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1 **Preconception exposure of adult male rats to bisphenol S impairs insulin**  
2 **sensitivity and glucose tolerance in their male offspring**

3

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18 **Running title:** Paternal bisphenol S exposure affects glucose homeostasis in offspring

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## 32 **Abstract**

33 Since the use of bisphenol A (BPA) has been restricted because of its endocrine disruptor  
34 properties, bisphenol S (BPS) has been widely used as a substitute of BPA. However, BPS exerts  
35 similar effects on metabolic health as BPA. The effects of maternal exposure to BPA and BPS on  
36 the metabolic health of offspring have been largely documented during the past decade. However,  
37 the impact of preconception paternal exposure to BPS on progenies remains unexplored. In this  
38 study we investigated the impact of paternal exposure to BPS before conception, on the  
39 metabolic phenotype of offspring. Male Wistar rats were administered BPS through drinking  
40 water at the dose of 4 µg/kg/day (BPS-4 sires) or 40 µg/kg/day (BPS-40 sires) for 2 months  
41 before mating with females. The progenies (F1) were studied at fetal stage and in adulthood. We

42 showed that preconception paternal exposure to BPS for 2 months did not alter the metabolic  
43 status of sires. The female offspring of sires exposed to lower or higher doses of BPS showed no  
44 alteration of their metabolic phenotype compared to females from control sires. In contrast, male  
45 offspring of BPS-4 sires exhibited increased body weight and body fat/lean ratio, decreased  
46 insulin sensitivity and increased glucose-induced insulin secretion at adult age, compared to the  
47 male offspring of control sires. Moreover, male offspring of BPS-4 sires developed glucose  
48 intolerance later in life. None of these effects were apparent in male offspring of BPS-40 sires.  
49 In conclusion, our study provides the first evidence of the non-monotonic and sex-specific effects  
50 of preconception paternal exposure to BPS on the metabolic health of offspring, suggesting that  
51 BPS is not a safe BPA substitute regarding the inter-generational transmission of metabolic  
52 disorders through the paternal lineage.

53 **Key words:** Paternal programming, bisphenol S, insulin sensitivity, glucose tolerance, insulin  
54 secretion

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## 60 **Introduction**

61 The endocrine system plays an important role in maintaining body's homeostasis. The disruption  
62 of the endocrine system adversely affects physiological activities in our body. It is estimated that

63 there are about 800 man-made chemicals potentially acting as endocrine disruptors, including  
64 bisphenols (Encarnaç o et al., 2019).

65 Bisphenol A (BPA) is a compound widely used in plastic products, food packaging, thermal  
66 paper, dental materials, *etc* (Holtcamp, 2011; Vandenberg et al., 2007). BPA mimics estrogen  
67 action by binding to estrogen receptors and is considered as an endocrine disruptor (Barros et al.,  
68 2006; Livingstone and Collison, 2002). Based on toxicological assessments, in 2015, the  
69 tolerable daily intake (TDI) for BPA has been lowered from 50  $\mu\text{g}/\text{kg}/\text{day}$  to 4  $\mu\text{g}/\text{kg}/\text{day}$  by  
70 European Food Safety Authority (EFSA). Further, in 2017, European Chemicals Agency  
71 (ECHA) added BPA to the candidate list of Substances of Very High Concern (SVHC) because  
72 of its endocrine disruptor properties (Beausoleil et al., 2018). In addition, experimental and  
73 epidemiological evidence show that BPA exposure induces metabolic disorders (Azevedo et al.,  
74 2019; Lind and Lind, 2018; Moon et al., 2015). To face the increasing concern regarding BPA,  
75 several substitutes have been used by the industry to replace this bisphenol in various products.  
76 Bisphenol S (4,4'-solfonyldiphenol, abbreviated as BPS), one of the substitutes of BPA, is  
77 ubiquitous in environment and can be readily found in plastic and epoxy resin products labeled  
78 "BPA-free" (Wu et al., 2018). The concentration and frequency of BPS detected in human urine  
79 was almost comparable to that of BPA, as BPS was detectable in 81% of 315 urine samples  
80 collected from different countries (Liao et al., 2012; Zhou et al., 2014). BPS has higher  
81 bioavailability and 3.5 times lower plasma clearance compared to the same exposure level of  
82 BPA, suggesting that BPS may be more hazardous than BPA for human health (Gayrard et al.,  
83 2019). BPS binds to estrogen receptors  $\text{ER}\alpha$  and  $\text{ER}\beta$  with a 1000 to 2000-fold lower affinity  
84 than estradiol, but induces similar or even stronger estrogen-like effects (Acconcia et al., 2015).

85 The effects of BPS on glucose and energy metabolism have been investigated during the past  
86 decade. It has been shown that BPS has a higher capacity than BPA in promoting body weight

87 increase (Azevedo et al., 2019), adipocyte differentiation and lipid accumulation (Martínez et al.,  
88 2020). Further, investigation of generational transmission of metabolic disorders revealed that  
89 maternal exposure to BPA (Alonso-Magdalena et al., 2010; Angle et al., 2013; Bansal et al.,  
90 2017; García-Arevalo et al., 2014; Susiarjo et al., 2015) or BPS (Ivry Del Moral et al., 2016; da  
91 Silva et al., 2019; Ahn et al., 2020; Brulport et al., 2021) increased the predisposition to obesity  
92 and metabolic disorders in the progeny. Importantly, in these studies, the effects of maternal  
93 exposure to BPA or BPS on their descendants are sex-specific, with a higher vulnerability of  
94 male progenies. More recently, the question of the effects of paternal exposure to BPA on the  
95 metabolic health of offspring has been raised (Mao et al., 2015; Rashid et al., 2020), but to date,  
96 the information regarding this question remains scarce and no studies have investigated the  
97 effects of other bisphenols such as BPS on the transmission of metabolic disorders through the  
98 paternal lineage.

99 In this study, we investigated the consequences of paternal BPS exposure at environmentally  
100 relevant doses, on glucose and energy metabolism of their descendants. We demonstrated for the  
101 first time that the exposure of adult male rats to BPS exerted sex- and dose-specific effects on  
102 insulin sensitivity and glucose tolerance of their descendants at adult age, providing yet  
103 additional evidence for the detrimental effects of paternal exposure to BPS on the metabolic  
104 health of their progeny.

## 105 **Materials and Methods**

### 106 *Animals and diets*

107 Male and female Wistar rats were obtained from our local colony maintained at Université Paris  
108 Cité animal core facility. Rats were housed in a controlled environment (temperature  $20\pm 1^\circ\text{C}$ ,  
109 humidity 60%) with 12 h light/dark cycle, and were given *ad libitum* access to diet and water.

110 The diet used in this study was SAFE D113, composed of 45% carbohydrate, 18.9% protein and  
111 4.5% fat (SAFE, Villemoisson-sur-Ogre, France). Experimental protocol was approved by  
112 Université Paris Cité Buffon ethical committee (n° CEB 09-2018). Animal experiments have  
113 been carried out in accordance with France Directive n° 2013-118 in application of EU Directive  
114 2010/63/EU.

### 115 *Experimental design*

116 Fourteen weeks old male Wistar rats of equal body weight (approximately 400 g) were randomly  
117 allocated into three groups and given water containing bisphenol S (BPS) diluted in 0.1% v/v  
118 ethanol or water containing 0.1% v/v ethanol (vehicle, Ctrl sires). BPS (SIGMA-ALDRICH, St.  
119 Louis, USA) was used at the dose of 4 µg/kg/day (BPS-4 sires) or 40 µg/kg/day (BPS-40 sires).  
120 Converted to human equivalents (Nair and Jacob, 2016), the above doses are respectively 0.648  
121 µg/kg/day and 6.48 µg/kg/day, after applying the conversion coefficient. Knowing that the TDI  
122 for humans is 4 µg/kg/day, the doses we used in this study are much below the TDI (for the dose  
123 4 µg/kg/day) or similar to human TDI (for the dose 40 µg/kg/day). The concentration of BPS in  
124 drinking water was calculated according to the daily water consumption of rats, determined in a  
125 group of rats of the same age and body weight in the previous two weeks. Thus, throughout the  
126 study, the amount of BPS added to water was calculated every two weeks based on the water  
127 consumption and body weight of the rats. Fresh BPS solution was bottled twice a week. To ensure  
128 the absence of bisphenol in the environment, the cages and drinking bottles were made of  
129 polypropylene and polyphenylsulfone, respectively.

130 Body weight, blood glucose levels, food and water consumption were monitored weekly. Blood  
131 glucose was tested on samples from the tail vein, by Accu-Check® blood glucose test strips  
132 (Roche Diagnostics, Meylan, France) and Accu-Check® Aviva glucometers (Roche Diagnostics,  
133 Meylan, France). After 2 months of continuous BPS administration, rats (F0: Ctrl sires, BPS-4

134 sires and BPS-40 sires) were mated with 2-month-old virgin female Wistar rats fed with regular  
135 BPS-free water. The progenies (F1) were studied at fetal stage and at 3, 6 or 9 months (Fig. 1).

136 For fetal studies, male and female fetuses from Ctrl, BPS-4 and BPS-40 sires were recovered at  
137 the embryonic day 18 post-conception (E18). Body weight and body nose-to-tail length of  
138 fetuses were measured. Pancreases were collected and weighed, then processed for histological  
139 analyses.

140 At birth, litters were restricted to 8-9 pups per dam. After weaning, the progenies of sires from  
141 each specific group were randomly mixed to minimize possible litter effect. They were fed with  
142 standard diet (SD) and had regular BPS-free water. For different analyses, the progenies from at  
143 least four different fathers were used for each experimental group (Ctrl, BPS-4, BPS-40) and  
144 were named as Ctrl/F1, BPS-4/F1 and BPS-40/F1, respectively. Male and female progenies were  
145 studied separately. Body weight, blood glucose and food consumption were monitored weekly  
146 from weaning until the day of euthanasia. Liver, soleus muscle and epididymal adipose tissue of  
147 10-month-old male F1 from BPS-4 and Ctrl groups were dissected, then immediately frozen in  
148 liquid nitrogen and stored at -80°C until analysis. Pancreases were dissected and processed for  
149 histological analyses.

#### 150 ***Body composition***

151 Body composition of sires and offspring was determined by a positron emission tomographic  
152 scanner (EchoMRI, Houston Texas, USA). Calibration was performed according to the  
153 manufacturer's guidelines before each measurement.

#### 154 ***Insulin tolerance test***

155 After 6 h of fasting with access to water, insulin tolerance tests (ITT) were performed on non-  
156 anaesthetized Wistar rats. Human insulin (Novo Nordisk A/S, Puteaux, France) was injected

157 intraperitoneally at the dose of 0.5 U/kg body weight. Blood glucose concentrations were  
158 determined using an Accu-Check® Glucometer (Roche Diagnostics, Meylan, France) in blood  
159 samples collected at the tip of the tail at times 0, 15, 30, 60, 90, 120 min after administration of  
160 insulin.

#### 161 *Glucose tolerance test*

162 One week after insulin tolerance test, rats underwent an intraperitoneal glucose tolerance test  
163 (IPGTT). IPGTT (2 g glucose/kg body weight) was performed on non-anaesthetized rats. Blood  
164 was collected at the tail vein before (0 min), and 15, 30, 60, 90 and 120 min after administration  
165 of glucose. Blood glucose concentration was determined using an Accu-Chek® Glucometer  
166 (Roche Diagnostics, Meylan, France). Moreover, plasma samples from blood collected at 0, 15,  
167 30 and 90 minutes were stored at -20°C for insulin determination using an enzyme-linked  
168 immunosorbent assay ELISA (ALPCO/Eurobio, Les Ulis, France).

#### 169 *Pyruvate tolerance test*

170 The rats were fasted overnight prior to pyruvate tolerance test (PTT). Intraperitoneal pyruvate  
171 tolerance test (1 g sodium pyruvate/kg body weight) was performed on non-anaesthetized male  
172 F1 rats at the age of 9 months. Blood was collected at the tail vein before (0 min), and 15, 30,  
173 60, 90, and 120 min after administration of pyruvate. Blood glucose concentrations were  
174 determined using an Accu-Check® Glucometer (Roche Diagnostics, Meylan, France).

