The role of polycarbonate monomer bisphenol-A in insulin resistance

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Bisphenol A (BPA) is a synthetic unit of polycarbonate polymers and epoxy resins, the types of plastics that could be found in essentially every human population and incorporated into almost every aspect of the modern human society. BPA polymers appear in a wide range of products, from liquid storages (plastic bottles, can and glass linings, water pipes and tanks) and food storages (plastics wraps and containers), to medical and dental devices. BPA polymers could be hydrolyzed spontaneously or in a photo- or temperature- catalyzed process, providing widespread environmental distribution and chronic exposure to the BPA monomer in contemporary human populations. Bisphenol A is also a xenoestrogen, an endocrine-disrupting chemical (EDC), that interferes with the endocrine system mimicking the effects of an estrogen and could potentially keep our endocrine system in a constant perturbation that parallels endocrine disruption arising during pregnancy, such as insulin resistance (IR). Gestational insulin resistance represents a natural biological phenomenon of higher insulin resistance in peripheral tissues of the pregnant females, when nutrients are increasingly being directed to the embryo instead of being stored in peripheral tissues. Gestational diabetes mellitus may appear in healthy non-diabetic females, due to gestational insulin resistance that leads to increased blood sugar levels and hyperinsulinemia (increased insulin production from the pancreatic beta cells). The hypothesis states that unnoticed and constant exposure to this environmental chemical might potentially lead to the formation of chronic low-level endocrine disruptive state that resembles gestational insulin resistance, which might contribute to the development of diabetes. The increasing body of evidence supports the major premises of this hypothesis, as exemplified by the numerous publications examining the association of BPA and insulin resistance, both epidemiological and mechanistic. However, to what extent BPA might contribute to the development of diabetes in the modern societies still remains unknown. In this review, I discuss the chemical properties of BPA and the sources of BPA contamination found in the environment and in human tissues. I provide an overview of

mechanisms for the proposed role of bisphenol A in insulin resistance and diabetes, as well as other related diseases, such as cardiovascular diseases. I describe the transmission of BPA effects to the offspring and postulate that gender related differences might originate from differences in liver enzyme levels, such as UDP-glucuronosyltransferase, which is involved in BPA processing and its elimination from the organism. I discuss the molecular mechanisms of BPA action through nuclear and membrane-bound ER receptors, non-monotonic dose response, epigenetic modifications of the DNA and propose that chronic exposure to weak binders, such as BPA, may mimic the effects of strong binders, such as estrogens.

1 **The role of polycarbonate monomer bisphenol A in insulin resistance**

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18
19 **Abstract**

21 Bisphenol A (BPA) is a synthetic unit of polycarbonate polymers and epoxy resins, the
22 types of plastics that could be found in essentially every human population and
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25 linings, water pipes and tanks) and food storages (plastics wraps and containers), to
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31 Bisphenol A is also a xenoestrogen, an endocrine-disrupting chemical (EDC), that
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34 disruption arising during pregnancy, such as insulin resistance (IR). Gestational insulin
35 resistance represents a natural biological phenomenon of higher insulin resistance in
36 peripheral tissues of the pregnant females, when nutrients are increasingly being
37 directed to the embryo instead of being stored in peripheral tissues. Gestational
38 diabetes mellitus may appear in healthy non-diabetic females, due to gestational insulin
39 resistance that leads to increased blood sugar levels and hyperinsulinemia (increased
40 insulin production from the pancreatic beta cells). The hypothesis states that unnoticed
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42 formation of chronic low-level endocrine disruptive state that resembles gestational
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64 **Introduction**
65
66 Bisphenol A (BPA, IUPAC ID: 4,4'-(propane-2,2-diyl)diphenol) is one of the most
67 extensively used synthetic monomers that in a polymerized state constitutes
68 polycarbonate plastics and epoxy resins and makes up the significant part of the plastic
69 environment that surrounds modern human species. Bisphenol A is also a molecule that
70 behaves as an endocrine-disrupting chemical (EDC) that is mimicking the effects of an
71 estrogen (i.e. a xenoestrogen molecule). BPA could interfere with the endocrine system
72 and promote a chronic imbalance that parallels endocrine disruption that arises during
73 pregnancy under the influence of estrogens, such as insulin resistance (IR).
74
75 Human populations are being continuously exposed to bisphenol A to the extent that it
76 could be considered a common environmental factor present since 1957, when the first
77 production of BPA started. With over 6 billion pounds (2.7 million tonnes) of BPA
78 produced in 2003 and incorporated into polycarbonate polymers, and estimated 4.5
79 million tonnes in 2015, BPA represents one of the most abundant chemicals that come
80 in direct contact with human populations world-wide (Welshons et al., 2006). The
81 volume of BPA production in the world is predicted to increase continuously, and it is
82 presently estimated to surpass 5.4 million tonnes (Merchant Research & Consulting Ltd,
83 2017). BPA is found in plastic bottles, plastic food containers, baby and water bottles,
84 can and glass linings, various medical and dental devices, sealants for dental fillings,
85 compact disks and electronics, eyeglass lenses, and even in the lining of water pipes
86 and tanks (Talsness et al., 2009; Huang et al., 2012). BPA is the main monomer of
87 epoxy resins which are being used as coating agents on the interior of many water
88 storage tanks. Hence, BPA leaching from such widely used polymers may influence
89 human health inadvertently through consuming water or food. In addition, canned food
90 might represent one of the significant global sources of BPA, as it has been shown that
91 the canned food is significantly more contaminated with BPA compared to the non-
92 canned one and that the BPA levels from canned food did not differ between continents
93 (Repossi et al., 2016). In addition to dietary ingestion, a significant source of BPA
94 exposure in modern human populations represents thermal paper used for supermarket
95 and ATM receipts, that could efficiently transfer BPA to skin when holding the source for
96 as long as 5 s, and it remains largely extractable after 2 h, indicating perfusion into the
97 skin to such depths that it could no longer be removed or washed off easily
98 (Biedermann, Tschudin & Grob, 2010; Ehrlich et al., 2014). Furthermore, one of the
99 sources that could provide constant exposure to BPA is orthodontic material, as it has
100 been shown that the eluents from orthodontic adhesives immersed in saline for 1 month
101 at 37°C exhibit estrogenic action through the induction of MCF-7 cell proliferation, an
102 estrogen-responsive cell line (Gioka et al., 2009).
103
104 In addition, BPA is found as an additive to various polyvinyl chloride (PVC) products,
105 including food packaging stretch films (López-Cervantes & Paseiro-Losada, 2003).
106 Food and drink products are estimated to be the major source of BPA contamination,
107 with detected concentrations reaching microgram (µg) levels. BPA and several
108 bisphenol A analogues, including bisphenol-F (BPF) and bisphenol-S (BPS), have been

109 found in various categories of food and drink products including fruits, vegetables, dairy
110 products, cereals, beverages, fats and oils, fish and seafood, meat and meat products
111 and "other products" category using high-performance liquid chromatography-tandem
112 mass spectrometry (HPLC-MS/MS) (Liao & Kannan, 2013). BPA was detected in 75%
113 of the tested samples, and the detected concentration was ranging from the very limit of
114 quantification (LOQ) to 1.13 µg/g weight (mean value, 4.38 ng/g). BPA and its
115 analogues have been shown to appear in the indoor dust, and dust ingestion may be
116 another significant source of contamination with BPA (Liao et al., 2012). The potential to
117 use BPA analogues, BPS and BPF, as substitutes in the production of plastics has been
118 debated, as BPA analogues have shown similar effects to BPA on the cellular level
119 (Verbanck et al., 2017).
120
121 The global industrial trend of polycarbonate consumption is increasing from 1.6 million
122 tonnes in 2000 up to 3.4 million tonnes in 2010, and 4.5 million tonnes estimated in
123 2015 (Sevak Publications, 2008). Significant demand of BPA originates from East Asia
124 (predominantly China), that accounted for 59% of the polycarbonate consumption in
125 2010 and 68% estimated in 2015. On the other side, Europe contributed with 32% of
126 polycarbonate consumption in 2000, 30% in 2010, and estimated 25% in 2015. Similar
127 decreasing trend was observed for the United States (28% in 2000, 22% in 2010, and
128 18% in 2015). Particularly, the production of BPA in China has grown extensively and
129 this continuous trend will lead to the increasing BPA contamination in the global
130 environment (Huang et al., 2012). The Environmental Protection Agency (EPA) in the
131 US has established the tolerable daily intake (TDI) for BPA at 50 µg per kg (body
132 weight) per day in 1988 (Rubin, 2011). In January 2015, European Food Safety
133 Authority has reduced TDI for BPA from 50 to 4 µg per kg (body weight) per day. In
134 addition, EPA has established the oral reference dose (RfD) for BPA at 100 µg/L as a
135 standard for the total allowable concentration (TAC) in drinking water. Whether the
136 extent of such exposure is significant has been debated (Nohynek et al., 2013), and
137 various evidence have been presented supporting that the tight regulation of BPA is
138 necessary. This is especially important considering the new knowledge about BPA
139 action, including the numerous instances of experimental non-monotonic dose response
140 to the BPA treatment, indicating narrow effective range of concentrations and the
141 absence of effects on higher doses.
142
143 In this review, I present a global overview of the chemical properties of BPA and its
144 polymers, their hydrolytic reaction and leaching BPA concentrations present in the
145 environment and the corresponding concentrations in human tissues. Next, I define the
146 physiological gestational insulin resistance (GIR), its pathophysiological progression to
147 gestational diabetes and the role of estrogens in promoting these disorders. I elaborate
148 on the proposed mechanism of BPA endocrine disruption, including non-monotonic
149 dose response, and its transgenerational effects on male and female offspring, including
150 epigenetic modifications transmitted to the next generation. Finally, I review the
151 literature on biological effects of BPA in mice and humans including insulin resistance
152 and diabetes, as well as in cardiovascular diseases such as coronary artery disease
153 and other physiological disorders that BPA might contribute to.
154

