A Review of Sex Differences in Immune Function after Aerobic Exercise

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ABSTRACT

When menstrual phase and oral contraceptives are controlled for, males and females display marked differences in immune response to an exercise stress. In highly controlled research studies, sex differences in immune cell changes, cytokine alterations, along with morbidity and mortality after inoculation are apparent. Exercise has been hypothesized to serve as a model of various clinical stresses by inducing similar hormonal and immunological alterations. Thus, a greater understanding of sex differences in post exercise non-specific immune function may provide insight into more effective clinical approaches and treatments. This paper reviews the recent evidence supporting sex differences in post exercise immune response and highlights the need for greater control when comparing the post exercise immune response between sexes.

Key Words: Immune Function, Sex, Cytokines, Aerobic Exercise.

INTRODUCTION

Exercise as a model to assess immune function

Exercise modulates the non-specific (innate) (52) and specific (acquired or adaptive) (12) arms of the immune system with an intensity dependent response. Moderate bouts of exercise have been shown to enhance immunity (51). However, intense exercise depresses the immune system (8, 52). More specifically, during

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moderate and intense bouts of exercise there are transient increases in circulating pro- and anti- inflammatory cytokine levels (55), concentration of lymphocytes and lymphocyte sub-sets (46), and macrophage activity (22). Recently, researchers (9, 53, 75, 76,) have suggested there are sex differences in the immune response to moderate and intense exercise.

Exercise has been hypothesized to serve as a model for certain clinical stresses. In a review article, Dr. BK Pederson wrote:

> "Physical exercise can be regarded as a prototype of physical stress. Many clinical physical stressors (e.g. surgery, trauma, burn, sepsis) induce a pattern of hormonal and immunological responses that have similarities to that of exercise (60)."

Clinical physical injury, similar to exercise injury, displays marked sex differences (4). For example, females have higher levels of mortality than males in response to burns of similar size (31). Females have a lower incidence of multiple organ dysfunction syndrome (MODS) and sepsis in response to shock compared to males (17). It is thought that the disparity in sex outcomes results from interactions of sex hormones with various aspects of the immune system. Since exercise induces similar immune responses, it may provide a useful model to study sex differences in immune response to clinical stressors. However, to understand this relationship, studies that control for menstrual phase, oral contraceptive (OC) use, and fitness levels between men and women are needed. The focus of this narrative review will be to discuss what is currently known about sex differences in non-specific immune responses to aerobic exercise. This review will discuss both animal and human studies that have examined the post exercise immune response.

Sex Difference in Immune Function in Non-Exercising Conditions

Several aspects of immunity have marked sex differences in non-exercising conditions. T cells, macrophages, and monocytes possess estrogen receptors (4) with two different subtypes, ERα and ERβ (61). ERα is mainly found in the uterus and mammary glands, while ERβ prevails in the central nervous, cardiovascular, and immune systems (32). Through these receptors, estrogen led to greater survival against herpes simplex virus 1 (HSV-1) in inoculated rats (9). In addition, in vitro stimulation of lymphocytes with phytohemagglutinin, a toxin used to elicit cytokine production from immune competent cells, found that females produce more Th2 (IL-4, IL-10) cytokines than males (29). Th2 cytokines are responsible for secretion of antibodies and this may play a role in the higher incidence of autoimmune diseases in women (85). Furthermore, females have a higher percentage of T lymphocytes within the total lymphocyte pool (5), and have more active circulating polymorphonuclear leukocytes (neutrophils) and macrophages (64, 65). Overall, physiologic levels of estrogen stimulate humoral and cell-mediated immune responses, but large increases in estrogen (either from pregnancy or supraphysiologic doses) can suppress cell-mediated immunity (54). Taken together, results imply that females of reproductive age have a more active immune system than age matched males. This could account for females having a lower incidence of, and mortality rates from, certain types of infection (bacteria septlcemai, pneumonia/influenza, bacterial meningitis) (28) and lower rates of atherosclerosis (79). Similarly, this could also explain the increased incidence of autoimmune diseases.