#### 175 *Plasma lipid analysis*

176 Plasma samples of male F1 at the age of 3 and 9 months were collected at 9:00 a.m. for the  
177 measurement of cholesterol, high-density lipoprotein (HDL), triglycerides and non-esterified  
178 fatty acids (NEFA). Samples were tested in CRI-Plateforme de Biochimie in UMR 1149  
179 Université Paris Cité.

180 ***Histological studies***

181 Pancreases were fixed in aqueous Bouin solution for 24 h and embedded in paraffin (Labonord,  
182 Templemars, France). Each paraffin block was serially sectioned (5  $\mu$ m) throughout its full-  
183 length. The entire pancreas was homogeneously sampled using a fix-interval sampling method  
184 to avoid bias due to regional variations in islets distribution, as previously described (Movassat  
185 et al., 1997). Sections at a fixed interval were mounted on slides and stored at room temperature  
186 for future immunostaining. Hydrated pancreatic sections were incubated with 10% normal goat  
187 serum in Tris Buffer Saline (TBS) for 1 hour, then probed for insulin using primary mouse anti-  
188 insulin antibody (Santa Cruz Biotechnology/Cliniscience, Nanterre, France, 1:200) and glucagon  
189 in the neighboring section using primary rabbit anti-glucagon antibody (eBioscience/  
190 Cliniscience, Nanterre, France, dilution 1:200). Sections were then incubated with an HRP  
191 conjugated anti-mouse (Jackson immunoResearch Laboratories/Interchim, Montlucon, France,  
192 dilution 1:200) or anti-rabbit secondary antibody (Boster Biological Technology/Interchim,  
193 Montlucon, France, dilution 1:200). The area of  $\alpha$  cells and  $\beta$  cells were determined by  
194 morphometric analysis in pancreatic sections stained for glucagon or insulin respectively, using  
195 an OLYMPUS BX60 microscope, equipped with a computer-assisted image analysis system  
196 software Histolab 10.5.1 (Microvision Instrument, Evry, France). The relative  $\alpha$  cell or  $\beta$  cell  
197 area in each stained section was calculated by the ratio of glucagon or insulin positive cells area  
198 over the total pancreatic cells area (Movassat et al., 1997; Figeac et al., 2009). The mass of  $\alpha$  and  
199  $\beta$  cells was calculated by multiplying the  $\alpha$  cell or  $\beta$  cell area ratio by the pancreas weight. At  
200 least six homogeneously distributed sections were analyzed for each pancreas.

201 ***Histological analysis of the epididymal adipose tissue***

202 To investigate the effect of BPS on adipocyte, histological analysis of epididymal white adipose  
203 tissue (eWAT) was performed in male Ctrl/F1, BPS-4/F1 and BPS-40/F1. The eWAT sections

204 were fixed in aqueous Bouin solution for 24 h and embedded in paraffin (Labonord, Templemars,  
205 France). Each paraffin block was serially sectioned (5 µm) throughout its length, as previously  
206 described. The sections were deparaffinized and hydrated, then stained with Papanicolaou's  
207 solution 1a Harris' hematoxylin solution. Stained slides were analyzed using Histolab 10.5.1  
208 (Microvision Instrument, Evry, France) to measure the adipocyte diameter. Six rats were  
209 analyzed in each group. Six sections were analyzed for each rat.

### 210 *Pancreatic insulin content*

211 For the determination of insulin content, pancreases were homogenized in acid/alcohol solution  
212 (75% ethanol, 1.5% 32.5 mol/L HCl and 23.5% distilled water) and centrifuged (1500 g) at 4°C,  
213 then incubated at 4°C overnight. The supernatant fraction was stored at -20°C until insulin was  
214 assayed by ELISA (ALPCO/Eurobio; Les Ulis, France).

### 215 *Islet isolation*

216 Pancreases from 10-month-old male Ctrl/F1 rats and BPS-4/F1 rats (six rats in each group) were  
217 digested by Liberase TL Research Grade (Roche LifeScience/Sigma-Aldrich, St. Quentin  
218 Fallavier, France) in Hank's balanced salt solutions (137 mM NaCl, 5.36 mM KCl, 4.17 mM  
219 NaHCO<sub>3</sub>, 0.88 mM MgSO<sub>4</sub>, 0.44 mM KH<sub>2</sub>PO<sub>4</sub>, 0.34 mM Na<sub>2</sub>PO<sub>4</sub>, 1.27 mM CaCl<sub>2</sub>, 10 mM  
220 Hepes) at 37°C for 13 min. Islets of Langerhans were purified from exocrine tissue by  
221 discontinuous density-gradient centrifugation on Histopaque 1077 (Sigma-Aldrich, St. Quentin  
222 Fallavier, France). Islets were hand-picked under a stereo microscope and divided into different  
223 batches for insulin secretion studies.

### 224 *Measurement of ex vivo glucose-induced insulin secretion*

225 Islets were isolated from 10-month-old male Ctrl/F1 and BPS-4/F1 rats. Batches of 10 islets  
226 were pre-incubated in KRBH (Krebs-Ringer/bicarbonate/Hepes) containing 0.2% fatty acid free

227 BSA (Roche LifeScience/Sigma-Aldrich, St. Quentin Fallavier, France) with 2.8 mM glucose  
228 for 1 h, followed by 1 h incubation in KRBH 0.2% fatty acid free BSA with 2.8 or 16.7 mM  
229 glucose to measure glucose-induced insulin secretion (GIIS). Supernatants were then collected  
230 and stored at -20°C until the determination of insulin concentration by ELISA (ALPCO/Eurobio,  
231 Les Ulis, France). Islets were collected and stored at -80°C for DNA content determination (Liu  
232 et al., 2015).

### 233 *Insulin signaling pathway*

234 Male Ctrl/F1 and BPS-4/F1 rats of 10 months old were fasted overnight. The right-side soleus  
235 muscle and epididymal adipose tissue of F1 rats were collected under anesthesia, followed by  
236 intraperitoneal insulin (Novo Nordisk A/S, Puteaux, France) injection at 10 U/kg body weight.  
237 After 25 minutes, the left-side soleus muscle and epididymal adipose tissue were collected. The  
238 tissues were stored at -80°C for future western blots analysis.

### 239 *Western blotting*

240 Muscle and adipose tissue lysates were prepared by sonication in RIPA lysis buffer (Cell  
241 Signaling Technology, Saint-Cyr-L'École, France). Fifteen µg protein were resolved on 10%  
242 SDS PAGE gel and then transferred to PVDF membranes. Membranes were blocked for non-  
243 specific binding with 5% BSA in Tris Buffered Saline-Tween (TBST) and incubated with anti-  
244 phospho-Akt (Abcam, Paris, France, dilution 1:2000) antibody overnight at 4°C. After washes  
245 in TBST, membranes were incubated with appropriate horseradish peroxidase-conjugated  
246 secondary antibody (dilution 1:10000). The membrane was incubated in Amersham™ ECL™  
247 Prime solution (GE healthcare, Beaucozé, France) for 3 min at room temperature, then placed  
248 in a film-cassette with protein side facing up. The chemiluminescence emitted from targeted  
249 protein band was acquired by Amersham imager 600 imaging system (GE healthcare, Beaucozé,

250 France) using implement method. The exposed image was visualized every 30 seconds until  
251 reaching an image with the highest signal-to-noise ratio. Membranes were then stripped using  
252 Re-Blot Plus Strong solution (Sigma-Aldrich, St. Quentin Fallavier, France) and then re-probed  
253 with anti-AKT (Abcam, Paris, France, dilution 1:1000) and anti-cyclophilin A (Cell Signaling  
254 Technology, Saint-Cyr-L'École, France, dilution 1:2000) antibody for 1 h at room temperature.  
255 Following incubation with appropriate secondary antibody, protein bands revelation was  
256 performed as described before. The images of blots were quantified using ImageJ software.  
257 Target proteins bands intensity were normalized to cyclophilin A in each lane.

### 258 *Data and statistical analysis*

259 Results are expressed as means  $\pm$  S.E.M. The statistical significance between means was  
260 assessed by Student's t test when two groups were compared, or by ordinary one-way ANOVA  
261 followed by Fisher's LSD test when more than two groups were compared. Two-way ANOVA  
262 followed by Tukey correction was used when comparing two parameters. All analyses were  
263 performed using the GraphPad Prism 9 (GraphPad Software, San Diego, USA). A *p* value less  
264 than 0.05 ( $p < 0.05$ ) was considered statistically significant.

## 265 **Results**

### 266 *Impact of BPS exposure on metabolic characteristics of sires before mating*

267 We exposed 14-week-old male Wistar rats to BPS at the dose of 4  $\mu\text{g}/\text{kg}/\text{day}$  (BPS-4 sires) or 40  
268  $\mu\text{g}/\text{kg}/\text{day}$  (BPS-40 sires) for at least 2 months to cover one full cycle of spermatogenesis.

269 The body weight and body fat/lean mass ratio were similar between BPS-4, BPS-40 and Ctrl  
270 sires (Fig. 2A and B). No significant difference was found between the groups regarding daily  
271 food and water intake (Fig. 2C and D).

272 The blood glucose levels, monitored between 9:00 and 10:00 a.m., were not altered by the  
273 administration of BPS (Fig. 2E). Furthermore, the glycemic profiles during ITT (Fig. 2F) and  
274 IPGTT (Fig. 2G) were similar between the three groups of sires. Finally, no statistical difference  
275 was found between the 3 groups of animals regarding the *in vivo* glucose-induced insulin  
276 secretion (GIIS) (Fig. 2H). However, we found a significant decrease in plasma cholesterol levels  
277 and high-density lipoprotein (HDL) levels in BPS-4 ( $p < 0.01$  and  $p < 0.001$ , respectively) and  
278 BPS-40 groups ( $p < 0.05$  and  $p < 0.01$ , respectively) compared to the Ctrl group (Fig. 2I and J).  
279 Further analysis showed a significantly higher plasma cholesterol/HDL ratio in BPS-4 group  
280 compared to Ctrl ( $p < 0.0001$ ) and BPS-40 ( $p < 0.01$ ) groups (Fig. 2K). Increased non-esterified  
281 fatty acids (NEFA) levels were only observed in the BPS-4 group, when compared to Ctrl ( $p <$   
282  $0.05$ ) (Fig. 2L). No significant difference was found in plasma triglycerides levels between the  
283 groups (Fig. 2M).

#### 284 ***Impact of paternal BPS exposure on offspring during fetal life***

285 After mating BPS-4, BPS-40 and Ctrl male rats with females, we analyzed the anthropometric  
286 and pancreatic parameters of their fetuses at the embryonic day 18 post-conception (E18).  
287 Because the period of gestation and subsequent growth and development of fetuses are closely  
288 dependent on maternal physiology, we took caution to use virgin females that were similar in  
289 terms of age and body weight. Fetuses in each group were derived from at least four sires of the  
290 corresponding group. The number of pups per litter was not significantly different between Ctrl,  
291 BPS-4 and BPS-40 groups. We found 8 to 12 fetuses per dam in the three groups. The sex ratios  
292 in the litters were not significantly different between the three groups:  $47\% \pm 5\%$  male and  $53\%$   
293  $\pm 5\%$  female fetuses in the Ctrl group;  $53\% \pm 6\%$  male and  $47\% \pm 6\%$  female fetuses in the BPS-  
294 4 group;  $49\% \pm 4\%$  male and  $51\% \pm 4\%$  female fetuses in the BPS-40 group.

295 The body weight and pancreas weight of male and female fetuses were measured separately. No

296 significant differences were found between the groups of fetuses from Ctrl, BPS-4 and BPS-40  
297 fathers, irrespective of their sex (Fig. 3A and B). Intriguingly, male fetuses from BPS-4 and BPS-  
298 40 fathers had smaller nose-to-tail body length compared to those from Ctrl sires (not shown),  
299 which resulted in higher body mass index (BMI) in male fetuses derived from BPS-4 and BPS-  
300 40 sires ( $p < 0.05$  and  $p < 0.001$ , respectively) (Fig. 3C). There were no significant differences  
301 in body length and BMI in female fetuses from different groups (Fig. 3C).

302 We also measured the pancreatic  $\alpha$  and  $\beta$  cell mass by histomorphometry in male fetuses of Ctrl  
303 and BPS-4 group and found no significant differences in these parameters between the two  
304 groups (Fig. 3D).

