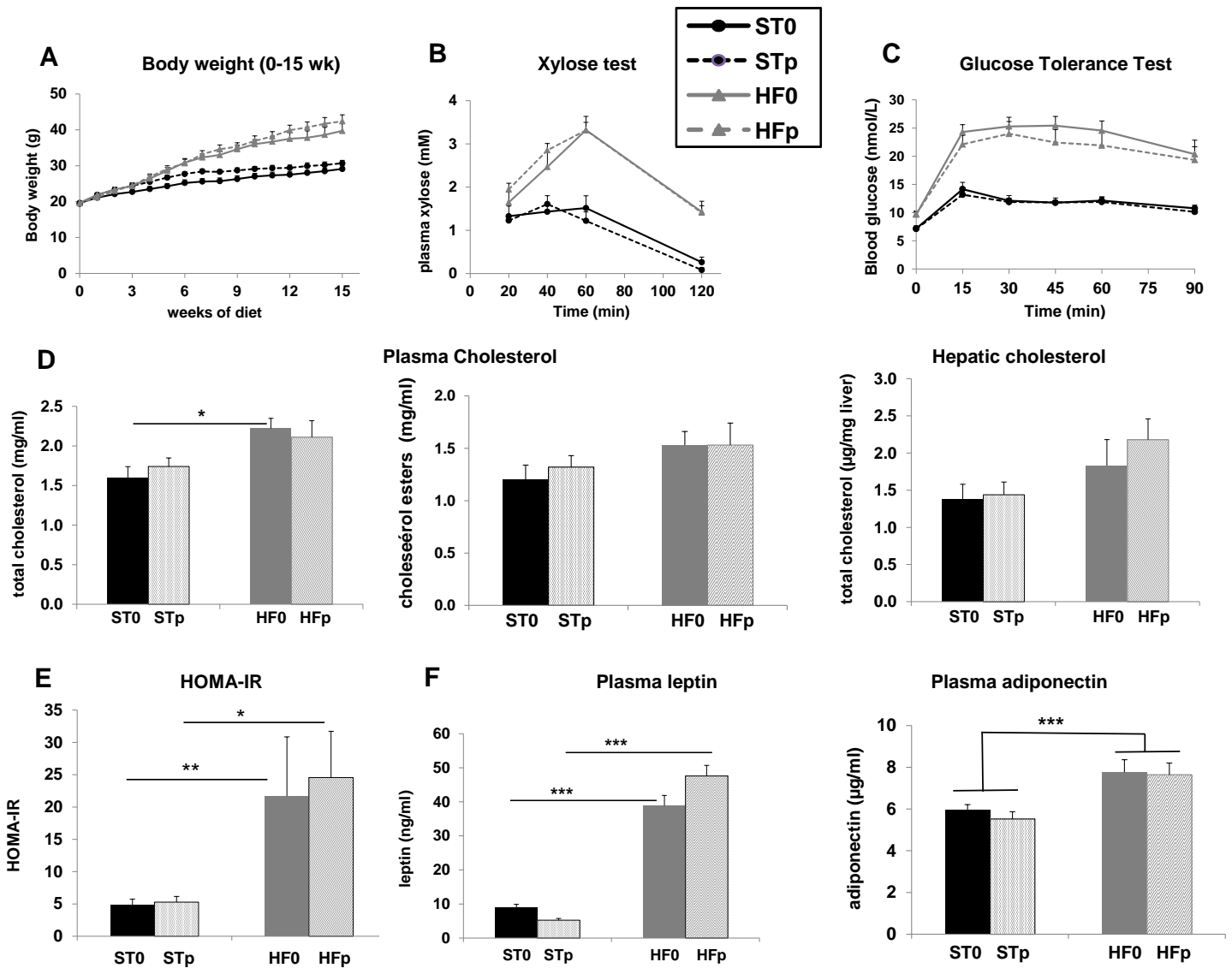
880 mixture and doses used in the diets (all per kg and per day).

