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Research Article

Transient exposure to bisphenol F in early life affects the metabolic health of adults

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Abstract

Although bisphenol F (BPF) is widely used in plastic products, there are concerns about its potential health risks. Here, we aimed to understand the long-term effects of a brief BPF exposure during development. We treated zebrafish larvae 7 days post-fertilization with 1 mg/L BPF for 48 h. We then maintained the animals under standard conditions for 5 months and compared them to control adults never exposed to BPF. In addition to sequencing the gut microbiome, we profiled six different tissues and serum by metabolomics and lipidomics. Strikingly, we found widespread alterations in metabolites and lipids throughout the animal that were both sex and tissue specific. For example, over 60 lipid species were depleted in the livers of BPF-treated females but no changes were observed in male livers. At the tissue level, BPF treatment altered fatty acid oxidation uniquely in skeletal and cardiac muscle. This study shows that transient exposures limited to the developmental phase of life can induce metabolic abnormalities later in adulthood. Our findings highlight the importance of profiling specimens from early life by exposomics and suggest that, even with the introduction of regulatory measures, the adverse effects of BPF could persist in the population for a generation.

Key words: exposomics; mass spectrometry; metabolomics; lipidomics; zebrafish.

Lay summary Limited data are available on the long-term effects of bisphenol F. We show that a brief exposure in early life adversely affects adult animals. This raises concerns about population health as the first children exposed to bisphenol F grow older.

Introduction

Bisphenol A (BPA) is a well-known endocrine disruptor that adversely affects human health. Increasing evidence indicates that even low concentrations of BPA can act on hormonally mediated pathways to disrupt growth and development in children.[1] Given these concerns, BPA regulations have increased around the world and BPA use in infant products has been banned in the US and Europe.[2] Unfortunately, increased regulatory actions and growing public concern have led to the development of BPA substitutes such as bisphenol F (BPF), which is now in widespread use. In 2014, BPF was measured in the urine of nearly 70% of individuals sampled from the US population.[3]

Like BPA, BPF is used in the manufacturing of plastic products such as food containers, water bottles, toys, and medical devices.[4,5] It is also used to produce epoxy resins that coat metal food cans, bottle tops, and water supply pipes.[6] During the production of plastic and epoxy materials, BPF and other chemicals are polymerized, but the process is not complete. Over time, particularly in the presence of heat and other stresses, polymers can also be degraded. The unbound monomer BPF molecules in the material are released into surrounding substances, including food and beverages that are consumed by humans.[7] Although marketed as "BPA free" to give the impression of safety, the only difference between BPF and BPA is two methyl groups. Considering the high degree of structural similarity, it is not surprising that BPA and BPF have similar biochemical activities.[8] The number of studies examining the effects of BPF remain relatively limited, but emerging *in vitro* and *in vivo* data suggest that BPF adversely impacts cellular and physiological functions through mechanisms that are similar to BPA. Thus far, BPF has been associated with a number of deleterious effects including developmental abnormalities, oxidative stress, inflammation, obesity, immunotoxicity, endocrine disruption, and neurotoxicity.[9]

The questions raised about the safety profile of BPF have led to calls for its regulation, especially in children's products.[10] Already, some regulatory measures have been introduced in both

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the US and Europe that include compounds structurally related to BPA.[11-13] As measurement technologies and exposomics approaches continue to advance, our understanding of which chemicals are hazardous to human health will only progress. It is inspiring to imagine that improved knowledge will lead to more regulatory controls of chemicals such as BPF.[14] A major problem, however, is that millions of people have already been exposed to the chemicals in use today. Future bans against the use of BPF cannot reverse previous exposures. Hence, there is an important need to characterize the biochemical imprint of chemicals beyond an exposure period.

In this work, we aimed to understand whether temporary exposure to BPF in early life leads to long-lasting effects that persist into adulthood. Zebrafish larvae were exposed to BPF for a period of 48 h. We then maintained the animals under standard, BPF-free conditions for 5 months and evaluated their metabolic health with metabolomics. Strikingly, compared to vehicle-treated controls, there were system-wide changes in the metabolites and lipids of BPF-treated animals that spanned the brain, eye, liver, heart, muscle, fin, and serum. These findings suggest that, irrespective of future regulatory measures, historic exposure to BPF over the past two decades might lead to an increase in metabolic disease as infants born during this time age into adults.

Materials and methods

Animal husbandry and BPF exposure

Experiments were carried out in accordance with Washington University Institutional Animal Care and Use Committee (IACUC) regulations (protocol 24-0312). All experiments were conducted with an inbred AB strain of zebrafish, maintained according to standard husbandry practices (28 ± 1°C, 14:10 light: dark cycle).[15] Zebrafish larvae 7 days post-fertilization (dpf) were transferred to six-well plates in facility water. Half of the larvae were exposed to BPF for 48 h, while the other half served as controls. We treated zebrafish with BPF at 7 dpf because these animals are no longer embryos but rather larvae with fully developed organs. Moreover, prior to 7 dpf, metabolic detoxification systems are not yet fully activated.[16,17] A limitation of our study design is that we only investigated animals 7 dpf. Other periods of development may have different sensitivities to BPF exposure. Larvae were treated with 1 mg/L BPF for 48 h. This exposure concentration was selected based on prior studies and the LC_{50} value reported previously, which was 8.93 mg/L over 48 h.[18,19] Future work might benefit from the testing of additional concentrations. Larvae were fed rotifers starting at 5 dpf but were not fed during the 48 h of BPF treatment. After BPF exposure, fish were raised for 5 months following normal husbandry practices without any further exposure. Adult zebrafish were first anesthetized in a beaker containing 100 mL of facility water at 17 °C. The beaker was placed in a shallow ice bath, and the temperature lowered to 12 °C, when stage 3, phase 2 anesthesia was reached. Adult zebrafish were subsequently euthanized by maintaining the animals in beakers in a shallow ice bath for at least 10 min.

