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Eunhan Cho
AdventHealth Orlando
Stephanie Compton
AdventHealth Orlando
Abigail Tice
AdventHealth Orlando
Shenping Yang
Pennington Biomedical Research Center
Tejani Mohamedtaki
AdventHealth Orlando
Zakari Ahmed
AdventHealth Orlando
Brian Iriving
University of Virginia
Justin Brown
AdventHealth Orlando
Guillaume Spielmann
gspielmann@lsu.edu

Louisiana State University

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Acute and Chronic Effects of Aerobic Exercise on Circulating T-Cell Phenotypes and Mitochondrial Bioenergetics in Colorectal Cancer Survivors: A Pilot Ancillary Study of a Randomized Trial

Eunhan Cho, PhD,[1] Stephanie L E Compton, PhD, RD,[1] Abigail L Tice, PhD,[1] Shengping Yang, PhD,[2] Mohamedtaki A. Tejani, MD,[1] Ahmed Zakari, MD,[1] Brian A. Irving, PhD,[2,3,4] Justin C. Brown, PhD,[1] Guillaume Spielmann, PhD. [2,4]

[1]AdventHealth, Orlando, Florida USA; [2]Pennington Biomedical Research Center, Baton Rouge, LA, USA; [3]University of Virginia Department of Kinesiology, School of Education and Human Development, Charlottesville, VA, USA; [4]Louisiana State University School of Kinesiology, Baton Rouge, LA, USA

Co-Corresponding Authors

Justin C. Brown, Ph.D.

301 E Princeton St

Orlando, FL 32804

Phone: (407) 609-0449

Email: Justin.Brown4@AdventHealth.com

Guillaume Spielmann, Ph.D.

LSU School of Kinesiology

112 Huey P Long Field House,

Baton Rouge, LA 70803

Email: gspielmann@lsu.edu

Abstract

Background

Acute exercise mobilizes circulating T-cells in healthy adults and other cancer populations [1, 2], but evidence in colorectal cancer (CRC) survivors is limited, and whether aerobic exercise training modifies these acute responses remains unclear.

Methods

In this pilot ancillary study of a randomized trial, 20 CRC survivors were randomized to 12 weeks of moderate-intensity aerobic exercise (n=11) or a stretching control program (n=9). Blood was collected before and after an acute bout of moderate-vigorous intensity exercise at baseline and week 12. Circulating CD4+ and CD8+ T-cell subsets were quantified by flow cytometry, and T-cell mitochondrial oxygen flow was assessed by high-resolution respirometry at the cellular and tissue (blood-normalized) level.

Results

At baseline, acute exercise increased the number of circulating CD4+ naïve [64.54 cells/μL (95% CI: 27.79, 101.30); p=0.001], central memory [78.02 cells/μL (95% CI: 44.88, 111.16); p<0.001], effector memory [51.95 cells/μL (95% CI: 35.77, 68.14); p<0.001], terminally differentiated effector memory [10.22 cells/μL (95% CI: 4.94, 15.49); p<0.001], and senescent [13.67 cells/μL (95% CI: 0.10, 27.24); p=0.048] T-cell subsets, along with equivalent CD8+ subsets [naïve: 26.57 cells/μL (95% CI: 16.56, 36.57); central memory: 15.34 cells/μL (95% CI: 8.41, 22.26); effector memory: 41.95 cells/μL (95% CI: 29.37, 54.53); terminally differentiated effector memory: 55.99 cells/μL (95% CI: 31.21, 80.76); senescent: 51.32 cells/μL (95% CI: 33.81, 68.83); all p<0.001], but not recent thymic emigrants. Acute exercise also increased tissue-level oxygen flow across routine [1.50 $pmol \cdot s^{-1} \cdot mL\ blood^{-1}$ (95% CI: 0.96, 2.04); p<0.001], LEAK [0.44 $pmol \cdot s^{-1} \cdot mL\ blood^{-1}$ (95% CI: 0.18, 0.71); p=0.001], and oxidative phosphorylation states (range: 1.52–4.54 $pmol \cdot s^{-1} \cdot mL\ blood^{-1}$; all p≤0.001), without change at the cellular level

(all p≥0.74). Twelve weeks of aerobic exercise training did not alter resting T-cell phenotypes, mitochondrial bioenergetics, or the magnitude of the acute response, though the largest between-group difference in tissue-level oxidative phosphorylation capacity approached significance [-1.76 pmol·s^{-1}·mL blood^{-1} (95% CI: -3.62, 0.11); p=0.065].

Conclusions

In this pilot ancillary study, a single bout of submaximal exercise mobilized circulating T-cells and increased tissue-level T-cell mitochondrial bioenergetic capacity in CRC survivors. Larger trials are needed to determine whether aerobic exercise training modifies the magnitude or bioenergetic features of acute immune responses to exercise in CRC survivors.

Clinical trial registration: ClinicalTrials.gov, NCT05789433, registered 2023-03-16

Keywords: Aerobic exercise; acute exercise; T-cells; mitochondrial bioenergetics; colorectal cancer; cancer survivorship.

1. Background

Regular exercise is associated with improved disease-free survival and overall survival among colorectal cancer (CRC) survivors [3]; however, the biological mechanisms underlying these benefits remain incompletely understood. One hypothesized mechanism involves exercise-related changes in the peripheral T-cell compartment. T-cells are central to immune surveillance, and their phenotype and functional capacity are influenced by aging [4], cancer history [5], cancer-directed treatment [6], and lifestyle factors, including physical activity [7-9].

Immunosenescence is a hallmark of age-related immune deterioration characterized by reduced naïve T-cell abundance, diminished thymic output, reduced diversity of the T-cell receptor repertoire, and accumulation of highly differentiated or senescent T-cell populations in circulation [10, 11]. These changes may limit the capacity to recognize and respond to novel antigens and to maintain a diverse T-cell repertoire [12, 13]. Cross-sectional studies have reported higher frequencies of naïve T-cells and recent thymic emigrants (RTE) in long-term physically active older adults than in age-matched inactive individuals [4]. Thus, regular exercise may attenuate selected features of immunosenescence and reshape the peripheral T-cell pool in ways that affect cancer prognosis.

Both acute and chronic exercise training may influence peripheral T-cell biology. A single bout of exercise mobilizes circulating T-cells, particularly effector-memory (EM), and terminally differentiated effector memory (TEMRA) populations through sympathetic activation and catecholamine-mediated β2-adrenergic receptor signaling [14, 15]. Limited evidence suggests that chronic aerobic exercise training increases CD8+ EM T-cell frequency [16]. Given that T-cell subsets differ in their metabolic characteristics, exercise-induced changes in T-cell distribution may also affect mitochondrial respiration.

T-cell bioenergetics can be assessed through mitochondrial respiration, which supports energy demands of T-cell activation, proliferation, migration, and effector function [17-20]. Exercise-related stimuli may affect mitochondrial respiration; lactate exposure changes T-cell mitochondrial respiration *in vitro* [17], and higher cardiorespiratory fitness has been associated with greater mitochondrial respiratory capacity in peripheral blood mononuclear cells from healthy women [19, 21]. However, most evidence comes from healthy adults or populations without cancer [16, 17, 19, 21], and CRC survivors may have persistent immune and metabolic alterations related to aging, surgery, chemotherapy, radiotherapy, and other treatment exposures [9, 22, 23]. Consequently, it remains unknown whether aerobic exercise training alters resting T-cell phenotypes or mitochondrial bioenergetic capacity in CRC survivors or modifies the magnitude of acute T-cell mobilization or mitochondrial oxygen-flow responses to a subsequent bout of exercise.

Therefore, we conducted a pilot ancillary study embedded within an ongoing randomized trial to examine the effects of acute submaximal exercise and 12 weeks of moderate-intensity aerobic exercise training on circulating T-cell phenotypes and mitochondrial bioenergetic capacity in CRC survivors. We hypothesized that: (1) acute exercise would mobilize circulating T-cell subsets and increase mitochondrial oxygen flow; (2) 12 weeks of aerobic exercise training would change T-cell phenotypes and mitochondrial oxygen flow compared with control; and (3) 12 weeks of aerobic exercise training would improve the acute T-cell and mitochondrial oxygen flow response to submaximal exercise compared with the baseline acute response.