Pollutants in the mixture	PCB153	BPA	TCDD	DEHP
NOAEL	40µg	5mg	2 ng	5mg
TDI	20ng	4µg	1-4pg	50µg
doses used	200ng	40µg	20pg	500µg

886 **Supplementary Table 2: List of the primers used in RT-qPCR**

gene	reference	sense	antisense
<i>Abca1</i>	NM_013454	CAG-GAG-GTG-ATG-TTT-CTG-ACC-A	TTG-GCT-GTT-CTC-CAT-GAA-GGT-C
<i>Abcg5</i>	NM_031884	AGC-CTC-GCT-CTG-AGC-TCT-TC	TTC-AGG-ACA-GGG-GTA-ACC-AC
<i>Abcg8</i>	NM_026180;NM_001286005	AGC-CTC-GCT-CTG-ACA-TCT-TC	GTC-AAG-TCC-ACG-TAG-AAG-TC
<i>Acaca</i>	NM_022193	GAG-CAA-GGG-ATA-AGT-TTG-AG	AGG-TGC-ATC-TTG-TGA-TTA-GC
<i>Ahr</i>	NM_013464.4	TCA-TCT-GGT-TTC-CTG-GCA-ATG-AAT	ATA-AGC-TGC-CCT-TTG-GCA-TC
<i>Angptl4</i>	NM_020581	GA-CCT-TAA-CTG-TGC-CAA-GAG	AAG-TCA-CTG-TCT-TCT-TAG-GC
<i>Apoa5</i>	NM_080434	AGA-GCC-TCT-GGG-ACT-ACT-TC	GTC-CCA-GCT-TTT-CTA-GGT-AA
<i>Apoa3</i>	NM_001289755;NM_001289756;NM_023114;NM_001289833	GCT-AAG-TAG-CGT-GCA-GGA-GT	TCC-TCA-GGG-TTA-GAA-TCC-CA
<i>Ccl2</i>	NM_011333	TGG-AGC-ATC-CAC-GTG-TTG-GC	ACT-ACA-GCT-TCT-TTG-GGA-CA
<i>Cd36</i>	NM_001159558.1	AAG-ATC-CAA-AAC-TGT-CTG-TA	GTC-CTG-GCT-GTG-TTT-GGA-GG
<i>Cidec</i>	NM_178373;NM_001301295	TGG-CAC-AAT-CGT-GGA-GAC-AG	AGA-GGG-TTG-CCT-TCA-CGT-TC
<i>Cpt1a</i>	NM_001876;NM_001031847	TAC-AAC-AGG-TGG-TTT-GAC-A	GGC-TGT-CAA-TGG-ACA-TGA-CG
<i>Cyp1a2</i>	NM_009993	TCA-TCC-TGG-AGA-TCT-ACC-GA	TGA-CCT-GCC-ACT-GGT-TTA-TG
<i>Cyp51</i>	NM_020010	AGT-GCC-TGG-ATG-GGC-TTC-TT	GGA-GGC-CTC-AGT-CTT-AGT-GT
<i>Cyp3a11</i>	NM_007818	ACG-CCT-CTC-CTT-GCT-GTC-ACC	TTG-CCT-TCT-GCC-TCA-AGT-AG
<i>Cyp7a1</i>	NM_007824.2	TAC-AGA-GTG-CTG-GCC-AAG-AG	AGT-GAA-GTC-CTC-CTT-AGC-TG
<i>Cyp2b10</i>	NM_009999	TTC-TGC-CCT-TCT-CAA-CAG-GA	CCT-TAG-GAG-CAA-CAT-GGC-TT
<i>Esr1</i>	NM_000125;NM_001122740;NM_001122741;NM_001122742	TGT-TTG-CTC-CTA-ACT-TGC-TC	CCT-TCT-CTT-CCA-GAG-ACT-TC
<i>Fabp1</i>	NM_017399	TGC-GAA-CTG-GAG-ACC-ATG-AC	CCA-ATG-TCA-TGG-TAT-TGG-TG
<i>Fabp4</i>	NM_024406	CAG-AAG-TGG-GAT-GGA-AAG-TGG	CGA-CTG-ACT-ATT-GTA-GTG-TTT-GA
<i>Got2</i>	NM_010325	ATG-TGC-AAG-GAT-GCC-TGG	TTC-ATC-CGC-ATC-TTT-GCA-GAC-C
<i>Gper1</i>	NM_029771	AGC-TGA-TCA-GAT-CTA-GGG-AG	GTC-CTG-GGA-GCC-TGT-TAG-TC
<i>Gusb</i>	NM_010368	CTT-CAT-GAC-GAA-CCA-GTC-AC	GCA-ATC-CTC-CAG-TAT-CTC-TC