In total, 25 fish were used (14 control, 11 treated). Intestines from all fish were used to assess the microbiome. Of the total 25 fish, 2 were used for method optimization and 23 were used for LC/MS-based metabolomics. The 23 fish used for metabolomics included 12 control fish (7 males, 5 females) and 11 treated fish (5 males, 6 females). BPF-treated larvae were collected from two independent experiments, using group spawning. The two experiments were performed nearly a year apart from one another. The first experiment consisted of 11 fish (6 control and 5 treated), and the second experiment consisted of 12 fish (6 control and 6 treated).

To assess the amount of BPF taken up by animals during the exposure period, we treated larvae 7 dpf with BPF at 1 mg/L for 48 h. We then harvested the larvae at 9 dpf and quantitated the concentration of BPF in μg per gram of larval weight (Figure S1). Larvae were euthanized in a similar manner to adults, except that they were placed on ice for 30 minutes. The samples were analyzed as four biological replicates, each containing 14-15 larvae.

Sample harvest

Blood was collected from anesthetized fish by using centrifugation, as previously described.[20] Briefly, a scalpel was used to excise the caudal fin and some attached muscle before immediately placing the fish in a 1.5 mL centrifuge tube with a small hole in the bottom. The 1.5 mL centrifuge tube was attached to a 0.5 mL centrifuge tube, which served as the collection tube for blood. Zebrafish were centrifuged at 40 g for 1 min at 15 °C. Blood in the collection tube was allowed to clot on ice for 10 min prior to centrifugation at 1600 x g for 10 min at 4 °C to separate serum. Serum was then pipetted into a new 0.5 mL centrifuge tube and snap frozen in liquid nitrogen. Organs (brain, eye, fin, heart, liver, and muscle) were harvested from fish and snap frozen in pre-weighed 1.5 mL centrifuge tubes. Table S1 provides a summary of the number of organs and serum samples included in the study. Figure S2 provides fish weight and liver weight for male and female fish. All samples were kept at −80 °C until metabolites and lipids were extracted as described below. Fish facility water was assessed for trace levels of BPF.

Metabolite and lipid extraction

Polar metabolites were extracted as previously described.[20] In brief, serum samples were treated with methanol:acetonitrile: water (2:2:1, v/v/v) at a ratio of 1 μL serum per 15 μL of methanol: acetonitrile:water. Samples were incubated at −20 °C for 1 h before centrifuging (20,000 x g for 10 min at 4°C). Supernatants were transferred to LC vials for analysis. Organ samples were homogenized in their pre-weighed 1.5 mL centrifuge tubes by using a disposable pestle and liquid nitrogen. For each 1 mg of frozen tissue, 40 μL of methanol:acetonitrile:water was added. Homogenized organs were subjected to two freeze-thaw cycles that included sonicating for 5 min, briefly vortexing, and snap freezing in liquid nitrogen. Samples were then incubated at −20°C for 1 h before centrifuging (20,000 x g for 10 min at 4°C). Supernatants were transferred to LC vials for polar metabolite analysis. Lipids were extracted from the remaining pellets by adding 15 μL isopropanol (IPA) for every 1 μL serum and 40 μL IPA for every 1 mg of organ tissue. Samples were then extracted with two rounds of vortexing and sonicating before they were incubated at −20°C for 1 h. Following incubation, samples were centrifuged at 20,000 x g for 10 min while maintained at 4°C. The extracts from methanol/acetonitrile/water and IPA were mixed in LC vials at a 1:1 ratio for lipid LC/MS analysis.

To assess the amount of BPF taken up over 48 h, pooled larvae were analyzed immediately after treatment with BPF. BPF was extracted from the pooled larvae by adding 200 μL of acetonitrile to each tube, followed by sonication for 5 min, vortexing for 10 min, and centrifugation (20,000 x g for 10 min at 4°C). The supernatant was then collected for LC/MS measurement.

16S sequencing

For 16S sequencing, intestines were isolated from euthanized fish and kept frozen at −80°C until DNA isolation. DNA was isolated by using the Qiagen PowerFecal Pro DNA kit following the manufacturer's guidelines. Sequencing was performed by the Genome Technology Access Center at Washington University in St Louis following previously described protocols.[21]

LC/MS analysis

Metabolite profiling was performed by using hydrophilic interaction liquid chromatography (HILIC) on a Vanquish Horizon UHPLC system interfaced with an Orbitrap ID-X Tribrid mass spectrometer (Thermo Scientific). Metabolites were separated with an iHILIC-(p)- classic column (100 mm × 2.1 mm, 5 μm, HILICON) at 40°C by using a 22-min gradient. Mobile phase A was 20 mM ammonium hydrogencarbonate, 0.1% ammonium hydroxide solution (25% ammonia in water), and 5 μM medronic acid in water:acetonitrile (95:5), and mobile phase B was acetonitrile and water (95:5). We applied the following linear gradient at a flow rate of 0.25 mL/min: 0–1 min, 90% B; 12 min, 35% B; 12.5–14.5 min, 25% B; 15–22 min, 90% B. The flow rate was increased to 0.4 mL/min from 16.5 to 20 min. Data were collected in polarity-switching mode with the following MS source settings: spray voltage, 2.8 kV (neg) or 3.5 kV (pos); sheath gas, 35; auxiliary gas, 10; sweep gas, 1; ion transfer tube temperature, 250 °C; vaporizer temperature, 300 °C; mass range, 67–900 Da; resolution, 120,000 (MS1), 15,000 (MS2); AGC target, 2e5 (MS1), 2.5e4 (MS2); maximum injection time, 100 ms (MS1), 40 ms (MS2); isolation window, 1.5 Da (MS2).

