The attenuation of diabetic nephropathy by ANXA1 *via* regulation of lipid metabolism through AMPK/PPAR α /CPT1b pathway

Liang Wu, Changjie Liu, Dong-Yuan Chang, Rui Zhan, Mingming Zhao, Sin Man Lam, Guanghou Shui, Ming-Hui Zhao, Lemin Zheng and Min Chen

Table of Contents

Supplemental Figure 1. ANXA1 deficiency exacerbated tubular injury in HFD/STZ-treated mice.

Supplemental Figure 2. Transcriptome analysis of kidney cortex

Supplemental Figure 3. Ac2-26 administration ameliorated renal lipotoxicity in diabetic *Anxa1* KO mice and db/db mice.

Supplemental Figure 4. Oxygen consumption rate (OCR), mitochondrial fatty acid oxidation (FAO) and extracellular acidification rate (ECAR) in HK-2 cells

Supplemental Figure 5. *ANXA1* knockdown aggravated the lipid disorders in podocytes

Supplemental Figure 6. The regulatory effects of hrANXA1 upon FPR2/ALX knockdown

Supplemental Figure 7. Upregulation of ADRP in renal biopsies from patients with diabetic nephropathy

Supplemental Table 1. Primer sequences used in real-time PCR analysis

Supplemental Table 2. Physical and biochemical parameters of experimental animals

Supplemental Table 3. mRNA levels of genes involved in fatty acid metabolism in kidney cortex of HFD/STZ *Anxa1*^{-/-} and HFD/STZ WT mice

Supplemental Table 4. Raw data of triglycerides detected in kidneys of HFD/STZ *Anxa1*^{-/-} and HFD/STZ WT mice

Supplemental Figure 1. ANXA1 deficiency exacerbated tubular injury in HFD/STZ-treated mice.

(A) Representative images and quantitative analysis of F4/80 staining in tubulointerstitium. Scale bar = 50 μ m. (B) Representative images and quantitative analysis of α -SMA staining in tubulointerstitium. Scale bar = 50 μ m. (C) Quantification of *Kim-1* and *Ngal* gene expression by real-time PCR. Data were expressed as mean $\pm$ s.d. * P < 0.05; ** P < 0.01; *** P < 0.001. SFD, standard fat diet; KO, knockout; WT, wild type.

Supplemental Figure 2. Transcriptome analysis of kidney cortex

(A) Volcano plots of significantly differentially expressed genes (DEGs). Red, up-regulated; green, down-regulated. (B) KEGG pathway enrichment analysis of DEGs. Gene set enrichment analysis highlighting strong enrichment for the AMPK pathway. (C) Representative images and quantification of p-AMPK, PPAR α and CPT1b using immunohistochemistry. Scale bar = 50 μ m. Data were expressed as mean $\pm$ s.d. **P* < 0.05; ***P* < 0.01; ****P* < 0.001. HFD, high fat diet; SFD, standard fat diet; KO, knockout; WT, wild type.

Supplemental Figure 3. Ac2-26 administration ameliorated renal lipotoxicity in diabetic *Anx1* KO mice and db/db mice.

(A) Representative TEM images and quantification of lipid droplet area, mitochondrial aspect ratio and form factor of proximal tubular cells from diabetic *Anx1*^{-/-} mice without and with Ac2-26 treatment. Scale bars = 2 μ m. (B) Representative TEM images and quantification of lipid droplet area, mitochondrial aspect ratio and form factor of proximal tubular cells from db/m mice, db/db mice and db/db mice treated with Ac2-26. Scale bars = 2 μ m. (C) Quantification of α -SMA, *Col1a1* and fibronectin (*Fn*) gene expression by real-time PCR among four groups. (D) Quantification of α -SMA, *Col1a1*, *Fn* and *Ngal* gene expression by real-time PCR among three groups. Data were analyzed by a one-way ANOVA followed by a Bonferroni correction and indicated as mean $\pm$ s.d. * P < 0.05; ** P < 0.01; *** P < 0.001. HFD, high fat diet; SFD, standard fat diet; KO, knockout; WT, wild type; Mt, mitochondria; LD, lipid droplet.

