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Supplemental Digital Content (SDC)

Serum Acylglycerols Inversely Associate with Muscle Oxidative Capacity in Severe COPD

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SUPPLEMENTAL METHODS

Clinical assessments

As part of the COPDGene study protocol, clinical data collected included demographics, vital signs, medical and smoking history, and current medications.

Spirometry was performed according to American Thoracic Society guidelines (21) using a dual beam Doppler ultrasound-based spirometer (EasyOne Pro, Ndd Medical, Zürich, Switzerland). Participants inhaled two puffs of metered dose albuterol sulfate (ProAir HFA, Teva Respiratory, Horsham, PA, USA) 15 minutes before spirometric testing. Forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were measured from the greatest FEV_1 and FVC from up to eight maximum expiratory maneuvers, where the greatest two measurements were within 150 mL.

Lung diffusing capacity for carbon monoxide (DL_{CO}) was measured after the post-bronchodilator spirometry assessment (EasyOne Pro DL_{CO}, Ndd Medical, Zürich, Switzerland) (22). DL_{CO} measurement was made following an exhalation to residual volume, followed by a rapid inspiration to total lung capacity and breath hold for 8-12 seconds. The maneuver ended with a complete exhalation to residual volume and resumption of normal breathing. This procedure was performed at least 2 and no more than 5 times. The test was accepted when 2 maneuvers were within 3 $mL.min^{-1}.mmHg$ or within 10% of the greatest value.

Resting arterial oxygen saturation was measured using pulse oximetry (SpO_2; Rad-5 Pulse Oximeter MasimoSET®, Masimo Co., Irvine, CA).

Muscle oxidative capacity

Oxidative capacity of the medial *gastrocnemius* muscle was assessed using near-infrared spectroscopy (NIRS) (23, 24). Briefly, a wireless, portable, continuous-wave, spatially-

resolved NIRS probe (PortaMon, Artinis, The Netherlands) was wrapped in plastic film and secured with an elastic bandage to the belly of the medial *gastrocnemius* to measure relative change in oxygenated and deoxygenated myoglobin + hemoglobin. From these, tissue saturation index (TSI, %), and the relative change in total tissue myoglobin + hemoglobin was calculated. A 13 x 85 cm rapid-inflation pressure-cuff (SC12D, Hokanson, USA) was placed proximally on the thigh of the same leg and attached to a rapid cuff-inflator (E20, Hokanson, USA). Participants were familiarized with rapid cuff inflation procedures. During familiarization the pressure required for arterial occlusion was identified (range 230–300 mmHg). Participants lay at rest for ~3 min to determine resting muscle TSI and SpO_2 at the fingertip (Rad-5 Pulse Oximeter MasimoSET®, Masimo Co., Irvine, CA). Participants then performed 10–12 cycles of ~1 Hz plantar-flexion muscle contractions, followed immediately by arterial occlusion until a stable minimum TSI was reached, or for 5 min (whichever came first). This was used to establish an individualized physiologic range (maximum and minimum) of muscle TSI(23) After at least 3 min recovery, participants performed ~10-15 s plantar-flexion muscle contractions to increase muscle oxygen uptake ($m\dot{V}O_2$) and desaturate the muscle to ~50% of physiologic range(23) ; this was followed immediately by a series of intermittent arterial occlusions (5 occlusions for 5 s; 10 for 10 s; each separated by 5–20 s recovery; total duration ~6 min). This last ~6 min phase was repeated after ~2 min rest. For each brief arterial occlusion, the rate of decline in TSI ($\%.s^{-1}$) was calculated to determine relative $m\dot{V}O_2$. The $m\dot{V}O_2$ recovery rate constant (k, min^{-1}) was measured by non-linear least-squares regression of the $m\dot{V}O_2$ exponential recovery (OriginPro v8.6, OriginLab Co., Northampton, USA) (23). k is directly proportional to muscle oxidative capacity (25). The average k of two repeats is reported.

Prime metabolomics and lipidomics

Post-prandial blood was collected from a peripheral vein using a serum separator tube (8.5 mL, BD Vacutainer) and the serum aliquoted (1 mL) and stored at -80°C for subsequent analysis.

