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Natural Killer Cell Mobilisation and Egress Following Acute Exercise in Men with Prostate Cancer

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New Findings

- Prostate cancer treatments, including androgen deprivation therapy (ADT), adversely target multiple physiological systems with minimal evidence on the immune response at rest and with acute exercise, which has implications on exercise prescription.
- Irrespective of ADT, smaller exercise-induced rises in epinephrine were observed in prostate cancer survivors compared to controls, yet natural killer (NK) cell mobilisation was similar between groups. Intracellular cytokine expression was altered during prostate cancer treatment, potentially affecting NK cell function. However, NK cell populations returned to resting levels 24 hours after exercise, suggesting that training on successive days will have no adverse immune consequences.

Abstract

Prostate cancer treatment affects multiple physiological systems, although the immune response during exercise has been minimally investigated. **Purpose:** To characterise the natural killer (NK) cell response following acute exercise in prostate cancer survivors. **Methods:** Prostate cancer survivors on androgen deprivation therapy (ADT) and those without (PCa) along with non-cancer controls (CON) completed a moderate intensity cycling bout. NK cells were phenotyped before and 0h, 2h, and 24h after acute exercise using flow cytometry. **Results:** CD56total NK cell frequency increased by 6.2% at 0h ($P<0.001$) and decreased by 2.5% at 2h ($P<0.01$) with similar findings in $CD56^{dim}$ cells. NK cell counts also exhibited a biphasic response. Independent of exercise, ADT had intracellular interferon gamma (IFNγ) expression that was nearly two-fold higher than CON ($P<0.01$). PCa perforin expression was reduced by 11.4% ($P<0.05$), suggesting these cells may be more prone to degranulation. $CD57^-$ NK cells demonstrated increased perforin and IFNγ frequencies after exercise with no change within the $CD57^+$ populations. All NK and leukocyte populations returned to baseline by 24h. **Conclusions:** NK cells mobilisation and egress with acute exercise appears normal, as cell counts and frequencies in prostate cancer survivors change similarly to CON. However, lower perforin proportions (PCa) and higher IFNγ expression (ADT) may alter NK cytotoxicity and requires

further investigation. The return of NK cell proportions to resting levels overnight suggests that consecutive training sessions can be used without adverse effects on the immune system during prostate cancer treatment.

Introduction

Regular exercise is recommended as a complementary therapy to minimize the side effects of prostate cancer treatment and to improve physical function, quality of life, and even survival (Campbell et al., 2019; Hayes, Newton, Spence, & Galvao, 2019; Wang et al., 2017). Exercise interventions in prostate cancer survivors result in improved strength, body composition, and physical function (Hanson, Wagoner, Anderson, & Battaglini, 2016; Yunfeng, Weiyang, Xueyang, Yilong, & Xin, 2017) with high adherence rates and few adverse incidents (Hayes et al., 2019). However, immune, endocrine, and inflammatory status following acute exercise during prostate cancer treatment have received minimal attention (Fairey, Courneya, Field, & Mackey, 2002; Khosravi, Stoner, Farajivafa, & Hanson, 2019; Kruijsen-Jaarsma, Revesz, Bierings, Buffart, & Takken, 2013) despite these systems playing critical roles in cancer progression and recurrence (Koelwyn, Wennerberg, Demaria, & Jones, 2015). This represents a key gap in the exercise oncology literature.

There are several factors linked with prostate cancer treatment that may alter immune cell counts, frequencies and function. Chronic, unresolved inflammation observed in prostate cancer survivors (De Marzo, Nakai, & Nelson, 2007) is linked to cardiovascular disease, increased risks of secondary cancers, and poor prognosis (Donin et al., 2016; Fujita, Hayashi, Matsushita, Uemura, & Nonomura, 2019; Shafique et al., 2012), as prostate tumour progression is accelerated by inflammation (Fujita et al., 2019; Koelwyn et al., 2015). Androgen deprivation therapy (ADT) is a common treatment but leads to loss of lean mass and increases in fat mass and body fat percentage (Galvao et al., 2008; van Londen, Levy, Perera, Nelson, & Greenspan, 2008). Changes in body composition further increase proinflammatory cytokine levels in older adults (Schmidt et al., 2015) and those undergoing ADT (Saylor et al., 2012) that are correlated with disease progression (Michalaki, Syrigos, Charles, & Waxman, 2004). Prostate cancer treatments also appear to directly influence immune function. For example, radiotherapy decreases leukocyte and lymphocyte counts (Hojan et al., 2016), but when combined with exercise training there was greater natural killer (NK) cell infiltration and reduced tumour volume (Dufresne et al., 2020). One year of ADT increased in naïve CD4 T cell proportions and overall proliferation initially but not long term (Morse & McNeel, 2010), while NK cell counts nearly doubled following short-term ADT that was prevented with testosterone replacement (Page et al., 2006).

In healthy individuals it is well-established that leucocytosis occurs with acute exercise in an intensity-dependent manner while moderate intensity exercise training reduces the risk of infection (Nieman, 1994; Walsh et al., 2011). However, the immune response to exercise in cancer survivors has only been superficially examined (Khosravi et al., 2019; Kruijsen-Jaarsma et al., 2013). Complete blood counts are the most common outcome (Galvao et al., 2008; Jonsson et al., 2011; Ladha, Courneya, Bell, Field, & Grundy, 2006; Shore & Shepard, 1999) with minimal work investigating changes in specific cell types (Evans et al., 2015; Zimmer et al., 2016). As the stress hormone response to acute exercise appears attenuated in prostate cancer (Hanson et al., 2018), this may diminish lymphocyte mobilisation. Previously, acute resistance exercise in prostate cancer survivors induced leucocytosis and lymphocytosis (Galvao et al., 2008). However, the effects of aerobic exercise and how different immune cell populations respond along with cellular egress during recovery are all unknown and have potential implications on exercise prescription and immune function during prostate cancer treatment.

Conventional NK cells represent 5-15% of all human circulating lymphocytes, are highly responsive to acute exercise (Rooney et al., 2018) and are frequently studied during cancer (Khosravi et al., 2019) given their role in tumour suppression. This makes them a logical initial choice for examining the immune system during prostate cancer treatment. NK cells are highly cytotoxic and express perforin and granzyme proteases to lyse target cells, with several factors appearing to influence their response to acute exercise. $CD56^{bright}$ cells, the less mature, less frequent subset responsible primarily for cytokine production (De Maria, Bozzano, Cantoni, & Moretta, 2011), have a smaller response to exercise relative to their $CD56^{dim}$ counterparts (Campbell et al., 2009), the subset responsible for cytotoxic function. $CD56^{dim}$ NK cells expressing CD57, a marker of maturation, have greater cytotoxic functions than $CD57^{neg}$ NK cells (Kared, Martelli, Ng, Pender, & Larbi, 2016; Lopez-Verges et al., 2010; Nielsen, White, Goodier, & Riley, 2013), and have preferential mobilisation and egress compared to other NK cells (Bigley et al., 2014). NK cells also produce interferon gamma (IFNγ) following activation (De Maria et al., 2011), which may enhance cytotoxic function (Wang, Jaw, Stutzman, Zou, & Sun, 2012).

Following exercise-induced increase of immune cells, lymphocyte numbers abruptly decline during the first few hours of recovery (Nieman, 1994). While reductions in immune cell number post-exercise has recently been questioned as a risk factor for opportunistic infections (Campbell & Turner, 2018), NK mobilisation and egress following acute exercise driven partly by alterations in hormonal and inflammatory markers increases immune cell infiltration that reduces tumour viability (Dethlefsen et al., 2016; Pedersen et al., 2016). However, it is currently unknown if circulating

immune counts and proportions return to baseline levels in prostate cancer survivors after an overnight rest. Repeated bouts of acute exercise without restoration of immune function may compromise the benefits of training during cancer treatment, through missed sessions or increased frequency of illness. As such, it is imperative that the use of exercise as a complementary therapy for cancer is thoroughly explored in all physiological systems, including immune function. This study is an initial step in understanding the specific immune responses to acute exercise in prostate cancer survivors and has implications on exercise prescription and recovery in oncology populations.

The purpose of this study was to characterize the NK cell response following acute moderate intensity aerobic exercise in prostate cancer survivors relative to age-matched controls with no history of cancer and to examine the relationship between stress hormones and NK cell mobilisation. As ADT may influence immune status, the response to exercise was determined separately in prostate cancer survivors on the basis of ADT use. We hypothesised that NK cell populations would exhibit a biphasic response following a single bout of exercise but overall responses would be smaller than non-cancer controls due to attenuated stress hormone release (Hanson et al., 2018). We further hypothesised that NK cells from prostate cancer survivors would have greater NK cell maturity and lower IFNγ and perforin expression in response to acute exercise.

Methods

Ethics Approval

Local ethics committees at Peter MacCallum Cancer Centre (protocol # 12/131), Victoria University, and Western Health approved this project. All procedures were conducted in accordance with standards set by the Declaration of Helsinki, except for registration in a database. Participants were informed of the study procedures and provided written informed consent.

Participants

Men with physician diagnosed prostate cancer on ADT [ADT; n=11, 67 (2yr)] and not on ADT [PCa; n=14, 67 (2y)] were recruited from local oncology practices and support groups in Melbourne, Australia along with non-cancer controls [CON; n=8, 64 (3y)]. All participants were sedentary (no regular exercise except for walking for previous 6 months) and were screened for conditions that would contraindicate participation in aerobic exercise. Exclusion criteria included uncontrolled prostate cancer, symptomatic cardiovascular disease, any conditions that caused severe pain with exertion, Type 1 diabetes, uncontrolled Type 2 diabetes, history of bone fractures, inability to engage safely in moderate exercise, or lack of medical clearance from their oncologist, urologist,

general practitioner or specialist physician. CON met all inclusion and exclusion criteria and reported no previous history of cancer.