305 ***Impact of paternal BPS exposure on physiological characteristics of male and female***  
306 ***offspring in adulthood***

307 After weaning, the offspring (F1) were fed with standard diet and used BPS-free water. From  
308 week 5 onward, the body weight of male F1 from BPS-4 sires (BPS-4/F1) was higher compared  
309 to male F1 from Ctrl sires (Ctrl/F1) (Fig. 4A). In contrast, in male F1 from BPS-40 group (BPS-  
310 40/F1), there was no significant difference in body weight in comparison to the Ctrl/F1 (Fig. 4A).  
311 Interestingly, the food intake was similar between the three groups of male F1 throughout the  
312 study, indicating that the increased body weight was not related to hyperphagia (Fig. 4B). At the  
313 age of 24 weeks, a significantly higher fat/lean ratio was found in male BPS-4/F1 compared to  
314 BPS-40/F1 ( $p < 0.05$ ) (Fig. 4C). The non-fasting glycemia was similar in male F1 of three groups  
315 of rats throughout the study (Fig. 4D).

316 From week 8 onward, female BPS-4/F1 showed higher body weight than female Ctrl/F1 and  
317 BPS-40/F1 (Fig. 4E). The food intake during the period of age 5 to 24 weeks was similar in the  
318 three groups of female F1 (Fig. 4F) and there was no difference in body fat/lean ratio between  
319 these groups (Fig. 4G). Finally, we found similar non-fasting glycemia in age-matched F1

320 females from Ctrl, BPS-4 and BPS-40 fathers, from the age of 5 to 24 weeks post-natal (Fig.  
321 4H).

### 322 *Sex-specific effects of paternal BPS exposure on glucose metabolism in offspring*

323 In order to assess the metabolic phenotype of offspring from fathers exposed to BPS, we  
324 measured insulin sensitivity and glucose tolerance through insulin tolerance test (ITT) and  
325 intraperitoneal glucose tolerance test (IPGTT), respectively.

326 Male and female offspring were studied separately. At 3 months post-natal, male BPS-4/F1  
327 exhibited insulin resistance compared to Ctrl/F1 ( $p < 0.05$ ), while no significant difference in  
328 insulin sensitivity was observed between male BPS-40/F1 and Ctrl/F1 (Fig. 5A). Regarding  
329 glucose tolerance, although no difference in the area under the curve (AUC) was apparent  
330 between the male offspring of 3 groups of fathers at the age of 3 months, during IPGTT, the  
331 glycemic value was higher at T15 min in BPS-4/F1 and BPS-40/F1 compared to controls (Fig.  
332 5B). Finally, male BPS-4/F1 had higher glucose-induced insulin secretion (GIIS) 15 min after  
333 glucose administration compared to Ctrl/F1 and BPS-40/F1 (Fig. 5C).

334 At the age of 6 months, the difference in insulin sensitivity of male offspring between the three  
335 groups did not reach statistical significance (Fig. 5D). However, male BPS-4/F1 exhibited  
336 impaired glucose tolerance compared to Ctrl/F1 ( $p < 0.05$ ), a feature that was not found in BPS-  
337 40/F1 animals (Fig. 5E). No significant difference in *in vivo* GIIS was observed between male  
338 BPS-4/F1 and Ctrl/F1 during IPGTT, but male BPS-40/F1 showed decreased insulin secretion  
339 during IPGTT compared to the other two groups ( $p < 0.01$ ) (Fig. 5F).

340 Regarding female F1 at the age of 3 months, we found enhanced insulin sensitivity in BPS-40/F1,  
341 compared to Ctrl/F1 and BPS-4/F1 ( $p < 0.05$ ), whereas insulin sensitivity was similar between  
342 BPS-4/F1 and Ctrl/F1 females (Fig. 5G). At the age of 6 months, insulin sensitivity was

343 comparable between the 3 groups of females F1 (Fig. 5J). There was no significant difference in  
344 glucose tolerance at 3 months or 6 months of age between the female offspring from the three  
345 groups (Fig. 5H and K), except for a higher glycemic value at T15 min in the BPS-4/F1 group  
346 at the age of 3 months. Intriguingly, insulin secretion was enhanced in 3-month-old BPS-4/F1  
347 females compared to Ctrl/F1 and BPS-40/F1 ( $p < 0.05$ ) (Fig. 5I). No such difference was found  
348 at 6 months of age (Fig. 5L).

349 Since impaired glucose tolerance was found only in male BPS-4/F1 group, male offspring were  
350 monitored until 9 months of age only in this group and compared to the Ctrl/F1. First, we  
351 measured the metabolic characteristics of the two groups. Male BPS-4/F1 showed higher body  
352 weight compared to Ctrl/F1 ( $p < 0.05$ ) (Fig. 6A). A significant difference in body fat/lean ratio  
353 was observed in male BPS-4/F1 compared to Ctrl/F1 ( $p < 0.05$ ) (Fig. 6B). In order to assess  
354 adipocyte morphologic characteristics, we performed morphometric analysis of the adipose  
355 tissue sections of males F1 from the Ctrl and BPS-4 sires. No difference in the size distribution  
356 of adipocytes was observed between the groups (not shown). Interestingly, male BPS-4/F1  
357 showed increased non-fasting plasma insulin compared to Ctrl/F1 ( $p < 0.01$ ) (Fig. 6C),  
358 suggesting a state of basal insulin resistance.

359 At 9 months, during ITTs, a decreased insulin sensitivity was apparent in male BPS-4/F1, when  
360 compared to Ctrl/F1 (Fig. 6D). Male BPS-4/F1 also showed glucose intolerance compared to  
361 Ctrl/F1 (Fig. 6E), although area under the curve (AUC) values did not reach statistical  
362 significance ( $p = 0.053$ ), likely due to the limited number of animals. In BPS-4/F1, there was a  
363 significant increase in insulin secretion in response to glucose, compared to male Ctrl/F1 ( $p <$   
364  $0.05$ ) (Fig. 6F). To test whether the decreased insulin sensitivity was due to hepatic insulin  
365 resistance (increased gluconeogenesis), we performed pyruvate tolerance test (PTT) in males F1.  
366 Although there was no significant difference in AUC between Ctrl/F1 and BPS-4/F1, it appeared

367 clearly that at T15 min post-pyruvate injection, the rise of glycemia in BPS-4/F1 group was  
368 significantly higher than the in Ctrl/F1 group, suggesting an increased gluconeogenic capacity  
369 in the former group (Fig. 6G). Interestingly, we observed higher fasting glycemia (18 hours  
370 fasting) in male BPS-4/F1 when compared to male Ctrl/F1 ( $93.67 \pm 2.99$  mg/dL *versus*  $84.50 \pm$   
371  $1.82$  mg/dL), reflecting further disturbance in glucose metabolism (Fig. 6G).

372 Further, to determine whether the whole-body insulin resistance observed in the BPS-4/F1 group  
373 was related to impaired muscle and/or adipose tissue insulin sensitivity, we assessed the  
374 activation status (phosphorylation) of AKT, one of the main intermediates of the insulin signaling  
375 pathway.

376 We found that, as expected, the administration of insulin significantly increased the p-AKT  
377 (ser473)/AKT ratio in the peri-gonadal adipose tissue of Ctrl/F1 ( $p < 0.01$ ) (Fig. 6H and I). A  
378 similar pattern of AKT phosphorylation was observed in the adipose tissue of BPS-4/F1 group  
379 in response to insulin ( $p < 0.01$ ) (Fig. 6H and I). In muscle, the insulin-stimulated AKT  
380 phosphorylation observed in the Ctrl/F1 group, failed to occur in the BPS-4/F1 group (Fig. 6J  
381 and K), suggesting impaired muscle insulin sensitivity in the BPS-4/F1 group.

382 As the increased *in vivo* GIIS was observed in male BPS-4/F1, we performed a static incubation  
383 test on isolated pancreatic islets to measure the intrinsic secretory capacity of islets in response  
384 to glucose (16.7 mM) *ex vivo*. Interestingly, no difference was observed in insulin release  
385 between islets from Ctrl/F1 and BPS-4/F1 males under either 2.8 or 16.7 mM glucose (Fig. 6L),  
386 suggesting that the secretory capacity of the islets was not affected in the BPS-4/F1 group.

387 Therefore, in order to find an explanation for the increased GIIS *in vivo*, we performed  
388 histomorphometric analysis of the pancreatic sections from male Ctrl/F1 and BPS-4/F1 (Fig. 6M  
389 and N) to evaluate the  $\beta$  cell mass. A significant increase of the pancreatic  $\beta$  cell mass was  
390 observed in male BPS-4/F1 compared to Ctrl/F1 ( $p < 0.05$ ) (Fig. 6O), suggesting an adaptive

391 mechanism to face the insulin resistance.

392 Finally, plasma samples were collected from 3 groups of male F1 at 3 months and in Ctrl/F1 and  
393 BPS-4/F1 at 9 months of age, to evaluate the lipid profiles (Table 1). The total plasma cholesterol  
394 levels were found to be similar in different groups of male F1 at 3 months, however, male BPS-  
395 4/F1 exhibited increased plasma cholesterol level at 9 months of age compared to Ctrl/F1 ( $p <$   
396 0.05). A significant decrease in plasma HDL levels were observed in male BPS-4/F1 and BPS-  
397 40/F1 compared to Ctrl/F1 group ( $p < 0.001$  and  $p < 0.01$ , respectively) at 3 months, which were  
398 not shown at 9 months of age. The elevated total cholesterol/HDL ratio in male BPS-4/F1  
399 compared to Ctrl/F1 observed at 3 months persisted at 9 months. We did not observe any  
400 significant difference in plasma non-esterified fatty acids (NEFA) and triglycerides levels  
401 between the groups, either at the age of 3 months or 9 months.

## 402 **Discussion**

403 Endocrine disruptors (ED) are now admitted to be among environmental risk factors for  
404 metabolic diseases. While most individuals are exposed to ED across the lifespan, specific time  
405 windows are suggested as periods of increased vulnerability (Sargis and Simmons, 2019). A  
406 large body of literature demonstrates that maternal exposure to bisphenols induces changes in  
407 glucose and energy homeostasis in the offspring of first (Alonso-Magdalena et al., 2010; Wei et  
408 al., 2011; Angle et al., 2013; Susiarjo et al., 2015; Bansal et al., 2017), and even second  
409 generation (Susiarjo et al., 2015; Bansal et al., 2017). While the transmission of deleterious  
410 effects of bisphenols through maternal lineage is well documented, the impact of paternal  
411 exposure to these compounds on the metabolic health of their descendants are only beginning to  
412 be addressed. To date, only a limited number of papers addressing the metabolic impact of  
413 paternal exposure to BPA (but not BPS) on offspring have been published with discrepant  
414 results, and in most of them the sires were exposed to BPA during development (Li et al., 2014;

415 Rashid et al., 2020). In this study, we evaluated the effect of the direct exposure of adult male  
416 genitors to BPS, a widely used BPA substitute on the metabolic health of their descendants.

417 We report for the first time that direct exposure of sires to 4  $\mu\text{g}/\text{kg}/\text{day}$  of BPS (corresponding  
418 to a dose about 10 times lower than the current TDI for BPA in humans) induces significant  
419 changes in body mass and glucose metabolism, mostly in their male progenies.

420 The exposure to BPS (either the lower or the higher dose) had no significant effect on the body  
421 weight of sires, in line with other studies in male mice (Moon et al., 2015; Rashid et al., 2020)  
422 and rats (Ding et al., 2014) exposed to dietary BPA at post-pubertal stage. Interestingly, in our  
423 study, paternal exposure to 4  $\mu\text{g}/\text{kg}/\text{day}$  BPS resulted in a significant increase of body weight in  
424 their offspring compared to the Ctrl group, suggesting a potential BPS-mediated programming  
425 of overweight/obesity through the paternal lineage.