Serum samples were shipped to West Coast Metabolomics Center at the University of California, Davis on dry ice for metabolomics analysis. For untargeted assessment of primary metabolites, gas chromatography time-of-flight mass spectrometry (GC-TOF MS) method was used as described (26). Briefly, 30 μL serum samples and internal standards were extracted and derivatized by silylation/methyloximation. Metabolites were separated using an Agilent 6809 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA). Mass spectrometry was performed with a Leco Pegasus IV time of flight mass spectrometer. Peak heights of quantifier ions for each metabolite were determined by comparing with internal standards. For untargeted lipidomics, serum samples were extracted in methyl tert-butyl ether with the addition of internal standards, followed by ultra-high pressure liquid chromatography on a Waters CSH column, interfaced to a quadrupole time-of-flight mass spectrometer (high resolution, accurate mass), with a 15-minute total run time. Peak areas of lipid species within the range of the calibration curves were analyzed by comparing the individual peak areas with those of corresponding internal standards for determining the final concentration of each metabolite. Data were collected in both positive and negative ion mode and analyzed using MassHunter (Agilent).

Supplemental Tables

Supplementary Table 1. Lipid metabolites that showed sex differences in one-way ANOVA. Nineteen out of 24 metabolites that were different between the sexes were sphingomyelins.

Metabolite	F value	P value	FDR	Fisher's LSD
SM d322 A	16.224	1.04E-08	4.39E-06	COPD-F vs. COPD-M; COPD-F vs. CON-M; CON-F vs. COPD-M; CON-F vs.CON-M
SM d322 B	13.068	2.68E-07	5.66E-05	COPD-F vs. COPD-M; COPD-F vs.CON-M; CON-F vs. COPD-M; CON-F vs.CON-M
SM d301A	10.025	7.45E-06	0.001048	COPD-F vs. COPD-M; COPD-F vs.CON-F; COPD-F vs.CON-M; Con-F vs.CON-M
SM d371 A	7.7381	0.000104	0.010925	COPD-F vs. COPD-M; COPD-F vs.CON-M; CON-F vs.CON-M
SM d412 A	7.2925	0.000175	0.011198	COPD-F vs.CON-M; COPD-M vs.CON-M; CON-F vs.CON-M
SM d402 A	7.2063	0.000194	0.011198	COPD-F vs. COPD-M; COPD-F vs.CON-M; CON-F vs.CON-M
SM d321 A	7.1344	0.000212	0.011198	COPD-Fvs. COPD-M; COPD-Fvs.CON-M; CON-F vs.CON-M
SM d412 B	7.1313	0.000212	0.011198	COPD-F vs.CON-M; CON-F vs.CON-M
SM d403	7.0219	0.000242	0.011338	COPD-F vs.CON-M; CON-F vs.COPD-M; CON-F vs.CON-M
SM d363 A	6.6337	0.000385	0.015247	COPD-F vs.CON-M; COPD-M vs.CON-M; CON-F vs.CON-M
SM d321 B	6.5523	0.000424	0.015247	COPD-F vs. COPD-M; COPD-F vs.CON-M; CON-F vs.CON-M
SM d342A	6.5343	0.000434	0.015247	COPD-F vs.CON-M; COPD-F vs.CON-M; CON-F vs.CON-M
SM d423 A	5.9594	0.000869	0.026405	COPD-F vs.CON-M; CON-F vs.CON-M
SM d342B	5.9524	0.000876	0.026405	COPD-F vs.CON-M; CON-F vs.CON-M
SM d371 B	5.7273	0.001152	0.032411	COPD-F vs.CON-M; CON-F vs. COPD-M; CON-F vs.CON-M
PC 343 A	5.6496	0.001267	0.033407	COPD-F vs.CON-M; CON-F vs.CON-M
SM d392 B	5.5296	0.001467	0.033769	COPD-F vs. COPD-M; COPD-F vs.CON-M; CON-F vs. COPD-M; CON-F vs.CON-M
PC 352 A	5.5039	0.001514	0.033769	COPD-F vs.CON-M; CON-F vs.CON-M
SM d363 B	5.4571	0.001603	0.033769	COPD-F vs.CON-M; CON-F vs.CON-M
SM d391	5.4289	0.001659	0.033769	COPD-F vs. COPD-M; COPD-F vs.CON-M; CON-F vs.CON-M
Ceramide d321	5.3837	0.001754	0.033769	COPD-F vs.CON-F; COPD-F vs.CON-M; COPD-M vs.CON-M; CON-F vs.CON-M
FA 141	5.3805	0.001761	0.033769	COPD-F vs. COPD-M; COPD-F vs.CON-F; COPD-F vs.CON-M

