Acute Bouts of Exercise Induce a Suppressive Effect on Lymphocyte Proliferation in Human

Subjects: A Meta-Analysis

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Abstract

Objective: Lymphocyte proliferative responses are commonly used to assess immune function in clinical settings, yet it is unclear how proliferative capacity is altered by exercise. This analysis aims to quantitatively assess the proliferative response of lymphocytes following an acute bout of exercise.

Methods: Electronic databases were searched for articles containing the keywords “exercise” OR “acute” OR “aerobic” OR “resistance training” OR “immune function” AND “proliferation” AND “lymphocyte.” Initial results yielded 517 articles of which 117 were reviewed in full. Twenty-four articles met the inclusion criteria. Calculated standardized mean difference (SMD) and corresponding standard errors (SE) were integrated using random-effect models.

Results: Analyses uncovered evidence for suppression of proliferative capacity following acute exercise in general (SMD = -0.18, 95% CI: -0.21, -0.16) with long duration, high intensity exercise exhibiting a moderate suppressive effect (SMD = -0.55, 95% CI: -0.86, -0.24). Discordant proliferative responses for long duration, high intensity exercise in competitive versus non-competitive settings were identified with enhanced proliferation (SMD = 0.46, 95% CI: 0.03, 0.89) observed following competitive events and a large suppressive effect detected for similar activities outside of a competitive environment (SMD: -1.28, 95% CI: -1.61, -0.96) ($p=0.02$).

Conclusion: Evidence suggests lymphocyte proliferation is suppressed following acute bouts of exercise, with exercise lasting longer than one hour having a greater magnitude of effect regardless of exercise intensity. Variations in observed effect sizes across intensity, duration, and competitive environment further highlight our need to acknowledge the impact of study designs in advancing our understanding of exercise immunology.

Key Words: DURATION; EXERCISE; IMMUNE FUNCTION; INTENSITY

1. Introduction

Exercise induced alterations of specific immune cell populations and hormonal concentrations are some of, if not the most, commonly reported effects in the area of exercise immunology. The most replicated of these effects is the rapid mobilization of leukocytes and lymphocytes into peripheral circulation during exercise [1-4]. Disruptions in leukocyte trafficking are thought to result from events including increased shear forces and hydrostatic pressure from surges in cardiac output [5, 6], additional lymphatic fluid pushed out by forceful muscle contractions [5] and as a response to increased concentrations of catecholamines [7] and glucocorticoids [1] in the blood.

Exercise associated lymphocytosis and lymphocytopenia have been reported on extensively [8-10]. The characteristic exercise response of blood leukocyte counts involves an initial increase as cells are mobilized from marginal pools and other organs followed by a decrease in total counts as those same cells move into areas like the skin, mucosa, and lymph nodes. Observations of this phenomenon coupled with epidemiological data have produced ideas like the "Open Window" hypothesis whereby individuals are thought to have decreased immune function following intense physical activity [10, 11].

Alterations in cell trafficking, however, contribute little information about the overall immunocompetence of an individual. Immunocompetence is better assessed by investigating the ability of lymphocytes to proliferate in response to cognate antigen. Upon encountering cognate antigen, a lymphocyte undergoes clonal expansion to increase the number of lymphocytes present with identical antigen specificity and defend the host from the threat [12]. This proliferative capacity of lymphocytes is a crucial feature of the adaptive immune response [13]. A failure to proliferate in response to foreign antigen is indicative of impaired immune function, and the host's ability to respond to an immunological challenge can be substantially diminished in these circumstances.

Lymphocyte proliferation can also be induced by factors other than cognate antigen. Plant lectins are carbohydrate-binding glycoproteins that agglutinate cells [14]. When added to a cell culture, lectins act as a mitogen to induce lymphocyte proliferation via cross-linking of glycoproteins on the cell surface [15]. For decades a simple assay for the ability of human T cells to proliferate in response to the T cell mitogen phytohaemagglutinin (PHA) has been used to evaluate immunocompetence. PHA assays have been routinely used to monitor immune function in potentially immunosuppressed patients including transplant recipients, HIV positive individuals, and others at risk for immunosuppression. Because lymphocytes play a pivotal role in the immune response, it is important that investigations of susceptibility to infection following exercise be conducted within the context of proliferative capacity. For this reason, we used measures of lymphocyte proliferation as a functional assessment of immunocompetence following exercise.