Lipidomics was performed by using a 1290 Infinity II UHPLC coupled to a 6495C triple quadrupole mass spectrometer (Agilent Technologies). The lipid extract was separated with a ZORBAX Eclipse Plus reversed-phase column (2.1 × 100 mm, 1.8 μm, Agilent Technologies) at 45°C. Mobile phase A was 10 mM ammonium formate and 5 μM deactivator additive (p/n 5191-3940, Agilent Technologies) in water:acetonitrile:isopropanol (5:3:2). Mobile phase B was 10 mM ammonium formate in water:acetonitrile:isopropanol (1:9:90). Both mobile phases were sonicated to ensure the solubility of buffers. The following linear gradient was applied at a flow rate of 0.4 mL/min: 0 min 15% B, 2.5 min 50% B, 2.6 min 57% B, 9 min 70% B, 9.1 min 93% B, 11 min 96% B, 11.1 min 100% B, 12 min 100% B, 12.2–16 min 15% B. A dual jet stream electrospray ionization source was used with the following parameters: gas temp 150 °C, gas flow 17 L/min, nebulizer pressure 20 psi, sheath gas temp 200 °C, sheath gas flow 10 L/min, capillary voltage 3.5 kV (positive mode), capillary voltage 3.0 kV (negative mode), and nozzle voltage 1000 V. Data were acquired in dynamic multiple reaction monitoring (dMRM) mode. All samples were analyzed in a randomized order.

Details on sample preparation and LC/MS methods for the measurement of BPF in zebrafish facility water are provided in the Supplementary Material (see Supplementary Methods and Figure S3).

Data processing and statistical analysis

Data processing was performed as previously described.[22] In brief, polar metabolites were identified by matching accurate mass, MS/MS data, and retention times to our in-house library created from authentic reference standards. Identifications are level 1 according to the Metabolomics Standard Initiative.[23] Lipid profiling was achieved by using a previously established dMRM method.[24] Lipid nomenclature was based on the LIPID MAPS consortium guidelines.[25] All metabolites and lipids were then analyzed in Skyline (20.1.0.155). Peak shapes and retention times were manually verified. For each experiment, metabolite and lipid peak areas were normalized to the median peak area of all samples in that batch. Statistical significance was determined with a Mann-Whitney U test (unpaired) by using Agilent Mass Profiler Professional (15.0). Fold changes, *p*-values, and FDR-corrected *p*-values are included in Table S3.

Results and discussion

Workflow to assess the metabolic effects of transient exposure

Over the past couple of decades, there has been a growing appreciation for the potentially deleterious effects of environmental chemicals on human health.[26] Metabolomics and lipidomics have become key technologies to evaluate the biochemical response of model systems to environmental chemicals. To date, however, metabolomics and lipidomics analyses have primarily been performed immediately after chemical exposure. In some experiments, exposure time is relatively short, on the order of minutes to hours.[27,28] In other studies, exposure times are extended to weeks or months.[29,30] Nonetheless, neither experimental design addresses an important question in environmental health: can a transient exposure in early life yield long-lasting effects that influence the metabolic health of adults?

Here, we exposed zebrafish larvae to 1 mg/L BPF for 48 h. At the end of the exposure period, BPF was present at ∼25 μg per gram of tissue (Figure S1). The dosing was designed to simulate a high situational exposure that occurs over a short duration. For the next 5 months, the animals were then maintained under standard conditions without any further exposure to BPF (Figure 1A). We validated that standard facility water did not contain trace levels of BPF, confirming that there was no exposure to BPF beyond the defined 48 h window (Figure S3C). In parallel to the BPF arm of experiments, control larvae were exposed to a vehicle treatment and allowed to develop into adults. Five months after BPF or vehicle treatment, adult fish were evaluated by metabolomics and lipidomics. In addition to serum, we examined brain, eye, liver, heart, muscle, and fin tissue (Figure 1B). Although adult zebrafish have a low blood volume and relatively small organs, using previously established protocols,[20,31] we were able to profile metabolites and lipids from the specimens of individual animals.

To analyze polar metabolites and lipids from the same specimens, we adopted a sequential two-step extraction (Figure 1C). First, polar metabolites were extracted by using a 2:2:1 mixture of acetonitrile, methanol, and water. Second, the remaining non-solubilized material was treated with ice-cold IPA to extract non-polar lipids. Polar metabolites were analyzed from the supernatant of the acetonitrile, methanol, and water mixture. Lipids were analyzed from both the supernatant of the acetonitrile, methanol, and water mixture as well as the supernatant from the IPA mixture. For the latter, the supernatants were mixed at a one-to-one ratio, which provided comprehensive coverage of polar lipid classes such as acylcarnitines and lyso-phospholipids as well as non-polar lipids such as triglycerides and cholesterol esters. We note that we were unable to detect BPF from the serum or tissue of adult zebrafish (Figure S4). These results indicate that BPF is cleared from the animals over the course of five months, without accumulating in any tissue. The metabolic alterations we observe are therefore not due to persistent BPF but instead a result of the long-term effects of transient BPF exposure during larval development.