426 Regarding the metabolic status of offspring, we found that only male F1 from BPS-4 fathers  
427 exhibited decreased insulin sensitivity and impaired glucose tolerance. Our data therefore clearly  
428 point to a dose-specific and sex-dependent effects in offspring of male genitors exposed to BPS  
429 in adulthood. These results are in contrast with the only other recently published report on post-  
430 puberty exposure to BPA in male mice, which showed no impairment of glucose metabolism in  
431 the offspring (Rashid et al., 2020). However, in that study, the offspring were studied at the age  
432 of 4 months. Because most metabolic impairments occur or aggravate with age, it is possible that  
433 in their model, investigation at a more advanced age would have shown metabolic disturbances  
434 in the animals. Another possible explanation is the difference in animal species (rat *versus*  
435 mouse) and the use of BPS *versus* BPA in the two studies. Indeed, studies in other disease-related  
436 conditions reported that BPA analogues such as BPS produced stronger effects than BPA  
437 (Ahmed and Atlas, 2016; Martínez et al., 2020; Thoene et al., 2020).

438 In the present study, we found that the *in vivo* insulin secretion in response to glucose was  
439 increased in male BPS-4/F1 compared to age-matched Ctrl/F1 group, probably to meet the  
440 increased insulin demands related to the state of insulin resistance in the former group. We  
441 therefore asked whether this adaptive mechanism was due to increased intrinsic secretory  
442 capacity of islets or the expansion of the  $\beta$  cell number. *Ex vivo* evaluation of the glucose-induced  
443 insulin secretion in isolated islets from BPS-4/F1 rats revealed no difference with islets from  
444 Ctrl/F1 animals. However, the  $\beta$  cell mass were increased in the pancreas of BPS-4/F1 rats  
445 compared to Ctrl/F1, suggesting that the increased insulin secretion during IPGTT is due to the  
446 increased number of  $\beta$  cells in this group. Moreover, this also suggests that paternal exposure to  
447 BPS at 4  $\mu\text{g}/\text{kg}/\text{day}$  did not alter the plasticity of pancreatic  $\beta$  cells in response to insulin  
448 resistance. In contrast, Mao et al. have reported that male offspring of sires exposed to BPA  
449 during fetal life have decreased  $\beta$  cell fraction and mass (Mao et al., 2015). The different results  
450 are most likely due to the different timing of exposure in male genitors, since fetal and neonatal  
451 stages are critical windows of susceptibility to BPA (Tudurí et al., 2018), during which defective  
452 pancreatic phenotype could be acquired and transmitted to the next generation. Finally, in order  
453 to gain further insights into the molecular mechanisms involved in insulin resistance observed  
454 in the BPS-4/F1 group, we investigated the insulin signaling pathway in muscle and peri-gonadal  
455 adipose tissue of male offspring from Ctrl and BPS-4 fathers. We showed that the insulin  
456 signaling pathway was impaired in muscle but not in peri-gonadal adipose tissue. Although the  
457 molecular mechanisms of insulin resistance transmitted through the paternal line have not been  
458 previously investigated elsewhere, it has been shown that insulin signaling in skeletal muscle  
459 was impaired in adult rodents directly exposed to BPA, while other peripheral tissues, including  
460 the liver and adipose tissue, were not affected in terms of insulin signaling (Batista et al., 2012;  
461 Moon et al., 2015).

462 In our study, the impairment of glucose metabolism observed in male offspring did not appear

463 in BPS-4/F1 females pointing to a gender specific transmission of metabolic traits through  
464 paternal exposure to BPS. Most studies dealing with fetal programming reveal sex-specific  
465 effects, but the reported changes are not always concordant among the studies. For instance, a  
466 recent study in zebrafish indicates a gender-specific effect of BPAF (a BPA analog) on the  
467 embryonic development of their offspring, depending whether the maternal or the paternal line  
468 was exposed to BPAF (Zhang et al., 2022).

469 In a study by Rashid et al., BPA exposure of sires during fetal life induced glucose intolerance  
470 in their female offspring (Rashid et al., 2020). It is not surprising that exposure to toxicants  
471 during adult life *versus* fetal life could cause different outcome in the next generation, since adult  
472 gametes may have different vulnerability to environmental pollutants as compared to primordial  
473 germ cells.

474 Of importance, in our study, we showed that the metabolic disturbances observed in male  
475 offspring occurred without notable impairment of glucose and energy metabolism in the fathers  
476 at the time of mating. Paternal diet-induced obesity has been shown to impair glucose  
477 metabolism in offspring (Grandjean et al., 2015; Ng et al., 2010; Wei et al., 2014). The sires  
478 exposed to BPS for 2 to 3 months maintained normal glucose and energy metabolism in our  
479 present study. This observation ruled out the contribution of the metabolic disorders of BPS-4  
480 sires to the metabolic phenotype of their offspring, suggesting potential direct effects of BPS on  
481 paternal gametes, which can be transmitted to the next generation.

482 It is important to note that deleterious effects of early exposure to bisphenols are not limited to  
483 metabolic disorders. Many other negative outcomes, such as neurological defects (Chen et al.,  
484 2018; Zhang et al., 2020; Meng et al., 2021), bone defects (Lind et al., 2017) and hepatic  
485 alterations (Strakovsky et al., 2015) have been reported. Interestingly, in these reports, the effects  
486 in offspring were also sex-specific, with a higher vulnerability of male descendants, similar to

487 what we observed in our study.

488 Because male genitors do not have physical interactions with their offspring, they contribute to  
489 the next generation only through sperm. Epigenetic modifications are the most likely mechanism  
490 for the transmission of altered metabolic phenotype to offspring. We (Gong et al., 2021) and  
491 others (Wei et al., 2014; Grandjean et al., 2015; de Castro Barbosa et al., 2015; Watkins et al.,  
492 2018) have reported that in rodents, changes in macro-nutrients composition of diet consumed  
493 by fathers influence the metabolic phenotype of their progenies through epigenetic modifications  
494 of spermatozoa. These observations were also reported in obese men who underwent bariatric  
495 surgery (Donkin et al., 2016). Mao et al. have shown that early-life exposure to BPA in F1  
496 generation impairs the glucose tolerance and pancreatic islet structure in F2 generation, which  
497 was associated with dysregulated methylation of *Igf2/H19* genes. The expression of *Igf2* gene  
498 was altered in the sperm of adult F1, suggesting that epigenetic changes in germ cells could  
499 contribute to the multigenerational effects of paternal BPA exposure (Mao et al., 2015).  
500 Therefore, in our study the transmission of dysmetabolic traits to the male offspring of BPS-4  
501 fathers might result from epigenetic modifications such as DNA methylation of the  
502 spermatozoon genome, or most probably, through differential expression of sperm small non-  
503 coding RNAs as reported elsewhere (Rodgers et al., 2015; Chen et al., 2016; Gong et al., 2021;  
504 Raad et al., 2021).

505 Finally, in this study we showed that the metabolic disorders observed in male offspring of BPS-  
506 exposed fathers occurred only in the progenies of fathers exposed to the lower dose of BPS (4  
507  $\mu\text{g}/\text{kg}/\text{day}$ ), pointing to the non-monotonic effects of BPS. The non-monotonic effects of  
508 endocrine disruptors have been largely documented especially in hormone-sensitive organs (vom  
509 Saal and Vandenberg, 2021). In line with this, Rashid et al. have also reported the negative effects  
510 of paternal exposure to lower but not higher dose of BPA during development on their offspring

511 (Rashid et al., 2020). These results together suggest that the lowering of the TDI of BPA by  
512 safety organizations is not necessarily protective against metabolic disorders. In conclusion, our  
513 study provides the first evidence of detrimental effects of post-puberty paternal exposure to BPS,  
514 one of the most widely used BPA analogues, on the metabolic health of offspring. These effects  
515 are sex-specific and appear with a dose of BPS much lower than the TDI of BPA, authorized by  
516 the European Food Safety Authority. While the awareness about dangers of exposure of pregnant  
517 women to environmental pollutants in general, and to endocrine disrupting chemicals in  
518 particular is growing, it is important to build solid knowledge on the risks of these compounds  
519 in transmitting negative metabolic and other disease-related effects through the paternal lineage.  
520 The protection of both women and men of reproductive age from these toxicants could help to  
521 interrupt the vicious circle of non-genetic inter- and trans-generational transmission of metabolic  
522 diseases.

## 523 **Abbreviations**

524 AOC: area over curve

525 AUC: area under curve

526 BMI: body mass index

527 BPA: bisphenol A

528 BPS: bisphenol S

529 ECHA: European Chemicals Agency

530 ED: endocrine disruptors

531 EFSA: European Food Safety Authority

532 GIIS: glucose-induced insulin secretion

533 HDL: high-density lipoprotein

534 IPGTT: intraperitoneal glucose tolerance test

535 ITT: inulin tolerance test  
536 KRBH: Krebs-Ringer/bicarbonate/Hepes  
537 NEFA: non-esterified fatty acids  
538 PTT: pyruvate tolerance test  
539 TBS: Tris Buffer Saline  
540 TBST: Tris Buffered Saline-Tween  
541 TDI: tolerable daily intake

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## 545 **Duality of interest**

546 The authors declare no conflict of interest.

## 547 **Author Contribution**

548 PG: Methodology, Formal analysis, Investigation, Visualization, Writing the original draft. DB:  
549 Methodology, Investigation, Project administration. ST, GP and JL: Formal analysis. JM:  
550 Conceptualization, Methodology, Funding acquisition, Writing the original draft, Review &  
551 editing of the Manuscript.

## 552 **References**

553 Acconcia, F., Pallottini, V., Marino, M., 2015. Molecular Mechanisms of Action of BPA. Dose Response 13.  
554 <https://doi.org/10.1177/1559325815610582>  
555 Ahmed, S., Atlas, E., 2016. Bisphenol S- and bisphenol A-induced adipogenesis of murine preadipocytes  
556 occurs through direct peroxisome proliferator-activated receptor gamma activation. International

557 Journal of Obesity 40, 1566–1573. <https://doi.org/10.1038/ijo.2016.95>

558 Ahn, Y.-A., Baek, H., Choi, M., Park, J., Son, S.J., Seo, H.J., Jung, J., Seong, J.K., Lee, J., Kim, S., 2020.  
559 Adipogenic effects of prenatal exposure to bisphenol S (BPS) in adult F1 male mice. *Science of The*  
560 *Total Environment* 728, 138759. <https://doi.org/10.1016/j.scitotenv.2020.138759>

561 Alonso-Magdalena, P., Vieira, E., Soriano, S., Menes, L., Burks, D., Quesada, I., Nadal, A., 2010. Bisphenol  
562 A Exposure during Pregnancy Disrupts Glucose Homeostasis in Mothers and Adult Male Offspring.  
563 *Environ Health Perspect* 118, 1243–1250. <https://doi.org/10.1289/ehp.1001993>

564 Angle, B.M., Do, R.P., Ponzi, D., Stahlhut, R.W., Drury, B.E., Nagel, S.C., Welshons, W.V., Besch-Williford,  
565 C.L., Palanza, P., Parmigiani, S., vom Saal, F.S., Taylor, J.A., 2013. Metabolic disruption in male mice  
566 due to fetal exposure to low but not high doses of bisphenol A (BPA): Evidence for effects on body  
567 weight, food intake, adipocytes, leptin, adiponectin, insulin and glucose regulation. *Reprod Toxicol*  
568 42, 10.1016/j.reprotox.2013.07.017. <https://doi.org/10.1016/j.reprotox.2013.07.017>

569 Azevedo, L.F., Porto Dechandt, C.R., Cristina de Souza Rocha, C., Hornos Carneiro, M.F., Alberici, L.C.,  
570 Barbosa, F., 2019. Long-term exposure to bisphenol A or S promotes glucose intolerance and changes  
571 hepatic mitochondrial metabolism in male Wistar rats. *Food and Chemical Toxicology* 132, 110694.  
572 <https://doi.org/10.1016/j.fct.2019.110694>

573 Bansal, A., Rashid, C., Xin, F., Li, C., Polyak, E., Duemler, A., van der Meer, T., Stefaniak, M., Wajid, S.,  
574 Doliba, N., Bartolomei, M.S., Simmons, R.A., 2017. Sex- and Dose-Specific Effects of Maternal  
575 Bisphenol A Exposure on Pancreatic Islets of First- and Second-Generation Adult Mice Offspring.  
576 *Environ Health Perspect* 125, 097022. <https://doi.org/10.1289/EHP1674>