SM d423 B	5.2575	0.002048	0.037568	COPD-F vs.CON-M; CON-F vs. COPD-M; CON-F vs.CON-M
FA 161	5.2165	0.002153	0.037862	COPD-F vs. COPD-M; COPD-F vs.CON-F; COPD-F vs.CON-M

M, male; F, Female; FDR, False discovery rate; COPD, chronic obstructive pulmonary disease; CON, controls.

Supplementary Table 2. Sphingomyelin (SM) metabolite concentrations that were significantly different between males and females in the smoker control group.

	T value	P value	Cohen's d	FDR
SM d322 A	5.0406	4.42E-06	1.315	0.000207
SM d322 B	4.9329	6.57E-06	1.290	0.000207
SM d403	4.2554	7.30E-05	1.085	0.001534
SM d423 A	4.0718	0.000137	1.048	0.002151
SM d412 A	3.9271	0.000221	1.011	0.00243
SM d363 A	3.9139	0.000231	1.008	0.00243
SM d402 A	3.8024	0.000334	0.985	0.002496
SM d371 B	3.7873	0.000351	0.979	0.002496
SM d412 B	3.782	0.000357	0.973	0.002496
SM d363 B	3.7262	0.000427	0.967	0.002692
SM d342 A	3.6856	0.000487	0.955	0.002788
SM d423 B	3.6023	0.000635	0.938	0.003135
SM d301 A	3.5964	0.000647	0.937	0.003135
SM d342 B	3.5445	0.000762	0.920	0.003428
SM d321 A	3.3925	0.001221	0.876	0.005128
SM d362 A	3.3449	0.001412	0.871	0.005415
SM d371 A	3.3336	0.001461	0.857	0.005415
SM d321 B	3.2448	0.00191	0.839	0.006683
SM d412 C	3.1006	0.002922	0.806	0.009211
SM d362 B	3.0916	0.003	0.802	0.009211
SM d320 A	3.0836	0.00307	0.801	0.009211
SM d382 A	2.9913	0.004004	0.772	0.011466
SM d392 B	2.971	0.004242	0.769	0.01162
SM d301 B	2.9399	0.004633	0.758	0.011918
SM d391	2.9325	0.004729	0.754	0.011918
SM d331 A	2.8927	0.005288	0.747	0.012814
SM d382 B	2.7557	0.007712	0.708	0.017995
SM d340 A	2.7387	0.008075	0.705	0.018169
SM d331 B	2.6503	0.01023	0.681	0.022224
SM d402 B	2.5422	0.013571	0.653	0.028499
SM d361 A	2.4758	0.016087	0.639	0.032692
SM d432 A	2.4614	0.016685	0.630	0.032729
SM d402 A	2.4506	0.017144	0.630	0.032729
SM d422 A	2.4276	0.018164	0.618	0.033641
SM d432 B	2.4163	0.018689	0.618	0.033641
SM d361 B	2.3764	0.020638	0.610	0.036116
SM d340 B	2.3105	0.024257	0.596	0.041302
SM d392 A	2.2419	0.028615	0.580	0.04744

Supplementary Table 3. Sphingomyelin (SM) metabolite concentrations that were significantly different between males and females in the COPD group.

	T value	P value	Cohen's d	FDR
SM d322 A	4.450	0.00006	1.350	0.00391
SM d301 A	3.769	0.00051	1.149	0.01593
SM d322 B	3.618	0.00079	1.101	0.01663
SM d301 B	3.264	0.00219	0.996	0.03445