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1 **Multigenerational study of the obesogen effects of bisphenol S after a perinatal exposure in**
2 **C57BL6/J mice fed a high fat diet.**

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21

22 **Abstract**

23 Background: Bisphenol S is an endocrine disruptor exhibiting metabolic disturbances,
24 especially following perinatal exposures. To date, no data are available on the
25 ~~transgenerational~~ obesogen effects of BPS in a multigenerational issue ~~on energy metabolism~~.

26 Objectives: We investigated obesogen effects of BPS in a multigenerational study by focusing
27 on body weight, adipose tissue and plasma parameters ~~related to energy metabolism~~ in male
28 and female mice.

29 Methods: Pregnant C57BL6/J mice were exposed to BPS (1.5 µg/kg bw/day *ie* a human
30 equivalent dose of 0.12 µg/kg bw/day) by drinking water from gestational day 0 to post natal
31 day 21. All offsprings were fed with a high fat diet during 15 weeks. Body weight was
32 monitored weekly and fat mass was measured before euthanasia. At euthanasia, blood
33 glucose, insuline, triglyceride, cholesterol and no esterified fatty acid plasma levels were
34 determined and gene expressions in visceral adipose tissue were assessed. F1 males and
35 females were mated to obtain the F2 generation. Likewise, the F2 mice were cross-bred to
36 obtain F3. The same analyses were performed.

37 Results: In F1 BPS induced an overweight in male mice associated to lipolysis gene
38 expressions upregulation. In F1 females, dyslipidemia was observed. In F2, BPS exposure
39 was associated to an increase in body weight, fat and VAT masses in males and females.
40 Several plasma parameters were increased but with a sex related pattern (blood glucose,
41 triglycerides and cholesterol in males and NEFA in females). We observed a down-regulation
42 in mRNA expression of gene involved in lipogenesis and in lipolysis for females but only in
43 the lipogenesis for males. In F3, a decrease in VAT mass and an upregulation of lipogenesis
44 gene expression occurred only in females.

45 Conclusions: BPS perinatal exposure induced sex-dependent obesogen ~~and metabolic~~
46 multigenerational effects, the F2 generation being the most impacted. Transgenerational
47 disturbances persisted only in females.

48

49 Bisphenol S perinatal exposure induces sex-dependant transgenerational effects in association
50 with a potentiation of the obesity development.

51 Keywords: transgenerational effects, obesogen, low dose exposure, peri-natal exposure

52 **1. Introduction**

53 Bisphenol S (BPS) is a chemical used as monomer in the manufacture of plastics and
54 resins by nearly sixty industrial sectors (Qiu et al., 2019). This compound is ubiquitously
55 present in the environment: indoor air, surface water, sediment, sewage sludge, paper, paints,
56 varnishes, inks, glues, electronic and electrical components, cosmetics, medical equipment,
57 food and food contact materials with a specific migration limit (SML) of 50 µg/kg (EU
58 10/2011) (Qiu et al., 2019). Observational study highlighted that BPS appears to be one of the
59 bisphenols that is the most resistant to biodegradability (Danzl et al., 2009; Frankowski et al.,
60 2020), present an important dermal penetration level (Yang et al., 2019), and is one with the
61 highest estimated daily intakes *via* indoor dust ingestion (Zhang et al., 2020). Although the
62 food is the main route of BPS contamination, these data show that non-dietary exposure may
63 also be significant to human exposure (Karrer et al., 2020).

64 The use of BPS is becoming of increasing concern since the restrictions on the use of
65 bisphenol A (BPA) in relation to its reprotoxic and endocrine disruptor properties, but also
66 due to its greater thermal stability compared to BPA (Karrer et al., 2020). To support this
67 recent increase in usage of BPS, the detection frequency of BPS in American adults urine
68 samples was about 20 % for the period 2000-2007 and about 70 % in years 2009-2014 (Ye et
69 al., 2015). Population-based cohort of BPS biomonitoring studies in urine samples have

70 shown that BPS was detected at concentrations ranging from 0.03 ng/ml (mean value of 33
71 samples, Korea, 2010-2011) to 1.18 ng/ml (mean value of 36 samples, Japan, 2010-2011)
72 (Qiu et al., 2019). Larger-scale studies show mean concentrations ranging from 0.1 ng/ml
73 (1800 samples, U.S., 2013-2014) to 0.36 ng/ml (1400 samples, Netherlands, 2004-2005)
74 (Karrer et al., 2020). A recent study measured urinary levels of BPS at 0.33 ng/ml and
75 estimated an exposure between 0.7 and 7.5 ng/kg bw/day (mean values of 144 samples,
76 Norway, 2016-2017) (Karrer et al., 2020). But, unlike BPA, the modelling of BPS exposure
77 underestimated measured amounts due to patchy exposure characterization (e.g. food and
78 textiles) and lack of data on the absorption and metabolism of BPS (Karrer et al., 2020).

79 From the early 2000s, the role of environmental pollution in the physiopathology of
80 obesity has been highlighted (Baillie-Hamilton, 2002; Heindel, 2003). This awareness has led
81 to the definition of the concept of obesogen, *i.e.* exogenous chemical substances disrupting
82 lipid metabolism and adipogenesis leading to potentiating a pre-existing imbalance in the
83 energy homeostasis (Grun et al., 2006; Le Corre et al., 2015; Papalou et al., 2019). In 2015,
84 the obesogen concept was requalified as a notion of metabolic disruptor considering the
85 globality of metabolic alterations associated with obesity (Heindel et al., 2017a; Papalou et
86 al., 2019). Most metabolic disruptors are known to be endocrine disruptors (EDs). BPS is a
87 structural analogue of BPA with similar ED properties (Rochester and Bolden, 2015). BPA is
88 the most studied metabolic disruptor and BPS appears to induce similar effects in rodents and
89 sheep (Ivry Del Moral et al., 2016; Meng et al., 2019, 2018; Thoene et al., 2020).

90 They mainly act following perinatal exposures (De Coster and van Larebeke, 2012).
91 The BPS crosses the placental barrier (Grandin et al., 2019). In women, BPS has been
92 detected in placenta (60 samples, China, 24.4 pg/g), in cord placenta (60 samples, China, 7.2
93 pg/g) (Pan et al., 2020) and in breast milk (1 sample, France, 0.23 µg/kg) (Deceuninck et al.,
94 2015). EDs are highly studied factors in the DOHaD concept (Developmental Origins of

95 Health and Disease) through potential effects of the gestational microenvironment during
96 early stages of development on the occurrence of obesity later in life (Heindel et al., 2017b;
97 Mochizuki et al., 2017). Usually, the DOHaD concept is applied to directly exposed
98 individuals, but the effects of ED can also impact the health of their children and of
99 subsequent generations (Diamanti-Kandarakis et al., 2009; Gore et al., 2015). For the
100 maternal exposure (F0), the F1 and F2 generations are exposed *in utero* and *via* the F1
101 gametes, respectively. These are considered multigenerational effects. From the F3
102 generation, the first unexposed generation, the observed effects are described as
103 transgenerational. If the exposure is paternal, we consider that transgenerational effects occur
104 in F2 (first unexposed generation) (Heard and Martienssen, 2014; Skvortsova et al., 2018).
105 BPA and BPS metabolic disruptor effects are well documented and highlighted disparities
106 according to the maternal exposure window, the diet, the doses, route of exposure and sex of
107 rodents in F1 generation (Le Corre et al., 2015; van Esterik et al., 2014). Only one study
108 showed carbohydrate metabolism alterations in F2 following perinatal exposure to BPA at 40
109 $\mu\text{g}/\text{kg}$ bw/day by gavage in male wistar rats (Li et al., 2014). Interestingly, a recent study
110 showed that another ED (Tributyltin) could induce a transgenerational obesogenic effect after
111 perinatal exposure at 50 nM in drinking water in C57BL6/J male mice (Chamorro-Garcia et
112 al., 2017). It has already been shown that other EDs (hydrocarbons,
113 dichlorodiphenyltrichloroethane (DDT), BPA and phthalates mixture, methoxychlor) can
114 induce a transgenerational obesogenic effect following perinatal exposure to non-
115 environmental doses (amounts to 25 milligrams or more per kilogram of body weight per day)
116 by intraperitoneal injection (Manikkam et al., 2014, 2013; Skinner et al., 2013; Tracey et al.,
117 2013). ~~To our knowledge, no studies on BPS induced multi- or transgenerational energy~~
118 ~~metabolism disturbances are available.~~ Here, we analyzed the obesogen effects induced by
119 BPS after perinatal exposure of the F1 generation to 1.5 $\mu\text{g} / \text{kg}$ bw / day. We also

120 investigated these disturbances on three generations (F1, F2 and F3) of male and female
121 C57BL6 / J mice fed with a high fat diet in the adult stage.

122

123 **2. Methods**

124 2.1. Animals and materials

125 Pregnant C57Bl/6J mice were purchased from Charles Rivers. Bisphenol S (BPS) was
126 provided by Sigma-Aldrich. The high fat diet (HFD) was based on the 4RF25 reproduction
127 diet (Mucedola) with the addition of palm oil (Huilerie de Vigan) and cholesterol from
128 Sigma-Aldrich (Table S3). The standard diet (SD) was based on the 4RF21 diet (Mucedola).
129 Mucedola 4RF25 and 4RF21 diets are certified as estrogen free and accurately tested for the
130 detection of estrogenic activities. In the diet, the percentage of phytoestrogens is certified to
131 be less than 4 ppb (parts per billion) according to international standards (U.S. Food and Drug
132 Administration National Center for Toxicological Research Standard No. 2, September 5,
133 1973). In the high fat diet, 0.042% cholesterol has been added (because it is missing in palm
134 oil and it is required by Research diet Inc. in diet-induced obesity). Triglyceride FSTM,
135 Cholesterol FSTM and No Esterified Fatty Acids (NEFA) FSTM kits were purchased from
136 DiaSys. Blood glucose test strips (Accu-Check®, Roche Diagnostics) and glucometers (Accu-
137 Check® Aviva, Roche Diagnostics) were purchased from the pharmacy of the University
138 (Dijon, France).

139

140 2.2. Experimental design

141 Mice were housed in a 12h light-dark cycle at a temperature of 22°C in one
142 conventional animal house and allowed free access to food and water. Cages and bottles were
143 made of polypropylene (bisphenol-free). Thirty-seven F0 pregnant C57Bl/6J mice were
144 individually housed. They were divided into two groups and exposed to BPS (20 females) or

145 not (17 females) in their drinking water at concentration of either 0 or 8.5 ng/ml from the first
146 day of gestation (GD0) to post-natal day 21 (PND21) of F1 offsprings. The determination of
147 GD0 was done in the morning by vaginal plug detection. Water intake was determined by
148 measuring the difference in the amount of water placed in the water bottle each week and the
149 amount remaining the following week. Consequently, the levels of BPS consumed weekly
150 were estimated. Under our conventional animal facility conditions, BPS is stable for more
151 than seven days in drinking water (verified by a SPE extraction and HPLC analysis; data not
152 shown). The average BPS intake was 1.78 (\pm 0.03) μ g/kg bw/d. BPS was dissolved in
153 absolute ethanol (0.1%). Control group drinking water contained only 0.1% ethanol. As food
154 is the major route of BPS exposure, in animal experiments, an exposure *via* drinking water
155 allows i) a continuous administration to be as close as possible to BPS detoxification
156 metabolism involved in environmental exposure ii) the homogeneity of the BPS dosage and
157 iii) minimize the degradation of BPS by microorganisms (filtered water and hermetic bottles)
158 (Kang et al., 2006; Pivnenko et al., 2015; Pottenger et al., 2000).