As we have noted above, proliferation assays are a mainstay of clinical diagnostics yet they represent a small proportion of the literature comprising the field of exercise immunology [9, 16]. Non-systematic review articles of lymphocyte proliferation report inconsistent findings regarding whether immune function is enhanced or suppressed by exercise [8-10, 16]. We are not aware of any systematic reviews of lymphocyte proliferation following acute bouts of exercise. Therefore, the purpose of this meta-analysis was to provide a quantitative review of the effects of acute exercise bouts on lymphocyte proliferation. To do this, we conducted a comprehensive review of published studies involving acute (minutes to hours) bouts of exercise and outcomes of lymphocyte proliferation in healthy individuals. We hypothesized that lymphocyte, specifically T cell, proliferative ability would be depressed immediately post exercise with higher intensity exercise having a greater suppressive effect.

2. Materials and Methods

Studies were identified by searching the following electronic databases: PubMed, Google Scholar and Web of Science. Subject specific search terms utilized either individually or jointly were: "exercise",

“acute”, “aerobic”, “resistance training”, “immune function”, “proliferation”, “lymphocyte.” We included studies that were published up to March 2016. No registered protocol exists for this meta-analysis. We adhered to the PRISMA Guidelines for manuscript preparation. A flow chart of studies evaluated and excluded during the review process is shown in Fig. 1. One hundred seventeen publications were identified as possibly relevant and the reference lists of these articles were forward and backward searched for pertinent articles not identified in the initial electronic searches. Of the 117 articles reviewed in full, 81 either did not have a measure of lymphocyte proliferation or did not utilize an acute training protocol and were excluded from analysis. Authors of papers that did not include sufficient data for calculation of standardized mean differences (SMD) were contacted to request further information. Twenty-four of the retrieved articles were determined to be suitable for inclusion [17-40] (Table 1).

2.1 Description of the studies

We included all studies that assessed changes in lymphocyte proliferation after an acute bout of exercise by comparison to a baseline sample (paired samples pre-test vs. post-test designs). For studies using placebo-controlled trials of individuals taking nutritional supplements for the purpose of mitigating immunosuppression following exercise, the data for the control groups were included in this analysis.

2.2 Criteria for considering studies for review

Types of participants: Healthy individuals able to perform exercise without restriction. No restrictions for age or sex were made.

Types of interventions: We considered studies reporting on the effects of sustained and interval style exercise, both aerobic and resistance training, on lymphocyte proliferation.

Types of outcome measures: Primary outcome measures were: phytohaemagglutinin (PHA), concanavalin A (ConA), pokeweed mitogen (PWM) and interleukin (IL)-2R stimulated lymphocyte proliferation. Also included in this analysis were the use of tumor-associated-antigen (TAA).

2.3 Statistical Analysis

Outcome measures were obtained from pre-exercise samples (baseline) and the sample obtained closest to exercise cessation (immediate post) for analysis. Standardized mean difference (SMD) and 95% confidence intervals (CI) were calculated for each study. SMD were calculated as the mean difference between time points divided by the pooled standard deviation [41]. Due to the small sample sizes of included studies, Hedges' adjustment for small study samples was applied [42, 43].

Standardized mean difference calculations were designed so that a negative value indicates a decrease in proliferative ability compared to baseline. Separate SMD were calculated for proliferation outcome measures when studies used multiple mitogens. Overall analysis was conducted using each mitogen outcome as a unit of analysis as each of the three commonly used mitogens (PHA, ConA, & PWM) act through distinct mechanistic processes. Similarly, TAA is specific to a particular cytotoxic T cell and different TAA were treated as their own unit of analysis. Cohen's classifications for evaluating the magnitude of the overall effect size were utilized with SMD = 0.2-0.49 small; 0.5-0.8 moderate; and >0.8 large effect sizes [44].