<i>Hmgcr</i>	NM_008255	CCG-GCC-TGT-GTG-TCG-CTG-GT	CCA-GCC-ACT-ATG-AGC-GTG-AA
<i>Hprt</i>	NM_013556	TTG-CTG-ACC-TGC-TGG-ATT-AC	AGT-TGAG-AGA-TCA-TCT-CCA-C
<i>Il1b</i>	NM_008361	ACT-GTT-CCT-GAA-CTC-AAC-TG	CTT-GTT-GAT-GTG-CTG-CTG-CG
<i>Il6</i>	NM_031168	AGT-TGC-CTT-CTT-GGG-ACT-GAT	TCC-ACG-ATT-TCC-CAG-AGA-AC
<i>Il10</i>	NM_010548	CAG-GGC-CCT-TTG-CTA-TGG-TG	CGG-CTG-GGG-GAT-GAC-AGT-AG
<i>Lipe</i>	NM_001039507;NM_010719	GTG-TGT-CAG-TGC-CTA-TTC-AG	GTC-AGC-TTC-TTC-AAG-GTA-TC
<i>Lpl</i>	NM_008509	GGT-CGA-AGC-ATT-GGA-ATC-CAG	TAG-GGC-ATC-TGA-GAA-CGA-GTC
<i>Mttp</i>	NM_008642;NM_001163457	GGA-GAA-GTA-ACC-TGA-ACA-TC	ACA-GGT-CTG-AGC-TGA-ACA-TC
<i>Npc1l1</i>	NM_207242	GCT-AGC-AGC-CAA-CAT-CAC-AG	CAG-TAG-GAG-GTA-GCA-GAC-CA
<i>Nr1c1</i>	NM_011144; NM_001113418.1	AAG-GGC-TTC-TTT-CGG-CGA-AC	GTT-CAT-GTT-GAA-GTT-CTT-CAG
<i>Nr1c3</i>	NM_001127330;NM_011146	TCT-CTC-CGT-AAT-GGA-AGA-CC	GCA-TTA-TGA-GAC-ATC-CCC-AC
<i>Nr3c4</i>	NM_013476.3	AGA-AGA-CCT-GCC-TGA-TCT-G	GCT-GGC-ACA-TAG-ATA-CTT-CTG
<i>Nr1h3</i>	NM_013839	CCG-GGA-AGA-CTT-TGC-CAA-AGC	GGA-GCT-GGT-CCT-GCA-CGT-TG
<i>Nr1i2</i>	NM_010936.3	AGG-AGG-AGT-ATG-TGC-TGA-TG	CTT-CAG-GAA-CAG-GAA-CCT-GTG
<i>Nr1i3</i>	NM_001243063;NM_001243062;NM_009803	GTC-CCA-TCT-GTC-CGT-TTG-C	AGG-GCT-TCT-GAC-AGT-ATC
<i>Pnpla2</i>	NM_025802;NM_001163689	TCA-TC-CAG-GCC-AAT-CTC-TGC	TGG-ATG-TTG-GTG-GAG-CTG-TC
<i>Sar1b</i>	NM_025535	ATT-GCT-GGC-ATG-ACG-TTT-AC	TGC-CAT-TGA-TAG-CAG-GAA-GG
<i>Scd1</i>	NM_009127	GAT-ACA-CTC-TGG-TGC-TCA-AC	AAC-GTG-GTG-AAG-TTG-ATG-TG
<i>Slc2a2</i>	NM_031197	TGG-GTA-CTC-TTC-ACC-AAC-TG	AGG-ATG-TGC-CAA-TGA-TCC-TG
<i>Slc5a1</i>	NM_019810	GCT-CCT-TGA-CCT-CCA-TCT-TC	CAG-GCA-ATG-CTG-ATG-CCA-AT
<i>Slc27a4</i>	NM_011989	GAC-ACC-TCA-AAG-GCA-CGA-GC	GAG-GG-TCC-AGA-TGC-TCT-GTG
<i>Sreb1f1</i>	NM_011480	ACG-GAG-CCA-TGG-ATT-GCA-CA	AAG-GGT-GCA-GGT-GTC-ACC-TT
<i>Tnfa</i>	NM_013693	CCA-GAG-CCT-CAC-ACT-CAG-ATC	CAC-TTG-GTG-GTT-TGC-TAC-GAC
<i>Tbp</i>	NM_013684	TGG-TGT-GCA-CAG-GAG-CCA-AG	TTC-ACA-TCA-CAG-CTC-CCC-AC
<i>Ugt1a1</i>	NM_201645	GCA-TCT-ATC-TGG-CTG-ATG-AG	CAG-AGG-CGT-TGA-CAT-AGG
<i>Vnn1</i>	NM_011704	CTG-AAG-TGT-TGC-TGA-GTG-AG	GAT-GCC-CAG-TCC-TTC-CCA-TA