155
156 **Survey Methodology**
157
158 In order to survey the effects of BPA on insulin resistance I searched for studies
159 analyzing BPA and insulin resistance on Pubmed or Pubmed Central (PMC) from their
160 inception through May 2, 2017 using the following search algorithm: (*bpa[Title/Abstract]*
161 *OR bisphenol-A[Title/Abstract]) AND ("insulin resistance"[MeSH Terms] OR*
162 *("insulin"[Title/Abstract] AND "resistance"[Title/Abstract]) OR "insulin*
163 *resistance"[Title/Abstract])*. This search yielded the list of 19 publications on Pubmed
164 Central and 86 publications on Pubmed. The survey of papers describing the influence
165 of BPA on cardiovascular diseases was performed by applying the search algorithm:
166 (*bpa[Title/Abstract] OR bisphenol-A[Title/Abstract]) AND "cardiovascular*
167 *disease*"[Title/Abstract],* which yielded 33 publications on Pubmed and no publications
168 on PMC. The search with algorithm: *(bpa[Title/Abstract] OR bisphenol-A[Title/Abstract])*
169 *AND "cardiovascular"[Title/Abstract]* yielded 97 publications on Pubmed and 5
170 publications on PMC. The survey of papers describing chemical properties of BPA
171 molecule was performed with the algorithm: *(bpa[Title/Abstract] OR bisphenol-*
172 *A[Title/Abstract]) AND "chemical properties"[Title/Abstract]*, which yielded 27
173 publications on Pubmed and no items on PMC. The survey of BPA concentration levels
174 in both environment and human tissues was performed with the algorithm:
175 *(bpa[Title/Abstract] OR bisphenol-A[Title/Abstract]) AND "concentration*"[Title]*, yielding
176 89 publications on Pubmed and 12 on PMC. Papers with subject relevant to the search
177 term and not present in the initial search were obtained through Similar Article Pubmed
178 function. Initial papers that describe the structural and functional properties of BPA as
179 an estrogen-like molecule from 1936 and 1938 were not present in the Pubmed or PMC
180 databases and were found on Wikipedia and obtained from the JStore database. In
181 addition, the search was performed using '*' symbol that denotes the truncated search
182 terms to increase the number of publications obtained. After reviewing, articles were
183 excluded from the study in case they were published in languages other than English or
184 if they described subjects that were not related to the main search topic. Papers that did
185 not contributed to the scientific understanding of the search topic were excluded, as well
186 as papers that were addressing the same or similar subjects in order to eliminate the
187 redundant studies. After filtering for each search term the number of papers retained
188 were: BPA chemical properties - 6, BPA and insulin resistance relationship - 11, BPA
189 and cardiovascular and other diseases - 4, and BPA and environmental and human
190 tissue concentrations - 18. Additional papers were introduced after the revision process.
191
192
193 **BPA chemical properties, polymerization and hydrolysis.**
194
195 Bisphenol A (BPA), is an organic synthetic molecule composed of the two hydroxy-
196 phenyl groups connected through a carbon atom and, therefore, belonging to the group
197 of diphenylmethane derivatives, with the formula $(CH_3)_2C(C_6H_4OH)_2$. BPA holds certain
198 steric resemblance to the estrogen molecule 17β-estradiol (E2), especially in the span
199 of outer hydroxyl groups, and although it does not have the structure of a complete
200 steroid ring, its behavior as a synthetic estrogen is based on the similar chemical

201 properties, steric mimicking of an estrogen ring and on a weak interaction with the
202 estrogen receptors. BPA has an average mass of 228.3 Da, while estrogen E2 has an
203 average mass 272.4 Da (Pence & Williams, 2010). BPA possesses experimental
204 melting point of 153-158 °C (Alfa Aesar), while E2 has a similar, but higher value 175-
205 178 °C (Alfa Aesar). Acidic dissociation constant, K_a, for BPA is 10.29 and for E2 is
206 10.27 (Bento et al., 2014). BPA cross-linking properties have propelled its utilization in
207 the manufacturing of polycarbonate plastics and epoxy resins. The polymer structural
208 properties and efficiency of polymerization and degradation when exposed to higher
209 than normal temperatures are essential for the degree of contamination in the
210 environment. The glass-liquid transition temperature (Tg, in amorphous materials
211 represents transition from a compact glassy state into a viscous state) of BPA
212 polycarbonate polymers is 147 °C, while heat deflection temperature (defining polymer
213 deformation under a specified load) is 128 °C under 1.8 MPa. Direct photochemical
214 effect on BPA involves irreversible photo-scission leading to bisphenol-like products
215 (Rivaton, Sallet & Lemaire, 1983; Collin et al., 2012) and only secondary photo-
216 reactions are influenced by oxygen and may involve Photo-Fries rearrangement of the
217 benzyl groups. On the other side, photo aging of the BPA polycarbonate has been
218 shown also to occur through ring oxidation, e.g. resin was photo-oxidized under both
219 sunlight (A > 300 nm) and Hg arc light (A > 280 nm) which indicated the loss of aromatic
220 groups (Clark & Munro, 1984). Capillary gas chromatogram showed BPA to be highly
221 prevalent in the photo-aged polycarbonate product mixture (Factor, Ligon & May, 1987).
222 BPA, therefore has the potential of leaching out from the food and liquid storage units,
223 as well as from the medical and dental materials, especially if exposed to higher
224 temperatures or through a photo-oxidation mechanism.
225
226
227 **BPA exposure levels in human tissues**
228
229 In the late 90s Japan can industry has changed its formula for plastic can linings, which
230 has been associated to over 50% decrease in human BPA levels and to the loss of
231 correlation between usage of canned drinks and urine BPA levels in Japan (Matsumoto
232 et al., 2003). A wider range of BPA levels has been detected in the adult and fetal
233 serum in humans. Only two studies did not detect any BPA in humans, while in all other
234 studies serum BPA was detected with the concentrations ranging from 0.32 to 4.4 ng
235 ml^{-1} (Vandenberg et al., 2007). Several studies testing various human tissues detected
236 even higher BPA concentrations than those present in the serum, with the highest
237 appearing in placenta 11.2 ng ml^{-1} (Schönfelder et al., 2002), umbilical cord 4.4 ng ml^{-1}
238 (Todaka & Mori, 2002), and amniotic fluid 8.3 ng ml^{-1} (Ikezuki et al., 2002), as well as in
239 colostrum (late pregnancy milk) 3.4 ng ml^{-1} (Kuruto-Niwa et al., 2007) and breast milk
240 7.3 ng ml^{-1} (Ye et al., 2006). These independent findings are the indication of potential
241 transmission of the effects of elevated BPA exposure from mothers to the progeny,
242 either through placental transfer or breast feeding. The highest concentrations of BPA
243 were found in human saliva immediately after the dental sealant application, 42.8 ng ml^{-}
244 ^{1}, with the levels dropping to 7.9 ng ml^{-1} 1h after the application (Joskow et al., 2006;
245 Vandenberg et al., 2007). Recent study found significant differences in BPA
246 concentrations in saliva between a group of patients with tooth surfaces filled with a

247 polymer-based dental material and a control group without any polymer-based materials
248 (p=0.044, Mann-Whitney U test) (Berge et al., 2017). These findings imply a potential
249 long term exposure of BPA after dental surgeries.
250
251 In the human urine, BPA was found with detection rates from 52-100% (Vandenberg et
252 al., 2007). In the studies from 2005 to 2007, BPA was found in human urine with
253 detection rates of 96% (Calafat et al., 2005), 89% (Liu, Wolff & Moline, 2005), 97% (Ye
254 et al., 2005), 97.5% (Yang et al., 2006) and 94% (Wolff et al., 2007). These results
255 indicate that BPA in human urine has been almost completely detectable in all tested
256 individuals and confirms broad human exposure to BPA. Another source of newborn
257 and infant exposure to BPA might be the persistent leaching from the baby bottles.
258 While a study from 1997 failed to detect any traces of BPA in baby bottles (Mountfort et
259 al., 1997), a study from 2001 found 2.1 ng ml^{-1} in distilled water that came in contact
260 with the baby bottles for 30 s at 100 °C (D'Antuono et al., 2001). Similarly, a study from
261 2003 found BPA leaching from the baby bottles in concentration of 0.23 ng ml^{-1} in the
262 distilled water after 1h at 100 °C, as well as increased BPA levels, ranging from 6.7 to
263 8.4 ng ml^{-1}, after repeated cycles of bottle washing and brushing (Brede et al., 2003).
264
265 A study from 2004, found BPA leaching levels from a polycarbonate tubing to be as high
266 as 3 ng ml^{-1} per day released into the passing water (Sajiki & Yonekubo, 2004). More
267 recent study found that BPA was detected in 46.9% of cardboard samples for the take-
268 out food that could potentially be leaching the chemical to the packaged food (Lopez-
269 Espinosa et al., 2007). These results indicate that water and food may be the dominant
270 sources of BPA contamination depending on the composition of material used for their
271 packaging and transport.
272
273
274 **Gestational insulin resistance and diabetes mellitus**
275
276 Gestational insulin resistance (IR) is a naturally occurring physiological phenomenon
277 that arises during pregnancy in order to direct the blood circulating nutrients of pregnant
278 females to the growing fetus (Mack & Tomich, 2017). Gestational IR appears as the
279 result of diabetogenic hormonal action originating from placenta, including hormones
280 such as growth hormone, corticotropin-releasing hormone, placental lactogen and
281 progesterone. Subsequently, as a response to increasing insulin resistance in the
282 peripheral tissues, beta-cells of pancreas undergo hypertrophy and start secreting
283 larger amounts of insulin. In certain cases, the inefficacy of larger levels of insulin to
284 compensate for insulin resistance leads to gestational diabetes mellitus (GDM) that
285 manifests as increased blood glucose levels in otherwise healthy pregnant females that
286 had not been suffering from diabetes previously (Chiefari et al., 2017).
287
288 Long term consequences of gestational diabetes mellitus exist for both mothers and
289 offspring. Even though in most cases the maternal blood glucose levels after pregnancy
290 are brought down to normal levels, women that experiences GDM have higher risk for
291 developing diabetes later in life (Damm et al., 2016). In fact, GDM is one of the
292 strongest predictors of diabetes mellitus. It has been estimated that approximately one