Sex Difference in Immune Response to Exercise: Inoculation Studies

Inoculating animals with viruses has previously been used as a model to study upper respiratory infections in animals by inducing illness (33). Inoculation purposefully infects the animal by transferring the causative agent into the animal. In this manner, whole body responses can be measured after inducing a specific illness. With this methodology, female mice experienced lower mortality after intranasal inoculation with herpes simplex virus 1 (HSV-1) at rest and after exercise than males. HSV-1 was delivered after the third bout of running to exhaustion or after 3 non-exercising control sessions. Though exercise resulted in greater morbidity (illness symptoms) than control, both sexes experienced the same degree of morbidity. Despite males and females having a similar rate of infection by HSV-1 after inoculation, fewer females died (9). Similarly, female mice that exercised at a moderate intensity had a greater macrophage resistance to HSV-1 than their male counterparts (8). However, both males and females experienced suppressed macrophage function after exhaustive exercise, and experienced this suppression to a similar degree. Thus, it is plausible that the decreased mortality after HSV-1 inoculation seen in female mice may be due to increased macrophage function. Since more females survived HSV-1 inoculation than males, the presence of estrogen could be an important determinant of this response. However, ovariectomized mice supplemented with estrogen experienced higher mortality than intact female mice after HSV-1 inoculation (7). Despite the better protection of intact mice, there was only a trend ($p=0.1$) toward intact females having greater macrophage resistance than the estrogen treated ovariectomized group. Therefore, the authors suggested that antiviral macrophage resistance is not responsible for the lower mortality (7). Since estrogen supplementation did not restore the protective effects of intact mice, other female hormones could be responsible for this added fortification of female mice. Taken together, animal research with HSV-1 inoculation demonstrates that male and female mice are equally susceptible to an infection at rest or after exhaustive exercise. However, more females survived. The greater macrophage activity may be responsible for this effect, but future studies should incorporate other immune parameters. The mechanism behind greater female survival with HSV-1 may be related to other ovarian hormones besides estrogen. It should be noted that the results from the experiments above were performed by a single research group and have yet to be replicated by others.

Sex Difference in the Cytokine Response to Exercise

The local response to a tissue injury involves the release of cytokines. Cytokines are released from the site of inflammation. The local response of cytokine release is supplemented by the release of cytokines from the liver, termed the acute phase response. The acute phase cytokines are TNF-α, IL-1β, and IL-6. These pro-inflammatory cytokines cause the movement of lymphocytes, neutrophils, and monocytes to the injured site. These leukocytes ultimately infiltrate the damaged muscle and serve to repair the tissue (2). Initially, exercise leads to increased release of pro-inflammatory cytokines (TNF-α, IL-1β,) and this is counteracted quickly by the release of cytokine inhibitors (IL-1ra, TNF receptors) and anti-inflammatory cytokines (IL-10), which limit the inflammatory response of exercise (60).

With chronic exercise and training, there is a decrease in cytokine production during an acute bout of exercise (69). Decreased cytokine release may contribute to immunosuppression and lead to a greater risk of bacteria and infection that is often evident in endurance-trained athletes (51). However, this decrease in inflammation could be a key link between exercise and health through a possible reduction in the risk of chronic disease.

Generally, cytokines are released after prolonged exercise or exercise that causes muscle damage (10, 60). The intensity and duration of exercise, along with fitness level, determines the cytokine profile (30). Interestingly, exercise does not cause an alteration in pro-inflammatory gene expression in peripheral blood mononucleated cells (PBMC) (81), suggesting that this is not a primary site for cytokine release. Recently, researchers demonstrated IL-6 is released from the exercising muscle (38, 67). IL-6 can increase 100 fold after exercise making it the most responsive cytokine to exercise and perhaps underscoring its biological significance. IL-6 has been shown to regulate metabolic factors such as glucose uptake and fatty acid oxidation (59). Recently, IL-6 released from the exercising muscle has been shown to have anti-inflammatory properties through its up-regulation of anti-inflammatory cytokines IL-1ra (56) and IL-10 (55), in addition to inhibiting TNF-α release (66). For a detailed review of IL-6 and exercise, see *Febbraio, 2005* (21).

