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Comparative lipid profiling dataset of the inflammation-induced optic nerve regeneration

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Abstract

In adult mammals, retinal ganglion cells (RGCs) fail to regenerate following damage. As a result, RGCs die after acute injury and in progressive degenerative diseases such as glaucoma; this can lead to permanent vision loss and, eventually, blindness. Lipids are crucial for the development and maintenance of cell membranes, myelin sheaths, and cellular signaling pathways, however, little is known about their role in axon injury and repair. Studies examining changes to the lipidome during optic nerve (ON) regeneration could greatly inform treatment strategies, yet these are largely lacking. Experimental animal models of ON regeneration have facilitated the exploration of the molecular determinants that affect RGC axon regeneration. Here, we analyzed lipid profiles of the ON and retina in an ON crush rat model using liquid chromatography–mass spectrometry. Furthermore, we investigated lipidome changes after ON crush followed by intravitreal treatment with Zymosan, a yeast cell wall derivative known to enhance RGC regeneration. This data is available at the NIH Common Fund’s Metabolomics Data Repository and Coordinating Center (supported by NIH grant, U01-DK097430) website, the Metabolomics Workbench, http://www.metabolomicsworkbench.org, where it has been assigned Project ID: PR000661. The data

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1. Data

Lipid profiling was performed from the retina and ON samples during Zymosan-induced retinal ganglion cells regeneration through extractive mass spectrometry-based lipidomics. The experimental groups were: intact control (control), optic nerve (ON) crush + vehicle (crush) and ON crush + Zymosan + CPT-cAMP (regeneration). Zymosan is a yeast cell wall preparation traditionally used to induce sterile inflammation experimentally. The addition of a cell-permeable cAMP analog (CPT-cAMP) potentiates Zymosan’s action but cannot induce ON regeneration when administered alone [1]. The ONs were collected 3, 7, 14 and the retinas 7, 14 days post-crush (Fig. 1a). Zymosan + CPT-cAMP treatment potently increased the amount of axon regeneration (Fig. 1b). Time points were chosen according to our and others previous reports. After axotomy, most RGCs die within 2 weeks [2]. The intravitreal inflammatory response presents a hazy vitreous on day 3 post-crush and concomitant Zymosan injection [3]. On day 7, the ON crush site is densely occupied by Iba1 positive macrophages/microglia [3]. Zymosan + CPT-cAMP doubles the number of live RGCs in retina 2 weeks after ON crush.
After a chloroform-methanol based extraction, lipid samples were analyzed using high-performance liquid chromatography (HPLC) with C30 column coupled to a Q Exactive mass spectrometer operated in a data-dependent mode (Fig. 1c). Peak identification and relative quantification were performed in LipidSearch software. Lists of species and their relative abundances were uploaded to MetaboAnalyst [4] for statistical analysis.

2. Experimental design, materials, and methods

2.1. ON crush and intravitreal injections

All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and policies of the UCLA Animal Research Committee. A rat model of inflammation-induced ON regeneration was established with intravitreal injection of a yeast cell wall preparation (Zymosan A) [5] and a cell-permeant CPT-cAMP [1], immediately after ON crush. Ten-week-old male Fischer rats were deeply anesthetized by inhalation of isoflurane, and the eyes were treated with topical anesthetic (proparacaine HCl 0.5% ophthalmic) and a cycloplegic...
(tropicamide 0.5% ophthalmic) to reduce pain and assist with visualization of intravitreal injections. The left ON was exposed by blunt dissection through a temporal, fornix-based conjunctival incision and crushed for 10 seconds with Dumoxel #N5 self-closing forceps (Dumont, Montignez, Switzerland). Post crush, an absence of injury to the retinal vascular supply was confirmed by funduscopic examination. Intravitreal injections (5 μL) of PBS vehicle or a suspension of finely ground, sterilized 4 Zymosan A (Z4250; Sigma-Aldrich, St. Louis, MO, USA; 12.5 mg/mL) plus CPT-cAMP (C3912; Sigma-Aldrich, St. Louis, MO, USA; 100 μM) were performed with a pulled glass pipette attached to a Hamilton syringe on a manual micromanipulator. Injections were made 2 mm posterior to the limbus, and care was taken to prevent lens injury, choroidal hemorrhage, or retinal detachment. Post intravitreal injection, an absence of lens injury, choroidal haemorrhage and retinal detachment was confirmed by fundoscopic examination. Conjunctival incisions were closed with 8–0 polyglactin sutures and petrolatum ophthalmic ointment was applied to the ocular surface. Schematic diagram of optic nerve crush and intravitreal injection is presented in Fig. 2.