Supplemental Figure 4. Oxygen consumption rate (OCR), mitochondrial fatty acid oxidation (FAO) and extracellular acidification rate (ECAR) in HK-2 cells

(A) Oxygen consumption rate (OCR) in HK-2 cells exposed to HGPA for 24 h (n=3). Where indicated, oligomycin (2 mM), FCCP (1 mM), and rotenone/antimycin A (AA) (0.5 mM) were added. The blank group was served as a normal control group. Mannitol and endotoxin-free BSA were used as the isotonic solvent control named as HGPA control group. (B) OCR in HK-2 cells exposed to HGPA for 24 h with or without hrANXA1 supplementation (n=3). (C) HK-2 cells treated with or without hrANXA1 received PA:BSA or BSA alone followed by FAO evaluation (n=3). (D) Extracellular acidification rate (ECAR) in *shANXA1* cells and *shCtrl* cells exposed to HGPA for 24 h. Where indicated, glucose (10 mM), oligomycin (1 mM), and 2-DG (50 mM) were added. Data analysis was performed by one-way ANOVA followed by a Bonferroni correction or student's *t* test. Data were expressed as mean±s.d. **P* < 0.05; ***P* < 0.01; ****P* < 0.001. *shANXA1*, shRNA against ANXA1; *shCtrl*, negative control shRNA; HGPA, high glucose plus palmitic acid; DG, deoxyglucose; OLI, oligomycin; FCCP, carbonyl cyanide p-trifluoromethoxy phenylhydrazine; Rot/anti-A, rotenone/antimycin A; hrANXA1, human recombinant ANXA1; PA, palmitic acid.

Supplemental Figure 5. *ANXA1* knockdown aggravated the lipid disorders in podocytes.

(A) Representative images and quantitative assessment of Oil Red O staining. Scale bar = 50 μ m. (B) Representative images and quantitative evaluation of TUNEL assay. Scale bar = 50 μ m. (C) Quantification of *COL4A1*, *TNF* and *IL-1 β* gene expression by real-time PCR in *siCtrl* cells and *siANXA1* cells exposed to HGPA. Data analysis was performed by one-way ANOVA followed by a Bonferroni correction or student's *t* test. Data were expressed as mean $\pm$ s.d. **P* < 0.05; ***P* < 0.01; ****P* < 0.001. *siANXA1*, siRNA against *ANXA1*; *siCtrl*, negative control siRNA; HGPA, high glucose plus palmitic acid.

Supplemental Figure 6. The regulatory effects of hrANXA1 upon FPR2/ALX knockdown

(A) RT-qPCR analyzing the expression level of FPR2 in HK-2 cells (n=6). Relative mRNA level of *PPARα* (B) and *CPT1b* (C) in *siFPR2* and *siCtrl* cells treated with hrANXA1. Data analysis was performed by one-way ANOVA followed by a Bonferroni correction or student's *t* test. Data were expressed as mean±s.d. **P* < 0.05; ***P* < 0.01; ****P* < 0.001. *siFPR2*, siRNA against FPR2; *siCtrl*, negative control siRNA; HGPA, high glucose plus palmitic acid; hrANXA1, human recombinant ANXA1 protein.

Supplemental Figure 7. Upregulation of ADRP in renal biopsies from patients with diabetic nephropathy

(A) Representative images of ADRP staining in human renal tissue (n=30) and the control (n=7). Scale bar = 50 μ m. (B) Quantitative analysis of ADRP staining. (C) Tubulointerstitial ADRP expression significantly correlated with eGFR. (D) Tubulointerstitial ADRP expression significantly correlated with ANXA1. Data were expressed as mean $\pm$ s.d. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. HC, healthy control; DN, diabetic nephropathy.

