

Supplementary material

Correlation of Serum Acylcarnitines with clinical presentation and severity of Coronary Artery Disease

Olga Deda^{†1,2*}, Eleftherios Panteris^{†1,2*}, Thomas Meikopoulos^{2,3}, Olga Begou^{2,3}, Thomai Mouskeftara^{1,2}, Efstratios Karagiannidis⁴, Andreas S. Papazoglou⁴, Georgios Sianos⁴, Georgios Theodoridis^{2,3}, Helen Gika^{1,2*}

¹Laboratory of Forensic Medicine and Toxicology, School of Medicine, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece

oliadmy@gmail.com, eleftherios.panteris@gmail.com, gkikae@auth.gr

²Biomic_AUTH, CIRI-AUTH Center for Interdisciplinary Research and Innovation Aristotle University of Thessaloniki, 57001, Thessaloniki, Greece

thomas_meik@hotmail.com, olina_18@hotmail.com, mouskeftara32@gmail.com, gtheodor@chem.auth.gr

³Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece

⁴First Department of Cardiology, AHEPA University Hospital, Aristotle University of Thessaloniki, St. Kiriakidi 1, 54636, Thessaloniki, Greece

stratoskarag@gmail.com, anpapazoglou@yahoo.com, gsianos@auth.gr

[†]Equal contribution

*Correspondence: oliadmy@gmail.com (O.D.); eleftherios.panteris@gmail.com (E.P), gkikae@auth.gr (H.G.)

Table S1. Median values (µg/L) of all measured acylcarnitines and 95% CIs for ACS and CCS patients. Statistically significant analogues are shown in bold.

	CCS			ACS			Mann-Whitney U test
	Median	↓95.0% CIs	↑95.0% CIs	Median	↓95.0% CIs	↑95.0% CIs	p
C2	2,970.44	2,846.84	3,111.70	2,933.31	2,761.05	3,059.40	0.949
C3	176.75	169.34	185.38	175.42	166.99	181.98	0.608
C4	38.65	36.74	41.17	38.76	36.31	40.90	0.736
C5	25.71	24.95	27.50	26.41	25.16	28.32	0.251
C6	29.98	28.79	31.46	28.51	26.86	29.77	0.265
C8	63.06	58.68	68.55	54.75	51.21	57.68	0.012
C10	106.12	96.74	116.37	88.51	83.51	93.92	0.007
C12	29.89	28.30	31.39	27.76	25.20	29.04	0.072
C14	19.23	18.51	19.79	17.98	17.25	19.01	0.061
C16	63.21	60.85	65.63	59.97	57.66	61.97	0.018
C18	19.01	18.35	19.52	18.27	17.63	18.84	0.060
C18:1	92.54	88.15	97.76	84.43	80.05	89.91	0.011
C18:2	60.22	57.89	63.29	51.89	50.44	54.67	<0.001

