Food and Chemical Toxicology 126 (2019) 142–151

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

In utero exposure to bisphenol-A disrupts key elements of retinoid system in male mice offspring

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ARTICLE INFO

Keywords:
Bisphenol-A
Endocrine disruption
Retinoid system
All-*trans*-retinoic acid
Fibroblast growth factor 21

ABSTRACT

The retinoid system controls essential cellular processes including mitosis, differentiation and metabolism among others. Although the retinoid-signalling pathway is a potential target for the action of several endocrine disrupting chemicals (EDCs), the information about the developmental effects of bisphenol-A (BPA) on the hepatic retinoid system is scarce. Herein, male mice were *in utero* exposed to BPA following maternal subcutaneous doses of 0, 10 and 100 μg/kg bw/day from gestational day 9–16 and they were sacrificed at post-natal day 30. Retinoid concentrations and gene expression of key elements involved in the retinoid system were determined in liver. BPA increased all-*trans*-retinoic acid concentration and expression of *Adh1*, *Aox1* and *Cyp1a2* (biosynthesis of retinoic acid), while reduced *Mrp3* (efflux from hepatocyte to blood), increased *Bcrp* expression (biliary excretion) and changed the retinoid-dependent signalling system after reducing expression of *Rxrβ* and increasing that of *Fgf21*. Furthermore, we found bivariate associations of *Rar*γ and *Rxr*γ expressions with all-*trans*-retinoic acid concentrations and of *Fgf21* expression with that of *Rar*γ. Those findings occurred in animals which showed altered pancreatic function and impaired glucose metabolism during adulthood. The present information should be useful for enhancing testing methods for the identification of EDCs.

1. Introduction

Humans are exposed to a wide number of chemicals including EDCs which have been proposed to play a contributing role in the aetiology of diseases, such as metabolic disorders, cancer and alterations in fertility and development (ECHA, 2017; Gore et al., 2015). One of the EDCs which has attracted major attention is BPA, due to its potential human health hazard (ECHA, 2017; Gore et al., 2015; NTP, 2018).

BPA is used in the production of polycarbonate plastic and epoxy resins and consequently it is present in many consumer products including food containers, water bottles, children toys, medical devices, dental sealants as well as the thermal paper of cash register receipts (Geens et al., 2012; Huang et al., 2018; Vandenberg et al., 2010, 2007).

Humans are continuously exposed to BPA from different sources, diet and dermal contact being the main routes of exposure (Bernier and Vandenberg, 2017; Huang et al., 2018). Thus, BPA has been measured in different human biological samples, including blood, urine, human milk (Mercogliano and Santonicola, 2018) and placenta (Vandenberg et al., 2010, 2007). Although BPA bans on products for children were associated with decreasing intake trends, those for adults were increasing and varied greatly among continents (Huang et al., 2018).

According to the European Chemical Agency (ECHA), BPA exposure has been related to alterations in reproduction, mammary gland development, cognitive function and metabolism, whereas other effects will deserve closer attention in the future (ECHA, 2017). A report of the perinatal and chronic extended-dose-range study of BPA by the National Toxicology Program is available (NTP, 2018) and final CLARITY-BPA conclusions are expected in 2019.

Although the risk was considered negligible as a result of BPA exposure in humans compared with the EFSA's temporal-Tolerable Daily Intake of 4 μg/kg bw/day (Sarigiannis et al., 2016), it was also stated that risk should not be ignored because 15.7–19.8% of pregnant women exceeded the exposure of 0.08 μg/kg bw/day which produced adverse effects on the mammary gland in rats (Bemrah et al., 2014).

At the moment, testing methods are being updated and there is consensus for the need to enhance testing methods aimed at the

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https://doi.org/10.1016/j.fct.2019.02.023
Received 21 December 2018; Received in revised form 8 February 2019; Accepted 9 February 2019
Available online 18 February 2019
0278-6915/

Abbreviations

9C4O13,14DHRA	9-*cis*-4-oxo-13,14-dihydro-retinoic acid
ABC	ATP-binding cassette
Abcb1	ATP-binding cassette B1
Abcc2	ATP-binding cassette C2
Abcc3	ATP-binding cassette C3
Abcc4	ATP-binding cassette C4
Abcg2	ATP-binding cassette G2
Adh1	alcohol dehydrogenase 1
AIC	Akaike information criterion
ALDH	aldehyde dehydrogenase
Aldh1a1	aldehyde dehydrogenase 1A1
Aldh1a2	aldehyde dehydrogenase 1A2
ANOVA	analysis of variance
Aox1	aldehyde oxidase 1
ATRA	all-*trans*-retinoic acid
Bcrp	breast cancer resistance protein
BPA	bisphenol-A
BW	body weight
cDNA	complementary DNA
Ces1c	carboxylesterase 1C
Crabp2	cellular retinoic acid binding protein II
Crbp1	cellular REOH binding protein 1
Cyp1a2	cytochrome P450 1A2
Cyp1b1	cytochrome P450 1B1
CYP26	cytochrome P450 26
Cyp26a1	cytochrome P450 26A1
ECHA	European Chemical Agency
EDC	endocrine disrupting chemicals
EFSA	European Food Safety Authority
Fgf21	fibroblast growth factor 21
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GD	gestational day
HPLC	high performance liquid chromatography
IU	international Unit
Lrat	lecithin:REOH acyltransferase
LXR	liver X receptor
Mdr1b1	multidrug resistance protein 1b1
mRNA	messenger RNA
Mrp2	multidrug resistance-associated protein 2
Mrp3	multidrug resistance-associated protein 3
Mrp4	multidrug resistance-associated protein 4
NTP	National Toxicology Program
Oatp2b1	organic anion transporter 2B1
OECD	Organisation for Economic Co-operation and Development
OF-1	Oncins France 1
PCR	polymerase chain reaction
PND	postnatal day
PPAR	peroxisome proliferator-activated receptor
PPARγ	peroxisome proliferator-activated receptor γ
RA	retinoic acid
RAR	retinoic acid receptor
Rarα	retinoic acid receptor α
Rarβ	retinoic acid receptor β
Rarγ	retinoic acid receptor γ
Rbp4	retinol binding protein 4
REOH	retinol
REPA	retinyl palmitate
RXR	retinoid X receptor
Rxrα	retinoid X receptor α
Rxrβ	retinoid X receptor β
Rxrγ	retinoid X receptor γ
SD	standard deviation
SLCO	solute carrier organic anion transporter
Stra6	stimulated by retinoic acid gene 6
TTR	transthyretin
Ugt1a1	UDP glucuronosyltransferase 1A1
Ugt1a6	UDP glucuronosyltransferase 1A6
Ugt1a9	UDP glucuronosyltransferase 1A9
Ugt2b35	UDP glucuronosyltransferase 2B35
USEPA	United States Environmental Protection Agency

identification of EDCs (Manibusan and Touart, 2017). A proposed approach is to implement observations on the retinoid-signalling pathway. In fact, a Detailed Review Paper on the retinoid system under the frame of the Organisation for Economic Co-operation and Development (OECD) is underway to propose new testing methods and relevant enhancements (Manibusan and Touart, 2017). Available adverse outcome pathways for alterations of the retinoid system described lipid accumulation and obesity (Manibusan and Touart, 2017) as well as defects on neural tube and axial patterning (Tonk et al., 2015).

The retinoid system plays an essential role in the homeostasis of physiological processes such as tissue differentiation and development, cell proliferation and apoptosis; immune response; development and organogenesis of the foetus among others which was reviewed elsewhere (Chelstowska et al., 2016; Kedishvili, 2013; Novak et al., 2008; Piersma et al., 2017; Rhinn and Dolle, 2012; Theodosiou et al., 2010). The retinoid system consists of: 1) chemical molecules derived from vitamin A, known as retinoids; 2) proteins involved in their transport, biosynthesis and biotransformation and 3) nuclear receptors involved in their signalling. Retinoids are not synthetized *de novo*, yet they are essential nutrients obtained from the diet, either from vegetables as β-carotene or animal sources as retinol (REOH) and retinyl esters, including retinyl palmitate (REPA). In addition, some retinoids have been tested as candidates for therapies in diseases such as cancer, acne and metabolic alterations, among others (Vaz and de Lera, 2012). The biological activity of functional retinoids, such as all-*trans*-retinoic acid (ATRA) and 9-*cis*-4-*oxo*-13,14-dihydro-retinoic acid (9C4O13,14DHRA) (Schuchardt et al., 2009), is mediated by binding to retinoic acid receptors (RARs) which is involved in the regulation of gene expression (Evans and Mangelsdorf, 2014; Rhinn and Dolle, 2012). RARs form heterodimers with retinoid X receptors (RXRs), which in turn are able to dimerise with many other nuclear receptors, including PPAR (Evans and Mangelsdorf, 2014).

Therefore, the alteration of key elements of the retinoid system is associated with severe adverse developmental effects, such as lethality and a wide array of abnormalities in different organ systems (Rhinn and Dolle, 2012).

The outcomes of the interaction between BPA exposure and the retinoid system revealed changes at the level of retinoid receptors (Li et al., 2008; Nishizawa et al., 2005, 2003), as well as toxic effects in the liver (Shmarakov, 2015; Shmarakov et al., 2017, 2016).

There is a data gap on lasting effects on the retinoid system as a result of BPA exposure *in* utero. Therefore, in the present work we have quantified retinoid concentrations as well as gene expression of target genes of enzymes, transport proteins, receptors and signalling hormones related with the retinoid system in liver several weeks after mice, exposed to BPA in utero, were born.