Design

Detailed methods have been published elsewhere (Hanson et al., 2018). Briefly, the trial consisted of 4 visits completed across 1-2 weeks. A familiarisation session preceded cardiopulmonary exercise testing (CPET) followed by the main testing sessions (visit 3 and 4), which were performed in the early morning and at the same time of day to minimize the effects of diurnal variations.

Familiarization (Visit 1)

Participants were fitted with all equipment and completed 3 to 4 submaximal CPET stages (0 watts up to 60 or 80 watts) using an electronically braked cycle ergometer (Lode, Gronigen, Netherlands). Pre-assessment guidelines, 2 or more hours fasted, no exercise in previous 24 hours, and no caffeine or alcohol for previous 12 and 48 hours, were discussed and used for all subsequent visits.

Preliminary Testing (Visit 2)

Body composition was determined using dual-energy x-ray absorptiometry (Hologic, Waltham, MA, USA). The scanner was calibrated daily and all scans were performed and analysed by the same densitometry technician. A CPET was used to determine peak oxygen consumption (VO_2peak) and exercise trials workloads. Participants completed 1-minute stages with 20 watt increases until volitional exhaustion. Expired gases were sampled every 15 seconds using automated gas analysers (Moxus Modular VO_2 System, AEI Technologies, Pittsburgh, PA, USA) calibrated prior to each test. VO_2peak was determined as the average oxygen consumption across the last minute of the test. Heart rate was assessed continuously via 12 lead electrocardiogram (GE Case Cardiosoft v6.6 ECG Diagnostic Systems, Palatine, IL, USA) and rate of perceived (RPE) exertion using the original Borg scale.

Trial Protocol (Visit 3 and 4)

Approximately 1 week later, participants returned to the laboratory between 0600 and 0900. A venous catheter was inserted for repeat blood sampling and a resting blood sample was obtained. Participants completed an acute, intermittent exercise bout consisting of 10 intervals of 3 minutes of cycling at 60% of peak wattage from the CPET followed by 1.5 minutes of passive recovery. This protocol was adapted from work in breast cancer survivors (Evans et al., 2015). Respiratory gases, heart rate, and RPE measurements were collected throughout the trial. Additional blood samples were obtained immediately (0h) and 2h after exercise. During recovery, participants

remained seated and consumed water *ad libitum*. Participants then went home before returning to the laboratory 24h after exercise for an additional blood sample. They were asked to consume an identical meal prior to visits 3 and 4.

Hormone Analysis

Prostate specific antigen levels (R & D Systems, Minneapolis, MN, USA) and total testosterone (Abnova, Taipei City, Taiwan) were determined at baseline only while epinephrine, norepinephrine and cortisol levels (Abnova, Taipei City, Taiwan) were determined at baseline and 0h, 2h, and 24h after exercise. All hormones were analysed in duplicate using ELISA and have been previously reported (Hanson et al., 2018).

Haematology Analysis

Duplicate complete blood counts were determined from each time point (Sysmex KX-21N, Kobe, Japan) with a maximal white blood cell difference of 0.1 cells/µL and values were averaged. Haemoglobin and haematocrit were used to estimate plasma volume shifts following exercise (Dill & Costill, 1974).

Peripheral blood mononuclear cells isolation and immunofluorescence.

Freshly isolated peripheral blood mononuclear cell (PBMC) were labelled as performed previously (Hanson et al., 2019; Hanson et al., 2017). Briefly, whole blood was diluted in PBS and isolated using SepMate-50 (Stemcell, Vancouver, BC Canada) following manufacturer instructions. Peripheral bound mononuclear cells were washed, counted via haemocytometer and 2×10^6 cells were aliquoted for analysis. NK cell phenotyping was determined using direct immunofluorescence labelling of cells by identifying surface markers with mouse anti-human monoclonal antibodies (Biolegend, San Diego, CA) in 100 µL of cell staining buffer for 15 min at 4°C in the dark. Lymphocytes were gated using forward and side scatter parameters, with NK cells identified as $CD3^-$ (APC-Cy7) and $CD56^+$ (AF647) to identify the dim, bright, and total populations. CD16 was omitted from the final analysis of the $CD56^{dim}$ due to inconsistent staining patterns. However, the use of $CD56^{dim}$ alone was able to identify 90-95% of the $CD16^+CD56^{dim}$ cells in a subset of individuals (data not shown). CD57 (Pacific Blue) was used as a marker of maturity and differentiation status. Intracellular NK cell cytokine expression was measured as an indicator of function. Cells were washed twice in 200 µL of PBS before fixation and permeabilization according to manufacturer instructions (Cytofix/Cytoperm kit, BD Biosciences, San Jose, CA). Intracellular perforin (PE, Biolegend, San Diego, CA) and IFNγ (PE-Cy7) were stained in 100 µL at 4°C in the dark for 30 min. After washing, cells were resuspended in 200 µL of cell staining buffer for flow cytometry analysis.

Flow Cytometry

500,000 events for each sample were acquired and analysed via flow cytometry on a BD Canto II running FACSDIVA v6.1 (BD Biosciences) software. Gating analyses (Figure 1) were done in duplicate by blinded investigators using FCS express v7.0 (Pasadena, CA, USA). Fluorescence minus one and single colour compensation tubes were used with every experiment. NK cell counts (cells/μL) were determined by multiplying the percentage of each population with the haematology lymphocyte count (Hanson et al., 2019; Hanson et al., 2017). NK cell subpopulation counts were determined using the NK cell count and the percentage of the respective daughter populations for each specific cell type. Levels of surface and intracellular marker expression amongst expressing subsets were estimated using Median Fluorescent Intensity (MFI).

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy or ethical restrictions.

Statistical Analysis

Group differences for participant characteristics were assessed using one-way ANOVA with Tukey post-hoc analysis. Two-way repeated measures ANOVA with Tukey post-hoc analyses were used to assess changes in stress hormone levels across time and between groups and are reported as the mean difference relative to baseline. All immune analyses were examined using linear mixed modelling with group and time as fixed factors with a random intercept. Group x time interactions were resolved with simple effects examining the group response at each time point. Paired t-tests were used to determine differences in cell proportions between $CD56^{dim}$ and $CD56^{bright}$ populations. Correlation coefficients were determined to assess the relationship between immune cell mobilisation and circulating stress hormones. Data are presented as mean ± SD, with model estimates being expressed as the mean difference relative to baseline or the CON group and include 95% confidence intervals. Percentage change was calculated as 100 × [(final – original)/(original)] where baseline or CON was the original. Effects sizes (es) were calculated using Cohen's D (d) such that 0.2, 0.5 and 0.8 represent small, medium, and large differences, respectively. All data were analysed using Jamovi v1.0.2.0 and figures were created in GraphPad Prism version 8 (La Jolla, CA, USA) with statistical significance set at $P<0.05$.

Results

Participants. Individuals in this study were sedentary and overweight but relatively healthy otherwise based on comorbidity index (Table 1). Men on ADT had greater body mass (P=0.019) and

% fat (P<0.001) compared to CON and lower total testosterone compared to PCa and CON (P<0.001, Table 1). Both ADT and PCa were similar in years post-diagnosis, with ADT having higher Gleason scores (P=0.007) with no apparent differences in the proportion of men treated with surgery or radiation.

Acute exercise responses. Absolute VO_2peak and peak power outputs were similar between groups with relative VO_2peak being significantly lower for ADT (Table 1) vs. CON (due to higher body mass). Average heart rate and oxygen uptake during acute exercise were 83.9% $\pm$ 10.2 and 80.7% $\pm$ 7.8 of maximum, respectively, as published previously (Hanson et al., 2018), with similar responses between groups.

Haematological Changes with Exercise. A group x time interaction (P=0.016) was present for leukocyte counts (Table 2). At 0h, CON increased by 46.3% ($2.5x10^3$ cells/μL [95% CI 1.8, 3.3], P<0.001), ADT by 56.7% ($3.1x10^3$ cells/μL [95% CI 2.4, 3.8], P<0.001) and PCa by 39.3% ($2.0x10^3$ cells/μL [95% CI 1.4, 2.6], P<0.001) compared to baseline. At 2h, leukocytes decreased slightly but remained elevated relative to baseline for CON by 33.2% ($1.8x10^3$ cells/μL [95% CI 1.0, 2.6], P<0.001) and 32.5% for ADT ($1.8x10^3$ cells/μL [95% CI 1.1, 2.4], P<0.001) while PCa increased to 46.3% ($2.4x10^3$ cells/μL [95% CI 1.8, 3.0], P<0.001). Lymphocytes increased by 58.1% at 0h ($0.9x10^3$ cells/μL [95% CI 0.7, 1.1], P<0.001) before returning to baseline by 2h with similar patterns for mixed cells. Neutrophils increased by 46.3% at 0h ($1.5x10^3$ cells/μL [95% CI 1.1, 1.9], P<0.001) and 63.9% at 2h ($2.1x10^3$ cells/μL [95% CI 1.7, 2.5], P<0.001). Plasma volume shifts were observed at all recovery timepoints. All counts returned to baseline by 24h.

