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Original Research

Post-Exercise T-Cell Response Influenced by Absolute Intensity in Resistance Exercise, But Not Cardiorespiratory Exercise in Middle-Aged to Older-Adults

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Abstract

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Exercise may be a method to improve desirable numbers of circulating T cells; however, the best exercise dose and type for increasing circulating T cells is still relatively unknown. The purpose of this study was to determine the role of absolute intensity during a single session of cardiorespiratory exercise or resistance exercise on exercise-induced changes in T cell subsets counts. In this randomized complete crossover design, healthy middle-aged-to-older adults (n=24; 63.0 ± 1.0 years) completed a single session of treadmill walking (cardiorespiratory exercise: CRE) for 30 minutes at 70% of heart rate reserve or resistance exercise (RE) at 70% 1 repetition maximum on separate days. Blood was collected at rest and immediately after exercise. Cells were stained and analyzed on a flow cytometer. Serum was stored for batch analyses of cortisol and cytomegalovirus seropositivity. Absolute RE intensity (total volume lifted) had a positive effect on the number of T cells in circulation for five of the T cell populations (CD3+, CD4+ CM, CD8+ CM, CD8+ EM, and Naïve CD8+; $p<0.05$), but small effect size may suggest a limited significance for some T cell subsets. Absolute CRE intensity (treadmill speed, grade) had no effect on any of the 16 T cell subsets. At the same relative intensity, circulating T cell response to acute exercise is influenced by absolute intensity in resistance exercise but not in cardiorespiratory exercise. This supports that RE absolute intensity may need to be considered with relative intensity to modify post-exercise T cell numbers.

Keywords: Immunity, Aerobic Exercise, Weight Training, Acute Exercise, CMV

Introduction

With increasing age is an increasing likelihood for developing acute and chronic illnesses.[1–5] Physical inactivity contributes to this increased chance of developing illnesses, by acting as a physiological disregulator to physical health.[6–9] Specifically, a lack of physical activity can lead to detrimental changes in the immune system. In addition to increased senescent T cells,[11–13] physical inactivity in aging is associated with lower quality of life (QoL), higher reported fatigue, lower physical function, and lower physiological systematic efficiency.[6–8,14–21] Despite the importance of physical activity for health, exercise prescription knowledge gaps exist.

T cells are a lymphocytic immune cell with many roles, including killing pathogens and diseased

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cells.[22] Two subsets of T cells are helper (CD4+) and cytotoxic T cells (CD8+). CD4+ helper T cells assist other immune cells, such as B cells and CD8+ T cells, to identify threats and increase the immune response. The CD4+ T cells also help to recognize and trigger cell death with CD8+ T cells killing mutated or dysregulated diseased cells and foreign pathogens throughout the human body.[23] Both CD4+ and CD8+ T cells can be further classified into additional subsets. Effector memory T cells (CD4+ EM, CD8+ CM, CD4+ EMRA, CD8+ EMRA) and central memory T cells (CD4+ CM, CD8+ CM) play a crucial role in immune surveillance and pathogen recognition for the lymphoid organs (CM) and peripheral organs (EM). Naïve T cells (Naïve CD4+, Naïve CD8+) also have a role in immune surveillance, in part by encountering new pathogens before differentiating into memory cells. Other subsets can denote higher inflammatory expression (Th17+, CD4+ CD57+, CD8+ CD57+) or increased senescent and exhaustive cells (CD4+ CD57+ EMRA, CD8+ CD57+ EMRA).[23–26] In short, T cells are a paramount component of immune health and are especially important in aging for their ability to recognize or fight off chronic illnesses and acute infections.[27] However, as age of the person increases so does the proportion of T cells in circulation that are senescent. These senescent T cells are not as efficient in proliferation and effector roles, leading to more pathogens and tumor cells evading immune surveillance.[23] There is some evidence that physical activity reduces the number of circulating senescent T cells with age.[11–13] This decrease in aging-linked senescence may result in increased immune function and less overall incidences of infectious diseases or cancers;[11,12] highlighting the potential importance of physical activity mediation in middle-aged-to-older adults.