293   third of women that suffer from diabetes mellitus had experienced GDM previously
294   (Cheung & Byth, 2003). Epidemiological studies indicate that the risk of developing type
295   2 diabetes after suffering from GDM can be largely attenuated by lifestyle intervention
296   and physical activity by 50% (Buchanan et al., 2002; Ratner et al., 2008). Long term
297   effects on the offspring of mothers with GDM have been less extensively studied,
298   however, in animal studies it has been shown that offspring of mothers with GDM have
299   increased risk of GDM, diabetes, obesity, cardiovascular disease and dysplasia of the
300   ventromedial hypothalamic nucleus, which is involved in the regulation of metabolism
301   and inhibits insulin secretion (Harder et al., 2001; Aerts & Van Assche, 2006). In fact,
302   induced lesions in hypothalamic ventromedial nucleus in rats resulted in significant
303   hyperinsulinemia and increased blood glucose levels (Satoh et al., 1997). Therefore,
304   malformation of the ventromedial nucleus may be in part responsible for the persisting
305   alterations of glucose homeostasis found in the offspring of mothers with GDM.
306
307   GDM is one of the most dominant pregnancy complications as it affects from 2% to 10%
308   of all pregnancies (Wedekind & Belkacemi, 2016). During pregnancy, peripheral insulin
309   resistance first appears as a physiological response to changes in steroid balance in the
310   organism (Vejrazkova et al., 2014). In fact, the similar effect could be observed with the
311   application of hormonal contraceptives, primarily those containing estrogens, that have
312   been associated with changes in carbohydrate metabolism and increased insulin
313   resistance (Lopez, Grimes & Schulz, 2012). For example, one study showed 43-61%
314   increase in plasma glucose levels on the oral glucose-tolerance test (OGTT) in women
315   taking oral contraceptives (Godsland et al., 1990), while other studies, in addition to
316   increased OGTT plasma glucose, have found elevated fasting and post-glucose insulin
317   levels and recommended that estrogen content of oral contraceptives should be
318   reduced to minimize the diabetogenic effects (Wynn et al., 1979).
319
320
321   **BPA-induced endocrine disruption and insulin resistance**
322
323   In a 2014 study, it has been demonstrated that the offspring from BPA-exposed mice
324   showed adverse symptoms of diabetes (García-Arevalo et al., 2014). In male offspring,
325   the BPA treated group, similar to the groups fed with high fat diet (HFD) and with high
326   fat diet plus BPA, showed fasting hyperglycemia, glucose intolerance and higher levels
327   of insulin and free fatty acids. In 17-week old male offspring, under the fasting
328   conditions, the BPA group of mice was hyperglycemic compared to the control, however
329   this effect of BPA was masked under the high fat diet (HFD vs HFD-BPA). In addition, at
330   17 weeks, the BPA, HFD and HFD-BPA groups showed higher insulin levels than the
331   control group. Intraperitoneal glucose tolerance test (ipGTT) showed higher glucose
332   intolerance in the HFD and HFD-BPA groups compared to the control, while the BPA
333   group showed a similar tendency without reaching the statistical significance. In 28-
334   week old male offspring, the BPA group had the highest fasting plasma glucose levels
335   and the highest insulin levels, even compared to HFD and HFD-BPA groups. At 28
336   weeks, ipGTT showed that all three tested groups (BPA, HFD and HFD-BPA) acquired
337   higher glucose intolerance compared to the control. In addition, at 28 weeks, insulin
338   sensitivity, measured by intraperitoneal insulin tolerance test (ipITT), showed high

339   tendency of impairment in BPA, HFD and HFD-BPA groups compared to the control
340   group. Remarkably, the BPA group gained more weight starting from the 18th week
341   compared to the control, and kept increasing the weight until it reached the levels of the
342   HFD and HFD-BPA groups.
343
344   In addition, BPA exposure in mice shows an effect on mothers' glucose metabolism
345   during pregnancy, as well as later in life (Alonso-Magdalena et al., 2010). During
346   pregnancy, the treatment with BPA at doses of 10 or 100 µg/kg/day on days of gestation
347   9-16 (BPA10 and BPA100 mice, respectively) induced a tendency to glucose
348   intolerance measured with ipGTT. In BPA10 mice increased fasting plasma insulin
349   levels were observed (1.38 times higher). In ipITT, both control and BPA10 treated
350   groups, showed only a slight decrease in serum glucose levels, consistent with the
351   physiological insulin resistance in late pregnancy. While BPA10 mice did not alter
352   naturally occurring insulin resistance during pregnancy, in BPA100 mice modestly
353   induced insulin sensitivity was observed. This indicates that BPA10 mice were unable to
354   compensate with their elevated insulin levels to counteract physiological insulin
355   resistance. In addition, in liver BPA treatment reversed insulin-stimulated Akt
356   phosphorylation that occurs in control F0 mice, indicating BPA effects on signaling
357   pathways in liver that are consistent with the strong insulin resistance in these mice.
358   Consequences for mothers later in life included increased body weight four months after
359   delivery, decreased insulin sensitivity measured using ipITT and 2.2 times higher
360   plasma insulin levels after fasting compared to controls. In addition, increased plasma
361   leptin, triglyceride, and glycerol levels were observed in mothers four months after
362   delivery relative to controls. Another study has reported 3% increase in the body weight
363   during the postpartum period and at 5 and 6 months postpartum found substantial
364   impairment of glucose tolerance and decreased insulin sensitivity. These alterations in
365   glucose metabolism appeared in pregnant, but not in non-pregnant female mice
366   (Alonso-Magdalena et al., 2015). Taken together these data indicate that exposure to
367   BPA during pregnancy produces dysregulated nutrient metabolism later in life. Model
368   animals, therefore, present a valuable source of information on the effect of BPA on
369   insulin resistance, type 2 diabetes and obesity and unveil the connection of
370   environmental estrogens to these phenotypes.
371
372
373   **Molecular mechanisms of BPA in promoting endocrine disruption, gestational**
374   **insulin resistance and diabetes mellitus**
375
376   BPA has been first reported to act as a synthetic estrogen in 1936 (Dodds & Lawson,
377   1936), well before scientists discovered that it could be polymerized into polycarbonate
378   plastics in the 1950s. As a xenoestrogen and an endocrine-disrupting chemical, BPA
379   has a potential to intervene with any aspect of the hormonal function, to change the
380   hormonal equilibrium and subsequently affect many physiological processes in different
381   tissues. The mechanism of BPA action as a xenoestrogen is thought to be through
382   binding and competing for estrogen receptors, ER-alpha (ERα) and ER-beta (ERβ)
383   (Figure 1) (Dodds & Lawson, 1938). However, the interaction of BPA with ER receptors
384   is relatively weak, ranging 2 to 3 orders of magnitude lower compared to estrogens

385 (relative recruitment ability – RRA; E2 and BPA recruitment to ERα, 100 and 0.073,
386 respectively; E2 and BPA recruitment to ERβ, 100 and 0.75 respectively) (Routledge et
387 al., 2000). Therefore, whether chronic and low-dose BPA exposures function through
388 the ER pathways is still debatable (Safe et al., 2002). Nevertheless, BPA, as well as
389 other xenoestrogens, are indeed able to efficiently and fully displace radioactively
390 labeled E2 from both ERα and ERβ receptors in a dose dependent manner using a
391 ligand binding assay (Routledge et al., 2000). In addition, recent findings indicate that
392 BPA may act through a receptor named estrogen-related receptor gamma (ERR-γ)
393 (Takayanagi et al., 2006; Okada et al., 2008). ERR-γ is a member of estrogen-related
394 receptor class of genes, a subfamily of orphan nuclear receptors, closely related to the
395 ERs. BPA was found to bind ERR-γ in both a direct receptor binding assays (FRET), as
396 potently as a tracer for ERR-γ, and in a cell-based reporter assay where it rescues high
397 constitutive ERR-γ activity in HeLa cells treated with an ERR-γ inverse agonist 4-
398 hydroxytamoxifen (4-OHT) (Okada et al., 2008). Whether BPA exerts its effect on
399 insulin resistance primarily through one of these mechanisms by mimicking estrogen
400 action remains still to be shown, especially since the risk assessment of xenoestrogens
401 based primarily on the reporter gene and binding assays may be insufficient.
402
403 In addition, while BPA is capable of displacing E2 when binding to ERα or ERβ in a
404 dose dependent manner, it has been shown that BPA interacts with a different set of
405 transcriptional co-regulators than E2 (Routledge et al., 2000). As ERs regulate target
406 gene transcription by the association with their co-regulators, interaction with a specific
407 set of transcriptional co-regulators may mean BPA binding induces a unique ER
408 conformation and subsequently recruits a unique set of co-regulators conferring specific
409 gene regulation pattern to the target genes. In addition, BPA demonstrated clear
410 preference for certain co-regulators when bound to ERβ compared to ERα. For
411 example, BPA-ERβ complex had 500-fold higher recruitment affinity for coactivator TIF2
412 than BPA-ERα complex (RRA for BPA-ERα and BPA-ERβ <0.0001 and 0.05
413 respectively), which was directionally equivalent to BPA showing almost 10-fold greater
414 binding affinity for ERβ than ERα (RRA for ERα and ERβ 0.073 and 0.75 respectively)
415 (Routledge et al., 2000). BPA can therefore confer specific conformational changes to
416 ERβ and enhance ERβ recruitment activity by the order of magnitude compared to its
417 binding effect on ERα. As the main function of E2 binding to ERs is the induction of ER
418 conformational change, in order for the subsequent recruitment of coactivators and
419 assembly of the basal transcription machinery factors, the finding that BPA can
420 efficiently displace E2 from the ER and induce a unique conformational structure of ER
421 (particularly potentiating specific co-factor recruitment to ERβ) and the fact that similar
422 has been shown for other xenoestrogens (Routledge et al., 2000), suggests an effective
423 mechanism of BPA action through ER binding.
424
425 Other than proposed ER-activation mechanism that involves binding to nuclear
426 receptors, it has been suggested that BPA may exert its effects through other
427 mechanisms, such as through rapid non-genomic (i.e. non-nuclear) pathways (Figure 1)
428 (Welshons et al., 2006; Alonso-Magdalena et al., 2012). Such systems are cell signaling
429 systems with receptors associated to the membrane and the response time of such
430 systems are generally shorter than those mediated by nuclear receptors (Judy &

