

# LIPIDOMIC PROFILING OF WILD TYPE AND FATTY ACID SYNTHASE (FASN) KNOCKOUT MICE WITH LC-CIMS-TOF MS **AND MS/MS TECHNIQUES**

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# INTRODUCTION

Fatty acid synthesis in mammalians is catalyzed by a multienzyme complex named fatty acid synthase (FAS) which is encoded by FASN gene.<sup>1,2</sup> It catalyzes acetyl-CoA polymerization into short chain fatty acids (SCFA) that undergo further elongation to long chain FA (LCFA) through a different enzymatic system. Although this mitochondrial fatty acid synthesis pathway was proposed a few decades ago, its contribution to the overall fatty acid production in the body has not been evaluated.

Alternative uses for acetyl-CoA include cholesterol and or steroids synthesis, fatty acid elongation, other anabolic pathways, or it oxidation to CO<sub>2</sub> and H<sub>2</sub>O for energy production. Thus, acetyl-CoA availability for these key pathways of structural and bioactive molecules production might depend upon the activity of FAS. However, FAS activity effect on these pathways has not been fully evaluated.

In the present study, we conditionally knocked out FASN in adult mice and performed lipidomics and <sup>3</sup>H labeling experiments to determine FASN KO effect on the major pathways for acetyl-CoA utilization.

#### **METHODS**

#### Animals

Homozygous FASN<sup>flox/flox</sup> mice (a generous gift from Dr. Semenkovich, Washington University) were crossed to UBC-Cre-ERT2 mice (Jackson Laboratory). Eight weeks old mice were treated with tamoxifen (i.p.) once a day for 6 days. Tissues were analyzed 5 days after last TAM injection. Livers and brains from FASN knockout mice (TG), as well as heterozygous (WT/TG) and wild type mice (WT) were extracted using the Folch method and diluted prior to chromatographic analysis.

#### Chromatography

Mobile Phase A: 60:40 ACN: 10 mM AmFormate 0.1% HFor

Mobile Phase B: 90:10 Isopropanol: ACN 10 mM AmFormate 0.1% HFor

Column: ACQUITY™ CSH C18 1.7 µm d<sub>p</sub> 2.1x100 mm

		-		
Gradient:				
	Time	Flow (mL/min)	%A	Curve
	0	0.4	50	6
	0.5	0.4	47	6
	4	0.4	45	6
	7	0.4	35	6
	7.5	0.4	20	1
	10	0.4	1	6

#### Mass Spectrometry

Waters Select Series<sup>™</sup> Cyclic<sup>™</sup> IMS

ES+ and ES– data was acquired in single and multipass HDMS<sup>E</sup> mode.<sup>3</sup>

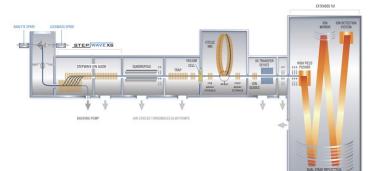


Figure 1. Schematic for Waters Select Series Cyclic IMS, Key components are the cyclic mobility separator and T-Wave Array used to change the direction of ion motion into and out of the mobility selector.

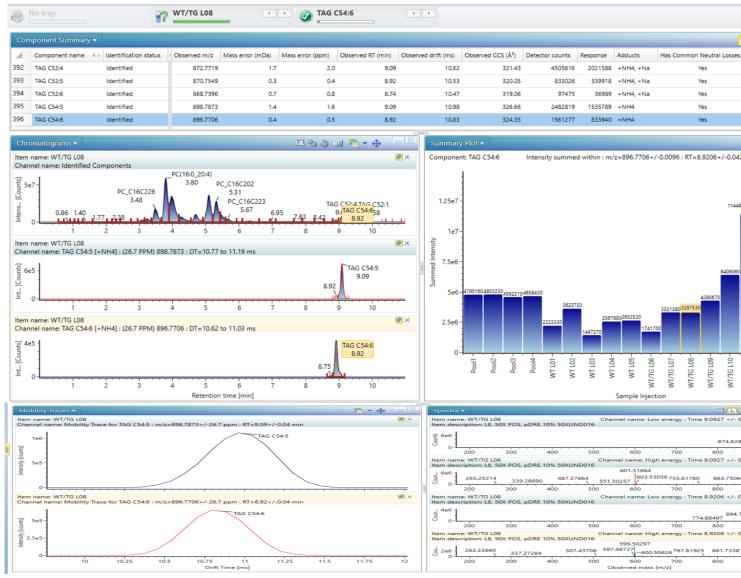
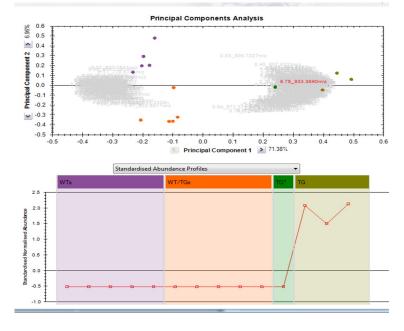