577 Barros, R.P.A., Machado, U.F., Gustafsson, J.-A., 2006. Estrogen receptors: new players in diabetes mellitus.  
578 *Trends Mol Med* 12, 425–431. <https://doi.org/10.1016/j.molmed.2006.07.004>

579 Batista, T.M., Alonso-Magdalena, P., Vieira, E., Amaral, M.E.C., Cederroth, C.R., Nef, S., Quesada, I.,  
580 Carneiro, E.M., Nadal, A., 2012. Short-Term Treatment with Bisphenol-A Leads to Metabolic  
581 Abnormalities in Adult Male Mice. *PLoS One* 7, e33814.  
582 <https://doi.org/10.1371/journal.pone.0033814>

583 Beausoleil, C., Emond, C., Cravedi, J.-P., Antignac, J.-P., Applanat, M., Appenzeller, B.R., Beaudouin, R.,  
584 Belzunces, L.P., Canivenc-Lavier, M.-C., Chevalier, N., Chevrier, C., Elefant, E., Eustache, F., Habert,  
585 R., Kolf-Clauw, M., Le Magueresse-Battistoni, B., Mhaouty-Kodja, S., Minier, C., Multigner, L.,

586 Schroeder, H., Thonneau, P., Viguié, C., Pouzaud, F., Ormsby, J.-N., Rousselle, C., Verines-Jouin, L.,  
587 Pasquier, E., Michel, C., 2018. Regulatory identification of BPA as an endocrine disruptor: Context  
588 and methodology. *Molecular and Cellular Endocrinology*, Identification of Bisphenol A as an  
589 endocrine disrupter based on its effects on the female reproductive system, the mammary gland, the  
590 metabolism and the neurodevelopment. 475, 4–9. <https://doi.org/10.1016/j.mce.2018.02.001>

591 Brulport, A., Le Corre, L., Maquart, G., Barbet, V., Dastugue, A., Severin, I., Vaiman, D., Chagnon, M.-C.,  
592 2021. Multigenerational study of the obesogen effects of bisphenol S after a perinatal exposure in  
593 C57BL6/J mice fed a high fat diet. *Environmental Pollution* 270, 116243.  
594 <https://doi.org/10.1016/j.envpol.2020.116243>

595 Carchia, E., Porreca, I., Almeida, P.J., D'Angelo, F., Cuomo, D., Ceccarelli, M., De Felice, M., Mallardo, M.,  
596 Ambrosino, C., 2015. Evaluation of low doses BPA-induced perturbation of glycemia by  
597 toxicogenomics points to a primary role of pancreatic islets and to the mechanism of toxicity. *Cell*  
598 *Death & Disease* 6, e1959–e1959. <https://doi.org/10.1038/cddis.2015.319>

599 Chen, Q., Yan, M., Cao, Z., Li, X., Zhang, Yunfang, Shi, J., Feng, G., Peng, H., Zhang, X., Zhang, Ying, Qian,  
600 J., Duan, E., Zhai, Q., Zhou, Q., 2016. Sperm tsRNAs contribute to intergenerational inheritance of  
601 an acquired metabolic disorder. *Science* 351, 397–400. <https://doi.org/10.1126/science.aad7977>

602 Chen, Z., Li, T., Zhang, L., Wang, H., Hu, F., 2018. Bisphenol A exposure remodels cognition of male rats  
603 attributable to excitatory alterations in the hippocampus and visual cortex. *Toxicology* 410, 132–141.  
604 <https://doi.org/10.1016/j.tox.2018.10.002>

605 da Silva, B.S., Pietrobon, C.B., Bertasso, I.M., Lopes, B.P., Carvalho, J.C., Peixoto-Silva, N., Santos, T.R.,  
606 Claudio-Neto, S., Manhães, A.C., Oliveira, E., de Moura, E.G., Lisboa, P.C., 2019. Short and long-  
607 term effects of bisphenol S (BPS) exposure during pregnancy and lactation on plasma lipids,  
608 hormones, and behavior in rats. *Environmental Pollution* 250, 312–322.  
609 <https://doi.org/10.1016/j.envpol.2019.03.100>

610 de Castro Barbosa, T., Ingerslev, L.R., Alm, P.S., Versteyhe, S., Massart, J., Rasmussen, M., Donkin, I., Sjögren,  
611 R., Mudry, J.M., Vetterli, L., Gupta, S., Krook, A., Zierath, J.R., Barrès, R., 2015. High-fat diet  
612 reprograms the epigenome of rat spermatozoa and transgenerationally affects metabolism of the  
613 offspring. *Mol Metab* 5, 184–197. <https://doi.org/10.1016/j.molmet.2015.12.002>

614 Ding, S., Fan, Y., Zhao, N., Yang, H., Ye, X., He, D., Jin, X., Liu, J., Tian, C., Li, H., Xu, S., Ying, C., 2014.

615 High-fat diet aggravates glucose homeostasis disorder caused by chronic exposure to bisphenol A.  
616 Journal of Endocrinology 221, 167–179. <https://doi.org/10.1530/JOE-13-0386>

617 Donkin, I., Versteyhe, S., Ingerslev, L.R., Qian, K., Mehta, M., Nordkap, L., Mortensen, B., Appel, E.V.R.,  
618 Jørgensen, N., Kristiansen, V.B., Hansen, T., Workman, C.T., Zierath, J.R., Barrès, R., 2016. Obesity  
619 and Bariatric Surgery Drive Epigenetic Variation of Spermatozoa in Humans. Cell Metabolism 23,  
620 369–378. <https://doi.org/10.1016/j.cmet.2015.11.004>

621 Encarnação, T., Pais, A.A., Campos, M.G., Burrows, H.D., 2019. Endocrine disrupting chemicals: Impact on  
622 human health, wildlife and the environment. Science Progress 102, 3–42.  
623 <https://doi.org/10.1177/0036850419826802>

624 Figeac, F., Uzan, B., Faro, M., Chelali, N., Portha, B., Movassat, J., 2009. Neonatal growth and regeneration  
625 of  $\beta$ -cells are regulated by the Wnt/ $\beta$ -catenin signaling in normal and diabetic rats. American Journal  
626 of Physiology-Endocrinology and Metabolism 298, E245–E256.  
627 <https://doi.org/10.1152/ajpendo.00538.2009>

628 García-Arevalo, M., Alonso-Magdalena, P., Santos, J.R.D., Quesada, I., Carneiro, E.M., Nadal, A., 2014.  
629 Exposure to Bisphenol-A during Pregnancy Partially Mimics the Effects of a High-Fat Diet Altering  
630 Glucose Homeostasis and Gene Expression in Adult Male Mice. PLOS ONE 9, e100214.  
631 <https://doi.org/10.1371/journal.pone.0100214>

632 Gayrard, V., Lacroix, M.Z., Grandin, F.C., Collet, S.H., Mila, H., Viguié, C., Gély, C.A., Rabozzi, B., Bouchard,  
633 M., Léandri, R., Toutain, P.-L., Picard-Hagen, N., 2019. Oral Systemic Bioavailability of Bisphenol  
634 A and Bisphenol S in Pigs. Environ. Health Perspect. 127, 77005. <https://doi.org/10.1289/EHP4599>

635 Gong, P., Bailbé, D., Bianchi, L., Pommier, G., Liu, J., Tolu, S., Stathopoulou, M.G., Portha, B., Grandjean,  
636 V., Movassat, J., 2021. Paternal High-Protein Diet Programs Offspring Insulin Sensitivity in a Sex-  
637 Specific Manner. Biomolecules 11, 751. <https://doi.org/10.3390/biom11050751>

638 Grandjean, V., Fourré, S., De Abreu, D.A.F., Derieppe, M.-A., Remy, J.-J., Rassoulzadegan, M., 2015. RNA-  
639 mediated paternal heredity of diet-induced obesity and metabolic disorders. Sci Rep 5, 18193.  
640 <https://doi.org/10.1038/srep18193>

641 Holtcamp, W., 2011. Prenatal Exposure to BPA and Sexually Selected Traits in Male Mice. Environmental  
642 Health Perspectives 119, a383. <https://doi.org/10.1289/ehp.119-a383>

643 Ivry Del Moral, L., Le Corre, L., Poirier, H., Niot, I., Truntzer, T., Merlin, J.-F., Rouimi, P., Besnard, P.,

644 Rahmani, R., Chagnon, M.C., 2016. Obesogen effects after perinatal exposure of 4,4'-  
645 sulfonyldiphenol (Bisphenol S) in C57BL/6 mice. *Toxicology* 357–358, 11–20.  
646 <https://doi.org/10.1016/j.tox.2016.05.023>

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

650 Liao, C., Liu, F., Alomirah, H., Loi, V.D., Mohd, M.A., Moon, H.-B., Nakata, H., Kannan, K., 2012. Bisphenol  
651 S in urine from the United States and seven Asian countries: occurrence and human exposures.  
652 *Environ. Sci. Technol.* 46, 6860–6866. <https://doi.org/10.1021/es301334j>

653 Lind, P.M., Lind, L., 2018. Endocrine-disrupting chemicals and risk of diabetes: an evidence-based review.  
654 *Diabetologia* 61, 1495–1502. <https://doi.org/10.1007/s00125-018-4621-3>

655 Lind, T., Lejonklou, M.H., Dunder, L., Rasmusson, A., Larsson, S., Melhus, H., Lind, P.M., 2017. Low-dose  
656 developmental exposure to bisphenol A induces sex-specific effects in bone of Fischer 344 rat  
657 offspring. *Environ Res* 159, 61–68. <https://doi.org/10.1016/j.envres.2017.07.020>

658 Liu, J.J., Raynal, S., Bailbé, D., Gausseres, B., Carbonne, C., Autier, V., Movassat, J., Kergoat, M., Portha, B.,  
659 2015. Expression of the kynurenine pathway enzymes in the pancreatic islet cells. Activation by  
660 cytokines and glucolipototoxicity. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*  
661 1852, 980–991. <https://doi.org/10.1016/j.bbadis.2015.02.001>

662 Livingstone, C., Collison, M., 2002. Sex steroids and insulin resistance. *Clin Sci (Lond)* 102, 151–166.  
663 <https://doi.org/10.1042/cs1020151>

664 Mao, Z., Xia, W., Chang, H., Huo, W., Li, Y., Xu, S., 2015. Paternal BPA exposure in early life alters Igf2  
665 epigenetic status in sperm and induces pancreatic impairment in rat offspring. *Toxicology Letters* 238,  
666 30–38. <https://doi.org/10.1016/j.toxlet.2015.08.009>

667 Martínez, M.Á., Blanco, J., Rovira, J., Kumar, V., Domingo, J.L., Schuhmacher, M., 2020. Bisphenol A  
668 analogues (BPS and BPF) present a greater obesogenic capacity in 3T3-L1 cell line. *Food and*  
669 *Chemical Toxicology* 140, 111298. <https://doi.org/10.1016/j.fct.2020.111298>

670 Meng, L., Liu, J., Wang, C., Ouyang, Z., Kuang, J., Pang, Q., Fan, R., 2021. Sex-specific oxidative damage  
671 effects induced by BPA and its analogs on primary hippocampal neurons attenuated by EGCG.  
672 *Chemosphere* 264, 128450. <https://doi.org/10.1016/j.chemosphere.2020.128450>

673 Moon, M.K., Jeong, I.-K., Oh, T.J., Ahn, H.Y., Kim, H.H., Park, Y.J., Jang, H.C., Park, K.S., 2015. Long-term  
674 oral exposure to bisphenol A induces glucose intolerance and insulin resistance. *Journal of*  
675 *Endocrinology* 226, 35–42. <https://doi.org/10.1530/JOE-14-0714>

676 Movassat, J., Saulnier, C., Serradas, P., Portha, B., 1997. Impaired development of pancreatic beta-cell mass  
677 is a primary event during the progression to diabetes in the GK rat. *Diabetologia* 40, 916–925.  
678 <https://doi.org/10.1007/s001250050768>

679 Nair, A.B., Jacob, S., 2016. A simple practice guide for dose conversion between animals and human. *J Basic*  
680 *Clin Pharm* 7, 27–31. <https://doi.org/10.4103/0976-0105.177703>

681 Ng, S.-F., Lin, R.C.Y., Laybutt, D.R., Barres, R., Owens, J.A., Morris, M.J., 2010. Chronic high-fat diet in  
682 fathers programs  $\beta$ -cell dysfunction in female rat offspring. *Nature* 467, 963–966.  
683 <https://doi.org/10.1038/nature09491>