159 To generate the mice of F2 generation, multiple couples of mice were mated at the age
160 of 10 weeks. Each litter came from a unique couple mice made up of male and female mice
161 derived from different litters and dams). ~~One male and one female mice (only fed with the~~
162 ~~standard diet) of the F1 generation were mated at the age of 10 weeks to obtain the F2~~
163 ~~generation.~~ Under the same conditions, the F2 mice were cross-bred to obtain the individuals
164 of the F3 generation (Graphical abstract). At weaning, a maximum of six mice were housed in
165 each cage according to the BPS exposure status of their mother, grandmother or great-
166 grandmother. The litters were mixed randomly after weaning to minimize a possible litter
167 effect. For each generation, 12 male and female mice were analysed. These mice fed a
168 standard diet from weaning to 8-weeks of age. C57BL/6 strain is an established model for diet
169 induced obesity. C57BL/6J mice will develop severe obesity, hyperglycemia, and insulin

170 resistance if weaned onto a high-fat diet (Rossmeisl et al., 2003; Surwit et al., 1995). For each
171 generation, at 8 weeks (sexual maturity), 12 male and 12 female puppies from the control and
172 BPS groups were fed a diet rich in fat (60% Kcal of lipids; composed of 4RF25 diet breeding
173 added with 30% palm oil and 0.42 g / kg cholesterol) or with a standard diet. At 8-weeks old
174 (sexual maturity), all pups were fed with a high fat diet (60% Kcal from lipids; composed by
175 4RF25 reproduction diet added with 30% palm oil and 0.42 g/kg of cholesterol. All mice
176 (fasted 4 h before) were euthanased at 23-weeks old. Mice were anesthetized with isoflurane,
177 blood was sampled by cardiac puncture and then the mice were euthanized by cervical
178 dislocation. Before euthanasia, the body weight was measured. At the euthanasia, adipose
179 tissue was weighed, then immediately frozen in liquid nitrogen and stored at -80°C.
180 Experimental protocol (Accreditation Number: #11422) was approved by the ministry and the
181 University of Burgundy's ethic committee. Animal experiments have been carried out in
182 accordance with EU Directive 2010/63/EU for animal experiments.

183

184 2.3. Body weight monitoring and corporal composition measurement

185 Body weight was monitored weekly. Just before (4h fasted) euthanasia, body weight,
186 fat and lean masses of 23-weeks old mice were measured. The fat and lean masses of each
187 mouse were determined individually using a quantitative EchoMRI 500T™ (EchoMRI,
188 Houston, USA). Before each measurement, calibration was performed in compliance with the
189 manufacturer's guidelines.

190

191 2.4. Food intake measurement

192 For each generation (F1; F2; F3), these experiments were performed six weeks after
193 the beginning of the high fat diet to allow an acclimation time of diet change. Measurements
194 were done by cage and then normalized to the number of mice per cage. The mice were not

195 individualized so as not generate stress which could modify the feeding behavior of the
196 animal. The amount of food was weighed daily during four consecutive days. Daily food
197 intake was determined by weighing out the difference in the amount of food placed and the
198 remaining amount after 24 h. Thus, the daily consumption per mouse was calculated from the
199 average of the data obtained for three consecutive days. Particular attention was paid to
200 spillage and/or hoarding. Spilled food was weighed and subtracted from the measurements
201 when necessary.

202

203 2.5. Biochemical analysis

204 Just before euthanasia (*i.e.* fasted 4h before), mouse blood samples were collected by
205 an intracardiac puncture using heparinized syringe. After centrifugation of 10 min at 2000g
206 and 4°C, the level of total plasma cholesterol, triacylglycerol and NEFA were measured using
207 respective kits mentioned above. The plasma insulin was determined using ELISA kit as
208 mentioned previously. Assays were performed according to the manufacturer's instructions.
209 The day of euthanasia, blood glucose levels were measured, using a glucometer, from a blood
210 sample collected at the animal's tail and before anaesthesia. The homeostatic model
211 assessment of insulin resistance (HOMA-IR) was calculated by a formula adapted to a
212 previous method in human (Matthews et al., 1985). For mice, reference values were
213 calculated using average fasting glucose (4.16 mmol/l) and plasma insulin (26.11 mU/l)
214 concentrations from the control mice fed with standard diet (van Dijk et al., 2013). The
215 homeostatic model assessment (HOMA) adapted to mice was calculated as $([\text{glucose}$
216 $(\text{mmol/l})] \times [\text{insulin (mU/l)}])/108.6$ and used as a surrogate measure of whole-body insulin
217 sensitivity.

218

219 2.6. Real-time PCR analysis

220 Lysis of visceral adipose tissue samples of each mouse were performed using Lysing
221 Matrix D™ tubes (MP Biomedical) and a tissue lyser Precellys® (Ozyme). Total RNA was
222 extracted using Tri-reagent™ (Sigma-Aldrich). cDNA was reverse-transcribed on 3 µg of
223 total RNA samples using the High Capacity cDNA reverse transcription kit™ (Life
224 Technologies). Real time PCR was done with 30 ng of cDNA, 5 µl of Taqman™ Universal
225 Master Mix (Life Technologies), 0.5 µl of Taqman™ gene expression assay (20X; Life
226 Technologies) for a final volume of 10 µl (qsp H2O). Taqman™ gene expression assay
227 references were: Mm01184322_m1 (*Ppary*), Mm00503040_m1 (*Pnpla2*), Mm00495359_m1
228 (*Lipe*), Mm00662319_m1 (*Fasn*), Mm00449274_m1 (*Mgll*), Mm01304257_m1 (*Acaca*) and
229 Mm99999915-g1 (*Gapdh*, reference gene). PCR was run on the Step-One plus™ system
230 (Applied Biosystems) using following conditions: 50°C for 2 min, 95°C for 10 min and 40
231 cycles of 95°C for 15s and 60°C for 30s. Gene expression was determined relative to the
232 control gene glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) using the $2^{-\Delta\Delta C_t}$ method
233 (Schmittgen and Livak 2008).

234

235 2.7. Statistical analysis

236 All data were expressed as mean \pm standard error of mean (SEM). To determine the
237 statistically significant difference between two groups, a Student's t test was used (two-tailed,
238 paired samples for means and equal variance). Statistical tests were performed with GraphPad
239 Prism7® software. Results were considered statistically significant at $p < 0.05$.

240

241 3. Results

242 3.1. Multigenerational effects of BPS on body weight and adiposity in male mice

243 In mice fed with a HFD for 15 weeks, BPS induced a significant overweight in F1 (a 9
244 % increase, $p = 0.002$) and F2 males (a 14 % increase, $p = 0.03$) (Figure 1A and 1B). In F3,

245 this shift did not reach statistical significance (2 % higher) (Figure 1C). Interestingly, for both
246 F1 and F2, overweight induced by BPS occurred after six weeks of HFD, but the body weight
247 gain at euthanasia was higher in F2 than F1 (29 % and 18 % higher, respectively) (Figure
248 1D,E). To investigate whether this overweight is related to an obesogenic effect of BPS, we
249 evaluated the mouse adiposity. Mouse fat mass and VAT mass significantly increased only in
250 F2 (a 21 % increase and a 27 % increase, $p = 0.05$ and $p = 0.008$, respectively) (Figure 1H and
251 1K).

252

253 3.2. Multigenerational effects of BPS on body weight and adiposity in female mice

254 Under the same experimental conditions BPS induced a significant body weight
255 increase in females, but only in F2 (a 17 % increase, $p = 0.03$) (Figure 2B). In F1 and F3, BPS
256 exposure was associated with a weak, but not significant body weight decrease (3 % and 8 %
257 lower, respectively) (Figure 2A and 2C). Like for F2 males, overweight induced by BPS
258 occurred after six weeks of HFD in F2 females, but the body weight gain at euthanasia was
259 higher in females (35 % higher) (Figure 2E). Regards to BPS obesogenic effect assessment, in
260 F2, fat and VAT masses were significantly increased to a greater extent compared to males (a
261 39 % increase and a 44 % increase, $p = 0.004$ and $p = 0.04$, respectively) (Figure 2H and 2K).
262 Unlike in F1, in F3, the small decrease in body weight was associated with a significant
263 decrease in VAT mass (a 40 % decrease, $p = 0.03$) (Figure 2L).

264

265 3.3. BPS multigenerational effect on food intake in male and female mice

266 The obesogenic effect of BPS observed in F2 males and females mice was associated
267 with a decrease in food intake which was significant only in male mice (a 15 % decrease and
268 a 6 % decrease, $p = 0.0007$ and $p = 0.52$, respectively) (Figure 3B and 3E). For F1 males, the
269 food intake of exposed mice with overweight was increased, but not significant (8 % higher).

270

271 3.4. Transgenerational effects of BPS on plasma parameters related to blood glucose and lipid
272 metabolism in male and female mice

273 In males exposed to BPS, overweight observed in F1 was associated with a
274 hypocholesterolemia (a 20 % decrease, $p = 0.007$) (Table 1). In F2, the obesogenic effect of
275 BPS induced a significant increase in triglyceride and total cholesterol plasma levels (a 12 %
276 increase and a 50 % increase, $p = 0.0003$ and $p = 0.01$, respectively) (Table 1). Regardless of
277 the generation, no significant changes were observed in HOMA-IR and glucose, insulin and
278 NEFA plasma level (Table 1).

279 In females, despite the absence of a significant effect on body weight, the metabolic disruptor
280 effects of BPS were manifested by a hypercholesterolemia and a hypertriglyceridemia in F1 (a
281 39 % increase and a 44 % increase, $p = 0.0002$ and $p = 0.02$, respectively) and a decrease in
282 blood glucose level in F3 (an 11 % decrease, $p = 0.04$) (Table 2). In F2, the obesogenic effect
283 of BPS was associated with a significant increase in NEFA plasma level (a 116 % increase, p
284 $= 0.02$) (Table 2). As in male and regardless the generation, no significant changes were
285 observed in HOMA-IR and insulin plasma level (Table 2).

286 ~~All no significant results for male and female mice F1, F2 and F3 fed with HFD (glucose,~~
287 ~~insulin and NEFA plasma level and HOMA-IR) are reported in supplementary data (Table~~
288 ~~S1).~~

289

290 3.5. BPS transgenerational effects on adipose tissue lipid metabolism marker genes expression
291 in male and female mice

292 In males exposed to BPS, overweight shown in F1 was associated with a significant
293 downregulation of the mRNA expression of Patatin Like Phospholipase Domain Containing 2
294 (*Pnpla2*) and Monoglyceride Lipase (*Mgl1*), two enzymes involved in the first and last stage

295 of lipolysis, respectively (a 30 % decrease and a 22 % decrease, $p = 0.005$ and $p = 0.03$,
296 respectively) (Figure 4). In F2, the BPS obesogenic effect was associated with a significant
297 decrease of *Acetyl-CoA Carboxylase Alpha (Acaca)* mRNA level, an enzyme involved in the
298 first step of lipogenesis (a 22 % decrease, $p = 0.04$) (Figure 4).