Supplemental Table 1. Primer sequences used in real-time PCR analysis

Gene	Primer Sequence 5' to 3'	
	Forward	Reverse
Human		
COL4A1	GCAAACGCTTACAGCTTTTGG	GGACGGCGTAGGCTTCTTG
MCP-1	CGCCTCCAGCATGAAAGTC	GGGAATGAAGGTGGCTGCTA
TNF	CCCAGGCAGTCAGATCATCTTC	GCTGCCCCCTCAGCTTGAG
ANXA1	GGCCTGCTTGAGGAATTCTG	GAGAAGAATTTCTCTTTAGTTCTTTGC
PPAR α	CGGTGACTTATCCTGTGGTCC	CCGCAGATTCTACATTCGATGTT
CPT1b	CATGTATCGCCGTAAACTGGAC	TGGTAGGAGCACATAGGCACT
FPR2	TTCACGGCCACATTACCATTC	AATCCAAGGTCCGACGATCAC
β -ACTIN	GAAGTGTGACGTGGACATCC	CCGATCCACACGGAGTACTT
Mouse		
Cpt1b	AAGAGACCCCGTAGCCATCAT	GACCCAAAACAGTATCCCAATCA
Ppar α	GCAGCTCGTACAGGTCATCA	CTCTTCATCCCCAAGCGTAG
Hsl	CAACGGATACCGTAGTTTGGTG	CGGGATTTGTGTAGTAGGTGTG
Fabp4	AAGGTGAAGAGCATCATAACCCT	TCACGCCTTTCATAACACATTCC
Ngal	CCATCTATGAGCTACAAGAGAAC AAT	TCTGATCCAGTAGCGACAGC
Kim-1	GGAATCCCATCCCATACTCCT	AAGTATGTACCTGGTGATAGCCAC
α -SMA	GAGGCACCACTGAACCCTAA	CATCTCCAGAGTCCAGCACA
Colla1	ATCTCCTGGTGCTGATGGAC	ACCTTGTTTGCCAGGTTCAC
Fn	CGAGGTGACAGAGACCACAA	CTGGAGTCAAGCCAGACACA
β -Actin	TCCATCATGAAGTGTGACGT	GAGCAATGATCTTGATCTTCAT

Supplemental Table 2. Physical and biochemical parameters of experimental animals

Variable	SFD WT	SFD KO	HFD/STZ WT	HFD/STZ KO
Weight [g]	26.23±1.37	29.72±1.57	27.15±0.85	28.22±1.92
Kidney weight [g]	0.19±0.01	0.19±0.01	0.19±0.02	0.21±0.01
Kidney/body weight	0.73±0.09	0.65±0.03	0.69±0.08	0.74±0.04
Blood glucose [mmol/L]	7.00±0.87	7.01±0.69	20.99±2.49	21.55±1.09 ^{b) c)}
Total cholesterol [mmol/L]	2.60±0.87	2.98±1.58	3.16±1.50	3.52±1.21
Triglycerides [mmol/L]	0.39±0.15	0.21±0.16	0.68±0.31	0.76±0.33 ^{b)}
uACR [μg/mg]	74.92±9.33	99.25±10.63	201.69±35.18	342.84±137.73 a) b) c)

[Abbreviations] Data are shown as mean±s.d. n = 6 per group. HFD/STZ, high-fat diet plus streptozotocin; KO, knockout; SFD, standard fat diet; uACR, urine albumin to creatinine ratio; WT, wild type.

^{a)} ANOVA $P < 0.05$ versus HFD/STZ WT mice .

^{b)} ANOVA $P < 0.05$ versus SFD *Anxa1*^{-/-} mice.

^{c)} ANOVA $P < 0.05$ versus SFD WT mice.

Supplemental Table 3. mRNA levels of genes involved in fatty acid metabolism in kidney cortex of HFD/STZ *Anxa1*^{-/-} and HFD/STZ WT mice