2.2. Immunohistochemistry

Optic nerves were fixed in PBS plus 4% paraformaldehyde and cryoprotected overnight at 4 °C in 30% sucrose. Cryoprotected tissue was embedded in optimal cutting temperature compound and flash frozen in liquid nitrogen. Longitudinal sections of nerve (14 μm) were cut with a cryostat, mounted on plus charged glass microscope slides, and permeabilized for 30 minutes at room temperature (RT) in TBS plus 0.25% Tween 20 (0.25%TBST). The sections were then blocked with 10% normal donkey serum in TBS for 1 hour at RT and incubated overnight at 4 °C with gentle shaking in 0.1%TBST plus 2% BSA (2% BSA-0.1%TBST) and rabbit polyclonal anti-GAP43 (Abcam, Cambridge, MA, USA; ab16053; 1:250). The sections were rinsed with 0.1%TBST and incubated for 1 hour at RT with Hoechst 33,258 in 2%BSA-0.1% TBST plus Alexa Fluor-conjugated donkey secondary antibody. Finally, the sections were rinsed with 0.1%TBST and coverslipped with an aqueous mounting medium. Images of immunostained tissues were obtained with a Fluoview FV1000 confocal microscope (Olympus, Center Valley, PA, USA).

2.3. Lipid extraction

Specimens were stored at −80 °C. 6 mL of methanol (LC-MS grade) and 3 mL of chloroform (LC-MS grade) were added to each sample. After 2 min of vigorous vortexing and 2 min of sonication in an ultrasonic bath, the samples were incubated at 48 °C overnight (in borosilicate glass vials, PTFE-lined caps). The following day, 3 mL of water (LC-MS grade) and 1.5 mL of chloroform were added, samples vigorously vortexed for 2 min and centrifuged at 3000 RCF, 4 °C for 15 min to obtain phase separation.
# Table 1

Samples list.

| Data    | Subject | Eye | Tissue | Day | ON crush | IVI injection | Lipid extraction protocol | LC-MS protocol | ESI mode |
|---------|---------|-----|--------|-----|----------|---------------|---------------------------|----------------|----------|
| PBS     | Zymosan | +CPT-cAMP |
| DS_1_POS | Rat_1 | OD | ON | 3 | - | - | - | + | + |
| DS_1_NEG | Rat_1 | OD | ON | 3 | - | - | - | + | - |
| DS_2_POS | Rat_2 | OD | ON | 3 | - | - | - | + | + |
| DS_2_NEG | Rat_2 | OD | ON | 3 | - | - | - | + | - |
| DS_3_POS | Rat_3 | OD | ON | 3 | - | - | - | + | + |
| DS_3_NEG | Rat_3 | OD | ON | 3 | - | - | - | + | - |
| DS_4_POS | Rat_1 | OS | ON | 3 | + | + | - | + | + |
| DS_4_NEG | Rat_1 | OS | ON | 3 | + | + | - | + | - |
| DS_5_POS | Rat_2 | OS | ON | 3 | + | + | - | + | + |
| DS_5_NEG | Rat_2 | OS | ON | 3 | + | + | - | + | - |
| DS_6_POS | Rat_3 | OS | ON | 3 | + | + | - | + | + |
| DS_6_NEG | Rat_3 | OS | ON | 3 | + | + | - | + | - |
| DS_7_POS | Rat_4 | OS | ON | 3 | + | + | - | + | + |
| DS_7_NEG | Rat_4 | OS | ON | 3 | + | + | - | + | - |
| DS_8_POS | Rat_5 | OS | ON | 3 | + | + | - | + | + |
| DS_8_NEG | Rat_5 | OS | ON | 3 | + | + | - | + | - |
| DS_9_POS | Rat_6 | OS | ON | 3 | + | + | - | + | + |
| DS_9_NEG | Rat_6 | OS | ON | 3 | + | + | - | + | - |
| DS_10_POS | Rat_7 | OD | ON | 7 | - | - | - | + | + |
| DS_10_NEG | Rat_7 | OD | ON | 7 | - | - | - | + | - |
| DS_11_POS | Rat_8 | OD | ON | 7 | - | - | - | + | + |
| DS_11_NEG | Rat_8 | OD | ON | 7 | - | - | - | + | - |
| DS_12_POS | Rat_9 | OS | ON | 7 | - | - | - | + | + |
| DS_12_NEG | Rat_9 | OS | ON | 7 | - | - | - | + | - |
| DS_13_POS | Rat_7 | OD | ON | 7 | + | + | - | + | + |
| DS_13_NEG | Rat_7 | OD | ON | 7 | + | + | - | + | - |
| DS_14_POS | Rat_8 | OS | ON | 7 | + | + | - | + | + |
| DS_14_NEG | Rat_8 | OS | ON | 7 | + | + | - | + | - |
| DS_15_POS | Rat_9 | OS | ON | 7 | + | + | - | + | + |
| DS_15_NEG | Rat_9 | OS | ON | 7 | + | + | - | + | - |
| DS_16_POS | Rat_10 | OS | ON | 7 | + | + | - | + | + |
| DS_16_NEG | Rat_10 | OS | ON | 7 | + | + | - | + | - |
| DS_17_POS | Rat_11 | OS | ON | 7 | + | + | - | + | + |
| DS_17_NEG | Rat_11 | OS | ON | 7 | + | + | - | + | - |
| DS_18_POS | Rat_12 | OS | ON | 7 | + | + | - | + | + |
| DS_18_NEG | Rat_12 | OS | ON | 7 | + | + | - | + | - |
| DS_19_POS | Rat_13 | OS | retina | 7 | - | - | - | + | + |
| DS_19_NEG | Rat_13 | OS | retina | 7 | - | - | - | + | - |
| DS_20_POS | Rat_14 | OD | retina | 7 | - | - | - | + | + |
| DS_20_NEG | Rat_14 | OD | retina | 7 | - | - | - | + | - |
| DS_21_POS | Rat_15 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_21_NEG | Rat_15 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_22_POS | Rat_16 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_22_NEG | Rat_16 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_23_POS | Rat_17 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_23_NEG | Rat_17 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_24_POS | Rat_18 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_24_NEG | Rat_18 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_25_POS | Rat_19 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_25_NEG | Rat_19 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_26_POS | Rat_20 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_26_NEG | Rat_20 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_27_POS | Rat_21 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_27_NEG | Rat_21 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_28_POS | Rat_22 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_28_NEG | Rat_22 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_29_POS | Rat_23 | OD | ON | 14 | ee | ee | ee | ee | ee |
| DS_29_NEG | Rat_23 | OD | ON | 14 | ee | ee | ee | ee | ee |