2. Methods

2.1. Study Design

The Effects of Aerobic Exercise on Skeletal Muscle Remodeling in Colorectal Cancer (RESTORE) trial is an ongoing randomized, parallel-group, single-blind, controlled study

conducted at a single site (Pennington Biomedical Research Center, Baton Rouge, LA, USA) and sponsored by the National Cancer Institute (NCI) [24]. The RESTORE trial was designed to examine the effects of 12 weeks of home-based moderate-intensity aerobic exercise, compared with a stretching control program, on whole-body intermuscular adipose tissue. The present study is a pilot ancillary study nested within the RESTORE trial that evaluated the effects of acute submaximal exercise and 12 weeks of aerobic exercise training on circulating T-cell phenotypes and mitochondrial oxygen flow in CRC survivors. The trial adhered to Good Clinical Practice guidelines and the ethical principles of the Declaration of Helsinki. The study protocol and informed consent documents, including procedures for collection and banking of blood samples, were approved by the Institutional Review Board at Pennington Biomedical Research Center, Baton Rouge, LA (2022-014-PBRC). All participants provided written informed consent. The study was registered on ClinicalTrials.gov (NCT05789433). The endpoints reported here are exploratory for hypothesis generation. The parent trial protocol and statistical analysis plan are available from the corresponding authors on reasonable request. No changes were made to the trial protocol after commencement. This pilot ancillary study is reported in accordance with the CONSORT 2025 statement. The completed CONSORT checklist is provided in **Additional file 1**.

2.1.1. Participants

Eligible participants were adults (aged ≥18 years) with histologically confirmed stage I–III colorectal cancer who had completed curative-intent complete surgical resection at least 30 days before baseline assessments. Participants had also completed all postoperative cancer-directed therapy (e.g., chemotherapy and/or radiation) at least 30 days before enrollment and had no additional cancer-directed surgery planned during the 12-week intervention period. Participants self-reported ≤150 minutes per week of moderate- to vigorous-intensity physical activity over the past month and had physician approval to participate. Additional eligibility

criteria included the ability to read and speak English, willingness to be randomized, and capacity to provide written informed consent. Key exclusion criteria included evidence of recurrent or metastatic colorectal cancer, or any cardiovascular, pulmonary, orthopedic, or other condition that could compromise safety or protocol adherence.

2.1.2. Submaximal exercise test

Submaximal exercise capacity (VO_2peak; mL•kg^{-1}•min^{-1}) was assessed using an incremental, symptom-limited submaximal cardiopulmonary exercise test on an electronically braked treadmill [25]. Participants selected a comfortable yet brisk walking speed, which also served as the warm-up. After the warm-up, the treadmill was set to the self-selected brisk speed at 0% grade, and the speed was then held constant while the grade increased by 2% every 2 minutes [26]. The test was terminated when participants reached 80% of age-predicted maximal heart rate or reported a rating of perceived exertion of 18, consistent with American College of Sports Medicine guidelines for exercise testing and prescription [27].

2.1.3. Randomization and blinding

Participants were randomized in a 1:1 ratio to aerobic exercise or stretching control using covariate-adaptive randomization with minimization. Randomization was stratified by sex, age, race, and prior chemotherapy. Randomization was administered by the study statistician, and allocation was concealed from enrollment staff until assignment. Outcome assessors remained blinded to treatment allocation. Participants and the certified cancer exercise specialists were unblinded.

2.1.4. Interventions

The aerobic exercise group completed a 12-week moderate-intensity aerobic exercise program, progressing to 225 minutes per week. Each participant received an in-home treadmill (T202, Horizon Fitness, Cottage Grove, WI, USA) and a heart rate monitor (ScanWatch, model

3700546706400 HWA09, Withings, Issy-les-Moulineaux, France). Exercise intensity was prescribed at 60%–80% of age-predicted maximal heart rate, and sessions included a 5-minute warm-up, 20–60 minutes of treadmill walking, and a 5-minute cool-down. Weekly exercise volume was progressively increased until the 225-minute target was achieved by week 8 and maintained through week 12. The exercise specialist downloaded the heart rate monitor information weekly to review the frequency, duration, and intensity of exercise to ensure adherence to the protocol. Adherence was calculated as the percentage of prescribed weekly exercise minutes completed, based on heart rate monitor–derived exercise data and averaged across the 12-week intervention. Participants assigned to the control group completed a home-based progressive stretching program targeting eight major muscle groups, with weekly contact matched to the exercise group. Stretching control was designed to match the aerobic intervention for setting, and contact frequency, and while avoiding an aerobic training stimulus. A certified cancer exercise specialist contacted participants in both groups weekly throughout the 12-week intervention to maintain study-related communication and monitor for adverse events.

2.2 Outcome measurements

2.2.1 Study time points and blood collection

Blood samples were collected at four time points before and after the submaximal exercise test at baseline and after the 12-week intervention: baseline pre-exercise (T1), baseline post-exercise (T2), week-12 pre-exercise (T3), and week-12 post-exercise (T4).

2.2.2 PBMC and T-cell isolation

At each study time point, venous blood was collected and complete blood counts were performed immediately after collection using a Sysmex XN-330 hematology analyzer (Sysmex Corporation, Kobe, Japan). Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation, as previously described (STEMCELL Technologies, Vancouver,

BC, Canada; Cat. No. 18060) [17]. Following isolation, PBMCs were counted using an automated cell counter (Countess 3 FL; Thermo Fisher Scientific, Waltham, MA, USA). T-cells were then isolated from PBMCs by negative selection using magnetic-activated cell sorting (MACS) separation beads (Miltenyi Biotec, Auburn, CA, USA; Cat. No. 130-096-535), as previously described [17]. Negatively selected T-cells were washed in RPMI medium at 400 × g for 10 minutes and counted again before downstream flow-cytometric and high-resolution respirometry analyses.

2.2.3. Antibody labeling and flow cytometry

PBMCs were stained using monoclonal antibodies (mAbs) diluted to the optimized concentrations in a multi-color direct immunofluorescence assay. The following fluorophore-conjugated mAbs were utilized: anti-CCR7 (Brilliant Violet 421, clone G043H7), anti-CD45RA (cFluor v450, clone HI100), anti-CD3 (BV570, clone UCHT1), anti-CD4 (cFluor BYG584, clone SK3), anti-KLRG1 (PE-Fire 640, clone 2F1/KLRG1), anti-CD57 (cFluor R668, clone HNK-1), anti-CD28 (BV650, clone CD28.2), anti-CD8 (cFluor B532, clone SK1), anti-PTK7 (PE, clone REA8361), anti-CD31 (PE/Dazzle 594, clone WM59), anti-CD103 (PE-Cyanine7, clone Ber-ACT8), anti-CD45 (cFluor R780, clone 2D1), and anti-CD27 (cFluor R840, clone QA17A18). Ghost Dye Violet 510 was used for viability staining. Anti-CD28, anti-CCR7, anti-KLRG1, anti-CD31, anti-CD103 and anti-CD3 were purchased from BioLegend (San Diego, CA, USA), anti-PTK7 was purchased from Miltenyi Biotec (Auburn, CA, USA). The remaining antibodies were sourced from Cytek Biosciences (Fremont, CA, USA).

2.2.4. Flow cytometry analysis

Following antibody labeling, cellular phenotypes were assessed using a 3-laser full-spectrum flow cytometer (Northern Light, Cytek Biosciences, Fremont, CA, USA). Data acquisition and spectral unmixing were performed using SpectroFlo® software (Cytek Biosciences), allowing for

real-time visualization. Subsequent data analyses were conducted using FCS Express (Version 7.0, De Novo Software, Pasadena, CA, USA).