Gene Name	Gene Symbol	Log2 Fold Change	FDR
carnitine palmitoyltransferase 2	Cpt2	-0.01147	8.80670E-01
carnitine palmitoyltransferase 1a, liver	Cpt1a	-0.04556	6.61852E-01
acyl-coenzyme A oxidase 1, palmitoyl	Acox1	-0.19395	6.50257E-02
acyl-coenzyme A dehydrogenase, very long chain	Acadvl	-0.25458	1.72328E-02
enoyl-coenzyme A delta isomerase 1	Eci1	-0.35260	4.59422E-02
acyl-coenzyme A synthetase long-chain family member 4	Acsl4	-0.44855	3.36325E-03
forkhead box O1	Foxo1	-0.52295	8.53064E-04
acetyl-coenzyme A carboxylase beta	Acacb	-0.70782	5.02068E-04
carnitine palmitoyltransferase 1b, muscle	Cpt1b	-0.96139	2.99789E-05
patatin-like phospholipase domain containing 2	Pnpla2	-1.00141	2.09850E-05
perilipin 5	Plin5	-1.05785	3.30357E-06
alkylglycerol monooxygenase	Agmo	-1.09698	5.61022E-04
lipase, hormone sensitive	Lipe	-1.27720	8.43239E-05
CCAAT/enhancer binding protein (C/EBP), beta	Cebpb	-1.38587	4.72826E-03
serine palmitoyltransferase, long chain base subunit 3	Sptlc3	-1.84188	5.86523E-04
phosphatase, orphan 1	Phospho1	-1.90859	1.16910E-19
pyruvate dehydrogenase kinase, isoenzyme 4	Pdk4	-2.03020	8.54242E-04
fatty acid binding protein 4, adipocyte	Fabp4	-2.03357	1.86983E-03
alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide	Adh7	-2.56037	1.74130E-04
adiponectin, C1Q and collagen domain containing	Adipoq	-2.63079	3.55370E-03
cell death-inducing DFFA-like effector c	Cidec	-2.65977	1.64128E-04
perilipin 1	Plin1	-2.76269	1.89866E-03
adipogenin	Adig	-3.14269	1.05593E-03
uncoupling protein 3 (mitochondrial, proton carrier)	Ucp3	-3.36105	2.03715E-04
adrenergic receptor, beta 3	Adrb3	-3.68813	1.74211E-07
cell death-inducing DNA fragmentation factor, alpha subunit-like effector A	Cidea	-5.00330	1.95223E-05
uncoupling protein 1 (mitochondrial, proton carrier)	Ucp1	-6.82017	4.97374E-08

Supplemental Table 4. Raw data of triglycerides detected in kidney of HFD/STZ *Anx1*^{-/-} and HFD/STZ WT mice

Lipid species	HFD/STZ WT	HFD/STZ KO	P value
TAG42:0(14:0)	1.54±0.91	2.34±0.91	0.196
TAG44:2(16:1)	0.66±0.41	1.47±0.65	0.039
TAG44:1(14:1)	1.07±0.68	1.53±0.74	0.332
TAG44:1(14:0)	1.43±0.91	3.07±1.71	0.086
TAG44:1(16:1)	1.32±1.13	3.06±1.31	0.048
TAG44:1(16:0)	3.59±2.55	8.79±5.8	0.096
TAG46:3(18:2)	1.87±1.41	4.46±1.66	0.024
TAG46:3(16:1)	1.87±1.15	3.31±2.13	0.213
TAG46:2(18:2)	4.96±3.28	15.25±10.75	0.068
TAG46:2(16:0)	7.57±5.23	22.1±15.16	0.07
TAG46:2(16:1)	3.45±2.2	10.17±4.15	0.01
TAG46:2(16:2)	1.32±1.39	1.46±1.38	0.876
TAG46:1(14:1)	3.27±2.83	6.24±2.8	0.126
TAG46:1(16:0)	17.32±12.7	50.17±40.13	0.112
TAG46:1(16:1)	7.77±5.89	19.08±9.03	0.041
TAG48:4(18:2)	2.99±3.12	6.12±3.73	0.181
TAG48:4(16:2)	1.97±1.52	1.85±0.43	0.863
TAG48:4(16:1)	1.77±1.87	2.22±1.02	0.644
TAG48:3(18:2)	10.25±6.81	24.99±10.8	0.027
TAG48:3(18:1)	6.35±4.66	16.43±7.52	0.029
TAG48:3(16:1)	10.44±7.57	22.07±11.71	0.092
TAG48:3(16:0)	6.96±7.36	11.49±5.92	0.308
TAG48:2(18:2)	41.83±27.55	102.03±74.38	0.121
TAG48:2(18:1)	22.9±17.52	63.99±28.45	0.02
TAG48:2(16:1)	30.83±21.66	82.12±44.06	0.042
TAG48:2(16:0)	62.65±42.14	161.42±102.3	0.074
TAG48:1(18:0)	4.48±2.76	13.96±11.73	0.109
TAG48:1(18:1)	76.58±51.95	218.08±164.46	0.096
TAG48:1(16:0)	191.85±131.08	603.02±451.45	0.079
TAG48:1(16:1)	60.79±41.35	159.91±109.64	0.088
TAG48:0(16:0)	384.05±208.96	1445.62±1215.36	0.083
TAG50:4(18:2)	29.51±21.93	58.52±29.46	0.108
TAG50:4(16:2)	8.41±8.59	10.64±6.75	0.658
TAG50:3(18:2)	125.73±84.47	314.74±195.05	0.075
TAG50:3(18:1)	77.92±59.89	203.28±119.32	0.062
TAG50:3(16:0)	116.72±73.16	350.66±223.64	0.05