Separate meta-analyses were conducted to compare the effect of intensity (moderate vs. high) and duration (short vs. long). Studies were further stratified so that individual meta-analyses could be conducted to examine intensity-duration combinations and mitogen specific responses. High intensity exercise was defined as: >80% of VO_{2max} ; a maximal test; interval training; multiple sets of 10-repetitions at 65%-85% of 1-repetition max (RM); multiple sets of 10-repetitions at 10-RM; or exercise to volitional exhaustion. Studies

that assessed proliferative responses to competition were included in the high intensity category. Moderate intensity exercise was defined as exercise $\leq 80\%$ of VO_{2max} . Exercise of short duration was defined as less than 60min with long duration encompassing all exercise lasting 60min or longer.

Differing effects of age and sex were accounted for across all intensity-duration combinations and are reported as separate meta-analyses. For studies where it was not possible to determine the sex characteristics for placebo groups those subjects are classified as 'unidentified' in the descriptive subject data.

Calculated SMD and corresponding standard errors (SE) were entered into Microsoft Excel (Excel 2013, Microsoft, Redmond, WA) and integrated using the random-effect model [45]. Sub group analyses were conducted using the random effect model with separate estimates of τ^2 [46]. Random-effect models view each study as a random observation from a population of possible studies and accounts for variance across studies related to sampling error and individual differences derived from study designs. Working within this context allows for more robust inferences from completed studies and provides a stronger foundation for generalizing to future research [47]. For all analyses, 95% confidence intervals were computed with the SMD determined to be statistically significant provided zero was not included in the corresponding interval. Mean effects for sub group differences were compared using a Z-test [46].

Statistical heterogeneity of studies was analyzed using Q and I^2 statistics. Together these are a measure of how much variance between studies is attributed to study difference rather than chance [48]. The magnitude of heterogeneity was categorized as: $I^2 = 0-24\%$ low; 25-49% moderate; 50-74% substantial; and 75-100% considerable [43, 48].

3. Results

Fifty-four SMD were retrieved from 24 published studies that included a total 345 subjects (males = 182; females = 48; sex not identified = 115). The weighted average for subject age across all studies included in these analyses was 31.7 yrs. The funnel plot of all SMD from the 24 studies did not indicate publication bias (Fig. 2). Meta-analysis discovered evidence for a small immunosuppressive effect of exercise (SMD = -0.18, SE: 0.01, 95% CI: -0.21, -0.16). There was substantial heterogeneity ($Q = 131.77$, $p < .0001$, $I^2: 59.78\%$) among studies. Given that the magnitude of changes in circulating leukocyte counts following exercise are proportional to intensity and duration, with intensity typically having a more pronounced effect [49, 50], we chose to examine how lymphocyte proliferative responses differ based on these factors. Sub group analyses to this effect were performed using random effect models with separate estimates of τ^2 .

3.1 Intensity & Duration

Twenty-eight SMD were retrieved from 10 studies investigating the proliferative response to moderate intensity exercise and 26 SMD retrieved from 14 studies investigating high intensity exercise. Evidence for the suppressive effects of exercise were found for both moderate (SMD = -0.15, SE: 0.05, 95% CI: -0.26, -0.04) and high intensity exercise (SMD = -0.21, SE: 0.03, 95% CI: -0.27, -0.16) (Fig. 3). There remained moderate-to-substantial heterogeneity among the studies. Contrary to what we would expect based on cell trafficking data, this analysis uncovered no substantial differences in proliferative capacity as intensity increased.

Examination of short versus long duration exercise utilized 34 SMD retrieved from 13 studies involving short duration exercise and 20 SMD retrieved from studies with long duration exercise. In these analyses short duration exercise generated a slight suppressive effect (SMD = -0.15, SE: 0.01, 95% CI: -0.17, -0.13) whereas a small effect size was observed for suppression of the proliferative response following exercise of long duration (SMD = -0.27, SE: 0.04, 95% CI: -0.34, -0.20) (Fig. 4). Although the

compared mean effects were not significantly different ($p = .57$), the difference in observed estimates is suggestive of a greater magnitude of suppression following long duration activity. Moderate-to-substantial heterogeneity was observed among the studies. We theorized that some of the heterogeneity observed in these analyses may be a result of intensity and duration combinations. Therefore, we undertook subgroups analyses examining all possible intensity and duration combinations: short, moderate intensity bouts; short-high; long-moderate; and long, high intensity.