NK Cell Frequencies and Counts. Group x time interactions for NK cell proportions trended toward significance in total CD56 (P=0.071, Figure 2A) and $CD56^{dim}$ cells (P=0.091, Figure 2B), with cell frequencies returning to baseline at 2h in ADT but declining below in CON and PCa. Across time only, total CD56 proportions increased at 0h (6.2% [95% CI 4.5, 7.9], P<0.001) and decreased at 2h (-2.5% [95% CI -4.2, -0.8], P=0.005) compared to baseline with similar values by 24h with nearly identical changes in $CD56^{dim}$ cells (Figure 2B). $CD56^{bright}$ cell proportions increased only at 0h (0.2% [95% CI 0.06 to 0.3], P=0.007, Figure 2C).

Total $CD56^{+}$ cell counts increased by 171% (247 cells/μL [95% CI 190, 305], P<0.001) at 0h and decreased below baseline by 38% (-54 cells/μL [95% CI -112, 4], d=0.7, P=0.074) at 2h, although this did not reach significance (Table 3). $CD56^{dim}$ cells increased by 185% (236 cell/μL [95% CI 180,

293], P<0.001) while $CD56^{bright}$ counts increased by 100% (10 cells/μL [95% CI 7, 14], P<0.001) at 0h only.

NK Cell Perforin. Perforin expression in total $CD56^{+}$ cells was lower in PCa (-11.4% [95% CI -21.6, -1.3], P=0.036) compared to CON with a trend to change over time (-4.4% [95% CI -10.0, 1.1], d=1.0, P=0.081, Figure 3A). $CD56^{dim}$ cells showed a similar pattern for group differences but did not reach significance (P=0.090, Figure 3B) whereas the proportion of cells positive for perforin was reduced at 24h (-6.8% [95% CI -13.5, -0.2], P=0.047). Relative to $CD56^{dim}$, $CD56^{bright}$ cells had a slightly lower percentage of perforin expressing cells (-3.5% ± 1.4, P<0.001) and did not change over time or across group (Figure 3C). Perforin MFI was also unchanged for group and time across all three NK populations (Figure 3D, E, F).

Total $CD56^{+}Perforin^{+}$ cell counts increased by 181% (240 cells/μL [95% CI 183, 297], P<0.001, Table 4) at 0h and tended to decrease below baseline by 39% (-52 cells/μL [95% CI -110, 6], d=0.7, P=0.081). $CD56^{dim}$ cells increased by 185% (229 cell/μL [95% CI 172, 286], P<0.001) and $CD56^{bright}$ counts increased by 90% (9 cells/μL [95% CI 6, 12], P<0.001) at 0h only.

NK Cell IFNγ. The proportion of total $CD56^{+}$, $CD56^{dim}$, and $CD56^{bright}$ cells expressing IFNγ did not change with exercise or with prostate cancer treatment (Figure 4A, B, C). $CD56^{bright}$ NK cells had lower IFNγ expression levels compared with $CD56^{dim}$ (-8.2% ± 1.3, P<0.001). IFNγ MFI was greater in ADT in all 3 NK cell populations (total $CD56^{+}$: 1503 [95% CI 766, 2241], P=0.002, Figure 4D; $CD56^{dim}$: 1404 [95% CI 643, 2165], P=0.003, Figure 4E: $CD56^{bright}$: 1035 [95% CI 272, 1800], P=0.020, Figure 4F) compared to CON.

Cell counts for total $CD56^{+}IFN\gamma^{+}$, $CD56^{dim}IFN\gamma^{+}$, $CD56^{bright}IFN\gamma^{+}$ did not change with acute exercise (Table 4).

NK CD57 Frequencies and Counts. CD57 was used as a marker of maturity within CD56 total, bright, and dim NK cell populations. The proportion of total $CD56^{+}$ expressing $CD57^{+}$ cells did not change in the hours immediately following exercise but was reduced at 24h (-6.5% [95% CI -11.7, -1.2], P=0.018, Figure 5A) compared to baseline. A similar trend was observed for $CD56^{dim}$ cells (P=0.069, Figure 5B). The proportion of $CD56^{bright}CD57^{+}$ cells was reduced at 2h (-5.3% [95% CI -10.0, -0.7], P=0.030, Figure 5C) and 24h (-5.6% [95% CI -10.3, -0.9], P=0.023).

Cell counts for total $CD56^{+}CD57^{+}$ increased by 198% (166 cells/μL [95% CI 122, 211], P<0.001, Table 3), $CD56^{dim}CD57^{+}$ by 190% (165 cells/μL [95% CI 122, 210], P<0.001), and $CD56^{bright}CD57^{+}$ by 200% (2 cells/μL [95% CI 1, 4], P<0.001) at 0h only.

Perforin and IFNγ Expression is Affected by CD57. To determine if maturity status affected intracellular perforin and IFNγ proportions, $CD57^+$ and $CD57^-$ NK cells were compared. Overall, $CD57^-$ NK cells had lower perforin proportions (CD56 total: -20.2% ± 1.6, $CD56^{dim}$: -14.2% ± 1.4, $CD56^{bright}$: -31.8% ± 3.1, all P<0.01) vs. $CD57^+$ cells. Across time, $CD56^+CD57^-$ perforin proportions increased at 0h (5.7% [95% CI 1.2, 10.3], P=0.016, Figure 6A) and decreased at 2h (-4.9% [95% CI -9.6, -0.4], P=0.037) while $CD57^+$ percentages showed a decrease at 24h only (-6.6 [95% CI -11.8, -1.3], P=0.017). $CD56^{dim}$ and $CD56^{bright}$ cells demonstrated similar but smaller effects with increases in $CD57^-$ perforin proportions at 0h only (Figure 6B & 6C).

Overall $CD57^-$ NK cell IFNγ expression was greater for CD56 total (7.5% ± 2.3, P=0.002) and $CD56^{dim}$ cells (4.9% ± 2.1 P=0.023) but lower in $CD56^{bright}$ cells (-9.9% ± 3.0, P=0.002). Across time, IFNγ expression was increased at 24h compared to baseline in $CD56^+CD57^-$ cells (9.4% [95% CI 1.6, 17.2], P=0.024, Figure 5D) but was unchanged in $CD57^+$ cells. $CD56^{dim}CD57^-$ cells trends for increased IFNγ proportions at 24h (P=0.059, d=0.5, Figure 5E) with no changes within $CD56^{bright}$CD57 cells (Figure 5F).

Stress Hormones and Correlations with Immune Changes. Cortisol levels were elevated at 0h and dropped below baseline at 2h (both P<0.05, Table 5). There was also a group differences between ADT and PCa (P=0.017). There was a group x time interaction (P<0.001) for epinephrine where the change at 0h was greater in CON vs. PCa (P=0.01) and ADT (P=0.008). Norepinephrine levels were elevated at 0h and 2h (both P<0.001). All stress hormone levels had returned to resting levels by 24h.

No correlations between epinephrine and any NK cell counts were observed at baseline or 0h (data not shown). The change (Base to 0h) in epinephrine was correlated (r=0.40, P=0.043, Figure 7A) with the change in $CD56^{bright}$ but not total CD56 of $CD56^{dim}$ cells. Baseline norepinephrine was also correlated with the change in $CD56^{bright}$ cells (r=0.51, P=0.008, Figure 7B). Cortisol demonstrated no relationship with any NK cell populations (data not shown).

Discussion

Prostate cancer treatments are associated with adverse effects, including altered immune function. With exercise being recommended to counteract treatment-related side effects, this study addresses a key knowledge gap by providing an initial characterization of NK cell mobilisation and egress following acute exercise in men being treated for PCa with and without ADT. We show, for

the first time, that prostate cancer survivors have NK cell mobilisation that is compared to CON following acute exercise, although the CD56 total cell egress tended to be attenuated in ADT. ADT also consistently showed a less mature phenotype with greater proportion of IFNγ expression and possibly lower cytotoxicity, although perforin levels were unchanged. However, the proportion of perforin expressing NK cells in PCa was reduced, suggesting these cells may be more prone to degranulation. By 24h, all NK and leukocyte populations returned to baseline, tentatively implying that consecutive training sessions could be used without adverse immune system effects during prostate cancer. However, acute exercise responses need to be examined across micro- and meso-cycles of training to support this hypothesis.

To contextualize these findings, the limitations and strengths of this study are reported here. Small sample sizes in each group may have limited the ability to detect the significant interactions or group differences (i.e. Figure 2). When statistical trends were present, effect sizes were also reported to aid with interpretation. The time since diagnosis was highly variable, possibly influencing baseline counts; however, diagnosis time and treatments were similar between PCa and ADT. This study did not include NK cytotoxicity assays, instead reporting markers (e.g. perforin, IFNγ) associated with function. Study strengths include reporting of cell counts and proportions for all outcomes, which are often missing in exercise and cancer studies (Khosravi et al., 2019). Multiple recovery time points were used, including 24h post exercise to assess if immune cells normalize after one night of rest. We also examined NK cell populations that have not previously been reported in prostate cancer survivors and subdivided these men on the basis of ADT use to further characterize the response to acute exercise.

Despite previous reports on changes in lymphocyte counts with prostate cancer treatments (Hojan et al., 2016; Page et al., 2006), we observed minimal haematological differences between groups (Table 2). In response to acute exercise, leukocyte counts increased similarly at 0h but remained elevated for PCa only at 2h. As neutrophil, monocyte (mixed), and lymphocyte counts all demonstrated comparable changes across groups, it is unclear what cell population was responsible for this interaction. We are aware of only one other acute exercise and immune study where men on ADT performing resistance exercise demonstrated 15% and 20% increases in leukocyte and neutrophil counts, respectively, with no change in lymphocyte or monocyte counts (Galvao et al., 2008). Although exercise modes confound the comparison, the attenuated haematological response compared to the current study is potentially due to differences in catecholamine and shear stress levels (Walsh et al., 2011), both of which were likely greater in aerobic vs. resistance exercise.