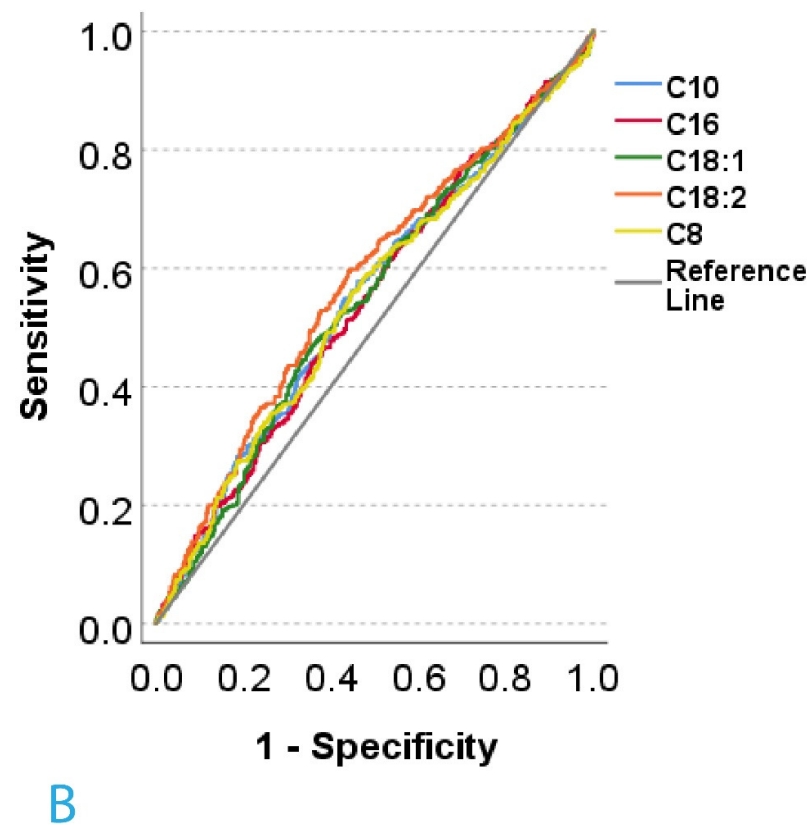
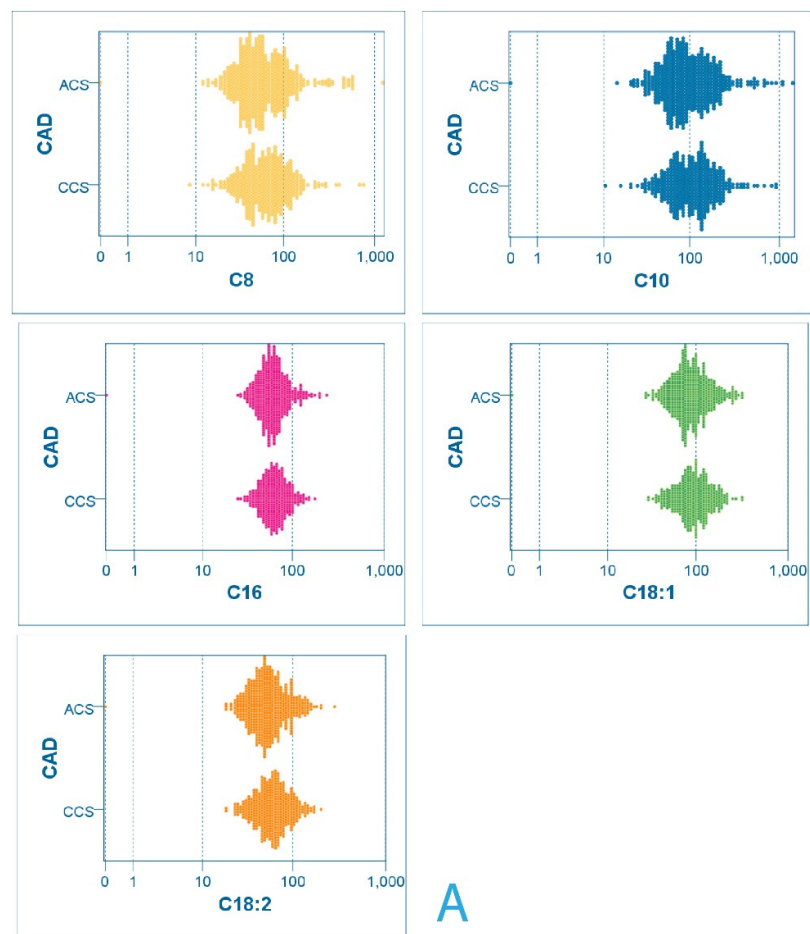


Figure S1. A) ACS *vs* CCS, log scaled box-plots of C8, C10, C16, C18.1 and C18.2 levels distribution and B) their respective ROC curves. Acylcarnitine C18.2 presented the highest, yet weak, discriminatory power AUC=0.576 (95% CI 0.539–0.612, $p < 0.001$).

Table S2. Diabetes Mellitus (DM) ROC areas for acylcarnitine C2, C3, C4 and C8.