684 Raad, G., Serra, F., Martin, L., Derieppe, M.-A., Gilleron, J., Costa, V.L., Pisani, D.F., Amri, E.-Z., Trabucchi,  
685 M., Grandjean, V., 2021. Paternal multigenerational exposure to an obesogenic diet drives epigenetic  
686 predisposition to metabolic diseases in mice. *eLife* 10, e61736. <https://doi.org/10.7554/eLife.61736>

687 Rashid, C.S., Bansal, A., Mesaros, C., Bartolomei, M.S., Simmons, R.A., 2020. Paternal bisphenol A exposure  
688 in mice impairs glucose tolerance in female offspring. *Food and Chemical Toxicology* 145, 111716.  
689 <https://doi.org/10.1016/j.fct.2020.111716>

690 Rodgers, A.B., Morgan, C.P., Leu, N.A., Bale, T.L., 2015. Transgenerational epigenetic programming via  
691 sperm microRNA recapitulates effects of paternal stress. *Proc Natl Acad Sci U S A* 112, 13699–13704.  
692 <https://doi.org/10.1073/pnas.1508347112>

693 Sargis, R.M., Simmons, R.A., 2019. Environmental neglect: endocrine disruptors as underappreciated but  
694 potentially modifiable diabetes risk factors. *Diabetologia* 62, 1811–1822.  
695 <https://doi.org/10.1007/s00125-019-4940-z>

696 Strakovsky, R.S., Wang, H., Engeseth, N.J., Flaws, J.A., Helferich, W.G., Pan, Y.-X., Lezmi, S., 2015.  
697 Developmental bisphenol A (BPA) exposure leads to sex-specific modification of hepatic gene  
698 expression and epigenome at birth that may exacerbate high-fat diet-induced hepatic steatosis. *Toxicol*  
699 *Appl Pharmacol* 284, 101–112. <https://doi.org/10.1016/j.taap.2015.02.021>

700 Susiarjo, M., Xin, F., Bansal, A., Stefaniak, M., Li, C., Simmons, R.A., Bartolomei, M.S., 2015. Bisphenol A  
701 Exposure Disrupts Metabolic Health Across Multiple Generations in the Mouse. *Endocrinology* 156,

702 2049–2058. <https://doi.org/10.1210/en.2014-2027>

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

706 Tudurí, E., Marroqui, L., Dos Santos, R.S., Quesada, I., Fuentes, E., Alonso-Magdalena, P., 2018. Timing of  
707 Exposure and Bisphenol-A: Implications for Diabetes Development. *Frontiers in Endocrinology* 9.

708 Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W.V., 2007. Human exposure to bisphenol A  
709 (BPA). *Reprod Toxicol* 24, 139–177. <https://doi.org/10.1016/j.reprotox.2007.07.010>

710 vom Saal, F.S., Vandenberg, L.N., 2021. Update on the Health Effects of Bisphenol A: Overwhelming  
711 Evidence of Harm. *Endocrinology* 162. <https://doi.org/10.1210/endocr/bqaa171>

712 Watkins, A.J., Dias, I., Tsuru, H., Allen, D., Emes, R.D., Moreton, J., Wilson, R., Ingram, R.J.M., Sinclair,  
713 K.D., 2018. Paternal diet programs offspring health through sperm- and seminal plasma-specific  
714 pathways in mice. *Proc Natl Acad Sci U S A* 115, 10064–10069.  
715 <https://doi.org/10.1073/pnas.1806333115>

716 Wei, J., Lin, Y., Li, Y., Ying, C., Chen, J., Song, L., Zhou, Z., Lv, Z., Xia, W., Chen, X., Xu, S., 2011. Perinatal  
717 Exposure to Bisphenol A at Reference Dose Predisposes Offspring to Metabolic Syndrome in Adult  
718 Rats on a High-Fat Diet. *Endocrinology* 152, 3049–3061. <https://doi.org/10.1210/en.2011-0045>

719 Wei, Y., Yang, C.-R., Wei, Y.-P., Zhao, Z.-A., Hou, Y., Schatten, H., Sun, Q.-Y., 2014. Paternally induced  
720 transgenerational inheritance of susceptibility to diabetes in mammals. *Proc Natl Acad Sci U S A* 111,  
721 1873–1878. <https://doi.org/10.1073/pnas.1321195111>

722 Wu, L.-H., Zhang, X.-M., Wang, F., Gao, C.-J., Chen, D., Palumbo, J.R., Guo, Y., Zeng, E.Y., 2018. Occurrence  
723 of bisphenol S in the environment and implications for human exposure: A short review. *Sci. Total*  
724 *Environ.* 615, 87–98. <https://doi.org/10.1016/j.scitotenv.2017.09.194>

725 Zhang, H., Wang, Z., Meng, L., Kuang, H., Liu, J., Lv, X., Pang, Q., Fan, R., 2020. Maternal exposure to  
726 environmental bisphenol A impairs the neurons in hippocampus across generations. *Toxicology* 432,  
727 152393. <https://doi.org/10.1016/j.tox.2020.152393>

728 Zhang, Y., Li, T., Pan, C., Khan, I.A., Chen, Z., Yue, Y., Yang, M., 2022. Intergenerational toxic effects of  
729 parental exposure to bisphenol AF on offspring and epigenetic modulations in zebrafish. *Sci Total*  
730 *Environ* 823, 153714. <https://doi.org/10.1016/j.scitotenv.2022.153714>

731 Zhou, X., Kramer, J.P., Calafat, A.M., Ye, X., 2014. Automated on-line column-switching high performance  
732 liquid chromatography isotope dilution tandem mass spectrometry method for the quantification of  
733 bisphenol A, bisphenol F, bisphenol S, and 11 other phenols in urine. J. Chromatogr. B Analyt. Technol.  
734 Biomed. Life Sci. 944, 152–156. <https://doi.org/10.1016/j.jchromb.2013.11.009>

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## 739 **Figure Legends**

740 **Fig. 1. Schematic diagram of the experimental design.**

741 **Fig. 2. Physiological characteristics, glycemia and lipid profiles in Ctrl, BPS-4 and BPS-40**  
742 **sires.** Fourteen weeks old male Wistar rats were given water containing BPS at doses of 4  
743  $\mu\text{g}/\text{kg}/\text{day}$  (BPS-4, n=15) or 40  $\mu\text{g}/\text{kg}/\text{day}$  (BPS-40, n=9) for up to 11 weeks. Control rats were  
744 given regular water (Ctrl, n=15). (A) Body weight monitored weekly and (B) body fat/lean mass  
745 ratio measured after the period of BPS administration. (C) Food intake, (D) water intake and (E)  
746 non-fasting glycemia were monitored weekly. (F) Insulin tolerance test (insulin, 0.5 U/kg), (G)  
747 intraperitoneal glucose tolerance test (glucose, 2 g/kg) and (H) glucose-induced insulin secretion  
748 *in vivo* were performed at end of BPS treatment (Ctrl, n=12; BPS-4, n=12; BPS-40, n=6). Plasma  
749 levels of (I) cholesterol, (J) high-density lipoprotein (HDL), (K) cholesterol/HDL ratio, (L) non-  
750 esterified fatty acids (NEFA) and (M) triglycerides were measured (Ctrl, n=9; BPS-4, n=6; BPS-  
751 40, n=6). Area over the curve (AOC,  $\text{mg}\cdot\text{dL}^{-1}\cdot 120\text{ min}$ ) and area under the curve (AUC,  $\text{mg}\cdot\text{dL}^{-1}\cdot 120\text{ min}$ ;  
752 insulin,  $\text{mg}\cdot\text{dL}^{-1}\cdot 90\text{ min}$ ) are shown in the insert. Results are expressed as means  $\pm$   
753 S.E M. \* Ctrl vs BPS-4 sires; # Ctrl vs BPS-40 sires; \$ BPS-4 vs BPS-40 sires. \*, #  $p < 0.05$ , \*\*, \$\$  $p$

754 < 0.01, \*\*\* $p$  < 0.001, \*\*\*\* $p$  < 0.0001.

755 **Fig. 3. Fetal body and pancreatic parameters of offspring of Ctrl, BPS-4 and BPS-40 sires.**

756 After 2 months of administration of BPS water, Ctrl, BPS-4 and BPS-40 sires were mated with  
757 virgin female Wistar rats. Male and female offspring were studied separately at the embryonic  
758 day 18 post-conception (E18). (A) Body weight (male, n=28-34; female, n=26-33), (B) pancreas  
759 weight (male, n=12-33; female, n=21-31) of male and female F1 fetuses at E18 were measured,  
760 and (C) body mass index (BMI) (male, n=12-33; female, n=15-31) was calculated. (D) Male  
761 fetuses from Ctrl and BPS-4 sires were randomly chosen and analyzed for pancreatic  $\alpha$  cell mass  
762 and  $\beta$  cell mass by histomorphometry (n=4-5). Results are expressed as means  $\pm$  S.E.M. \* Ctrl  
763 vs BPS-4 fetus. # Ctrl vs BPS-40 fetus. \* $p$  < 0.05, ### $p$  < 0.001.

764 **Fig. 4. Physiological characteristics of progenies of Ctrl, BPS-4 and BPS-40 sires in**

765 **adulthood.** Male and female offspring were studied separately in adulthood. (A) Body weight  
766 (n=6-8), (B) food intake (n=6-8), (C) body fat/lean mass ratio (n=6) and (D) non-fasting  
767 glycemia (n=6-8) of adult male offspring were monitored. (E) Body weight, (F) food intake, (G)  
768 body fat/lean mass ratio and (H) non-fasting glycemia of adult female F1 (n=6-8) were monitored.  
769 Results are expressed as means  $\pm$  S.E.M. \* BPS-4/F1 vs Ctrl/F1; \$ BPS-4/F1 vs BPS-40/F1. \*, \$ $p$   
770 < 0.05, \*\*, \$\$ $p$  < 0.01, \*\*\*, \$\$\$ $p$  < 0.001, \*\*\*\* $p$  < 0.0001.

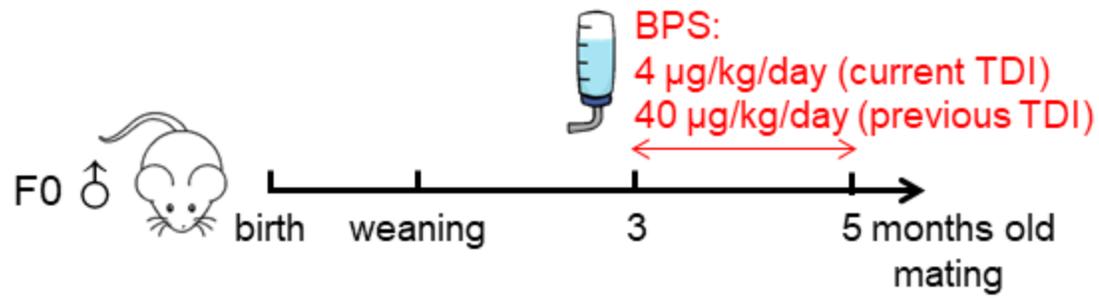
771 **Fig. 5. Parameters of glucose metabolism in 3- and 6-month-old F1 from Ctrl, BPS-4 and**

772 **BPS-40 sires.** Insulin tolerance test (ITT) (insulin, 0.5 U/kg) in (A) 3 months and (D) 6 months  
773 old male F1, and in (G) 3 months and (J) 6 months old female F1. Intraperitoneal glucose  
774 tolerance test (glucose, 2 g/kg) in (B) 3 months and (E) 6 months old male F1, and in (H) 3  
775 months and (K) 6 months old female F1. Glucose-induced insulin secretion *in vivo* in (C) 3  
776 months and (F) 6 months old male F1, and in (I) 3 months and (L) 6 months old female F1. (A)  
777 n=17-20; (B, C) n=10-15; (D) n=11-14; (E, F) n=10; (G, H, I) n=7-12; (J, K, L) n=6. Area over