Sex differences in the regulation of cytokines have been previously demonstrated in non-exercising conditions. After lymphocytes were stimulated with phytohemaglutinin, a toxin used to elicit cytokine production from immune competent cells, a greater Th1 profile, characterized by increased release of IFN-γ and IL-2, was shown in lymphocytes drawn from men compared to women. Women possessed a greater Th2 cytokine release (IL-4, IL-10) than men, but there were no differences across the menstrual cycle (29). Th2 cytokines are responsible for humoral mediated immunity and lead to increased secretion of antibodies. Similarly, IL-1 release from mononucleated cells is lower in males and is menstrual phase dependent in females (44). More specifically, the balance of the IL-1 family (IL-1-α, IL-β - agonist, IL-1ra - antagonist) is menstrual phase dependent. The ratio of agonist (IL-1-α, IL-β) to antagonist (IL-1ra) was equal during the follicular stage, but the agonist was ~45% higher in the luteal phase. Thus, the activity of IL-1α/β was greater in the luteal phase. IL-1β may influence reproductive functions like endometrial development and preparing the birth canal for parturition. IL-1β has also been shown to block luteinizing hormone and ovulation in rats (28). After trauma-hemorrhage injury, ovariectomized mice had decreased cytokine expression (IL-2, IL-3, and IFN-γ) from macrophages compared to ovariectomized mice treated with 17-β estradiol. The estradiol treated group maintained cytokine release after injury and this suggests that estrogen is capable of preventing immunosuppression that had been previously demonstrated with male mice and enhancing survival (41).

Currently, there are a handful of studies that have compared the cytokine response to exercise between sexes. There was no difference reported in serum IL-10, IL-1ra, IL-6, and IL-8 between men and women immediately and 1.5 hours after completing a marathon (50). The in-vitro production of IL-1, IFN-γ, and IL-4

from cultured whole blood showed no differences between sexes in response to continuous incremental cycling at 55%, 70%, and 85% VO_{2peak} (49). Similarly, 90 minutes of cycling at 65% VO_{2max} resulted in no difference in serum IL-6 levels between men and women (75). There was however, a trend ($p=0.06$) of increased IL-6 in women who took OC and those who were not taking OC and exercising in the follicular phase (75). The change in IL-6 values could be due to altered carbohydrate (CHO) oxidation rates. It was shown that whole body CHO oxidation during 50 min of cycling at 70-90% of lactate threshold is higher in the follicular phase (89). This higher rate of CHO oxidation could have lead to a greater depletion of CHO. In response to low CHO availability, IL-6 production will increase (38). In contrast, Edwards found that 60 minutes after a maximal cycling test, female IL-6 values were greater than men (18), although there were no differences between sexes at baseline, immediately, or 30 minutes post exercise. At 60 minutes post exercise, the male IL-6 values decreased towards baseline while the female values continued to rise. The exercise-induced IL-6 response is directly linked to the duration and intensity of exercise, along with the number of muscle fibers recruited (increased release) and the fitness level of subjects (decreased response) (57). Thus, methodological differences could account for the current disparity in the literature regarding IL-6.

At the transcriptional level, Northoff et al found a sex and menstrual phase difference in mRNA inflammatory gene expression in response to a 60 min run at 93% of the individual's anaerobic threshold (53). Women in the luteal phase demonstrated a greater condition of pro-inflammation than women in the follicular phase or men immediately after exercise. This pro-inflammatory state was characterized by an increase in inflammatory genes (interferon-γ, IL-12 receptor β1, and prostaglandin D2 receptor) and a decrease in anti-inflammatory genes (IL-6, IL1R2, IL1-ra) in PBMC. The authors state that the increase pro-inflammatory condition in the luteal phase could be a "mechanism designed to end a very early pregnancy in case of major external stress input. After all, human females get a new chance to conceive in the next month and nature may prefer to destabilize a pregnancy under influence of stress rather than carry it on under high risk." Furthermore, women in the luteal phase regulated over 200 genes (129 genes up-regulated, 143 genes down-regulated), while women in the follicular phase regulated 80 genes (48/32) and men regulated only 63 genes (34/29). Interestingly, post exercise IL-6 mRNA was down- regulated in the luteal phase, while up-regulated in the follicular phase after exercise. Future studies that control for menstrual cycle are needed to assess the expression of the specific proteins before any conclusions can be drawn.