2. Materials and methods

2.1. Chemicals

BPA (97% purity), also known as 4,4′-isopropylidenediphenol, was purchased from MP Biochemicals (Illkirch, France). The following retinoid standards were acquired from Sigma-Aldrich (Madrid, Spain):

all-*trans*-retinoic acid (ATRA) (purity > 98%), retinol (REOH) (purity > 95%), retinyl palmitate (REPA) (purity > 90%), acitretin (purity > 98%) and retinyl acetate (purity > 90%). The retinoic acid metabolite 9-*cis*-4-oxo-13,14-dihydro-retinoic acid (9C4O13,14DHRA) was not commercially available. All the other reagents and chemicals used were of HPLC or analytical grade.

2.2. Animals and treatment

Pregnant OF-1 mice were purchased from Charles River (Barcelona, Spain) and individually housed under standard conditions. Mice were maintained on 2014 Teklad Global 14% protein rodent maintenance diet (Harlan Laboratories, Barcelona, Spain), which does not contain alfalfa or soybean meal. The composition of the diet was as follows: vitamin A, 21000 IU/kg; crude protein, 14.3%; fat, 4%; carbohydrate, 48%; crude fiber, 4.1%; neutral detergent fiber, 18%; ash, 4.7%; energy density, 2.9 kcal/g (12.1 kJ/g); calories from protein, 20%; calories from fat, 13%; and calories from carbohydrate, 67%.

BPA was dissolved in tocopherol-stripped corn oil and subcutaneously administered from gestation day (GD) 9–16 in a volume of 100 µL. The daily dose applied was either 10 or 100 µg/kg. The lower dose was between the tolerable daily intake of 4 µg/kg/day established by the European Food Safety Authority (Bolognesi et al., 2015) and the reference dose of 50 µg/kg/day established by the Environmental Protection Agency (USEPA, 1988). In animal models, adverse effects were observed below the reference dose of 50 µg/kg/day (Vandenberg et al., 2012). Hence, the selected doses used in the present study were considered to be within the relevant range in terms of human health. Control animals received a daily dose of 100 µL of tocopherol-stripped corn oil vehicle, at the same time points.

After weighing at postnatal day (PND) 0, pups from the same treatment were pooled together and then placed in equal number with foster mothers of the same treatment group. The litter size was maintained at a constant number. Animals were sexed and weaned on PND21. They were housed (eight male mice/group) from weaning through the day the animals were euthanized. After weaning, they were maintained, *ad libitum*, on the diet described above. Animals were euthanized in a CO_2 chamber and decapitated on PND30, livers were dissected out, flash frozen in liquid nitrogen and stored at −80 °C until analysis. Only male offspring was used in this study.

The Ethical Committee of Miguel Hernandez University specifically reviewed and approved this study (approval identification IB-AN-001-11, UMH-IB-AN-01–14). Animals were treated humanely and with regard for alleviation of suffering.

2.3. Retinoid analysis in liver

ATRA, its non-commercially available metabolite 9C4O13,14DHRA (see Schmidt et al., 2003 for quantification details), REOH and REPA in livers were analyzed by HPLC (Agilent 1100 series, Agilent, CA, USA) as previously described (Mahiout et al., 2017; Schmidt et al., 2003). Acitetrin was used as an internal standard for the determination of ATRA and 9C4O13,14DHRA whereas retinyl acetate for that of REOH and REPA. Briefly, 300 mg of livers were homogenized in 300 mL of water. Separation of ATRA and 9C4O13,14DHRA (acidic fraction) from REOH and REPA (neutral fraction) was achieved by solid-phase extraction using an aminopropyl phase (Agilent SampliQ amino, Agilent, CA, USA). REOH and REPA were eluted in a first tube by using chloroform-isopropanol 2:1 (v/v). Then, ATRA and 9C4O13,14DHRA were eluted in a second tube with chloroform-isopropanol 2:1 (v/v) containing 3% glacial acetic acid. Both neutral and acidic fractions were evaporated and reconstituted in the corresponding tube and finally transferred to different HPLC micro-vials. Separation was achieved on a Poroshell 120 EC-C18 column (Agilent, CA, USA) and detection at 325 nm for REOH and REPA and at 340 nm for ATRA and 9C4O13,14DHRA.

2.4. Quantitative real-time PCR

PCR assays were performed using the CFX96 real-time system (Bio-Rad Laboratories). RNA extraction was performed with the RNeasy Micro Kit (Qiagen, USA) and RNA purity estimated from the 260/280 nm absorbance ratios with a mean (SD) value of 2.01 (0.05). A total RNA amount of 0.5 µg was used for the retrotranscription reaction (HighCapacity cDNA Reverse transcription, Applied Biosystems). Reactions were carried out in a final volume of 10 µL, containing 100 µM of each primer, 1 µL of cDNA, and 5 µL IQ SYBR Green supermix (Bio-Rad Laboratories). Samples were subjected to the following amplification conditions: 3 min at 95 °C, 40 cycles (5 s at 95 °C, 5 s at 60 °C, and 10 s at 72 °C), and a melting curve of 65–95 °C. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous control for quantification. The resulting values were analyzed with CFX Manager version 1.6 (Bio-Rad Laboratories) and shown as the relative expression with respect to control levels ($2^{-\Delta\Delta CT}$). mRNA expression of the genes was measured within the following categories: 1) Storage and mobilization of retinoids: carboxylesterase 1C (*Ces1c*), cellular REOH binding protein 1 (*Crbp1*), lecithin:REOH acyltransferase (*Lrat*); 2) RA biosynthesis: alcohol dehydrogenase 1 (*Adh1*), aldehyde dehydrogenases 1A1 and 1A2 (*Aldh1a1* and *Aldh1a2*), aldehyde oxidase 1 (*Aox1*), cytochromes P450 1A2 and 1B1 (*Cyp1a2* and *Cyp1b1*); 3) RA biotranformation: cellular retinoic acid binding protein II (*Crabp2*), cytochrome P450 26A1 (*Cyp26a1*), UDP glucuronosyltransferases 1A1, 1A6, 1A9 and 2B (*Ugt1a1, Ugt1a6, Ugt1a9* and *Ugt2b35*); 4) REOH carrier in the blood: REOH binding protein 4 (*Rbp4*). 5) Influx transport from blood to hepatocyte: stimulated by retinoic acid gene 6, (*Stra6*), solute carrier organic anion transporter (SLCO) 2B1 (*Oatp2b1*); 6) Efflux transport from hepatocyte to blood: multidrug resistance-associated proteins 3 and 4 (*Mrp3* and *Mrp4*), also known as ATP-binding cassette (ABC) transporters (*Abcc3* and *Abcc4*); 7) Biliary excretion: breast cancer resistance protein (*Bcrp*, also known as *Abcg2*); multidrug resistance protein (*Mdr1b1*, also known as *Abcb1*),

Table 1
Concentrations of retinoids in livers of male OF-1 mice offspring at PND 30 after exposure to bisphenol-A (BPA) in utero.

Retinoid	p-value[b]	Control[a]		BPA10[a]		BPA100[a]	
		N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
All-*trans*-retinoic acid (pmol/g)	0.013	8	9.40 ± 2,09	8	12.1* ± 2,4	8	8,7 ± 1,9
9-*cis*-4-*oxo*-13,14-dihydro-retinoic acid (pmol/g)	0.549	8	290 ± 180	8	366 ± 177	8	370 ± 123
Retinol (nmol/g)	0.835	8	604 ± 155	8	668 ± 185	8	668 ± 350
Retinyl palmitate (µmol/g)	0.285	8	12.1 ± 3.4	8	9.7 ± 3.0	8	9.9 ± 3.3

Data are expressed as mean ± SD from 8 independent samples per dose group (N).

[a] Pregnant mice were exposed subcutaneously to either control vehicle (100 µL of tocopherol-stripped corn oil) or commercial bisphenol-A (BPA) at doses of either 10 or 100 µg/kg bw/d from day 9 to day 16 of gestation. Male OF-1 mice offspring was sacrificed at PND30. Pairwise comparisons between means were performed by using analysis of variance and linear contrast tests.

[b] Statistical significance was considered for p-values < 0.05. * p-values < 0.05 vs control.

Mrp2, also known as *Abcc2*; 8) Signalling: fibroblast growth factor 21 (*Fgf21*), retinoic acid receptors α, β and γ (*Rarα*, *Rarβ*, *Rarγ*), retinoid X receptors α, β and γ (*Rxrα*, *Rxrβ*, *Rxrγ*). The primers used in these assays are described in Supplementary Table 1.

2.5. Statistical analysis

A descriptive analysis, which included calculations of mean and standard deviation, was made for retinoid concentrations and gene expressions since they showed a normal distribution as determined by the Levene's test and Q-Q plots. Pairwise multiple comparisons between means were performed by using analysis of variance (ANOVA) and linear contrast tests. Statistical significance was considered for p-values < 0.05 and tendency for p-values < 0.1. The associations between continuous variables, ie. hepatic concentrations of ATRA and expression of target genes involved in retinoic acid (RA) signalling were evaluated by using the package PROAST (version 65.5) for the analysis of dose-response data according to EFSA guidance (EFSA et al., 2017). The exponential model was considered to be better than the null model on the basis of the difference of their Akaike information criterion (AICs), ie. AIC null model – AIC exponential model > 2 (EFSA et al., 2017). Statistical tests were run in R version 3.5.0 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Hepatic retinoid concentrations following BPA exposure

Male offspring at PND30 following maternal BPA exposure from gestational day 9–16 at a dose of 10 µg/kg bw/day showed increased hepatic concentrations of ATRA compared with those of controls (Table 1). Hepatic concentrations of 9C4O13,14DHRA, REOH and REPA did not differ between groups (Table 1).

Table 3
Hepatic gene expression of transporters in livers of male OF-1 mice offspring at PND 30 after exposure to bisphenol-A (BPA) in utero.