Area Under the ROC Curve					
Test Result Variable(s)	Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Intervals	
				↓95.0% CIs	↑95.0% CIs
C2	0.591	0.019	0.000	0.553	0.629
C3	0.576	0.019	0.000	0.538	0.614
C4	0.544	0.020	0.029	0.505	0.583
C8	0.541	0.020	0.040	0.502	0.580
a. Under the nonparametric assumption					
b. Null hypothesis: true area = 0.5					

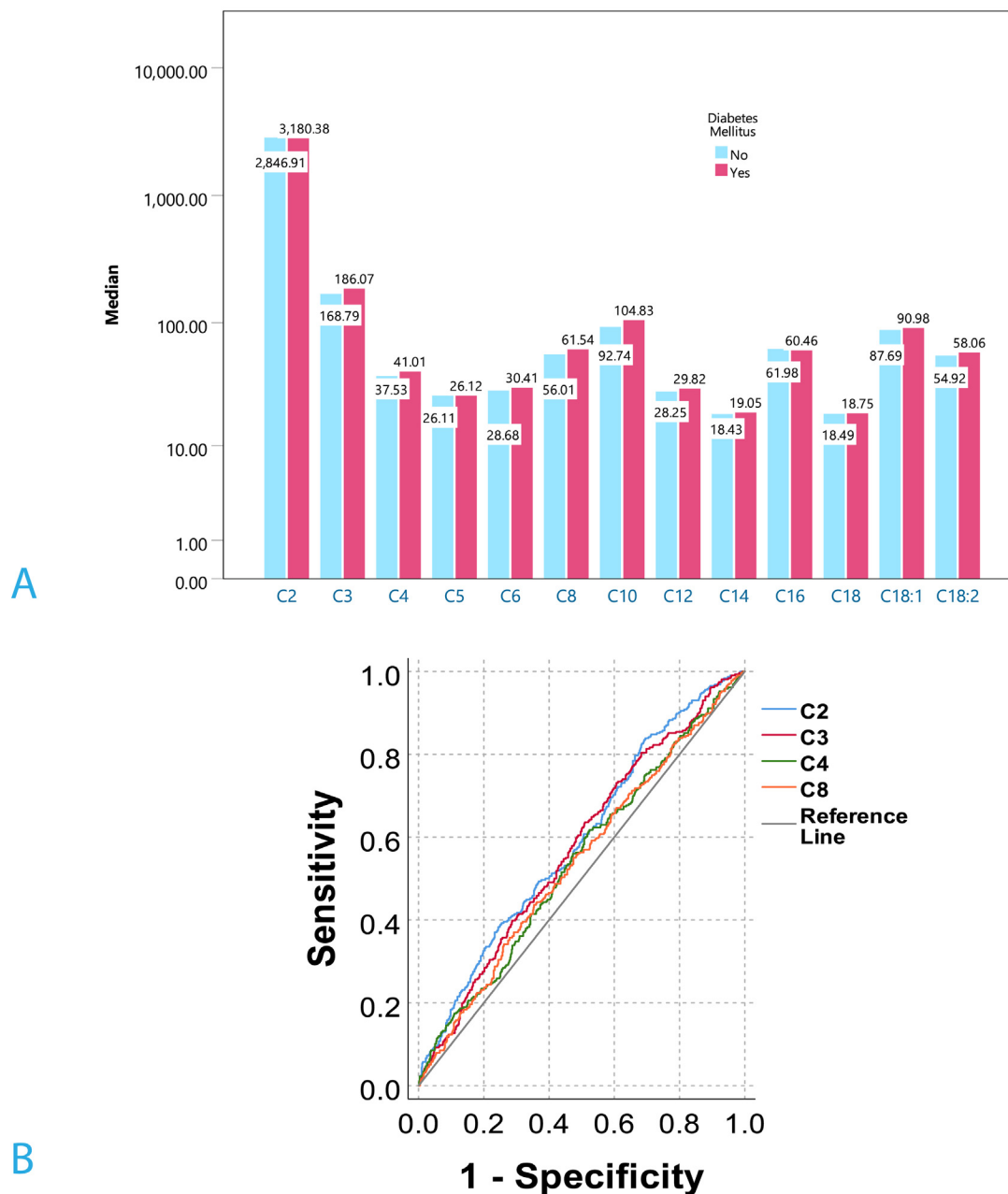


Figure S2. A) Log scaled bar graph for acylcarnitine median levels of DM and non-DM patients and B) ROC curves for C2, C3, C4, C8. Acylcarnitine C2 has the highest discriminatory power.

Table S3. Diabetes Mellitus (DM) ROC areas for acylcarnitines C2, C3, C4 and C8.