299 In females, the BPS obesogenic effect highlighted in F2 was associated with a
300 downregulation of *Pnpla2* and *Acaca* mRNA expressions (a 33 % decrease and a 39 %
301 decrease, $p = 0.04$ and $p = 0.008$, respectively) (Figure 5). For F3 females exposed to BPS,
302 the decrease in VAT mass was associated with an upregulation of *Acaca* and *Fatty Acid*
303 *Synthase (Fasn)* mRNA levels, two enzymes involved in lipogenesis (a 65 % increase and a
304 69 % increase, $p = 0.01$ and $p = 0.009$, respectively) (Figure 5).

305 All other marker genes mRNA expressions were not modified (Figure S1 and S2).

306

307 **4. Discussion**

308 In our previous one-generation study in C57BL/6J mice fed with a HFD, we
309 demonstrated obesogenic and metabolic effects resulting from perinatal and chronic exposure
310 (up to 23 weeks-old mice) to BPS at 1.5 $\mu\text{g}/\text{kg}$ bw/day in F1 male mice only (Ivry Del Moral
311 et al., 2016). Here, using the same experimental model, but a limiting window exposure
312 (gestation and lactation), we investigated transgenerational metabolic disturbances of BPS.
313 Thus, in this study, we carried out a similar analysis focused on the principal physiological
314 parameters associated with an obesogen effect (body weight gain associated with an alteration
315 of adipose tissue lipid metabolism).

316 ***Experimental strategy:***

317 To conduct a study on the obesogen effect of an endocrine disruptor such as bisphenol
318 S, choices had to be made on important parameters of this type of investigation: composition
319 of diets, dose of exposure, method of administration and exposure windows. These choices

320 were based on the data available on bisphenol A, the most studied obesogen endocrine
321 disruptor (Le Corre et al., 2015).

322 The use of an HFD is necessary because the BPS only potentiate the development of
323 obesity by exacerbating an HFD-induced energy imbalance. Indeed, BPS does not induce any
324 obesogen effect in mice having undergone the same exposure protocol but fed with a standard
325 diet (Ivry Del Moral et al., 2016). Here, we confirm that whatever the sex and the generation,
326 BPS did not alter body weight, corporal composition, food intake and plasma parameters
327 (supplementary data, table S1 and S2 for male and female mice, respectively).

328 ~~Concerning the HFD used, we previously described that it was very efficient to induce~~
329 ~~the obesity development in the same mouse strain (Ivry Del Moral et al. 2016), and also to~~
330 ~~alter physiological parameters involved in the obesity physiopathology (de Wit et al. 2008) in~~
331 ~~accordance with the emerging concept of metabolic disruptors (Heindel et al. 2017a; Papalou~~
332 ~~et al. 2019). giving rise to a metabolic disruptor effect in male mice as reported in our~~
333 ~~previous investigation (Ivry Del Moral et al. 2016). BPS concentration was based on a~~
334 ~~preliminary study evaluating the water consumption of mice during the gestation to obtain~~
335 ~~expected BPS exposure of 1.5 µg/kg body weight/day. To our knowledge, no studies have~~
336 ~~assessed human BPS exposure related to country-specific bisphenol use regulations. The~~
337 ~~choice was made to use the available data for BPA, the most studied metabolic disruptor,~~
338 ~~which is also a structural analogue of BPS. The dose of 1.5 µg/kg bw/d corresponds to the~~
339 ~~BPA exposure of adults (EFSA 2006). In our study, we used BPS at a dose of 1.5 µg/kg bw/d.~~
340 This is the lowest dose of BPS known to have an obesogen effect after perinatal exposure in
341 mice as we reported in our previous study using three different doses 0.2, 1.5 and 50 µg/kg
342 bw/d (Ivry Del Moral et al., 2016).

343

344 ~~In the present study, in order to better understand the role of BPS in the DOHaD~~
345 ~~concept, we have reduced the duration of exposure to the strict perinatal period and removed~~
346 ~~the notion of chronic exposure.~~

347 In this study, the window of exposure was limited to pregnancy and lactation to target key
348 stages of tissue development of interest i) germline as a vector of multi- and transgenerational
349 heritability, subjected to two reprogramming waves of DNA methylation during
350 gametogenesis (GD0.5-GD3.5 and GD8.5-GD16.5) (Heard and Martienssen, 2014) ii) and
351 adipose tissue that appears from the twelfth day of embryonic development in C57Bl/6J mice
352 (Mayeuf-Louchart et al., 2019). Moreover, there are likely distinct pathways for
353 developmental (in utero) and non-developmental exposures of obesogens, as well as persistent
354 versus non-persistent exposures (Griffin et al., 2020). In this context, we have focused only on
355 the early stages of development.

356 ***BPS induced obesogen effect in F1 generation in sex-dependent manner***

357 Interestingly, in comparison with the present study, Ivry del Moral *et al* research
358 showed a higher body weight gain (12 %) and adiposity (13 %), a hypercholesterolemia and
359 hyperinsulinemia only in male mice (Ivry Del Moral et al., 2016). These more marked
360 metabolic disturbances may be related to changes in the BPS window exposure. However,
361 ~~which nevertheless suggest that BPS exposure during gestation and lactation is sufficient to~~
362 ~~potentiate the obesity in adulthood.~~ as reported with BPA, it seems important and sufficient to
363 have an impregnation of BPS during the gestation period to observe significant obesogen
364 effects (Le Corre et al., 2015; Nikaido et al., 2004; Rubin et al., 2001). More generally, EDs
365 act on the same basis as endogenous hormones and consequently can interfere with
366 development processes (Gore et al., 2015).

367 In accordance with our data about BPS, one other study showed an increase in body
368 weight and adiposity in ICR F1 male mice, with or without nutritional stress (HFD),

369 following perinatal exposure to a higher dose of BPS (100 µg/kg bw/day) by gavage (Meng et
370 al., 2019). An increase in body weight was also observed in Swiss F1 male mice fed a
371 standard diet and following a BPS exposure in adulthood at doses ranging from 25 to 100
372 µg/kg bw/day by drinking water (Rezg et al., 2018). With the two experimental protocols
373 (BPS perinatal or perinatal and chronic exposure) used in the lab, we failed to induce an
374 obesogenic effect in F1 female mice (Ivry Del Moral et al., 2016). In F1, only one study
375 shows a body weight increase in ICR adolescent female mice (from 3 to 5 weeks of age) fed a
376 standard diet and following a BPS perinatal exposure (100 µg/kg bw/day) by gavage (Meng et
377 al., 2018). These mice also show an increase in triglyceride and cholesterol hepatic levels
378 (Meng et al., 2018). This is consistent with the dislipidemia observed without change in
379 adiposity in our F1 females, in which, under a HFD and at an older age, BPS appears to
380 induce a potential deregulation of energy metabolism in the liver (Titchenell et al., 2017).

381 The sex-dependent effect suggests an impact on sex hormones which are powerful regulators
382 of energy metabolism (Mauvais-Jarvis et al., 2013; Navarro et al., 2015). For example,
383 suboptimal or insufficient estrogen levels have been reported to be linked to fat accumulation
384 and obesity in women (Litwak et al., 2014). Likewise, in men, obesity is strongly associated
385 with low testosterone levels (Navarro et al., 2015). The action of endogenous hormones is
386 highly dose dependent. Thus, endocrine disruptors could reverse their effects on energy
387 metabolism. In addition, BPS is described both as a weak estrogenic compound in vitro
388 (Kuruto-Niwa et al., 2005; Molina-Molina et al., 2013) and as an anti-androgen receptor
389 antagonist. It is also reported as an inhibitor of the steroidogenesis in MA-10 Leydig murine
390 cells (Kitamura et al., 2005; Roelofs et al., 2015) and to decrease the basal secretion of
391 testosterone by the testes of mice and human fetuses (Eladak et al., 2015). Thus, a
392 combination of estrogenic and antiandrogenic properties of BPS could cause an imbalance of
393 sex hormones in humans leading to deregulation of lipid homeostasis. Conversely, in women,

394 the potential BPS-induced disruption of estrogen homeostasis might be negligible regards to
395 endogenous estrogen levels.

396 ***The obesogen effect of BPS is multigenerational (F2) but not transgenerational (F3)***

397 Our results showed difference between generations (Figures 1 and 2). However, it is not
398 relevant to compare the generations between them. Indeed, the variation in measurements can
399 be explained by the high fat diet. The diet-induced obesity (DIO) in mice shows significant
400 variability (Burcelin et al., 2002; Vaanholt et al., 2015). Likewise, the range values of the
401 plasma parameters are consistent with the data provided by Charles River in C57Bl / 6J mice.
402 (Jax Phenom 3 study, in 2006). In 16 week-old and 4h-fasted mice, it reported that the glucose
403 plasma levels are 159 ± 25.1 mg/dl in females and 152 ± 29.2 mg/dl in males; triglyceride
404 levels are 0.667 ± 0.219 g/l in females and 0.707 ± 0.16 mg/dl in males; total cholesterol
405 levels are 0.698 ± 0.605 g/l in females and 0.933 ± 0.102 g/l in males.

406 In male and female mice, BPS induced multigenerationnal (*i.e.* only in exposed animals)
407 obesogen effects under our experimental conditions. In both sexes, F2 appears to be the most
408 impacted generation regarding analyses focused on body weight, adipose tissue, blood
409 glucose and insulinemia. No transgenerational effect is observed because the F3 mice did not
410 show any gain in body weight.

411 The worsening obesity status in F2 male and female mice suggests different mechanisms of
412 action compared to the F1 generation. Only germ cells of F2 mice were exposed to BPS. In
413 this case, hereditary transmission vectors are believed to be primarily epigenetic changes such
414 as DNA methylation, histone acetylation, or non-coding RNA (Bird, 2007; Desai et al., 2015).
415 Interestingly, BPS has been described as inducing an obesogenic effect in male F1 mice in
416 association with a change in hepatic DNA methylation profile (Brulport et al., 2020).

417 ***Obesogen phenotype induced by the BPS in male mice is generation-dependent***

418 In males, individuals showed different phenotypes following the F1 and F2 generations.
419 Unlike in F2, in F1 males, adiposity increases insignificantly and has been associated with a
420 downregulation of genes involved in lipolysis - *Plnpla2* and *Mgll* -. The storage capacity of
421 adipose tissue does not seem to be affected. ~~With the same insulin sensitivity than control~~
422 ~~group, the liver of these animals can normally still store fatty acids in the form of triglycerides~~
423 ~~(Titchenell et al. 2017). It seems consistent not to observe dyslipidemia in F1 males. The~~
424 ~~explanation of the lower plasma cholesterol level requires further investigation. It would be~~
425 ~~interesting to study fluctuations in bile acid levels, which are cholesterol dependent for their~~
426 ~~biosynthesis, and whose role in the physiopathology of obesity and its associated~~
427 ~~comorbidities has been the aim of many recent studies (Chavez-Talavera et al. 2017).~~

428 In F2 males, the significant increase in fat mass and visceral adipose tissue weight and the
429 downregulation of *Acaca* mRNA level, a gene involved in lipogenesis, may suggest that the
430 adipose tissue storage capacity was reaching saturation. Indeed, it has already been shown that
431 in C57BL6/J mice fed a high fat high sucrose diet, fat accumulation reaches a plateau after 17
432 weeks (Karunakaran and Clee, 2018). Without sucrose, but with BPS, our animals probably
433 reached this plateau as early as the 15th week of diet. All these data may explain dyslipidemia
434 observed in these animals. Although our control and treated groups have the same insulin
435 levels, as all animals fed a HFD, we cannot exclude that they have an organ specific insulin
436 resistance preventing inhibition of hepatic glucose production resulting in hyperglycemia and
437 a worsening of dyslipidemia in F2 males (Titchenell et al., 2017).