Gene	p-value[b]	Control[a]		BPA10[a]		BPA100[a]	
		N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Retinol carrier in the blood							
Rbp4	*0.063*	6	1.00 ± 0.25	7	0.72 ± 0.37	8	0.59* ± 0.26
Influx transport from blood to hepatocyte							
Stra6	0.660	5	1.00 ± 0.40	6	1.20 ± 0.74	8	0.93 ± 0.45
Oatp2b1	*0.081*	6	1.00 ± 0.74	6	1.15 ± 0.32	8	1.65* ± 0.46
Efflux transport from hepatocyte to blood							
Mrp3	**0.015**	6	1.00 ± 0.46	7	0.95 ± 0.54	8	0.38* ± 0.11
Mrp4	0.557	6	1.00 ± 0.53	7	0.78 ± 0.51	8	1.45 ± 1.83
Biliary excretion from hepatocyte to bile duct							
Mrp2	0.889	5	1.00 ± 0.84	7	1.11 ± 0.54	8	0.98 ± 0.24
Bcrp	**0.005**	5	1.00 ± 1.03	7	1.71 ± 0.67	8	2.56** ± 0.54
Mdr1b1	0.311	6	1.00 ± 0.07	6	0.54 ± 0.26	6	2.16 ± 3.06

Data are expressed as mean ± SD from 5 to 8 independent samples per dose group (N).

[a] Pregnant mice were exposed subcutaneously to either control vehicle (100 µL of tocopherol-stripped corn oil) or commercial bisphenol-A (BPA) at doses of either 10 or 100 µg/kg bw/d from day 9 to day 16 of gestation. Male OF-1 mice offspring was sacrificed at PND30. Pairwise comparisons between means were performed by using analysis of variance and linear contrast tests.

[b] Statistical significance was considered for p-values < 0.05 and tendency for p-values < 0.1. * p-values < 0.05 or ** p-values < 0.01 vs control. *Rbp4*, Retinol binding protein 4; *Stra6*, Stimulated by retinoic acid gene 6; *Oatp2b1*, Solute carrier organic anion transporter (SLCO) 2B1; *Mrp3*, multidrug resistance-associated protein 3, also known as ATP-binding cassette (ABC) transporter ABC C3; *Mrp4*, also known as ABC C4; *Mrp2*, also known as ABC C2; *Bcrp*, breast cancer resistance protein, also known as ABC G2; *Mdr1b1*, multidrug resistance protein 1B1/P-glycoprotein, also known as ABC B1.

Table 2
Hepatic expression of genes involved in storage, mobilization, biosynthesis and biotransformation of all-*trans*-retinoic acid (ATRA) in livers of male OF-1 mice offspring at PND 30 after exposure to bisphenol-A (BPA) in utero.

Gene	p-value[b]	Control[a]		BPA10[a]		BPA100[a]	
		N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Storage and mobilization							
Lrat	0.157	6	1.00 ± 0.62	7	1.83 ± 1.10	7	1.95 ± 0.88
Ces1c	0.996	6	1.00 ± 0.70	7	0.98 ± 0.37	7	0.98 ± 0.34
Crbp1	0.414	6	1.00 ± 0.51	7	1.33 ± 0.79	7	1.45 ± 0.46
ATRA Biosynthesis							
Adh1	**0.041**	6	1.00 ± 0.35	6	1.44* ± 0.19	7	1.22 ± 0.26
Aox1	**0.010**	6	1.00 ± 0.57	7	1.95* ± 0.70	7	2.38** ± 0.86
Aldh1a1	0.369	6	1.00 ± 0.54	7	1.03 ± 0.30	7	1.32 ± 0.48
Aldh1a2	0.274	6	1.00 ± 0.66	7	1.49 ± 0.54	7	1.47 ± 0.57
Cyp1a2	**0.029**	5	1.00 ± 0.58	7	2.57* ± 1.34	6	2.47* ± 0.59
Cyp1b1	0.117	5	1.00 ± 0.62	6	1.70 ± 0.95	7	0.97 ± 0.20
Crabp2	0.763	5	1.00 ± 0.52	6	1.30 ± 0.74	7	1.22 ± 0.73
ATRA Biotransformation							
Cyp26a1	0.789	5	1.00 ± 0.77	7	0.81 ± 0.46	6	0.81 ± 0.27
Ugt1a1	0.120	6	1.00 ± 0.17	7	1.11 ± 0.61	7	0.64 ± 0.30
Ugt1a6	0.349	6	1.00 ± 0.36	7	1.00 ± 0.39	6	0.76 ± 0.16
Ugt1a9	0.714	5	1.00 ± 0.33	7	1.18 ± 0.47	7	1.22 ± 0.54
Ugt2b35	0.428	5	1.00 ± 1.08	7	1.50 ± 0.50	8	1.41 ± 0.45

Data are expressed as mean ± SD from 5 to 8 independent samples per dose group (N).

[a] Pregnant mice were exposed subcutaneously to either control vehicle (100 µL of tocopherol-stripped corn oil) or commercial bisphenol-A (BPA) at doses of either 10 or 100 µg/kg bw/d from day 9 to day 16 of gestation. Male OF-1 mice offspring was sacrificed at PND30. Pairwise comparisons between means were performed by using analysis of variance and linear contrast tests.

[b] Statistical significance was considered for p-values < 0.05. * p-values < 0.05 or ** p-values < 0.01 vs control. *Lrat,* Lecithin-retinol acyltransferase (phosphatidylcholine-retinol-O-acyltransferase)*; Ces1c,* Carboxylesterase 1C*; Crbp1,* Cellular retinol binding protein 1; *Adh1,* Alcohol dehydrogenase 1*; Aox1,* Aldehyde oxidase 1*; Aldh1a1,* Aldehyde dehydrogenase 1A1*; Aldh1a2,* Aldehyde dehydrogenase 1A2*; Cyp1a2,* Cytochrome P450 1A2*; Cyp1b1,* Cytochrome P450 1B1*; Crabp2,* Cellular retinoic acid binding protein II*; Cyp26a1,* Cytochrome P450 26A1*; Ugt1a1,* UDP-glucuronosyltransferase 1A1*; Ugt1a6,* UDP-glucuronosyltransferase 1A6*; Ugt1a9,* UDP-glucuronosyltransferase 1A9*; Ugt2b35,* UDP-glucuronosyltransferase 2B35.

3.2. Hepatic gene expression of retinoid system components following BPA exposure

There was an increment in the level of expression of genes involved in ATRA biosynthesis in hepatic cells. *Adh1* expression showed a significant increase in the BPA 10 group compared with those of controls (Table 2). *Aox1* and *Cyp1a2* gene expressions were increased in both BPA10 and BPA100 animals compared with those of controls (Table 2). Expressions of *Aldh1a1*, *Aldh1a2*, *Cyp1b1* and *Crabp2* did not differ between groups following exposure to BPA (Table 2).

The hepatic expression of *Lrat*, *Ces1c*, and *Crbp1* genes, involved in storage and mobilization of retinoids, did not show significant differences between groups following prenatal exposure to BPA (Table 2).

Hepatic expressions of *Cyp26A1*, *Ugt1a1*, *Ugt1a6*, *Ugt1a9*, and *Ugt2b35* genes, involved in ATRA biotransformation in hepatic cells, did not differ between groups (Table 2).

We, then, evaluated the hepatic gene expression of REOH binding protein in serum (*Rbp4)* and transporters involved in the disposition of retinoid metabolites, ie. hepatocyte influx and efflux transporters, and those for biliary excretion (Table 3). There were two tendencies with p-values < 0.1, ie. reduced expression of *Rbp4* (p-value = 0.063) and increased one for *Oatp2b1* (p-value = 0.081; Table 3). At the dose of 100 μg/kg bw/day, the hepatic gene expression of *Mrp3* was significantly reduced, whereas *Bcrp* was increased compared with those of controls according (Table 3). Expressions of *Stra6*, *Mrp4, Mrp2* and *Mdr1b1* did not differ between groups following exposure to BPA (Table 3).

Gene expression of nuclear receptors *Rar α, β*, and γ, and *Rxr α, β*, and γ as well as those of the hormone *Fgf21* was measured as indicators of modulated retinoid-dependent signalling in hepatic cells. Among all those genes, mRNA expression of *Rxr β* showed a significant reduction in BPA100 group, whereas *Fgf21* expression was increased in BPA10 compared with those of controls (Table 4).

Hepatic levels of ATRA were associated with those of *Rar* γ (Fig. 1A) and *Rxr* γ (Fig. 1B) as well as those of *Rar* γ with *Fgf21* expression (Fig. 1C). Neither expression of *Rar α* or *β*, nor the expression of *Rxr α* or *β* was associated with ATRA concentrations. No association was observed between *Fgf2*1 mRNA expression and *Rxr* γ.

4. Discussion

In the current work, we found lasting effects at PND30 following gestational exposure to BPA, including: increased retinoid-dependent signalling changes involving increased *Rar* γ, *Rxr* γ and *Fgf21* (BPA10), but reduced *Rxr β* expression (BPA100); induced ATRA biosynthesis based on its hepatic concentrations and expressions of *Adh1*, *Aox1* and *Cyp1a2*; and modulation of transporter expressions, including *Mrp3* and *Bcrp* towards the biliary excretion of glucuronide metabolites. Animals from the same experimental design in mice showed adverse metabolic effects as a result of BPA exposure *in utero* at environmentally relevant doses (Alonso-Magdalena et al., 2010; Garcia-Arevalo et al., 2016, 2014). With regard to the selected doses and route of exposure, livers removed at birth from pups of pregnant rats treated with 50 μg/kg bw/day showed total BPA levels (Xia et al., 2014) within the range found in human foetal livers (Cao et al., 2012). Additionally, non-oral routes such as transdermal exposures are considered to be significant sources of BPA (Bernier and Vandenberg, 2017; Geens et al., 2012). As a matter of fact, BPA pharmacokinetics studies reported that subcutaneous administration of BPA may be a better delivery route than oral administration, leading to a ratio of conjugated vs unconjugated BPA within the range reported in human biomonitoring studies (Taylor et al., 2011; vom Saal et al., 2014). Thus, subcutaneous exposure as herein employed may provide a better model for human exposure to BPA than oral route (Vandenberg et al., 2014).