Area Under the ROC Curve					
Test Result Variable(s)	Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Intervals	
				↓95.0% CIs	↑95.0% CIs
C2	0.591	0.019	0.000	0.553	0.629
C3	0.576	0.019	0.000	0.538	0.614
C4	0.544	0.020	0.029	0.505	0.583
C8	0.541	0.020	0.040	0.502	0.580
a. Under the nonparametric assumption					
b. Null hypothesis: true area = 0.5					

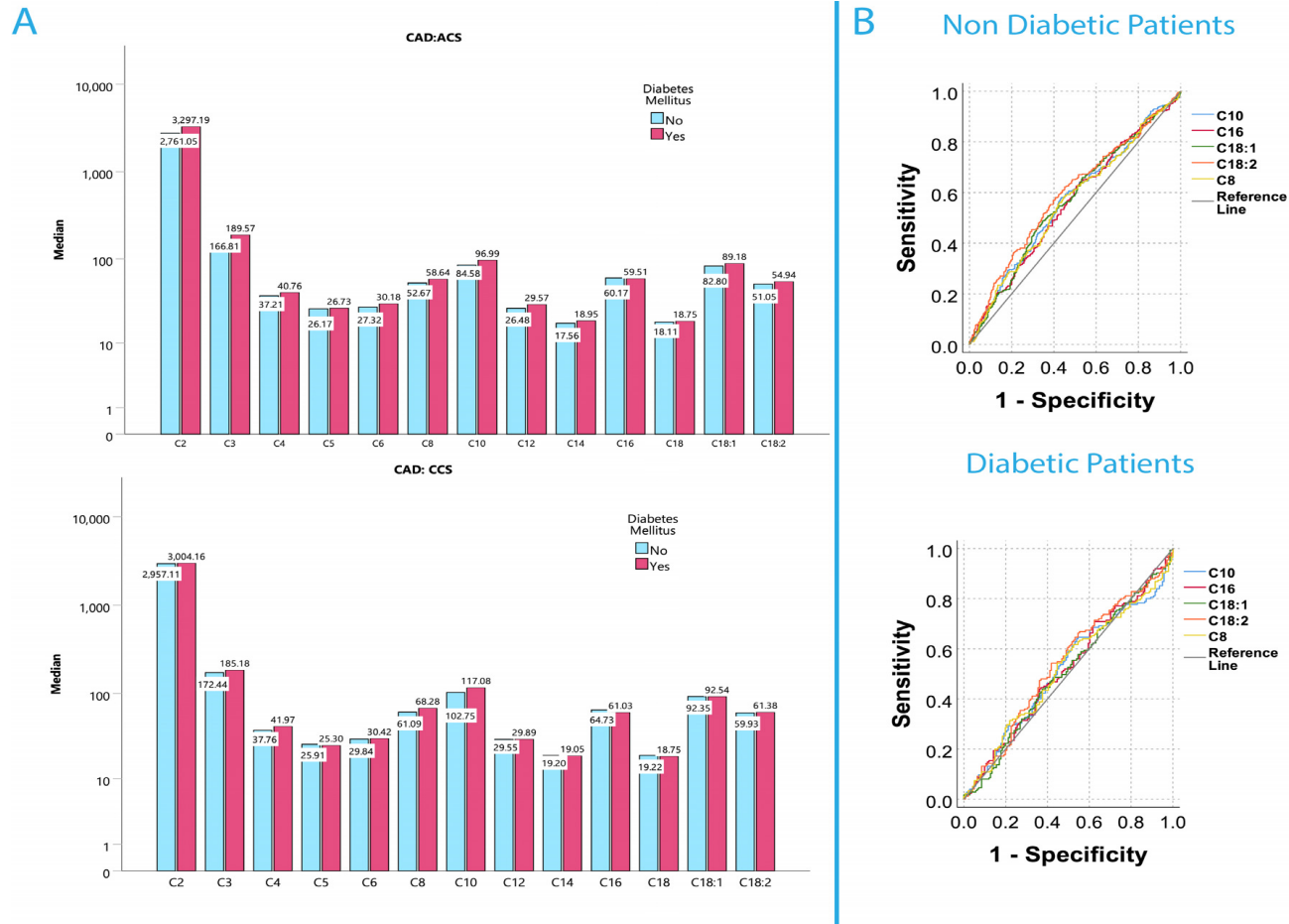


Figure S3. A) Log scaled bar graphs for acylcarnitine median levels for both ACS and CCS, DM and non-DM patients and B) ROC analysis of C8, C10, C16, C18.1 and C18.2 showed that for non-diabetics, AUCs were improved especially for C18.2.