T-cell subset frequencies were expressed as the percentage of CD3+CD4+ or CD3+CD8+ T-cell population. Absolute T-cell subset counts (cells/μL) were calculated by multiplying the absolute lymphocyte count obtained from the complete blood count by the corresponding subset frequency. Absolute circulating total T-cell counts (CD3+ cells/μL) were calculated in the same manner, by multiplying the absolute lymphocyte count by the CD3+ T-cell frequency, and converted to counts per mL blood for normalization of systemic (tissue-level) mitochondrial oxygen flow.

2.2.5. Gating strategy

Flow cytometry data were first gated to exclude debris and doublets using forward and side scatter parameters. Viable cells were identified by exclusion of Ghost Dye Violet 510–positive events, and lymphocytes were selected by light scatter characteristics. Within the lymphocyte gate, T-cells were defined as CD45+CD3+ events. CD4+ and CD8+ T-cell subsets were then identified within the CD45+CD3+ gate based on CD4 and CD8 expression.

Within CD4+ and CD8+ T-cells, differentiation subsets were defined according to CCR7 and CD45RA expression as naïve T-cells (naïve; CCR7+CD45RA+), central memory (CM; CCR7+CD45RA-), effector memory (EM; CCR7-CD45RA-), and effector memory T-cells re-expressing CD45RA (TEMRA; CCR7-CD45RA+). Recent thymic emigrants (RTE) were defined as CD31+ cells within the PTK7+ naïve CD4+ gate, and as CD103+ naïve CD8+ T-cells. Senescent T-cell populations were further evaluated within total CD4+ and total CD8+ T-cells based on expression of CD57 and KLRG1 (CD57+ and KLRG1+).

2.2.6. High-Resolution Respirometry

Mitochondrial respiration in T-cells was assessed using high-resolution respirometry (Oroboros Instruments, Innsbruck, Austria). Chambers were calibrated at room air in mitochondrial respiratory medium 5 (MiR05: 0.5 mM EGTA, 20 mM taurine, 3 mM $MgCl_2$, 110 mM sucrose, 60 mM K-lactobionate, 10 mM KH_2PO_4, 20 mM K-HEPES, 1 g/L fatty acid-free BSA, pH 7.1; MiR05 Kit, Oroboros: 60101-01). All experiments were performed at 37°C, with oxygen concentrations maintained between approximately 50 and 190 µM O_2. Following calibration, 3-6×10^6 T-cells were added to each 0.5 mL chamber. After signal stabilization, routine oxygen flow (I_{O2}; pmol·s^{-1}·10^6 T-$cells^{-1}$) was measured in intact T-cells (CE). Cells were then permeabilized by titration of α-chaconine (final concentration 30 µM; Sigma, 80075). Palmitoyl-L-carnitine (Pal; 10 µM; Sigma, P1645) and malate (M; 1 mM; Sigma, M1000) were added to assess I_{O2} in the LEAK state supported by fatty acid–linked substrates ($PalM_L$ / F_L). ADP (2.5 mM; Sigma, A5285) with $MgCl_2$·$6H_2O$ (1.5 mM; Sigma, M2670) were then titrated to measure I_{O2} in the OXPHOS capacity with fatty acid–linked substrates ($PalM_P$ / F_P), followed by cytochrome c (cytc; 10 µM; Sigma, C7752) to test outer mitochondrial membrane integrity. Pyruvate (Pyr; 5 mM; Sigma, P2256) and glutamate (G; 10 mM; Sigma, G1626) were added sequentially to evaluate the additive effect of NADH-linked respiration on I_{O2} in the OXPHOS state ($PalMPyr_P/FN_P$ and $PalMPyrG_P/FN_P$). Succinate (S; 10 mM; Sigma, S2378) was then titrated to measure the additive effect of succinate-linked respiration on I_{O2} in the OXPHOS state ($PalMPyrGS_P/FNS_P$). Rotenone (R; 0.5 µM; Sigma, R8875) was subsequently added to inhibit complex I and to measure succinate-linked I_{O2} in the OXPHOS state ($PalMPyrGSR_P/S_P$). Finally, antimycin A (2.5 µM, Sigma: A8674) was added to measure residual oxygen consumption (ROX), which was subtracted from all I_{O2} measurements [17]. All I_{O2} measurements were corrected for daily air calibration, instrumental background, and zero oxygen calibrations. For each condition, values represent the mean of duplicate measurements. Mitochondrial oxygen flow was expressed at two levels. Cellular-level oxygen flow (I_{O2}; pmol·s^{-1}·10^6 T-$cells^{-1}$) reflects the intrinsic respiratory capacity of a fixed number of T-cells, whereas tissue-level (blood-normalized; pmol·s^{-1}·mL blood-

[1]) oxygen flow reflects per-cell respiratory capacity scaled by the number of T-cells circulating per mL of blood, and was calculated by multiplying cellular-level oxygen flow by the circulating T-cell count per mL blood (Section 2.2.4).

2.3. Statistical analysis

All inferential analyses were conducted on an intention-to-treat basis. The analytic strategy used multilevel mixed models for repeated measures (MMRM) to account for the longitudinal within-participant correlation. The baseline value of the dependent variable was included as a covariate in the regression models to improve model precision [28]. The regression model included group-by-visit-by-time interaction terms as fixed effects with subject-specific intercepts. Contrasts of interest were estimated from the MMRM as the mean difference and corresponding 95% confidence interval (CI). The 95% CIs were not adjusted for multiplicity; thus, the results should be interpreted accordingly. Model fit was assessed using a combination of numeric and graphical techniques. All statistical testing was two-sided. The sample size was determined by the parent trial, and this ancillary study was not powered a priori for the present endpoints. No interim analyses or formal statistical stopping guidelines were planned for this ancillary study. Analyses were done using Stata/MP v.19.5 (StataCorp, LLC). All 20 participants were included in the analyses. Mixed models used all available observations from each participant under a missing at random assumption, and no imputation was performed.

2.4. Patient and public involvement

Patients and members of the public were not involved in the design, conduct, reporting, or dissemination plans of this research.

3. Results

3.1 Participant flow and baseline characteristics

Participant flow is shown in **Supplementary Figure S1**. Enrollment of participants included in this ancillary study began in August 2023, and all week-12 assessments were completed by April 2025. The parent trial remains ongoing. Twenty colorectal cancer survivors participated in this ancillary study (exercise, n=11; control, n=9). Participants had a mean age of 59.3±9.5 years; 55% were female, and 55.0% and 45.0% had stage I/II and III disease, respectively. Median time since completion of cancer treatment was 1.8 years (IQR: 0.8, 4.5) (**Table 1**). The mean adherence to the 12 weeks of prescribed exercise intervention was 95.4±3.3%. No participant received cancer directed therapy during the intervention. No adverse events occurred in either group during the study.

Table 1. **Participant demographic and clinical characteristics at baseline**

Characteristics	**Control (n=9)**	**Exercise (n=11)**
Age, years	60.4±8.6	58.3±10.2
Sex, n %		
Male	5 (55.6%)	4 (36.4%)
Female	4 (44.4%)	7 (63.6%)
Primary tumor, n (%)		
Colon	4 (44.4%)	7 (63.6%)
Rectal	5 (55.6%)	4 (36.4%)
Cancer treatment, n (%)		
Chemotherapy	5 (55.6%)	5 (45.5%)
Radiotherapy	3 (33.3%)	2 (18.2%)
Time since completion of cancer treatment, year, median (IQR)	1.9 (0.8, 4.6)	1.6 (1.0, 3.9)
Cancer stage, n (%)		
I/II	4 (44.4%)	7 (63.6%)
III	5 (55.6%)	4 (36.4%)

*Values are mean ± SD for other continuous variables, median (IQR) for time since treatment completion, and n (%) for categorical variables.

