Supporting Information

**Metabolomics Reveals that Aryl Hydrocarbon Receptor Activation by Environmental Chemicals Induces Systemic Metabolic Dysfunction in Mice** Limin Zhang,†,‡ Emmanuel Hatzakis,§ Robert G. Nichols,† Ruixin Hao,† Jared Correll,† Philip B. Smith,& Christopher R. Chiaro,† Gary H. Perdew,† Andrew D. Patterson\*,†

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There are two figures and three tables in the supplemental information.

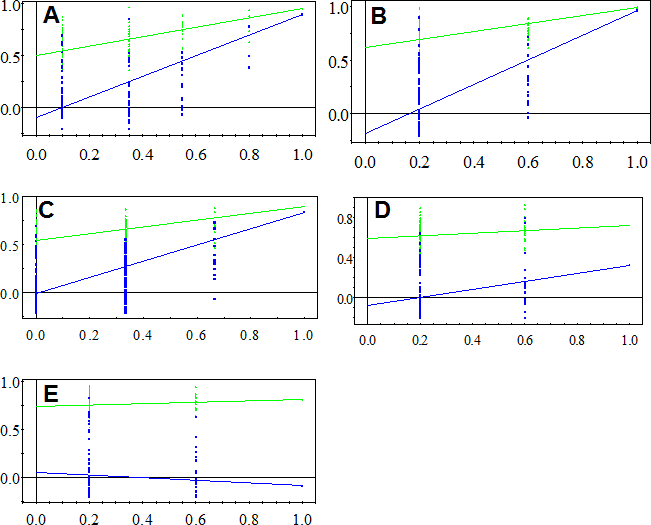


Figure S1. Permutation tests (200 tests) for the PLS-DA models of liver (A), serum CPMG (B) and serum diffusion (C) NMR data obtained from *Ahr+/+* mice and liver (D), serum CPMG (E) NMR data from *Ahr-/-* mice.

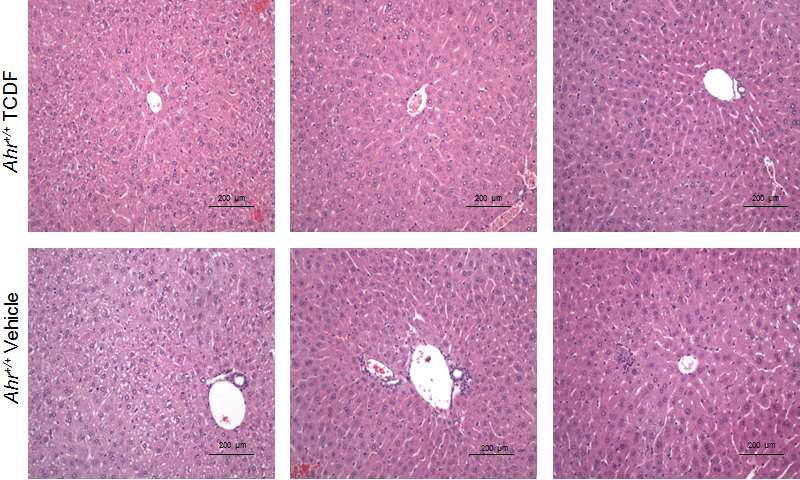


Figure S2. Hepatic histopathology. Light micrographs (×200) of liver from wild type vehicle and TCDF-treated mice (5 µg kg-1).

Table S1. 1H NMR chemical shifts for metabolites assigned in liver extracts and serum.