Figure 2. UNIFI™ Summary for Selected TAGs observed in Liver. Tabulated data includes mass accuracy, observed Collisional Cross Section (CCS), and detector counts. Arrival Time Distributions (ATD) and Mobility filtered low and high energy spectra for TAGS C54:5 and C54:6 are shown. Neutral loss is consistent with a C18:2 acyl chain.



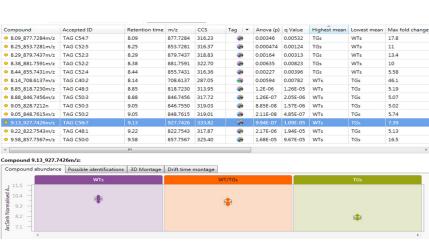


Figure 4. Selected TAGs identified in liver. Data was filtered on the basis of ANOVA < 0.005. Combined Carbon chain and number of unsaturations are reported along with CCS, Highest/lowest group, and fold change. Bar plot is reported for TAG C56:7 showing standard deviations.

Figure 3. PCA Scores Bi-Plot for WT, WT/TG and TG animal groups, showing trend plot for selected species.

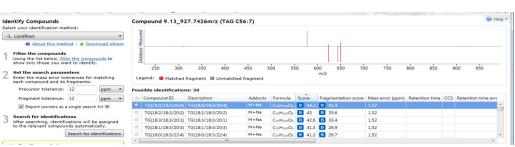


Figure 5. Results of LipidBlast database search for TAG 56:7. Based on the spectral match, the three acyl chains are C18:0, C18:3, and C20:4.

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#### RESULTS

Filters # 🚯 🖣 4505616 2021588 +NH4, +Na 833026 97475 36989 +NH4, +Na 2482819 1535789 +NH4 339.28890 487.37864 551.50257 603.53 b 2e6 263.23660 337.27284 507.45708 597.48727 -600.50626 767.61925 861.7381 901.72597 1011.64369