431 Welshons, 2010). In addition, responsiveness can usually be achieved by smaller initial
432 concentrations of the hormone, indicating high level of signal amplification. In 2000,
433 Nadal et al. demonstrated for the first time that in pancreatic beta cells xenoestrogens,
434 such as BPA, as well as E2, both occupy a common membrane binding site that is
435 distinct from the cytoplasmatic/nuclear ER receptors (Nadal et al., 2000). Subsequently,
436 it has been shown that BPA at picomolar concentrations can cause rapid influx of
437 calcium ion (within 30 sec of administration) followed with a prolactin release in pituitary
438 tumor cells (characterized by the high levels of ERα receptor in the plasma membrane)
439 (Wozniak, Bulayeva & Watson, 2005). Evidence suggested that BPA alters the
440 conformation of voltage-dependent calcium channels through membrane depolarization,
441 and the prolactin release was fully preventable by removing calcium from the
442 extracellular solution. Similar was detected in mouse pancreatic cells where BPA
443 treatment promoted calcium influx at the nanomolar concentrations (as low as 1nm),
444 with a depolarization-induced influx subsequently causing CREB phosphorylation
445 (Quesada et al., 2002). Cellular responsiveness to lower than nanomolar BPA
446 concentrations (0.1 nm) was detected also in MCF-7 human breast cancer cells (Walsh,
447 Dockery & Doolan, 2005). Similarly, it has been found that the BPA exposure in mouse
448 Leydig cells induced activation of protein kinase A and phosphorylation of MAPK only 5
449 minutes after administration and subsequent rapid induction of Nur77 gene expression
450 that regulates testosterone synthesis (Figure 1), which is an effect that could not be
451 mediated by the prolonged response of nuclear receptors (Song, Lee & Choi, 2002).
452 Rapid membrane BPA response was confirmed by protein kinase inhibitor H-89 that
453 strongly inhibited BPA-mediated Nur77 gene activation.
454
455 The present concept of BPA estrogenicity (mainly based on nuclear receptor activation)
456 demands substantial refinement to incorporate such rapid responses of estrogen
457 receptors found in the membrane (Nadal et al., 2017). It has been shown that ERα and
458 ERβ could be expressed in both nuclear and membrane cell fractions and could be
459 functional outside the nucleus (Razandi et al., 1999). Transfection of ERα cDNA in CHO
460 cells resulted in the presence of ERα in nuclear and membrane associated cellular
461 fractions and in specific binding of labeled E2 to the CHO cells. The membrane bound
462 ER fractions had similar dissociation constant as nuclear receptors (0.287 and 0.283
463 nM, respectively), however they represented minor fractions (3% and 2%, ERα and
464 ERβ, respectively) of total receptors in the cell. E2 binding to the membrane ERs
465 resulted in activation of G proteins in the membrane followed by increased adenylate
466 cyclase activity. In 2005, the specific class of steroid membrane receptors named
467 orphan receptor GPR30 (now denoted as G-protein-coupled estrogen receptor, GPER)
468 was discovered in the human breast cancer cells, which could propagate the non-
469 classical estrogen effects localized at the cell surface (Thomas et al., 2005). Specific E2
470 binding was detected in SKBR3 breast cancer cells that express GPER, but are missing
471 estrogen receptors, an effect that was reversible with siRNA-mediated decrease in
472 GPER expression. GPER pre-genomic signaling activity was confirmed to be conducted
473 through the plasma membrane G-protein coupled receptor activities, specifically, the
474 activation of adenylyl cyclase (Filardo & Thomas, 2012). GPER knock-out mice are
475 viable, however they show impaired glucose tolerance, potentiating the role of ER non-
476 genomic signaling in glucose homeostasis (Mårtensson et al., 2009). In addition, it is

477   speculated that estrogens could bind a large variety of receptors at the plasma
478   membrane surface (Kow & Pfaff, 2016) and that their effects almost always reproduce
479   the effects of the estrogenic agents that permeate the cell.
480
481   It has been demonstrated that BPA treatment of isolated pancreatic islets, similar to E2,
482   shows the non-monotonic dose response (i.e. an inverted U-shape curve) when insulin
483   content is measured after 48-hour treatment (Alonso-Magdalena et al., 2008). Similar
484   response was observed in vivo when mice were treated with 100 µg/kg or 1 mg/kg of
485   BPA during 4 days, as low doses of BPA increased insulin content, while the higher
486   doses of BPA exhibited no effect. This non-monotonic dose response was ERα
487   dependent as ERα agonists showed similar pattern of activation. It has been estimated
488   that BPA non-monotonic dose response occurs in 20% of experiments conducted using
489   BPA and in 30% of the literature related to BPA the non-monotonic dose-response has
490   been detected (Vandenberg, 2014). The non-monotonic dose response to BPA, while
491   contradicting traditional concepts of toxicology, at the molecular level indeed does not
492   involve BPA acting as a standard toxic chemical, but rather as a hormonal molecule that
493   initiates signaling cascades through the interaction with a hormone receptor. The
494   molecular mechanisms behind such non-linear relationship between the dose and
495   response of EDC may involve receptor saturation, negative feedback loop mechanisms,
496   and/or non-linear process of receptor homodimerization. It has been shown that
497   computational models can accurately predict the appearance of non-monotonic dose
498   response in gene expression within the standard genomic niche of E2 and EDC
499   signaling (Li et al., 2007). Indeed, since a critical step in steroid hormonal action is the
500   dimerization of ligand-bound receptor monomers, computational modeling showed
501   homodimerization of ligand-bound receptors to be an inherently nonlinear process
502   (Figure 2). Furthermore, heterodimerizaton (L_{en}ER-ERL_{ex}; ER receptors bound to both
503   endogenous- L_{en} and exogenous ligands- L_{ex}) can also induce U-shaped responses with
504   the magnitude of response varying with the transcriptional activity of the heterodimer.
505   Model shows that U-shaped response arises when the exogenous ligand L_{ex} is an
506   agonist, regardless of the heterodimer being absent, or being pure/partial activator or
507   repressor. On the other side, inverted U-shaped response appears when the
508   heterodimer is a pure or partial activator, regardless whether the exogenous ligand is an
509   agonist or antagonist. Monotonic response, however, only occurs is two simulated
510   scenarios i.e. with the treatment with antagonist when mixed-ligand heterodimer is a
511   repressor or absent.
512
513
514   **Role of estrogens in gestational insulin resistance**
515
516   During pregnancy gestational insulin resistance develops naturally to direct nutrients to
517   the embryo, however the plasma glucose levels may rise to promote gestational
518   diabetes. Insulin resistance perturbs the glucose metabolism and manifests itself in
519   attenuated glucose uptake in the skeletal muscle, white adipose tissue and liver and
520   inadequate suppression of glucose production in the liver (Catalano et al., 2003). Body
521   adapts to this disturbance by increasing the biosynthesis and secretion of insulin
522   through beta-cells located in the pancreatic islets (islets of Langerhans) and by

523 increasing their cell mass. In the case of GDM beta-cells do not respond adequately to
524 the changes in the organism which leads to elevated blood glucose levels. It is possible
525 that in case of GDM beta cells fail to respond adequately to the natural hormonal
526 signaling during pregnancy, particularly to estrogens (estradiol, estrone and estriol) and
527 progesterone during the second trimester, when both GDM and increase in
528 estrogens/progesterone concentrations might co-occur (10 fold increase in
529 progesterone, 30 fold increase in estrogens) (Nadal et al., 2009b). In addition, both
530 estrogen and progesterone receptors are expressed in the pancreatic beta-cells (Nadal
531 et al., 2009a) indicating their responsiveness to the pregnancy hormones.
532
533 Three major mechanisms that are involved in counteracting insulin resistance during
534 pregnancy are increasing the biosynthesis of insulin, enhancing the sensitivity of
535 pathways for glucose stimulated insulin secretion, and hypertrophy of the beta-cells.
536 Indeed, E2 has been shown to increase glucose stimulated insulin secretion (Nadal et
537 al., 1998). E2 interacts with a membrane-associated receptor and closes ATP-
538 dependent K^+ channels. Subsequently, this depolarizes the plasma membrane and
539 allows the influx of Ca^{2+}, which in turn triggers the release of insulin. When E2 was
540 applied to isolated mouse islets of Langerhans in the absence of glucose stimulation it
541 produced no effect, while in the presence of 8.3mM glucose an increase in oscillatory
542 electrical activity followed with an oscillatory pattern of Ca^{2+} concentration were
543 observed, indicating glucose dependent E2 effect. This pattern of Ca^{2+} oscillations
544 induced pulsatile insulin secretion (Martin, Sanchez-Andres & Soria, 1995) and a total
545 increase in insulin secretion of 30%. Therefore, E2 exerts its effect on pancreatic beta
546 cells that parallels those observed during normal pregnancy. Wide range of
547 physiological E2 concentrations (100pM-1M) were shown to increase intracellular
548 calcium concentrations, however, even the suboptimal pM concentrations (10pM) were
549 able to show a slight effect. The same effect was observed in vivo (Alonso-Magdalena
550 et al., 2006), when adult male mice were injected twice a day with E2 or a vehicle for
551 four days. Insulin content measured with immunocytochemistry showed that beta-cells
552 from E2 mice had increased staining compared to the vehicle group and therefore
553 possessed higher insulin content in each individual cell that was measured (similar
554 effect was observed for the BPA treatment).
555
556 In addition, it has been shown that stimulation of islets isolated from adult mice with E2
557 at physiological concentrations increases insulin content through promoting insulin
558 mRNA synthesis (Alonso-Magdalena et al., 2008). Insulin mRNA levels increased 1.6
559 fold only 6 hours after treatment of the islets with E2 (10nM). In the long term
560 stimulation (48 hours) E2 showed similar increase in total insulin content, which fully
561 paralleled the effect of BPA at concentrations of 1nM. BPA titration displayed inverted
562 U-shape dose-response curve indicating the existence of an active concentration range.
563 Similar narrow concentration window of physiological activity was observed for E2, as
564 islets treated with the increasing doses of E2 for 48 hours showed non-monotonic dose
565 response (inverted U-shape) with the maximal response at E2 concentrations 1-10nM.
566 Consistently, the treatment of pancreatic beta-cell line MIN6 for 2 h with various
567 concentrations of E2 produced an inverted U-shaped curve (maximum of insulin
568 secretion at E2 concentrations 1-10nM) (Sharma & Prossnitz, 2011) This indicates that

569   stabilizing effect on gestational insulin resistance through increasing insulin biosynthesis
570   and secretion in the islets may be highly regulated biological process directed through a
571   narrow range of hormonal concentrations.
572
573   Glucose homeostasis is primarily controlled at the level of glucose uptake by the
574   skeletal muscle (SM) and white adipose tissues (WAT). Estrogen receptors ERα and
575   ERβ are expressed in SM and WAT and have opposing effects on expression of GLUT4
576   glucose transporter. GLUT4 is the main glucose transporter in SM and WAT, and it
577   accelerates the transport of glucose under insulin stimulation (Barnard & Youngren,
578   1992; Barros et al., 2009). With insulin binding to its receptors and activation of the
579   tyrosine kinase signaling pathway, GLUT-4 receptors are being transported from the
580   intracellular depos to the sarcolemmal membrane (SL) in SM or plasma membrane in
581   WAT. With GLUT4 being deposited in the membrane, glucose can diffuse into the cell
582   (facilitated diffusion). GLUT4 defective synthesis, glycosylation, translocation and
583   anchorage to the membrane could promote development of insulin resistance. The
584   effect of estrogen on insulin resistance at the level of peripheral tissues is mediated by
585   its two receptors, ERα and ERβ, and sometimes perplexing and contradictory role of
586   estrogen in IR might be explained by the opposing functions of these receptors. ERβ
587   knockout mice show signs of hypoglycemia, islet hypertrophy and elevated plasma
588   insulin after glucose stimulation, while ERα knock-out mice show completely opposite
589   phenotypes of hyperglycemia and glucose intolerance. In addition, expression of
590   GLUT4 in ERβ knockout mice is increased, while ERα knockout mice possess lower
591   GLUT4 expression (Barros et al., 2006; Barros, Machado & Gustafsson, 2006). This
592   indicated that ERβ activation could have a diabetogenic effect, while ERα action is
593   important for the maintenance of normal glucose homeostasis. Therefore, in case that
594   ERα and ERβ are exhibiting diametrically opposed effects, cells and tissues with both
595   receptors expressed would respond to E2 in opposite ways depending primarily on the
596   ERα to ERβ ratio (net effect). In addition, it has been shown that ERα is dominating in
597   the white adipose tissue, while ERβ is more distributed in the skeletal muscle, where E2
598   as a net effect might promote insulin resistance (Barros et al., 2009).
599
600   The role of estrogens in developing insulin resistance during pregnancy, a feature that
601   chronic bisphenol A exposure is predicted to be mimicking, is somewhat dichotomous,
602   as some studies show protective effect of estrogen on insulin resistance. In a 2012
603   study, male and ovariectomized female C57BL/6J mice had higher propensity to
604   developing insulin resistance compared to non-ovariectomized females when exposed
605   to a high-fat diet, while the administration of E2 to ovariectomized females reduced
606   insulin resistance in both high and low fat diet groups, as measured by area under curve
607   (AUC) in the glucose tolerance test (Stubbins et al., 2012). The study did not show the
608   effect of E2 on non-ovariectomized females, therefore drawing conclusions only on the
609   stabilizing effect of estrogen on hormone-deficient mice. Similarly, ArKO mice
610   (transgenic mice with inactivated aromatase enzyme, essential for E2 synthesis)
611   developed glucose intolerance and insulin resistance, an effect reversible by E2 (Barros
612   & Gustafsson, 2011). Likewise, ERα -/- mice are glucose intolerant and insulin resistant
613   (Heine et al., 2000). A study in rats found that treatment of male rats with E2 protected
614   against accumulation of fatty acids in pancreatic islets and against pancreatic beta cell