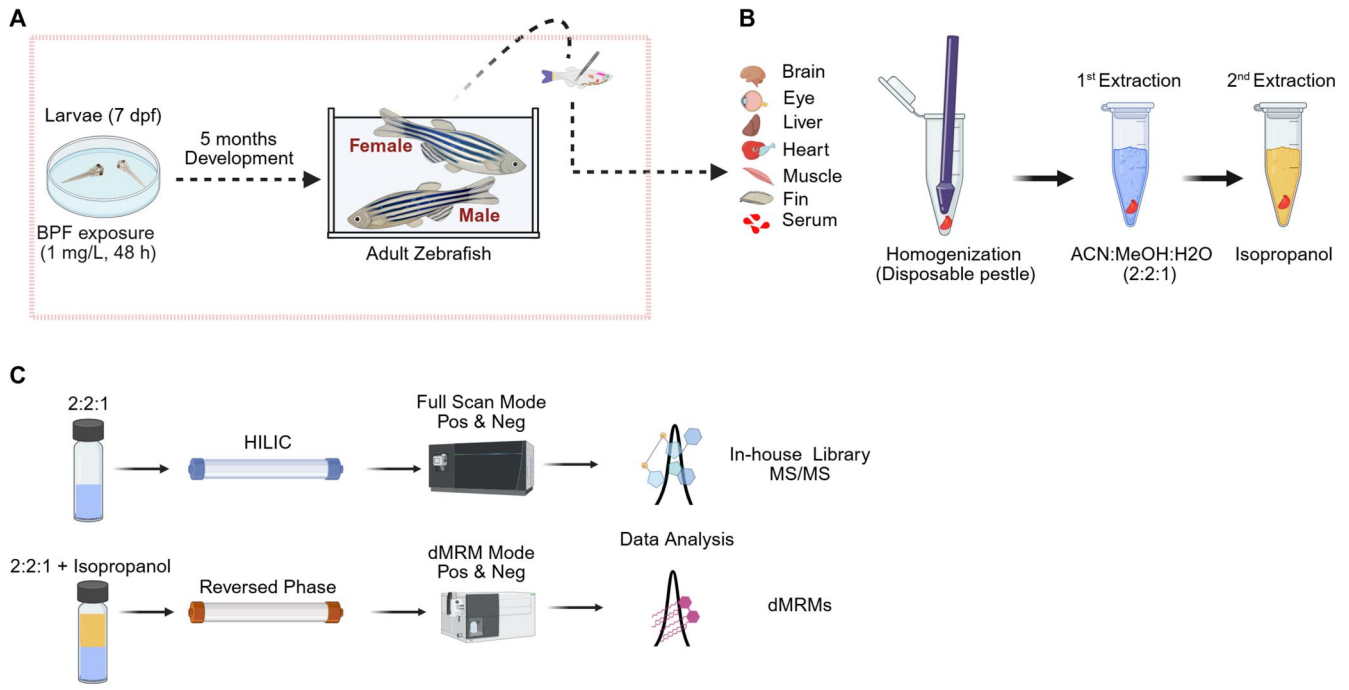

Figure 1. Workflow to evaluate metabolic changes due to a transient exposure of BPF during the developmental phase of life. (A) Zebrafish larvae (7 dpf) were transiently exposed to BPF (1 mg/L, 48 h) and then maintained in standard tank water without BPF for 5 months. (B) Brain, eye, liver, fin, heart, and muscle tissue as well as serum were subjected to two sequential extractions. The first extraction was performed with 2:2:1 (acetonitrile:methanol: water), followed by a second extraction with isopropanol (100%). (C) Polar metabolites were profiled from the 2:2:1 extract by using hydrophilic interaction liquid chromatography (HILIC) coupled to high-resolution mass spectrometry. Lipids were profiled from a 1:1 mixture of the 2:2:1 and isopropanol extracts by using reversed-phase (RP) liquid chromatography coupled to triple quadrupole mass spectrometry. Created with BioRender.com.

Transient BPF exposure causes long-term alterations in metabolites and lipids

Our data show that a 48-hour exposure to BPF during the early developmental phase of zebrafish life is sufficient to cause system-wide alterations in the metabolism of adult animals (Figures 2 and 3). Interestingly, some metabolic changes are organ specific while others are shared across the animal. A subset of BPF-induced alterations only occurs in females or males (Figures 2 and 3). The latter is notable because larvae were exposed to BPF from 7 to 9 dpf, prior to gonad development and sex differentiation.[32] These results indicate that the effects of transient BPF exposure uniquely influence the metabolism of females and males.

To the best of our knowledge, the results presented herein represent the first comprehensive assessment of metabolites and lipids that are altered in response to BPF at the individual organ level of zebrafish. To advance our understanding of the mode of action of BPF, all of the data have been made publicly available as a resource for the environmental health community. Given the large quantity of data collected, it is not practical to discuss each of the altered metabolites and lipids across every tissue. Instead, we highlight three patterns of BPF-induced changes from the liver, muscle, and brain that are most interesting in the context of prior work.

Hepatic imprint of transient BPF exposure is sex specific

We first focused on the liver because it is considered to be one of the main target organs for bisphenols.[33-36] Indeed, a number of studies have demonstrated that weeks to months of exposure to BPF disrupts hepatic lipid metabolism.[37] Unlike prior analyses where animals were administered BPF for prolonged periods of time, which ultimately led to the accumulation of hepatic lipids and steatotic liver disease,[36,38,39] our zebrafish were only exposed to BPF for 48 h. Additionally, instead of evaluating the liver lipidome immediately after exposure to BPF, we waited 5 months. Although we still observed substantial changes in liver lipids (Figure 3), the directionality of the alterations was opposite compared to prior studies. Rather than lipids accumulating, nearly all of the disrupted lipids had decreased concentration due to BPF. While additional work is needed to understand the mechanisms underlying the disparity in lipid trends, prior research has suggested that BPF exposure has two opposing effects.[39] On the one hand, it stimulates autophagy and thereby activates lipid degradation. On the other hand, it impairs the breakdown of lipid droplets by disrupting the pH of lysosomes. During chronic BPF exposure, the effects of the latter overshadow the effects of the former and cause lipids to accumulate in the liver. It is possible, however, that removal of BPF after a transient exposure restores the pH of lysosomes but that the signaling pathways activated by BPF remain stimulated and therefore ultimately cause a decrease in lipids. A pathway analysis using our metabolite and lipid profiling data supports this idea (Table S4). It suggests that retinoic acid-related orphan receptor α signaling processes, which have been linked to lysosomal acidification and autophagic flux, are highly enriched in the liver of BPF-exposed animals.[40]