A strict regulation of RA levels is essential for life maintenance since both a detriment (Wiseman et al., 2017) and an excess (Hathcock et al., 1990) of retinoid-dependent signalling are associated with adverse effects. During development, a balance between synthesis and degradation is required for a RA homeostasis in the vertebrate gastrula, which determines vertebrate body axis pattern formation in the embryo (Piersma et al., 2017). With respect to the liver, repressed RA signalling triggered a progression of the histopathology, ie. steatosis, steatohepatitis and hepatocellular carcinoma along with biochemical changes in mice (Yanagitani et al., 2004). Similarly, lower levels of RA in serum and of *Rxrα* mRNA in liver were associated with hepatic steatosis severity and liver injury in humans (Liu et al., 2015). Complementary to that, RA signalling was considered as a protective factor as, for instance, its administration to mice increased lipid metabolism and reduced lipogenesis (Amengual et al., 2010), repressed obesity and insulin resistance (Berry and Noy, 2009) and reversed the occurrence of liver tumours (Yanagitani et al., 2004). Similarly, specific RARβ2 agonists improved hyperglycemia and peripheral insulin resistance and promoted the reduction of lipid levels (Trasino et al., 2016).

In this context, the increase of ATRA at BPA10 but unchanged levels at BPA100, along with the corresponding bivariate associations with *Rar* γ, *Rxr* γ and *Fgf21* (Fig. 2) could be understood as a protective response to counteract a direct action of BPA on the liver. The endogenous ligand ATRA activates RARs for the regulation of cellular processes via RXR-RAR heterodimers (Allenby et al., 1993; Bastien and Rochette-Egly, 2004; Conaway et al., 2013; Mic et al., 2003; Shmarakov, 2015). Furthermore, ATRA induced the expression of *Rars α, β* and γ (Balmer and Blomhoff, 2002), which might be related to the induction of *Rar* γ shown in the present paper (Fig. 2), for which RAR-dependent responses might be anticipated. One of those effects might be the increased expressions of *Fgf21* (Fig. 2), which is a hormone produced in liver and fat, released into the circulation for energy regulation via glucose and lipid metabolism and regarded as a protective factor against steatosis (Li et al., 2014; Xu et al., 2009). *Fgf21* expression was not only induced by RXR-PPAR heterodimers formed with RXRs and PPARα in liver (Badman et al., 2007; Inagaki et al., 2007; Lundasen et al., 2007) and with PPARγ in adipose tissue (Dutchak et al., 2012; Evans and Mangelsdorf, 2014; Muise et al., 2008), but also via RA signalling by RARs (Li et al., 2013). Complementary to that, ATRA was able to activate PPARs (Berry and Noy, 2007; Conaway et al., 2013; Shaw et al., 2003; Wolf, 2010). Regarding the reduced gene expression of *Rxr β*, although none of the retinoids assayed herein could *a priori* explain such a change at BPA100, it might be relevant for different findings found later in life. An RXRβ active in its transcriptional

Table 4
Hepatic gene expression of nuclear signalling receptors in livers of male OF-1 mice offspring at PND 30 after exposure to bisphenol-A (BPA) in utero.

Gene	p-value[b]	Control[a]		BPA10[a]		BPA100[a]	
		N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Rarα	0.219	6	1.00 ± 0.34	7	1.69 ± 1.06	7	1.26 ± 0.76
Rarβ	0.216	6	1.00 ± 0.44	7	0.63 ± 0.21	7	0.73 ± 0.43
Rarγ	0.184	6	1.00 ± 0.34	6	1.31 ± 0.38	6	0.98 ± 0.23
Rxrα	0.296	6	1.00 ± 0.38	7	0.83 ± 0.44	7	0.69 ± 0.18
Rxrβ	**0.034**	6	1.00 ± 0.38	7	0.75 ± 0.32	7	0.54* ± 0.13
Rxrγ	0.101	6	1.00 ± 0.32	7	0.83 ± 0.34	7	0.65 ± 0.12
Ffg21	**0.023**	5	1.00 ± 1.43	6	4.62* ± 3.62	8	1.46 ± 0.67

Data are expressed as mean ± SD from 5 to 8 independent samples per dose group (N).

[a] Pregnant mice were exposed subcutaneously to either control vehicle (100 μL of tocopherol-stripped corn oil) or commercial bisphenol-A (BPA) at doses of either 10 or 100 μg/kg bw/d from day 9 to day 16 of gestation. Male OF-1 mice offspring was sacrificed at PND30. Pairwise comparisons between means were performed by using analysis of variance and linear contrast tests.

[b] Statistical significance was considered for p-values < 0.05. * p-values < 0.05 vs control. *Rar α, β,* γ, retinoic acid receptors α, β and γ; *Rxr α, β,* γ, retinoid X receptors α, β and γ; *Fgf21,* fibroblast growth factor 21.

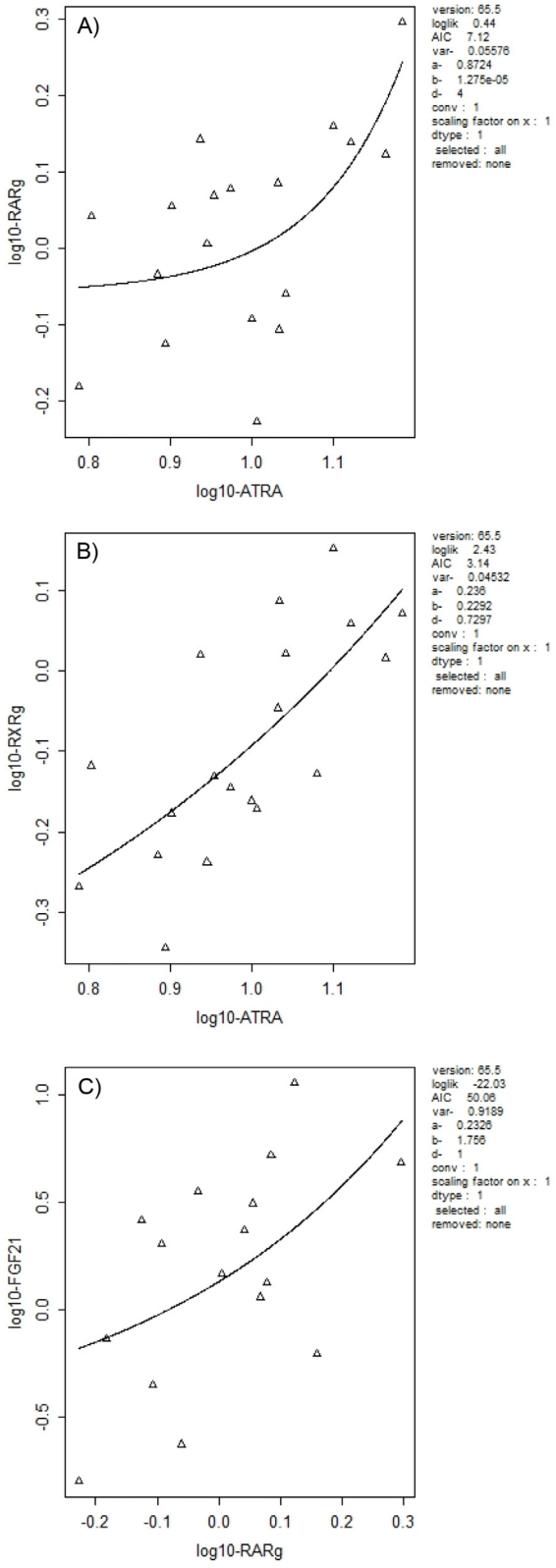

Fig. 1. Exponential models fitted to retinoid-dependent signalling data in livers of male OF-1 mice offspring at PND 30 after exposure to bisphenol-A (BPA) in utero.

Pregnant mice were exposed subcutaneously to either control vehicle (100 μL of tocopherol-stripped corn oil) or commercial bisphenol-A (BPA) at doses of either 10 or 100 μg/kg bw/d from day 9 to day 16 of gestation. Male OF-1 mice offspring was sacrificed at PND30. Bivariate associations describe relative mRNA expressions of A) *Rar* γ and B) *Rxr* γ *vs* all-*trans* retinoic acid concentrations (in pmol/g), and C) *Fgf21* vs *Rar* γ mRNA expressions, where *Rar* γ *is* retinoic acid receptor γ, *Rxr* γ *is* retinoid X receptors γ and *Fgf21 is* fibroblast growth factor 21. Both *x* and *y* axis are represented in log scale. The exponential models (*E3:* $y = a*\exp(bx\hat{}d)$) were better than the null model (*E1:* $y = a$) as the Akaike information criterion (AIC) difference of null model minus AIC of exponential model $>$ 2 (EFSA et al., 2017), ie. 4.76 for A), 10.04 for B) and 3.10 for C. In model C), *d* parameter was constrained to 1, while default settings for E3 model provided an AIC difference of 1.94. Further details on retinoid concentrations and mRNA gene expressions are described under "2.4. Quantitative real-time PCR".

activation function AF-2 was found to be essential to control lipid stores in Sertoli cells (Mascrez et al., 2004) for which a reduced signalling could be responsible for lipid overloads.