615   failure (Tiano et al., 2011), therefore preparing the islets for increased insulin production
616   during the pregnancy and gestational insulin resistance. Concurrently, the study
617   proposes ERα or ERβ receptors as promising therapeutics to prevent beta cell failure in
618   T2D. Estrogens, although with a protective role on beta-pancreatic cells through ER
619   receptors signaling, in peripheral tissues like SM were predicted to promote insulin
620   resistance via ERβ net effect (ratio ERβ/ERα) (Barros et al., 2009). However, some
621   studies showed that E2 treatment might result only in slight decrease in glucose uptake
622   in SM without insulin and with significant improvement of muscle glucose uptake with
623   insulin treatment, which suggests ERα dominance over ERβ in SM (Gorres et al., 2011;
624   Inada et al., 2016), leading to contention within the field.
625
626   On the other side, insulin resistance and hyperinsulinemia are common among women
627   consuming oral contraceptives containing estrogens and during pregnancy, and
628   diminished peripheral glucose uptake was observed among normal subjects treated
629   with ethinyl estradiol (Polderman et al., 1994). Association has been found between
630   insulin resistance and E2 levels in obese children (Lin & Ji, 2016). Studies suggest that
631   in humans the lipolytic effect of placental lactogen directs maternal metabolism toward
632   lipids, rather than glucose utilization, in the same time adding to the preservation of
633   glucose for the fetus (Baz, Riveline & Gautier, 2016). In non-diabetic women, employing
634   the intravenous glucose tolerance test in 296 oral contraceptive users and 95 nonusers,
635   estrogen based contraceptives reduced the glucose elimination constant and reduced
636   insulin sensitivity by 30-40% (Godsland et al., 1992). Other studies showed that users of
637   oral contraceptives with synthetic estrogen had up to 61% higher plasma glucose levels,
638   up to 40% higher insulin response and up to 40% higher C peptide response, a method
639   to determine residual insulin secretion (Godsland et al., 1990). More recent meta-
640   analysis showed less disturbance in carbohydrate metabolism (Lopez, Grimes &
641   Schulz, 2014), potentially due to the change in composition of contraceptives that
642   contain fewer estrogen content.
643
644
645   **Strong cross-generational transmission of BPA effects to male offspring**
646
647   In 2010 came the first study in mice that showed that treatment of pregnant females
648   with low doses of BPA could in F1 male offspring provoke glucose intolerance, insulin
649   resistance and altered beta-cell function (Alonso-Magdalena et al., 2010). The
650   pancreatic islets from male offspring showed abnormal Ca^{2+} signaling and increased
651   insulin secretion. Decreased bromodeoxyuridine (BrdU) incorporation into insulin-
652   producing cells was detected in the male offspring, indicating absence of proliferation.
653   An indication of non-monotonic dose response was observed as insulin sensitivity was
654   evidently impaired in F1-BPA10 male offspring (slight alteration in 3-months old mice,
655   significant alteration in 6-months old mice), however no significant alteration of insulin
656   sensitivity was detected in F1-BPA100 males. Similarly, both serum insulin levels and
657   glucose-stimulated insulin secretion in vivo and ex vivo (using isolated islets) were
658   higher in F1-BPA10 males compared to the control, while the same effect was not
659   observed in F1-BPA100 male mice, again suggesting non-monotonic dose response.
660   Similar non-monotonic dose response was observed in the increase of intracellular Ca^{2+}

661 in isolated islets after glucose stimulation (both 7mM and 16mM glucose) that was
662 detected in the F1-BPA10, but not F1-BPA100 males. In addition, F1-BPA10 male mice
663 weighted 3% more than the control mice, while F1-BPA100 were 4.5% lighter at birth
664 than controls. On the other side, the glucose tolerance was altered in both F1-BPA10
665 and F1-BPA100 male mice, however with a slight decrease in AUC in F1-BPA100
666 males. The phenotype of altered glucose metabolism in male offspring was confirmed
667 subsequently in additional studies (Wei et al., 2011; Angle et al., 2013; García-Arevalo
668 et al., 2014; García-Arévalo et al., 2016).
669
670 In addition, the interaction of two environmental stimuli, BPA and high fat diet (HFD) has
671 been examined (García-Arevalo et al., 2014). The group of F1 male mice exposed to
672 BPA in utero increased their weight starting from 18 weeks old and reached the HFD
673 and HFD-BPA groups at week 28. The BPA group showed similarities to both HFD and
674 HFD-BPA groups in exhibiting fasting hyperglycemia and glucose intolerance (slight
675 increase in AUC at 17 weeks, and reaching HFD and HFD-BPA groups at 28
676 weeks). Other studies have found similar tendencies of increased body weight, elevated
677 serum insulin levels and impaired glucose tolerance in F1-BPA offspring, which were
678 elevated and accelerated with the introduction of a high fat diet (Wei et al., 2011).
679 Consistent with the BPA-HFD interaction, F1-HFD-BPA mice showed severe metabolic
680 syndrome, including obesity, dyslipidemia, hyperglycemia, hyperinsulinemia, and
681 glucose intolerance. However, the effects were present on lower BPA doses, but were
682 absent on higher BPA doses indicating that, even though it triggered adverse metabolic
683 effects of BPA, HFD did not influence the non-monotonic dose response to BPA.
684 Another study showed that the fetal and perinatal treatment with BPA was more
685 detrimental than neonatal (Liu et al., 2013). The effect of BPA on blood glucose
686 homeostasis in 6 months old male mice was pronounced in the group with fetal
687 exposure from day 6 of pregnancy until postnatal day (PND) 0, while only modest
688 effects were observed in the group with neonatal exposure (PND0–PND21), suggesting
689 a critical timeframe when BPA can exert its effects during the embryological
690 development.
691
692 Furthermore, the a plethora of diverse effects on male offspring were detected
693 (including a decrease in glucose tolerance and serum adiponectin and an increase in
694 body and liver mass, abdominal adipocyte number, and serum leptin and insulin levels).
695 This occurred at the doses of BPA below the predicted 'no adverse effect level'
696 (NOAEL), while doses 10-fold above NOAEL did not show any significant effect,
697 confirming the non-monotonic dose response (Angle et al., 2013). In addition, the BPA
698 exposure might predispose offspring to the fatty liver disease, a part of the metabolic
699 syndrome symptoms (Jiang et al., 2014). In Wistar rats, with perinatal exposure to BPA,
700 at 26 weeks of age extensive fat accumulation in liver was observed, as well as
701 elevated serum alanine aminotransferase (ALT), the indicator of liver damage. Milder
702 effects were observed at 3 weeks, i.e. a decrease in mitochondrial respiratory complex
703 (MRCI, MRCIII) activity and altered expression levels of genes involved in mitochondrial
704 fatty acid metabolism, while at 15 weeks an infiltration of liver cells with fat (steatosis),
705 as well as upregulation of lipogenesis genes and increased levels of reactive oxygen
706 species (ROS, indicative of the loss of mitochondrial function) were observed.

707
708 It has been shown, using different animal models, that the prenatal exposure to BPA
709 leads to severe glucose intolerance, insulin resistance and hyperinsulinemia during
710 postnatal life (Alonso-Magdalena et al., 2015). In mice, BPA-treated pregnant females
711 (10 µg/kg on days 9–16 of gestation) produced male progeny that showed altered
712 glucose metabolism at 17 and 28 weeks of age (García-Arevalo et al., 2014), therefore
713 confirming that the treatment with endocrine-disrupting chemicals leads to the
714 perturbation of glucose metabolism of pregnant females that is being efficiently
715 transmitted to the offspring. Recently, it has been shown that BPA treatment of pregnant
716 female mice (10 and 100 µg/kg per day) in male offspring promotes increased
717 expression of cell division genes in the beta cells of pancreas followed with increased
718 pancreatic beta-cell growth and increased insulin levels at postnatal days 0, 21 and 30
719 (García-Arévalo et al., 2016). Conversely, at postnatal day 120 beta cell mass
720 diminished and mice showed increased fasting glucose levels and tendency towards
721 glucose intolerance. Therefore, parental BPA exposure in mice leads to the surplus of
722 insulin signaling during early life in male offspring that could advance into the impaired
723 glucose tolerance of adulthood. Such perturbations in glucose metabolism induced by
724 EDC could therefore be actively transmitted to the developing mouse embryo and
725 appear as long-term consequences later in life (Figure 3). A 2016 study in humans
726 showed association of prenatal creatinine-adjusted urinary BPA concentrations with BMI
727 levels and waist circumference in male children of 1-4 years of age (Vafeiadi et al.,
728 2016). For female offspring, prenatal urinary BPA was inversely associated with BMI
729 and adiposity measures, confirming similar gender-related trends that were observed in
730 animal studies.
731
732
733 **BPA effects detected in female offspring in mice**
734
735 Interestingly, even though the effects of BPA exposure in mice are usually observed in
736 the male offspring, multiple studies have reported the occurrence of strong effects in the
737 female offspring. In one study, female offspring showed altered blood glucose
738 homeostasis, as measured with intraperitoneal glucose tolerance test (ipGTT) (Liu et
739 al., 2013). At 3 months, the response to glucose challenge was evident in the group with
740 fetal BPA exposure from day 6 of pregnancy until PND 0 (P6–PND0) compared to the
741 groups with preimplantation (P1-P6), neonatal (PND0-PND26) or fetal plus neonatal
742 (P6- PND26) exposure, indicating the existence of the critical window of exposure
743 similar to the effect on male offspring. At 6 months, the effects are significant for both
744 P1-P6 and P6–PND0 groups, however the intensity of alterations in glucose
745 homeostasis decreases. Finally, at 8 months, AUC in ipGTT in all groups did not differ
746 from the control group of mice. In addition, P6-PND0 female offspring at 3 months
747 showed increasing fasting insulin levels, while at 6 months they showed increased
748 fasting glucose levels. The insulinogenic index ($\Delta I_{0-30}/\Delta G_{0-30}$), which is measuring
749 insulin release in response to the glucose load, was decreased in 3 months old females
750 from the P6–PND0 group, and then improved in 6 and 8 months old females. A study in
751 rats showed similar profiles between male and female offspring rats in fasting blood
752 glucose and fasting serum insulin at 3, 9, 15, and 26 weeks after perinatal exposure to