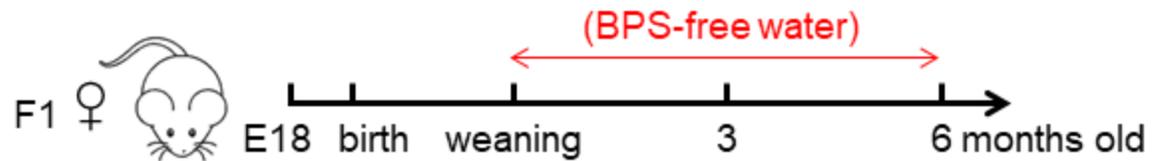
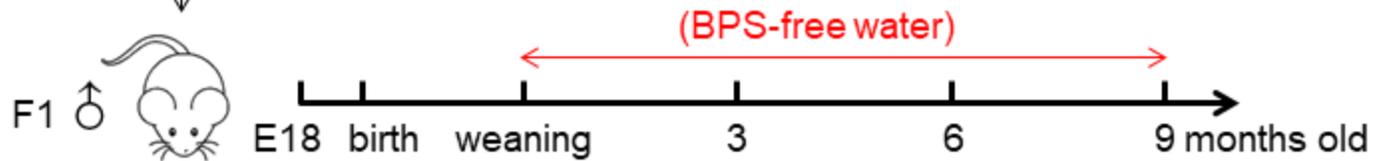
778 curve (AOC,  $\text{mg}\cdot\text{dL}^{-1}\cdot 120 \text{ min}$ ) and area under the curve (AUC,  $\text{mg}\cdot\text{dL}^{-1}\cdot 120 \text{ min}$ ; insulin,  
779  $\text{mg}\cdot\text{dL}^{-1}\cdot 90 \text{ min}$ ) are shown in the insert. Results are expressed as means  $\pm$  S.E.M. \* Ctrl/F1 vs  
780 BPS-4/F1; # Ctrl/F1 vs BPS-40/F1; \$ BPS-4/F1 vs BPS-40/F1. \*, #, \$  $p < 0.05$ , \*\*, ##, \$\$  $p < 0.01$ , \*\*\*,  
781 ###, \$\$\$  $p < 0.001$ , \*\*\*\*, \$\$\$\$  $p < 0.0001$ .

782 **Fig. 6. Physiological characteristics in male F1 from Ctrl and BPS-4 sires at 9 months of**  
783 **age.** Comparison of (A) body weight, (B) fat/lean mass ratio and (C) non-fasting plasma insulin  
784 in 9-month-old male Ctrl/F1 and BPS-4/F1. (D) Insulin tolerance test (insulin, 0.5 U/kg), (E)  
785 intraperitoneal glucose tolerance test (glucose, 2 g/kg), (F) glucose-induced insulin secretion *in*  
786 *vivo* and (G) intraperitoneal pyruvate tolerance test (sodium pyruvate, 1 g/kg) were performed  
787 in male Ctrl/F1 and BPS-4/F1 at 9 months of age. (n=5-8). Area over curve (AOC,  $\text{mg}\cdot\text{dL}^{-1}\cdot 120$   
788 min) and area under the curve (AUC,  $\text{mg}\cdot\text{dL}^{-1}\cdot 120 \text{ min}$ ; insulin,  $\text{mg}\cdot\text{dL}^{-1}\cdot 90 \text{ min}$ ) are shown in  
789 the insert. Adipose tissue and soleus muscle were dissected before and after insulin  
790 administration. Protein levels of AKT and p-AKT (ser473) in (H, I) adipose tissue and (J, K)  
791 soleus muscle were determined and quantified. Males F1 were sacrificed at 10 months of age.  
792 (L) Isolated islets of 10-month-old male Ctrl/F1 and BPS-4/F1 were incubated with 2.8 or 16.7  
793 mM glucose to measure the glucose-induced insulin secretion *ex vivo*. Morphological analysis  
794 of  $\beta$  cells was performed on pancreatic sections of 10-month-old male Ctrl/F1 and BPS-4/F1.  $\beta$   
795 cell were identified by immunostaining of pancreatic sections for insulin (brown) in (M) Ctrl/F1  
796 and (N) BPS-4/F1 groups, and (O) pancreatic  $\beta$  cell mass was measured. (n=6). Results are  
797 expressed as means  $\pm$  S.E.M. \*, &  $p < 0.05$ , \*\*, &&  $p < 0.01$ .

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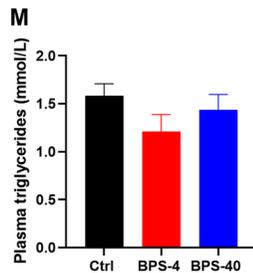
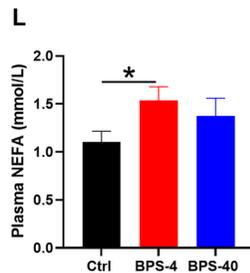
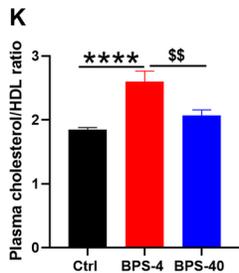
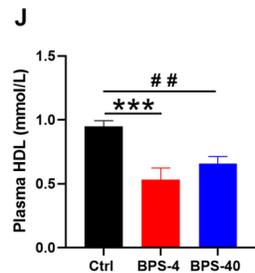
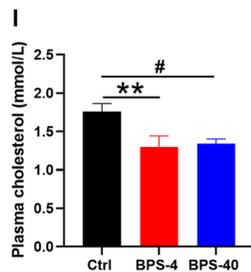
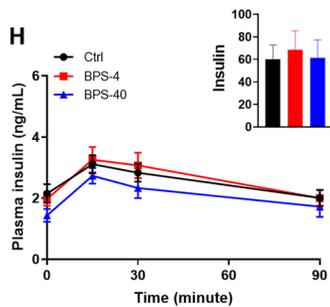
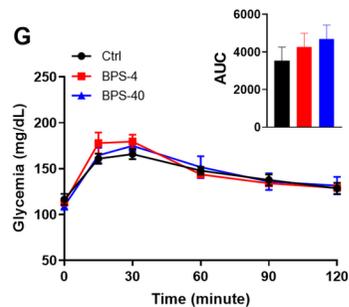
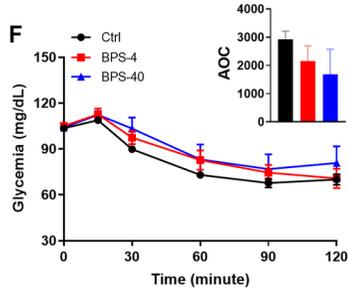
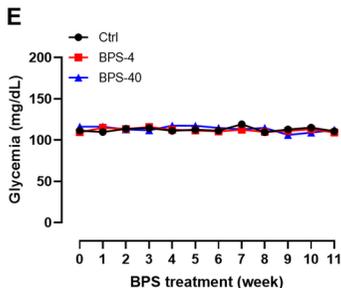
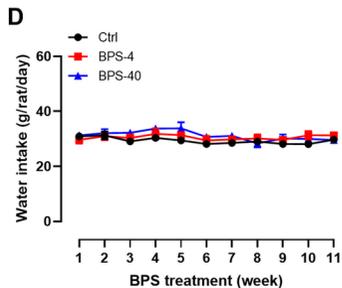
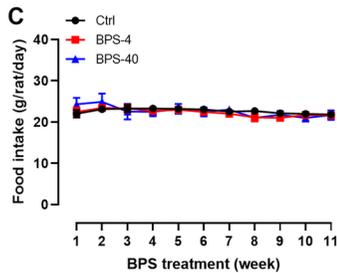
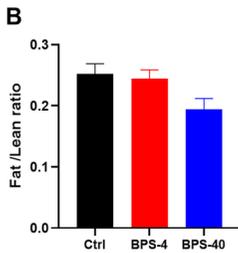
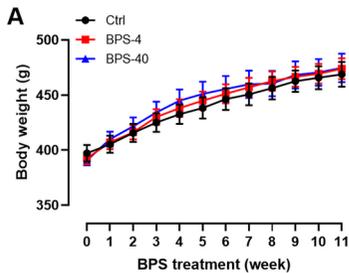


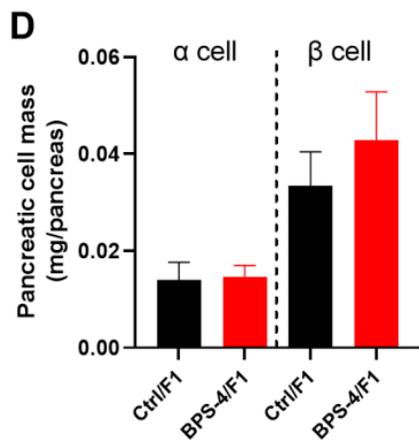
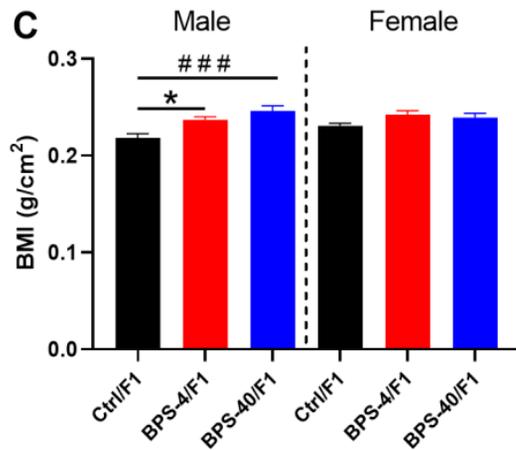
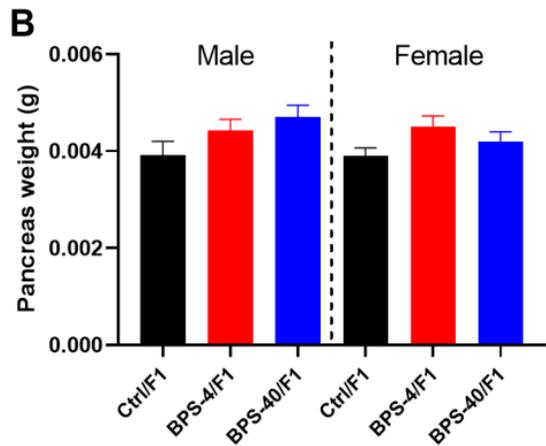
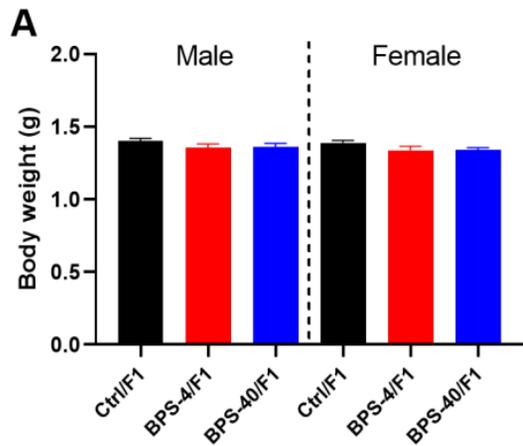
<u>Group F0:</u>	<u>Group F1</u>
Ctrl sire	Ctrl/F1
BPS-4 sire	BPS-4/F1
BPS-40 sire	BPS-40/F1



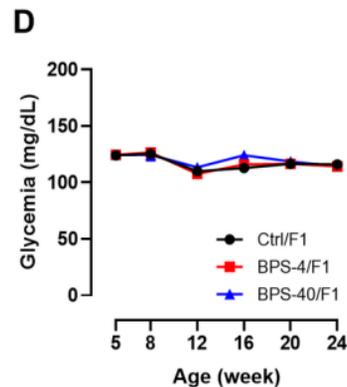
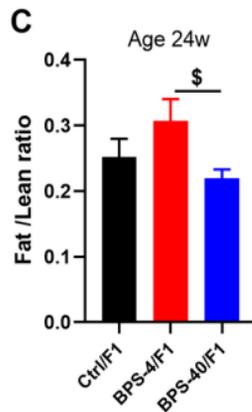
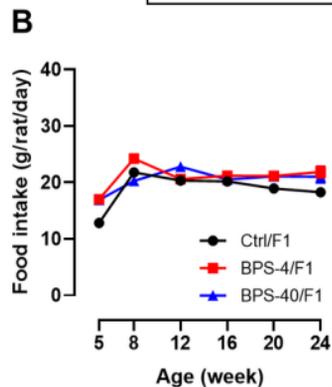
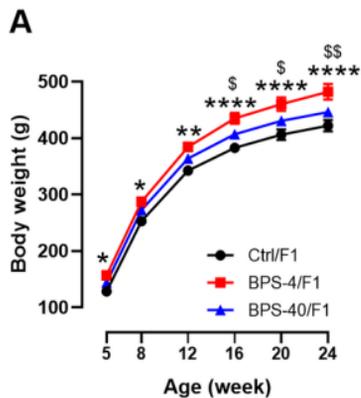
Tolerable daily intake (TDI)