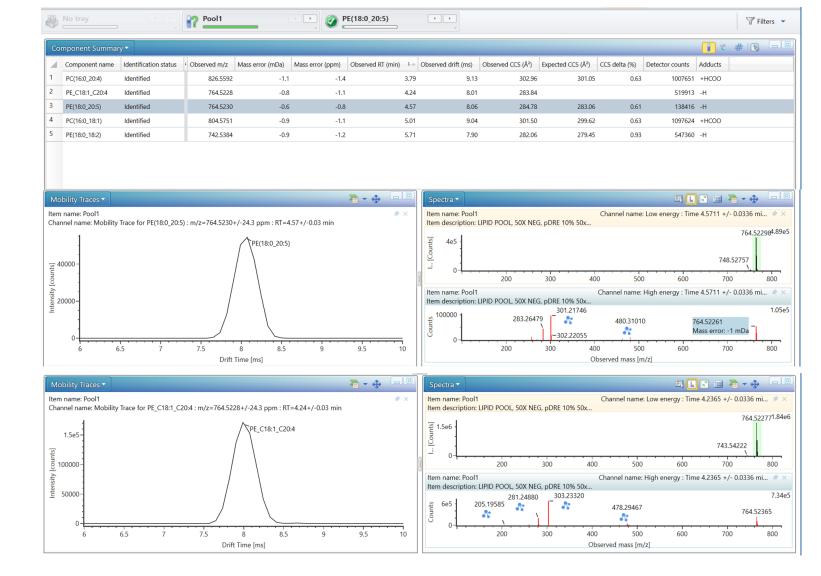


Figure 6. UNIFI Summary isomeric PEs observed in Liver. Tabulated data includes mass accuracy, observed CCS and difference from expected values, and detector counts. Arrival Time Distributions (ATD) and Mobility filtered low and high energy spectra for PE C18:0 C20:5 and C18:1 C20:4 with identified fragment ions are annotated.

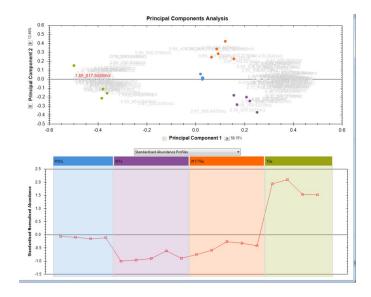


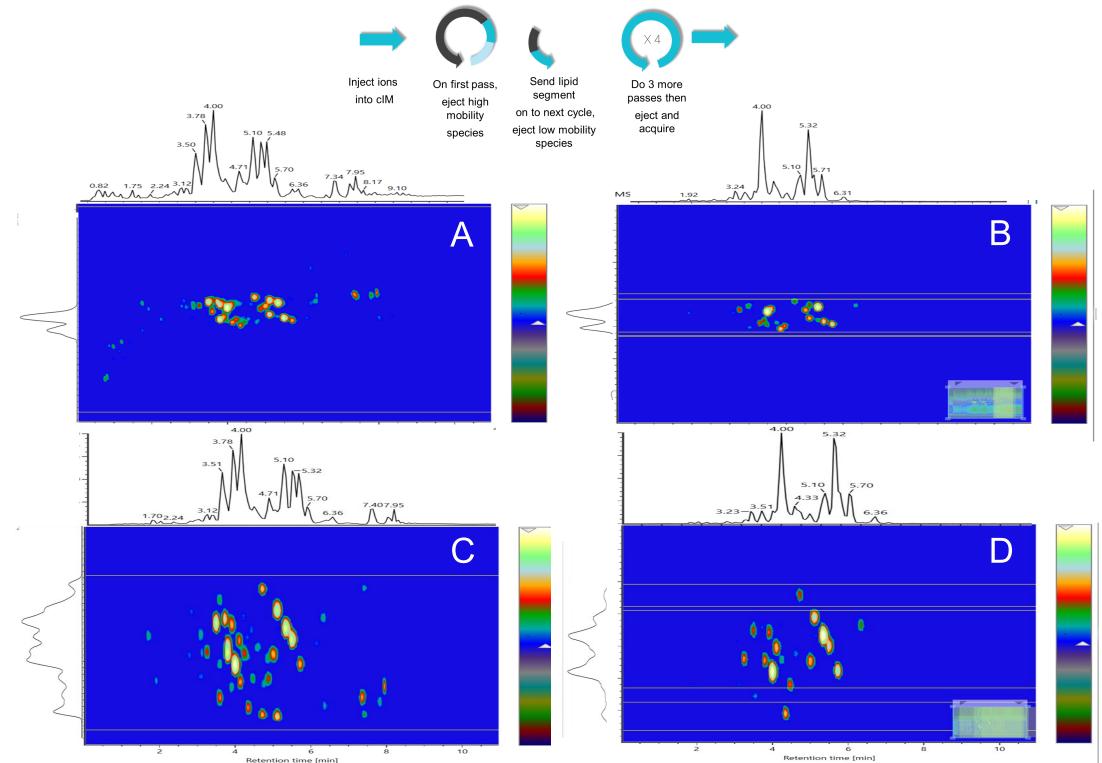
Figure 7. PCA Scores Bi-Plot for WT, WT/TG and TG animal groups, showing trend plot for selected species. Replicate assays of a pooled sample cluster tightly in the center of the plot.

