Serum Acylglycerols Inversely Associate with Muscle Oxidative Capacity in Severe COPD

Rongsong Li, Alessandra Adami, Chih-Chiang Chang, Chi-Hong Tseng, Tzung K. Hsiai, Harry B. Rossiter

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 (FEV₁) and forced vital capacity (FVC) were measured from the greatest FEV₁ and FVC from up to eight maximum expiratory maneuvers, where the greatest two measurements were within 150 mL.

Lung diffusing capacity for carbon monoxide (DLCO) was measured after the post-bronchodilator spirometry assessment (EasyOne Pro DLCO, Ndd Medical, Zürich, Switzerland) (22). DLCO 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 (SpO₂; 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 SpO₂ 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 (mVO₂) 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 mVO₂. The mVO₂ recovery rate constant (k, min⁻¹) was measured by non-linear least-squares regression of the mVO₂ 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).