Thus, in limited research on aerobic exercise, it appears the overall cytokine response to exercise is not markedly different between sexes. However, few studies controlled for either menstrual phase or oral contraception. Some work has demonstrated a greater up-regulation of inflammation (129 genes up-regulated, 143 genes down-regulated) in the luteal phase at the transcriptional level after exercise (53). Potential sex differences in IL-6 may exist after maximal exercise (18) and further research is needed to confirm the IL-6 response at longer time points after exercise while controlling for menstrual phase and oral contraceptive use.

Sex Differences in Leukocyte Response to Exercise

Moderate aerobic exercise results in a transient increase in both innate (monocytes, macrophages, neutrophils, NK cells) and specific (B and T lymphocytes) cells of the immune system. The effector cells of the innate immune system are monocytes, macrophages, neutrophils, and a subset of lymphocytes called natural killer (NK) cells. These cells represent the first line of defense against infections by neutralizing microbes or pathogens through phagocytosis (monocytes, macrophages, neutrophils) or by directly lysing the pathogen (NK cells). T cells recognize specific antigens presented to them to create memory cells, and B cells secrete antibodies to kill extracelluar pathogens. B cells are fundamental for eradicating bacterial infections. The number of total leukocytes, lymphocytes, granulocytes (neutrophils), and monocytes increase in a biphasic response (46). The immediate increase of leukocytes is characterized by increases in lymphocytes, monocytes, macrophages, and neutrophils, and is then followed by a delayed response of additional neutrophils 2 hours post exercise (46, 87).

Both the duration and intensity of exercise combine to determine the specific increase in leukocytes with exercise. Exercising for up to 30 minutes leads to increased lymphocytes (CD4+T cells, CD8+T cells, CD19+ B cells, CD16+ NK cells, CD56+ NK cells), which return to baseline values within 10-30 minutes after cessation of exercise (46). Longer duration exercise requires longer time periods for leukocytes to return to baseline. Specifically, CD8+ lymphocytes increase more with exercise than CD4+ cells (60). CD8+ lymphocytes can directly kill foreign or infected cells, whereas CD4+ are helper cells that mainly produce cytokines to magnify the immune response. Also, memory lymphocytes are recruited into the circulation more so than naïve lymphocytes (27). Memory cells are more likely than naïve cells to relocate to non-lymphoid tissues or possible locations of infection, like the vasculature of the skin, lung, liver, and gut.

The increases in epinephrine release and cardiac output associated with exercise are thought to contribute to the exercise-induced leukocytosis through de-margination from vascular pools and immune organs (24, 26, 80). The delayed increase in neutrophils may be mediated by an increase in Granulocyte colony-stimulating factor (G-CSF) more so than epinephrine or cardiac output (87). Epinephrine release in response to submaximal exercise has been shown to be sex dependent, with males demonstrating a greater release compared to mid-follicular females (11, 15, 34). However, an overall greater expression of β_2-adrenergic receptors on lymphocyte has been found in women compared to men (43, 84). The majority of previous research suggests there are no post exercise sex differences in leukocytes (1, 49), lymphocytes (1, 49), natural killer cells (6, 48) monocytes (1) or neutrophils (1). However, the above studies did not control for menstrual cycle phase, oral contraceptives, or matching male and female subjects for activity or fitness level.