(continued on next page)
Lower phases were collected and dried in a centrifugal vacuum concentrator. Samples were stored at 
−20 °C until reconstituted in 60 µL of chloroform:methanol (1:1) prior to mass spectrometric analysis.

2.4. High-performance liquid chromatography (HPLC)

Reversed-phase chromatographic separation was achieved with the Accela Autosampler, Accela 600 pump and Acclaim C30 column; 3 µm, 2.1 × 150 mm (Thermo Fisher Scientific, Waltham, MA). The column temperature was maintained at 30 °C (negative mode) or 45 °C (positive mode) and tray temperature at 20 °C. Solvent A was composed of 10 mM ammonium acetate (LC-MS grade) in 60:40 methanol:water (LC-MS grade) with 0.2% formic acid (LC-MS grade). Solvent B was composed of 10 mM ammonium acetate with 60:40 methanol:chloroform with 0.2% formic acid. The flow rate was 260 µL/min, and the injection volume was 5 µL. The gradient was 35–100% solvent B over 13.0 min, 100% solvent B over 13.0–13.8 min, 100-35% solvent B over 13.8–14.5 min, 35% solvent B over 14.5–18.0 min, 0% solvent B over 18.0–20.0 min.

Table 1 (continued)

| Data     | Subject | Eye | Tissue | Day | ON crush | IVI injection | Lipid extraction protocol | LC-MS protocol | ESI mode |
|----------|---------|-----|--------|-----|----------|---------------|---------------------------|----------------|----------|
|          |         |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_29_NEG Rat_14 OD ON 14 – – – + + – |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_30_POS Rat_15 OD ON 14 – – – + + – |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_30_NEG Rat_15 OD ON 14 – – – + + – |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_31_POS Rat_13 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_31_NEG Rat_13 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_32_POS Rat_14 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_32_NEG Rat_14 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_33_POS Rat_15 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_33_NEG Rat_15 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_34_POS Rat_16 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_34_NEG Rat_16 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_35_POS Rat_17 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_35_NEG Rat_17 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_36_POS Rat_18 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_36_NEG Rat_18 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_37_POS Rat_19 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_37_NEG Rat_19 OS ON 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_38_POS Rat_13 OD retina 14 – – – + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_38_NEG Rat_13 OD retina 14 – – – + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_39_POS Rat_14 OD retina 14 – – – + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_39_NEG Rat_14 OD retina 14 – – – + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_40_POS Rat_15 OD retina 14 – – – + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_40_NEG Rat_15 OD retina 14 – – – + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_41_POS Rat_13 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_41_NEG Rat_13 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_42_POS Rat_14 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_42_NEG Rat_14 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_43_POS Rat_15 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_43_NEG Rat_15 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_44_POS Rat_16 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_44_NEG Rat_16 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_45_POS Rat_17 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_45_NEG Rat_17 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_46_POS Rat_18 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_46_NEG Rat_18 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_47_POS Rat_19 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
| DS_47_NEG Rat_19 OS retina 14 + – + + + + |          |     |        |     |          | PBS Zymosan +CPT-cAMP  |               |            |          |
2.5. Mass spectrometry