Besides endogenous retinoids, it was proved that xenobiotics are able to modulate retinoid receptors (Novak et al., 2008), including BPA *in vitro* and *in vivo* (Iwamuro et al., 2006; Li et al., 2008; Nishizawa et al., 2005, 2003). The decreased hepatic expression of *Rxrβ* (BPA100, Fig. 2) could potentially alter glucose homeostasis and be connected with the metabolic outcomes observed later in life. The potential association between retinoids and glucose homeostasis relies partly on the fact that RXR is crucial for the function of other nuclear receptors such as PPARγ and LXR. Both PPARγ and LXRs form obligate heterodimers with the RXR and can be activated by RXR ligands (Mukherjee et al., 1997). Along with that, specific agonists of RXRs have been shown to display antidiabetic effects in several animal models (Rhee and Plutzky, 2012). On the contrary, it has been largely demonstrated that developmental exposure to BPA is a programming factor for metabolic disorders including diabetes and obesity (Alonso-Magdalena et al., 2015; Gore et al., 2015; Heindel et al., 2017). In particular, administration of BPA following the same experimental design used in the current study lead to a rise in pancreatic β-cell division and mass together with marked hyperinsulinemia at PND30 (Garcia-Arevalo et al., 2016). This excessive insulin signalling was connected with later metabolic abnormalities. After 6 months of age, they showed severe glucose intolerance and decreased insulin sensitivity; alterations that were more pronounced in BPA10 (Alonso-Magdalena et al., 2010; Garcia-Arevalo et al., 2014).

The increased expression of retinoid-dependent signalling at BPA10 but reduced at BPA100 occurred along with the induction of both *Adh1* and *Aox1* genes (Fig. 2). Those genes are involved in a reversible oxidation of REOH to retinal and an irreversible oxidation of retinal for the biosynthesis of ATRA, respectively (Kedishvili, 2013; Rhinn and Dolle, 2012; Shmarakov, 2015; Theodosiou et al., 2010), as well as, the non-specific and high capacity hepatic *Cyp1a2* enzyme (Chen et al., 2000; Tomita et al., 1996; Zhang et al., 2000) (Fig. 2).

Preserving hepatic REOH levels would also be required for homeostatic ATRA levels (Fig. 2), which might explain the decreasing tendency (BPA100) of *Rbp4* expression (Senoo et al., 2017), a carrier of REOH in blood which forms a complex with transthyretin (TTR) to deliver hepatic REOH to target tissues through STRA6 (Chelstowska et al., 2016; Novak et al., 2008; Theodosiou et al., 2010). Marked RBP4 reductions in patients were associated with chronic liver disease and a reduced hepatic *Rbp4* gene expression was linked to an impaired hepatic function after induction of experimental cirrhosis in male Sprague-Dawley rats (Yagmur et al., 2007). Although both *Aox1* and *Cyp1a2* mRNA levels were upregulated, ATRA concentration (BPA100) and the expression of enzymes involved in the biotransformation of ATRA were unchanged (Fig. 2), so that the observed modulations were considered to represent an attempt to counteract the effects of BPA. On the contrary, the induction of genes involved in both biosynthesis and biotransformation of ATRA were found in humans diagnosed with steatosis and steatohepatitis of non-alcoholic origin (Ashla et al., 2010).

Moreover, the absence of changes on hepatic REPA and

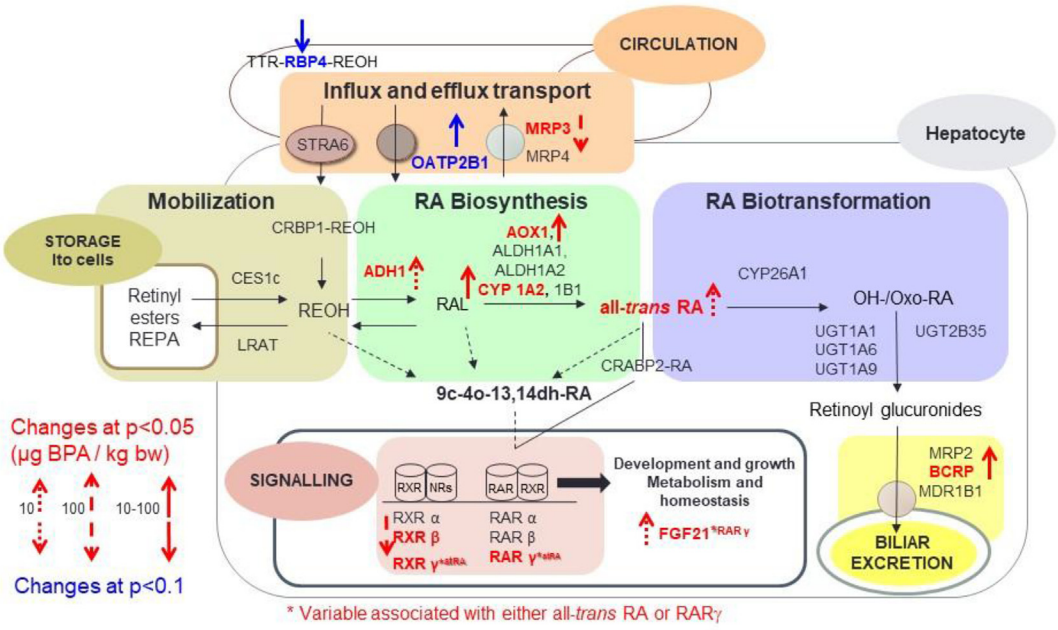

Fig. 2. Modulation of the retinoid-signalling pathway in livers of male OF-1 mice offspring at PND 30 after exposure to bisphenol-A (BPA) in utero.
Pregnant mice were exposed subcutaneously to either control vehicle (100 µL of tocopherol-stripped corn oil) or commercial bisphenol-A (BPA) at doses of either 10 or 100 µg/kg bw/d from day 9 to day 16 of gestation. Male OF-1 mice offspring was sacrificed at PND30. A summary of results reported in Tables 1–4 and Fig. 1 are depicted for supporting the discussion section. Abbreviations of key elements of the retinoid system are provided in alphabetical order within the following categories: **Retinoids:** all-*trans* RA, all-*trans* retinoic acid; 9c-4o-13,14dh-RA, 9-*cis*-4-oxo-13,14-dihydro-retinoic acid; REOH, retinol; REPA, retinyl palmitate (Table 1). **Storage and mobilization of retinoids:** CES1C, carboxylesterase 1C; CRBP1, cellular retinol binding protein 1; LRAT, lecithin:retinol acyltransferase (Table 2). **RA biosynthesis:** ADH1, alcohol dehydrogenase 1; ALDH1A1 and 1A2, aldehyde dehydrogenases 1A1 and 1A2; AOX1, aldehyde oxidase 1; CYP1A2 and 1B1, cytochromes P450 1A2 and 1B1 (Table 2). **RA biotransformation:** CRABP2, cellular retinoic acid binding protein II; CYP26A1, cytochrome P450 26A1; UGT1A1, 1A6, 1A9 and 2B35, UDP-glucuronosyltransferases 1A1, 1A6, 1A9 and 2B35 (Table 2). **Retinol carrier in the blood:** RBP4, Retinol binding protein 4 (Table 3). **Influx transport from blood to hepatocyte:** STRA6, Stimulated by retinoic acid gene 6; OATP2B1, solute carrier organic anion transporter (SLCO) 2B1 (Table 3). **Efflux transport from hepatocyte to blood:** MRPs 3 and 4, multidrug resistance-associated proteins 3 and 4, also known as ATP-binding cassette (ABC) transporters ABC C3 and ABC C4 (Table 3). **Biliary excretion:** BCRP, breast cancer resistance protein, also known as ABC G2; MDR1B1 multidrug resistance protein, also known as ABC B1; MRP2, also known as ABC C2 (Table 3). **Signalling:** FGF21, fibroblast growth factor 21; RAR α, β, γ, retinoic acid receptors α, β and γ; RXR α, β, γ, retinoid X receptors α, β and γ (Table 4, Fig. 1). Further descriptions of the key elements involved in the retinoid system along with references are provided in the 4. Discussion section. Statistical analysis: Changes were observed at 10 (dotted arrow), 100 (dashed arrow) or both 10 and 100 µg/kg bw/day (solid arrow). The sign of the given change is determined by the direction of the arrow, ie. up or down. Red colour is set when $p < 0.05$, whereas blue colour for tendencies with $p < 0.1$, ie. RBP4 and OATP2B1 in the influx and efflux transport category. * represent significant bivariate associations within the signalling category, ie. both RAR γ and RXR γ gene expressions with all-*trans*-retinoic acid concentrations and Fgf21 with RAR γ gene expressions.

9C4O13,14DHRA might reveal that the hepatic function was not severely affected as retinoids in the form of retinyl esters, being the main one REPA, are stored in healthy stellate cells of the liver (50–80% of all vitamin A in the body), whereas such levels are depleted after a morphological change of stellate cells towards myofibroblasts (Senoo et al., 2017). Such a consideration is supported by the absence of responses on 9C4O13,14DHRA, which shows a similar behaviour to REPA (Schmidt et al., 2002), and LRAT, the enzyme involved in the esterification of retinol into REPA (Senoo et al., 2017). However, it does not imply necessarily the absence of deleterious effects by BPA on the liver at longer time points. Similarly, metabolic disorders were apparent in old mice whereas young mice showed compensatory responses, such as fatty acid oxidation (Ke et al., 2016). Moreover, long-term changes with direct effects on mitochondria following perinatal exposure to BPA were apparent in livers of rats after several weeks of treatment (Xia et al., 2014).

Another factor which influences ATRA concentrations is the expression of a number of genes that code for transporters involved in the transport of glucuronide metabolites, since its oxidised metabolites are known to be conjugated with glucuronic acid generating both 4-oxo-all-*trans*- and 4-oxo-13-*cis*-retinoyl-β-glucuronide isomers by UDP-glucuronosyltransferases (UGTs) (Li et al., 1996).

The disposition of glucuronide metabolites was modulated towards biliary excretion on the basis of decreased expression of *Mrp3*, a sinusoidal transporter which is involved in the efflux transport from hepatocytes to blood, and increased expression of *Bcrp*, a canalicular transporter which is involved in the biliary excretion from hepatocyte to bile duct (Quesnot et al., 2014) (Fig. 2). The expression of both genes, belonging to the ABC transporter family, was increased in HepaRG cells and primary human hepatocytes after incubation with 5 µM ATRA for 48 h (Le Vee et al., 2013), whereas RXRα:RARα supressed *Mrp3* expression (Chen et al., 2007).