903 **Supplementary Figures**



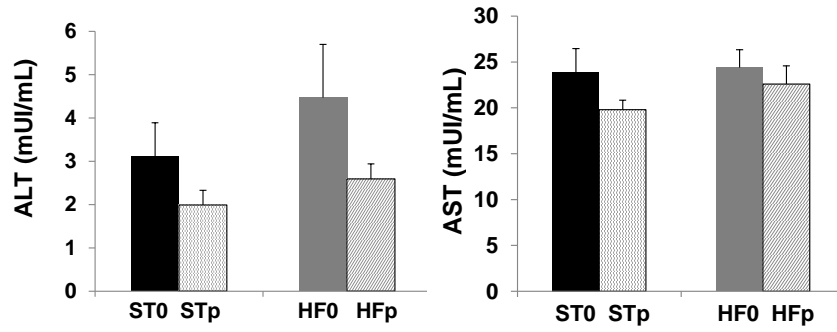
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905 **Supplementary Figure 1:** (A) Body weight measured along the 15 weeks of diet (B/C) Tests
 906 performed after 13 wks of diet (Xylose absorption test) and after 14 wks of diet (Glucose
 907 tolerance test) (D) Plasma total cholesterol and cholesteryl esters and hepatic total cholesterol
 908 (E) HOMA-IR calculated from blood glucose and plasma insulin measurements.