In one of the few studies to examine immune cell changes that controlled for menstrual phase, oral contraception, and fitness, Timmons *et al* showed that women taking OC had a greater post exercise increase in lymphocytes and neutrophils compared to men and non-OC users after 90 min of cycling at 65% of VO_{2max}

(75). Women taking OC experienced cycle specific (follicular and luteal phases that corresponded to triphasic OC) exercise induced changes in total leukocytes, neutrophils, monocytes, and lymphocytes, whereas non OC users had no fluctuations across the menstrual cycle. The increase in immune cells after exercise were greater in OC users on days taking the pill, and these increases were always greater than the post-exercise changes seen in men. There were no differences in total leukocytes, neutrophils, and monocytes between men and regularly menstruating women not taking OC. However, non-OC users had a greater post exercise increase in lymphocytes than men. Taken together, this study demonstrated immune cell changes between men and women that are specific to OC use. There was a greater increase in immune cells after exercise in the high progesterone phase of women taking OC than men and non OC using women. Also, non OC using women had more lymphocytes circulating post exercise than men.

Since there were no changes in lymphocyte number across the menstrual cycle in non-OC users, sex hormones probably do not account for sex differences. While the authors corrected for exercise-induced changes in plasma volume, there was no mention of correcting for contraceptive induced changes in plasma volume. Previous research has found an increase in plasma volume in women taking OC (83). A difference in plasma volume between woman taking OC and those who did not could influence the results not only of the previous study, but also much of the preceding literature.

Thus, with moderate to intense aerobic exercise, the circulating leukocyte populations change dramatically. However, the majority of research suggests that there is no difference between sexes in the leukocyte response to aerobic exercise. Currently, Timmons *et al* is the only study to control for OC use, and the only study to show a difference between men, OC, and non OC users. Future research is warranted.

Sex Differences in Natural Killer Cell Response to Exercise

Natural Killer (NK) cells are a subset of lymphocytes produced in the bone marrow and are part of the innate immune system. NK cells kill virally infected cells or tumor cells through direct cytolytic mechanisms, without activation. NK cells account for 10-15% of circulating blood mononuclear cells. During exercise, NK cells are transiently increased by 186- 344% of initial resting value, following both maximal and sub-maximal bouts (63). NK cells are the most responsive leukocyte to exercise due to their catecholamine sensitivity (25). The magnitude of increase in NK cells is more responsive to the intensity than duration of exercise. Generally, NK cell number and activity will decline only in intense exercise lasting at least 1 hour (58). At rest, men have a higher NK cell activity despite no difference in NK cell numbers than regularly menstruating women or women using OC. Women using OC had the lowest NK cell activity (88). Furthermore, IL-1 release from monocytes, an activator of NK cell activity, has been shown to be both sex and menstrual phase dependent (44).

Previous research supports the notion that there are no sex differences in NK cell number or activity in response to incremental or continuous exercise (6, 48).

However, neither study controlled for menstrual phase or OC use. In contrast, adolescent girls not taking OC and tested in the mid-follicular phase had a greater increase in NK cell count than adolescent boys during (77) and after (78) cycling exercise for 60 min at 70% VO_2**.** Also, NK cell subset expression was significantly different between sexes (77). NK cells can be divided into 2 unique groups: $CD56^{dim}$, representing 90% of the circulating NK cells, and $CD56^{bright}$ cells that are more responsible for inflammation (13). The ratio of $CD56^{dim}$: $CD56^{bright}$ have been shown to play a role in reproduction as the concentration of NK cells in the uterine mucosa changes across the menstrual cycle and with pregnancy (40). For an in depth review of NK cell subset changes with exercise see *Timmons, 2008* (74). NK cell activity was not assessed in either study. Since results from Yovel 2001 (88) suggest there is both a sex and OC effect on NK cell activity at rest, future controlled studies are needed to quantify NK cell activity during and after exercise in an adult population.