Additionally, *in vitro* studies reported differences in gene expression among the transporters mentioned herein in response to BPA exposure (Dankers et al., 2013; Hanet et al., 2008; Jin and Audus, 2005).

Furthermore, the expression of *Oatp2b1*, a SLCO transporter located in the sinusoidal surface of the hepatocytes which is involved in the influx transport from blood to hepatocyte, showed a tendency to increased levels (BPA100). Remarkably, such an increase is associated with the uptake of glucuronide conjugates (Fig. 2). Interestingly, *Oatp2b1* levels were supressed by knocking-down expression of RARα and RXRα (Le Vee et al., 2013).

Overall, the expression of transporter genes was modulated for the uptake of their substrates from the circulation (tendency to increased levels of *Oatp2b1*, $p < 0.1$), in order to minimize their efflux to the circulation (*Mrp3*), and to increase their excretion in the biliary tract (*Bcrp*), which might be involved in the reduction of glucuronide metabolites (Fig. 2). Such a modulation on the gene expression of

transporters of glucuronide metabolites might be also involved in the regulation of BPA levels. Yet, extrapolation of those results from animal studies to humans should be made with caution since location of transporters might differ between species (Mazur et al., 2012).

Taken together, the current work showed modulations on the retinoid system at PND30 following *in utero* exposure to environmentally relevant doses of BPA, supporting its role as a biomarker for the identification of EDCs (Manibusan and Touart, 2017). Remarkably, the experimental animal model used in the current study exhibit metabolic abnormalities in the long-term.

Acknowledgements

We are thankful to Jose Manuel Haro Moreno and Sergio Pomares Perez for their technical assistance. This work was supported by Ministerio de Economía y Competitividad, Agencia Estatal de Investigación and Fondo Europeo de Desarrollo Regional, EU Grants SAF2014-58335-P, BFU2017-86579-R and Generalitat Valenciana PROMETEO II/2015/016. Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas (CIBERDEM) is an initiative of the Instituto de Salud Carlos III.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.02.023.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.02.023.

References

Allenby, G., Bocquel, M.T., Saunders, M., Kazmer, S., Speck, J., Rosenberger, M., Lovey, A., Kastner, P., Grippo, J.F., Chambon, P., 1993. Retinoic acid receptors and retinoid X receptors: interactions with endogenous retinoic acids. Proc. Natl. Acad. Sci. 90, 30–34. https://doi.org/10.1073/pnas.90.1.30.

Alonso-Magdalena, P., Garcia-Arevalo, M., Quesada, I., Nadal, A., 2015. Bisphenol-A treatment during pregnancy in mice: a new window of susceptibility for the development of diabetes in mothers later in life. Endocrinology 156, 1659–1670. https://doi.org/10.1210/en.2014-1952.

Alonso-Magdalena, P., Vieira, E., Soriano, S., Menes, L., Burks, D., Quesada, I., Nadal, A., 2010. Bisphenol a exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environ. Health Perspect. 118, 1243–1250. https://doi.org/10.1289/ehp.1001993.

Amengual, J., Ribot, J., Bonet, M.L., Palou, A., 2010. Retinoic acid treatment enhances lipid oxidation and inhibits lipid biosynthesis capacities in the liver of mice. Cell. Physiol. Biochem. 25, 657–666. https://doi.org/10.1159/000315085.

Ashla, A.A., Hoshikawa, Y., Tsuchiya, H., Hashiguchi, K., Enjoji, M., Nakamuta, M., Taketomi, A., Maehara, Y., Shomori, K., Kurimasa, A., Hisatome, I., Ito, H., Shiota, G., 2010. Genetic analysis of expression profile involved in retinoid metabolism in non-alcoholic fatty liver disease. Hepatol. Res. 40, 594–604. https://doi.org/10.1111/j.1872-034X.2010.00646.x.

Badman, M.K., Pissios, P., Kennedy, A.R., Koukos, G., Flier, J.S., Maratos-Flier, E., 2007. Hepatic fibroblast growth factor 21 is regulated by PPAR alpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metabol. 5, 426–437. https://doi.org/10.1016/j.cmet.2007.05.002.

Balmer, J.E., Blomhoff, R., 2002. Gene expression regulation by retinoic acid. J. Lipid Res. 43, 1773–1808. https://doi.org/10.1194/jlr.RI00015-JLR200.

Bastien, J., Rochette-Egly, C., 2004. Nuclear retinoid receptors and the transcription of retinoid-target genes. Gene 328, 1–16. https://doi.org/10.1016/j.gene.2003.12.005.

Bemrah, N., Jean, J., Riviere, G., Sanaa, M., Leconte, S., Bachelot, M., Deceuninck, Y., Le Bizec, B., Dauchy, X., Roudot, A.C., Camel, V., Grob, K., Feidt, C., Picard-Hagen, N., Badot, P.M., Foures, F., Leblanc, J.C., 2014. Assessment of dietary exposure to bisphenol A in the French population with a special focus on risk characterisation for pregnant French women. Food Chem. Toxicol. 72, 90–97. https://doi.org/10.1016/j.fct.2014.07.005.

Bernier, M.R., Vandenberg, L.N., 2017. Handling of thermal paper: implications for dermal exposure to bisphenol A and its alternatives. PLoS One 12, 14. https://doi.org/10.1371/journal.pone.0178449.

Berry, D.C., Noy, N., 2009. All-trans-retinoic acid represses obesity and insulin resistance by activating both peroxisome proliferation-activated receptor beta/delta and retinoic acid receptor. Mol. Cell Biol. 29, 3286–3296. https://doi.org/10.1128/mcb.01742-08.

Berry, D.C., Noy, N., 2007. Is PPAR beta/delta a Retinoid Receptor? PPAR Res. 5. https://doi.org/10.1155/2007/73256.

Bolognesi, C., Castle, L., Cravedi, J.P., Engel, K.H., Fowler, P., Franz, R., Grob, K., Gurtler, R., Husoy, T., Mennes, W., Milana, M.R., Penninks, A., Roland, F., Silano, V., Smith, A., Pocas, M.D.T., Tlustos, C., Toldra, F., Wolfle, D., Zorn, H., Materials, E.P.F.C., 2015. Scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: PART II - Toxicological assessment and risk characterisation. Efsa J. 13, 621.

Cao, X.L., Zhang, J., Goodyer, C.G., Hayward, S., Cooke, G.M., Curran, I.H.A., 2012. Bisphenol A in human placental and fetal liver tissues collected from Greater Montreal area (Quebec) during 1998-2008. Chemosphere 89, 505–511. https://doi.org/10.1016/j.chemosphere.2012.05.003.

Chelstowska, S., Widjaja-Adhi, M.A.K., Silvaroli, J.A., Golczak, M., 2016. Molecular basis for vitamin A uptake and storage in vertebrates. Nutrients 8, 24. https://doi.org/10.3390/nu8110676.

Chen, H., Howald, W.N., Juchau, M.R., 2000. Biosynthesis of all-trans-retinoic acid from all-trans-retinol: catalysis of all-trans-retinol oxidation by human P-450 cytochromes. Drug Metab. Dispos. 28, 315–322.

Chen, W.S., Cai, S.Y., Xu, S.H., Denson, L.A., Soroka, C.J., Boyer, J.L., 2007. Nuclear receptors RXR alpha : RAR alpha are repressors for human MRP3 expression. Am. J. Physiol. Liver Physiol. 292, G1221–G1227. https://doi.org/10.1152/ajpgi.00191.2006.

Conaway, H.H., Henning, P., Lerner, U.H., 2013. Vitamin A metabolism, action, and role in skeletal homeostasis. Endocr. Rev. 34, 766–797. https://doi.org/10.1210/er.2012-1071.

Dankers, A.C.A., Roelofs, M.J.E., Piersma, A.H., Sweep, F., Russel, F.G.M., van den Berg, M., van Duursen, M.B.M., Masereeuw, R., 2013. Endocrine disruptors differentially target ATP-binding cassette transporters in the blood-testis barrier and affect leydig cell testosterone secretion in vitro. Toxicol. Sci. 136, 382–391. https://doi.org/10.1093/toxsci/kft198.

Dutchak, P.A., Katafuchi, T., Bookout, A.L., Choi, J.H., Yu, R.T., Mangelsdorf, D.J., Kliewer, S.A., 2012. Fibroblast growth factor-21 regulates PPAR gamma activity and the antidiabetic actions of thiazolidinediones. Cell 148, 556–567. https://doi.org/10.1016/j.cell.2011.11.062.

ECHA, 2017. Member State Committee Support Document For Identification Of 4,4'-Isopropylidenediphenol (Bpa, Bisphenol A) as a Substance Of Very High Concern Because of its Endocrine Disrupting Properties Which Cause Probable Serious Effects To Human Health Which Give [WWW Document]. https://echa.europa.eu/documents/10162/908badc9-e65d-3bae-933a-3512a9262e59, Accessed date: 25 January 2019.

EFSA, S.C., Hardy, A., Benford, D., Halldorsson, T., Jeger, M.J., Knutsen, K.H., More, S., Mortensen, A., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Silano, V., Solecki, R., Turck, D., Aerts, M., Bodin, L., Davis, A., Edler, L., Gundert-Remy, U., Sand, S., Slob, W., Bottex, B., Abrahantes, J.C., Marques, D.C., Kass, G., Schlatter, J.R., 2017. Update: use of the benchmark dose approach in risk assessment. Eur. Food Saf. Auth. J. 15, 41. https://doi.org/10.2903/j.efsa.2017.4658.