3.2. Acute exercise mobilized circulating T-cell phenotypes at baseline

At baseline (i.e., in the untrained state, before randomization to a study group), the acute exercise response was assessed by comparing post-submaximal exercise (T2) with pre-submaximal exercise (T1). Acute exercise increased absolute circulating CD4+ naïve T-cells

[64.54 cells/μL (95% CI: 27.79, 101.30); p=0.001], CD4+ CM T-cells [78.02 cells/μL (95% CI: 44.88, 111.16); p<0.001], CD4+ EM T-cells [51.95 cells/μL (95% CI: 35.77, 68.14); p<0.001], CD4+ TEMRA T-cells [10.22 cells/μL (95% CI: 4.94, 15.49); p<0.001], and CD4+ senescent T-cells [13.67 cells/μL (95% CI: 0.10, 27.24); p=0.048]. Acute exercise did not change circulating CD4+ RTE T-cell counts [9.34 cells/μL (95% CI: −3.44, 22.12); p=0.152] (**Figure 1A**). Acute exercise also increased absolute numbers of circulating CD8+ naïve T-cells [26.57 cells/μL (95% CI: 16.56, 36.57); p<0.001], CD8+ CM T-cells [15.34 cells/μL (95% CI: 8.41, 22.26); p<0.001], CD8+ EM T-cells [41.95 cells/μL (95% CI: 29.37, 54.53); p<0.001], CD8+ TEMRA T-cells [55.99 cells/μL (95% CI: 31.21, 80.76); p<0.001], and CD8+ senescent T-cells [51.32 cells/μL (95% CI: 33.81, 68.83); p<0.001]. CD8+ RTE T-cell counts were not changed by acute submaximal exercise [1.08 cells/μL (95% CI: -1.98, 4.15); p=0.489] (**Figure 1A**).

Acute exercise changed the frequency of circulating T-cell subsets. CD4+ naïve T-cell frequency decreased [−2.70% (95% CI: −4.67, −0.74); p=0.007], while CD4+ EM T-cells [2.13% (95% CI: 0.65, 3.60); p=0.005], CD4+ TEMRA T-cells [0.92% (95% CI: 0.22, 1.62); p=0.01], CD4+ senescent T-cells [1.53% (95% CI: 0.25, 2.82); p=0.019] frequencies increased. Similarly, CD8+ naïve T-cell frequency decreased [−4.11% (95% CI: −5.66, −2.57); p<0.001], whereas CD8+ TEMRA T-cells [4.31% (95% CI: 1.97, 6.66); p<0.001] and CD8+ senescent T-cells [4.17% (95% CI: 0.87, 7.46); p=0.013] frequencies increased (**Figure 1B**).

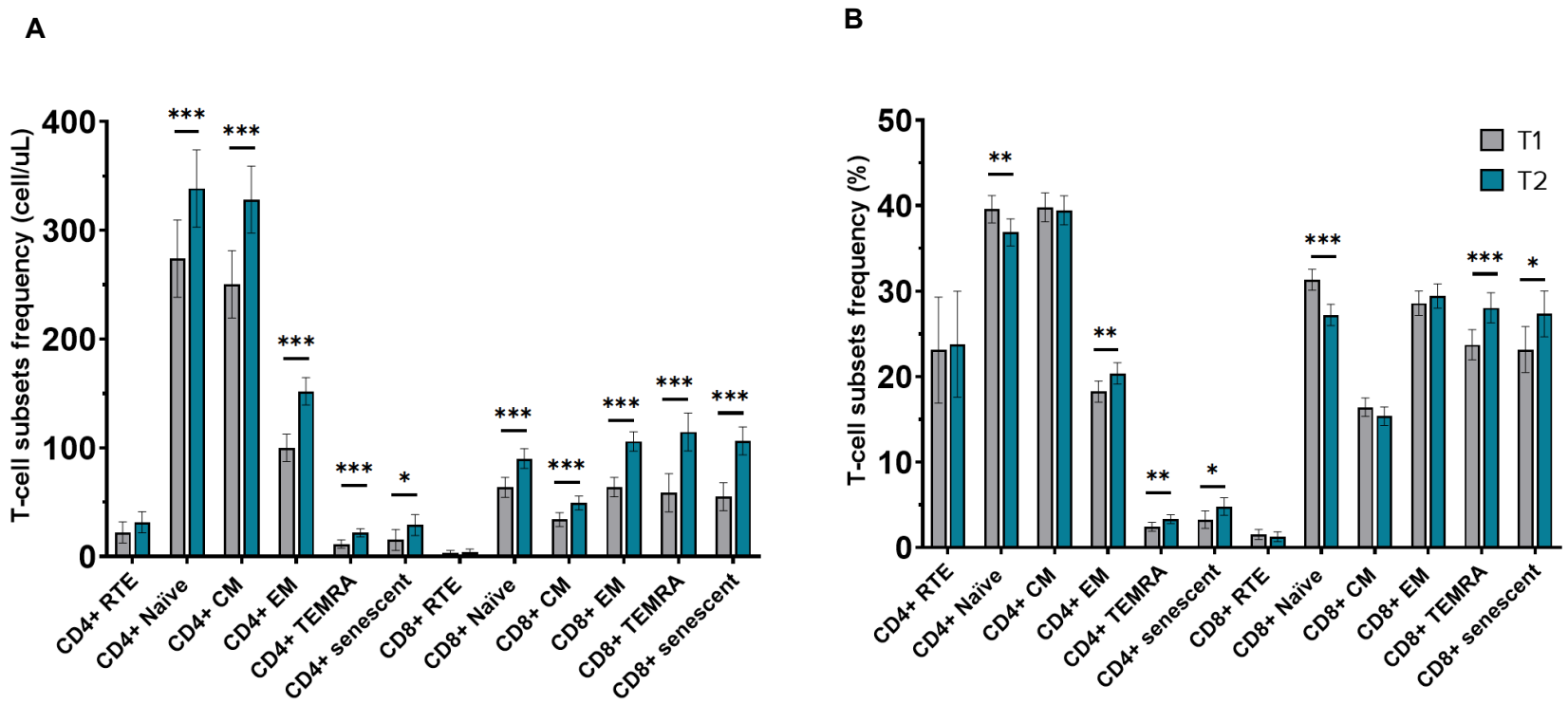


Figure 1. **Acute exercise increases circulating CD4+ and CD8+ T-cell subset counts and frequencies.** Absolute counts (A) and frequencies (B) CD4+ and CD8+ T-cell subsets immediately before (T1) and after (T2) an acute bout of aerobic exercise at baseline. Counts are expressed as cells/μL and frequencies as percentage of the parent CD4+ or CD8+ gate. Data are estimated marginal means with 95% confidence intervals from mixed-effects models. *p ≤0.05, **p ≤0.01, ***p ≤0.001 for the T2−T1 difference.

3.3. Acute exercise increased tissue-level T-cell mitochondrial bioenergetic capacity at baseline.

At baseline, acute submaximal exercise increased tissue-level oxygen flow across routine, LEAK, and OXPHOS respiratory states (**Figure 2A**). Routine respiration in intact T-cells (CE) increased [1.50 pmol·s^{-1}·mL blood^{-1} (95% CI: 0.96, 2.04); p<0.001]. LEAK state (PalM$_L$/F$_L$) oxygen flow increased [0.44 pmol· s^{-1}·mL blood^{-1} (95% CI: 0.18, 0.71); p=0.001]. Tissue-level oxygen flow also increased in all OXPHOS states including PalM$_P$/F$_P$ [1.52 pmol· s^{-1}·mL blood^{-1} (95% CI: 0.90, 2.14); p<0.001], PalMPyr$_P$/FN$_P$ [1.55 pmol· s^{-1}·mL blood^{-1} (95% CI: 0.73, 2.37); p<0.001], PalMPyrG$_P$/FN$_P$ [1.61 pmol· s^{-1}·mL blood^{-1} (95% CI: 0.68, 2.54); p=0.001], PalMPyrGS$_P$/FNS$_P$ [4.54 pmol· s^{-1}·mL blood^{-1} (95% CI: 2.02, 7.06); p<0.001], PalMPyrGSR$_P$/S$_P$ [3.92 pmol· s^{-1}·mL blood^{-1} (95% CI:1.71, 6.12); p<0.001]. However, cellular-level oxygen flow did not change in CE [0.05 pmol·s^{-1}·10^6 T-cells^{-1} (95% CI: -0.25, 0.35); p=0.74], or LEAK state [PalM$_L$/F$_L$ -0.01 pmol·s^{-1}·10^6 T-cells^{-1} (95% CI: -0.13, 0.12); p=0.91], or any OXPHOS state (**Figure 2B**).