438 In this context of metabolic disturbances associated with an obesity phenotype, it's
439 unexpected to observe a decrease in food intake in F2 males. Although not investigated in this
440 study, it appears unlikely that this observation was related to a decrease in energy expenditure
441 or locomotor activity. In the same model, following perinatal and chronic exposure, BPS does
442 not lead to changes in food intake and energy expenditure in F1 males (Ivry Del Moral et al.,

443 2016). Interestingly, perinatal exposures to BPS (10 or 50 µg/kg bw/day) induced a decrease
444 in food intake with no change in locomotor activity in F1 wistar male rats (da Silva et al.,
445 2019). As highlighted by Rezg *et al.* (2018) under other experimental conditions (adulthood
446 exposure), the deregulation of orexigenic and anorexigenic hypothalamic neuropeptides
447 appears to be an area of investigation (Rezg et al., 2018). Likewise, it cannot be excluded that
448 at an early stage, before the appearance of resistance to leptin and insulin, high plasma levels
449 of leptin may inhibit food intake (Thon et al., 2016).

450 ***Multigenerational obesogen effect of BPS in female mice***

451 Interestingly, the obesogen effect of BPS appears only in F2 female mice. ~~In F2, like for~~
452 ~~males, BPS leads to an obesogenic phenotype in females. However,~~ Compared to male mice,
453 the storage capacity of adipose tissue seems to be greater in females. Indeed, BPS induced the
454 downregulation of *Pnpla2* -a key gene for lipolysis- and of *Acaca* mRNA levels -first enzyme
455 involved in lipogenesis-. Likewise, the alteration of *Acaca* mRNA expression could be related
456 to the increase in NEFA plasma level ~~could be related to the downregulation of *Acaca* mRNA~~
457 ~~level, the first enzyme involved in lipogenesis.~~

458 ***Transgenerational effect of BPS in female, an adaption mechanism?***

459 Only F3 female from BPS-exposed F0 dams exhibited a phenotype different of control group.
460 ~~Concerning the F3 females, Mice of the F3 generation are the first to be unexposed.~~
461 ~~generation.~~ F3 female mice ~~they~~ presented lower body weight and adiposity without
462 metabolic disturbances, despite an upregulation of the expression of genes involved in
463 lipogenesis (*Acaca* and *Fasn*). Thus, the hypothesis of a transgenerational adaptation
464 mechanism is not excluded in females and could result in a shift from a thrifty phenotype to
465 an energy over-consumption phenotype. However, while this hypothesis requires further

466 investigation, it's interesting to see that this particularity appears to be present only in females
467 while in F3 males no metabolic disruption appears to persist.

468 ***Is the multigenerational obesogen effect of BPS of concern in humans?***

469 To our knowledge, in humans, no epidemiology studies have shown an association between
470 maternal BPS exposure ~~during the pregnancy and lactation~~ and the occurrence of metabolic
471 disease in subsequent generations. A cross-sectional epidemiology study (U.S. National
472 Health and Nutrition Examination Surveys: 2013-2016) described an association between
473 urinary BPS concentrations and prevalence of general and abdominal obesity (Jacobson et al.,
474 2019). These observations performed on the first generation highlight the critical points
475 already known to modulate the actions of EDs (window exposure, sex, doses, route of
476 exposure, diet) (Le Corre et al., 2015) and we will now address the question of
477 transgenerational effects, for which this study is innovative on the consequences of exposure
478 to BPS.

479 The toxicokinetics of BPS are also different between mice and human. Little data is available
480 on this point. A physiologically based pharmacokinetic modeling highlighted that BPS is the
481 BPA substitute which has the most important enterohepatic recirculation leading to poorer
482 urinary excretion and it's also the BPA analog leading to the highest internal concentrations
483 of unconjugated bisphenols (Karrer et al., 2018). It is also important to note that the
484 metabolism of BPS differs between human and mice. In female mice, 50% of the
485 administered BPS is excreted in the urine in 6 hours in metabolized form (detoxification
486 mainly in glucuro-conjugated form) (Song et al., 2017) . In humans after oral absorption,
487 Khmiri et al. (2020) reported limited amounts of BPS and its glucuronide conjugate were
488 recovered in the urine and maximum excretion rates were reached between 5 and 11 h after
489 administration with a poor recovery rate in the urine for BPS and its conjugate -
490 approximately 0.004 ± 0.003 and $0.09 \pm 0.07\%$, respectively -. The authors shown higher oral

491 bioavailability and subsequent higher systemic levels of active BPS than BPA (Khmiri et al.,
492 2020). Taken together, these data suggest that BPS toxicological impacts might be more
493 critical in Humans than in mice.

494 At molecular level, *in vitro* were performed. It has been shown that low concentrations of
495 BPS (at fM to μ M levels) can induce lipid accumulation in human HepG2 cell line (Helies-
496 Toussaint et al., 2014) as in murine 3T3L1 preadipocyte cell line (Ahmed and Atlas, 2016;
497 Ramskov Tetzlaff et al., 2020). *Ex vivo*, a recent study reported that BPS at low doses (0.1
498 and 25 μ M) promotes adipogenesis in visceral and subcutaneous human preadipocytes
499 (Peshdary et al., 2020).

500 In conclusion, our study shows that BPS is of concern for its ability to induce
501 obesogenic effects in the offspring (F2 or even F3) of mice exposed perinatally at low doses.
502 These effects were also sex-dependent. These phenotypes were observed in relatively young
503 adult mice (23 weeks of age) and in perspective, it would be interesting to see the impact of
504 aging on the phenotypes described as obesity contributes to the aging process and *vice versa*.
505 It would be necessary to investigate the disorders associated with obesity such as the
506 inflammatory state, the gut microbiota and lipid homeostasis (lipid metabolism and associated
507 protein activities), notably in adipose tissue or the liver. Likewise, the processes related to the
508 heredity of the observed effects should be clarified by studying epigenetic modifications such
509 as DNA methylation or histone acetylation.

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517

518 References

519 Ahmed, S., Atlas, E., 2016. Bisphenol S- and bisphenol A-induced adipogenesis of murine
520 preadipocytes occurs through direct peroxisome proliferator-activated receptor gamma
521 activation. *Int J Obes (Lond)* 40, 1566–1573. <https://doi.org/10.1038/ijo.2016.95>

522 Baillie-Hamilton, P.F., 2002. Chemical toxins: a hypothesis to explain the global obesity
523 epidemic. *J Altern Complement Med* 8, 185–92.
524 <https://doi.org/10.1089/107555302317371479>

525 Bird, A., 2007. Perceptions of epigenetics. *Nature* 447, 396–398.
526 <https://doi.org/10.1038/nature05913>

527 Brulport, A., Vaiman, D., Chagnon, M.-C., Le Corre, L., 2020. Obesogen effect of bisphenol
528 S alters mRNA expression and DNA methylation profiling in male mouse liver. *Chemosphere*
529 241, 125092. <https://doi.org/10.1016/j.chemosphere.2019.125092>

530 Burcelin, R., Crivelli, V., Dacosta, A., Roy-Tirelli, A., Thorens, B., 2002. Heterogeneous
531 metabolic adaptation of C57BL/6J mice to high-fat diet. *Am J Physiol Endocrinol Metab* 282,
532 E834-842. <https://doi.org/10.1152/ajpendo.00332.2001>

533 Chamorro-Garcia, R., Diaz-Castillo, C., Shoucri, B.M., Käch, H., Leavitt, R., Shioda, T.,
534 Blumberg, B., 2017. Ancestral perinatal obesogen exposure results in a transgenerational
535 thrifty phenotype in mice. *Nat Commun* 8, 2012. <https://doi.org/10.1038/s41467-017-01944-z>

536 da Silva, B.S., Pietrobon, C.B., Bertasso, I.M., Lopes, B.P., Carvalho, J.C., Peixoto-Silva, N.,
537 Santos, T.R., Claudio-Neto, S., Manhães, A.C., Oliveira, E., de Moura, E.G., Lisboa, P.C.,

538 2019. Short and long-term effects of bisphenol S (BPS) exposure during pregnancy and
539 lactation on plasma lipids, hormones, and behavior in rats. *Environ Pollut* 250, 312–322.
540 <https://doi.org/10.1016/j.envpol.2019.03.100>

541 Danzl, E., Sei, K., Soda, S., Ike, M., Fujita, M., 2009. Biodegradation of bisphenol A,
542 bisphenol F and bisphenol S in seawater. *Int J Environ Res Public Health* 6, 1472–84.
543 <https://doi.org/10.3390/ijerph6041472>

544 De Coster, S., van Larebeke, N., 2012. Endocrine-disrupting chemicals: associated disorders
545 and mechanisms of action. *J Environ Public Health* 2012, 713696.
546 <https://doi.org/10.1155/2012/713696>

547 Deceuninck, Y., Bichon, E., Marchand, P., Boquien, C.-Y., Legrand, A., Boscher, C.,
548 Antignac, J.P., Le Bizec, B., 2015. Determination of bisphenol A and related
549 substitutes/analogues in human breast milk using gas chromatography-tandem mass
550 spectrometry. *Anal Bioanal Chem* 407, 2485–2497. [https://doi.org/10.1007/s00216-015-8469-](https://doi.org/10.1007/s00216-015-8469-9)
551 9

552 Desai, M., Jellyman, J.K., Ross, M.G., 2015. Epigenomics, gestational programming and risk
553 of metabolic syndrome. *Int J Obes (Lond)*. <https://doi.org/10.1038/ijo.2015.13> ijo201513 [pii]

554 Diamanti-Kandarakis, E., Bourguignon, J.-P., Giudice, L.C., Hauser, R., Prins, G.S., Soto,
555 A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-disrupting chemicals: an Endocrine Society
556 scientific statement. *Endocr. Rev.* 30, 293–342. <https://doi.org/10.1210/er.2009-0002>

557 Eladak, S., Grisin, T., Moison, D., Guerquin, M.J., N'Tumba-Byn, T., Pozzi-Gaudin, S.,
558 Benachi, A., Livera, G., Rouiller-Fabre, V., Habert, R., 2015. A new chapter in the bisphenol
559 A story: bisphenol S and bisphenol F are not safe alternatives to this compound. *Fertil Steril*
560 103, 11–21. <https://doi.org/10.1016/j.fertnstert.2014.11.005> S0015-0282(14)02351-6 [pii]

561 Frankowski, R., Zgoła-Grześkowiak, A., Smułek, W., Grześkowiak, T., 2020. Removal of
562 Bisphenol A and Its Potential Substitutes by Biodegradation. *Appl Biochem Biotechnol* 191,
563 1100–1110. <https://doi.org/10.1007/s12010-020-03247-4>