It is interesting to consider why the decrease in lipids is more prominent in female livers compared to male livers. The liver is one of the most sexually dimorphic organs. The physiological basis of this difference is driven by the unique metabolic needs of female and male livers, many of which are associated with reproductive functions. For oviparous animals such as zebrafish in particular, the female liver must synthesize a large quantity of biomass for the egg yolk, including protein and lipids.[41] The high abundance of lipids in female liver might provide more substrate

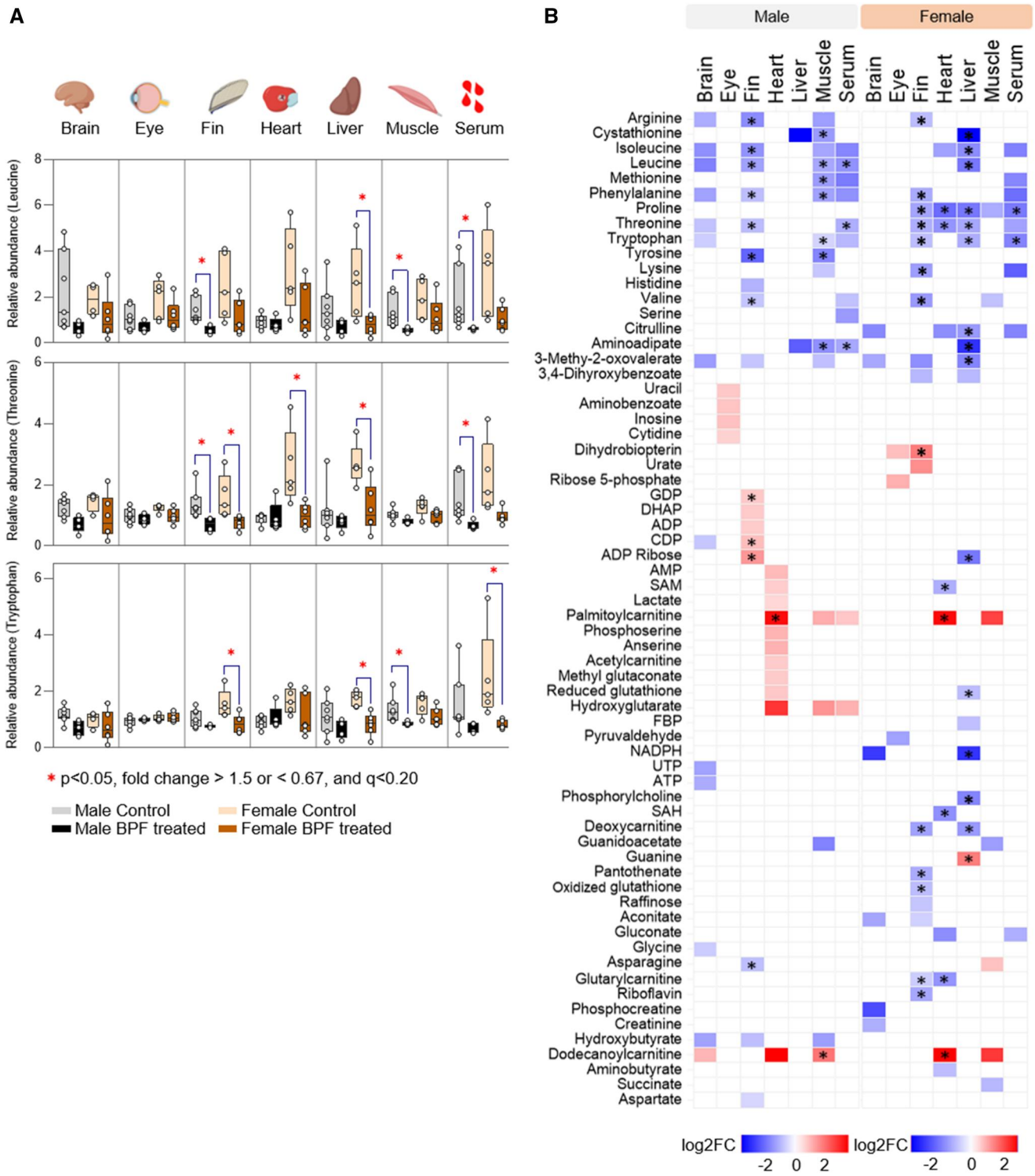

Figure 2. Transient exposure of zebrafish larvae to BPF caused system-wide perturbations in adult metabolites that were specific to sex and tissue. (A) Box plots show relative levels of leucine, threonine, and tryptophan. (B) Heat map shows fold change (treated/control) for selected metabolites across organs for male and female fish. The experiment compared 12 control animals (7 males, 5 females) to 11 treated animals (5 males, 6 females). Metabolites that decrease as a result of BPF exposure have negative log2FC values and metabolites that increase following BPF exposure have positive log2FC values. For clarity, metabolites having a *p*-value greater than 0.05 or a fold change between 0.67 and 1.5 are displayed without color on the heatmap. Metabolites with a *q*-value <0.20 are indicated by an asterisk. *P*-values were calculated by using a Mann Whitney *U*-test (unpaired). Q-values were calculated by using the Benjamini-Hochberg Procedure to control for false discovery rate. Fold changes, *p*-values, and *q*-values are provided in Table S3.