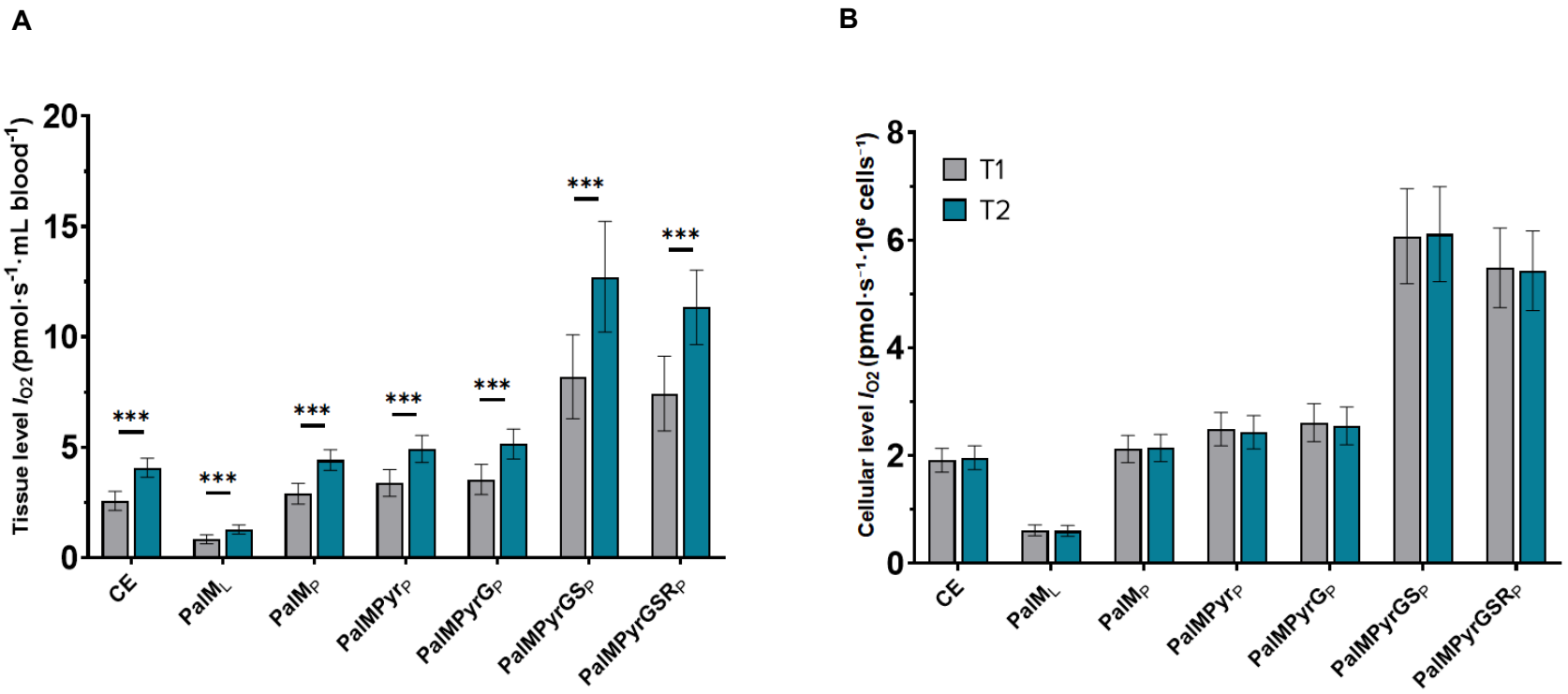


Figure 2. **Acute exercise increases tissue-level, but not cellular-level T-cell mitochondrial oxygen flux in untrained CRC survivors**. Tissue level (A; per mL whole blood) and cellular level (B; per million T-cells) immediately before (T1) and after (T2) a single bout of aerobic exercise at baseline. Fluxes are expressed as pmol·s^{-1}·mL blood^{-1} (A) and pmol·s^{-1}·10^6 T-cells^{-1} (B) across sequential substrate-uncoupler-inhibitor titration states. Tissue-level flux was calculated by multiplying cell-normalized flux by the concentration of circulating CD3+ T-cells per mL of whole blood at the corresponding timepoint. Data are estimated marginal means with 95% confidence intervals from mixed-effects models. *p ≤0.05, **p ≤0.01, ***p ≤0.001 for the T2−T1 difference.

3.4 12-weeks of aerobic exercise did not impact resting T-cell phenotypes or mitochondrial bioenergetics.

Intervention effects at rest were assessed by comparing baseline pre-submaximal exercise at baseline (T1) and week 12 (T3) between exercise and control groups. Changes in the frequencies of CD4+ and CD8+ subsets, including RTE, naïve, CM, EM, TEMRA, and senescent subsets, were not different between groups (**Table 2**). Similarly, changes in absolute CD4+ and CD8+ T-cell subset counts from T1 to T3 were not different between groups (**Supplement Table S1**).

Cellular-level oxygen flow did not differ between groups in CE, LEAK, or all OXPHOS states (**Table 3**). Similarly, tissue-level oxygen flow did not differ between groups across respiratory states (**Supplement Table S2**).

Table 2. **Resting T-cell subset frequencies before (T1) and after (T3) 12 weeks of aerobic training, by group.**

Phenotype	Group	T1 Mean (95% CI)	T3 Mean (95% CI)	Δ T3-T1 Mean (95% CI)	Δ from Control Mean (95% CI)	p-value
CD4+ RTE	Control	23.32 (14.08, 32.56)	24.00 (14.75, 33.24)	0.68 (-10.77, 12.12)	0.00—Referent	—
	Exercise	22.89 (14.53, 31.25)	13.62 (5.26, 21.99)	-9.27 (-19.62, 1.09)	-9.94 (-25.38, 5.49)	0.21
CD4+ Naïve	Control	38.99 (36.59, 41.39)	39.38 (36.98, 41.78)	0.39 (-2.55, 3.32)	0.00—Referent	—
	Exercise	40.07 (37.90, 42.24)	42.51 (40.34, 44.68)	2.44 (-0.21, 5.09)	2.06 (-1.90, 6.01)	0.31
CD4+ CM	Control	39.77 (37.24, 42.30)	40.65 (38.12, 43.18)	0.88 (-2.09, 3.85)	0.00—Referent	—
	Exercise	39.82 (37.53, 42.11)	37.69 (35.41, 39.98)	-2.12 (-4.81, 0.57)	-3.00 (-7.01, 1.01)	0.14
CD4+ EM	Control	18.49 (16.59, 20.39)	16.90 (15.01, 18.80)	-1.59 (-3.78, 0.61)	0.00—Referent	—
	Exercise	18.02 (16.31, 19.74)	17.61 (15.89, 19.32)	-0.42 (-2.40, 1.57)	1.17 (-1.79, 4.13)	0.44
CD4+ TEMRA	Control	2.06 (1.27, 2.85)	2.38 (1.59, 3.17)	0.32 (-0.72, 1.36)	0.00—Referent	—
	Exercise	2.69 (1.97, 3.40)	2.78 (2.07, 3.50)	0.10 (-0.85, 1.04)	-0.22 (-1.63, 1.18)	0.76
CD4+ senescent	Control	2.83 (1.32, 4.35)	2.05 (0.54, 3.56)	-0.78 (-2.65, 1.08)	0.00—Referent	—
	Exercise	3.65 (2.22, 5.09)	4.17 (2.74, 5.61)	0.52(-1.25, 2.29)	1.31 (-1.26, 3.88)	0.32
CD8+ RTE	Control	1.52 (0.66, 2.39)	2.52 (1.65, 3.39)	0.99 (-0.07, 2.06)	0.00—Referent	—
	Exercise	1.48 (0.69, 2.26)	1.98 (1.19, 2.76)	0.50 (-0.47, 1.46)	-0.50(-1.93, 0.94)	0.50
CD8+ Naïve	Control	30.98 (29.12, 32.84)	32.38 (30.52, 34.24)	1.40 (-0.90, 3.70)	0.00—Referent	—
	Exercise	31.61 (29.92, 33.29)	32.31 (30.62, 34.00)	0.70(-1.38, 2.78)	-0.70(-3.80, 2.41)	0.66
CD8+ CM	Control	16.38 (14.79, 17.97)	17.07 (15.48, 18.66)	0.69 (-1.12, 2.51)	0.00—Referent	—
	Exercise	16.44 (15.00, 17.88)	16.43 (14.99, 17.87)	-0.01(-1.65, 1.63)	-0.70 (-3.14, 1.74)	0.57
CD8+ EM	Control	28.67 (26.54, 30.81)	27.65 (25.51, 29.79)	-1.02 (-3.71, 1.67)	0.00—Referent	—
	Exercise	28.48 (26.54, 30.42)	27.19 (25.25, 29.13)	-1.29 (-3.72, 1.14)	-0.27(-3.89, 3.35)	0.88
CD8+ TEMRA	Control	23.75 (21.12, 26.37)	22.68 (20.06, 25.31)	-1.07 (-4.57, 2.42)	0.00—Referent	—
	Exercise	23.69 (21.31, 26.07)	24.40 (22.02, 26.79)	0.71 (-2.45, 3.87)	1.78 (-2.93, 6.50)	0.46
CD8+ senescent	Control	22.14 (18.10, 26.18)	18.59 (14.42, 22.76)	-3.55 (-8.52, 1.43)	0.00—Referent	—
	Exercise	24.07 (20.29, 27.85)	22.54 (18.76, 26.32)	-1.53 (-6.08, 3.02)	2.02 (-4.72, 8.76)	0.56