Evans, R.M., Mangelsdorf, D.J., 2014. Nuclear receptors, RXR, and the big bang. Cell 157, 255–266. https://doi.org/10.1016/j.cell.2014.03.012.

Garcia-Arevalo, M., Alonso-Magdalena, P., Dos Santos, J.R., Quesada, I., Carneiro, E.M., Nadal, A., 2014. Exposure to bisphenol-A during pregnancy partially mimics the effects of a high-fat diet altering glucose homeostasis and gene expression in adult male mice. PLoS One 9, 13. https://doi.org/10.1371/journal.pone.0100214.

Garcia-Arevalo, M., Alonso-Magdalena, P., Servitja, J.M., Boronat-Belda, T., Merino, B., Villar-Pazos, S., Medina-Gomez, G., Novials, A., Quesada, I., Nadal, A., 2016. Maternal exposure to bisphenol-A during pregnancy increases pancreatic beta-cell growth during early life in male mice offspring. Endocrinology 157, 4158–4171. https://doi.org/10.1210/en.2016-1390.

Geens, T., Aerts, D., Berthot, C., Bourguignon, J.P., Goeyens, L., Lecomte, P., Maghuin-Rogister, G., Pironnet, A.M., Pussemier, L., Scippo, M.L., Van Loco, J., Covaci, A., 2012. A review of dietary and non-dietary exposure to bisphenol-A. Food Chem. Toxicol. 50, 3725–3740. https://doi.org/10.1016/j.fct.2012.07.059.

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015. EDC-2: The endocrine society's second scientific statement on endocrine-disrupting chemicals. Endocr. Rev. 36, E1–E150. https://doi.org/10.1210/er.2015-1010.

Hanet, N., Lancon, A., Delmas, D., Jannin, B., Chagnon, M.C., Cherkaoui-Malki, M., Latruffe, N., Artur, Y., Heydel, J.M., 2008. Effects of endocrine disruptors on genes associated with 17 beta-estradiol metabolism and excretion. Steroids 73, 1242–1251. https://doi.org/10.1016/j.steroids.2008.06.005.

Hathcock, J.N., Hattan, D.G., Jenkins, M.Y., McDonald, J.T., Sundaresan, P.R., Wilkening, V.L., 1990. Evaluation of Vitamin-A toxicity. Am. J. Clin. Nutr. 52, 183–202.

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

Huang, R.P., Liu, Z.H., Yin, H., Dang, Z., Wu, P.X., Zhu, N.W., Lin, Z., 2018. Bisphenol A concentrations in human urine, human intakes across six continents, and annual trends of average intakes in adult and child populations worldwide: A thorough literature review. Sci. Total Environ. 626, 971–981. https://doi.org/10.1016/j.scitotenv.2018.01.144.

Inagaki, T., Dutchak, P., Zhao, G.X., Ding, X.S., Gautron, L., Parameswara, V., Li, Y., Goetz, R., Mohammadi, M., Esser, V., Elmquist, J.K., Gerard, R.D., Burgess, S.C., Hammer, R.E., Mangelsdorf, D.J., Kliewer, S.A., 2007. Endocrine regulation of the fasting response by PPAR alpha-mediated induction of fibroblast growth factor 21. Cell Metabol. 5, 415–425. https://doi.org/10.1016/j.cmet.2007.05.003.

Iwamuro, S., Yamada, M., Kato, M., Kikuyama, S., 2006. Effects of bisphenol A on thyroid