Within NK cells, CD56 total and CD56^{dim} frequencies demonstrated biphasic responses to acute exercise, which supported our hypothesis. There was a trend (P=0.074) in CD56 total for CON NK cells to have a greater egress at 2h, with a 3.7% decline in frequency below baseline whereas ADT showed a minimal (-0.4%) change (Figure 2A). This partially supports our hypothesis that the response magnitude would be lower in prostate cancer survivors, although PCa NK cell frequencies closely align with the response in CON. NK cell counts all robustly increased at 0h but did not demonstrate a true biphasic response, as the declines at 2h were not reduced relative to baseline (Table 3). With a lack of NK and exercise data in prostate cancer, we compared our findings to a similar study in breast cancer. Immediately following exercise, NK cell counts in breast cancer survivors increased by 126%, which was similar to healthy controls (Evans et al., 2015) and also the current study, particularly after accounting for small differences in exercise intensity. To the contrary, NK cell proportions in breast cancer survivors decreased below baseline at 15 min and 24h after a half marathon (Zimmer et al., 2016). Decreased NK cell counts at 15 min are likely due to a rapid egress that occurs almost immediately following exercise cessation (Rooney et al., 2018), whereas we and other (Evans et al., 2015) completed the post-exercise draw immediately after stopping cycling. The sustained suppression at 24h is interesting and may be related to the longer duration (and more exhausting) exercise session, although cell counts were not reported to confirm this. Collectively, there is little evidence that NK cell frequency or number respond substantially differently to acute exercise in hormone-sensitive cancers. This is despite attenuated stress hormone responses (Hanson et al., 2018), although CD56^{bright} cell mobilisation correlates with resting norepinephrine and exercise-induced increases in epinephrine. While this initial characterization of NK cell mobilisation and egress are intriguing, our findings warrant confirmation using larger samples sizes.

While changes in NK cell counts are important, functional outcomes in NK cells have greater clinical significance and are less commonly reported. The frequency of CD56 total cells expressing perforin was reduced in PCa compared to CON, suggesting that PCa were more prone to degranulation and a lower cytotoxic capacity (Figure 3A). Perforin expression has previously been shown to be lower in circulating peripheral lymphocytes (He et al., 2015) and NK CD56^{dim} cells isolated from malignant prostate tissue (Tokmadzic et al., 2011). Interestingly, ADT perforin levels were unchanged and the reasons for this are unclear. One possibility is a change in the ratio of testosterone to beta-oestradiol. During 4 weeks of medically induced castration in healthy men, both sex hormones decreased but to a greater extent in testosterone such that this ratio was much less pronounced (Page et al., 2006). Testosterone suppression and NK cell function is complex, as

older women appear to have greater NK cytotoxic responses than older men (Al-Attar, Presnell, Peterson, Thomas, & Lutz, 2016) while NK cytotoxicity is greater in young men vs. young women (Yovel, Shakhar, & Ben-Eliyahu, 2001). Short-term ADT increases NK cell number but not homing or activation markers (Page et al., 2006). Moreover, high doses of beta-oestradiol inhibited murine NK cell cytotoxic function (Curran et al., 2001), providing additional evidence of the effects of the sex hormone on NK cells. We propose that a critical balance may exist between testosterone to beta-oestradiol as it relates to cytotoxic function, although different outcomes and immune populations prevent direct comparisons.

While acute exercise has increased NK cell activity in other cancer survivors (Kruijsen-Jaarsma et al., 2013), we observed a small decrease in perforin proportions within $CD56^{dim}$ cells only. Although somewhat unexpected, this was consistent with decreased perforin expression following strenuous acute exercise in young endurance athletes (Staats et al., 2000). Because greater than 90% of NK cells expressed perforin (potential ceiling effect) and changes occurred only at 24h, perforin MFI was also determined (Figure 3D, E, & F). The lack of change in perforin expression suggests that any potential improvements in cytotoxicity would likely be the result of greater cell numbers. In support of this, we report that the absolute number of NK cells expressing perforin increases nearly 2-fold at 0h (Table 4). Moreover, a methodological review of NK cell activity with acute exercise indicates increases in function that are no longer present but when adjusted on a per cell basis in non-cancer populations (Zimmer et al., 2017). Alternatively, acute exercise may also be improving other functional markers that contribute to cytotoxic function were not examined in the current study (e.g. granzyme B).

Although unaffected by acute exercise, IFNγ MFI levels were elevated in ADT across all NK cell populations (Figure 4). IFNγ has multiple functional roles within NK cells, including cellular migration and activation (De Maria et al., 2011). $CD56^{bright}$ cells are less mature and have poorer cytotoxic function (Al-Attar et al., 2016; Kared et al., 2016) with their principal role being cytokine production (De Maria et al., 2011), including IFNγ. Despite not reaching statistical significance, the proportion of NK cells expressing IFNγ appears lower with ADT. As such, the greater MFI may be a compensatory response and perhaps explains the consistent response across both CD56 bright and dim cells. Interestingly, systemic IFNγ levels are elevated with obesity (Schmidt et al., 2015) and we report higher body mass index and % fat in ADT (Table 1). However, inflammatory cytokines from excess body fat are likely secreted from infiltrating macrophages or the adipocytes directly (Deng, Lyon, Bergin, Caligiuri, & Hsueh, 2016) rather than NK cells. We postulate that greater IFNγ production may be the result of the reduced CD57 expression and lower NK cell maturity after ADT.

This immature status may be particularly relevant within $CD56^{dim}$ cells, as any loss of cytotoxic function could compromise control of infections or tumours.

Next, we examined if cancer treatment or acute exercise alters maturity status and subsequent expression of intracellular cytokines. The lack of group differences for CD57 proportions (Figure 5) or cell numbers (Table 3) initially suggests that increased IFNγ MFI during ADT (Figure 4) was unrelated to NK cell maturation. However, when $CD57^+$ and $CD57^-$ populations were examined separately (Figure 6), the $CD57^+$ fraction appears to be driving the increase within ADT as the $CD57^-$ cells lacked group differences. Moreover, $CD57^-$ cells had greater IFNγ production that is consistent with higher expression levels in less mature cells (De Maria et al., 2011). Looking at acute exercise, NK $CD57^+$ counts increased by ~2-fold (Table 3), which supports previous work in healthy cyclists (Bigley et al., 2014), but did not immediately alter $CD57^+$ cell proportions. Instead, lower percentages of total CD56 and $CD56^{bright}CD57^+$ cells occurred during recovery (Figure 5A & C). This NK cell redistribution may represent a greater egress of highly cytotoxic cells into the tissues following exercise. Considering that greater $CD57^+$ NK cell numbers have been measured within tumour tissues (Kared et al., 2016; Nielsen et al., 2013), an additional influx of cells following acute exercise could provide additional benefit via greater killing capacity. Furthermore, the decrease at 24h in $CD57^+$ was coupled with a decrease in perforin expression, which is also suggestive of a shift in cytotoxicity. Acute exercise induces a biphasic response for perforin proportions within $CD57^-$ but not $CD57^+$ cells, with ceiling effects being less likely in the former given the lower relative expression levels. It is unknown if increased perforin expression within these less mature $CD57^-$ NK cells has functional consequences.

From a practical standpoint, there is very limited evidence examining immune function during recovery from training bouts that follow current exercise oncology guidelines (Campbell et al., 2019; Hayes et al., 2019). While decreased cell numbers post-exercise are now largely viewed as a redistribution of cells out of circulation (Campbell & Turner, 2018), we demonstrate that leukocyte, lymphocyte, and NK cell counts and proportions to return to baseline by 24h after exercise and is consistent with breast cancer survivors (Evans et al., 2015). These collective works provide preliminary evidence that NK cell numbers normalise following overnight rest and suggests that consecutive training sessions could be used without adverse affecting the immune system. Exercise training reduces tumour progression via NK cell infiltration (Pedersen et al., 2016), with the acute exercise that stimulates large but transient alterations in circulating factors (e.g. NK cells, catecholamine and inflammatory markers) being a key stimulus for attenuated tumour growth and viability (Dethlefsen, Pedersen, & Hojman, 2017). However, careful consideration of total training

volumes with adequate recovery period are prudent to minimise possible reductions in immunity over time (Peake, Neubauer, Walsh, & Simpson, 2017). With only limited evidence to date (Khosravi et al., 2019), high intensity training programs may be a double-edged sword. Increasing the training stimulus may provide greater adaptation within select physiological systems (e.g. cardiorespiratory, musculoskeletal) but potentially at the expense of others (e.g. immune and endocrine) if training loads are too high (Peake et al., 2017; Walsh et al., 2011).

Conclusions

In summary, NK cell numbers and proportions changed similarly between prostate cancer survivors and CON following acute exercise. However, compared to CON and independent of exercise, ADT had greater IFNγ expression and PCa demonstrated lower perforin proportions. This suggests that prostate cancer treatments may alter maturity and degranulation status and therefore cytotoxic function. Importantly, NK cell proportions mostly returned to resting levels by 24h, which suggests that consecutive training sessions using moderate intensity are unlikely to adversely affect immune function. While this is an initial step examining how immune function responds to acute exercise and supports current guidelines, larger trials that examine repeated acute exercise bouts and include direct measures of NK cell function are required.