Exercise is frequently linked to reducing age-associated cardiometabolic disease risk factors, such as insulin resistance and blood lipids. However, exercise can also improve overall immune health and T cell function.[11–13] Both acute, single session and chronic exercise training interventions support the ability of exercise to improve immunity.[1,11–13,28–31] For example, in a study of breast cancer survivors, a six-month combined resistance exercise (RE) and cardiorespiratory exercise (CRE) training program improved lymphocyte activation.[28] Further, after a walking program breast cancer survivors had a lower number of CD4+ EMRA T cells.[29] Similarly, 6-and-12 week resistance-specific programs were beneficial to lower exhausted CD8+ T cells from accumulating in sedentary older adults.[15,16] For these reasons, different types of exercise may provide different health benefits. Although these examples are all from chronic exercise training studies, to best understand how exercise is linked to immune health it is important to also understand the effect of each individual exercise session.

To date, no research study that measured the number of circulating T cells post-exercise in middle-aged-to-older adults, has included both cardiorespiratory and resistance exercise as separate acute exercise sessions. Such a study design allows the direct comparison of the effect of these two exercise types. Further, most published exercise studies focus on CRE or mixed CRE and RE exercise, which contributes to a limited understanding of the RE specific effects on circulating T cells.[13,30] CRE, such as biking or running, may provide more lower-body and cardiovascular-intensive benefits while RE may provide further musculoskeletal and hypertrophic benefits; underscoring the importance of understanding potential differences in the health benefits. In addition, exercise immunology studies almost universally rely solely on relative intensity, suggesting that a relative effort threshold is the primary initiation to elicit an immune response. Studies that include absolute intensity to examine the exercise-induced circulating T cell numbers are much sparser.[13,30] In addition, comparing the response of participants of various fitness levels will help ensure a wide range of absolute exercise intensities at a given relative intensity, and may also elucidate if there is an exercise fitness effect. Together, a study comparing two types of

exercise and two exercise training statuses will support future exercise training studies, and lead to future studies of the exercise dose required for minimal and maximal immune and physical health benefits.

The purpose of this study was to understand the effect of absolute exercise intensity on the number of circulating T cells, following an acute bout of exercise, in middle-aged-to-older adults. Though previous studies support the effect of relative intensity on circulating T cell response to both acute CRE and RE bouts,[31,32] it is unknown if absolute workload also has a significant influence on exercise-induced circulating T cell responses. For this reason, this analysis is highly exploratory, and aims to examine whether absolute weight lifted, or speed and grade walked, may further compound immune cell changes seen by exercise. This study will compare, at the same relative intensity, the influence of TM grade, TM speed, and RE volume on the exercise-induced increase in circulating T cell response to separate acute cardiorespiratory and resistance exercise bouts. We hypothesize that absolute intensity (TM speed, TM grade, RE volume) will not influence circulating T cell response to acute exercise in separate resistance and cardiorespiratory exercise bouts at the same relative intensity.

Methods

Participants

Twenty-four (71% female) community-dwelling healthy middle-aged-to-older adults of a variety of exercise training backgrounds participated in this study. All participant characteristics can be found in Table 4. Power analysis data, retention and recruitment flow chart (CONSORT figure) are previously reported.[31] All data collection occurred in the Laboratory of Integrative Physiology in the Department of Health & Human Performance at the University of Houston.

This study was approved by the Institutional Review Board at the University of Houston. Written informed consent was obtained by each participant prior to study. The datasets generated during and/or analyzed during the current study are not publicly available due to manuscript preparation on a secondary analyzes with overlapping variables, but are available from the corresponding author on reasonable request. The authors have no relevant financial or non-financial or competing interests to disclose. This research was carried out fully in accordance with the ethical standards of the *International Journal of Exercise Science*.[22]

Protocol

As previously reported,[31,32] all participants self-reported being in good health, and were recruited as being either physically active (PA) or physically inactive (PI) in accordance with ACSM definitions[33] and PA and PI participants were included in equal distribution (n=12 each classification). Contraindication to moderate or vigorous exercise; immune-influencing mediations (NSAID, statins, bisphosphonates, steroids, etc.), cardiovascular, metabolic, renal, or respiratory diseases; and a BMI > 30 kg • m^{-2} or BMI < 18.5 kg • m^{-2} were excluded from this study. Additional exclusions included inability to refrain from recreational drugs and/or alcohol within 24 hours prior to study visits, excessive travel that would impact study visit scheduling, and, for females, pre-menopausal. If the subject agreed to take part in this study, the data was collected: PA frequency questionnaire, height, weight, medical history, and body composition. If all inclusion criteria were met, follow-up visits were scheduled.