* Frequencies are expressed as percentage of the CD4+ or CD8+ gate. Values are estimated marginal means with 95% confidence intervals from linear mixed-effects models adjusted for baseline values.

Table 3. **Resting T-cell mitochondrial oxygen flux before (T1) and after (T3) at the cellular level 12 weeks of aerobic training, by group.**

Substrate State	Group	T1 Mean (95% CI)	T3 Mean (95% CI)	Δ T3-T1 Mean (95% CI)	Δ from Control Mean (95% CI)	p-value
CE	Control	1.86 (1.53, 2.19)	1.73 (1.36, 2.10)	-0.13 (-0.63, 0.36)	0.00—Referent	—
	Exercise	1.95 (1.68, 2.22)	1.85 (1.56, 2.14)	-0.10 (-0.50, 0.30)	0.03 (-0.61, 0.67)	0.93
$PalM_L/F_L$	Control	0.59 (0.45, 0.73)	0.63 (0.47, 0.79)	0.03 (-0.18, 0.24)	0.00—Referent	—
	Exercise	0.62 (0.50, 0.74)	0.54 (0.40, 0.68)	-0.08 (-0.25, 0.09)	-0.11 (-0.39, 0.16)	0.42
$PalM_P/F_P$	Control	2.03 (1.64, 2.42)	1.76 (1.35, 2.17)	-0.31 (-0.86, 0.25)	0.00—Referent	—
	Exercise	2.19 (1.86, 2.52)	1.60 (1.27, 1.93)	-0.59 (-1.05, -0.12)	-0.28 (-1.01, 0.44)	0.44
$PalMPyr_P/FN_P$	Control	2.42 (1.95, 2.89)	2.19 (1.68, 2.70)	-0.23 (-0.91, 0.45)	0.00—Referent	—
	Exercise	2.54 (2.13, 2.95)	2.22 (1.79, 2.65)	-0.32 (-0.89, 0.25)	-0.09 (-0.97, 0.80)	0.84
$PalMPyrG_P/FN_P$	Control	2.52 (1.99, 3.05)	2.36 (1.79, 2.93)	-0.16 (-0.92, 0.60)	0.00—Referent	—
	Exercise	2.67 (2.21, 3.13)	2.22 (1.74, 2.70)	-0.45 (-1.09, 0.19)	-0.29 (-1.28, 0.71)	0.57
$PalMPyrGS_P/FNS_P$	Control	5.82 (4.45, 7.19)	5.35 (3.90, 6.80)	-0.47(-2.37, 1.44)	0.00—Referent	—
	Exercise	6.26 (5.10, 7.42)	4.91 (3.69, 6.13)	-1.36 (-2.96, 0.25)	-0.89 (-3.38, 1.60)	0.48
$PalMPyrGSR_P/S_P$	Control	5.29 (4.11, 6.47)	4.88 (3.61, 6.15)	-0.41 (-2.04, 1.22)	0.00—Referent	—
	Exercise	5.62 (4.61, 6.63)	4.53 (3.47, 5.59)	-1.09 (-2.46, 0.28)	-0.68 (-2.81, 1.44)	0.53

* Fluxes are expressed as $pmol \cdot s^{-1} \cdot 10^6$ T-$cells^{-1}$ across sequential substrate-uncoupler-inhibitor titration states. Values are estimated marginal means (95% CI) from linear mixed-effects models adjusted for baseline values.

3.5 12-week of aerobic exercise did not impact the acute T-cell or mitochondrial bioenergetic response to submaximal exercise.

Training effects on acute submaximal exercise responses were assessed by comparing the baseline acute exercise response (T2-T1) with the week-12 acute exercise response (T4-T3) between exercise and control groups. Changes in the acute exercise response were not different between groups for any CD4+ or CD8+ T-cell subset frequency or absolute cell count (**Table 4**). Similarly, changes in the acute mitochondrial respiratory response did not differ between groups at the cellular level or tissue level (**Table 5**). The largest tissue level difference was observed in $PalM_P/F_P$ [-1.76 pmol·s^{-1}·mL $blood^{-1}$ (95%CI: -3.62, 0.11); p=0.065].

Table 4. Acute T-cell subset response to exercise after 12 weeks of training (T4−T3) compared with before training (T2−T1), by group.