3.2 Intensity & Duration Combinations

Meta-analysis of studies investigating short duration, moderate intensity exercise did not identify a significant change in proliferative ability post-exercise (SMD = -0.11, SE: 0.08, 95% CI: -0.26, 0.05). There was, however, evidence for a small suppressive effect following short, high intensity activity (SMD = -0.16, SE: 0.02, 95% CI: -0.19, -0.12) and long, moderate activity (SMD = -0.24, SE: 0.02, 95% CI: -0.28, -0.21). Long, high intensity activity had the greatest suppressive effect (SMD = -0.55, SE: 0.16; 95% CI: -0.86, -0.24) (Fig. 5). This finding would suggest that while intensity and duration are by themselves each significant factors for analysis, it is the different combinations of the two that will provide the most relevant standardizations for future analyses. Sub group differences between long, high intensity exercise and either short, high intensity ($p = 0.35$) or long, moderate intensity ($p = 0.06$) were not statistically significant. Significant heterogeneity was observed in all sub group analyses.

Within the context of long, high intensity exercise we did identify a discordant result ($p=0.02$) with the long duration, high intensity competitive events being associated with a moderate effect for increased proliferation (SMD: 0.46, 95% CI: 0.03, 0.89) and long duration, high-intensity exercise outside of competition having a large suppressive effect (SMD: -1.28, 95% CI: -1.61, -0.96). It should be noted that Henson, *et al.* [27] was included as a long duration, high-intensity event but not a competitive one as the

average finish time for the marathon was 4.57 h which suggests the participants were mainly recreational runners.

3.3 Effects of Age & Sex

Sub-groups analyses by age did not reveal any statistically significant deviations from the results presented above. Table 2 shows specific SMD estimates for each age group. Also highlighted in Table 2 is the lack of data for specific age groups; namely, the sparsity of research examining exercise and immune function in individuals over the age of 30. Although the estimates vary, there are no statistically significant differences in SMD presented in Table 2.

Twenty-three SMD were calculated from 13 studies examining only male subjects, and 9 SMD were calculated from 2 studies examining only females. Male subjects experienced a small, suppressive effect of exercise similar to the results reported above (SMD = -0.30, SE: 0.02, 95% CI: -0.34, -0.26). Females, however, exhibited a lesser suppression (SMD = -0.11, SE: 0.002, 95% CI: -0.11, -0.10). These measures are not statistically different from each other ($p=.22$) and are further limited in their value based on the small number of studies focused on female subjects.

4. Discussion

We conducted a quantitative review examining the effects of exercise on lymphocyte proliferation in healthy individuals. The accumulated evidence indicates that acute bouts of exercise generally have a slight, suppressive effect on lymphocyte proliferation (0.18 standard deviations relative to baseline measures) when conducted on trained individuals. Due to the variation in exercise modalities, duration, and intensity among the collected studies it seemed prudent to investigate differential effects in respect to confounding variables. Sub group analyses revealed decreases in lymphocyte proliferation are greater

following exercise lasting 60min or longer (SMD = -0.27 vs. -0.15 for long and short duration activities respectively) but not at a level of statistical significance. No appreciable differences were observed comparing across exercise intensities with moderate and high intensity exercise exhibiting similar suppressive effects (SMD = -0.15 vs. -0.21 respectively). Our findings expand upon and quantify data from previous qualitative reviews [8-10, 16, 51, 52] providing point estimates for the magnitude of suppression of lymphocyte proliferative capacity following acute bouts of exercise training. These analyses inform current and future research by providing a defined picture of how measures of immunocompetence change following exercise of varied duration and intensity.

