

**Chronic exposure to paraben promotes nonalcoholic fatty liver disease in
association with the changes of gut microbiota and lipid metabolism**

Yilin Ren^{1‡}, Xinyi Shi^{1‡}, Jing Mu¹, Shenyin Liu¹, Xin Qian¹, Wenlong Pei¹, Shanhong
Ni¹, Zhengduo Zhang¹, Lei Li^{1, 2*}, Zhan Zhang^{1, 2*}

Supplementary data

Figure S1. The effects of parabens exposure on intestine. The weight of (A) heart, (B) spleen and (C) kidney. The length of small intestine (D) and colon (E). (F) Representative histopathological observation of colon (4 μm). The colon was stained with hematoxylin and eosin (H&E) and observed using optical microscopy, scale bar: 100 μm . * $P < 0.05$, compared with the respective control; # $P < 0.05$, ## $P < 0.01$ compared with the ND group.

Figure S2. Effect of Mep or EtP on inflammation in mice fed with ND or HFD. Serum levels of (A) alanine transaminase (ALT) and (B) aspartate aminotransferase (AST). The relative expression of inflammatory genes, including (C) *IL1 β* , (D) *IL6*, (E) *TNF α* , (F) *Adgre1* and (G) *MCP-1*. Data were presented as the mean \pm SEM and analyzed by ordinary one-way ANOVA with Tukey's multiple comparisons. * $P < 0.05$, ** $P < 0.01$, compared with the respective control; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, compared with the ND group.

Figure S3. The effects of parabens exposure on the metabolic profile in HFD-fed mice. (A) The projection to latent structures discriminant analysis (PLS-DA) score plot among control, Mep and EtP groups fed with ND or HFD. (B) The numbers of significantly altered metabolites among control, Mep and EtP groups fed with ND or HFD. Heat map analysis of differential metabolites involved in enriched pathways after exposure to MeP (C) and EtP (D). Red color represents an upregulation of the metabolite while green color represents a downregulation. Enrichment of pathways was indicated by coloured bars on the left.

Figure S4. Taxonomic differences of fecal microbiota at the genus level. (A)

Taxonomic differences between ND and HFD groups. Taxonomic differences between control and Mep (B) or EtP (C) groups fed with ND at the genus level. Taxonomic differences between control and Mep (E) or EtP (F) group fed with HFD at the genus level. The statistical difference was analyzed by Wilcoxon rank-sum test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, compared with the respective control.

Figure. S5. Predicted functions of the metagenome from 16S rRNA sequencing.

Functional annotation of the predicted metagenome based on 16S rRNA data was performed by PICRUSt 2 and STAMP was used to identify significant pathways. (A) The numbers of significantly enriched pathways among control, MeP and EtP groups fed with ND or HFD. (B) The common enriched pathways between ND and HFD fed mice after MeP exposure. (C) The common enriched pathways between MeP and EtP exposure in HFD fed mice. Enriched pathways with same trend in ND (D) and HFD (E) fed mice after MeP exposure. Enriched pathways with same trend in HFD fed mice after MeP (F) and EtP (G) exposure. Differences between groups were determined using the Welch's t-test.

Figure. S6. Association analysis of genera with serum chemistry or metabolites.

Association analysis of differential genera with serum chemistry or metabolites in AA metabolism pathway between HFD and HFD+ MeP (A) or (B) HFD+ EtP groups. Significant correlations are denoted by stars ($*P < 0.05$; $**P < 0.01$, Spearman test).

Higher taxonomy of genera (phyla) and superpathways of metabolites were indicated by coloured bars.

Supplementary Table 1. Diet composition

Diet Formulas	Normal diet (ND)	High fat diet (HFD)
	kcal%	kcal%
Fat	10.00	60.00
Carbohydrate	70.00	20.00
Protein	20.00	20.00
Total	100.00	100.00
Ingredient	g/Kg	g/Kg
Casein	189.56	258.45
L-Cystine	2.84	3.88
Corn Starch	479.78	0.00
Maltodextrin 10	118.48	161.53
Sucrose	69.00	94.08
Cellulose, BW200	47.39	64.61
Soybean Oil	23.70	32.30
Lard	18.96	316.60
Mineral Mix S10026B	47.39	64.61
Vitamin Mix V10001C	0.95	1.29
Choline Bitartrate	1.90	2.58
FD&C Red Dye #40	0.00	0.00
FD&C Yellow Dye #5	0.04	0.00
FD&C Blue Dye #1	0.01	0.06