TAG50:3(16:1)	123.67±90.88	360.83±205.67	0.04
TAG50:3(16:2)	12.59±11.45	21.42±13.15	0.284
TAG50:2(18:2)	310.81±172.73	1222.31±1078.89	0.092
TAG50:2(18:1)	294.28±283.12	888.32±656.13	0.093
TAG50:2(16:0)	946.41±678.28	3409.42±2918.03	0.096
TAG50:2(16:1)	206.49±191.92	635.85±486.71	0.096
TAG50:2(16:2)	1.28±1.13	1.78±1.5	0.568
TAG50:1(16:1)	9.97±7.83	43.33±35.3	0.066
TAG50:1(16:0)	1459.2±1084.39	5157.83±3990.99	0.073
TAG50:1(18:0)	28.06±24.02	113.04±93.44	0.077
TAG50:1(18:1)	624.34±421.4	2351.88±1982.57	0.086
TAG50:0(18:0)	34.78±17.77	159.34±138.39	0.074
TAG51:3(17:1)	5.13±3.77	15.67±10.31	0.057
TAG51:3(15:0)	4.67±4.25	12.54±9	0.108
TAG51:2(17:0)	8.18±6.03	27.05±22.32	0.098
TAG51:2(17:1)	8.32±6.47	26.63±19.15	0.07
TAG51:2(15:0)	6.7±4.62	19.19±15.43	0.114
TAG51:0(17:0)	10.09±7.25	39.08±32.87	0.083
TAG52:6(18:3)	2.45±1.3	6.66±4.69	0.082
TAG52:6(16:1)	2.04±1.42	4.97±2.02	0.024
TAG52:6(16:2)	2.7±2.47	2.57±1.27	0.921
TAG52:6(16:3)	2.32±1.53	3.23±1.5	0.369
TAG52:5(18:2)	38.64±24.41	95.76±57.13	0.067
TAG52:5(18:3)	15.85±9.83	45.37±30.7	0.068
TAG52:5(16:0)	12.72±7.5	38.81±29.83	0.087
TAG52:5(16:1)	20.44±13.33	49.82±25.02	0.043
TAG52:5(16:2)	5.65±4.55	9.15±5.23	0.286
TAG52:5(16:3)	1.16±0.68	2.61±1.52	0.081
TAG52:4(16:0)	164.46±102.61	539.21±421.53	0.082
TAG52:4(16:1)	110.58±92.05	296.4±161.72	0.05
TAG52:4(16:2)	7.15±5.07	16.17±11.49	0.14
TAG52:4(18:1)	141.95±109.03	394.01±254.77	0.069
TAG52:4(18:2)	343.06±229.6	960.4±674.4	0.081
TAG52:4(18:3)	34.47±20.26	114.76±88.6	0.076
TAG52:3(16:0)	1001.77±859.24	3502.4±2909.84	0.095
TAG52:3(16:1)	198.25±213.28	587.07±453.69	0.114
TAG52:3(16:2)	2.24±1.94	7.88±6.72	0.101
TAG52:3(18:1)	1245.79±1125.24	4198.65±3050.66	0.07
TAG52:3(18:2)	863.85±789.44	2995.88±2333.04	0.082
TAG52:2(18:2)	139.23±95.55	672.61±619.08	0.086