564 Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J.,
565 Zoeller, R.T., 2015. EDC-2: The Endocrine Society's Second Scientific Statement on
566 Endocrine-Disrupting Chemicals. *Endocr. Rev.* 36, E1–E150. <https://doi.org/10.1210/er.2015->
567 1010

568 Grandin, F.C., Lacroix, M.Z., Gayrard, V., Viguié, C., Mila, H., de Place, A., Vayssière, C.,
569 Morin, M., Corbett, J., Gayrard, C., Gely, C.A., Toutain, P.-L., Picard-Hagen, N., 2019. Is
570 bisphenol S a safer alternative to bisphenol A in terms of potential fetal exposure? Placental
571 transfer across the perfused human placenta. *Chemosphere* 221, 471–478.
572 <https://doi.org/10.1016/j.chemosphere.2019.01.065>

573 Griffin, M.D., Pereira, S.R., DeBari, M.K., Abbott, R.D., 2020. Mechanisms of action,
574 chemical characteristics, and model systems of obesogens. *BMC Biomed Eng* 2, 6.
575 <https://doi.org/10.1186/s42490-020-00040-6>

576 Grun, F., Watanabe, H., Zamanian, Z., Maeda, L., Arima, K., Cubacha, R., Gardiner, D.M.,
577 Kanno, J., Iguchi, T., Blumberg, B., 2006. Endocrine-disrupting organotin compounds are
578 potent inducers of adipogenesis in vertebrates. *Mol Endocrinol* 20, 2141–55.
579 <https://doi.org/me.2005-0367> [pii] 10.1210/me.2005-0367

580 Heard, E., Martienssen, R.A., 2014. Transgenerational epigenetic inheritance: myths and
581 mechanisms. *Cell* 157, 95–109. <https://doi.org/10.1016/j.cell.2014.02.045>

582 Heindel, J.J., 2003. Endocrine disruptors and the obesity epidemic. *Toxicol Sci* 76, 247–9.

583 Heindel, J.J., Blumberg, B., Cave, M., Machtinger, R., Mantovani, A., Mendez, M.A., Nadal,
584 A., Palanza, P., Panzica, G., Sargis, R., Vandenberg, L.N., Vom Saal, F., 2017a. Metabolism
585 disrupting chemicals and metabolic disorders. *Reprod Toxicol* 68, 3–33.
586 <https://doi.org/10.1016/j.reprotox.2016.10.001>

587 Heindel, J.J., Skalla, L.A., Joubert, B.R., Dilworth, C.H., Gray, K.A., 2017b. Review of
588 developmental origins of health and disease publications in environmental epidemiology.
589 *Reprod Toxicol* 68, 34–48. <https://doi.org/10.1016/j.reprotox.2016.11.011>

590 Helies-Toussaint, C., Peyre, L., Costanzo, C., Chagnon, M.C., Rahmani, R., 2014. Is
591 bisphenol S a safe substitute for bisphenol A in terms of metabolic function? An in vitro
592 study. *Toxicol Appl Pharmacol* 280, 224–35. <https://doi.org/10.1016/j.taap.2014.07.025>
593 S0041-008X(14)00291-9 [pii]

594 Ivry Del Moral, L., Le Corre, L., Poirier, H., Niot, I., Truntzer, T., Merlin, J.F., Rouimi, P.,
595 Besnard, P., Rahmani, R., Chagnon, M.C., 2016. Obesogen effects after perinatal exposure of
596 4,4'-sulfonyldiphenol (Bisphenol S) in C57BL/6 mice. *Toxicology* 357–358, 11–20.
597 <https://doi.org/10.1016/j.tox.2016.05.023> S0300-483X(16)30077-4 [pii]

598 Jacobson, M.H., Woodward, M., Bao, W., Liu, B., Trasande, L., 2019. Urinary Bisphenols
599 and Obesity Prevalence Among U.S. Children and Adolescents. *J Endocr Soc* 3, 1715–1726.
600 <https://doi.org/10.1210/js.2019-00201>

601 Kang, J.H., Kondo, F., Katayama, Y., 2006. Human exposure to bisphenol A. *Toxicology*
602 226, 79–89. [https://doi.org/S0300-483X\(06\)00405-7](https://doi.org/S0300-483X(06)00405-7) [pii] 10.1016/j.tox.2006.06.009

603 Karrer, C., Andreassen, M., von Goetz, N., Sonnet, F., Sakhi, A.K., Hungerbühler, K., Dirven,
604 H., Husøy, T., 2020. The EuroMix human biomonitoring study: Source-to-dose modeling of
605 cumulative and aggregate exposure for the bisphenols BPA, BPS, and BPF and comparison

606 with measured urinary levels. *Environ Int* 136, 105397.
607 <https://doi.org/10.1016/j.envint.2019.105397>

608 Karrer, C., Roiss, T., von Goetz, N., Gramec Skledar, D., Peterlin Mašič, L., Hungerbühler,
609 K., 2018. Physiologically Based Pharmacokinetic (PBPK) Modeling of the Bisphenols BPA,
610 BPS, BPF, and BPAF with New Experimental Metabolic Parameters: Comparing the
611 Pharmacokinetic Behavior of BPA with Its Substitutes. *Environ Health Perspect* 126, 077002.
612 <https://doi.org/10.1289/EHP2739>

613 Karunakaran, S., Clee, S.M., 2018. Genetics of metabolic syndrome: potential clues from
614 wild-derived inbred mouse strains. *Physiol Genomics* 50, 35–51.
615 <https://doi.org/10.1152/physiolgenomics.00059.2017>

616 Khmiri, I., Côté, J., Mantha, M., Khemiri, R., Lacroix, M., Gely, C., Toutain, P.-L., Picard-
617 Hagen, N., Gayrard, V., Bouchard, M., 2020. Toxicokinetics of bisphenol-S and its
618 glucuronide in plasma and urine following oral and dermal exposure in volunteers for the
619 interpretation of biomonitoring data. *Environ Int* 138, 105644.
620 <https://doi.org/10.1016/j.envint.2020.105644>

621 Kitamura, S., Suzuki, T., Sanoh, S., Kohta, R., Jinno, N., Sugihara, K., Yoshihara, S.,
622 Fujimoto, N., Watanabe, H., Ohta, S., 2005. Comparative study of the endocrine-disrupting
623 activity of bisphenol A and 19 related compounds. *Toxicol Sci* 84, 249–59.
624 <https://doi.org/kfi074> [pii] 10.1093/toxsci/kfi074

625 Kuruto-Niwa, R., Nozawa, R., Miyakoshi, T., Shiozawa, T., Yoshiyasu, Y., 2005. Estrogenic
626 activity of alkylphenols, bisphenol S, and their chlorinated derivatives using a GFP expression
627 system. *Environmental Toxicology and Pharmacology* 19, 121–130.

628 Le Corre, L., Besnard, P., Chagnon, M.C., 2015. BPA, an energy balance disruptor. *Crit Rev*
629 *Food Sci Nutr* 55, 769–77. <https://doi.org/10.1080/10408398.2012.678421>

630 Li, G., Chang, H., Xia, W., Mao, Z., Li, Y., Xu, S., 2014. F0 maternal BPA exposure induced
631 glucose intolerance of F2 generation through DNA methylation change in Gck. *Toxicol Lett*
632 228, 192–199. <https://doi.org/10.1016/j.toxlet.2014.04.012>

633 Litwak, S.A., Wilson, J.L., Chen, W., Garcia-Rudaz, C., Khaksari, M., Cowley, M.A.,
634 Enriori, P.J., 2014. Estradiol prevents fat accumulation and overcomes leptin resistance in
635 female high-fat diet mice. *Endocrinology* 155, 4447–60. <https://doi.org/10.1210/en.2014-1342>

636 Manikkam, M., Haque, M.M., Guerrero-Bosagna, C., Nilsson, E.E., Skinner, M.K., 2014.
637 Pesticide methoxychlor promotes the epigenetic transgenerational inheritance of adult-onset
638 disease through the female germline. *PLoS One* 9, e102091.
639 <https://doi.org/10.1371/journal.pone.0102091>

640 Manikkam, M., Tracey, R., Guerrero-Bosagna, C., Skinner, M.K., 2013. Plastics derived
641 endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance
642 of obesity, reproductive disease and sperm epimutations. *PLoS One* 8, e55387.
643 <https://doi.org/10.1371/journal.pone.0055387>

644 Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., Turner, R.C.,
645 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting
646 plasma glucose and insulin concentrations in man. *Diabetologia* 28, 412–9.

647 Mauvais-Jarvis, F., Clegg, D.J., Hevener, A.L., 2013. The role of estrogens in control of
648 energy balance and glucose homeostasis. *Endocr. Rev.* 34, 309–338.
649 <https://doi.org/10.1210/er.2012-1055>

650 Mayeuf-Louchart, A., Lancel, S., Sebti, Y., Pourcet, B., Loyens, A., Delhay, S., Duhem, C.,
651 Beauchamp, J., Ferri, L., Thorel, Q., Boulinguez, A., Zecchin, M., Dubois-Chevalier, J.,
652 Eeckhoutte, J., Vaughn, L.T., Roach, P.J., Dani, C., Pederson, B.A., Vincent, S.D., Staels, B.,
653 Duez, H., 2019. Glycogen Dynamics Drives Lipid Droplet Biogenesis during Brown
654 Adipocyte Differentiation. *Cell Rep* 29, 1410-1418.e6.
655 <https://doi.org/10.1016/j.celrep.2019.09.073>

656 Meng, Z., Wang, D., Liu, W., Li, R., Yan, S., Jia, M., Zhang, L., Zhou, Z., Zhu, W., 2019.
657 Perinatal exposure to Bisphenol S (BPS) promotes obesity development by interfering with
658 lipid and glucose metabolism in male mouse offspring. *Environ Res* 173, 189–198.
659 <https://doi.org/10.1016/j.envres.2019.03.038>

660 Meng, Z., Wang, D., Yan, S., Li, R., Yan, J., Teng, M., Zhou, Z., Zhu, W., 2018. Effects of
661 perinatal exposure to BPA and its alternatives (BPS, BPF and BPAF) on hepatic lipid and
662 glucose homeostasis in female mice adolescent offspring. *Chemosphere* 212, 297–306.
663 <https://doi.org/10.1016/j.chemosphere.2018.08.076>

664 Mochizuki, K., Hariya, N., Honma, K., Goda, T., 2017. Relationship between epigenetic
665 regulation, dietary habits, and the developmental origins of health and disease theory.
666 *Congenit Anom (Kyoto)* 57, 184–190. <https://doi.org/10.1111/cga.12213>

667 Molina-Molina, J.M., Amaya, E., Grimaldi, M., Saenz, J.M., Real, M., Fernandez, M.F.,
668 Balaguer, P., Olea, N., 2013. In vitro study on the agonistic and antagonistic activities of
669 bisphenol-S and other bisphenol-A congeners and derivatives via nuclear receptors. *Toxicol*
670 *Appl Pharmacol* 272, 127–36. <https://doi.org/10.1016/j.taap.2013.05.015> S0041-
671 008X(13)00224-X [pii]