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Author Contributions

Each author contributed to the manuscript in the following way. Conception and design: EH, SS, JV, CB, GK, AH. Data acquisition: EH, SS, SQ, EK, AH. Data analysis and interpretation: EH, EC, GS, DB, LS. Drafted the manuscript: EH, AH. Writing revisions and editing: SS, SQ, EC, GS, EK, JV, CB, LS, DB, GK. All authors approved the final manuscript.

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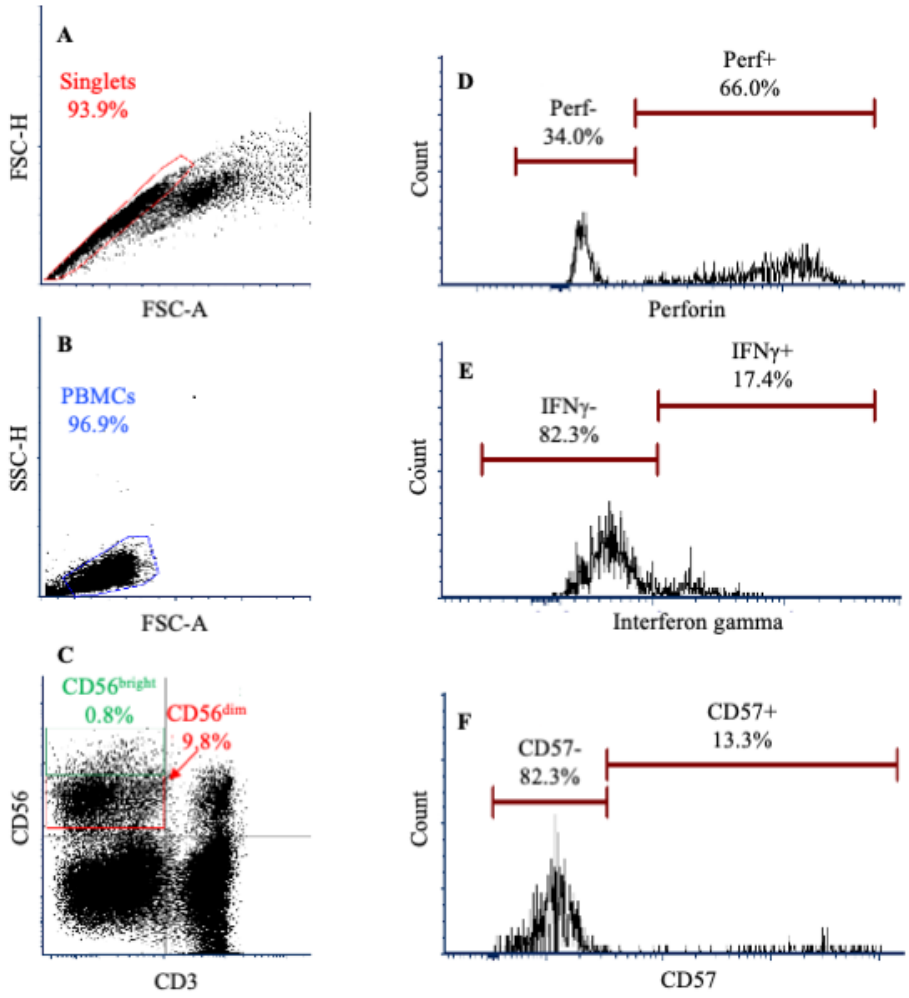

Figure 1. The gating strategy for the natural killer (NK) cells was initially performed on A) single cells, followed by B) peripheral blood mononuclear cells (PBMCs). NK cells were identified as C) $CD3^{-}CD56^{+}$ and included the $CD56^{dim}$ and $CD56^{bright}$ populations that were summed to determine total $CD56^{+}$ cells. From each of these populations, the frequency of D) perforin (Perf), E) interferon gamma (IFNγ) and F) CD57 positive cells were determined. Perforin and IFNγ were also determined in $CD57^{-}$ vs. $CD57^{+}$ cells separately (not shown) by first gating on CD57 status and then determining intracellular levels.

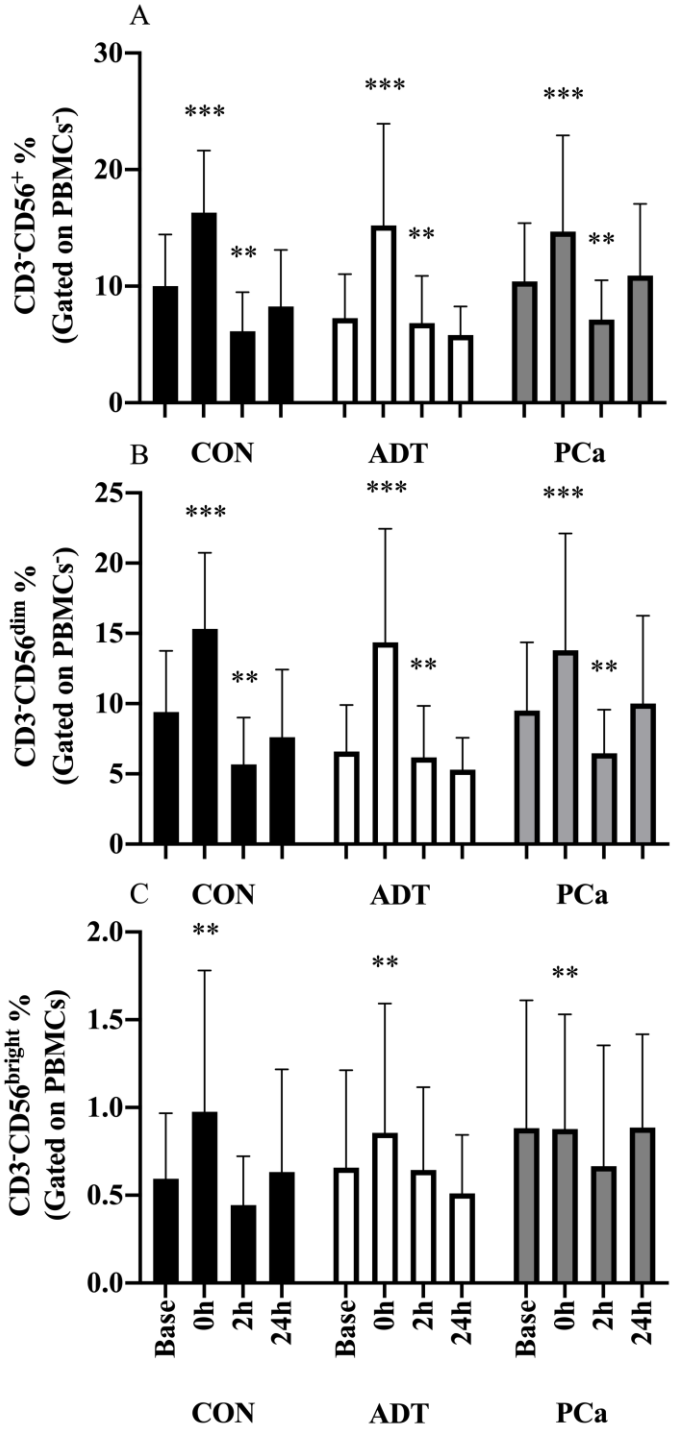

Figure 2. Changes in natural killer (NK) cell proportions with acute exercise for **A)** total $CD3^{-}CD56^{+}$, **B)** $CD3^{-}CD56^{dim}$, and **C)** $CD3^{-}CD56^{bright}$ NK cells. Data are presented as mean ± SD.