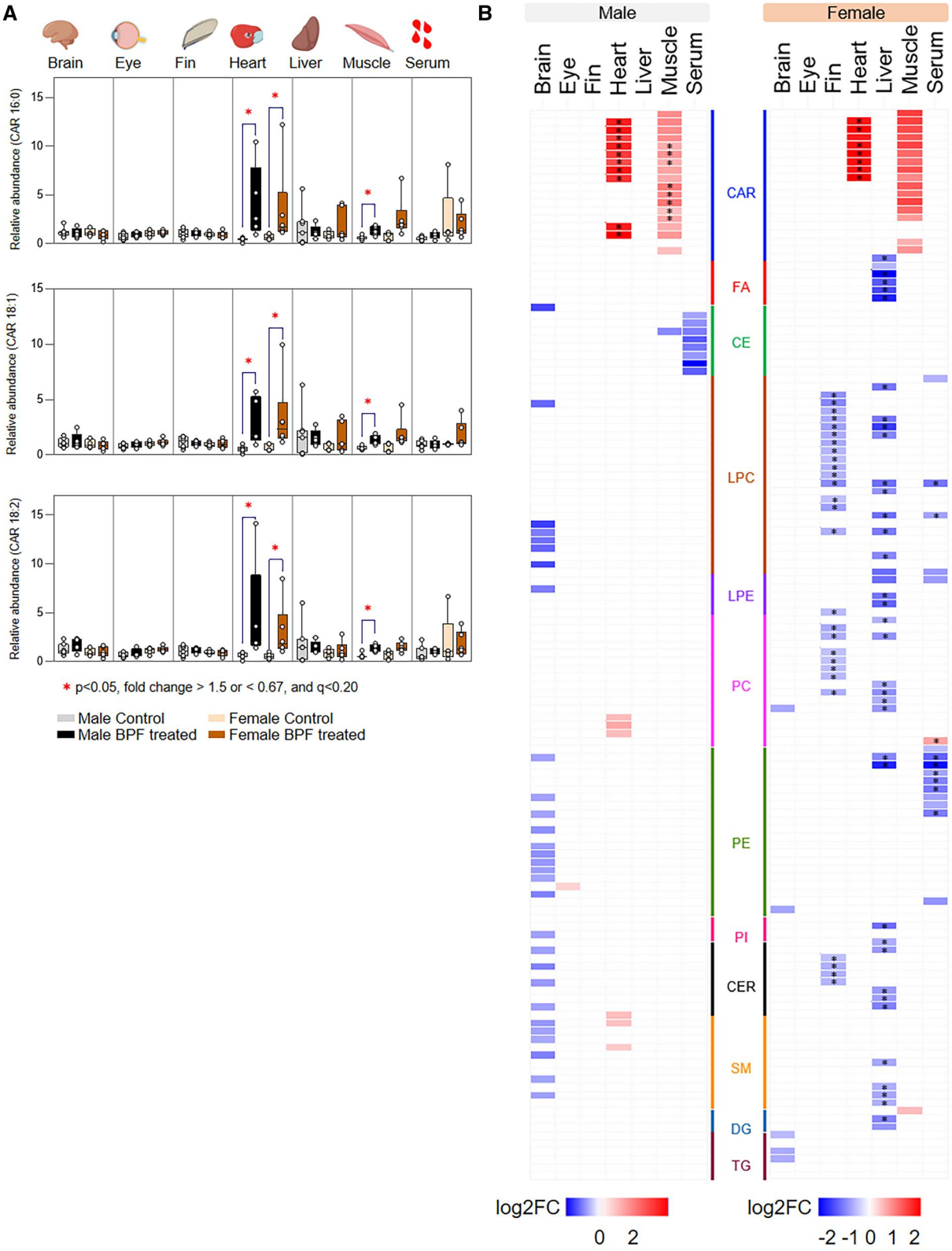

Figure 3. Transient exposure of zebrafish larvae to BPF caused system-wide perturbations in adult lipids that were specific to sex and tissue. (A) Box plots show relative levels of acylcarnitine 16:0 (CAR 16:0), acylcarnitine 18:1 (CAR 18:1), and acylcarnitine 18:2 (CAR 18:2). (B) Heat map shows fold change (treated/control) for selected lipid species across organs for male and female fish. The experiment compared 12 control animals (7 males, 5 females) to 11 treated animals (5 males, 6 females). Lipids that decrease as a result of BPF exposure are shown with negative log2FC values and lipids that increase following BPF exposure with positive log2FC values. For clarity, lipids having a *p*-value greater than 0.05 or a fold change between 0.67 and 1.5 are displayed without color on the heatmap. Lipids with a *q*-value <0.20 are indicated by an asterisk. *P*-values were calculated by using a Mann Whitney *U*-test (unpaired). *Q*-values were calculated by using the Benjamini-Hochberg Procedure to control for false discovery rate. A more detailed heatmap including all the names of each lipid species is shown in Figure S5. A combined analysis of acylcarnitines from males and female is shown in Supplementary Figure S6. Fold changes, *p*-values, and *q*-values are provided in Supplementary Table S3.

for degradation, thereby leading to larger changes following BPF treatment.

Fatty acid oxidation is uniquely impaired in muscle

Skeletal and cardiac muscle rely on long-chain fatty acids as a major source of energy.[42] Fatty acids must be oxidized in the mitochondrial matrix, but neither long-chain fatty acids nor their activated acyl-CoA analogues can freely cross the inner mitochondrial membrane. Accordingly, to harvest energy from long-chain fatty acids, they must be transformed into acylcarnitines.[43] Acylcarnitines are then translocated across the inner mitochondrial membrane to the matrix, where they are converted into acyl-CoAs and subsequently oxidized to yield ATP. Our results show that when zebrafish larvae are transiently exposed to BPF for 48 h, it leads to long-term dysregulation of fatty acid oxidation during adulthood (Figure 3; Figure S6 and Table S5). Specifically, we observe an accumulation of acylcarnitines in adult skeletal and cardiac muscle, which is often a signature of impaired fatty acid oxidation and may be reflective of cardiac lipotoxicity.[44,45] Dysregulated acylcarnitine levels are observed in adult skeletal and cardiac muscle, but no other tissues that we evaluated. There were no major differences in the levels of circulating lipid substrates between animal groups, suggesting that impaired fatty acid oxidation is not due to limited substrate availability but rather a result of some metabolic abnormality that is specific to skeletal and cardiac muscle. Notably, while acylcarnitines accumulate in the skeletal and cardiac muscle of both sexes, lactate and AMP are only elevated in male heart. These findings indicate that elevated fatty acid oxidation could be insufficient to maintain energy levels in the male heart following BPF exposure.