Participants were shown proper use of the exercise equipment during a follow-up visit, and subsequently completed a set of all exercises at a predicted 50% of 1RM, estimated from weights lifted during the exercise initialization. This session was an exercise protocol "familiarization" session for the participant. In addition, a 2-stage VO_2max estimation treadmill test, 8RM strength testing on each exercise (to estimate 1RM), and a body composition estimation (DEXA; Horizon A, Hologic, Marlborough, MA USA) were conducted. The two exercise testing days occurred at least one week after the familiarization session and at least 7 days apart from each other.

After initialization, participants completed separate CRE and RE trials, in a randomized order. These two trials were scheduled at least a week apart. During CRE training sessions, participants walked on a treadmill at an intensity of 60-70% of their heart rate reserve (HRR) for 30 minutes, plus a 5-minute warm-up and 2-minute cool down. During the test, the 6-20 Borg Scale was used for rating of perceived exertion.

On the RE trial day, participants completed 3 sets of 8 resistance exercises on stacked-weight machines. The resistance exercises were: lateral pull down, seated row, triceps extensions, chest press, leg extension, leg curl, leg press, and weighted calf raise. Each set consisted of 8-12 repetitions at 70% 1RM, taken to volitional stop. Participants were given 30-120 seconds of intraset rest, and 1-2 minutes in between sets, as an individualized cadence. The duration of this bout was approximately 30-minute, plus the same 5-minute TM warm-up and 2-minute TM cool-down as the CRE bout. CRE and RE exercise bouts were matched at 70% relative intensity (moderate-to-vigorous), commonly used in exercise prescription, to represent an individual-adjusted bout of challenging, well-tolerated exercise that can elicit a physiological response.

The Exercise Frequency Questionnaire (EFQ) and a 7-day accelerometer (ActiGraph GTx, ActiGraph, Pensacola, FL USA) recording were used to subjectively and objectively determine amounts of physical activity by the participants. A two-stage treadmill test was used to estimate VO_2max and cardiorespiratory fitness. An 8RM tests on each of the 8 stacked weight machines was utilized to estimate 1RM to for the 70% intensity prescription. Heart rate was measured using a Polar heart rate monitor (Polar V800). Heart rate data was gathered at rest and post exercise for both bouts. Body composition estimates were collected by using the dual energy x-ray absorptiometry (DEXA; Horizon A, Hologic, Marlborough, MA USA). All DEXA tests were conducted in the morning after an overnight fast and after refraining from fluids for 2 hours.

Participant blood was drawn pre-exercise (PRE) and immediately post-exercise (POST). As previously described,[31] fresh whole blood was stained, lysed, washed, and analyzed to quantify T cells using flow cytometry (Miltenyi MACSQuant10 Analyzer). A detailed gating strategy is described in the primary paper.[31] Separated serum was stored at -80°C for batch analyses. Cytomegalovirus (CMV) seropositivity was determined with a commercially available kit, following kit instructions (CalBiotech; CMV IgG ELISA, El Cajon, CA USA).

Statistical Analysis

A linear mixed-effects model was used to assess the relationship between predictor variables (TM grade, TM speed, RE volume) on the outcome variable (differences in T cell counts pre and post exercise), with and without potential covariates. Time and its interaction with the primary predictor were also included in the model; a random intercept by subject was included to model repeated observations with subjects. Potential covariates considered in the variable select models were resting heart rate, cortisol, body weight, relative body fat (percent), BMI, age,

and sex. Covariates were considered both as univariate variables and for their joint predictive value in each model. Variable selection models (Tables 2 and 3) were used to identify important variables and drop correlated but less predictive variables in predicting the POST value. Stepwise regression was used to define the model space, and the Bayesian Information Criterion (BIC) was minimized to choose the best model. All analyses were done in R (version 4.0.2), excluding PRE-to-POST T cell changes in Microsoft Excel (version 16.80). Data are reported as mean ± SE. As there was only one missing covariate value (resting heart rate) in one subject, all models were fit using subjects for which the model variables were completely observed.