** P<0.01 *** P<0.001 vs. baseline (main effect)

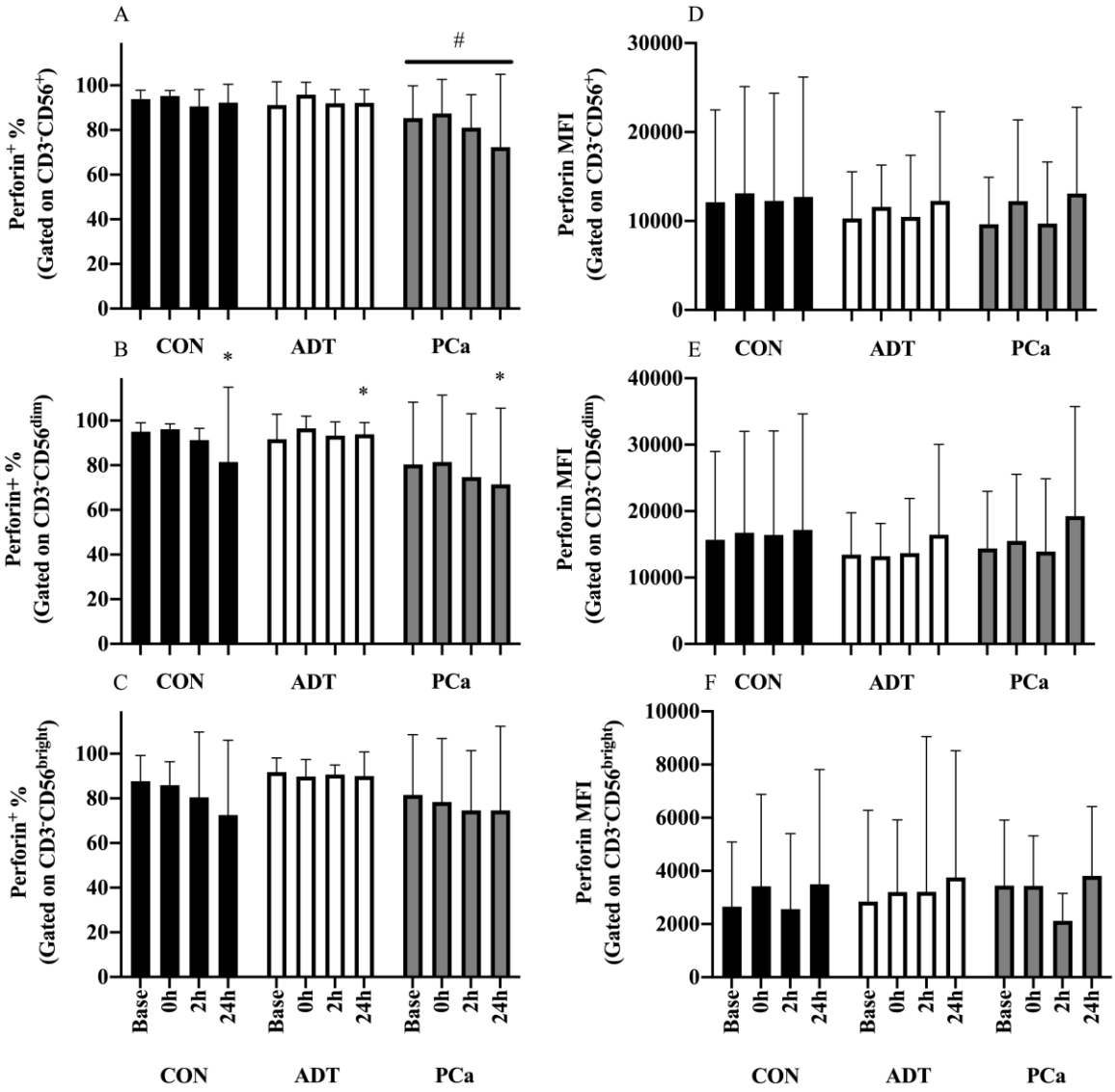

Figure 3. Effects of acute exercise on the proportion of NK cell expressing perforin for **A)** $CD3^{-}CD56^{+}$, **B)** $CD3^{-}CD56^{dim}$, or **C)** $CD3^{-}CD56^{bright}$ cells and for perforin median fluorescent intensity (MFI) in **D)** $CD3^{-}CD56^{+}$, **E)** $CD3^{-}CD56^{dim}$ and **F)** $CD3^{-}CD56^{bright}$ cells. Data are presented as mean ± SD.

* P<0.05 vs. baseline (main effect)

# P<0.05 vs. CON (main effect)

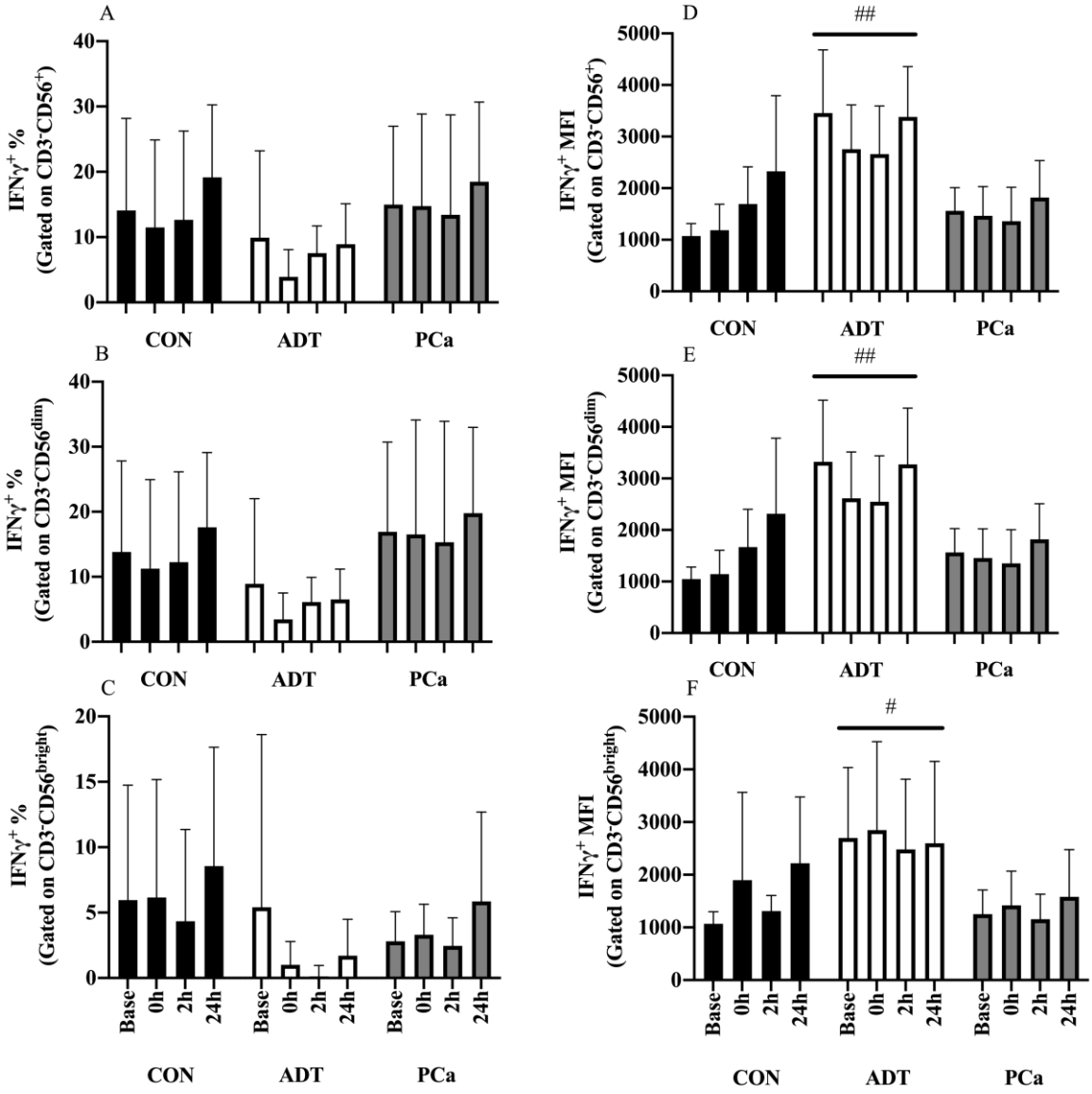

Figure 4. Effects of acute exercise on the proportion of NK cell expressing IFNγ for **A)** $CD3^{-}CD56^{+}$, **B)** $CD3^{-}CD56^{dim}$, or **C)** $CD3^{-}CD56^{bright}$ cells and for IFNγ median fluorescent intensity (MFI) for **D)** $CD3^{-}CD56^{+}$, **E)** $CD3^{-}CD56^{dim}$ and **F)** $CD3^{-}CD56^{bright}$ cells compared to CON. IFNγ analyses were conducted on a subset of participants (CON: n=4, ADT: n=7, PCa: n=5). Data are presented as mean ± SD.

# P<0.05 ## P<0.01 vs. CON (main effect)

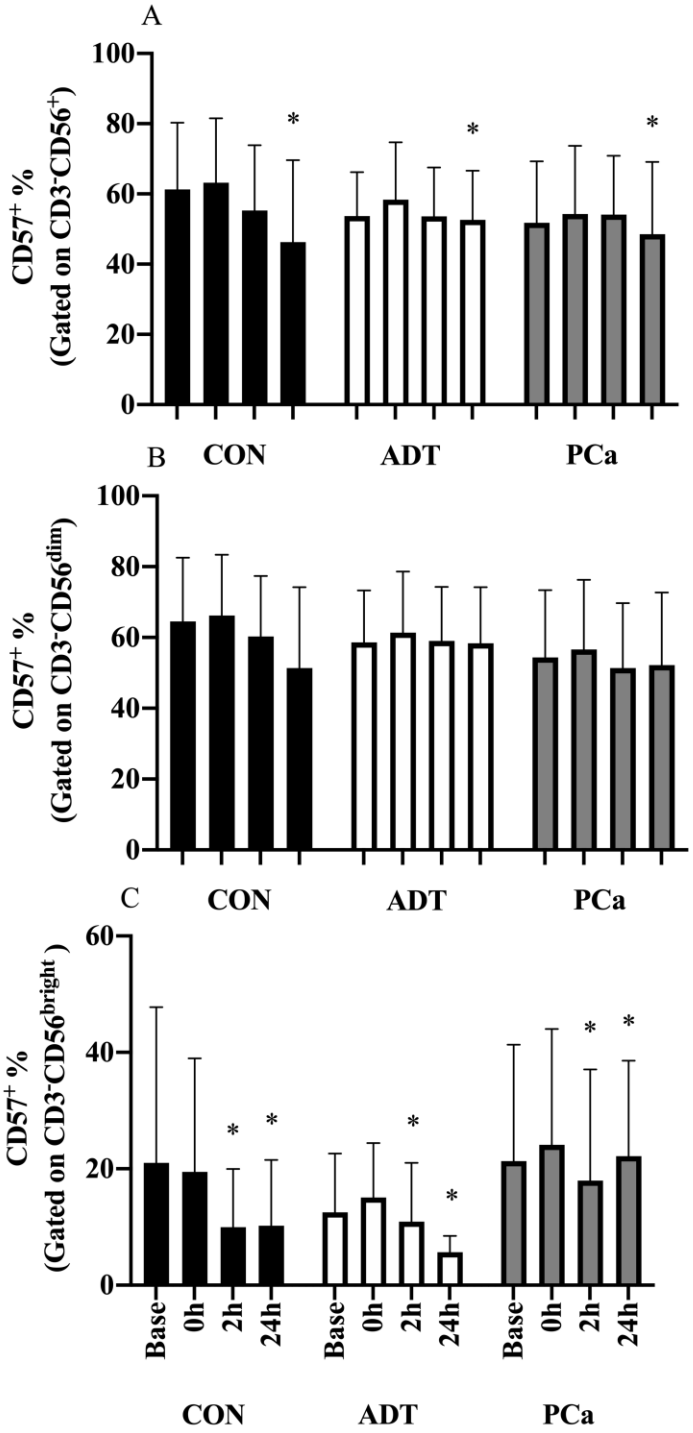

Figure 5. The percentage of NK cells expressing CD57 within the **A)** total $CD3^{-}CD56^{+}$ **B)** $CD3^{-}CD56^{dim}$ and **C)** $CD3^{-}CD56^{bright}$ populations. Data are presented as mean ± SD.