The Q Exactive (Thermo) mass spectrometer was operated under heated electrospray ionization (HESI) in positive and negative modes separately for each sample. The spray voltage was 4.4 kV, the heated capillary was held at 310 °C (negative mode) or 350 °C (positive mode) and heater at 275 °C (positive mode). The S-lens radio frequency (RF) level was 70. The sheath gas flow rate was 30 (negative mode) or 45 units (positive mode), and auxiliary gas was 14 (negative mode) or 15 units (positive mode). Full scan (m/z 150–1500) used resolution 70,000 at m/z 200 with automatic gain control (AGC) target of 1 × 10^6 ions and maximum ion injection time (IT) of 100 ms. Data-dependent MS/MS (top 10) were acquired with the following parameters: resolution 17,500; AGC 2 × 10^5 (negative mode) or 1 × 10^5 (positive mode); maximum IT 100 ms (negative mode) or 75 ms (positive mode); 1.3 m/z isolation window. Normalized collision energy (NCE) settings were 40 ± 30% for the negative mode and 30, parallel with 19 ± 5% for the positive mode. Samples list is available in Table 1.

2.6. Lipid identification and relative quantification

Lipid identification and relative quantification were performed with LipidSearch 4.1 software (Thermo). The search criteria were as follows: product search; parent m/z tolerance 5 ppm; product m/z tolerance 10 ppm; product ion intensity threshold 1%; filters: toprank, main isomer peak, FA priority; quantification: m/z tolerance 5 ppm, retention time tolerance 1 min. The following adducts were

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| Group                | Class | Key    | Lipid name                        |
|----------------------|-------|--------|-----------------------------------|
| phospholipid         | BisMePA| bismethyl phosphatidic acid       |
|                      | cPA   | cyclic phosphatic acid            |
|                      | dMePE | dimethylphosphatidylethanolamine  |
|                      | LdMePE| lysodimethylphosphatidylethanolamine |
|                      | LPC   | lysophosphatidylcholine           |
|                      | LPE   | lysophosphatidylethanolamine      |
|                      | LPI   | lysophosphatidylinositol          |
|                      | PA    | phosphatic acid                   |
|                      | PC    | phosphatidylcholine               |
|                      | PE    | phosphatidylethanolamine          |
|                      | PEt   | phosphatidylethanol               |
|                      | PG    | phosphatidylglycerol              |
|                      | PI    | phosphatidylinositol              |
|                      | PMe   | phosphatidylmethanol              |
|                      | PS    | phosphatidylerine                 |
| sphingolipid         | Cer   | ceramide                          |
|                      | SM    | sphingomyelin                      |
|                      | So    | sphinogosine                       |
| glycosphingolipid    | CerG1 | hexosyl ceramide                  |
|                      | CerG3 | trihexosyl ceramide               |
|                      | SoG1  | hexosyl sphingosine               |
|                      | ST    | sulfatide                          |
| cardiolipin          | CL    | cardiolipin                        |
| neutral glycerolipid | DG    | diglyceride                        |
|                      | MG    | monoglyceride                      |
|                      | TG    | triglyceride                       |
| steroid              | ChE   | cholesterol ester                  |
| coenzyme             | Co    | coenzyme                           |
| fatty esters         | AcCa  | acyl carnitine                     |
| glycoglycerolipid    | MGDG  | monogalactosyldiacylglycerol       |
|                      | SQDG  | sulfoquinovosyldiacylglycerol      |
Fig. 3. Dataset overview. (a–b) Abundance (average area) and (c–d) variability (% CV) distribution among treatment groups. Retina samples: (a–c). Optic nerve samples: (b–d). (e) Pearson’s correlation coefficients between biological replicates within each treatment group. Mean ± SD. (f–j) Principal component analysis (PCA) with samples plotted in 2 dimensions using their projection onto the first 2 principal components (in brackets % of total variance explained).
allowed in positive mode: +H, +NH4, +H–H2O, +H–2H2O, +2H, and negative mode: −H, +HCOO, +CH3COO, -2H. All classes were selected for search. LipidSearch nomenclature is used (Table 2).