Sex Differences in Neutrophil Response to Exercise

Neutrophils are a large subset of granulocytes, comprising ~90% of all granulocytes. Granulocytes are characterized by the granules in their cytoplasm and consist also of basophils and eosinophils. Neutrophils are members of the innate immune system. They are part of the acute inflammatory response and are the first cells recruited from the blood to the site of injury or infection (5). Neutrophils attack microbes that have entered the circulation by phagocytosing the microbe or releasing oxidative bursts to destroy the pathogen. Neutrophils also produce cytokines to recruit more neutrophils and other immune cells to the site of injury and enhance both specific and innate immunity. Granulocytes are higher in the luteal phase compared to the follicular phase (19) and have been shown to increase during pregnancy (82). There is evidence that with pregnancy there is a decrease in cell-mediated immunity (36). As a compensation mechanism, the pregnant women increase activity of the innate system, most notably granulocytes.

Acute exercise causes a mild inflammatory response to repair damaged tissue, which is characterized first by neutrophil infiltration, followed by macrophage infiltration several hours later (23). While the current data on sex differences in neutrophil infiltration after exercise are equivocal (45, 70, 71), generally females rats have a blunted post exercise inflammatory response that leads to less neutrophils infiltrating skeletal muscles and less muscle soreness (70, 72). From animal studies, it seems that estrogen is limiting neutrophil infiltration by acting as a cell membrane stabilizer and antioxidant. However, data from human studies are less compelling. For a review of sex differences in neutrophil infiltration *see Point – Counterpoint, Tiidus & Hubal 2009 (35, 73).*

Higher numbers of circulating neutrophils were observed both at rest and after 90 min of cycle ergometry in women taking OC compared to men and non-users. Furthermore, the greatest increase in neutrophils after exercise in OC users was seen in the luteal phase when estradiol levels were lowest (75). Since estrogen has been shown to inhibit the inflammatory response to exercise (70, 72), it makes sense that neutrophils would be highest when estrogen was lowest. Previously,

data from males suggested that increased IL-6 levels during exercise lead to increases in cortisol, which ultimately are responsible for exercise neutrophilia (68). However, data from sex comparison studies suggest there is no correlation between IL-6 levels and cortisol during exercise (18, 75). OC users had higher cortisol and neutrophil levels compared to men and non-users, but equivalent resting and post exercise levels of IL-6 (75). This could potentially highlight differences in regulation of anti-inflammatory mediators between men and women and future research should be conducted to understand this response.

Potential Mechanisms of Action for Sex Differences in Immune Response to Exercise Given the post-exercise sex differences in immune function, estrogen may be responsible for this disparity. However, results from a few well-controlled studies suggest other physiologic variables account for the sex discrepancies. The sex differences in IL-6 during maximal exercise could potentially be mediated by a difference in the amount of adipose tissue (42). Mohamed-Ali showed that adipose tissue released IL-6 (47). Furthermore, increases in catecholamines during exercise are related to IL-6 release from adipose tissue (39). Thus, the greater IL-6 response in women could be due to their greater fat content (18).

The disparity in post-exercise leukocyte and neutrophil responses between women who took OC, non-OC users, and men could be related to differences in growth hormone and cortisol levels. Both growth hormone (3) and cortisol levels (75) are higher in women taking OC. Furthermore, both growth hormone (37) and cortisol (14) have been shown to increase circulating neutrophil levels. However, in Timmons *et al* (75), cortisol levels did not differ between menstrual cycle phases, only between groups. Thus, cortisol alone could not be responsible for the increased post exercise immune cell response of the OC users. Exercise induced leukocytosis seen in both men and women appear to be associated with the increased circulating catecholamines (60). Thus, as noted by Timmons *et al*, the greater increase in lymphocytes in women during exercise may be due to their greater density of lymphocyte β_2-adrenergic receptors (84, 43). Furthermore, the number of β_2-adrenergic receptors on lymphocytes decreases over 10 wks of aerobic training (62). Thus differences in training also may be responsible for some of the sex differences reported in studies that did not control for fitness.