Amino acid levels are altered in the male brain

Recent evidence from animal models has demonstrated that exposure to BPF can negatively impact brain function.[46] The mechanisms of neurotoxicity remain incompletely characterized, but BPF is known to cross the blood-brain barrier and alter the expression of L-type amino acid transporter 1 in the male brain.[47,48] In our study, we found that the levels of numerous amino acids were decreased in male animals following transient BPF exposure (see Figure 2 and Table S3). Some of these amino acids are biosynthetic precursors for neurotransmitters such as dopamine and serotonin, and their limited availability could contribute to disrupted behavior and cognition. While they did not pass the multiple hypothesis testing correction, the same effect is observed for multiple amino acids and the calculated confidence intervals show that the differences are significant in male brain but not female brain (Figure S7).

BPF disrupts intestinal microbiota

Prior studies have revealed that the diversity, composition, and function of the gut microbiome is disrupted in both mice and zebrafish due to long-term BPF exposure.[36,49] Given the influence of intestinal bacteria on host metabolism, we aimed to explore whether some of the metabolic alterations observed in our fish were a result of changes in the gut microbiome. To examine whether transient exposure to BPF during the larval stage of development affects the microbiome of adults, we performed 16S sequencing on intestinal tissue harvested from adults that were treated with either BPF or vehicle as larvae. The major phyla measured from our animals included actinobacteria, proteobacteria, fusobacteria, and firmicutes, which is consistent with those measured from other zebrafish facilities[50] (Figure 4A). The relative abundance of these phyla did not change between control and BPF-treated fish. Alpha diversity (Figure 4B), which is a measure of microbial richness, showed features that were significantly downregulated after BPF treatment. Shannon entropy, which accounts for the richness and evenness of bacteria in a sample, was downregulated after BPF treatment, although the change was not statistically significant ($P = 0.12$). Beta diversity (Figure 4C), which examines diversity within groups, showed better separation via Principal Coordinates Analysis (PCoA) plots by using Jacaard than Bray Curtis. When analyzing the deeper taxonomic level differences, we found proteobacterial genera such as gemmobacter, rhodobacter, and reyranella that were essentially absent after BPF treatment (Table S2). In contrast, vibrio was only present in animals exposed to BPF. These results are consistent with the observations of an earlier report, which also found a decrease in gemmobacter and rhodobacter.[36] Of note, however, the previous study exposed zebrafish embryos to BPF for 180 days. The agreement between our findings suggests that even a transient early-life exposure is sufficient to alter gut bacteria and that the disruption of the microbiome persists into adulthood.

In contrast to the sex-specific alterations observed in metabolite and lipid profiles following transient BPF exposure, no corresponding sex-dependent differences were measured in the taxonomic composition of the gut microbiome (Figure S8). Notwithstanding, we cannot rule out the possibility that the same bacterial species exhibit a distinct phenotype in male and female hosts. For instance, secondary bile acids are known to influence hepatic lipid metabolism and could be produced at different levels by the same bacterial species in males and females following BPF treatment.[51,52]

Broader implications

The exposome refers to the totality of exposures encountered throughout a lifetime. Although the concept remains daunting, recent innovations in mass spectrometry enable measurement of the chemical component of the exposome with unprecedented resolution. Using cutting-edge technologies, it has become routine to profile hundreds to thousands of distinct chemicals (such as bisphenols) from a relatively modest amount of biofluid or tissue. The temporal dimension of the exposome, however, remains a considerable challenge. Due to practical considerations, most exposomics analyses performed to date have focused on specimens collected at a single time point. Even when multiple time points[53] have been incorporated into a study, it still only provides a narrow snapshot of the participant's total lifetime exposure profile. While it may be possible to infer some chemical exposures by using techniques such as geospatial mapping, there will inevitably be blind spots in time and space.

Children are particularly susceptible to the adverse effects of chemical toxicants. Compared to adults, they tend to have increased exposures per kilogram of body weight. Additionally, rapidly growing and developing organs are more sensitive to toxins than adult tissue. This raises an important question as to whether an exposure that is confined to the early window of life can induce disease during adulthood. Indeed, there is precedent for such reasoning. According to the "Barker Hypothesis", now more commonly referred to as the Developmental Origins of Health and Disease (DOHaD) theory, early-life exposures influence patterns of growth, body composition, and later risk of noncommunicable chronic disease in adults.[54] There is increasing evidence for the DOHaD concept at the epidemiological level. Barker and colleagues showed that starvation of pregnant women correlated with increased risk of cardiovascular and metabolic diseases in their offspring at adulthood.[55] More than 300 additional epidemiological studies also find an association