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| --- | --- | --- | --- | --- |
| key | metabolites | moieties | δ 1H (ppm) and multiplicitya | Samplesb |
| 1  2 | Lipid  Isoleucine | CH3, (CH2)n, CH2-C=C, CH2- C=O,C-CH2-C=,-CH=CH-  αCH, βCH, γCH3, δCH3 | 0.89(m), 1.27(m), 2.0(m),  2.3(m), 2.78(m), 5.3(m)  3.65(d), 1.95(m), 0.99(t), 1.02(d) | L, S  L, S |
| 3 | Leucine | αCH, βCH2, γCH3, δCH3 | 0.94(d), 3.72(t), 1.96(m), 0.91(d) | L, S |
| 4 | Valine | αCH, βCH, γCH3 | 3.6(d), 2.26(m), 0.98(d), 1.04(d) | L, S |
| 5 | D-3-hydroxybutyrate | CH, CH2, γCH3, CH2 | 4.16(dt),2.41(dd),1.20(d),2.31(dd) | L, S |
| 6 | Lactate | αCH, βCH3 | 4.11(q), 1.32(d) | L, S |
| 7 | Alanine | αCH, βCH3 | 3.77(q), 1.48(d) | L, S |
| 8 | Acetate | CH3 | 1.91(s) | L, S |
| 9 | HDL | CH3 | 0.82(m) | S |
| 10 | LDL | CH3 | 0.85(m) | S |
| 11 | VLDL | CH3 | 0.88(m) | S |
| 12 | Glutamate | αCH, βCH2, γCH2 | 2.08(m), 2.34(m), 3.75(m) | L, S |
| 13 | Glutamine | αCH, βCH2, γCH2 | 2.15(m), 2.44(m), 3.77(m) | L, S |
| 14 | Glutathione | CH2, CH2, S-CH2, N-CH, CH | 2.16(m), 2.55(m), 2.95(dd),  3.78(m), 4.56(q) | L |
| 15 | *N*-acetyl-glycoproteins | CH3 | 2.04(S) | S |
| 16 | *O*-acetyl-glycoproteins | CH3 | 2.14(S) | S |
| 17 | Acetoacetate | CH3 | 2.26(S) | S |
| 18 | Choline | N(CH3)3, OCH2, NCH2 | 3.2(s), 4.05(t), 3.51(t) | L, S |
| 19 | Phosphocholine(PC) | N(CH3)3, OCH2, NCH2 | 3.22(s), 4.21(t), 3.61(t) | L, S |
| 20 | Glycerophosphocholine | N(CH3)3, OCH2, NCH2 | 3.22(s), 4.32(t), 3.68(t) | L, S |
| 21 | β-Glucose | 1-CH | 4.66(d) | L, S |
| 22 | α-Glucose | 1-CH | 5.23(d) | L, S |
| 23 | Unsaturated fatty acid | CH=CH | 5.3(m) | L, S |
| 24 | TMAO | CH3 | 3.27(s) | L |
| 25 | Tyrosine | CH, CH | 6.89(dd), 7.18(dd) | L, S |
| 26 | Histidine | 2-CH, 4-CH, CH2 | 7.75(t), 7.08(d), 6.05(d) | L, S |
| 27 | Phenylalanine | Ring-CH | 7.40(m), 7.33(m), 7.35(m) | L, S |
| 28 | Formate | CH | 8.45(s) | L, S |
| 29 | Betaine | CH2, CH3 | 3.27(s), 3.93(s) | L |
| 30 | Glycogen | 1-CH | 5.38-5.45(m) | L |
| 31 | Bile acid | CH3 | 0.73(m) | L |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 32 | Lysine | αCH, βCH2, γCH2, δCH2 | 3.76(t), 1.89(m), 1.72(m), 3.01(t) | L, S |
| 33 | N-acetyl aspartate | CH3 | 2.01(s) | L |
| 34 | PUFA | CH3 | 2.73(m) | S |
| 35 | Succinate | CH3 | 2.41(s) | L, S |
| 36 | Taurine | S-CH2, N-CH2 | 3.26(t), 3.40(t) | L |
| 37 | Glycine | CH2 | 3.57(s) | L, S |
| 38 | Inosine | 14-CH, 1-CH, 8-CH, 4’-CH, | 8.34(s), 6.09(d), 8.22(s), 4.76(t), | L |
| 39 | Uridine | 5’-CH, CH2(1/2), CH2(1/2)  11-CH, 7-CH, 12-CH, 6-CH, | 4.47(m)  7.88(d), 5.92(d), 5.9(d), 4.36(m), | L |
| 40 | Fumarate | 5-CH, 4-CH, CH2, CH2  CH | 4.24(t)  6.53(s) | L, S |
| 41 | Nicotinurate | 2-CH, 6-CH, 4-CH, 5-CH | 8.93(s),8.62(d), 8.25(d),7.60(dd), | L |
| 42 | Adenosine | 14-CH | 8.32(s) | L, C |
| 43 | Uracil | 1-CH, 2-CH | 5.81(d), 7.54(d) | L |
| 44 | Citrate | CH2(1/2), CH2(1/2) | 2.55(d), 2.65(d) | S |
| 45 | Creatine | CH2, CH3 | 3.03(s), 3.92(s) | S |
| 46 | Glucose & amino acids | αCH resonances | 3.3-3.9 | L, S |
| 47 | Pyruvate | CH3 | 2.38(s) | S |
| 48 | Triglycerides | CH | 4.08(m), 4.21(m), 5.18(m) | S |