TAG52:2(18:1)	2533.89±2358	7402.83±5013.11	0.078
TAG52:2(16:0)	1527.06±1465.12	4914.19±3778.95	0.091
TAG52:2(16:1)	38.72±37.57	137.57±113.18	0.093
TAG52:1(18:1)	163.49±107.33	633.98±509.37	0.071
TAG52:1(16:1)	1.22±0.76	3.54±2.99	0.124
TAG52:0(18:0)	13.54±7.01	59.69±49.59	0.066
TAG53:5(18:3)	2.06±2.55	1.95±1.09	0.932
TAG53:5(17:1)	3.72±4.54	6.32±4.2	0.369
TAG53:5(17:2)	2.62±2.62	4.77±3.7	0.314
TAG53:4(19:2)	0.92±0.65	1.7±0.89	0.145
TAG53:4(18:3)	1.83±1.8	2.57±1.52	0.497
TAG53:4(17:0)	3.69±2.66	9.02±6.3	0.112
TAG53:4(17:1)	10.62±8.01	21.72±11.97	0.116
TAG53:4(17:2)	2.28±2.04	5.49±4.4	0.17
TAG53:3(19:1)	4.24±3.61	12.54±7.77	0.055
TAG53:3(19:2)	2.37±1.89	4.17±1.82	0.156
TAG53:3(17:0)	14.61±12.13	44.71±37.55	0.119
TAG53:3(17:1)	13.43±10.62	36.96±21.48	0.053
TAG53:3(16:0)	6.55±4.31	12.62±10.45	0.258
TAG53:2(19:0)	1.36±1.48	5.44±4.02	0.059
TAG53:2(19:1)	6.13±5.01	14.33±10.57	0.148
TAG53:2(17:0)	14.17±12.74	44.94±34.74	0.093
TAG53:2(17:1)	2.09±1.4	6.52±4.74	0.073
TAG53:2(16:0)	7.49±8.04	18.44±12.54	0.131
TAG53:1(19:0)	2.32±2.13	6.59±4.63	0.09
TAG54:8(20:4)	1.5±0.99	1.67±1.01	0.795
TAG54:8(18:3)	0.81±0.58	2.42±1.23	0.025
TAG54:8(22:6)	4.42±4.56	3.5±1.4	0.674
TAG54:7(22:6)	11.94±10.74	12.42±7.92	0.938
TAG54:7(20:4)	2.81±1.47	6.46±2.53	0.019
TAG54:7(20:5)	2.32±1.43	4.97±2.19	0.047
TAG54:7(18:2)	5.34±3.65	19.05±12.99	0.046
TAG54:7(18:3)	5.25±3.56	15.71±10.14	0.054
TAG54:6(20:4)	19.57±11.15	55.92±37.38	0.064
TAG54:6(18:2)	77.38±56.71	240.11±179.87	0.083
TAG54:6(18:3)	20.42±16.71	77.06±66.62	0.095
TAG54:5(20:4)	38.59±29.28	125.39±108.83	0.116
TAG54:5(18:1)	332.4±333.74	1105.98±931.51	0.111
TAG54:5(18:2)	463.43±488.23	1535.18±1324.57	0.12
TAG54:5(18:3)	42.45±39.17	163.16±140.28	0.094