Table S5. Acylarnitine levels (µg/ L) for CAD groups

CAD Groups					Kruskal Wallis
		Median	↓95.0% CIs	↑95.0% CIs	p value*
C2	NSTEMI	2,906.50	2,649.44	3,154.25	0.219
	STEMI	2,770.56	2,539.30	3,045.37	
	UA	3,147.27	2,839.91	3,452.40	
	SA	2,969.54	2,846.84	3,111.70	
C3	NSTEMI	168.35	151.99	182.39	0.824
	STEMI	173.55	157.15	191.74	
	UA	181.15	167.93	196.96	
	SA	176.80	169.84	185.38	
C4	NSTEMI	39.92	36.20	44.81	0.782
	STEMI	38.10	35.60	42.41	
	UA	37.99	34.83	43.02	
	SA	38.62	36.74	40.81	
C5	NSTEMI	24.79	23.46	28.80	0.026
	STEMI	29.08	26.36	30.73	
	UA	25.13	22.86	27.70	
	SA	25.72	24.95	27.50	
C6	NSTEMI	27.46	25.83	31.00	0.329
	STEMI	27.58	25.65	29.10	
	UA	30.69	27.77	33.83	
	SA	29.98	28.87	31.46	
C8	NSTEMI	54.66	49.00	60.09	0.053
	STEMI	52.99	49.11	58.13	
	UA	56.60	51.90	66.67	
	SA	63.06	58.68	68.55	
C10	NSTEMI	89.88	78.25	105.42	0.019
	STEMI	86.53	79.36	94.45	
	UA	91.50	83.49	110.42	
	SA	106.09	96.74	116.37	
C12	NSTEMI	26.54	23.70	30.20	0.377
	STEMI	27.96	25.11	29.83	
	UA	29.17	24.86	31.54	
	SA	29.88	28.26	31.39	
C14	NSTEMI	17.51	16.27	19.55	0.301
	STEMI	17.78	17.04	18.86	
	UA	19.28	17.52	20.69	
	SA	19.17	18.43	19.79	
C16	NSTEMI	58.38	55.03	63.64	0.012
	STEMI	58.29	55.82	60.89	
	SA	63.21	60.85	65.63	
	UA	62.90	60.82	66.52	
C18	NSTEMI	17.35	16.09	18.35	0.137
	STEMI	18.63	17.76	19.54	
	UA	18.63	17.49	19.29	
	SA	19.01	18.45	19.59	
C18:1	NSTEMI	85.82	78.64	94.91	0.013
	STEMI	82.80	76.43	88.61	
	SA	92.53	88.15	97.76	
	UA	91.30	79.31	97.06	
C18:2	NSTEMI	54.86	50.74	59.54	<0.001
	STEMI	50.26	47.34	52.41	
	SA	60.21	57.89	63.29	
	UA	53.75	50.00	60.48	

* Bonferroni corrected

Table S6. Acylcarnitines levels (µg/L) and Syntax Score groups.