Compound	Accepted ID	m/z	Retention time	CCS	Anova (p)	q Value	Max fold change	Highest mean	Lowest mean	Max Abundance	Min CV%	j
2.26_795.5170m/z	GPGro(16:0/22:5)	795.5170	2.26	293.83	5.49E-05	0.00017	2.48	TGs	WT/TGs	924.9941	15.90	
• 3.26_773.5335m/z	GPGro(18:0/18:2)	773.5335	3.26	292.50	1.12E-05	6.29E-05	2.31	TGs	WT/TGs	11827.1847	4.97	
• 2.96_821.5335m/z	GPGro(18:0/22:6)	821.5335	2.96	300.91	0.000177	0.000402	5.28	TGs	WT/TGs	680.0836	20.63	
• 2.61_771.5180m/z	GPGro(18:1/18:2)	771.5180	2.61	291.03	3.52E-06	2.71E-05	2.06	TGs	WT/TGs	14985.8683	9.55	
• 2.49_795.5179m/z												l
• 2.31_819.5180m/z	GPGro(18:1/22:6)	819.5180	2.31	299.49	1.44E-06	1.39E-05	2.19	TGs	WTs	29787.3373	10.04	
2.11_769.5027m/z	GPGro(18:2/18:2)	769.5027	2.11	289.56	1.32E-06	1.37E-05	3.06	TGs	WTs	11952.5462	11.06	
2.58_769.5026m/z	GPGro(18:2/18:2)	769.5026	2.58	286.57	0.0425	0.0259	30.5	TGs	WTs	455.2750	70.85	
2.02_793.5025m/z	GPGro(18:2/20:4)	793.5025	2.02	293.84	3.86E-07	5.2E-06	4.04	TGs	WTs	7313.0202	8.15	
2.80_821.5323m/z	GPGro(18:2/22:4)	821.5323	2.80	299.48	8.4E-05	0.000231	5.58	TGs	WTs	1421.9975	18.83	
• 1.89_817.5026m/z	GPGro(18:2/22:6)	817.5026	1.89	298.06	3.83E-08	1.03E-06	3.52	TGs	WTs	53692.7563	7.84	
2.02_843.5178m/z	GPGro(20:3/22:6)	843.5178	2.02	302.21	2.49E-07	3.74E-06	3.2	TGs	WTs	3053.7665	7.57	
1.65_815.4868m/z	GPGro(20:4/20:5)	815.4868	1.65	296.62	0.000512	0.000908	2.12	TGs	WTs	3262.5232	11.00	
2.18_843.5186m/z	GPGro(20:4/22:5)	843.5186	2.18	302.21	0.00264	0.00326	4.85	TGs	WTs	2074.5151	29.61	
• 1.81_841.5026m/z	GPGro(20:4/22:6)	841.5026	1.81	302.23	4.52E-05	0.000157	3.86	TGs	WTs	14745.5446	12.67	
1.55_839.4869m/z	GPGro(20:5/22:6)	839.4869	1.55	300.81	3.95E-05	0.000144	2.54	TGs	WT/TGs	3394.8355	12.83	
2.19_869.5342m/z	GPGro(22:4/22:6)	869.5342	2.19	307.72	0.000798	0.00131	4.09	TGs	WTs	7003.4423	20.60	
• 1.85_867.5181m/z	GPGro(22:5/22:6)	867.5181	1.85	306.33	1.84E-07	2.91E-06	2.79	TGs	WTs	8718.9802	7.93	
2.02_867.5182m/z	GPGro(22:5/22:6)	867.5182	2.02	306.33	0.00342	0.00393	3.27	TGs	WTs	2925.9514	28.39	
• 1.70_865.5030m/z	GPGro(22:6/22:6)	865.5030	1.70	304.93	1.3E-05	7.18E-05	2.89	TGs	WTs	128761.4181	9.82	
• 2.78_857.5188m/z	GPIns(16:0/20:4)	857.5188	2.78	300.71	6.2E-05	0.000186	2.31	WTs	TGs	52885.3767	6.96	
• 3.24_871.5344m/z	GPIns(17:0/20:4)	871.5344	3.24	304.90	0.0372	0.0231	1.56	WTs	TGs	3706.2460	9.11	
2.30 855.5021m/z	GPIns(18:1/18:4)	855.5021	2.30	300.72	0.000658	0.00112	5.37	WTs	WT/TGs	982.2737	28.90	

*Figure 8. Selected Phosphatidylglycerols (GPGro)* identified in liver. Data was filtered on the basis of ANOVA <0.005. CCS, Highest/lowest group, fold change, Maximum abundance and minimum CV % are reported.