TAG54:5(16:0)	22.72±13.98	71.38±59.3	0.104
TAG54:5(16:1)	4.81±4.48	13±6.1	0.036
TAG54:5(16:2)	1.11±1.07	2.74±2.46	0.204
TAG54:4(18:3)	11.9±10.38	64.98±62.64	0.091
TAG54:4(18:2)	1220.85±1530.47	4469.76±4228.08	0.137
TAG54:4(18:1)	2572.55±3381.67	8378.49±6956.07	0.124
TAG54:4(18:0)	71.03±65.59	369.18±346.15	0.088
TAG54:4(16:0)	32.35±31.81	134.4±119.62	0.095
TAG54:4(16:1)	9.32±12.83	28.17±22.03	0.129
TAG54:4(16:2)	1.75±2.61	6.54±5.28	0.099
TAG54:3(16:0)	65.03±84.66	234.38±211.74	0.128
TAG54:3(16:1)	11.03±15.74	29.42±26.02	0.206
TAG54:3(18:1)	4143.72±5400.16	11897.97±8966.47	0.129
TAG54:3(18:2)	352.75±393.03	1649.89±1493.63	0.09
TAG54:2(18:2)	20.57±16.42	124.13±113.86	0.072
TAG54:2(18:1)	690.6±737.72	2625.49±2356.83	0.11
TAG54:2(18:0)	410.35±399.13	1693.96±1519.42	0.098
TAG54:1(18:0)	85.67±63.49	385±331.07	0.075
TAG54:1(18:1)	35.98±28.49	174.51±143.28	0.06
TAG54:0(18:0)	5.2±3.58	16.76±10.24	0.039
TAG55:5(19:1)	1.24±0.99	4.85±3.82	0.068
TAG55:5(19:2)	1.35±1.57	3.91±3.45	0.162
TAG55:5(19:3)	0.56±0.61	1.68±0.56	0.013
TAG55:5(18:2)	4.48±3.65	8.56±5.65	0.205
TAG55:5(18:3)	0.77±0.51	1.76±0.97	0.071
TAG55:4(19:0)	0.99±1.04	3.02±2.53	0.127
TAG55:4(19:1)	7.61±10.5	22.07±17.09	0.138
TAG55:4(19:2)	1.8±1.72	5.07±4.76	0.179
TAG55:4(18:1)	9.12±8.08	27.16±20.45	0.096
TAG55:4(18:2)	8.02±8.28	24.54±17.27	0.083
TAG55:3(19:0)	4.64±5.49	14.08±11.17	0.121
TAG55:3(19:1)	10.73±12.19	33.13±24.84	0.1
TAG55:3(18:1)	21.4±24.06	65.54±47.29	0.093
TAG55:3(17:0)	0.85±0.89	2.7±1.82	0.068
TAG55:2(19:0)	6.68±8.05	19.82±15.44	0.122
TAG55:2(17:0)	1.13±0.8	2.79±1.66	0.072
TAG56:8(22:6)	25.48±21.9	22.45±13.63	0.799
TAG56:8(22:5)	1.66±1.05	2.19±1.95	0.604
TAG56:8(18:2)	7.85±5.35	9.84±5.08	0.559
TAG56:7(22:6)	38.79±27.97	62.93±46.49	0.343

TAG56:7(22:5)	8.92±5.92	19.31±11.8	0.109
TAG56:7(20:4)	14.88±10.68	46.68±33.27	0.069
TAG56:7(20:5)	1.85±1.72	4.17±2.01	0.078
TAG56:7(18:2)	10.63±6.72	23.39±16	0.131
TAG56:7(18:3)	1.16±1.01	2.85±2.29	0.163
TAG56:6(22:5)	14.97±8.93	48.44±36.4	0.074
TAG56:6(22:4)	5.04±4.33	13.04±8.15	0.081
TAG56:6(20:1)	0.66±0.61	2.97±2.38	0.061
TAG56:6(20:2)	3.11±2.76	10.9±8.79	0.088
TAG56:6(20:3)	13.17±14.93	33.74±24.87	0.144
TAG56:6(20:4)	31.71±29.5	109.41±89.88	0.096
TAG56:6(18:2)	15.94±12.15	47.54±34	0.079
TAG56:6(18:3)	1.27±1.1	4.65±2.86	0.033
TAG56:5(22:4)	8.73±8.85	25.34±17.13	0.083
TAG56:5(22:1)	1.55±1.14	3.32±0.76	0.016
TAG56:5(20:0)	0.25±0.23	1.25±0.68	0.01
TAG56:5(20:1)	13.12±17.45	36.95±28.05	0.138
TAG56:5(20:2)	24.4±31.79	74.38±58.74	0.125
TAG56:5(20:3)	23.17±28.29	68.35±52.97	0.123
TAG56:5(18:1)	59.83±75.9	180.66±141.02	0.122
TAG56:5(18:2)	33.28±42.99	119.56±96.98	0.099
TAG56:5(18:3)	2.05±2.06	7.26±5.65	0.082
TAG56:4(22:1)	1.85±1.63	3.97±1.93	0.09
TAG56:4(20:0)	2.79±2.64	7.92±6.44	0.13
TAG56:4(20:1)	82.26±122.29	231.27±189.93	0.171
TAG56:4(20:2)	42.79±54.37	147.6±128.42	0.124
TAG56:4(19:1)	0.96±0.78	2.46±1	0.025
TAG56:4(18:1)	139.65±194.69	395.07±324.31	0.162
TAG56:4(18:2)	70.38±101.62	208.81±177.19	0.161
TAG56:4(18:3)	1.04±0.81	2.98±2.63	0.145
TAG56:3(22:0)	0.33±0.66	0.55±0.57	0.585
TAG56:3(22:1)	1.01±1.46	4.29±3.07	0.056
TAG56:3(20:0)	7.89±8.18	27.49±22.58	0.098
TAG56:3(20:1)	66.81±83.87	175.52±135.08	0.157
TAG56:3(18:1)	111.46±147.77	312.08±262.75	0.168
TAG56:3(18:2)	13.38±15.41	43.01±32.37	0.094
TAG56:2(22:1)	2.57±2.81	7.42±6	0.133
TAG56:2(20:1)	12.92±12.53	39.47±30.66	0.103
TAG56:2(18:1)	29.5±34.46	87.33±73.09	0.141
TAG56:1(22:0)	1.83±1.64	3.7±2.46	0.189