Syntax Groups		Median	↓95.0% CIs	↑95.0% CIs	Kruskal Wallis (Pair) p value
C2	0 (a)	3,013	2,848.26	3,146.54	NS
	1-22 (b)	2,841	2,652.45	3,047.79	NS
	>22 (c)	2,951.55	2,798.57	3,325.86	NS
C3	0 (a)	171.49	162.86	179.56	NS
	1-22 (b)	175.68	167.86	184.94	NS
	>22 (c)	186.56	170.45	209.04	NS
C4	0 (a)	36.96	34.21	40.46	(a-c) 0.002
	1-22 (b)	37.95	35.60	40.18	(b-c) 0.005
	>22 (c)	45.16	38.94	49.61	
C5	0 (a)	25.25	23.99	26.36	(a-c) 0.024
	1-22 (b)	26.41	24.95	28.63	
	>22 (c)	27.82	25.34	30.79	
C6	0 (a)	29.81	28.07	33.04	NS
	1-22 (b)	28.89	27.63	30.08	NS
	>22 (c)	28.88	27.10	31.34	NS
C8	0 (a)	60.09	56.03	66.36	NS
	1-22 (b)	57.65	53.41	61.45	NS
	>22 (c)	55.13	49.58	62.70	NS
C10	0 (a)	99.63	90.03	109.52	NS
	1-22 (b)	94.48	86.47	101.15	NS
	>22 (c)	92.32	80.54	104.81	NS
C12	0 (a)	29.55	27.41	31.17	NS
	1-22 (b)	28.53	26.63	30.33	NS
	>22 (c)	27.73	25.11	29.71	NS
C14	0 (a)	19.24	18.12	20.36	NS
	1-22 (b)	18.54	17.59	19.27	NS
	>22 (c)	18.21	17.06	19.69	NS
C16	0 (a)	65.18	62.57	67.90	(c-a) 0.031
	1-22 (b)	60.28	57.95	62.48	(b-a) 0.044
	>22 (c)	59.27	56.28	61.94	NS
C18	0 (a)	19.11	18.27	20.08	NS
	1-22 (b)	18.39	17.70	19.03	NS
	>22 (c)	18.18	17.50	19.08	NS
C18:1	0 (a)	91.70	85.27	98.82	NS
	1-22 (b)	88.03	82.80	92.55	NS
	>22 (c)	87.63	80.05	93.49	NS
C18:2	0 (a)	60.48	56.37	64.61	(c-a) 0.019
	1-22 (b)	53.83	51.35	56.62	(b-a) 0.012
	>22 (c)	53.28	49.37	57.57	NS

NS= non-significant, p > 0.05

Table S7. Acylcarnitine levels (μg/L) and SS metrics (Heavy calcification and LVEF%).

Heavy calcification		Median	↓95.0% CIs	↑95.0% CIs	Mann-Whitney P value
C2	No	2,901.34	2,728.44	3,017.08	0.002
	Yes	3,121.71	2,933.37	3,325.86	
C3	No	169.84	165.03	176.21	<0.001
	Yes	200.02	183.58	213.75	
C4	No	36.74	35.23	38.67	<0.001
	Yes	45.77	41.23	50.19	
C5	No	25.55	24.85	26.69	0.002
	Yes	29.08	26.65	30.90	
C6	No	28.78	27.65	29.84	0.006
	Yes	30.33	28.66	32.17	
C8	No	57.71	54.65	60.51	0.104
	Yes	58.01	54.41	67.42	
C10	No	93.40	86.47	100.78	0.149
	Yes	97.62	92.32	113.59	
C12	No	28.70	27.33	29.95	0.468
	Yes	28.58	26.74	30.72	
C14	No	18.64	17.88	19.28	0.651
	Yes	18.96	17.28	20.02	
C16	No	62.34	60.31	64.11	0.365
	Yes	58.64	55.76	61.94	
C18	No	18.76	18.24	19.19	0.207
	Yes	18.10	17.38	19.02	
C18:1	No	87.65	83.89	92.22	0.327
	Yes	91.51	85.58	97.33	
C18:2	No	55.82	54.11	58.50	0.937
	Yes	54.23	51.18	59.94	
LVEF groups		Median	↓95.0% CIs	↑95.0% CIs	Kruskal Wallis (Pair) P value
C2	<40 (a)	3,255.34	3,022.59	4,185.32	(b-c) 0.006
	40-49 (b)	2,639.96	2,416.06	3,044.11	(a-c) 0.002
	>50 (c)	2,905.99	2,700.97	3,021.90	
C3	<40 (a)	206.90	175.46	243.44	(a-c) 0.001
	40-49 (b)	174.62	157.34	206.79	
	>50 (c)	171.25	165.03	178.98	(b-c) 0.006
C4	<40 (a)	52.70	39.71	58.60	(a-c) 0.001
	40-49 (b)	41.15	36.35	49.85	
	>50 (c)	36.60	34.83	38.94	
C5	<40 (a)	29.47	25.09	37.60	(b-c) 0.025
	40-49 (b)	28.92	25.43	31.66	(a-c) 0.006
	>50 (c)	25.13	24.02	26.14	
C6	<40 (a)	34.14	30.30	43.81	(a-b) 0.020
	40-49 (b)	29.25	26.86	32.84	(a-c) <0.001
	>50 (c)	27.81	26.62	29.26	
C8	<40 (a)	59.00	50.92	73.91	NS
	40-49 (b)	58.15	51.21	74.69	NS
	>50 (c)	55.22	51.17	59.64	NS
C10	<40 (a)	95.87	80.14	121.95	NS
	40-49 (b)	98.47	86.44	120.75	NS
	>50 (c)	92.34	84.57	98.89	NS
C12	<40 (a)	29.84	24.63	35.27	NS
	40-49 (b)	28.44	24.46	32.16	NS
	>50 (c)	27.55	26.17	29.02	NS
C14	<40 (a)	19.81	17.72	21.54	NS
	40-49 (b)	18.05	16.75	19.55	NS
	>50 (c)	18.39	17.43	19.14	NS
C16	<40 (a)	57.57	54.59	68.83	NS
	40-49 (b)	59.20	56.10	63.27	NS
	>50 (c)	61.33	58.85	63.55	NS
C18	<40 (a)	17.92	17.10	19.31	NS
	40-49 (b)	19.03	17.06	20.45	NS
	>50 (c)	18.22	17.63	18.76	NS
C18:1	<40 (a)	87.09	76.43	106.84	NS
	40-49 (b)	84.36	76.22	93.48	NS
	>50 (c)	86.04	81.90	90.19	NS
C18:2	<40 (a)	56.04	53.04	63.51	NS
	40-49 (b)	55.39	50.31	60.64	NS
	>50 (c)	53.63	50.74	57.07	NS