753 50 µg/kg per day of BPA (Wei et al., 2011). The group of HFD-BPA female rats showed
754 higher fasting blood glucose and fasting serum insulin levels starting from 9 weeks of
755 age compared to the HFD group, indicating the interaction of BPA exposure with the
756 HFD phenotype. In addition, at 26 weeks, female BPA-exposed offspring on a normal
757 diet, similar to the male offspring, had greater glucose and insulin AUC in OGTT and
758 ITT compared to controls. Concordantly, the interaction of HFD phenotype and BPA
759 exposure could be observed in females at 26 weeks in OGTT AUC.
760
761 In a 2017 study, fetal livers were collected from the F1 generation (embryonic day
762 E18.5) exposed to BPA in utero from E7.5 to E18.5, and gene expression of key
763 hepatocyte maturation markers was assessed (DeBenedictis, Guan & Yang, 2016).
764 Particularly, in female mice changes were prominent and involved decreasing levels of
765 mature hepatocyte markers, such as albumin and glycogen synthase (reduced 65% and
766 40%, respectively), decreased levels of C/EBP-alpha, the master transcription factor of
767 hepatocyte maturation (reduced 50%), and increased levels of immature hepatocyte
768 marker, α-fetoprotein (increased 43%). In addition, the markers of proliferation (PCNA)
769 were elevated (40% increase) and the markers of apoptosis (caspase-3) were
770 decreased (40% decrease). This suggests that BPA reduces maturation and alters the
771 balance of proliferation and apoptosis in fetal hepatocytes, in a similar direction as
772 observed in the pancreatic islets in F1 mice (García-Arévalo et al., 2016), where
773 upregulated genes enrich in gene ontologies related with cell cycle, mitosis and cell
774 division.
775
776
777 **Epigenetic modifications of DNA as a mechanism of BPA-induced**
778 **transgenerational effects**
779
780 In another study, it has been shown that BPA might promote epigenetic changes that
781 are associated with the transmission of perturbed carbohydrate metabolism phenotype
782 to offspring (Susiarjo et al., 2015). Exposure of BPA in C57BL/6 mothers produced
783 multigenerational metabolic abnormalities and stable inheritance of changes in DNA
784 methylation at the imprinted *Igf2* gene locus, a well-studied gene locus involved in fetal
785 growth and recently shown to alter adult energy metabolism, fat deposition and obesity
786 (Jones, Levorse & Tilghman, 2001). Study established altered body mass, glucose
787 tolerance, and insulin secretion in male, but not female, F1 mice and the effect on F2
788 generation was estimated to originate from the exposure to BPA in developing germ
789 cells. Previous study has shown that BPA can alter the methylation levels of
790 differentially methylated regions (DMR) and that it promotes loss of monoallelic
791 expression of the *Snrpn, Igf2 and Kcnq1ot1* genes in a tissue specific manner in F1
792 offspring (placental and embryonic tissue) (Susiarjo et al., 2013). Loss of imprinting was
793 highly specific for placenta as 13/28 placentas showed loss of imprinting (LOI)
794 compared to 0/23 controls for the *Snrpn* gene. On the contrary, at the *Igf2* gene locus,
795 LOI was found in 7/28 embryos compared to 0/23 controls, while it has been absent in
796 placentas. Repressed maternal *Igf2* allele was shown to increase its expression up to
797 68.9% of the total *Igf2* expression. Analysis of DNA methylation at the 16 CpG sites in
798 the *Snrpn* promoter, which is hypermethylated on the maternal allele, showed reduction

799 in methylation levels in upper dose BPA exposed placentas (observed methylation
800 levels of the maternal allele were 53.8% lower than those of controls), while similar
801 trend was observed in the embryos. On the Igf2 locus, DMR1 region showed
802 significantly increased methylation levels in embryos (45.6% in controls vs. 55.7% in
803 upper dose BPA in embryos, p<0.05; trend not observed in placentas), which was
804 consistent with previous association of the gain of DNA methylation with
805 biallelic *Igf2* expression (Figure 4). To determine whether genomic imprinting effects
806 were present in the F2 offspring in the absence of further exposure, F1 females were
807 mated to unexposed males, however biallelic expression of the *Igf2* gene was not
808 observed (Susiarjo et al., 2015). Nevertheless, BPA treated embryos in F2 generation
809 showed significant overexpression of *Igf2* gene compared to controls. Similar to the
810 BPA phenotype, $H19^{\Delta3.8/+}$ male mice with the imprinted control locus (ICR) deletion
811 showed increased glucose intolerance, increased body weight and body fat content,
812 indicating that BPA might exert its effect partly through epigenetic changes at the *Igf2*
813 locus.
814
815 Similarly, another study has shown that BPA can exert multigenerational effects, as the
816 F2 generation rats were found to exhibit glucose intolerance and insulin resistance in
817 ipGTT and ipITT and decreased expression of *Gck* (glucokinase) gene in the liver, and
818 this effect was estimated to be epigenetic. The *Gck* promoter in the hepatic tissue in F2
819 generation rats exhibited fully methylated status in the all five CpG sites upstream of the
820 promoter (located up to 314bp upstream), compared to the unmethylated status in the
821 control rats (Li et al., 2014). Similar trend of BPA induced *Gck* promoter
822 hypermethylation was observed in F1 generation in 3 week old offspring rats and more
823 pronounced increase of *Gck* methylation was observed in 21 week old rats (Ma et al.,
824 2013). In addition, in both studies, BPA was shown to induce changes in global DNA
825 methylation (decrease in DNA methylation, 84.3% BPA-treated F2 offspring vs 90.1%
826 controls) implicating long term epigenetic effects.
827
828
829 **Epidemiological studies connecting BPA and T2D**
830
831 Multiple epidemiological studies have linked BPA exposure (mainly measured as urinary
832 BPA concentrations) with T2D or prediabetes occurrence (diagnosed using lab tests of
833 fasting blood glucose or HbA1c, self-reports, previous doctor diagnosis, or current use
834 of diabetic drugs) (Sowlat et al., 2016). A study of 3,516 subjects from the National
835 Health and Nutritional Examination Survey (NHANES) 2003–2008 found a positive
836 correlation between higher urinary BPA and prediabetes (fasting plasma glucose), and
837 correlation was independent of confounders including body mass index, alcohol intake,
838 blood pressure and serum cholesterol (Sabanayagam, Teppala & Shankar, 2013).
839 When T2D was examined within the participants of NHANES, a correlation was
840 observed with odds ratio (OR) of 1.39, however only for the cycle 2003–2004 (Lang et
841 al., 2008; Silver et al., 2011, pp. 2003–2008). Another study of NHANES participants
842 found multivariable-adjusted OR of 1.68 when comparing 1st quartile of urinary BPA
843 concentrations with 4th quartile (p=0.002) (Shankar & Teppala, 2011). In addition to
844 measured urinary BPA concentrations, a study of 2581 subjects in Thailand that

845 involved blood (serum) BPA levels has found a significant correlation between serum
846 BPA levels and T2D (OR=1.83 and 1.88, for women and men respectively) (Aekplakorn,
847 Chailurkit & Ongphiphadhanakul, 2015). However, other epidemiological studies failed
848 to find a statistically significant correlation of BPA levels and T2D. For example, a study
849 involving 3423 subjects in China measured urinary BPA concentrations and found
850 slightly increased OR for T2D (OR=1.30, 1.37, in second and fourth BPA quartile,
851 respectively), but not third quartile, and the overall association was not significant (Ning
852 et al., 2011). Similarly, a cross-sectional study of Korean adults showed increased
853 adjusted odds ratio for T2D in the upper BPA quartile (OR=1.89), however, this
854 association did not reach statistical significance (Kim & Park, 2013). Therefore, the
855 epidemiological evidence for the connection between human BPA concentrations and
856 T2D requires further assessment.
857
858
859 **BPA effects in promoting cardiovascular diseases**
860
861 The previous studies on BPA treatment in animal-models found evidence of interference
862 on the mechanisms underlying insulin signaling and diabetes, while the underlying
863 mechanisms of association of cardiovascular diseases are not evident. In a 2008 study
864 (Lang et al., 2008), 1455 adults from the NHANES 2003-2004 study (694 men and 761
865 women), 18-74 years of age, have had their urinary BPA and creatinine levels
866 measured. Regression association was adjusted for the creatinine concentration in
867 urine, as well for a set of standard factors, such as age, sex, ethnicity, education, and
868 body mass index. Tested samples provided 80% power and detected that higher BPA
869 concentrations in urine were associated with cardiovascular diagnoses in models
870 adjusted for age and sex and in a fully adjusted model (OR=1.39 per 1-SD increase in
871 BPA, 95% confidence interval: 1.18-1.63, full adjustment pval=0.001). Similarly, in the
872 study of 1493 participants of the NHANES 2005-2006 elevated urinary BPA
873 concentrations were correlated with coronary artery disease (CAD) (OR=1.33) (Melzer
874 et al., 2010, p. 6). In mice, BPA exposure has been linked to the increase in production
875 of a strong helper T type 1 (Th-1) type cytokine (IFN-gamma) in lymphocytes (Youn et
876 al., 2002), which given the role of Th-1 immunity and inflammation in atherosclerosis
877 and CAD (Engelbertsen & Lichtman; Pjanic et al., 2016; Kim et al., 2017) might
878 mechanistically bridge the link to CAD. Whether BPA exerts it effect on cardiovascular
879 diseases through its loose binding to the estrogen receptor or via binding to the
880 estrogen related receptors, and whether this is a shared downstream mechanism with
881 the effect on insulin resistance remains to determined, especially considering that
882 estrogen receptor signaling exhibits pleiotropic effects on the cardiovascular system.
883
884
885 **Proposed BPA involvement in other phenotypes**
886
887 In epidemiological studies, bisphenol A exposure has been linked to various disorders in
888 humans, such as insulin resistance and diabetes (Shankar & Teppala, 2011),
889 cardiovascular diseases (Lang et al., 2008), and obesity (Wang et al., 2012). In a 2008
890 study (Lang et al., 2008), higher BPA concentrations were associated with diabetes