Phenotype	Group	(T4-T3) - (T2-T1) Mean (95% CI)		Δ from Control Mean (95% CI)		p-value	
		Frequency, %	Count, cells/μL	Frequency	Count	Frequency	Count
CD4+ RTE	Control	−1.63 (−17.82, 14.56)	−9.97 (−37.29, 17.34)	0.00—Referent	0.00—Referent	—	—
	Exercise	−0.80 (−15.91, 14.31)	−4.40 (−29.43, 20.64)	0.83 (-21.32, 22.97)	5.58 (-31.47, 42.63)	0.94	0.77
CD4+ Naïve	Control	1.49 (−2.66, 5.63)	10.40 (−67.08, 87.87)	0.00—Referent	0.00—Referent	—	—
	Exercise	1.60 (−2.27, 5.47)	−7.29 (−79.75, 65.16)	0.12 (-5.55, 5.79)	-17.69 (-123.76, 88.38)	0.97	0.74
CD4+ CM	Control	0.44 (−3.76, 4.64)	11.63 (−58.22, 81.48)	0.00—Referent	0.00—Referent	—	—
	Exercise	−0.24 (−4.17, 3.68)	−23.50 (−88.80, 41.80)	-0.68 (-6.44, 5.07)	-35.13 (-130.75, 60.49)	0.82	0.47
CD4+ EM	Control	−2.07 (−5.18, 1.04)	−13.44 (−47.55, 20.67)	0.00—Referent	0.00—Referent	—	—
	Exercise	−1.22 (−4.12, 1.68)	−17.52 (−49.33, 14.28)	0.85 (-3.40, 5.10)	-4.08 (-50.72, 42.55)	0.70	0.86
CD4+ TEMRA	Control	0.15 (−1.33, 1.62)	−2.24 (−13.36, 8.88)	0.00—Referent	0.00—Referent	—	—
	Exercise	−0.13 (−1.50, 1.24)	−1.16 (−11.51, 9.19)	−0.28 (−2.29, 1.73)	1.08 (-14.11, 16.28)	0.79	0.89
CD4+ senescent	Control	−0.72 (−3.36, 1.91)	2.20 (−25.67, 30.07)	0.00—Referent	0.00—Referent	—	—
	Exercise	0.44 (−2.15, 3.03)	8.40 (−18.85, 35.66)	1.16 (−2.54, 4.86)	6.21 (-32.78, 45.19)	0.54	0.76
CD8+ RTE	Control	−0.33 (−3.59, 2.94)	3.79 (−5.35, 12.94)	0.00—Referent	0.00—Referent	—	—
	Exercise	−0.33 (−3.38, 2.72)	0.43 (−8.10, 8.96)	0.01 (-4.45, 4.46)	-3.36 (-12.20, 5.48)	1.00	0.46
CD8+ Naïve	Control	0.23 (−2.42, 2.88)	5.08 (−24.79, 34.95)	0.00—Referent	0.00—Referent	—	—
	Exercise	−0.10 (−2.58, 2.38)	−16.19 (−44.17, 11.79)	−0.33 (−3.98, 3.31)	-21.27 (-50.14, 7.61)	0.86	0.15
CD8+ CM	Control	0.52 (−2.17, 3.21)	3.72 (−16.94, 24.37)	0.00—Referent	0.00—Referent	—	—
	Exercise	−0.50 (−3.01, 2.00)	−4.37 (−23.70, 14.96)	-1.01 (-4.52, 2.50)	-8.09(-28.06, 11.88)	0.57	0.43
CD8+ EM	Control	−2.66 (−6.61, 1.29)	−11.25 (−38.83, 16.33)	0.00—Referent	0.00—Referent	—	—
	Exercise	−0.88 (−4.57, 2.82)	−9.11 (−34.91, 16.70)	1.78 (-3.42, 6.97)	2.15(-34.05, 38.35)	0.50	0.91
CD8+ TEMRA	Control	2.48 (−2.67, 7.63)	10.06 (−44.17, 64.29)	0.00—Referent	0.00—Referent	—	—
	Exercise	1.88 (−2.94, 6.70)	5.66 (−44.81, 56.13)	-0.59 (-7.35, 6.16)	-4.41 (-75.69, 66.87)	0.86	0.90
CD8+ senescent	Control	0.46 (−5.84, 6.75)	2.73 (−35.71, 41.18)	0.00—Referent	0.00—Referent	—	—
	Exercise	−0.87 (−6.76, 5.03)	−2.54 (−37.79, 32.70)	-1.33 (-10.98, 8.33)	-5.28 (-57.44, 46.88)	0.79	0.84

Table 5. Acute T-cell mitochondrial cellular- and tissue-level respiratory response to exercise after 12 weeks of training (T4−T3) compared with before training (T2−T1), by group.

Substrate State	Group	(T4-T3) - (T2-T1) Mean (95% CI)		Δ from Control Mean (95% CI)		p-value	
		Cell pmol·s^{-1}·10^{6} T-cells^{-1}	Tissue pmols·s^{-1} ·mL blood^{-1}	Cell	Tissue	Cell	Tissue
CE	Control	0.45 (−0.24, 1.14)	0.76 (−0.49, 2.01)	0.00—Referent	0.00—Referent	—	—
	Exercise	0.30 (−0.29, 0.89)	0.58 (−0.48, 1.64)	-0.15 (-1.05, 0.75)	-0.17 (-1.82, 1.47)	0.75	0.84
$PalM_L/F_L$	Control	0.34 (0.05, 0.63)	0.73 (0.10, 1.36)	0.00—Referent	0.00—Referent	—	—
	Exercise	0.21 (−0.04, 0.46)	0.36 (−0.17, 0.89)	-0.13 (-0.52, 0.26)	-0.36 (-1.18, 0.45)	0.50	0.38
$PalM_P/F_P$	Control	0.96 (0.18, 1.74)	1.73 (0.32, 3.14)	0.00—Referent	0.00—Referent	—	—
	Exercise	0.21 (−0.48, 0.90)	−0.02 (−1.25, 1.21)	-0.75 (-1.79, 0.28)	-1.76 (-3.62, 0.11)	0.15	0.065
$PalMPyr_P/FN_P$	Control	1.15 (0.19, 2.11)	2.37 (0.53, 4.21)	0.00—Referent	0.00—Referent	—	—
	Exercise	0.33 (−0.51, 1.17)	0.35 (−1.26, 1.96)	-0.82 (-2.08, 0.45)	−2.01 (−4.35, 0.44)	0.221	0.11
$PalMPyrG_P/FN_P$	Control	1.14 (0.06, 2.22)	2.53 (0.43, 4.63)	0.00—Referent	0.00—Referent	—	—
	Exercise	0.44 (−0.50, 1.38)	0.41 (−1.43, 2.25)	-0.74 (-2.16, 0.69)	-2.12 (-4.91, 0.67)	0.31	0.14
$PalMPyrGS_P/FNS_P$	Control	1.64 (−1.05, 4.33)	3.13 (−2.55, 8.81)	0.00—Referent	0.00—Referent	—	—
	Exercise	1.01 (−1.34, 3.36)	1.53 (−3.43, 6.49)	-0.63 (-4.19, 2.94)	-1.59 (-9.14, 5.95)	0.73	0.68
$PalMPyrGSR_P/S_P$	Control	1.38 (−0.91, 3.67)	2.63 (−2.34, 7.60)	0.00—Referent	0.00—Referent	—	—
	Exercise	1.06 (−0.94, 3.06)	1.78 (−2.58, 6.13)	-0.32 (-3.36, 2.72)	-0.85 (-7.46, 5.76)	0.84	0.80

4. Discussion

In this pilot ancillary study, an acute bout of submaximal exercise increased absolute circulating CD4+ and CD8+ T-cell counts, shifted subset frequencies toward more differentiated phenotypes, and increased tissue-level mitochondrial bioenergetic capacity. However, 12 weeks of moderate-intensity aerobic exercise training did not change resting T-cell frequencies or absolute counts, mitochondrial bioenergetic capacity, or the magnitude of the acute T-cell and mitochondrial bioenergetic response to submaximal exercise. Although the direction of the acute response we observed is consistent with reports in healthy populations [14, 15, 19], it was previously unknown whether CRC survivors, many of whom have completed chemotherapy and/or radiotherapy, retain this capacity for exercise-induced immune mobilization.

Structured exercise improves disease-free and overall survival among individuals with colon cancer [3], but the biological mechanisms underlying these benefits remain incompletely understood. One proposed pathway is that acute exercise mobilizes circulating T-cells via catecholamine-mediated β2-adrenergic receptor signaling and modifies their mitochondrial bioenergetic capacity within circulating T-cells [15, 18]. We therefore designed this study to test whether this acute response is present in CRC survivors and whether it is modified by a structured aerobic training program.

The baseline acute exercise response (T2-T1), increased the absolute number of most CD4+ and CD8+ T-cell subsets, including naïve, CM, EM, TEMRA, and senescent T-cells. At the same time, the frequency of naïve CD4+ and CD8+ T-cells decreased, whereas frequencies of more differentiated T-cell subsets increased. This pattern mirrors observations in healthy populations after acute moderate-to-high-intensity exercise [19], likely because differentiated T-cells express greater densities of β2-adrenergic receptors than naïve T-cells, making them preferentially responsive to catecholamine-driven demargination during exercise [15].

Acute exercise also increased tissue-level bioenergetic capacity across routine, LEAK, and OXPHOS states, while cellular-level oxygen flow remained unchanged. Because blood is a dynamic tissue in which circulating T-cell numbers rise rapidly during exercise, unlike skeletal muscle, where cell number remains fixed in response to acute exercise, this pattern suggests that the acute increase in systemic mitochondrial bioenergetic capacity was driven primarily by additional T-cell mobilization into circulation rather than by enhanced mitochondrial function within individual cells. This finding extends our prior work in young, healthy individuals, in which maximal exercise increased tissue-level oxygen flow through a similar mobilization mechanism [18]. Direct comparisons should be made cautiously given differences in population (CRC survivors with sedentary lifestyles vs. highly trained young adults), cancer-treatment history, and exercise type and intensity (submaximal vs. maximal), any of which may influence the cellular mitochondrial response to acute exercise. In addition to catecholamine-mediated mobilization, exercise-induced increases in circulating lactate may have contributed to the tissue-level, but not cellular-level, mitochondrial response. Our prior *in vitro* work showed that lactate concentrations reached during vigorous, but not submaximal, exercise are sufficient to precondition cellular mitochondrial respiration [17].