Acute exercise generates a stereotypical biphasic response in circulating lymphocytes. The magnitude of the changes in circulating cell counts are proportional to the intensity and duration of exercise with the intensity typically having a more pronounced effect [49, 50]. For this reason, we chose to examine how proliferative response differ based on changes in intensity and duration. Interestingly this meta-analytic review uncovered no substantial differences in proliferative capacity as intensity increased, but did find different SMD estimates for exercise of different durations. Contrary to our hypothesis, these data suggest that while exercise intensity has a profound effect on alterations in immune cell trafficking it is likely not the primary factor influencing the reduced lymphocyte proliferative response. While this meta-analysis provides a general foundation for future work in exercise immunology, identification of potential mechanisms for the observed changes will need to be examined within the confines of experiments designed to capture immunologically relevant physiological measures specific to long versus short duration activity.

The subtle nuances specific to exercise interventions are many and the deviation observed in exercise intensities and durations across studies will continue to pose a challenge for future research designs. For example, Figure 4 shows two studies with comparatively large effect sizes for depressed proliferation. It turns out these two are unique in the population of studies analyzed in that they are the only

two examining the impact of high intensity training intervals lasting two-hours or more. Bacurau *et al.* [17] investigated the immune response in trained cyclists participating in an exercise bout composed of cycling intervals (6x20 min with equal rest intervals) at 90% of their anaerobic threshold. Additionally, Nieman *et al.* [31] examined the proliferative responses of elite adolescent tennis athletes who maintained a mean HR of over 80% of their predicted HR_{max} throughout 2 h of high-intensity tennis drills performed in 15 min intervals with 4-5 min rest. While these studies have work intervals greater than 60 min, they also had a higher sustained intensity level and their large changes in the proliferative response may be unique to high intensity, long-duration activity. Removal of these studies from the analysis of long duration exercise does alter the weighted mean difference (SMD: -0.21 relative to the reported SMD: -0.27) but not in a statistically significant manner ($p = .81$). A larger sample size of studies of this nature would allow for a more direct comparison of exercise effects, and we recommend that as researchers design new exercise protocols for examination of immunocompetence they pay close attention to the specific exercise stimulus they are hoping to investigate.

In light of the discordant relationship observed between long, high intensity competitive versus non-competitive exercise, we would suggest the psychological stress of competition or the anticipatory response to a difficult task is likely a modulating factor of immunocompetence. As an example, Bassit, *et al.* [18] examined immune responses after an Olympic distance triathlon (1.5 km swim, 40 km bike, 10 km run) and noted an increase in lymphocyte proliferation immediately post-race. This is supported by a report that evaluated lymphocyte proliferation immediately after an Adventure Spring Race consisting of 12 km of hiking, 100 m of vertical skills, and 30 km of mountain biking [34]. As you can see in Figure 5, these studies conflict with the observed effects following long duration, high-intensity exercise in a non-competitive setting and suggest that the psychological stress unique to competition may regulate the immune response via a different mechanism.

The reason for the observed differences in proliferative responses between long, high intensity exercise in a competitive versus non-competitive environment is unclear at this time. It is known that exercise, particularly exercise of long duration, leads to increased circulating concentrations of hormones and cytokines that may modulate the immune response. Radom-Aizik, *et al.* [53] observed that Jurkat cells, an immortalized T cell line, exhibited increased proliferative ability following stimulation by proliferating nuclear cell antigen when treated with sera obtained from healthy donors following a bout of exercise (30 min cycling at 80% peak work rate). This supports the hypothesis that serum factors are able to modulate T cell function *in vitro*, and suggests that secreted soluble factors are at least partially accountable for changes in immunocompetence following exercise. The identification of these secretory factors and their mechanisms of action for the immunomodulatory responses has not yet been elucidated.