Results

Of the 24 total participants, twelve physically active (PA) and 12 physically inactive (PI) participants were included in the data analysis. Eleven participants were CMV seronegative, and 13 participants were seropositive. Serum cortisol pre CRE (159.83 ± 8.39 ng/mL) concentration was not different from pre RE (148.61 ± 7.73 ng/mL). RE volume was used for absolute RE intensity, and was calculated as *number of sets x repetitions x weight lifted* for each of the eight exercises, then the eight exercises summed for total volume. Treadmill speed and treadmill grade were used for CRE absolute intensity, and varied by participant as well. The walking speed was selected as a comfortable walking pace for the participant, and the grade adjusted to increase the participant's heart rate to 70% HRR.

Changes in the number of circulating T cells from PRE to POST for each subtype and exercise type are listed in Table 1. The first analyses only included the absolute intensity values as predictors (no covariates). When considering absolute CRE intensity (TM grade and TM speed) in the initial predictor model, the TM grade and TM speed did not predict the post-exercise change in the number of circulating T cells for any subset (Table 1). In the initial RE predictor model, total volume lifted was a significant predictor of PRE-POST changes in circulating T cells for CD4+ central memory (CM) and naïve CD4+ T cell subsets (Table 1).

After the first model, predictor variables from a variable selection model with potential covariates were selected. The variable selection model results for the CRE bout are detailed in Table 2. As expected, the number of T cells PRE was a significant predictor for all cell subsets. CMV seropositivity was a predictor for CD4+CD57+, CD8+, CD8+CD57+, CD8+ CD57+ EMRA (effector memory cell re-expressing CD45RA), CD8+ CM, CD8+ EM (effector memory), and CD8+ EMRA. Most of these cell types are highly differentiated T cells, which retain antigen memory to better help recognize and attack foreign pathogens. PRE cortisol was also a predictor for some cell subsets: CD8+ CM, CD4+ EM, and CD4+ EMRA. However, these effects were much smaller, as indicated by the small linear regression estimates.

The variable selection model results for the RE bout are detailed in Table 3. All of the same covariates of interest for CRE (Table 2) were analyzed in RE (Table 3) as well. No covariates other than PRE T cell number, including CMV status or serum cortisol concentrations, significantly predicted exercise-induced changes in T cell number. When all potential covariates were included in the variable selection model, only absolute intensity (total volume lifted) affected the outcome variable.

Discussion

These results support that the absolute intensity of a single session of RE performed at the same

relative intensity influences the degree of mobilization of T cells into circulation. When accounting for all covariates, total volume (weight lifted) was a significant predictor of the pre-to post-exercise change in circulating numbers of CD3+, CD4+ CM, CD8+ CM, CD8+ EM, and Naïve CD8+. There was no evidence of an effect on relative intensity (treadmill grade or speed) for cardiorespiratory treadmill walking. If one of the goals of a resistance exercise session is to increase circulating T cell numbers post-exercise, which in turn could temporarily increase immune surveillance after each exercise session, then it may be prudent to consider higher absolute volume when designing the exercise prescription. This could be achieved by increasing the number of sets or repetitions, allowing the participant to not increase the weight lifted. However, it is important to also stress that our observations are for an exercise session of 70% of 1RM. It is currently unknown if, at other intensities of relative exercise, total volume lifted is a factor in T cell response to exercise. Total volume at other intensities of % 1RM should be studied before extrapolating these results.

Although the results do support a significant effect of absolute resistance exercise volume on the number of circulating T cells after an acute bout of resistance exercise, the effect size for some T cell subsets is small. The small effect size for volume is especially notable when comparing the effect size of the pre-exercise circulating T cell count. Pre-exercise circulating T cell count as a significant predictor is not unexpected, as in the primary study[31,32] pre-exercise circulating T cell count was a covariate in the liner-effects mixed model analyses and because of this the change in cell count was used in the current statistical analyses. However, even with pre-exercise circulating T cell count in the model, the absolute intensity (total volume) of the acute resistance exercise bout still influenced T cell response to resistance exercise in CD3+, CD4+ CM, CD8+ CM, CD8+ EM and naïve CD4+ cell subsets. This aligns with previous systematic review findings on acute RE and leukocytes,[30] which also saw a post-acute exercise dose-response relationship for mobilization of leukocytes.