* P<0.05 vs. baseline (main effect)

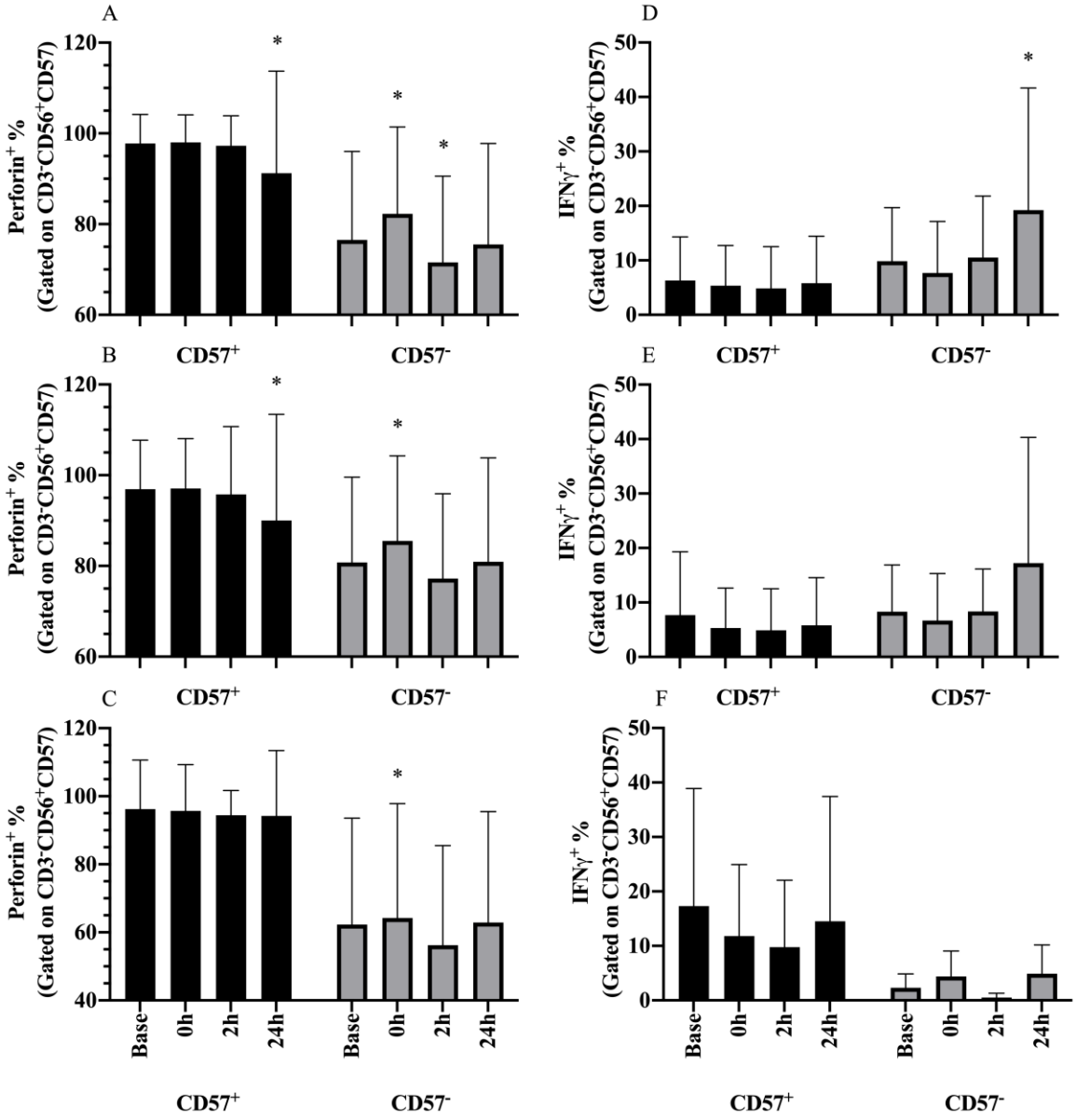

Figure 6. The effect of acute exercise and CD57 expression on **A)** $CD3^-CD56$ total, **B)** $CD3^-CD56^{dim}$, and **C)** $CD3^-CD56^{bright}$ NK cell perforin expression and **D)** $CD3^-CD56$ total, **E)** $CD3^-CD56^{dim}$, and **F)** $CD3^-CD56^{bright}$ NK cell IFNγ expression. As there were no group x time interactions or group effects, data were collapsed so that $CD57^+$ and $CD57^-$ populations could be presented together across time. Data are presented as mean ± SD.

* P<0.05 vs. baseline (main effect)

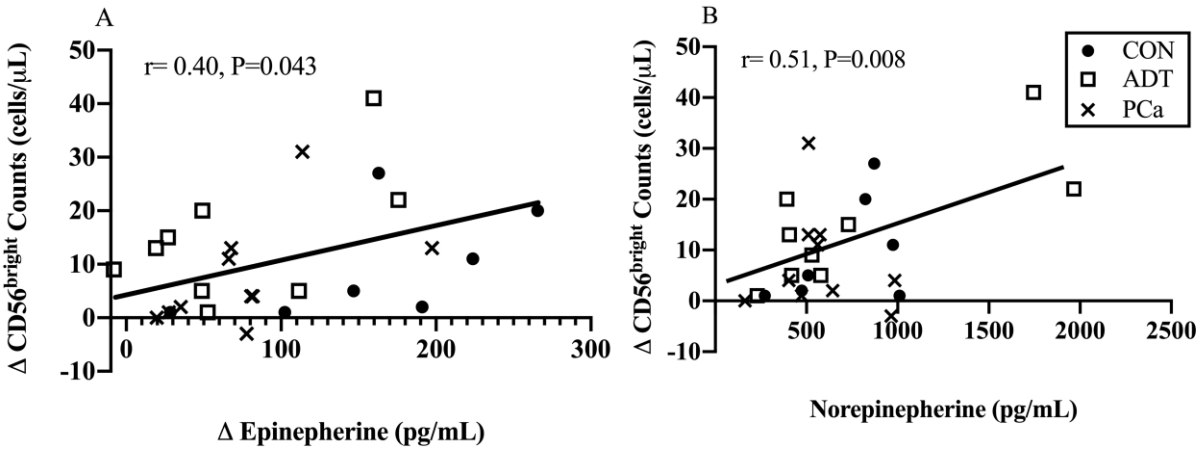

Figure 7. Changes in $CD3^{-}CD56^{bright}$ natural killer cell counts with acute exercise were correlated with **A)** the change in epinephrine from baseline to 0h and also with **B)** baseline norepinephrine levels.

Table 1. Participant characteristics.

	ADT (n=11)	PCa (n=14)	CON (n=8)	P value
Age (y)	67 ± 7	67 ± 7	64 ± 8	0.378
Mass (kg)	91.9 ± 19.8†	81.0 ± 8.1	75.1 ± 6.4	***0.019***
Height (cm)	173.1 ± 8.1	174.9 ± 6.0	173.1 ± 3.9	0.730
% Fat	29.9 ± 6.7†	25.3 ± 3.9	21.0 ± 3.5	***0.001***
Lean Mass (kg)	59.6 ± 8.9	56.5 ± 5.7	55.7 ± 4.7	0.370
Comorbidities	1.5 ± 1.1	1.4 ± 1.1	0.6 ± 1.1	0.988
VO_2peak (l/min)	2.1 ± 0.6	2.0 ± 0.6	2.4 ± 0.6	0.320
VO_2peak (ml/kg/min)	23.9 ± 8.4†	25.1 ± 5.8	32.4 ± 8.1	***0.040***
Maximal HR (bpm)	157 ± 25	152 ± 13	158 ± 11	0.659
Trial Workload (w)	175 ± 72	166 ± 58	206 ± 40	0.285
PSA (ng/mL)	9.3 ± 26.8	1.6 ± 2.5	1.9 ± 1.2	0.438
Testosterone (ng/dL)	46.3 ± 23.5†,‡	640.7 ± 354.2	669.2 ± 231.1	***<0.001***
Gleason Score	8 ± 1‡	7 ± 1	-	**0.007**
Diagnosis Days	1241 ± 1365	1307 ± 1099	-	0.894
Prostatectomy (%)	36	43	-	-
Radiation (%)	55	50	-	-
ADT Length (d)	596 ± 383	-	-	-

Mean ± SD. ADT = Androgen deprivation therapy group; PCa = prostate cancer group; CON = controls; Comorbidities is the sum of the following conditions being present: hypertension, hypercholesterolemia, Type II diabetes, smoker, former smoker, and regular alcohol consumption. Peak oxygen uptake = VO_2peak; HR = heart rate; bpm = beats per min; w = watts; PSA = prostate specific antigen;

† P<0.05 vs. CON

‡ P<0.05 vs PCa

Table 2. Complete blood counts and plasma volume (PV) shifts before and after acute exercise.