Intact female mice had lower mortality rates to post-exercise HSV-1 inoculation compared to males or ovariectomized females (7, 8). Yet, when estrogen was replaced after ovariectomy, ovariectomized females were still more susceptible than the intact group. Therefore, the authors concluded that physiologic doses of estrogen (1μg/day) are not responsible for the enhanced immunity seen in intact female animals. Further research is warranted to confirm this finding and to identify the cause for the greater immune response of the female animals. Similarly, 8 days of supplementing men with estradiol had no effect on resting or post exercise cortisol, IL-6, or neutrophil counts after 90 min of cycle ergometry at 60% of aerobic capacity (76). This study reinforces the suggestion that estrogen alone is not responsible for immune sex differences, and could potentially point to a difference in the expression of estrogen receptors (ER) on cells throughout the body. Both males and females have ERα and ERβ in skeletal muscle, with ERα mRNA

Table 1. Sex differences in immune function in studies that controlled for menstrual phase and OC use.

Author	N size	Exercise	Immune changes
Timmons, 2005	12 women (6 OC users, 6 NOC users), 12 men	90 min cycling, 65 % VO_{2max}	38% > lymphocyte increase post exercise in NOC women compared to men.
Northoff, 2008	9 women, 12 men	60 min treadmill run, 93% AT	>Pro-inflammatory gene expression in LP compared to men or FP.
Brown, 2004	89 female mice, 86 male mice.	3 consecutive days of treadmill running after HSV-1 inoculation until volitional fatigue.	>morbidity for males (28%) compared to females (16%).
Brown, 2006	36 female mice, 36 male mice	3 days of moderate (90 min) or exhaustive (volitional fatigue) treadmill running after HSV-1 inoculation.	>macrophage antiviral resistance in moderately exercised females compared to males.
Gonzalez, 1998	9 women	80 min walking, 32% VO_{2max} in cold (-5°C) environment.	41% decrease in IL1-β after exercise in LP compared to FP. No change in IL-6 or TNFα.
Timmons, 2006a	25 girls, 33 boys	60 min cycling, 70% VO_{2max}.	>Leukocyte count at 30 & 60 min post exercise in T5 boys compared to T4/5 girls. >NK cell response immediately post exercise in T4/5 girls compared to T3/4 boys.
Timmons, 2006b	11 girls, 11 boys.	60 min cycling, 70% VO_{2max}.	> Lymphocyte count in girls at 30 min (29%) and 60 min (23%) of exercise. $CD56^{dim}$ cells (105%) and $CD56^{dim}$ expressed as proportions (67%) greater in girls. $CD56^{bright}$ cell counts 82% greater in girls but not $CD56^{bright}$ proportions.
Ferrandez, 1999	60 female, 60 male mice	Swimming until exhaustion	>chemotaxis index in females compared to age matched male mice

OC – oral contraceptive user. NOC – non oral contraceptive user. LP – Luteal Phase. FP-Follicular Phase. T5 – Tanner stage 5. T4/5 – Tanner stage 4 and 5.

180 fold greater than ERβ (86). Females exhibit greater ERβ expression in the lungs than men (20), while ERβ mRNA is higher on adipocytes in women (16). Taken together, these data suggests a sex difference not only in ER quantities, but also a site-specific preferential expression of ER isotypes.

Future Research Considerations and Conclusions

When menstrual phase and oral contraceptives are controlled, males and females display marked differences in immune response to exercise (Table 1). Sex differences in immune cell changes, cytokine alterations, along with morbidity and mortality are apparent after submaximal and maximal aerobic exercise stressors. The primary mechanism for many of the sex differences does not appear to involve the presence of estrogen. Thus, future research should clarify which specific ovarian-related changes are responsible for these immune response differences and their specific actions. Future work should address the impact of site-specific ER isotypes on the post exercise immune response, as this may mediate sex differences. Also, while transcriptional evidence suggests a menstrual and sex-dependent effect on the cytokine response to running (53), there have been no studies that have examined serum cytokine responses in a similar, well controlled manner. Studies examining cytokines should carefully control intensity with regards to metabolic thresholds, as the exercising muscle may be a main source of serum cytokines. By using exercise to model the stress responses to certain clinical traumas, this avenue of research may provide valuable insight into new approaches and sex-specific treatments.

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