672 Navarro, G., Allard, C., Xu, W., Mauvais-Jarvis, F., 2015. The role of androgens in
673 metabolism, obesity, and diabetes in males and females. *Obesity (Silver Spring)* 23, 713–9.
674 <https://doi.org/10.1002/oby.21033>

675 Nikaido, Y., Yoshizawa, K., Danbara, N., Tsujita-Kyutoku, M., Yuri, T., Uehara, N., Tsubura,
676 A., 2004. Effects of maternal xenoestrogen exposure on development of the reproductive tract
677 and mammary gland in female CD-1 mouse offspring. *Reprod Toxicol* 18, 803–11.
678 <https://doi.org/10.1016/j.reprotox.2004.05.002> S0890623804000899 [pii]

679 Pan, Y., Deng, M., Li, J., Du, B., Lan, S., Liang, X., Zeng, L., 2020. Occurrence and Maternal
680 Transfer of Multiple Bisphenols, Including an Emerging Derivative with Unexpectedly High
681 Concentrations, in the Human Maternal-Fetal-Placental Unit. *Environ Sci Technol* 54, 3476–
682 3486. <https://doi.org/10.1021/acs.est.0c00206>

683 Papalou, O., Kandaraki, E.A., Papadakis, G., Diamanti-Kandarakis, E., 2019. Endocrine
684 Disrupting Chemicals: An Occult Mediator of Metabolic Disease. *Front Endocrinol*
685 (Lausanne) 10, 112. <https://doi.org/10.3389/fendo.2019.00112>

686 Peshdary, V., Styles, G., Gagné, R., Yauk, C.L., Sorisky, A., Atlas, E., 2020. Depot-Specific
687 Analysis of Human Adipose Cells and Their Responses to Bisphenol S. *Endocrinology* 161.
688 <https://doi.org/10.1210/endocr/bqaa044>

689 Pivnenko, K., Pedersen, G.A., Eriksson, E., Astrup, T.F., 2015. Bisphenol A and its structural
690 analogues in household waste paper. *Waste Manag* 44, 39–47.
691 <https://doi.org/10.1016/j.wasman.2015.07.017>

692 Pottenger, L.H., Domoradzki, J.Y., Markham, D.A., Hansen, S.C., Cagen, S.Z., Waechter,
693 J.M., 2000. The relative bioavailability and metabolism of bisphenol A in rats is dependent
694 upon the route of administration. *Toxicol Sci* 54, 3–18.

695 Qiu, W., Zhan, H., Hu, J., Zhang, T., Xu, H., Wong, M., Xu, B., Zheng, C., 2019. The
696 occurrence, potential toxicity, and toxicity mechanism of bisphenol S, a substitute of
697 bisphenol A: A critical review of recent progress. *Ecotoxicol Environ Saf* 173, 192–202.
698 <https://doi.org/10.1016/j.ecoenv.2019.01.114>

699 Ramskov Tetzlaff, C.N., Svingen, T., Vinggaard, A.M., Rosenmai, A.K., Taxvig, C., 2020.
700 Bisphenols B, E, F, and S and 4-cumylphenol induce lipid accumulation in mouse adipocytes
701 similarly to bisphenol A. *Environ Toxicol* 35, 543–552. <https://doi.org/10.1002/tox.22889>

702 Rezg, R., Abot, A., Mornagui, B., Aydi, S., Knauf, C., 2018. Effects of Bisphenol S on
703 hypothalamic neuropeptides regulating feeding behavior and apelin/APJ system in mice.
704 *Ecotoxicol Environ Saf* 161, 459–466. <https://doi.org/10.1016/j.ecoenv.2018.06.001>

705 Rochester, J.R., Bolden, A.L., 2015. Bisphenol S and F: A Systematic Review and
706 Comparison of the Hormonal Activity of Bisphenol A Substitutes. *Environ Health Perspect.*
707 <https://doi.org/10.1289/ehp.1408989>

708 Roelofs, M.J., Berg, M.V., Bovee, T.F., Piersma, A.H., Duursen, M.B., 2015. Structural
709 bisphenol analogues differentially target steroidogenesis in murine MA-10 Leydig cells as
710 well as the glucocorticoid receptor. *Toxicology* 329C, 10–20. [https://doi.org/S0300-](https://doi.org/S0300-483X(15)00004-9)
711 [483X\(15\)00004-9](https://doi.org/S0300-483X(15)00004-9) [pii] 10.1016/j.tox.2015.01.003

712 Rossmeisl, M., Rim, J.S., Koza, R.A., Kozak, L.P., 2003. Variation in type 2 diabetes--related
713 traits in mouse strains susceptible to diet-induced obesity. *Diabetes* 52, 1958–1966.
714 <https://doi.org/10.2337/diabetes.52.8.1958>

715 Rubin, B.S., Murray, M.K., Damassa, D.A., King, J.C., Soto, A.M., 2001. Perinatal exposure
716 to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH
717 levels. *Environ Health Perspect* 109, 675–80. https://doi.org/sc271_5_1835 [pii]

718 Skinner, M.K., Manikkam, M., Tracey, R., Guerrero-Bosagna, C., Haque, M., Nilsson, E.E.,
719 2013. Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic
720 transgenerational inheritance of obesity. *BMC Med* 11, 228. [https://doi.org/10.1186/1741-](https://doi.org/10.1186/1741-7015-11-228)
721 7015-11-228

722 Skvortsova, K., Iovino, N., Bogdanović, O., 2018. Functions and mechanisms of epigenetic
723 inheritance in animals. *Nat Rev Mol Cell Biol* 19, 774–790. [https://doi.org/10.1038/s41580-](https://doi.org/10.1038/s41580-018-0074-2)
724 018-0074-2

725 Song, Y., Xie, P., Cai, Z., 2017. Metabolism of bisphenol S in mice after oral administration.
726 *Rapid Commun Mass Spectrom.* <https://doi.org/10.1002/rcm.8051>

727 Surwit, R.S., Feinglos, M.N., Rodin, J., Sutherland, A., Petro, A.E., Opara, E.C., Kuhn, C.M.,
728 Rebuffé-Scrive, M., 1995. Differential effects of fat and sucrose on the development of
729 obesity and diabetes in C57BL/6J and A/J mice. *Metabolism* 44, 645–651.
730 [https://doi.org/10.1016/0026-0495\(95\)90123-x](https://doi.org/10.1016/0026-0495(95)90123-x)

731 Thoene, M., Dzika, E., Gonkowski, S., Wojtkiewicz, J., 2020. Bisphenol S in Food Causes
732 Hormonal and Obesogenic Effects Comparable to or Worse than Bisphenol A: A Literature
733 Review. *Nutrients* 12. <https://doi.org/10.3390/nu12020532>

734 Thon, M., Hosoi, T., Ozawa, K., 2016. Possible Integrative Actions of Leptin and Insulin
735 Signaling in the Hypothalamus Targeting Energy Homeostasis. *Front Endocrinol (Lausanne)*
736 7, 138. <https://doi.org/10.3389/fendo.2016.00138>

737 Titchenell, P.M., Lazar, M.A., Birnbaum, M.J., 2017. Unraveling the Regulation of Hepatic
738 Metabolism by Insulin. *Trends Endocrinol Metab* 28, 497–505.
739 <https://doi.org/10.1016/j.tem.2017.03.003>

740 Tracey, R., Manikkam, M., Guerrero-Bosagna, C., Skinner, M.K., 2013. Hydrocarbons (jet
741 fuel JP-8) induce epigenetic transgenerational inheritance of obesity, reproductive disease and
742 sperm epimutations. *Reprod Toxicol* 36, 104–116.
743 <https://doi.org/10.1016/j.reprotox.2012.11.011>

744 Vaanholt, L.M., Sinclair, R.E., Mitchell, S.E., Speakman, J.R., 2015. Factors influencing
745 individual variability in high fat diet-induced weight gain in out-bred MF1 mice. *Physiol*
746 *Behav* 144, 146–155. <https://doi.org/10.1016/j.physbeh.2015.03.029>

747 van Dijk, T.H., Laskewitz, A.J., Grefhorst, A., Boer, T.S., Bloks, V.W., Kuipers, F., Groen,
748 A.K., Reijngoud, D.J., 2013. A novel approach to monitor glucose metabolism using stable
749 isotopically labelled glucose in longitudinal studies in mice. *Lab Anim* 47, 79–88.
750 <https://doi.org/10.1177/0023677212473714> 0023677212473714 [pii]

751 van Esterik, J.C.J., Dollé, M.E.T., Lamoree, M.H., van Leeuwen, S.P.J., Hamers, T., Legler,
752 J., van der Ven, L.T.M., 2014. Programming of metabolic effects in C57BL/6JxFVB mice by
753 exposure to bisphenol A during gestation and lactation. *Toxicology* 321, 40–52.
754 <https://doi.org/10.1016/j.tox.2014.04.001>

755 Yang, Yunjia, Yang, Yi, Zhang, J., Shao, B., Yin, J., 2019. Assessment of bisphenol A
756 alternatives in paper products from the Chinese market and their dermal exposure in the
757 general population. *Environ Pollut* 244, 238–246.
758 <https://doi.org/10.1016/j.envpol.2018.10.049>

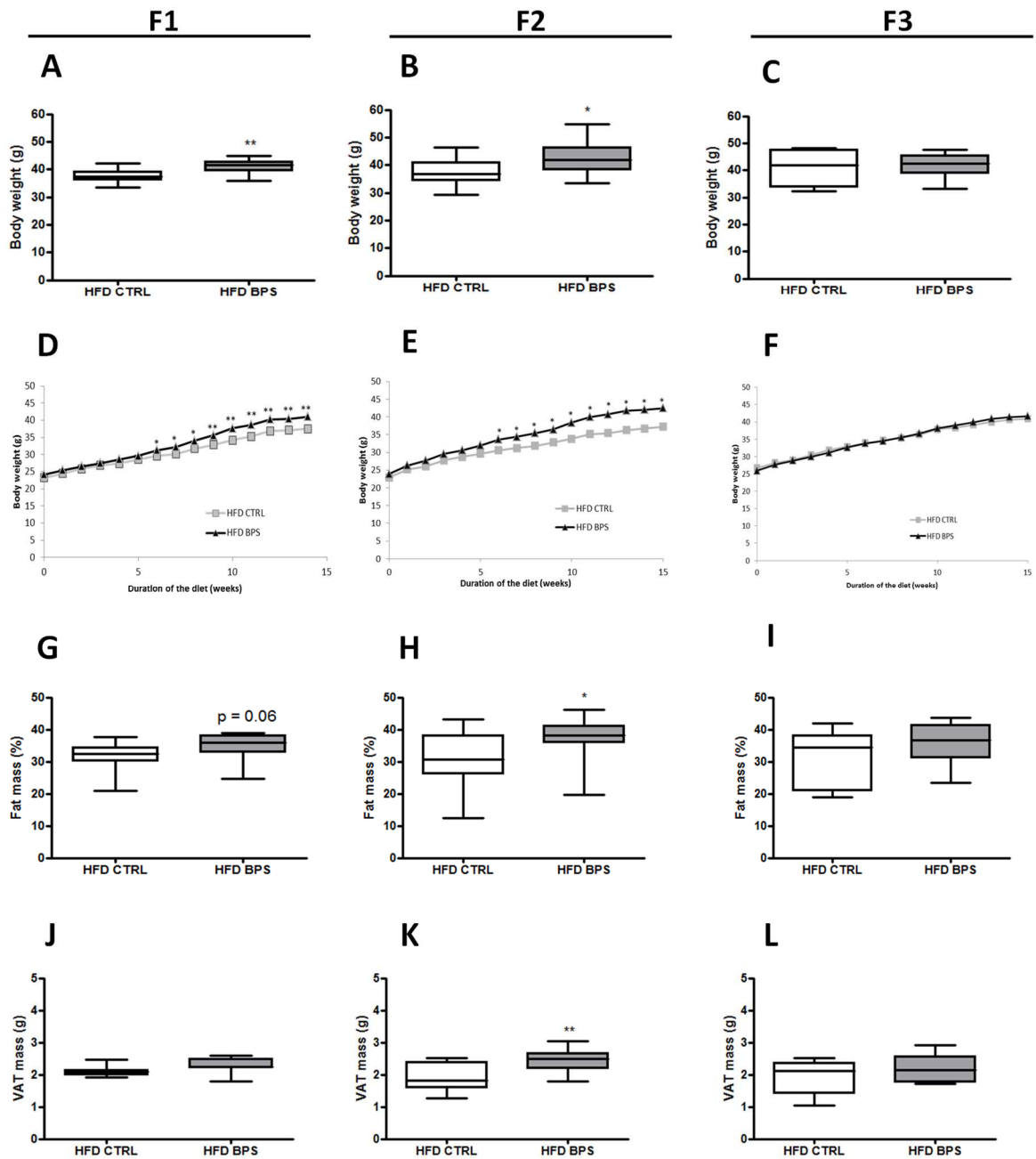
759 Ye, X., Wong, L.-Y., Kramer, J., Zhou, X., Jia, T., Calafat, A.M., 2015. Urinary
760 Concentrations of Bisphenol A and Three Other Bisphenols in Convenience Samples of U.S.
761 Adults during 2000-2014. *Environ Sci Technol* 49, 11834–11839.
762 <https://doi.org/10.1021/acs.est.5b02135>

