Supplemental Data

Methods

Intervention Description:

The intervention was described in detail in the protocol paper.[1] Briefly, it consisted of assessment and treatment of traditional cardiovascular risk factors by health care providers, individualized phone counselling from a registered dietitian, and optional exercise training. The dietitian counselling was focused on symptom management with follow-up sessions every 6 weeks for 9 possible sessions over 52 weeks. The exercise programming was supervised twice weekly for up to 24 weeks and was optional to accommodate patients from out of town or without transportation, or those experiencing high symptom burden. It was individualized for patient preferences and experience but consisted of 60-90 minutes of moderate-vigorous intensity aerobic and moderate-intensity whole-body resistance training. Usual care consisted of best supportive cancer care and clinical monitoring.

Outcome Measures

Custom semi-automated MATLAB software was used for all MRI analyses (The MathWorks, Natick, USA).

Skeletal Muscle and Fat Composition

Multi-slice (30 slices total) multi-echo axial images (5 mm thickness + 5 mm gap, 1.46 mm in-plane resolution) of the right lower leg and thigh were used to reconstruct water and fat separated images using the chemical-shift encoded MRI approach.[2] For the thigh, 5 consecutive slices starting from 10 cm proximal to the most distal point of the femur (identified via sagittal localizer image) were included in analysis. For the lower leg, a subset of 20 slices included in the final dataset were selected for each participant by aligning the cross-sectional profile of the lower leg between the four study time points, to ensure consistent spatial coverage.

For both the thigh and lower leg, absolute volumes of muscle, intermuscular fat, and subcutaneous fat were segmented using custom semi-automated MATLAB software. The automated segmentation of regions was manually checked for accuracy and adjusted if needed. Volumes were quantified using the disk summation method. Myosteatosis, which is relative fatty infiltration of muscle, was calculated as the ratio of intermuscular fat to skeletal muscle as well as the skeletal muscle fat fraction (intermuscular fat/(intermuscular fat + skeletal muscle)*100%) for each region.

Exercise test 1 - Incremental-to-maximum workload with ^{31}P spectroscopy

The exercise and MRI acquisition protocol has been previously reported.[3] Briefly, ^{31}P spectra were acquired over 12-second intervals for two minutes at rest, followed by an incremental-to-maximum workload unilateral (left leg), plantarflexion exercise test inside the bore using a commercially available MRI ergometer (Trispect Module, Ergospect, Austria). The exercise test protocol was initiated at 4 watts with 2-watt increments every minute until exhaustion with verbal encouragement provided by a tester to continue until volitional exhaustion. Acquisition of ^{31}P spectra continued during four minutes of recovery following cessation of exercise. The area under the individual spectra of phosphocreatine (PCr) and inorganic phosphate (Pi) were measured to represent relative concentrations, while intracellular pH was determined from the chemical shift between the Pi and PCr signals.[4] The Pi:PCr ratio and pH were determined for a low intensity and high intensity workload for each test for comparison of exercise metabolic responses. Low intensity was defined as 5.5 watts (occurring ~90 seconds into test), which corresponded to ~40% of peak power, and was chosen as it was the lowest intensity where all participants had available data. High intensity was chosen posthoc as 80% of each individual participant's pre-chemotherapy peak power output because this was a submaximal intensity for all subsequent tests and was associated with Pi:PCr <1.0 in 95% of tests. The PCr recovery time constant was calculated by fitting a mono-exponential curve to the four minutes of PCr recovery data.

Exercise test 2 - Steady state at 60% of peak workload with evaluation of O_2 consumption determinants

Following 15-20 minutes of rest, participants performed a second plantarflexion test on the opposite (right) leg for four minutes at a constant workload corresponding to 60% of peak workload achieved on the first test. The exercise and MRI acquisition protocol has been previously reported.[3,5–7] Briefly, axial images were prescribed perpendicular to the superficial femoral vein just superior to the knee to measure blood flow and venous O_2 saturation (S_vO_2) at rest and immediately (<1 second) after cessation of exercise. A custom acquisition enabled simultaneous measurement of blood flow (ml/min) and S_vO_2 in the vein with a temporal resolution of 2.6 seconds.[3,5–7]

<u>Calculations</u>:

1) Lower leg O_2 consumption (mL/min) = *leg blood flow (ml/min)* $\cdot$ *[($S_aO_2 - S_vO_2$, %)* $\cdot$ *hemoglobin g/dL]* $\cdot$ *1.34 mL O_2/g hemoglobin*

Resting S_aO_2 (arterial oxygen saturation) was measured by finger pulse oximetry, and hemoglobin was measured by complete blood count clinically acquired for the breast cancer patients typically within a few days of the scan and extracted from medical records. Missing blood counts at the one-year time point were replaced with the end-chemotherapy measurement. For the controls, a complete blood count was performed within 0-7 days of the scan.

2) Lower leg O_2 delivery (mL/min) = *blood flow (dL/min) · arterial O_2 content (mL/dL)*

Arterial O_2 content = *[hemoglobin (g/dL) · 1.34 mL O_2/g hemoglobin · S_aO_2 (%)] + [0.0031 mL O_2/dL/mmHg · P_aO_2 (mmHg)],*

with P_aO_2 estimated as 100 mmHg.

3) Lower leg O_2 extraction = *$(S_aO_2 - S_vO_2)/S_aO_2 \cdot 100\%$.*

Pulmonary VO_2peak Exercise Test

VO_2peak was measured with an incremental to-maximum workload cardiopulmonary exercise test (Encore229 Vmax; SensorMedics, Yorba Linda, USA) on a cycle ergometer (Ergoselect II 1200 Ergoline, Germany) as the highest 20-second average volume of O_2 uptake. The initial workload and ramp increment used for all tests for a given participant was chosen at baseline by the tester based on the participant's previous experience with the goal of an 8 to 15-minute test (e.g., 5-15 watt/minute ramp). The tester provided verbal encouragement to continue until volitional exhaustion, continuously monitored heart rate, S_aO_2, and electrocardiography, and collected blood pressure and Borg rating of perceived exertion every two minutes.

References

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