In the aforementioned systematic review,[30] Szlezak et al. state that resistance exercise increases circulating catecholamines (adrenaline), which may contribute to the post-resistance exercise mobilization of leukocytes. Specifically, acute CD4+ and CD8+ T cells were greater elicited through higher post-exercise adrenaline and lactate levels.[30] In our study, we measured cortisol, another hormone produced by the adrenal glands, but there was no relationship between cortisol T cell change in response to resistance exercise. This may have been due to the acute nature of exercise, or that all participants exercising at the same relative intensity, as there is a known relationship between relative exercise intensity and cortisol.[34] Further, when adults aged 60-70 years completed a resistance exercise session at 30% 1RM, 50% 1RM, or 80% 1RM there were only changes in cortisol after the 30% 1RM and 50% 1RM sessions, and not a change in cortisol after the 80% 1RM session [35]. The mean age of the participants in our study is 63 years, and the resistance exercise sessions closest to the 80% 1RM group. The results of the Taha et al.[35] study support our finding that cortisol was not a significant factor in resistance exercise.

In the present study, no analyzed T cell types or subtypes were significantly impacted by absolute intensity (TM speed, TM grade) of the cardiorespiratory exercise session. This underscores the current exercise prescription teachings that relative intensity, usually related to heart rate, should be used when designing a CRE routine for general health. Relative intensity is also a more practical exercise training prescription, since prescribing a plan by heart rate, compared to a possibly complicated algorithm of speed and grade, is easier for the client to follow and thus be successful with the training plan.

CMV seropositivity was selected as a significant variable for pre to post-exercise T cell response for six cell types, specifically CD4+CD57+, CD8+, CD8+CD57+, CD8+ CD57+ EMRA, CD8+ CM, and CD8+ EMRA. The CRE variable selection models with CMV seropositivity were specific to memory and cytotoxic subset types, including EMRA T cells which can represent senescent T cells. CMV seropositivity being related to the senescent T cells response to CRE could be related to people who are CMV seropositive having more senescent T cells.[36–41] However, CMV seropositivity was also related to an increase in other non-senescent circulating T cells, such as effector memory cells and cytotoxic CD8+ T cells. As this study did not measure the function of T cells, it is difficult to speculate on CMV seropositivity and the post-exercise immune function of these memory and CD8+ T cells. Nevertheless, if a goal of acute exercise is to increase immune surveillance by increasing circulating T cells, then it is possible that was achieved in this study.

The relationship between exercise-induced T cell release with CMV seropositivity status are similar to previous findings.[36,41] For example, after running on a treadmill at 80% $\dot{V}O_2max$ participants who were CMV seropositive had a greater increase in CD8+ T cells than those who were CMV seronegative, and the effector memory cells had the largest increase in response to treadmill running.[41] Seropositivity for human CMV, a beta-herpesvirus, could influence cytotoxic and memory T cell responses to aerobic exercise. It has previously been speculated that since people who are CMV positive have a greater number of memory-type T cells at rest then they will also have a greater number of memory-type T cells after exercise, and this could be what was observed in our study as well. Given the relatively high (40-70%) prevalence of latent CMV infection in the adult US population,[36] it is worth bringing attention to considering CMV sero-status when conducting immune cell-related exercise studies.

Of possible interest for CRE is cortisol. However, given the low effect size of cortisol, it is likely that cortisol—at least in this study—is an artifact rather than a meaningful influence on T cell response to acute exercise. There is limited literature available analyzing cortisol, the immune system, and the role of exercise intensity in the same research study. For example, the above referenced study by Taha et al. [35] was with participants of a similar age as our study. The authors compared three different resistance exercise intensities but did not measure any immune cells. Additional studies with a larger sample, as well as chronic training, may help to further investigate if these minor cortisol exercise variations do affect circulating T cell response to exercise.