891   mellitus (OR=1.39 per 1-SD increase in BPA, 95% confidence interval: 1.21-1.60, full
892   adjustment pval < 0.001). In addition, in the same study, out of 8 blood serum analytes,
893   urinary BPA was associated with clinically abnormal concentrations of the liver enzymes
894   γ-glutamyltransferase (OR=1.29 per 1-SD increase in BPA, 95% CI: 1.14-1.46, full
895   adjustment pval < 0.001), alkaline phosphatase (OR=1.48, 95% CI: 1.18-1.85, pval =
896   0.002) and lactate dehydrogenase (pval = 0.04). As no significant associations with the
897   other common disorders were found, the specificity of the associations to insulin
898   resistance, diabetes and cardiovascular diseases implicated BPA in modulation of
899   common mechanisms perturbed in these diseases. On the other side, the association of
900   BPA and the enzymes present in liver, specifically γ-glutamyltransferase and lactate
901   dehydrogenase, was preserved in a cohort without cardiovascular diseases or diabetes
902   (glutamyltransferase OR=1.22, 95% CI: 1.02-1.45, pval= 0.03; lactate dehydrogenase
903   OR=1.31, 95% CI: 1.06-1.62, pval= 0.01) (Lang et al., 2008), suggesting that
904   mechanisms underlying the BPA effect on liver are distinct from the cardiovascular and
905   insulin resistance/diabetes effects and therefore exclude reverse causation of these
906   diseases. In addition, in patients with BMI less than 25, BPA preserved significant
907   association with γ-glutamyltransferase (pval= 0.03).
908
909   **Discussion**
910
911   In the last two decades, bisphenol A has been a target of strong public and scientific
912   scrutiny. The number of papers on BPA available on Pubmed reaches 10,668, with
913   several hundred papers published each year. An overwhelming body of knowledge has
914   accumulated since, both mechanistic, in animal models, and epidemiological, that has
915   contributed to our better understanding of the implications that the widespread and
916   chronic exposure of human population to BPA carries. Even though BPA properties as
917   estrogen mimicking molecule have been discovered in 1936 (Dodds & Lawson, 1936)
918   and 1938 (Dodds & Lawson, 1938), its widespread use as a synthetic polymer unit,
919   starting from the late 1950s, hasn't been influenced by the fact that it might behave as
920   an endocrine-disrupting chemical. Driven by the industrial tendencies and novel
921   emerging markets, BPA based polycarbonate polymers have infiltrated almost every
922   aspect of the human life, including food containers, baby and water bottles, can and
923   glass linings, various medical and dental devices, eyeglass lenses, and finally the epoxy
924   lining of water pipes and tanks, making the large majority of human population
925   chronically exposed to the low levels of this synthetic chemical.
926
927   Whether the widespread use of BPA in the contemporary human environment is related
928   to the expansion of insulin resistance, diabetes and obesity-related diseases is unclear.
929   One can contemplate that this is most probably not a direct or unique cause of the
930   elevated fasting plasma glucose levels, insulin resistance and diabetes expansion in the
931   human populations, however the time frames of the diabetes expansion and the use of
932   plastic bottles coincide, hence, given the experimental findings, the question becomes
933   more quantitative than qualitative. The prevalence of hyperglycemia and diabetes are
934   rising globally since 1980 with a mean fasting plasma glucose level increasing 0.09
935   mmol/L per decade, while the number of people with diabetes increased from 153
936   million in 1980 to 347 million in 2008, more than doubling in size during 3 decades

937 (Danaei et al., 2011). The level of BPA exposure in human populations depends
938 primarily on how chemically effective the hydrolysis and photo-degradation of
939 polycarbonate polymers are in their natural environment and that depends on the
940 content, stability and storage conditions of plastic polymers and coating materials. On
941 higher temperatures, increased hydrolysis leads to the excess of leached BPA in the
942 neighboring environment. Certain polycarbonate plastics and coatings may represent
943 common sources of leaching BPA levels, e.g. food containers that will be exposed to
944 higher temperatures in microwave ovens. Therefore, unknowingly humans may further
945 increase the hydrolysis of polycarbonates and subsequently their exposure to BPA by,
946 e.g. microwaving food in plastic containers, refilling plastic water bottles or leaving
947 plastic water bottles in the sun exposed to light, with BPA polymers undergoing photo-
948 oxidative degradation.
949
950 BPA traces have been detected leaching from the polycarbonate plastic products, as
951 well as present in various human tissues. BPA environmental levels correspond to the
952 tissue levels, appearing in the concentrations of the same order of magnitude (ng ml^{-1}),
953 indicating effective transfer from the environment to the human internal organs and
954 tissues. BPA has been detected in human serum with concentrations up to 4.4 ng ml^{-1}
955 (Vandenberg et al., 2007) and urine with detection rates up to 97.5% (Yang et al.,
956 2006). As tissues that exhibited the highest BPA concentrations up to the levels of 11.2
957 ng ml^{-1} were related to the embryo development, such as placenta, umbilical cord, and
958 amniotic fluid, as well as to the maternal influence of postnatal development of infants,
959 like breast milk and colostrum, it may not be surprising that experimental studies in
960 mice, as well as epidemiological studies in humans, showed pronounced
961 transgenerational effects of BPA. The question of mechanism for gender-related
962 differences of the BPA effects in offspring still remains open, as to why predominantly
963 male offspring exhibit increased insulin resistance, while female offspring show negative
964 BMI correlation. The explanation may come from a gender-related differences in BPA-
965 processing liver enzyme levels and subsequent BPA clearance from the organism. For
966 instance, it has been shown that female rats harbor higher UDP-
967 glucuronosyltransferase liver levels, as well more effective BPA glucuronidation reaction
968 that eliminates BPA from the organism (Takeuchi et al., 2004).
969
970 Skepticism could emerge due to the fact that BPA has much lower affinity for estrogen
971 receptors, therefore questioning whether its effects are indeed negligible. However, it
972 may be possible that effects of prolonged exposure to low affinity binders mimic the
973 short term effect of high affinity binders, providing mechanistic explanation for direct
974 BPA action. Indeed, low affinity binders may have profound effects on the pathogenesis
975 of obesity and insulin resistance, as shown in the case of insulin-like growth factor
976 binding proteins (IGFBPs) comprising both insulin-like growth factor (IGF) high-
977 and low-affinity binders (Kim et al., 1997; Ruan & Lai, 2010).
978
979 As it has been shown that in various in vivo and in vitro experiments BPA effects follow
980 the non-monotonic dose response (i.e. the inverted U-shape curve), a narrow
981 concentration range might exist that is critical for the BPA action. Similarly, the narrow
982 window of developmental stages exists during which BPA will exert its maximal effect