763 Zhang, H., Quan, Q., Zhang, M., Zhang, N., Zhang, W., Zhan, M., Xu, W., Lu, L., Fan, J.,
764 Wang, Q., 2020. Occurrence of bisphenol A and its alternatives in paired urine and indoor
765 dust from Chinese university students: Implications for human exposure. *Chemosphere* 247,
766 125987. <https://doi.org/10.1016/j.chemosphere.2020.125987>

767

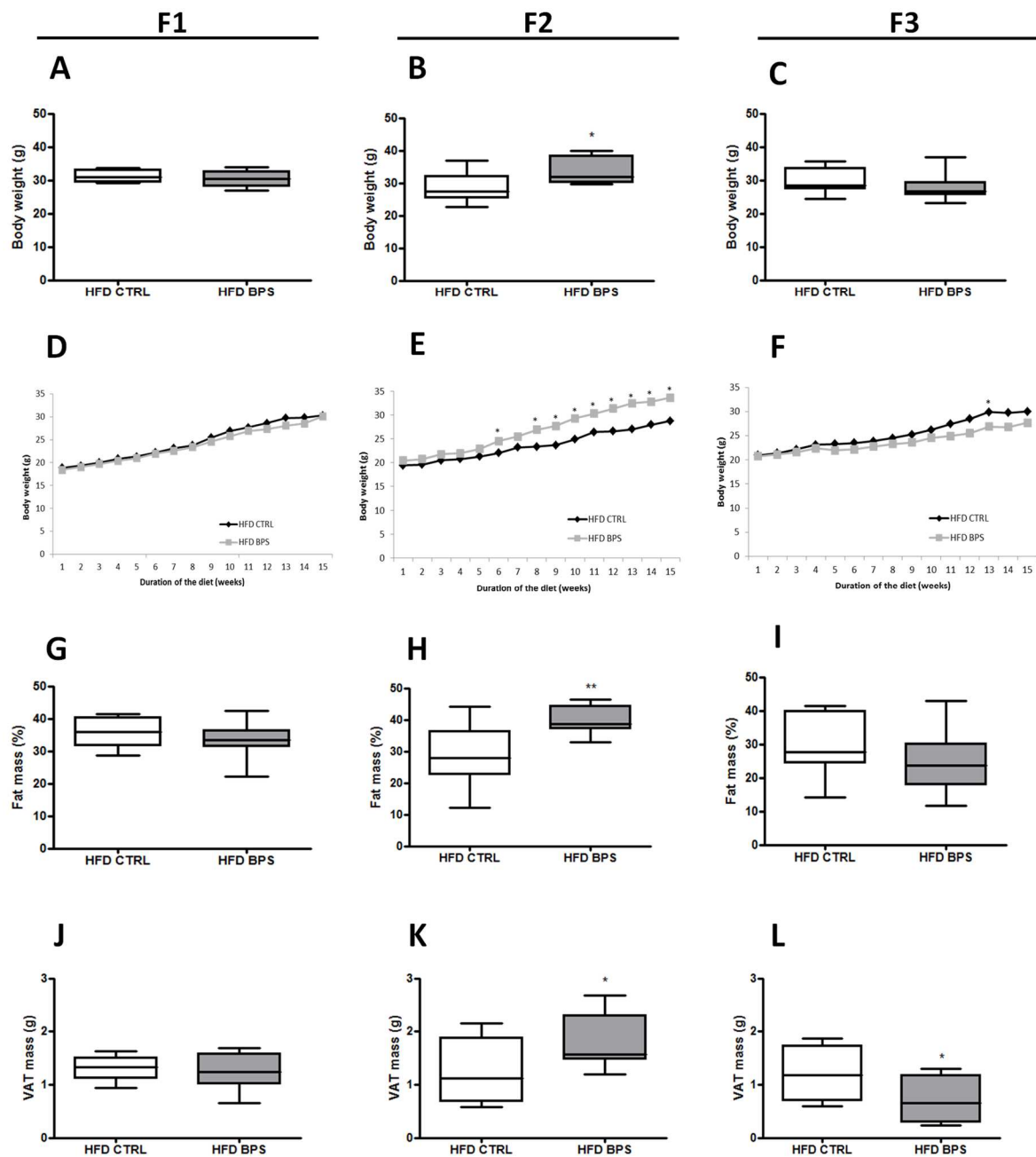
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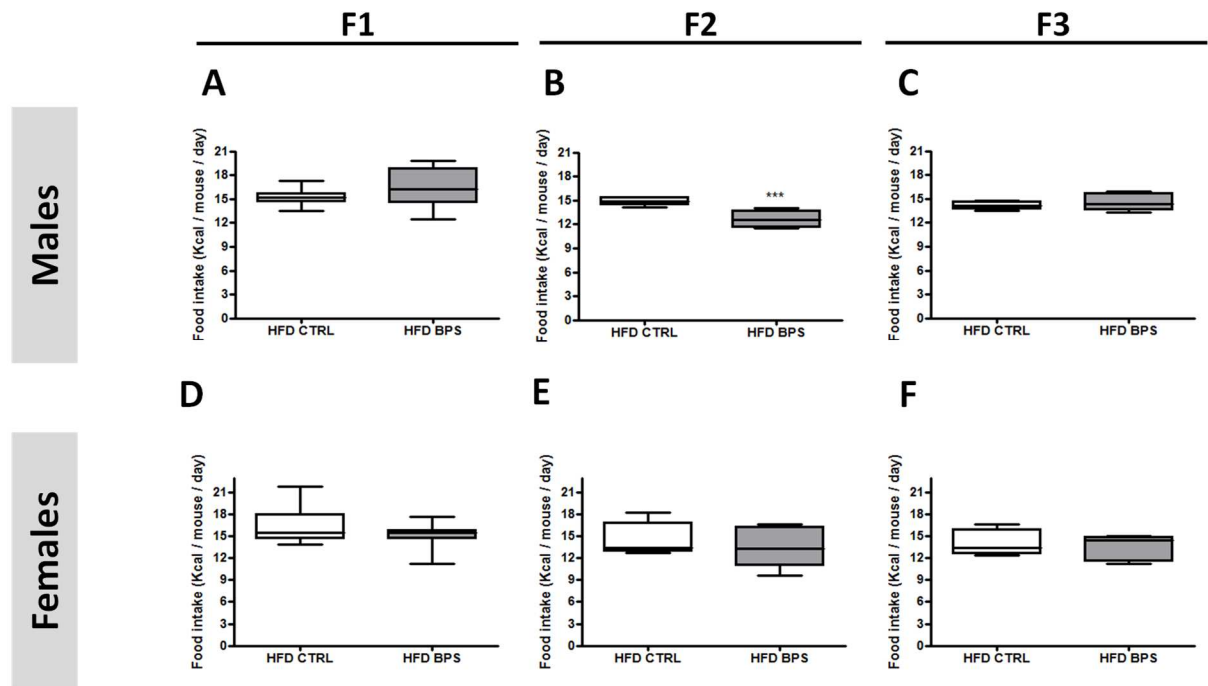
770 Figure 1: Transgenerational study of the BPS effects on body weight and adiposity in C57Bl/6J male
 771 mice: box plot displaying the 1st (lower line of the box), 2nd (horizontal line inside the box), 3rd
 772 quartiles (upper line of the box) and the interquartile (used as dispersion indicator) for long term
 773 follow-up of body weights (A - C), kinetics of weight gains (D – F), adiposity (G – I) and visceral
 774 adipose tissue (VAT) mass (J – L) in C57Bl/6J male mice after 15 weeks of high-fat diet and which
 775 are derived from F0 females exposed or not to BPS at 1.5 $\mu\text{g}/\text{kg}$ body weight/day. Measurements were

776 performed at 23 week-old. Values represent means \pm SEM. Unpaired *t*-test were performed; * $p \leq$
 777 0.05, ** $p \leq 0.01$. n = 12/group

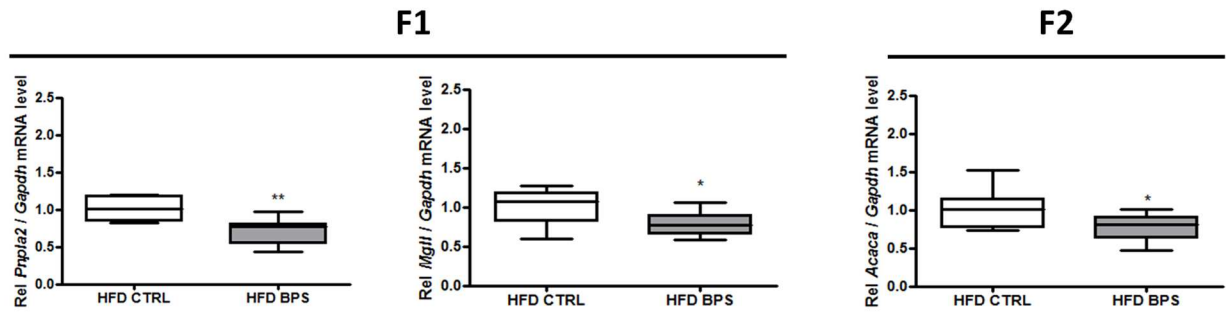


778 Figure 2: Transgenerational study of the BPS effects on body weight and adiposity in C57Bl/6J female
 779 mice: box plot displaying the 1st (lower line of the box), 2nd (horizontal line inside the box), 3rd
 780 quartiles (upper line of the box) and the interquartile (used as dispersion indicator) for long term
 781 follow-up of body weights (A - C), kinetics of weight gains (D – F), adiposity (G – I) and visceral
 782 adipose tissue (VAT) mass (J – L) in C57Bl/6J female mice after 15 weeks of high-fat diet and which
 783 are derived from F0 females exposed or not to BPS at 1.5 μ g/kg body weight/day. Measurements were