	817.5026m/z (GPGr	o(18:2/22:	6))							🕜 He
_										
nred										
Meas										
Database								1 1	1	
0 20 40 Legend: <b>B</b> Matche			0 180 2	00 220	240 260 280 m/z	300 320 340	360 380 4	00 420 440 460 48	0 500 520	540
Possible identificati	ons: 10									
+ Compound ID	Description	Adducts	Formula	Score	Fragmentation score	Mass error (ppm)	Isotope similarity	Retention time error (mins)	Retention time	CCS
GPGro(18:2/22:6)	GPGro(18:2/22:6)	M-H	C46H75O10P	C 45.4	C 42.1	0.10	84.82			
* GPGro(22:6/18:2)	GPGro(22:6/18:2)	M-H	C46H75O20P	C 45.4	C 42.1	0.10	84.82			
* GPA(18:4/24:4)	GPA(18:4/24:4)	M+FA-H	CasH23OaP	C 37.2	<b>C</b> 0	0.10	86.17			
	GPA(20:2/22:6)			-	=	0.10	86.17			
				-		0.10	86.17			
	CD4/20.4/22.5)					0.10	00.17			
	0 20 40 Legend: ● Matche  Possible identificati     ☆ Compound ID     GPGro(18:2/22:6)     ☆ GPA(22:6)/82     GPA(20:2/2:6)     ☆ GPA(20:3/22:5)	0         20         40         60         90         100         12           Legend:         Matched fragment         © Unmatch           Possible identifications:         10           ☆         GPGroutB2/25/16         GPGroutB2/25/16           ☆         GPGroutB2/25/16         GPGroutB2/25/16           ☆         GPA(184/244)         GPA(184/244)           ☆         GPA(124/244)         GPA(124/244)           ☆         GPA(1202/256)         GPA(202/226)           ☆         GPA(203/225)         GPA(203/225)	0         20         40         60         80         100         120         140         16           Legend:         ●         Hatched fragment         ●         Immatched fragment         ●         Immatched fragment           Possible identifications:         10          Compound ID         Description         Adducts           ©         GPGroct25/15/2)         GPGroct25/15/2)         M+H          M+H           ©         GPAQC02/25/16/2)         GPGroct25/16/20         M+H          GPAQ20/22/26         GPAQ20/22/26         M+FA-H           ©         GPAQ20/22/26)         GPAQ20/22/26         GPAQ20/22/26         M+FA-H          GPAQ20/22/26         GPAQ20/22/26         M+FA-H           ©         GPAQ20/22/26)         GPAQ20/22/26         M+FA-H          GPAQ20/22/26         M+FA-H	0         20         40         60         80         1.00         1.20         1.40         1.60         1.80         2           Legend:         ■ Matched fragment         ■ Unmatched fragment         ■         Unmatched fragment           Possible identifications:         10         ■         Cercentiso:2226)         M41         Celthious:         6           Grid Grid State         Grid Grid State         Grid State         Grid State         Celthious:         6           Grid Grid State         Grid State         Grid State         Grid State         Celthious:         6           Grid Grid State         Grid State         Grid State         Grid State         Celthious:         Celthious:           Grid Grid State         Grid State         Grid State         Grid State         Celthious:         Celthious:           Grid Grid State         Grid State         Grid State         Grid State         Celthious:         Celthious:         Celthious:         Grid State         Grid State         Grid State         Grid State	0         20         40         60         80         100         120         140         160         20         20         20           Legend:              •             Matched fragment              Unmatched fragment              Unmatched fragment              Volumetric fragment           Possible identifications: 10              Celencytics              Adducts              Formula              Screeting fragmetric fragmetri fragmetri fragmetric fragmetri fragmetric fragmetri fragmetric	0         20         40         60         80         100         120         140         160         180         200         240         260         280         m/2           Lepend:         Matched fragment         Unmatched fragment         Unmatched fragment         0         m/2         m/2           Possible identifications: 10           Image: Compound ID         Description         Adducts         Formula         Score         Fragmentation score           Image: Compound ID         Description         Adducts         Formula         Core         Fragmentation score           Image: Compound ID         Description         Adducts         Formula         Score         Fragmentation score           Image: Compound ID         Description         Adducts         Formula         Core         Fragmentation score           Image: Compound ID         Description         Adducts         H=         CuHnology IC         454         C 421           Image: Compound ID         Description         M=         CuHnology IC         454         C 421           Image: Compound ID         Description         M=         CuHnology IC         454         C 421           Image: Compound ID         Description         M=	20         40         60         80         100         120         140         160         180         200         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20	0         20         40         60         80         100         120         140         160         180         200         220         240         260         250         340         360         380         4           Legend:         •         Matched fragment         •         Unmatched fragment         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         <	0         20         40         60         80         100         120         140         160         180         200         220         240         260         250         340         360         380         400         420         440         460         440           m/2           Possible identifications: 10           (m/2)         (m/2)	20         40         60         80         100         120         140         160         180         200         220         240         260         280         300         320         340         360         380         400         420         440         460         450         500         200         m/2           Possible Identifications: 10           ☆         Grompund ID         Description         Adducts         Formula         Sore         Fragmentation score         Mass error (ppm)         Inotape similarity         Retention time error (mins)         Retention time           ★         Gradual State         Gradual State         Gradual State         Gradual State         100         84.82         1            ☆         GRADUAL State         Gradual State         Gradual State         100         84.82         1             ☆         GRAUSAZZAN         GRAUSAZZAN         MHFAH         Cuthrough C         332         2         0         0.10         84.51         1<