983 (e.g. P6–PND0). Combined with the gender related differences, there might exist a
984 specific set of conditions under which BPA exerts its maximal biological and
985 physiological effect (Figure 5). Therefore, the complete elucidation of the maximal BPA
986 effects on offspring may be limited to the very specific set of experimental conditions. In
987 addition, the discovery of BPA mechanism that involves epigenetic modifications (i.e.
988 DNA methylation) reveals the complexity of the mechanism responsible for the
989 appearance of BPA phenotype in subsequent generations.
990
991 Even though at present day BPA-free plastic products are getting more available, e.g.
992 BPA-free water bottles, the use of BPA-free polymers has not reached widespread
993 levels and in many cases BPA is simply substituted with one of its analogues, BPS or
994 BPF, that may exhibit similar behavior to BPA. In addition, the level of public education
995 on this subject remains relatively poor and the amount of BPA present in the
996 environment still remains at the levels of a substantial health threat. Consistently, it will
997 take years of regulation of environmental and industrial BPA levels to achieve reduced
998 BPA concentrations to the pre-industrial levels or its complete elimination.
999
1000 BPA may have an effect that needs to be deciphered from the existing data to prevent
1001 its long-term negative impact. As once, unaware of the health risks, Roman populations
1002 had been poisoned gradually by an increased lead content in the water, through
1003 utilization of leaded pipes in their water distribution network (Delile et al., 2014), which
1004 subsequently contributed to the decline of Roman empire, the environmental and health
1005 toll of BPA plastics in the human environment needs to be addressed thoughtfully in the
1006 modern world. Further experimental and epidemiological efforts are necessary to fully
1007 establish a magnitude of potentially hazardous effects of BPA in humans, and its
1008 association to insulin resistance and diabetes, as well as other human diseases.
1009
1010
1011
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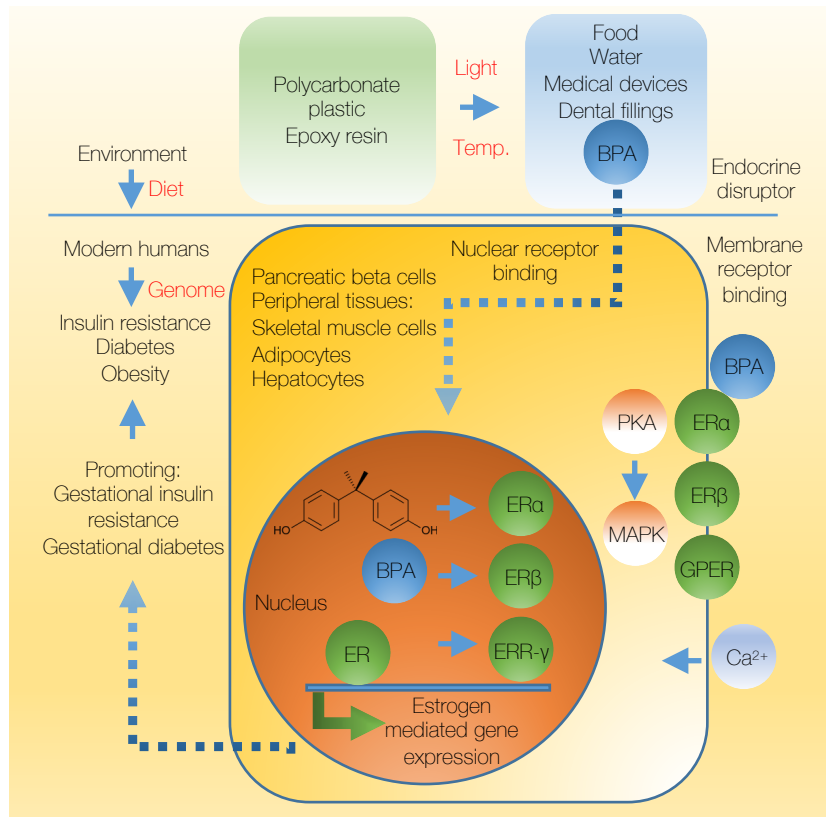
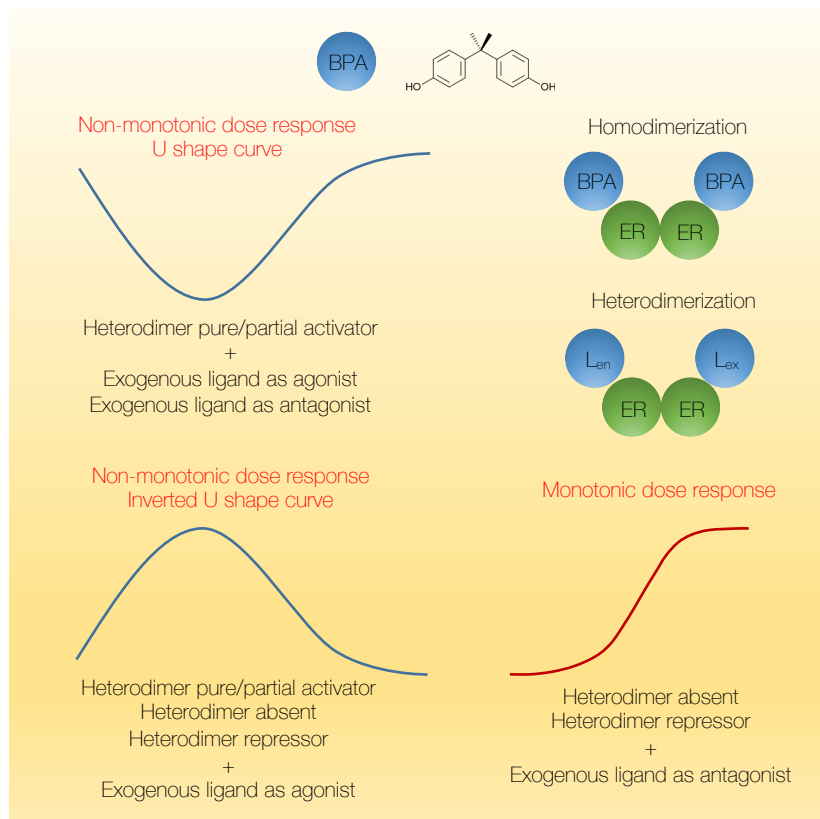
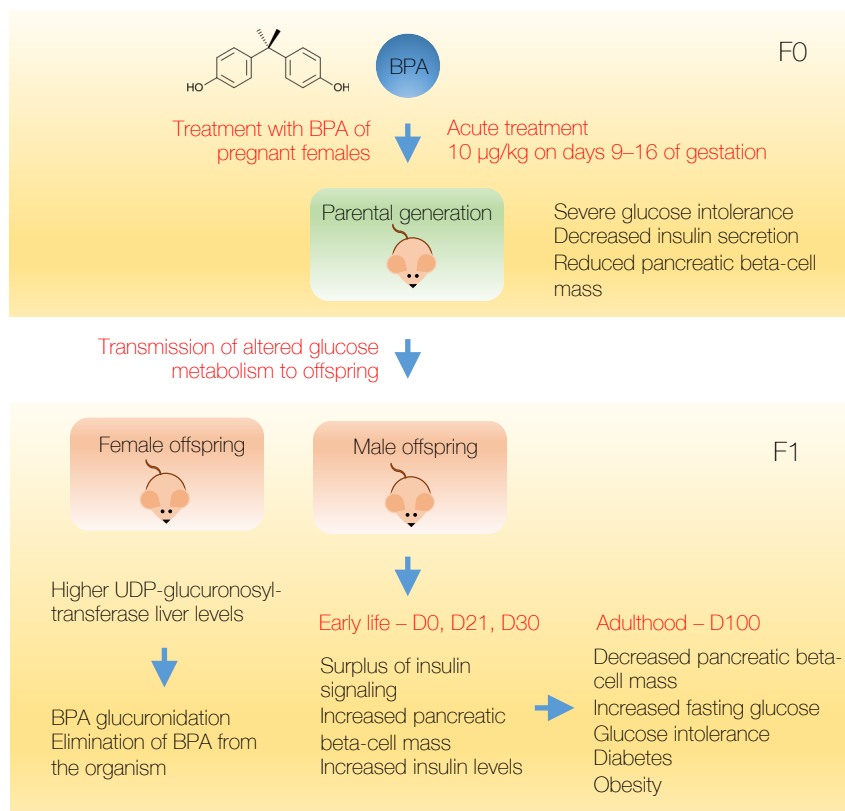
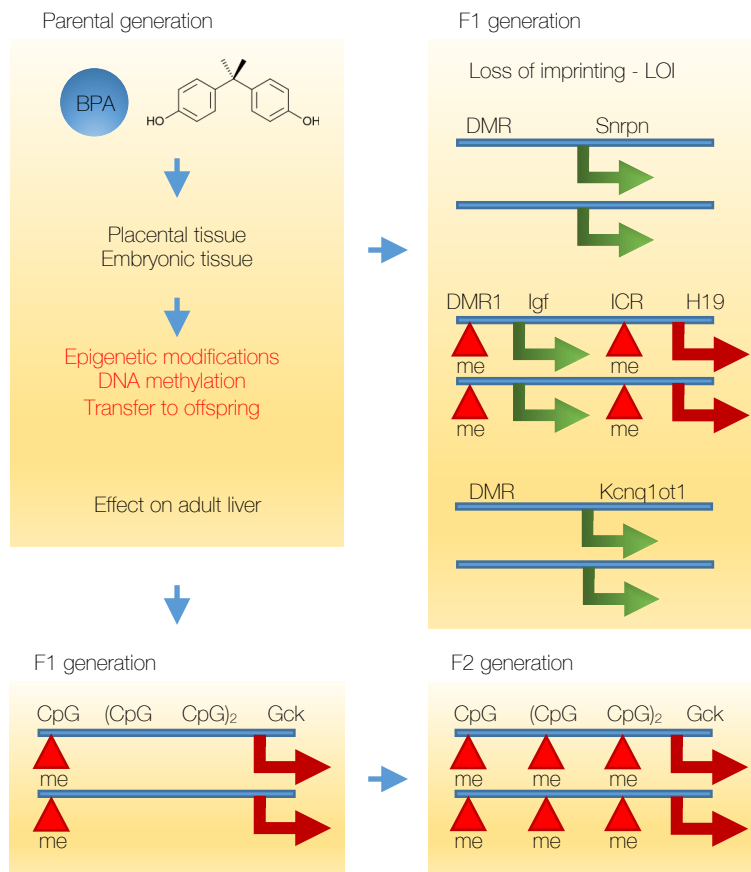
The model of BPA effect on insulin resistance and diabetes

Global model of the contribution of endocrine-disrupting chemical BPA to the development of insulin resistance and diabetes in humans. Light and temperature might induce the hydrolysis of polycarbonate plastics and subsequent leaching of BPA into the water and food sources. Once in human tissues BPA exerts its effects through ER receptors alpha, beta and gamma and estrogen mediated gene expression. In addition to the nuclear receptors, BPA can exert its effects through non-genomic, membrane associated receptors. Model indicates that BPA induced endocrine disruption may partially contribute to the development of insulin resistance, with major contributors being modern human diet and genomic composition. ER - estrogen receptor. ERR - estrogen-related receptor. GPER - G-protein-coupled estrogen receptor.

The prediction of non-monotonic dose response to BPA

BPA may induce non-monotonic dose response as manifested by the appearance of the inverted U-shape curve, e.g. when insulin content is measured in isolated pancreatic islets after treatment with increasing concentrations of BPA (0.1-1000 nM) for 48 hours (Alonso-Magdalena et al, 2008). Computational modeling that takes into account the dimerization kinetics of the estrogen receptors and binding to either endogenous or exogenous ligand, or both (heterodimerization) accurately predicts the appearance of non-monotonic dose responses (both the U-shape and inverted U-shape curves). U-shaped dose response appears in case of the heterodimer $L_{en}ER$-ERL_{ex} (also named LXXR) acting as a pure or partial activator, regardless of the nature of exogenous ligand. Non-monotonic inverted U-shape curve appears when exogenous ligand is an agonist, regardless of the activity of the heterodimer. Finally, monotonic response can also arise in two cases. L_{en} – endogenous ligand, L_{ex} - exogenous ligand.

Effective transmission of BPA effects to male offspring in mice

Acute BPA treatment during gestation leads to severe glucose intolerance, decreased insulin production, and altered glucose metabolism that is being transferred to the male offspring. During the early life in male offspring there is a surplus in insulin signaling and insulin production that ultimately leads to decreased pancreatic beta mass and glucose intolerance in adulthood. Female offspring is protected from the BPA effects due to the higher levels of the enzyme involved in BPA glucuronidation process and elimination of BPA from the organism.

BPA induced epigenetic modifications are actively transmitted to offspring

BPA can induce changes in DNA methylation in the placental tissue, as well as in the embryo of the F1 generation. Modifications in DNA methylation at the DMR - differentially methylated regions induce changes in gene expression and LOI – loss of imprinting (loss of monoallelic expression) of at least three imprinted genes (*Snrpn*, *Igf2* and *Kcnq1ot1*). In the case of *Igf2* increased methylation leads to the loss of expression of the neighboring *H19* gene from the maternal allele and subsequent biallelic expression of *Igf2*. In the case of *Snrpn* and *Kcnq1ot1* the loss of methylation at the DMR leads to biallelic expression of these genes. In the liver in F1 and F2 generations, DNA methylation of the *Gck* (glucokinase) gene promoter decreases its expression. In F1 generation *Gck* contains hypermethylated one CpG island (out of 5 CpG islands in total present in the promoter of the *Gck* gene). In F2 generation, DNA methylation is increased as all 5 CpG islands become methylated.

Maximal biological effect of BPA is confined to a narrow range and dependent on dose, gender and developmental stage

BPA exerts effects that follow non-monotonic dose response (e.g. the inverted U-shape curve), therefore a narrow concentration window might exist that is essential for the BPA action. Concordantly, BPA will show its maximal effect in the narrow window of developmental stages (e.g. P6–PND0). These confined ranges of maximal BPA effects, together with the gender related differences, comprise a specific set of conditions for the maximal biological and physiological effect of BPA.