Embryonic day 18 post-conception (E18)

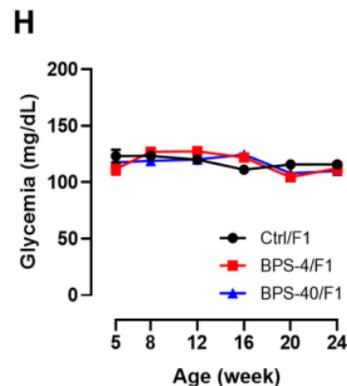
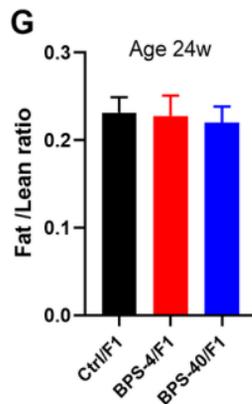
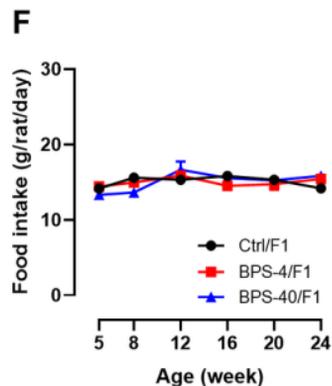
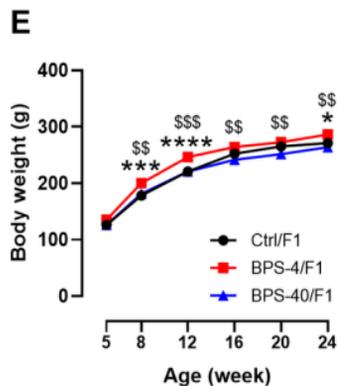


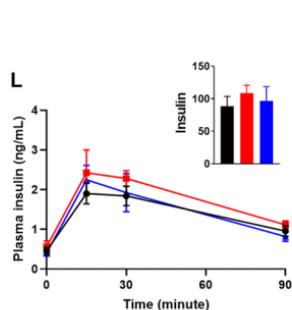
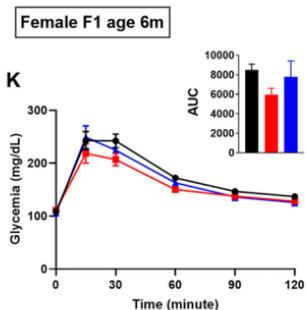
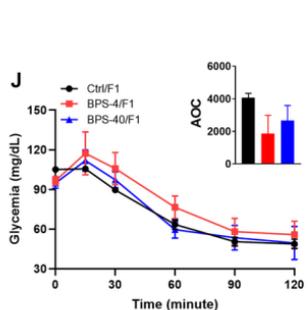
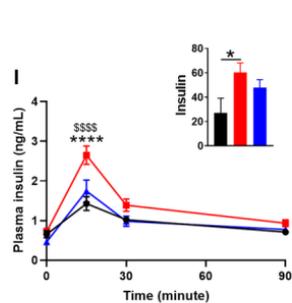
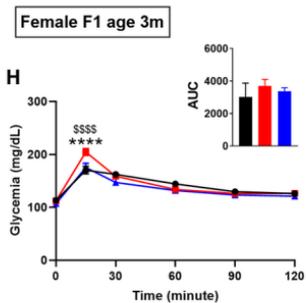
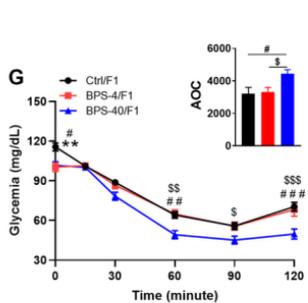
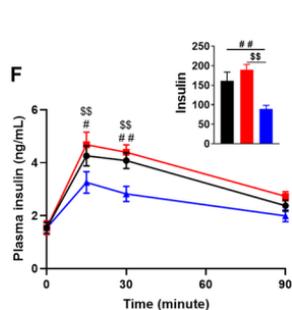
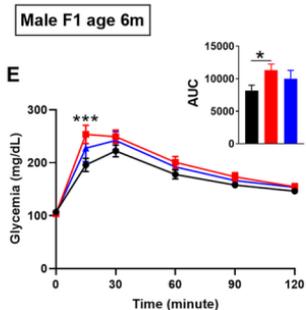
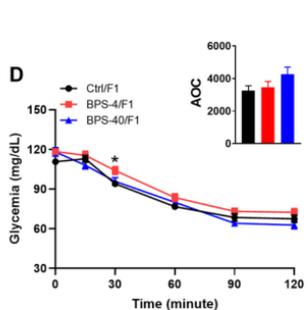
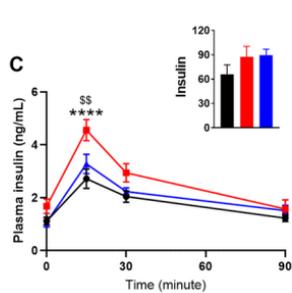
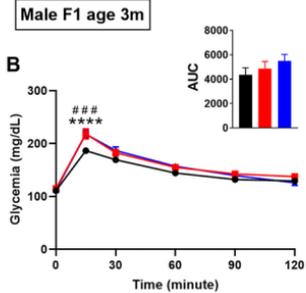
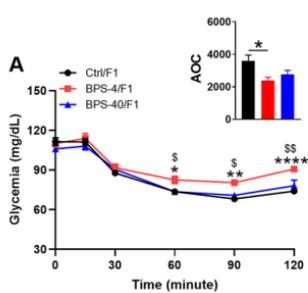


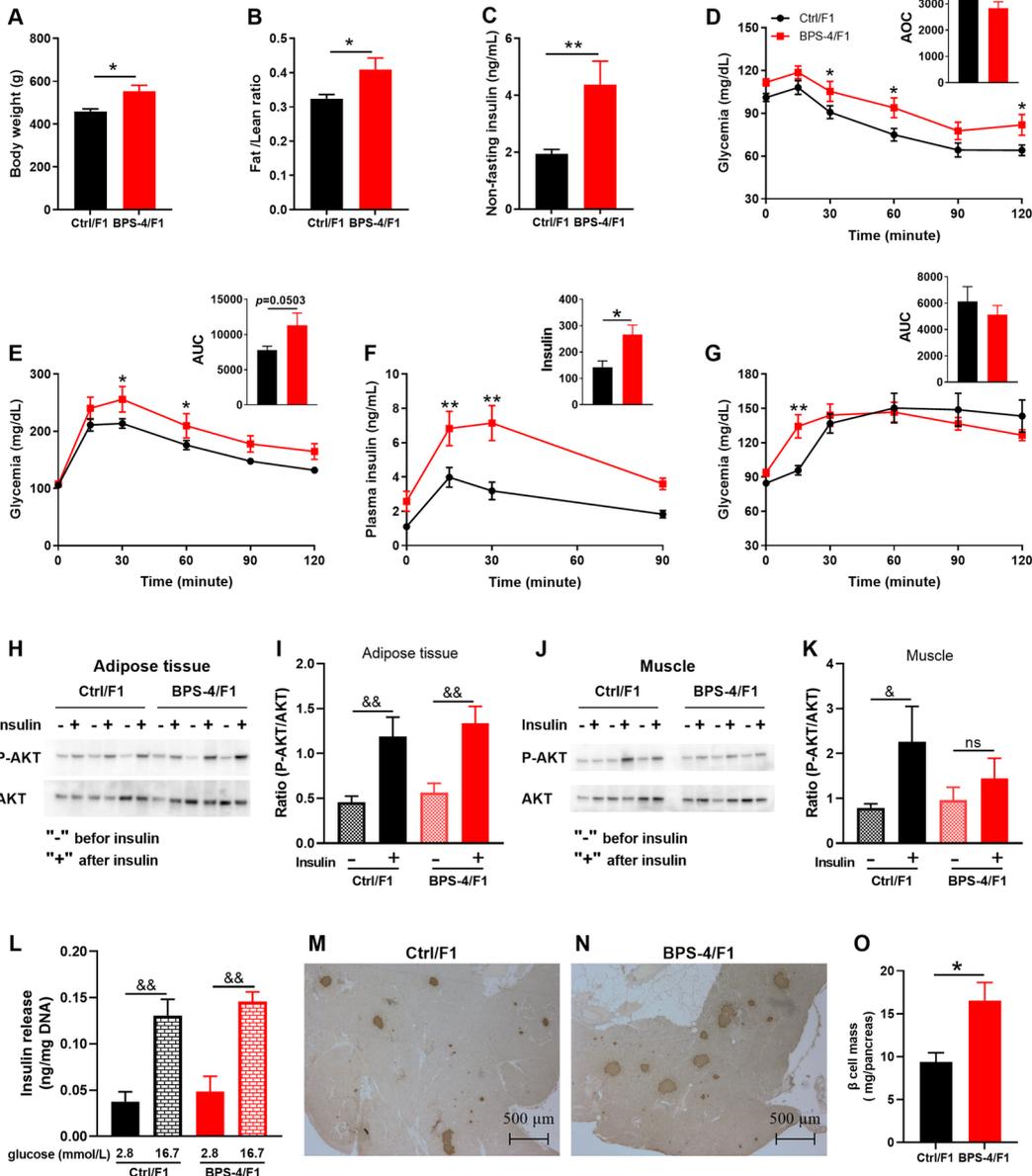
### Adult F1 male



### Adult F1 female





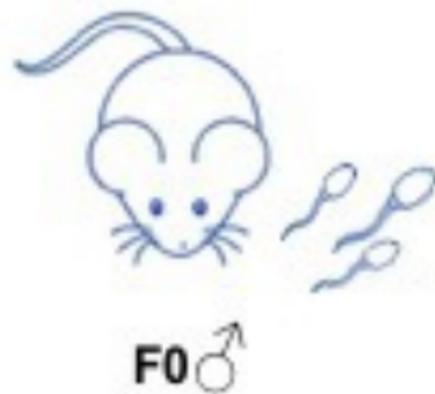


**Table 1. Plasma lipid profiles in male offspring at 3 and 9 months of age**

Lipid profiles (mmol/L)	Age	Ctrl/F1	BPS-4/F1	BPS-40/F1
<b>Cholesterol</b>	3 months old	1.77 ± 0.14	1.45 ± 0.15	1.55 ± 0.06
	9 months old	1.67 ± 0.10	2.01 ± 0.14*	—
<b>HDL</b>	3 months old	0.95 ± 0.06	0.58 ± 0.06**, \$	0.74 ± 0.04##
	9 months old	0.86 ± 0.03	0.88 ± 0.03	—
<b>Cholesterol/HDL</b>	3 months old	1.85 ± 0.05	2.92 ± 0.30**, \$	2.11 ± 0.10
	9 months old	1.93 ± 0.05	2.28 ± 0.08**	—
<b>NEFA</b>	3 months old	1.04 ± 0.12	0.92 ± 0.14	0.92 ± 0.13
	9 months old	0.81 ± 0.06	0.83 ± 0.07	—
<b>Triglycerides</b>	3 months old	1.75 ± 0.12	1.78 ± 0.27	1.80 ± 0.16
	9 months old	1.57 ± 0.07	1.99 ± 0.45	—

Data are expressed as means ± S.E.M. (n=5-7). \* Ctrl/F1 vs BPS-4/F1; # Ctrl/F1 vs BPS-40/F1; \$ BPS-4/F1 vs BPS-40/F1. \*, \$  $p < 0.05$ , \*\*, ##  $p < 0.01$ .

**BPS exposure**



Mating



Normal



Body weight ↑  
Body fat/lean ↑  
Insulin sensitivity ↓  
Glucose tolerance ↓