

Methods

Collection Summary:

Blood was collected via cardiac puncture at the time of tissue harvest. For separating serum from the blood cells, the samples were allowed to clot at room temperature for 20-30 minutes, followed by centrifugation for 10 min at 1000 x g, and storage of the separated serum at -80°C.

Treatment Summary:

Neonatal rats were treated with vehicle (sesame oil) or bisphenol A (BPA; 50 µg/kg dissolved in sesame oil) orally via pipette tip on post-natal days 1, 3, and 5. Littermates were randomly assigned to the treatment groups. BPA was obtained from the National Institute of Environmental Health Sciences (NIEHS). The dose and route of administration recapitulates human exposure to BPA. At day 180, adult rats in both treatment groups were fed a diet high in fat (40% kcal), fructose (20% kcal) and cholesterol (2%) (Western-style diet) for 60 days (D09100301, Research Diets, Inc). Rats were fasted overnight prior to tissue collection.

Sample Preparation Summary:

Lipids were extracted using a modified Bligh-Dyer method. Fifty µL of serum and 25 mg of crushed liver was used for the extraction. The extraction was carried out using 2:2:2 volume ratio of water/methanol/dichloromethane at room temperature after spiking internal standards 17:0 LPC, 17:0 PC, 17:0 PE, 17:0 PG, 17:0 ceramide, 17:0 SM, 17:0PS, 17:0PA, 17:0 TAG, 17:0 MAG, 16:0/18:1 DAG, 17:0 CE. The organic layer was collected and completely dried under nitrogen. Before MS analysis, the dried extract was resuspended in 100 µL of Buffer B (10:5:85 Acetonitrile/water/Isopropyl alcohol) containing 10 mM NH₄OAc and subjected to LC/MS. The lipidome was separated using reverse-phase chromatography.

MS acquisition and Data Processing Comments:

For data acquisition through LC/MS analysis, we used a Shimadzu CTO-20A Nexera X2 UHPLC system equipped with a degasser, binary pump, thermostatted auto sampler, and a column oven for chromatographic separation. The column heater temperature was set at 55°C. For lipid separation, the 5 µL of the lipid extract was injected into a 1.8 µm particle 50 × 2.1 mm Acquity HSS UPLC T3 column (Waters, Milford, MA) which heats to 55°C. Acetonitrile/water (40:60, v/v) with 10 mM ammonium acetate was solvent A and acetonitrile/water/isopropanol (10:5:85 v/v) with 10 mM ammonium acetate was solvent B. For chromatographic elution we used a linear gradient over a 20 min total run time, with 60% solvent A and 40% solvent B gradient in the first 10 minutes, then the gradient was ramped in a linear fashion to 100% solvent B which was maintained for 7 minutes. After that the system was switched back to 60% solvent B and 40% solvent A for 3 minutes. The flow rate used for these experiments was 0.4 mL/min and the injection volume was 5µL. The column was equilibrated for 3 min before the next injection and run at a flow rate of 0.4 mL/min for a total run time of 20 min. The data acquisition of each sample was performed in both positive and negative ionization modes using a TripleTOF 5600 equipped with a Turbo VTM ion source (AB Sciex, Concord, Canada). The column effluent was directed to the electrospray ionization source. The voltage of source was set to 5500 V for positive ionization and 4500 V for negative ionization mode, the declustering potential was set

to 60 V, and the source temperature to 450°C for both modes. The curtain gas flow, nebulizer, and heater gas were set to 30, 40, and 45 units, respectively. The instrument performed one TOF MS survey scan (150 ms) and 15 MS/MS scans with a total duty cycle time of 2.4 s. The mass range in both modes was 50-1200 m/z. We controlled the acquisition of MS/MS spectra by data-dependent acquisition (DDA) function of the Analyst TF software (AB Sciex, Concord, Canada) with the following parameters: dynamic background subtraction, charge monitoring to exclude multiply charged ions and isotopes, and dynamic exclusion of former target ions for 9 s. Rolling collision energy spread was set whereby the software calculated the collision energy value to be applied as a function of m/z. Mass accuracy was maintained by the use of an automated calibrant delivery system interfaced to the second inlet of the DuoSpray source.