Figure 9. Results of LipidBlast database search for GPGro C18:2 C22:6.



Sequence	
1 Inject	
2 Separate	
3 Eject	
4 Separate	
5 Eject	
6 Separate	
7 Eject and Acquire	

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Figure 10A. ATD vs RT plot for single pass ES– analysis of liver phospholipids 10B. Extracted fragment ion single pass ATD vs RT for C18:0, C18:1 and C18:2 fatty acyl fragments. 10C. ATD vs RT plot for 4 total pass ES– analysis of liver phospholipids 10D. Extracted fragment ion ATD vs RT plot for 4 total pass for C18:0, C18:1 and C18:2 fatty acyl fragments.

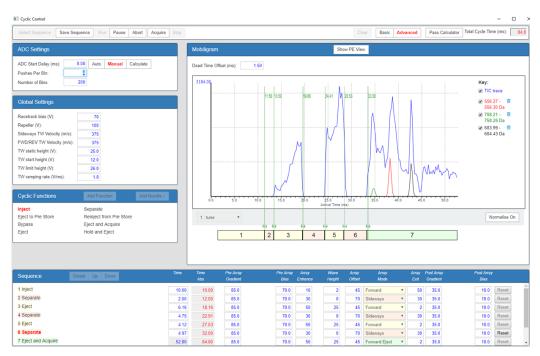


Figure 11. Cyclic Sequence Editor for 'Top and Tail' experiment. High and low mobility non-target ions are ejected in Steps 3 and 5, respectively. Target ions are further separated in Step 6.

# CONCLUSIONS

- Lipidomics experiments for characterization of differential metabolism for WT vs WT/TG and TG mice including identification of lipid species with altered metabolism
- Changes to Triacylglycerol and Phosphatidylglycerol lipid pools between wild type and transgenic groups suggest restructuring of liver metabolism
- Use of multipass IMS experiments to increase peak capacity for lipidomics experiments

#### References

- 1. Smith, S. FASEB J. 8, 1248-1259, 1994.
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- 3. Paglia, G and Astarita, G., Nature Protocols. 12, 797-813, 2017.