Table S8. Linear regression for Syntax Score.

Model Summary ^a										
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	0.404 ^a	0.164	0.157	12.3377	0.164	25.773	7	923	0.000	1.566
a. Predictors: (Constant), STATIN, Sex, Ratio C4/C18.2, CAD Groups, Diabetes Mellitus, Age, Smoking										
b. Dependent Variable: Syntax Score										
Bootstrap for Model Summary										
Model	Durbin-Watson		Bootstrap ^a							
			Bias	Std. Error	95% Confidence Interval					
					Lower		Upper			
1	1.566		-0.534	0.063	0.906		1.152			
a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples										
ANOVA ^a										
Model		Sum of Squares		df	Mean Square		F	Sig.		
1	Regression		27,462.184	7	3,923.169		25.773	0.000 ^b		
	Residual		140,499.070	923	152.220					
	Total		167,961.254	930						
a. Dependent Variable: Syntax Score										
b. Predictors: (Constant), STATIN, Sex, Ratio C4/C18.2, CAD Groups, Diabetes Mellitus, Age, Smoking										
Coefficients ^a										
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B			
		B	Std. Error	Beta			Lower Bound	Upper Bound		
1	(Constant)		5.432	2.85		1.904	0.057	-0.168	11.032	
	Sex		2.968	0.950	0.098	3.126	0.002	1.105	4.832	
	Diabetes Mellitus		2.056	0.438	0.145	4.689	0.000	1.195	2.916	
	Smoking		0.368	0.897	0.014	0.411	0.681	-1.392	2.129	
	Age		0.156	0.036	0.143	4.356	0.000	0.086	0.226	
	Ratio C4/C18.2		2.010	0.473	0.130	4.250	0.000	1.082	2.938	
	CAD Groups		-3.091	0.357	-0.269	-8.657	0.000	-3.791	-2.390	
STATIN		2.192	0.825	0.082	2.656	0.008	0.572	3.811		
a. Dependent Variable: Syntax Score										
Bootstrap for Coefficients										
Model			B	Bootstrap ^a						
				Bias	Std. Error	Sig. (2-tailed)	95% Confidence Interval			
							Lower	Upper		
1	(Constant)		5.432	0.009	2.714	0.038	0.308	10.510		
	Sex		2.968	-0.004	0.988	0.005	1.048	4.883		
	Diabetes Mellitus		2.056	0.002	0.470	0.001	1.150	2.978		
	Smoking		0.368	-0.062	0.848	0.651	-1.428	1.985		
	Age		0.156	-0.001	0.035	0.001	0.087	0.219		
	Ratio C4/C18.2		2.010	0.124	0.613	0.002	1.159	3.587		
	CAD Groups		-3.091	0.007	0.370	0.001	-3.803	-2.334		
STATIN		2.192	-0.007	0.849	0.011	0.489	3.870			
a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples										