TAG56:1(18:1)	2.86±2.65	7.08±5.63	0.161
TAG58:9(22:6)	9.59±6.94	13.05±6.03	0.42
TAG58:9(22:5)	2.38±1.38	4.34±2.55	0.161
TAG58:8(22:6)	14.07±9.84	22.75±15.57	0.317
TAG58:8(22:5)	9.1±5.37	19.18±12.79	0.135
TAG58:8(20:4)	2.94±1.72	2.84±1.92	0.935
TAG58:7(22:6)	10.51±6.48	19.71±13.52	0.2
TAG58:7(22:5)	12.48±11.17	33.17±26.73	0.141
TAG58:7(20:4)	2.16±1.54	3.79±2.72	0.271
TAG58:5(22:1)	0.98±1.27	3.56±1.67	0.021
TAG58:5(20:1)	1.31±1.46	4.04±3.32	0.123
TAG58:5(18:2)	2.3±3.13	7.16±5.72	0.127
TAG58:4(22:0)	0.34±0.23	1.14±1.03	0.121
TAG58:4(22:1)	2.72±3.5	6.62±5.5	0.21
TAG58:4(18:1)	5.33±7.45	10.7±8.6	0.316
TAG58:4(18:2)	2.9±3.5	7.47±6.9	0.216
TAG58:4(18:3)	0.06±0.11	0.23±0.23	0.178
TAG58:3(18:2)	1.51±1.24	4.89±4.19	0.114
TAG58:3(18:1)	6.8±9.95	17.79±15.25	0.207
TAG58:3(22:1)	3.06±3.92	8.32±6.83	0.166
TAG58:3(24:1)	0.8±1.01	1.9±1.1	0.13
TAG58:3(24:0)	0.07±0.07	0.06±0.08	0.795
TAG58:2(24:0)	0.44±0.36	1.52±1.62	0.176
TAG58:2(24:1)	0.55±0.52	1.64±1.55	0.166
TAG58:2(22:1)	1.34±1.13	1.9±1.61	0.541
TAG58:2(18:1)	3.4±4.12	10.34±6.73	0.077
TAG60:5(18:1)	0.58±0.34	2.45±0.78	0.001
TAG60:5(18:2)	0.4±0.26	1.15±0.82	0.081
TAG60:5(18:3)	0.3±0.21	0.43±0.23	0.397
TAG60:4(24:1)	0.55±0.71	1.68±1.36	0.131
TAG60:4(18:1)	1.3±1.3	4.54±2.8	0.041
TAG60:4(18:2)	0.68±0.62	2.67±1.74	0.036
TAG60:3(24:0)	0.49±0.38	1.68±1.4	0.099
TAG60:3(24:1)	1.02±1.04	2.56±2.07	0.168
TAG60:3(18:1)	2.31±2.48	5.82±4.06	0.13
TAG60:3(18:2)	0.96±0.99	1.36±1.22	0.583