Increases in sera hormone concentrations under competitive conditions are not unexpected, but represent a potential confounding variable in studies where participants are placed in anxiety-arousing situations due to the nature of the task they are asked to complete. As examples of augmented hormonal responses, significant elevations in cortisol concentrations have been observed in judoka [54] and ballroom dancers [55] prior to competition than is typical during time matched training sessions. Glucocorticoids (i.e. cortisol) are thought to be generally immunosuppressive both in term of cell trafficking [56] and proliferation [57] and disproportionate increases derived from psychological stimuli potentially magnify the exercise induced alterations. Similar research investigating endocrine responses to combined physical and psychological stress challenges support this augmented response with increased sympathetic and adrenal activation observed in the dual stress challenge compared to exercise alone trials [58-62]. Contrary to the current literature, our meta-analytic results suggest that there may be an immunomodulatory factor that mediates the exacerbated hormone response in combined stress challenges allowing for the observed increases in lymphocyte proliferation. To our knowledge no dual stress challenge studies have examined changes in immunocompetence. Inclusion of anxiety measurements pre/post high intensity training

293 sessions may help define areas where exercise induced immunological and physiological responses are
294 being augmented by a psychological component.

295 Perception of the physical exertion is likely another factor involved in the physiological response
296 [63]. Specifically, the idea that a higher level of fitness will moderate the physiological response to physical
297 challenges [64-66] will need to be better addressed in future studies. With the exception of one study that
298 investigated sedentary individuals [19], the majority of the studies included in this meta-analysis recruited
299 “trained” individuals. Unfortunately trained status as it is commonly used can refer to a continuum of fitness
300 ranging from recreational to elite athletes. We expect that there were appreciable differences in the
301 populations studied, and, unfortunately, subject demographics were not reported in a manner allowing for
302 stratification based on fitness level. Potteiger, et al undertook an excellent study looking at functional
303 immune responses in resistance trained versus untrained females [66]. This study found trained females
304 participating in an acute bout of resistance training did not see a significant reduction in T cell proliferative
305 capacity. Conversely, untrained females exhibited suppressed proliferative ability up to 3 hours post-
306 exercise. These data suggest training status influences T cell proliferative capacity following high-intensity
307 resistance training and that differences in training backgrounds will need to be accounted for in further
308 studies. Indeed, differential immune responses based on fitness level are an interesting area of research
309 and we believe our data will provide a foundation for analysis of functional differences.

310 **4.1 Methodological Considerations**

311 While this study represents a comprehensive review of the current literature, there are a number of
312 studies that were not quantitatively assessed due to a lack of summary statistics. A more significant issue,
313 however, is the variability in methods used to measure lymphocyte proliferation. The primary mitogens
314 used for proliferative assays (PHA, ConA and PWM) work through functionally distinct mechanisms and
315 consequently stimulate different subsets of lymphocytes. PHA is primarily thought of as T cell mitogen [67,

68] that cross-links CD2, CD3 and the T cell receptor (TCR) complex, among other surface glycoproteins, to initiate an activation signal into the cell [69]. This is considered a good measure for T cell proliferation with only a small percentage of B cells concomitantly stimulated [70]. ConA is best known to stimulate mouse T cell subsets but has been shown stimulate human B cells and a subset of human T cells [67, 71]. Cell activation with ConA is similar to PHA in that it cross-links surface glycoproteins but it would more accurately describe total lymphocyte proliferation. Pokeweed mitogen is primarily a B cell mitogen [67].

Pooled measures give an accurate representation of total proliferative capacity, but provides a limited ability to discuss specific mechanisms of exercise derived immunosuppression. As an example, the suppressive effect of short duration exercise measured only by PHA or ConA alone is trivial (-0.13 and -0.15 respectively). Looking at mitogen specific suppression of long duration exercise however provides an estimated SMD = -0.36 (95% CI: -0.47, -0.26) for PHA and a SMD = -0.21 (95% CI: -0.31, -0.12) for ConA. Although not statistically significant from each other ($p = 0.64$) it would suggest that different cellular processes are at work; namely, some sort of suppressive mechanism may be preferentially targeting the T-cell compartment during exercise lasting greater than 60min. In light of the existing literature [67, 68, 70, 71] and in agreement with clinical immunology practices, we would suggest that PHA is a more accurate assessment of T cell proliferation and future research investigating proliferative responses to exercise should consider the mitogenic properties of possible lectins and target lymphocyte subsets accordingly.