Table S9. Acylcarnitine levels ($\mu\text{g/L}$) comparison between CKD and non-CKD patients

	CKD patients (N = 127) vs Non CKDN = 818).						
	Non CKD			CKD			Mann Whitney test
	Median	↓95.0% CIs	↑95.0% CIs	Median	↓95.0% CIs	↑95.0% CIs	p value
C2	2,800.68	2,654.27	2,924.54	4,186.94	3,926.20	4,584.89	<0.001
C3	169.97	164.87	175.74	232.15	209.04	250.07	<0.001
C4	36.74	35.30	38.39	58.42	53.10	67.68	<0.001
C5	25.55	24.82	26.60	35.92	30.03	40.57	<0.001
C6	27.95	26.99	28.87	43.68	39.96	49.39	<0.001
C8	54.41	51.75	56.90	92.21	83.67	100.58	<0.001
C10	89.64	83.69	94.45	153.27	137.40	171.24	<0.001
C12	27.40	26.10	28.60	35.56	32.81	45.20	<0.001
C14	18.15	17.41	18.85	21.87	20.46	23.82	<0.001
C16	60.43	58.47	62.35	66.04	61.84	73.46	0.003
C18	18.35	17.86	18.86	19.24	18.49	21.36	<0.001
C18:1	87.22	83.38	90.38	97.50	91.44	105.93	<0.001
C18:2	54.92	52.75	57.07	60.04	54.99	65.12	0.016

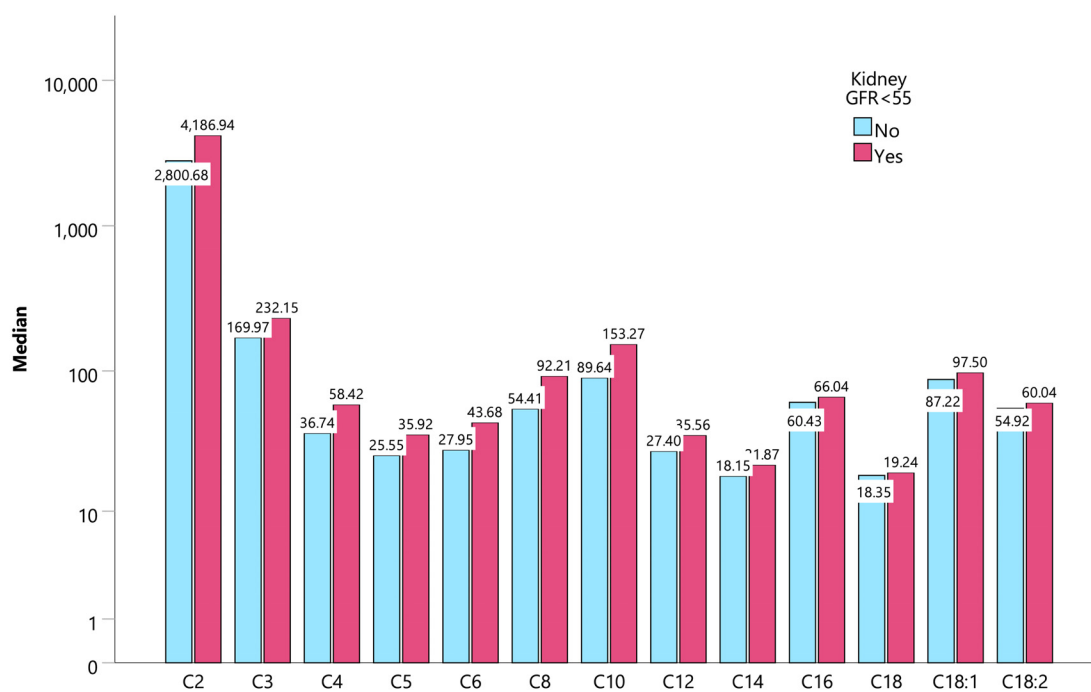


Figure S4.) Log scaled bar graphs of acylcarnitine levels comparison between kidney failure and non-kidney failure patients (GFR<55).