Aerobic exercise training over 12 weeks did not alter the frequency or absolute count of any T-cell subset at rest, nor resting T-cell mitochondrial bioenergetic capacity, between groups. We tested this hypothesis that training-induced changes in T-cell phenotype could mitigate the hallmark features of immunosenescence, namely expansion of senescent T-cells and reduced naïve T-cell frequency and RTE output [10, 11]. A prior randomized trial in postmenopausal women at high risk of breast cancer reported that 12 weeks of moderate-intensity continuous training increased EM CD8+ T-cell frequency. Compared with high-intensity interval training, moderate-intensity continuous training also preserved or increased total T-cells, naïve CD4+ T-cells, CM CD4+ T-cells, and RTE CD4+ T-cells. These findings raised the possibility that

longitudinal, moderate-intensity exercise training could favorably remodel the peripheral T-cell pool by preserving naïve T-cell frequency and limiting senescent T-cell expansion [16]. However, participants in that trial were women at elevated breast cancer risk without a history of cancer treatment, whereas participants in our study were CRC survivors who had completed curative-intent surgery followed by chemotherapy and/or radiotherapy. Since cancer-directed cytotoxic treatment can contribute to immunosenescence alterations in the peripheral T-cell pool [6], CRC survivors may have greater potential for exercise-related remodeling than treatment-naïve populations. Despite this, we observed no between-group differences in resting T-cell subset frequency or count after 12 weeks of training. One possible explanation is that treatment-related immune alterations may limit the capacity of the peripheral T-cell compartment to adapt to aerobic exercise training. Given that our present study did not include a treatment-naïve comparison or evaluate treatment-by-exercise interactions, this interpretation is speculative.

We tested this hypothesis based on the premise that aerobic exercise training may shape the acute physiological response that drives T-cell mobilization. Acute exercise increases sympathetic activity and hemodynamic shear stress, which promote the demargination and mobilization of differentiated T-cell subsets through catecholamine-mediated β2-adrenergic receptor signaling [8]. Highly differentiated CD8+ T-cell subsets, including EM and TEMRA T-cell subsets, have greater cytotoxic and tissue-migratory potential than naïve T-cells [14]. Their transient, repeated redistribution into the circulation during exercise has been proposed as a mechanism by which acute exercise enhances tumor immune surveillance [29]. We therefore hypothesized that 12 weeks of exercise would amplify the magnitude of acute T-cell mobilization and mitochondrial bioenergetic responses to submaximal exercise from week 12. Contrary to our third hypothesis, 12 weeks of aerobic exercise training did not change the magnitude of the acute T-cells or mitochondrial bioenergetic response to submaximal exercise from baseline to week 12 [(T4−T3) − (T2−T1)]. The between-group estimate for the change in acute tissue-level

$PalM_P/F_P$ oxygen flow suggested a trend toward a lower response in the exercise group relative to the control group [-1.76 pmol·s^{-1}·mL blood^{-1} (95% CI: -3.62, 0.11); p=0.065]. Given the number of comparisons performed, this finding should be interpreted as hypothesis-generating rather than as evidence that aerobic exercise training fails to modify the bioenergetic capacity.

There are several limitations to this study. First, this was a pilot ancillary analysis with a small sample size, thus it was not powered to establish the absence of training effects on T-cell phenotypes and mitochondrial bioenergetic capacity, and larger randomized trials are needed to determine whether aerobic training modifies the magnitude, phenotype, or mitochondrial characteristics of the acute T-cell response to exercise in CRC survivors. Non-significant findings should be interpreted as insufficient evidence of an effect, rather than proof of no effect. Second, the single-site sample with variable treatment histories may limit generalizability. Third, we did not measure circulating catecholamines or lactate, precluding direct assessment of whether training altered these mediators of the acute response. Therefore, these results are hypothesis-generating and require replication in larger, adequately powered trials.

This study also has important strengths. The four-timepoint design allowed us to evaluate resting, acute, and training-related T-cell and mitochondrial responses within a single randomized ancillary study nested in an NCI-sponsored parent trial. To our knowledge, this is the first study to characterize the acute T-cell mobilization and mitochondrial bioenergetic response to a submaximal exercise test specifically in CRC survivors, a design that has not previously been applied in this population. CRC survivors, all of whom had completed cancer-directed treatment, were able to complete a symptom-limited submaximal exercise test and mobilize circulating T-cells and tissue-level mitochondrial bioenergetic capacity to a degree qualitatively consistent with reports in healthy populations, demonstrating that acute T-cell mobilization and mitochondrial oxygen flow responses remain measurable after cancer-directed treatment. Furthermore, our combined use of comprehensive flow cytometric phenotyping,

absolute lymphocyte-derived T-cell counts, and high-resolution respirometry allowed us to distinguish cellular-level from tissue-level mitochondrial oxygen flow, providing a more mechanistically robust account of the acute mitochondrial bioenergetic response than one method alone. In particular, high-resolution respirometry resolves oxygen flux across multiple mitochondrial respiratory states, providing a more detailed, state-specific profile of bioenergetic function.

5. Conclusions

An acute bout of submaximal exercise, rather than 12 weeks of aerobic exercise training, was the main driver of T-cell mobilization and mitochondrial bioenergetic change in this cohort of CRC survivors. These findings suggest that repeated acute exercise-induced immune responses, rather than lasting changes in resting T-cell phenotypes or mitochondrial oxygen flow, may underlie the biological effects of regular aerobic exercise. However, larger, adequately powered trials are needed to confirm the acute exercise findings reported here and to determine whether resting T-cell phenotype and mitochondrial bioenergetics are modifiable with longer or higher-intensity aerobic exercise training in CRC survivors.

6. List of abbreviations

ACSM: American College of Sports Medicine

ADP: Adenosine diphosphate

BSA: Bovine serum albumin

CD: Cluster of differentiation

CE: Routine respiration in intact cells

CI: Confidence interval

CM: Central memory

CRC: Colorectal cancer

EM: Effector memory

I_{O2}: Oxygen flow

IQR: Interquartile range

KLRG1: Killer cell lectin-like receptor G1

LEAK: Leak (non-phosphorylating) respiratory state

MACS: Magnetic-activated cell sorting

mAb: Monoclonal antibody

MiR05: Mitochondrial respiration medium 5

MMRM: Mixed models for repeated measures

NCI: National Cancer Institute

OXPHOS: Oxidative phosphorylation

PBMC: Peripheral blood mononuclear cell

ROX: Residual oxygen consumption

RTE: Recent thymic emigrant

SD: Standard deviation

TEMRA: Terminally differentiated effector memory T-cells re-expressing CD45RA

VO2peak: Peak oxygen consumption

7. Declarations

Ethics approval and consent to participate: The study protocol and informed consent documents, including procedures for collection and banking of blood samples, were approved by the Institutional Review Board at Pennington Biomedical Research Center, Baton Rouge, LA (2022-014-PBRC). All participants provided written informed consent prior to enrollment. The parent trial was registered on ClinicalTrials.gov (NCT05789433).

Consent for publication: Not applicable. Availability of data and materials: The datasets generated and/or analysed during the current study are not publicly available due to participant confidentiality in an ongoing randomized trial but are available from the corresponding authors on reasonable request.

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Authors' contributions: EC and GS conceived and designed the study. EC, BAI, and SY performed the formal analyses. ALT contributed to data collection, and BAI contributed to data curation. EC and GS drafted the original manuscript. JCB contributed to study methodology, formal analysis, funding acquisition, project administration, resources, supervision, and critical revision of the manuscript. GS also contributed to methodology, formal analysis, project administration, resources, and supervision. SLEC, ALT, SY, MAT, AZ, and BAI critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Reference

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