The question of what lymphocyte subpopulation is being activated is further complicated by the cell culture techniques utilized for proliferation assays. The majority of studies use either peripheral blood mononuclear cells or whole blood cell cultures. Use of these cultures would preclude a true assessment of a specific cell type's functional capacity unless a T cell specific mitogen (i.e. PHA) is used for the assay. For this reason, it is problematic to interpret data pairing these culturing methods with either ConA or PWM. While outcome measures in these assays are similar in nature (i.e. counts per minute (CPM) or

disintegrations per minute (DPM)), they do vary by method. By standardizing the mean differences in the studies we examined we are able to provide an index that is comparable across different metrics. This does not, however, entirely address the issues of how cellular function is being assessed.

Some researchers have argued that whole blood assays mimic in vivo conditions and are the best method assessing proliferative responses [29]. The primary concern with this method, however, is that the combination of lymphocyte cell types, other leukocytes, and immunomodulatory factors such as cytokines and hormones present in the blood preclude a mechanistic analysis of proliferative capacity. As all the above listed cells and molecules have the ability to influence proliferation either separately or through a number of different interactions it is difficult to address a specific lymphocyte's function through this method. Peripheral blood mononuclear cell assays typically isolate the white blood cells for analysis but do not allow for either T- or B-cell specific analyses. For these reasons, it is difficult to draw conclusions as to the clinical implications of these findings as they relate to increased risk of infection. Isolation of either T or B cells for analysis in combination with the appropriate mitogen would better define a lymphocyte specific immune response.

Finally, there is a lack of randomized controlled trials (RCT) in this area of research. The majority of these studies are non-blinded pre-post designs that allow for researcher bias in analysis. Acknowledging that RCT are more difficult to perform, future research designs would be best served to utilize RCT designs to maximize the generalizability and impact of their findings.

5. Conclusions

We conducted a meta-analysis of studies examining the effect of acute exercise on peripheral blood lymphocyte proliferative capacity. Exercise was associated with a small, global suppression of lymphocyte proliferation with this effect being more prominent following exercise lasting longer than one

361 hour. It is our goal that future studies will utilize these results to investigate the clinical applications of the
362 immune response with a focus on the physical well-being of the individual following various training
363 protocols especially as it relates to exercise prescription for special populations. This study provides the
364 first quantitative assessment of lymphocyte proliferation in response to exercise and the point estimates
365 identified here will provide a foundation for advancements in the area of exercise immunology.

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Table 1. Summary of data included in meta-analytic review.

Study	Exercise	Mitogen
Bacurau, 2002	Twelve trained cyclists cycling at 90% of anaerobic threshold. Six intervals of 20 min working and 20 min rest.	ConA
Bassit, 2000	Twelve elite male triathletes. Olympic distance triathlon.	ConA
Ceddia, 1999	Sedentary elderly (n=33) and young (n=14) participated in Balke Maximal Exercise Test.	PHA, ConA
Dohi, 2001	Eight high- and eight low-strength females performed 6 sets of 10RM squats with 2 min rest.	PHA, ConA, PWM
Field, 1991	Twelve healthy males cycled to exhaustion at 80% of maximal workload.	PHA, ConA, PWM
Frisina, 1994	Seven healthy males performed 25 one-minute treadmill bouts at 112% of maximal work capacity separated by 2min rest intervals.	ConA
Fry, 1992	Eighteen healthy males performed 25 one-minute work periods of kayaking followed by 2min rest interval.	ConA
Gray, 1991	Ten competitive male cyclists and 6 untrained males completed a 60sec anaerobic cycling test.	CD25+CD5
Henson, 1998	Thirteen experienced marathon runners completed 2.5 hr of treadmill running at 75-80% of VO_{2max} .	PHA, ConA, PWM
Henson, 1999	Ten experienced triathletes (8-males, 2-females) underwent two 2.5 hr training sessions at 75% VO_{2max} one cycling and one running.	PHA, ConA
Henson, 2004	Twenty-five experienced marathoners completed a marathon.	PHA
Koch, 2001	Ten resistance trained males performed 5 set of 10 squats at 65% 1RM followed by 3 sets of 10 half squats at 85% 1RM with 1 min rest.	PHA
Krzywkowski, 2001	Ten male athletes completed 2 hr cycling at 75% VO_{2max} .	PHA
LaVoy, 2015	Nineteen healthy adults (9-males, 10-females) ascended 260 stairs (10-flights) as quickly as possible.	MAGE-A4, PRAME, WT-1
Nehlsen-Cannarella, 1991	Twelve healthy females walked for 45 min at 60% VO_{2max} .	PHA, ConA, CD25
Nieman, 1994	Ten healthy resistance trained males performed sets of 10 squats at 65% of their 1RM. 3 min rest between sets until failure.	ConA
Nieman, 1997	Six experienced marathon runners completed 2.5 hr of treadmill running at 75-80% of VO_{2max} .	PHA, ConA, PWM
Nieman, 2000	Ten male and 10 female elite tennis athletes underwent 2 hr of intensive tennis drills.	PHA

