**Dataset name: t\_cpetvc02\_ex03\_3b\_1383**

**Cardiopulmonary Exercise Test**

**Protocol/Methods**

For this study we established the first cardiopulmonary exercise testing (CPET) laboratory within the Framingham Heart Study and performed CPETs in 3117 FHS Gen3 Exam 3/Omni 2 participants. Participants were asked to abstain from all exercise for 24 hours prior to testing. CPET was performed on participants willing to perform exercise in the absence of active symptoms of uncontrolled cardiopulmonary or neurologic disease. Participants utilized maximal effort-limited protocol with continuous electrocardiography (ECG, Mortara Instruments, Milwaukee, WI) as described.1 In brief, a 4-min period of resting gas exchange data collection was followed by 3-min of low-level exercise then a 15W/min or 25W/min incremental ramp based on estimated fitness level at the time of pre-CPET participant interview by our exercise physiologists. The ramp was selected based on anticipated exercise capacity to target 8-12 min of incremental exercise to optimize peak VO2 ascertainment.2 VO2, carbon dioxide output (VCO2), tidal volume (Vt), and respiratory rate was measured breath-by-breath by a commercially available metabolic cart (Medgraphics, St. Paul, MN). This protocol permits uniform measurements at workloads and takes advantage of continuous ramping to optimize pattern recognition. While peak VO2 is currently accepted as the gold standard fitness metric, submaximal CPET variables are particularly attractive to study based on ease of ascertainment during low-level exercise,3 relevance to ability to perform of activities of daily living,4 independence from volitional effort,5 and close relationship to prognosis in overt CVD.5-8 O2 uptake efficiency slope (OUES) is highly reproducible,6, 9 strongly correlated with peak VO2 (r>0.8), and differs by <2% if derived from 75%, 90% or 100% of exercise duration.6 We and others have related exercise oscillatory ventilation (EOV) to impaired cardiac output responses during exercise.10, 11 EOV is present in up to 50% of patients with symptomatic LVSD10 and is known to occur in heart failure with preserved LVEF12 but has not been studied in the general population. Additional submaximum CPET variables that have been shown to reflect CV reserve capacity in small studies include: relative to external work (aerobic efficiency, ΔVO2/ΔW). Quality Control: We implemented comprehensive QC procedures related to metabolic cart and cycle calibration, data acquisition and analysis developed by the contact PI.

**Assessment of Plasma Metabolite Levels at Rest and with Exercise.** FHS/Omni: The baseline plasma samples for metabolite profiling were derived from the resting blood draw during the general exam visit which was separate from this study. This study involved repeated blood sampling immediately following peak exercise (5 ml EDTA plasma sample).

Detailed analytical methods including sample collection procedures, LC-MS methodology, and a list of metabolites assayed, have been previously published by the applicant and have been described in publications from this project as well.13-16 In brief, we have established a metabolomics platform that analyzes ~290 human metabolites in ~60 minutes from <200 μl of plasma in a targeted manner. A metabolite can be identified by its parent mass and dominant daughter mass on a high resolution mass spectrometer in combination with its retention time on an appropriate liquid chromatography column. The platform provides nearly uniform coverage of all pathways in the Kyoto Encyclopedia of Genes and Genomes Database (KEGG).

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