		Base	0h	2h	24h
Leukocytes	Total	5.3 ± 1.2	7.9 ± 1.5	7.3 ± 1.5	5.4 ± 1.2
($x10^3$ cells/µL)	ADT	5.5 ± 1.2	8.5 ± 1.5***	7.2 ± 1.7***	5.3 ± 0.8
	PCa	5.1 ± 1.3	7.1 ± 1.4***	7.5 ± 1.7***	5.4 ± 1.6
	CON	5.4 ± 0.8	7.9 ± 1.3***	7.2 ± 1.1***	5.3 ± 1.1
Lymphocytes	Total	1.6 ± 0.5	2.5 ± 0.9***	1.5 ± 0.5	1.6 ± 0.5
($x10^3$ cells/µL)	ADT	1.6 ± 0.5	2.8 ± 0.7	1.6 ± 0.4	1.6 ± 0.5
	PCa	1.5 ± 0.4	2.3 ± 0.9	1.4 ± 0.4	1.5 ± 0.4
	CON	1.8 ± 0.6	2.5 ± 1.0	1.4 ± 0.7	1.7 ± 0.7
Mixed	Total	0.6 ± 0.2	0.7 ± 0.3***	0.6 ± 0.2	0.6 ± 0.2
($x10^3$ cells/µL)	ADT	0.5 ± 0.1	0.7 ± 0.2	0.5 ± 0.2	0.5 ± 0.1
	PCa	0.6 ± 0.3	0.7 ± 0.3	0.6 ± 0.3	0.6 ± 0.3
	CON	0.5 ± 0.2	0.8 ± 0.3	0.6 ± 0.2	0.6 ± 0.1
Neutrophils	Total	3.2 ± 0.9	4.7 ± 1.3***	5.3 ± 1.5***	3.2 ± 0.9
($x10^3$ cells/µL)	ADT	3.1 ± 1.1	4.2 ± 1.5	5.5 ± 1.6	3.3 ± 0.5
	PCa	3.2 ± 1.0	4.8 ± 1.2	5.3 ± 1.6	3.2 ± 1.3
	CON	3.5 ± 0.7	5.2 ± 1.1	5.2 ± 1.0	3.2 ± 0.5
PV Shift	Total	-	-14.3 ± 5.5***	-5.6 ± 5.0***	2.7 ± 5.8*
(%)	ADT	-	-13.7 ± 4.6	-4.6 ± 4.7	0.1 ± 5.0
	PCa	-	-14.8 ± 7.0	-7.0 ± 4.2	-4.3 ± 5.9
	CON	-	-14.2 ± 4.0	-4.8 ± 6.5	-3.6 ± 5.9

Mean ± SD. When statistical significance is indicated on the Total, this represents a time main effect.

* P<0.05 *** P<0.001 vs. baseline

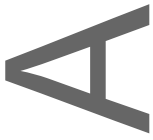

Table 3. Changes in NK cell counts before and after acute exercise.

		Base	0h	2h	24h
$CD3^-CD56^+$	Total	144 ± 94	391 ± 236***	90 ± 57	126 ± 112
(cells/µL)	ADT	104 ± 52	410 ± 197	103 ± 52	82 ± 40
	PCa	156 ± 101	361 ± 275	90 ± 62	163 ± 139
	CON	171 ± 123	401 ± 264	78 ± 62	135 ± 130
$CD3^-CD56^{dim}$	Total	133 ± 90	379 ± 232***	82± 52	117 ± 110
(cells/µL)	ADT	95 ± 47	387 ± 185	93 ± 48	75 ± 39
	PCa	143 ± 92	340 ± 272	80 ± 54	150 ± 137
	CON	163 ± 123	383 ± 269	74 ± 61	127 ± 130
$CD3^-CD56^{bright}$	Total	10 ± 11	20 ± 18***	8 ± 9	9 ± 8
(cells/µL)	ADT	9 ± 5	23 ± 16	10 ± 6	7 ± 4
	PCa	13 ± 16	21 ± 24	9 ± 13	12 ± 11
	CON	9 ± 5	18 ± 14	5 ± 3	8 ± 4
$CD3^-CD56^+CD57^+$	Total	81 ± 67	249 ± 188***	51 ± 34	74 ± 82
(cells/µL)	ADT	59 ± 35	260 ±165	57 ± 29	46 ± 27
	PCa	86 ± 66	220 ± 194	49 ± 33	106 ± 107
	CON	108 ± 99	273 ± 235	48 ± 46	81 ± 99
$CD3^-CD56^{dim}CD57^+$	Total	80 ± 68	247 ±189***	51 ± 35	74 ± 83
(cells/µL)	ADT	59 ± 36	258 ± 163	56 ± 29	46 ± 28
	PCa	83 ± 66	217 ± 195	48 ± 32	105 ± 107
	CON	108 ± 101	272 ± 239	48 ± 47	81 ± 100
$CD3^-CD56^{bright}CD57^+$	Total	2 ± 2	4 ± 5***	1 ± 1	1 ± 1
(cells/µL)	ADT	1 ± 1	4 ± 5	1 ± 1	0 ± 1
	PCa	2 ± 3	4 ± 3	1 ± 1	2 ± 2
	CON	1 ± 1	4 ± 7	0 ± 1	1 ± 2

Mean ± SD. When statistical significance is indicated on the Total, this represents a time main effect. *** P<0.001 vs. baseline

Table 4. Changes in NK cell counts expressing perforin and IFNγ before and after acute exercise.

		Base	0h	2h	24h
$CD3^-CD56^+Perf^+$	Total	132 ± 86	372 ± 239***	81 ± 49	113 ± 108
(cells/μL)	ADT	97 ± 52	399 ± 200	93 ± 44	74 ± 43
	PCa	139 ± 88	332 ± 281	77 ± 50	147 ± 144
	CON	161 ± 117	385 ± 262	71 ± 56	117 ± 116
$CD3^-CD56^{dim}Perf^+$	Total	122 ± 86	351 ± 242**	73± 47	107 ± 108
(cells/μL)	ADT	89 ± 48	379 ± 189	85 ± 40	69 ± 42
	PCa	123 ± 89	304 ± 291	68 ± 51	136 ± 143
	CON	155 ± 119	371 ± 268	65 ± 51	115 ± 120
$CD3^-CD56^{bright}Perf^+$	Total	9 ± 10	19 ± 17***	7 ± 7	8 ± 8
(cells/μL)	ADT	8 ± 5	21 ± 15	9 ± 5	6 ± 4
	PCa	13 ± 15	18 ± 21	8 ± 10	11 ± 11
	CON	8 ± 4	16 ± 13	4 ± 3	6 ± 7
$CD3^-CD56^+IFN\gamma^+$	Total	20 ± 27	29 ± 37	13 ± 20	20 ± 21
(cells/μL)	ADT	7 ± 5	9 ± 5	9 ± 9	10 ± 8
	PCa	31 ± 41	52 ± 60	18 ± 33	26 ± 24
	CON	21 ± 24	25 ± 18	10 ± 11	24 ± 27
$CD3^-CD56^{dim}IFN\gamma^+$	Total	18 ± 26	26 ± 35	11 ± 18	16 ± 18
(cells/μL)	ADT	5 ± 5	7 ± 5	7 ± 8	7 ± 5
	PCa	30 ± 40	50 ± 56	17 ± 31	24 ± 23
	CON	20 ± 22	22 ± 15	9 ± 9	18 ± 20
$CD3^-CD56^{bright}IFN\gamma^+$	Total	0.4 ± 0.5	0.6 ± 0.8	0.2 ± 0.4	0.5 ± 0.6
(cells/μL)	ADT	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.2	0.1 ± 0.1
	PCa	0.5 ± 0.6	1.0 ± 1.0	0.4 ± 0.5	0.8 ± 0.8
	CON	0.4 ± 0.6	0.6 ± 0.7	0.2 ± 0.3	0.5 ± 0.5

Mean ± SD. When statistical significance is indicated on the Total, this represents a time main effect. *** P<0.001 vs. baseline

Table 5. Changes from baseline of circulating stress hormones with acute exercise.

		Δ0h	Δ2h	Δ24h
Cortisol	Total	59.2 ± 105.0*	-32.2 ± 33.1*	-14.2 ± 40.5
(ng/mL)	ADT	50.3 ± 82.7‡	-28.2 ± 13.8‡	-17.0 ± 23.5‡
	PCa	99.8 ± 142.0	-21.1 ± 33.1	3.1 ± 49.3
	CON	15.8 ± 48.6	-53.2 ± 44.7	-34.9 ± 35.3
Epinephrine	Total	96.2 ± 73.7	-0.6 ±12.6	0.0 ± 16.2
(pg/mL)	ADT	70.6 ± 63.8*,†	5.1 ± 11.6	7.0 ± 8.6
	PCa	69.6 ± 54.0*,†	-3.0 ± 12.6	-5.7 ± 6.0
	CON	162.0 ± 72.8*	-2.6 ± 13.4	1.0 ± 27.2
Norepinephrine	Total	2602 ± 1862***	799 ± 703***	160 ± 367
(pg/mL)	ADT	2544 ± 2213	714 ± 473	221 ± 376
	PCa	2292 ± 1769	746 ± 542	138 ± 447
	CON	3095 ± 1695	967 ± 1090	122 ± 256

Data are presented as the mean difference from baseline ± SD. When statistical significance is indicated on the Total, this represents a main effect.

* P<0.05 *** P<0.001 vs. baseline

† P<0.05 vs. CON

‡ P<0.05 vs. PCa