It was surprising that absolute intensity was a predictor of changes in the number of circulating T cells in RE but not CRE. However, further study is needed to fully explain this finding. For example, whether this finding is specific to treadmill exercise. In addition to the results only being applicable to treadmill walking; another study limitation is the homogenous age demographic. The population included only healthy individuals between 55-75 years of age, and the findings can only be applied to a healthy, middle-aged-to-older immune response, and not generalized to a broader health or age demographic. Additionally, any cell changes in this publication are limited to immediately post-exercise changes. This limits our findings to post-exercise effects in middle-aged/older adults and a portion of the general healthy population. These post exercise effects are also limited to circulating changes in cell counts in this publication, not functional effects. It would be of interest to compare younger participants with older participants, as older adults, such as the participants in this study, are more likely to have been exposed to more pathogens and therefore have a greater proportion of highly differentiated and senescent T cells.[36]

Although immune health can be positively impacted by exercise, the understanding of best

exercise for improving immune health remains surprisingly limited.[13,30,37,42] A recent metanalysis of exercise training provided evidence that the mechanisms for understanding what dosage and typing of exercise are best for T cell health and function, are yet to be understood fully.[13] For example, exercise was noted to elicit transient leukocytosis, but senescent and exhausted T cells were mobilized in different conditions. Some mobilized only through high intensity mixed-type exercises, and another only elicited senescent subsets that mobilized during acute aerobic exercise.[13] Typical adult recommendations for exercise training are "moderate" intensity exercise. The studies included in recent metanalyses on exercise and immune health[13,30] were predominantly in this moderate intensity range. To our knowledge, this is the first study to isolate the effect of both acute CRE and RE absolute intensity on post-exercise T cell responses.

Although absolute exercise intensity was a predictor of exercise-induced changes to T cells in RE, this does not discount the use of relative intensity. Similar to what was previously discussed above regarding relative intensity for CRE prescription, instructing a person to lift weights at a specific relative intensity—such as "use a weight you can only lift 8-10 times"—is more accessible to someone interested in exercise for general health than having to calculate a total volume based on weight lifted, sets, and repetition numbers. Further, typically in research exercise studies a relative intensity exercise prescription is used as the method to elicit a post-resistance exercise increase in circulating immune cells.[13,30] Beyond of the typical relative exercise prescription, the present study lends support for considering total volume of weight lifted to increase or decrease exercise-induced response of CD3+, CD4+ CM, CD8+ CM, CD8+ EM, and naïve CD4+ subsets of T cells in 55-75 year old healthy adults. For example, if a study aims to elicit a high number of one of these T cell subsets in response to 70% 1RM resistance exercise, then the research team may want to consider increasing the volume of weight lifted by increasing sets and/or reps. Any prescriptive commentary however is speculative, and would benefit from future studies which analyze further clinical immune endpoints such as cytokine production and functional assays.

Sex was not a variable selected in the models for either RE or CRE. This is important, because males have higher absolute strength and cardiorespiratory fitness.[33] One of the advantages to using relative intensity for exercise prescription is that the exercise load is proportionate to the individual's fitness. While we acknowledge the disproportion in women to men in this study, and the potential effect of underpowered findings; our finding of no significant contribution of sex in the model also likely supports the inclusion of both males and females in exercise immunology research studies. However, the current study only included females who were post-menopausal, and menstrual cycle phase may still need consideration in exercise immunology study design.[43–45]

In summary, we observed a significant effect of absolute intensity (volume) on post-exercise RE T cell response to exercise in healthy middle-aged-to-older adults. In contrast, there was not an effect of absolute intensity (treadmill speed and grade) on post-exercise CRE T cell response to exercise. For CRE, the variable selection modeling identified CMV seropositivity and cortisol as contributing to circulating T cell response post-exercise. Further, the result that sex was not a predictor of change in circulating T cells in response to moderate intensity exercise in the 16 cell types studies supports including both males and females in exercise immunology research.

To our knowledge, this is the first study to examine the influence of absolute intensity on exercise-induced T cell response for both RE and CRE acute bouts in the same study, especially in middle-aged-to-older adults. Future studies should focus on comparing different relative intensities to determine if the reported results are specific to 70% relative intensities. Research on intensity of

exercise and outcomes expand our understanding of best practices for exercise typing and dosage programming when attempting to induce an exercise-induced increase in circulating T cells.