hormone-dependent up-regulation of thyroid hormone receptor alpha and beta down-regulation of retinoid X receptor gamma in Xenopus tail culture. Life Sci. 79, 2165–2171. https://doi.org/10.1016/j.lfs.2006.07.013.
Jin, H., Audus, K.L., 2005. Effect of bisphenol a on drug efflux in BeWo, a human trophoblast-like cell line. Placenta 26, S96–S103. https://doi.org/10.1016/j.placenta.2005.01.016.
Ke, Z.H., Pan, J.X., Jin, L.Y., Xu, H.Y., Yu, T.T., Ullah, K., Rahman, T.U., Ren, J., Cheng, Y., Dong, X.Y., Sheng, J.Z., Huang, H.F., 2016. Bisphenol a exposure may induce hepatic lipid accumulation via reprogramming the DNA methylation patterns of genes involved in lipid metabolism. Sci. Rep. 6, 13. https://doi.org/10.1038/srep31331.
Kedishvili, N.Y., 2013. Enzymology of retinoic acid biosynthesis and degradation. J. Lipid Res. 54, 1744–1760. https://doi.org/10.1194/jlr.R037028.
Le Vee, M., Jouan, E., Stieger, B., Fardel, O., 2013. Differential regulation of drug transporter expression by all-trans retinoic acid in hepatoma HepaRG cells and human hepatocytes. Eur. J. Pharm. Sci. 48, 767–774. https://doi.org/10.1016/j.ejps.2013.01.005.
Li, J., Ma, M., Wang, Z.J., 2008. A two-hybrid yeast assay to quantify the effects of xenobiotics on retinoid X receptor-mediated gene expression. Toxicol. Lett. 176, 198–206. https://doi.org/10.1016/j.toxlet.2007.11.006.
Li, S., Barua, A.B., Huselton, C.A., 1996. Quantification of retinoyl-beta-glucuronides in rat urine by reversed-phase high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B Biomed. Appl. 683, 155–162. https://doi.org/10.1016/0378-4347(96)00108-9.
Li, Y., Wong, K., Giles, A., Jiang, J.W., Lee, J.W., Adams, A.C., Kharitonenkov, A., Yang, Q., Gao, B., Guarente, L., Zang, M.W., 2014. Hepatic SIRT1 attenuates hepatic steatosis and controls energy balance in mice by inducing fibroblast growth factor 21. Gastroenterology 146, 539. https://doi.org/10.1053/j.gastro.2013.10.059.
Li, Y., Wong, K., Walsh, K., Gao, B., Zang, M.W., 2013. retinoic acid receptor beta stimulates hepatic induction of fibroblast growth factor 21 to promote fatty acid oxidation and control whole-body energy homeostasis in mice. J. Biol. Chem. 288, 10490–10504. https://doi.org/10.1074/jbc.M112.429852.
Liu, Y., Chen, H.G., Wang, J.J., Zhou, W.J., Sun, R.F., Xia, M., 2015. Association of serum retinoic acid with hepatic steatosis and liver injury in nonalcoholic fatty liver disease. Am. J. Clin. Nutr. 102, 130–137. https://doi.org/10.3945/ajcn.114.105155.
Lundasen, T., Hunt, M.C., Nilsson, L.M., Sanyal, S., Angelin, B., Alexson, S.E.H., Rudling, M., 2007. PPAR alpha is a key regulator of hepatic FGF21. Biochem. Biophys. Res. Commun. 360, 437–440. https://doi.org/10.1016/j.bbrc.2007.06.068.
Mahiout, S., Linden, J., Esteban, J., Sanchez-Perez, I., Sankari, S., Pettersson, L., Hakansson, H., Pohjanvirta, R., 2017. Toxicological characterisation of two novel selective aryl hydrocarbon receptor modulators in Sprague-Dawley rats. Toxicol. Appl. Pharmacol. 326, 54–65. https://doi.org/10.1016/j.taap.2017.04.020.
Manibusan, M.K., Touart, L.W., 2017. A comprehensive review of regulatory test methods for endocrine adverse health effects. Crit. Rev. Toxicol. 47, 433–481. https://doi.org/10.1080/10408444.2016.1272095.
Mascrez, B., Ghyselinck, N.B., Watanabe, M., Annicotte, J.S., Chambon, P., Auwerx, J., Mark, M., 2004. Ligand-dependent contribution of RXR beta to cholesterol homeostasis in Sertoli cells. EMBO Rep. 5, 285–290. https://doi.org/10.1038/sj.embor.7400094.
Mazur, C.S., Marchitti, S.A., Dimova, M., Kenneke, J.F., Lumen, A., Fisher, J., 2012. Human and rat ABC transporter efflux of bisphenol A and bisphenol A glucuronide: interspecies comparison and implications for pharmacokinetic assessment. Toxicol. Sci. 128, 317–325. https://doi.org/10.1093/toxsci/kfs167.
Mercogliano, R., Santonicola, S., 2018. Investigation on bisphenol A levels in human milk and dairy supply chain: A review. Food Chem. Toxicol. 114, 98–107. https://doi.org/10.1016/j.fct.2018.02.021.
Mic, F.A., Molotkov, A., Benbrook, D.M., Duester, G., 2003. Retinoid activation of retinoic acid receptor but not retinoid X receptor is sufficient to rescue lethal defect in retinoic acid synthesis. Proc. Natl. Acad. Sci. U.S.A. 100, 7135–7140. https://doi.org/10.1073/pnas.1231422100.
Muise, E.S., Azzolina, B., Kuo, D.W., El-Sherbeini, M., Tan, Y.J., Yuan, X.L., Mu, J., Thompson, J.R., Berger, J.P., Wong, K.K., 2008. Adipose fibroblast growth factor 21 is up-regulated by peroxisome proliferator-activated receptor gamma and altered metabolic states. Mol. Pharmacol. 74, 403–412. https://doi.org/10.1124/mol.108.044826.
Mukherjee, R., Davies, P.J.A., Crombie, D.L., Bischoff, E.D., Cesario, R.M., Jow, L., Hamann, L.G., Boehm, M.F., Mondon, C.E., Nadzan, A.M., Paterniti, J.R., Heyman, R.A., 1997. Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists. Nature 386, 407–410. https://doi.org/10.1038/386407a0.
Nishizawa, H., Manabe, N., Morita, M., Sugimoto, M., Imanishi, S., Miyamoto, H., 2003. Effects of In utero exposure to bisphenol A on expression of RAR alpha and RXR alpha mRNAs in murine embryos. J. Reprod. Dev. 49, 539–545. https://doi.org/10.1262/jrd.49.539.
Nishizawa, H., Morita, M., Sugimoto, M., Imanishi, S., Manabe, N., 2005. Effects of in utero exposure to bisphenol a on mRNA expression of arylhydrocarbon and retinoid receptors in murine embryos. J. Reprod. Dev. 51, 315–324. https://doi.org/10.1262/jrd.16008.
Novak, J., Benisek, M., Hilscherova, K., 2008. Disruption of retinoid transport, metabolism and signaling by environmental pollutants. Environ. Int. 34, 898–913. https://doi.org/10.1016/j.envint.2007.12.024.
NTP, 2018. NTP research report on the CLARITY-BPA core study: a perinatal and chronic extended-dose-range study of bisphenol A in rats. Natl. Toxicol. Progr. 9, 223.
Piersma, A.H., Hessel, E.V., Staal, Y.C., 2017. Retinoic acid in developmental toxicology: Teratogen, morphogen and biomarker. Reprod. Toxicol. 72, 53–61. https://doi.org/10.1016/j.reprotox.2017.05.014.
Quesnot, N., Bucher, S., Fromenty, B., Robin, M.A., 2014. Modulation of metabolizing enzymes by bisphenol A in human and animal models. Chem. Res. Toxicol. 27, 1463–1473. https://doi.org/10.1021/TX500087p.
Rhee, E.-J., Plutzky, J., 2012. Retinoid metabolism and diabetes mellitus. Diabetes Metab. J. 36, 167–180. https://doi.org/10.4093/dmj.2012.36.3.167.
Rhinn, M., Dolle, P., 2012. Retinoic acid signalling during development. Development 139, 843–858. https://doi.org/10.1242/dev.065938.
Sarigiannis, D.A., Karakitsios, S.P., Handakas, E., Simou, K., Solomou, E., Gotti, A., 2016. Integrated exposure and risk characterization of bisphenol-A in Europe. Food Chem. Toxicol. 98, 134–147. https://doi.org/10.1016/j.fct.2016.10.017.
Schmidt, C.K., Brouwer, A., Nau, H., 2003. Chromatographic analysis of endogenous retinoids in tissues and serum. Anal. Biochem. 315, 36–48. https://doi.org/10.1016/s0003-2697(02)00662-0.
Schmidt, C.K., Volland, J., Hamscher, G., Nau, H., 2002. Characterization of a new endogenous vitamin A metabolite. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1583, 237–251. https://doi.org/10.1016/s1388-1981(02)00212-3.
Schuchardt, J.P., Wahlstrom, D., Ruegg, J., Giese, N., Stefan, M., Hopf, H., Pongratz, I., Hakansson, H., Eichele, G., Pettersson, K., Nau, H., 2009. The endogenous retinoid metabolite S-4-oxo-9-cis-13,14-dihydro-retinoic acid activates retinoic acid receptor signalling both in vitro and in vivo. FEBS J. 276, 3043–3059. https://doi.org/10.1111/j.1742-4658.2009.07023.x.
Senoo, H., Mezaki, Y., Fujiwara, M., 2017. The stellate cell system (vitamin A-storing cell system). Anat. Sci. Int. 92, 387–455. https://doi.org/10.1007/s12565-017-0395-9.
Shaw, N., Elholm, M., Noy, N., 2003. Retinoic acid is a high affinity selective ligand for the peroxisome proliferator-activated receptor beta/delta. J. Biol. Chem. 278, 41589–41592. https://doi.org/10.1074/jbc.C300368200.
Shmarakov, I.O., 2015. Retinoid-xenobiotic interactions: the Ying and the Yang. Hepatobiliary Surg. Nutr. 4, 243–267. https://doi.org/10.3978/j.issn.2304-3881.2015.05.05.
Shmarakov, I.O., Borschovetska, V.L., Blaner, W.S., 2017. Hepatic detoxification of bisphenol A is retinoid-dependent. Toxicol. Sci. 157, 141–155. https://doi.org/10.1093/toxsci/kfx022.
Shmarakov, I.O., Borschovetska, V.L., Ivanishchuk, L.P., Marchenko, M.M., 2016. Hepatotoxicity of bisphenol A under conditions of differential supplementation with retinoids. Ukrainian Biochem. J. 88, 99–105. https://doi.org/10.15407/ubj88.03.099.
Taylor, J.A., vom Saal, F.S., Welshons, W.V., Drury, B., Rottinghaus, G., Hunt, P.A., Toutain, P.L., Laffont, C.M., VandeVoort, C.A., 2011. Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. Environ. Health Perspect. 119, 422–430. https://doi.org/10.1289/ehp.1002514.
Theodosiou, M., Laudet, V., Schubert, M., 2010. From carrot to clinic: an overview of the retinoic acid signaling pathway. Cell. Mol. Life Sci. 67, 1423–1445. https://doi.org/10.1007/s00018-010-0268-z.
Tomita, S., Okuyama, E., Ohnishi, T., Ichikawa, Y., 1996. Characteristic properties of a retinoic acid synthetic cytochrome P-450 purified from liver microsomes of 3-methylcholanthrene-induced rat. Biochim. Biophys. Acta Gen. Subj. 1290, 273–281. https://doi.org/10.1016/0304-4165(96)00030-x.
Tonk, E.C.M., Pennings, J.L.A., Piersma, A.H., 2015. An adverse outcome pathway framework for neural tube and axial defects mediated by modulation of retinoic acid homeostasis. Reprod. Toxicol. 55, 104–113. https://doi.org/10.1016/j.reprotox.2014.10.008.
Trasino, S.E., Tang, X.H., Jessurun, J., Gudas, L.J., 2016. Retinoic acid receptor beta 2 agonists restore glycaemic control in diabetes and reduce steatosis. Diabetes Obes. Metab. 18, 142–151. https://doi.org/10.1111/dom.12590.
USEPA, 1988. Integrated Risk Information System (IRIS). Bisphenol A; CASRN 80-05-7 [WWW Document]. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0356_summary.pdf, Accessed date: 24 January 2019.
Vandenberg, L.N., Chahoud, I., Heindel, J.J., Padmanabhan, V., Paumgartten, F.J.R., Schoenfelder, G., 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ. Health Perspect. 118, 1055–1070. https://doi.org/10.1289/ehp.0901716.
Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Lee, D.H., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W.V., Zoeller, R.T., Myers, J.P., 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr. Rev. 33, 378–455. https://doi.org/10.1210/er.2011-1050.
Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W.V., 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 24, 139–177. https://doi.org/10.1016/j.reprotox.2007.07.010.
Vandenberg, L.N., Welshons, W.V., vom Saal, F.S., Toutain, P.L., Myers, J.P., 2014. Should oral gavage be abandoned in toxicity testing of endocrine disruptors? Environ. Health 13, 7. https://doi.org/10.1186/1476-069x-13-46.
Vaz, B., de Lera, A.R., 2012. Advances in drug design with RXR modulators. Expert Opin. Drug Discov. 7, 1003–1016. https://doi.org/10.1517/17460441.2012.722992.
vom Saal, F.S., VandeVoort, C.A., Taylor, J.A., Welshons, W.V., Toutain, P.L., Hunt, P.A., 2014. Bisphenol A (BPA) pharmacokinetics with daily oral bolus or continuous exposure via silastic capsules in pregnant rhesus monkeys: Relevance for human exposures. Reprod. Toxicol. 45, 105–116. https://doi.org/10.1016/j.reprotox.2014.01.007.
Wiseman, E.M., Bar-El Dadon, S., Reifen, R., 2017. The vicious cycle of vitamin a deficiency: A review. Crit. Rev. Food Sci. Nutr. 57, 3703–3714. https://doi.org/10.1080/10408398.2016.1160362.
Wolf, G., 2010. Retinoic acid activation of peroxisome proliferation-activated receptor delta represses obesity and insulin resistance. Nutr. Rev. 68, 67–70. https://doi.org/10.1111/j.1753-4887.2009.00261.x.
Xia, W., Jiang, Y., Li, Y.Y., Wan, Y.J., Liu, J., Ma, Y., Mao, Z.X., Chang, H.L., Li, G.Q., Xu, B., Chen, X., Xu, S.Q., 2014. Early-life exposure to bisphenol a induces liver injury in rats involvement of mitochondria-mediated apoptosis. PLoS One 9, 9. https://doi.

org/10.1371/journal.pone.0090443.
Xu, J., Lloyd, D.J., Hale, C., Stanislaus, S., Chen, M., Sivits, G., Vonderfecht, S., Hecht, R., Li, Y.S., Lindberg, R.A., Chen, J.L., Jung, D.Y., Zhang, Z.Y., Ko, H.J., Kim, J.K., Veniant, M.M., 2009. Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. Diabetes 58, 250–259. https://doi.org/10.2337/db08-0392.
Yagmur, E., Trautwein, C., Weiskirchen, R., Tacke, F., Gressner, A.M., 2007. Insulin resistance in liver cirrhosis is not associated with circulating retinol-binding protein 4. Diabetes Care 30, 1168–1172. https://doi.org/10.2337/dc06-2323.
Yanagitani, A., Yamada, S., Yasui, S., Shimomura, T., Murai, R., Murawaki, Y., Hashiguchi, K., Kanbe, T., Saeki, T., Ichiba, M., Tanabe, Y., Yoshida, Y., Morino, S., Kurimasa, A., Usuda, N., Yamazaki, H., Kunisada, T., Ito, H., Shiota, G., 2004. Retinoic acid receptor alpha dominant negative form causes steatohepatitis and liver tumors in transgenic mice. Hepatology 40, 366–375. https://doi.org/10.1002/hep.20335.
Zhang, Q.Y., Dunbar, D., Kaminsky, L., 2000. Human cytochrome P-450 metabolism of retinals to retinoic acids. Drug Metab. Dispos. 28, 292–297.