784 performed at 23 week-old. Values represent means \pm SEM. Unpaired *t*-test were performed; * $p \leq$
785 0.05, ** $p \leq 0.01$. $n = 12/\text{group}$
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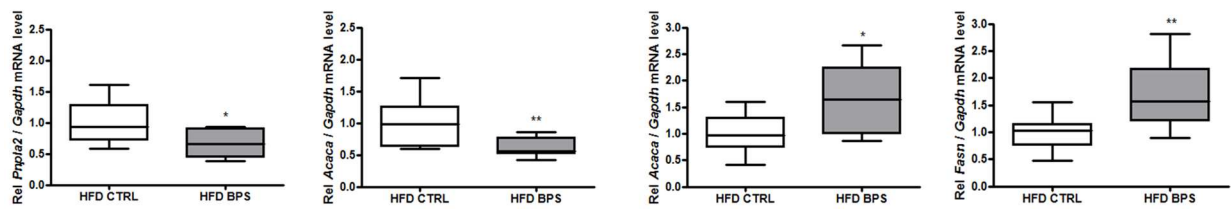


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788 Figure 3: Transgenerational study of the BPS effects on food intake in C57Bl/6J female and male
789 mice: box plot displaying the 1st (lower line of the box), 2nd (horizontal line inside the box), 3rd
790 quartiles (upper line of the box) and the interquartile (used as dispersion indicator) in C57Bl/6J male
791 (A – C) or female (D – F) mice after 8 weeks of high-fat diet and which are derived from F0 females
792 exposed or not to BPS at 1.5 $\mu\text{g}/\text{kg}$ body weight/day. Measurements were performed at 14 week-old.
793 Values represent means \pm SEM. Unpaired *t*-test were performed; *** $p \leq 0.001$. $n = 12/\text{group}$



794 Figure 4: Transgenerational study of the BPS effects on adipose tissue lipid metabolism marker genes
 795 expression in C57Bl/6J male mice: box plot displaying the 1st (lower line of the box), 2nd (horizontal
 796 line inside the box), 3rd quartiles (upper line of the box) and the interquartile (used as dispersion
 797 indicator) in C57Bl/6J male mice after 15 weeks of high-fat diet and which are derived from F0
 798 females exposed or not to BPS at 1.5 $\mu\text{g}/\text{kg}$ body weight/day. Measurements were performed at 23
 799 week-old. Values represent means \pm SEM. Unpaired *t*-test were performed; * $p \leq 0.05$, ** $p \leq 0.01$. n
 800 = 12/group.

801

F2**F3**

802 Figure 5: Transgenerational study of the BPS effects on adipose tissue lipid metabolism marker genes
803 expression in C57Bl/6J female mice: box plot displaying the 1st (lower line of the box), 2nd (horizontal
804 line inside the box), 3rd quartiles (upper line of the box) and the interquartile (used as dispersion
805 indicator) in C57Bl/6J female mice after 15 weeks of high-fat diet and which are derived from F0
806 females exposed or not to BPS at 1.5 $\mu\text{g}/\text{kg}$ body weight/day. Measurements were performed at 23
807 week-old. Values represent means \pm SEM. Unpaired *t*-test were performed; * $p \leq 0.05$, ** $p \leq 0.01$. n
808 = 12/group.

809 Table 1: Transgenerational study (F1; F2; F3) of BPS effects on plasma parameters related to
 810 blood glucose and lipid metabolism in male mice. NEFA: No Esterified Fatty Acids.
 811 Measurements were performed at 23 week-old. Values represent means \pm SEM. Paired
 812 student *t* test was performed; ***p* < 0.01; ****p* < 0.001. n = 12/group

	F1		F2		F3	
Plasma level at euthanasia	HFD CTRL	HFD BPS	HFD CTRL	HFD BPS	HFD CTRL	HFD BPS
Blood glucose (mg/dl)	181.92 \pm 11.44	185.58 \pm 8.66	159.5 \pm 6.32	183 \pm 5.72**	175.9 \pm 9.34	176.25 \pm 8.33
Insulin (μ g/l)	2.23 \pm 0.50	3.03 \pm 0.43	1.39 \pm 0.28	1.39 \pm 0.30	1.69 \pm 0.46	1.15 \pm 0.23
HOMA-IR	2.36 \pm 0.73	3.69 \pm 0.53	0.91 \pm 0.2	1.05 \pm 0.2	2.77 \pm 0.89	2.15 \pm 0.55
NEFA (mmol/l)	0.35 \pm 0.20	0.29 \pm 0.07	0.75 \pm 0.18	0.99 \pm 0.23	0.49 \pm 0.03	0.51 \pm 0.10
Cholesterol (g/l)	1.31 \pm 0.05	1.05 \pm 0.07**	1.07 \pm 0.04	1.2 \pm 0.04**	1.25 \pm 0.07	1.35 \pm 0.07
Triglycerides (g/l)	0.7 \pm 0.06	0.64 \pm 0.04	0.5 \pm 0.04	0.75 \pm 0.06***	0.57 \pm 0.06	0.7 \pm 0.05

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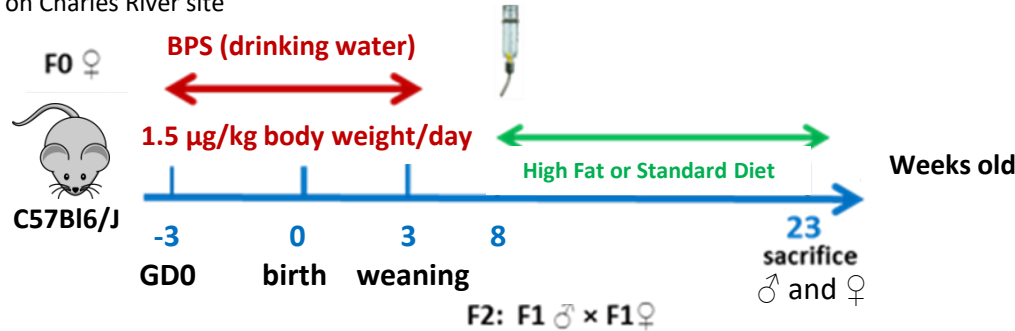
814 Table 2: Transgenerational study (F1; F2; F3) of BPS effects on plasma parameters related to
 815 blood glucose and lipid metabolism in female mice. NEFA: No Esterified Fatty Acids.
 816 Measurements were performed at 23 week-old. Values represent means \pm SEM. Paired
 817 student *t* test was performed; **p* < 0.05; ****p* < 0.001. n = 12/group

	F1		F2		F3	
Plasma level at euthanasia	HFD CTRL	HFD BPS	HFD CTRL	HFD BPS	HFD CTRL	HFD BPS
Blood glucose (mg/dl)	159.36 \pm 6.12	156.5 \pm 7.34	156 \pm 6.65	158.11 \pm 6.16	146.92 \pm 6.13	131.5 \pm 3.38*
Insulin (μ g/l)	0.94 \pm 0.18	0.88 \pm 0.15	1.58 \pm 0.29	1.42 \pm 0.26	0.83 \pm 0.23	0.78 \pm 0.21
HOMA-IR	1.29 \pm 0.22	1.27 \pm 0.21	1.49 \pm 0.34	1.48 \pm 0.27	1.43 \pm 0.75	1.47 \pm 0.39
NEFA (mmol/l)	0.47 \pm 0.12	0.47 \pm 0.18	0.44 \pm 0.13	0.95 \pm 0.12*	0.63 \pm 0.11	0.67 \pm 0.22
Cholesterol (g/l)	0.62 \pm 0.04	0.86 \pm 0.03***	0.75 \pm 0.06	0.9 \pm 0.05	0.92 \pm 0.03	0.92 \pm 0.04
Triglycerides (g/l)	0.34 \pm 0.04	0.49 \pm 0.05*	0.4 \pm 0.03	0.45 \pm 0.02	0.5 \pm 0.03	0.46 \pm 0.04

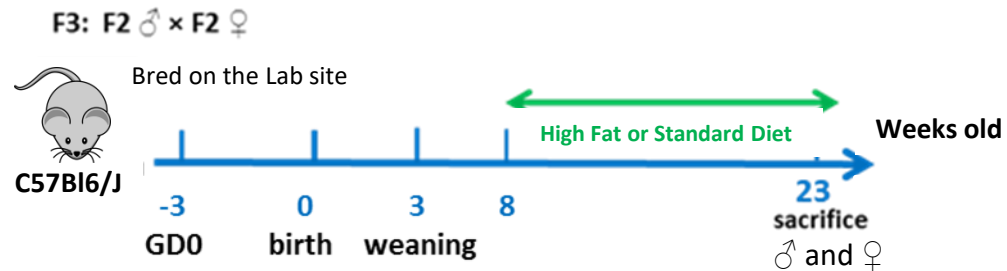
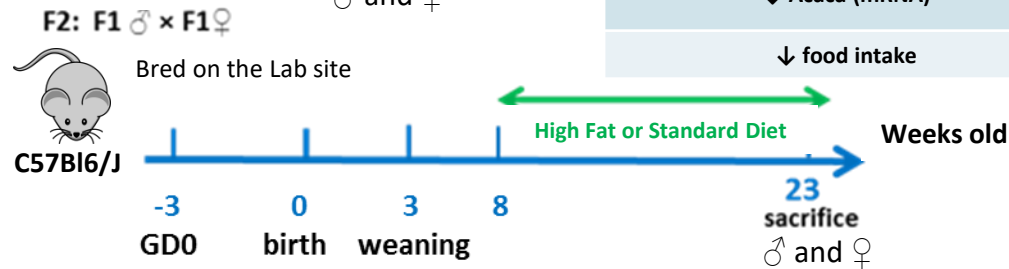
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F1 Males	F1 Females
↑ bw	↑ cholesterol / TG (plasma)
↓ cholesterol (plasma)	↓ estradiol (plasma)
↓ Pnpla2 and MglI (mRNA)	

Bred on Charles River site



F2 Males	F2 Females
↑ bw, fat mass, VAT mass	↑ bw, fat mass, VAT mass
↑ blood glucose, TG, cholesterol (plasma)	↑ NEFA, estradiol (plasma)
↓ Acaca (mRNA)	↓ Pnpla2 and Acaca (mRNA)
↓ food intake	



F3 Females
↓ VAT mass
↑ Acaca and Fasn (mRNA)

bw : body weight, VAT : Visceral Adipose Tissue, NEFA : No Esterified Fatty Acid, TG : Triglyceride, Pnpla2 : Patatin Like Phospholipase Domain Containing 2, MglI : Monoglyceride Lipase, Acaca : Acetyl-CoA Carboxylase Alpha, Fasn : Fatty Acid Synthase, GDO : Gestational Day 0