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Tables and Figures

Table 1. Absolute Intensity - Predictor Model.
Changes in mean T-cells from pre-exercise following separate acute cardiorespiratory exercise (CRE) and resistance exercise (RE) bouts. Treadmill absolute intensity (speed, grade) was not a predictor in any of the 16 T cell subsets examined. Resistance absolute intensity (volume) was a significant predictor in two T cell subsets ($p<0.05$). Changes reported as mean ± SE cells/µL.

Cell	CRE Difference between PRE to POST	Speed		Grade		RE Difference between PRE to POST	Volume	
		Coefficient	P	Coefficient	P		Coefficient	P
CD3+ (x10^3 cells/µL)	83.35 ± 31.96	30.300	0.542	5.740	0.557	214.39 ± 48.82	-0.0195	0.260
CD4+ (cells/µL)	17.07 ± 23.59	20.800	0.568	6.140	0.394	126.59 ± 36.76	-0.0238	0.061
CD4+ CD57+ (cells/µL)	5.72 ± 2.06	-0.302	0.924	0.424	0.501	10.9 ± 3.46	-0.0012	0.340
CD4+ EMRA CD57+ (cells/µL)	1.62 ± 0.69	-0.983	0.361	-0.073	0.729	2.86 ± 1.23	-0.0006	0.147
CD8+ (cells/µL)	43.28 ± 11.90	10.500	0.572	1.030	0.778	65.82 ± 15.12	-0.0032	0.554
CD8+ CD57+ (cells/µL)	22.08 ± 4.00	3.830	0.537	0.809	0.508	27.05 ± 6.31	-0.0012	0.594
CD8+ EMRA CD57+ (cells/µL)	15.62 ± 2.91	2.950	0.516	0.413	0.643	16.94 ± 4.19	-0.0006	0.689
CM CD4+ (cells/µL)	0.86 ± 9.92	12.200	0.417	3.470	0.246	48.23 ± 16.49	-0.0115	0.042
CM CD8+ (cells/µL)	0.76 ± 1.48	1.840	0.420	0.365	0.416	7.01 ± 2.34	-0.0011	0.193
EM CD4+ (cells/µL)	15.28 ± 6.73	8.980	0.386	1.800	0.376	40.7 ± 8.37	-0.0029	0.333
EM CD8+ (cells/µL)	10.23 ± 3.33	0.374	0.943	0.367	0.721	17.85 ± 5.16	-0.0020	0.276
EMRA CD4+ (cells/µL)	6.45 ± 1.71	-2.770	0.291	-0.532	0.302	5.44 ± 1.56	-0.0005	0.357
EMRA CD8+ (cells/µL)	29.83 ± 6.10	7.610	0.423	0.325	0.861	29.37 ± 6.23	-0.0014	0.534
Naïve CD4+ (cells/µL)	-5.47 ± 9.91	2.320	0.881	1.390	0.649	32.25 ± 12.76	-0.0089	0.043
Naïve CD8+ (cells/µL)	0.24 ± 4.47	1.480	0.833	0.0073	0.996	11.6 ± 4.11	-0.0015	0.296
Th17+ (cells/µL)	-3.19 ± 3.83	2.830	0.634	0.8320	0.478	11.11 ± 3.50	-0.0013	0.301

Table 2. CRE - Variable Selection Model.
Mean T cells coefficients change from PRE-to-POST exercise following an acute cardiorespiratory exercise (CRE) bout. Absolute intensity (TM speed, grade) was still not a predictor in any of the 16 T cell subsets examined ($p>0.05$). However, CMV seropositivity was a predictor ($p<0.05$) of 7 T cell subsets, all of which were CD8+ expressing with exception for one subset. Cortisol and resting heart rate were also predictors ($p<0.05$), but with minimal effect sizes, presenting possible artifacts. Pre-exercise resting cell count (Pre count) was a predictor for all cell types. Other variables that were included in the final variable-selected model but were not significant are not shown.