a Key: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublet.

b Liver aqueous extracts (L) and serum (S).

Table S2. Cross-validation with permutation test and CV-ANOVA for PLS-DA and OPLS-DA models from NMR spectra of serum and liver extracts.

TCDF-*Ahr+/+* Vs Vehicle-*Ahr+/+* TCDF-*Ahr-/-* Vs Vehicle-*Ahr-/-*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Samples  Liver | OLPS-DA CV-ANOVAa  2.33×10-3 | PLS-DA  Permutation testsb  √ | OLPS-DA CV-ANOVA  0.219 | PLS-DA  Permutation tests  × |
| Serum from CPMG NMR | 0.035 | √ | 0.571 | × |
| Serum from diffusion NMR | 2.06 ×10-3 | √ | 1.392 | × |

a *P* values of CV-ANOVA for OPLS-DA models;

b √ Pass and × fail for PLS-DA models.

Table S3. Primer sequences for qRT-PCR, related to the experimental procedures.

|  |  |  |
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| **Gene** | **Abbreviation** | **Sequence** |
| Cytochrome P450, family 1,  subfamily A, polypeptide 1 | *Cyp1a1* | AGAATACGGTGACAGCCAGG  TTTGGGAGGAAGTGGAAGG |
| Phosphoenolpyruvate  carboxykinase | *Pepck* | GGCCACAGCTGCTGCAG  GGTCGCATGGCAAAGGG |
| Glucokinase | *Gck* | TAT GAA GAC CGC CAA TGT GA  TTT CCG CCA ATG ATC TTT TC |
| Glucose-6-phosphatase | *G6pase* | CTGTGAGACCGGACCAGGA  GACCATAACATAGTATACACCTGCTGC |
| Glucose transporter 2 | *Glut2* | GTCCAGAAAGCCCCAGATACC  GTGACATCCTCAGTTCCTCTTAG |
| Lipocalin-2 | *Lcn-2* | ATTTCCCAGAGTGAACTGGC  AATGTCACCTCCATCCTGGT |
| Interleukin-1 beta | *IL-1β* | GGTCAAAGGTTTGGAAGCAG  TGTGAAATGCCACCTTTTGA |
| Tumor necrosis factor alpha | *TNF-α* | AGGCTGCCCCGACTACGT  GACTTTCTCCTGGTATGAGATAGCAAA |
| Serum amyloid A 1 | *Saa1* | TCATGTCAGTGTAGGCTCGC  GTCTTCTGCTCCCTGCTCC |
| Stearoyl-CoA desaturase-1 | *Scd1* | TTCTTGCGATACACTCTGGTGC  CGGGATTGAATGTTCTTGTCGT |
| Fatty acid synthase | *Fasn* | GGTGTGGTGGGTTTGGTGAATTGT  TCACGAGGTCATGCTTTAGCACCT |
| Acetyl-CoA  carboxylase alpha | *Acaca* | TAACAGAATCGACACTGGCTGGCT  ATGCTGTTCCTCAGGCTCACATCT |