Nieman, 2006	Twelve trained male cyclists underwent 2 hr cycling at 75% VO_{2max} .	PHA
Smith, 1993	25 healthy, adult runners ran for 30 min on an indoor track.	PHA, PWM
Tossige-Gomes, 2014	7 healthy males completed an Adventure Sprint Race.	PHA
Verde, 1992	10 male distance runners underwent a 30 min treadmill run at 80% of VO_{2max} at three different times in their training cycle.	PHA, ConA
Verde, 1992	10 male distance runners underwent a 30 min treadmill run at 80% of VO_{2max} .	PHA
Vider, 2001	19 males endurance trained athletes completed an incremental treadmill test to volitional exhaustion.	PHA, ConA

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Table 2. Effect size estimates for changes in lymphocyte proliferation following an acute bout of exercise accounting for age.

	<u>20-29 yrs</u>			<u>30-39 yrs</u>			<u>40-49 yrs</u>		
	SMD	SE	95% CI	SMD	SE	95% CI	SMD	SE	95% CI
Overall	-0.16	0.02	-0.20, -0.13	-0.19	0.09	-0.36, -0.01	-0.3	0.05	-0.39, -0.22
<u>Intensity</u>									
Moderate	-0.12	0.08	-0.28, 0.04	-0.19	0.09	-0.36, -0.01	--	--	--
High	-0.19	0.03	-0.24, -0.14	--	--	--	--	--	--
<u>Duration</u>									
Short	-0.17	0.01	-0.19, -0.14	--	--	--	--	--	--
Long	-0.25	0.07	-0.39, -0.11	--	--	--	-0.3	0.05	-0.39, -0.22
<u>Combinations</u>									
Short Moderate	-0.05	0.1	-0.25, 0.15	-0.18	0.12	-0.42, 0.05	--	--	--
Short High	-0.21	0.02	-0.26, -0.17	--	--	--	--	--	--
Long Moderate	-0.5	0.17	-0.83, -0.18	-0.19	0.14	-0.46, 0.07	-0.14	0.04	-0.22, -0.06
Long High	-0.28	0.08	-0.43, -0.12	--	--	--	-0.85	0.22	-1.34, -0.42

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Legends to Figures

Fig. 1. Flow Chart of Selection Process of Reviewed Studies.

Fig. 2. Funnel Plot for Evaluation of Potential Publication Bias.

Fig. 3. Effect of Exercise Intensity on Measures of Lymphocyte Proliferative Capacity. a) Moderate intensity exercise. b) High intensity exercise. Data are presented as mean effect size (boxes) for each study, 95% confidence intervals (horizontal lines), and summary of overall proliferative effect (diamonds).

Fig. 4. Effect of Short and Long Duration Exercise on Measures of Lymphocyte Proliferative Capacity. a) Short duration. B) Long duration. Data are presented as mean effect size (boxes) for each study, 95% confidence intervals (horizontal lines), and summary of overall proliferative effect (diamonds).

Fig. 5. Effect of Long Duration, High Intensity Exercise on Measures of Lymphocyte Proliferative Capacity. Data are presented as mean effect size (boxes) for each study, 95% confidence intervals (horizontal lines), and summary of overall proliferative effect (diamonds).

Figure 1

Figure 2

Figure 3

Figure 4

Long, High Intensity Exercise

Figure 5