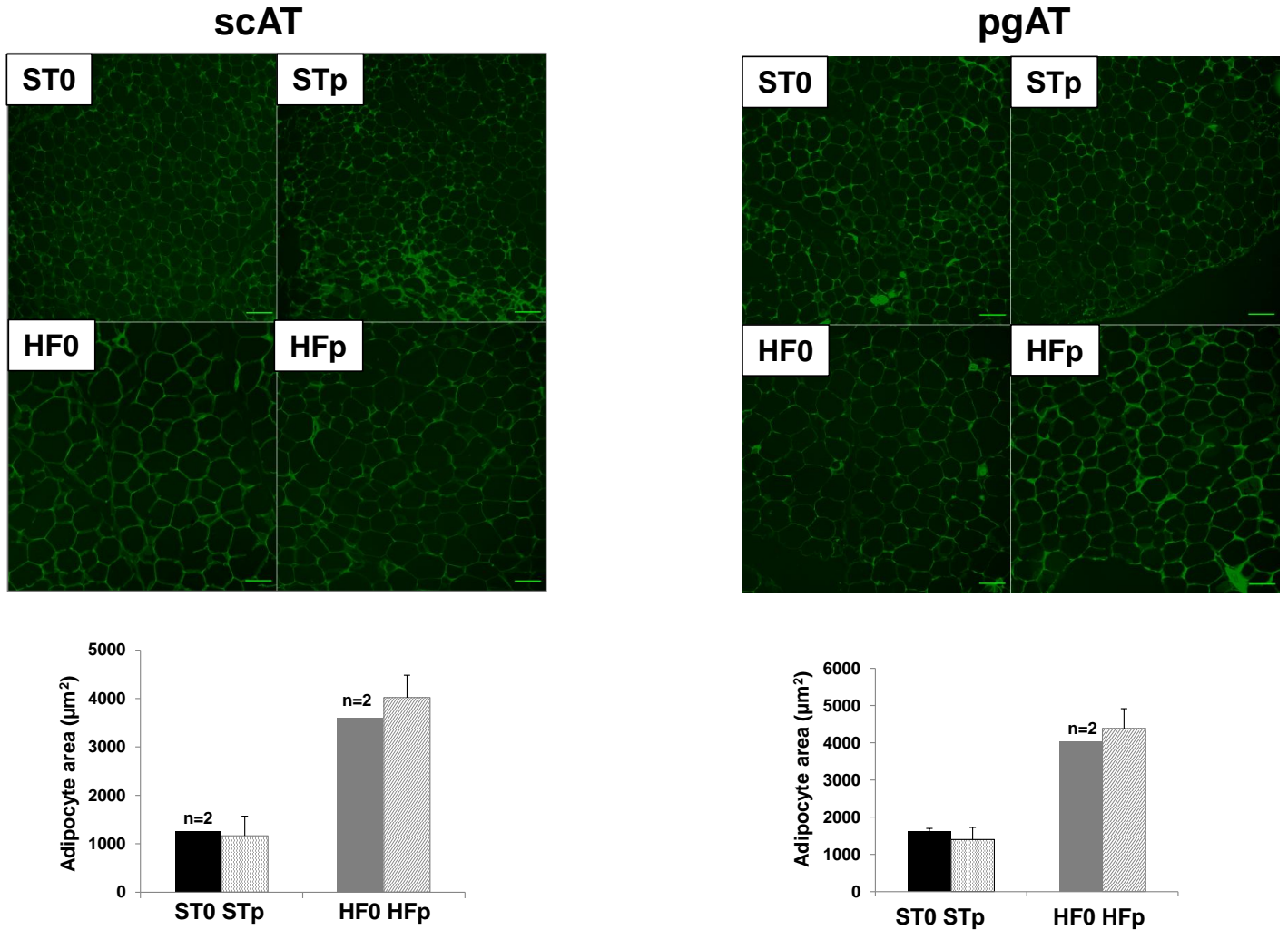
909 Values are means ± SEM with n=8. ***P<0.001; ** P<0.01; *P<0.05

910 (F) Plasma measurement of leptin and adiponectin by ELISA. Results are expressed as means
 911 ± SEM. Significant differences were assessed after One-way ANOVA followed by
 912 Bonferroni post-hoc test for leptin and overall HFHS effect by Two-way ANOVA using
 913 Bonferroni as post-hoc test for adiponectin. (***) P<0.001)

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Supplementary Figure 2: Plasma measurement of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase activities. Values are means \pm SEM with n=8.



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946 **Supplementary Figure 3:** Representative laminin immunostaining of paraffin section of
 947 adipose tissues obtained from mice fed standard diet without (ST0) or with the pollutant
 948 mixture (STp), and mice fed high fat high sucrose diet without (HF0) or with the pollutant
 949 mixture (HFp). Adipose tissue acquisition is represented for subcutaneous (scAT) and
 950 perigonadal (pgAT) depots using objective x10. Scale bar=100 μm . For adipocyte area
 951 measurement, at least 150 adipocytes for each sample were considered. Image analyses were
 952 performed with ImageJ software. Values are means \pm SEM with n=3. When n=2, the value is
 953 the arithmetic mean.

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