Cell	Pre Count		CMV		Pre Cortisol	
	Coefficient	P	Coefficient	P	Coefficient	P
CD3+ (x10^3 cells/µL)	0.982	p < 0.001	ns	>0.050	ns	>0.050
CD4+ (cells/µL)	0.873	p < 0.001	ns	>0.050	ns	>0.050
CD4+ CD57+ (cells/µL)	0.941	p < 0.001	12.600	0.002	ns	>0.050
CD4+ EMRA CD57+ (cells/µL)	0.818	p < 0.001	ns	>0.050	ns	>0.050
CD8+ (cells/µL)	1.120	p < 0.001	54.300	0.010	ns	>0.050
CD8+ CD57+ (cells/µL)	0.892	p < 0.001	32.300	<0.001	ns	>0.050
CD8+ EMRA CD57+ (cells/µL)	0.908	p < 0.001	22.600	<0.001	ns	>0.050
CM CD4+ (cells/µL)	0.980	p < 0.001	ns	>0.050	ns	>0.050
CM CD8+ (cells/µL)	0.999	p < 0.001	9.020	0.002	-0.0772	0.022

EM CD4+ (cells/µL)	1.150	p < 0.001	ns	>0.050	-0.5270	0.004
EM CD8+ (cells/µL)	1.100	p < 0.001	15.600	0.006	ns	>0.050
EMRA CD4+ (cells/µL)	1.140	p < 0.001	ns	>0.050	-0.1540	0.003
EMRA CD8+ (cells/µL)	1.080	p < 0.001	27.100	0.002	ns	>0.050
Naïve CD4+ (cells/µL)	0.834	p < 0.001	ns	>0.050	ns	>0.050
Naïve CD8+ (cells/µL)	0.874	p < 0.001	ns	>0.050	ns	>0.050
Th17+ (cells/µL)	0.765	p < 0.001	ns	>0.050	ns	>0.050

Table 3. RE – Variable Selection Model.
Mean T cells coefficients change from PRE-to-POST exercise following an acute resistance exercise (RE) bout, when accounting for covariates. Absolute intensity (volume) was still a significant predictor in five T cell subsets examined ($p<0.05$). Only pre-exercise resting cell count (Pre count) and absolute intensity total volume lifted (volume) were significant predictors, even accounting for covariates. All other variables that were included in the final variable-selected model but were not significant are not shown.

Cell	**Pre count**		**Volume**	
	Coefficient	**P**	**Coefficient**	**P**
CD3+ (x10^3 cells/µL)	1.160	P < 0.001	-0.0257	0.017
CD4+ (cells/µL)	1.070	P < 0.001	ns	>0.050
CD4+ CD57+ (cells/µL)	1.550	P < 0.001	ns	>0.050
CD4+ EMRA CD57+ (cells/µL)	1.810	P < 0.001	ns	>0.050
CD8+ (cells/µL)	1.180	P < 0.001	ns	>0.050
CD8+ CD57+ (cells/µL)	1.820	P < 0.001	ns	>0.050
CD8+ EMRA CD57+ (cells/µL)	2.010	P < 0.001	ns	>0.050
CM CD4+ (cells/µL)	1.020	P < 0.001	-0.0131	0.038
CM CD8+ (cells/µL)	1.350	P < 0.001	-0.0037	0.004
EM CD4+ (cells/µL)	1.280	P < 0.001	ns	>0.050
EM CD8+ (cells/µL)	1.070	P < 0.001	-0.0076	0.020
EMRA CD4+ (cells/µL)	1.280	P < 0.001	ns	>0.050
EMRA CD8+ (cells/µL)	1.360	P < 0.001	ns	>0.050
Naïve CD4+ (cells/µL)	0.994	P < 0.001	-0.0108	0.033
Naïve CD8+ (cells/µL)	1.240	P < 0.001	ns	>0.050
Th17+ (cells/µL)	1.310	P < 0.001	ns	>0.050

Table 4. Participant Characteristics.
Descriptive information for participants (Mean ± SE).

Characteristics	**n = 24**
Age (years)	63.00 (1.03)
Resting Heart Rate (BPM)	55.40 (1.80)
Height (inches)	65.53 (0.61)
Weight (kg)	68.76 (2.31)
BMI (kg·m^2)	24.73 (0.67)
Body Fat (%)	31.24 (1.65)
Fat Free Mass (kg)	47.33 (2.11)
VO_2max (mL·kg·min^2)	40.14 (2.84)
Physical Activity [MVPA] (minutes/week)	79.33 (7.54)