Supplementary Table 2. Primer sequence

Gene name	Forward sequence	Reverse sequence
<i>mGAPDH</i>	AACTTTGGCATTGTGGAAGG	GGATGCAGGGATGATGTTCT
<i>mIL1β</i>	TAGACA ACTGCACTACAGGCTCCGA	GGGTCCGACAGCACGAGGCT
<i>mIL6</i>	ACAAAGCCAGAGTCCTTCAGA	GGTCCTTAGCCACTCCTTCTG
<i>mTNFα</i>	GCTGAGCTCAAACCCTGGTA	AGTACTTGGGCAGATTGACCT
<i>mAdgre1</i>	ATACCCTCCAGCACATCCAG	AGTTTGCCATCCGGTTACAG
<i>mMcp-1</i>	TCCAATGAGTAGGCTGGAG	TCTGGACCCATTTCCTTCTTG
<i>mGlut2</i>	TCCCTTGTTTCATGGTTGCT	CCCAAGGAAGTCCGCAATGT
<i>mPfkfb1</i>	ATGAGCTGCCCTATCTCAAGT	GTCCCGGTGTGTGTTACAG
<i>mPfk1</i>	ACGTGAAGGATCTGGTGGTTC	ATTCGGTCGAAGGCTGAAGG
<i>mPfk2</i>	AGCATTCATACCTTGGGCAT	CCATGAAGAGCATCATGCAG
<i>mAldoA</i>	CAGATGAGTCCACCGGAAGC	AATGCAGGGATTACACGGT
<i>mPdha1</i>	TCATTTGCAAAATTACGGGA	AAGATGCTTGCCGCTGTATC
<i>mPdk1</i>	TTACTCAGTGGAACACCGCC	GTTTATCCCCGATTCAGGT
<i>mCs</i>	GGACAATTTTCCAACCAATCTGC	TCGGTTCATTCCCTCTGCATA
<i>mIdh3g</i>	GGTGCTGCAAAGGCAATGC	TATGCCGCCCACCATACTTAG
<i>mMe2</i>	GGCAGGTCAAGGTACAAGGA	CTGCTTGCTGCACCACCT
<i>mFbp1</i>	GCACAGCTCTATGGTATCGCT	CACAGGTAGCGTAGGACGAC
<i>mFbp2</i>	ACAGAAAGACCACGGAGGATG	TGTCCTGTGGAAAGAGCGAC
<i>mCD36</i>	GCTGTGTTTGGAGGCATTCT	TGGGTTTTGCACATCAAAGA
<i>mAcaa1a</i>	CCTGACTCCTATGGGGATGA	CCCTTGTCATCCAGGACAGT

<i>mCpt1a</i>	CCAGGCTACAGTGGGACATT	AAGGAATGCAGGTCCACATC
<i>mAdrp</i>	CTGTCTACCAAGCTCTGCTC	CGATGCTTCTCTTCCACTCC
<i>mPPARγ</i>	GATGGAAGACCACTCGCATT	CAACCATTGGGTCAGCTCTT
<i>mSrebf1</i>	TACTTCTTGTGGCCCGTACC	TCAGGTCATGTTGGAAACCA
<i>mAcaca</i>	GAGAGGGGTCAAGTCCTTCC	ACATCCACTTCCACACACGA
<i>mFasn</i>	CCCTTGATGAAGAGGGATCA	CAAGGCGTTAGGGTTGACAT
<i>mCYP4A14</i>	GGAGCAATATACGAGTCCTGC	CAGAGTCCGCCATGATTTTGA
<i>mCYP2J9</i>	CAAGAGGAGGCTCACTACCTT	TGATGACTCCAAACACATGGC
<i>mCYP2C50</i>	ACTGTGGTGTTCATGGATATG	GAGAAGCGCCTTGTGTTTTTC
<i>mEphx2</i>	ACCACTCATGGATGAAAGCTACA	TCAGGTAGATTGGCTCCACAG
<i>mCyp4A30B</i>	GTTACCAGGGTAGTGTCCAGT	GCACTCTCAAGTAAATCAGGTGT
<i>mCYP2C67</i>	TGTAGTCTTGGTGCTTTGTCTG	ACAATAGGCTGTGAGCCAAAATA
<i>hGAPDH</i>	ACAAC TTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC
<i>hAdrp</i>	TTGCAGTTGCCAATACCTATGC	CCAGTCACAGTAGTCGTCACA
<i>hCD36</i>	CTTTGGCTTAATGAGACTGGGAC	GCAACAAACATCACCACACCA
<i>hCpt1a</i>	ATCAATCGGACTCTGGAAACGG	TCAGGGAGTAGCGCATGGT
<i>hPPARγ</i>	GATGCCAGCGACTTTGACTC	ACCCACGTCATCTTCAGGGA
<i>hSrebf1</i>	CGGAACCATCTTGGCAACAGT	CGCTTCTCAATGGCGTTGT
<i>hAcaca</i>	CATGCGGTCTATCCGTAGGTG	GTGTGACCATGACAACGAATCT
<i>hFasn</i>	ACAGCGGGGAATGGGTACT	GACTGGTACAACGAGCGGAT
<i>hCYP4A11</i>	CCATCCCCATTGCACGACTT	CAGGTAGACAAGCAGGTAGGG
<i>hCYP2C8</i>	CATACTGACTTCCGTGCTACAT	CTCCTGCACAAATTCGTTTTCC

<i>hCYP2J2</i>	GAGCTTAGAGGAACGCATTCAG	GAAATGAGGGTCAAAGGCTGT
<i>hEphx2</i>	GACATCGGGGCTAATCTGAAG	GGCTTTACTGTCACGTACCCA

m, *Mus musculus*; *h*, *Homo sapiens*.