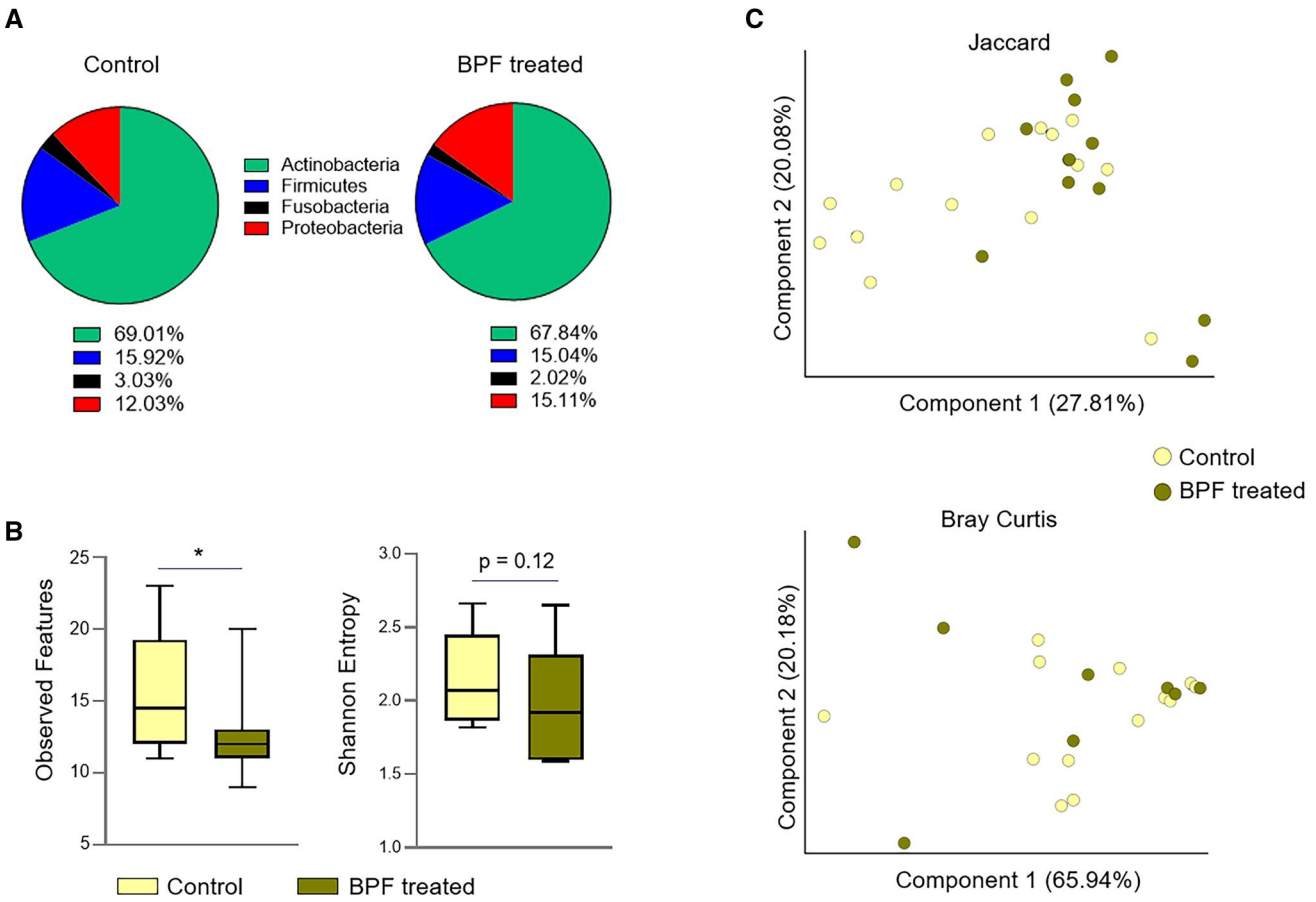

Figure 4. A transient exposure to BPF during the developmental phase of life altered the gut microbiome of adult animals. (A) Relative abundance of major phyla in control ($n = 14$) versus BPF-treated ($n = 11$) adult fish. (B) Alpha diversity as measured by using observed features and Shannon entropy. *P*-values were calculated by using a Kruskal-Wallis pairwise comparison (* indicates $P < 0.05$) (C) Beta diversity is displayed by using PCoA plots of Bray Curtis and Jaccard.

between developmental chemical exposures and later life diseases.[56] There are fewer reports from studies of model systems, but some compelling examples have been described.[57-61] Prenatal air pollution was identified as a cause of neuroinflammatory changes in adult mice.[62] Developmental exposure to xenoestrogens was found to disrupt epigenetic programming and promote tumor development in adult mice,[63] and an Alzheimer's disease-like pathology was discovered in aged monkeys after infantile exposure to environmental lead.[64]

Here, we build on prior work by providing additional support for the idea that a transient exposure confined to the developmental phase of life can disrupt the metabolic health of adults. Specifically, we show that when zebrafish larvae are exposed to BPF for only 48 h, it causes system-wide alterations in the metabolism of adult animals. Interestingly, the metabolic changes are tissue specific. For example, while skeletal and cardiac muscle show dysregulated fatty acid oxidation, other tissues do not. It is also notable that some metabolic changes were specific to females or males. The most striking observation of sexual dimorphism was in the liver, where transient exposure to BPF induced major changes in the hepatic lipidome of females but had minimal effect on male liver tissue.

Our findings add to the growing concern that, like BPA, BPF has adverse effects on health. Consistent with previous studies, we conclude that BPF exposure negatively impacts metabolic health. Performing metabolomics and lipidomics on six different tissues and serum, we provide the highest resolution analysis of the metabolic effects of BPF exposure to date and share these data as a resource for the environmental health community. Unlike earlier work, which evaluated animals immediately after BPF treatment, we examined zebrafish five months after a transient developmental exposure. This experimental design uniquely allowed us to isolate the long-term effects of BPF. Disturbingly, we find that even a brief exposure to BPF during development leads to an extensive metabolic reprogramming of adults. The implication is that, even with the introduction of immediate regulatory measures, BPF could negatively affect population health for a generation.

Author contributions

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Supplementary material

Supplementary material is available at *Exposome* online.

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Conflicts of interest

The Patti laboratory has research collaboration agreements with Thermo Fisher Scientific and Agilent Technologies. G.J.P. is the chief scientific officer of Panome Bio.

Data availability

Raw and processed LC/MS data have been uploaded to Metabolomics Workbench.

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