2.7. Data processing

Positive and negative mode identifications of the retina and ON samples were aligned in LipidSearch 4.1, allowing calculation of unassigned peaks. The following settings were applied: product search; alignment method max; retention time tolerance 0.1 min; filters: toprank, main isomer peak; M-score 5. Only peaks with molecular identification grade: A-B (A: lipid class and fatty acid completely identified or B: lipid class and some fatty acid identified) were accepted. Only peaks appearing in all biological replicates were accepted. Peaks with the same annotated lipid species were merged. Lists of

Fig. 4. Data analysis example. (a–b) The number of significant features at different FDR adjusted p value cut-offs for one-way ANOVA analysis: species (a) and classes (b). (c–d) Accumulation change patterns of dysregulated species (FDR adjusted p-values <0.05) in the retina (370 species; c) and ON (324 species; d) 14 days post-crush, presented as heatmaps. Ward clustering algorithm, Euclidean distance measure, autoscale features. Species are not labeled for clarity.
species and their relative abundances were uploaded to the MetaboAnalyst 4.0 [4] statistical analysis module.

2.8. Data availability

This data is available at the NIH Common Fund’s Metabolomics Data Repository and Coordinating Center (supported by NIH grant, U01-DK097430) website, the Metabolomics Workbench, http://www.metabolomicsworkbench.org, where it has been assigned Project ID: PR000661. The data can be accessed directly via its Project DOI: doi: 10.21228/M87D53. The submission includes 94. RAW files: Q Exactive output (description of each file is in Tables 1 and 2, txt files: LipidSearch alignment output for retina and ON, 1. xlsx file: list of peaks (filtered) and 1. docx file: description of the method.

In addition, 10. csv files have been uploaded to Figshare https://doi.org/10.6084/m9.figshare.7078253.v2. Species input: ON3_MetaboAnalyst.csv, ON7_MetaboAnalyst.csv, ON14_MetaboAnalyst.csv, retina7_MetaboAnalyst.csv and retina14_MetaboAnalyst.csv. Classes input: ON3_classMetaboAnalyst.csv, ON7_classMetaboAnalyst.csv, ON14_classMetaboAnalyst.csv, retina7_classMetaboAnalyst.csv and retina14_classMetaboAnalyst.csv.

2.9. Usage notes

Files in. csv format can be directly input to MetaboAnalyst: Statistical Analysis. The user should select the following format: samples in columns (unpaired). For the following analysis example, we replaced missing values with a small number (half of the minimum positive value in the original data), applied normalization to sum and log 2 transformation.

Data were obtained from 3 to 4 biological replicates for each group. In Fig. 3, distributions of the average area (Fig. 3a and b) and CV % values (Fig. 3c and d) for the experimental groups are presented. Biological replicates showed Pearson correlation coefficients ranging from 0.918 to 0.998 (Fig. 3e). In line, within each time point groups of samples were clearly distinguished from each other with 86.7–97.7% of variance accounted for by PC1 and PC2 (Fig. 3f and j).

To identify features undergoing the significant change between experimental groups, we used one-way ANOVA analysis with Tukey’s post-hoc test. We examined a number of significant features at different FDR adjusted p values (Fig. 4 species a and classes b). Next, we performed hierarchical clustering and heatmap visualization of the dysregulated species (FDR adjusted p values < 0.05). The heatmaps of significant species in the retina and ON 14 days post-crush are presented as Fig. 4c and d, respectively.

Author contributions

Conceptualization: D.S., S.K.B., J.C.
Methodology: D.S., M.P.
Investigation: A.T., D.S., J.K.
Analysis: A.T., D.S.
Writing — original draft: A.T., D.S.
Writing – review & editing: A.T., D.S., J.K., M.P., S.K.B., J.C.
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Transparency document

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Appendix A. Supplementary data

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