IMMUNE FUNCTION CHANGES IN DOWNHILL RUNNING SUBJECTS FOLLOWING ASCORBIC ACID SUPPLEMENTATION

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Purpose: To examine the effects of ascorbic acid (AA) supplementation on immune parameters in exercising individuals to assess the utility of pre-exercise / pre-event vitamin supplementation.
Methods: Fourteen physically active males who were 23 $\pm$ 2 years old (mean $\pm$ S.D.) with a body mass of 77.4 $\pm$ 8.3kg, height of 1.79 $\pm$ 0.1 m and a maximal oxygen uptake of 61.4 $\pm$ 5.3 $ml.kg^{-1}.min^{-1}$ participated in this investigation. Volunteers were divided into two matched groups and assigned to 9 days supplementation of either AA or placebo (PL) $800mg.d^{-1}$, in a double blind manner. On the seventh day individuals completed a 60 minute run on an incline (-10%) at a speed that elicited 70% VO_{2max} during level running. Antecubital venous samples were taken pre-supplementation, immediately pre-exercise, 1h, 24h and 48h post-exercise. Heparinised whole blood for phagocytosis and oxidative respiratory burst functional assays, EDTA for flow cytometric analysis (direct immunophenotyping and adhesion markers) and serum for cytokine assays was collected.
Results: The exercise induced inflammatory IL-6 response, monocyte respiratory burst and natural killer cell (NK)($CD3^{-}16^{+}56^{+}$) numbers were reduced in the AA group as compared to PL post-exercise. Monocyte and neutrophil adhesion markers were higher in the AA group, as were monocyte CD14 and monocyte counts (1h post-exercise). No difference was seen between groups with regard to CD45ro, CD4:8, lymphocyte CD18 and CD11b, monocyte and neutrophil phagocytosis, neutrophil respiratory burst and total and relative leucocyte subsets except as stated above.
Conclusions: These data indicate that AA supplementation confers anti-inflammatory immune benefits during exercise, as seen by reduced levels of IL-6. The increased levels of adhesion markers serve to facilitate and enhance the migration of leucocytes, particularly monocytes and NK cells through the vasculature post-exercise. This mode of exercise was not extreme enough to result in marked surface-marker changes or large changes in functional activity. In summary, AA supplementation could modulate the adverse immune effects of intense exercise.

EFFECTS OF RRR-alpha-TOCOPHEROL SUPPLEMENTATION ON THE EXPRESSION OF HSP72 AND HO-1 IN LEUKOCYTES AFTER EXHAUSTIVE TREADMILL RUNNING

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Recent research has shown that vigorous exercise is capable of inducing the expression of stress proteins (SP) in leukocytes (LE). With regard to the involvement of redox-sensitive regulatory mechanisms, we questioned whether RRR-alpha-tocopherol (TO, 500 IU daily over 8d) affects exercise-induced changes of heat shock protein 72 (HSP72) and heme oxygenase-1 (HO-1) in LE. Nine men were investigated in a double blind placebo controlled cross over study (washout period 28d). After the supplementation period the subjects performed an incremental treadmill test, followed by a continuous run (EX) until exhaustion at 110% of the individual anaerobic threshold (10.8±0.7 min, max. lactate 8.9±0.6 mmol/l). Expression of HSP72 and HO-1 was assessed on mRNA- and protein level using RT-PCR and flow cytometry, respectively. Plasma TO was determined in parallel and rose after supplementation with TO compared to placebo (69% vs. 3%). HSP72 mRNA rose 3h after EX, but the increase reach significance only in the placebo group (P). HO-1 mRNA was not affected by EX. Application of TO did not reveal significant effects of TO on both SP-mRNAs. Granulocyte HSP72-protein was elevated 3 and 48h after EX. It was lower in 8 of 9 TO-supplemented subjects 3h after EX. Cytoplasmic expression of HO-1 was increased in mono- (+24h and +48h) and granulocytes (+48h). Intraindividual differences exhibited slight, but not significant reducing effects of TO on HO-1 protein in monocytes (+48h). In conclusion, short-term exhaustive exercise augmented the expression of HSP72-mRNA in LE and induced a delayed response of HSP72 and HO-1 on protein level. However, these effects were less pronounced than after intensive endurance exercise. The slightly reducing effects of TO on exercise-induced expression of SP may suggest that reactive species are involved in exercise-related induction of SP synthesis in LE.

LYMPHOCYTE PROLIFERATION AND CYTOKINE PRODUCTION FOLLOWING A COMPETITIVE MARATHON RACE

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This randomized, double-blind, placebo-controlled study examined the influence of 6% carbohydrate ingestion and age on PHA-induced lymphocyte proliferation and in vitro cytokine production in 48 runners following a competitive marathon race. The marathoners were randomly assigned to carbohydrate (C) (N=23) and placebo (P) (N=25) groups, with blood samples taken before, immediately after, and 1.5h post-race. C versus P ingestion resulted in higher plasma glucose, lower plasma cortisol, and a reduced neutrophilia and monocytosis during recovery, but had no effect on the post-exercise reduction in T-lymphocytes or NK cells. No group differences were observed for PHA-induced lymphocyte proliferation or cytokine production. However, for all subjects combined, lymphocyte proliferation (unadjusted or adjusted per T-cell) and IFN- production (unadjusted or adjusted per T-cell) decreased significantly below pre-race values by 1.5h of recovery and these were negatively correlated with plasma cortisol at the same time point. Pre-race lymphocyte proliferation and IFN- production were 40% and 51% lower, respectively, in the 12 subjects 50 yrs compared to the 36 subjects <50 yrs of age, and this age-related depression in proliferation was observed at all post-race time points. In conclusion, PHA-induced lymphocyte proliferation and cytokine production decreased significantly following a competitive marathon race and this decrease was strongly linked to cortisol and only partially linked to T-cell changes. This decrease occurred in both younger and older marathon runners and was not influenced by carbohydrate.

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EFFECTS OF OAT BETA-GLUCAN ON SUSCEPTIBILITY TO INFECTION FOLLOWING BOTH MODERATE AND STRENUOUS EXERCISE

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Moderate exercise may decrease the risk of infection, whereas severe exercise appears to increase risk. Consumption of oat beta-glucan (Obg), a soluble fiber, is a mild immune system enhancer and may offset immune suppression associated with intense training and perhaps further enhance the benefits of moderate exercise. The purpose of this study was to test the effects of Obg consumption on susceptibility to infection following short-term exhaustive and moderate exercise training using our mouse model of respiratory infection (Davis et.al. J. Appl. Physiol. 83 (5): 1461-6 1997). Male CD-1 mice (n=192) were randomly assigned to one of six treatment groups. Moderate Exercise mice (ME-obg and ME-h2o) ran on a treadmill for 6 consecutive days for one hour at a speed of 36m/min at 8% grade. Fatiguing Exercise mice (FE-obg and FE-h2o) ran for 3 consecutive days to volitional fatigue at the same speed and grade (138min $\pm$43; X $\pm$SD). Control mice (C-obg and C-h2o) were exposed to the same environment of the treadmill room, but remained in their cages throughout the exercise period. Obg was consumed in the drinking water (~3.6mg/day) (ME-obg, FE-obg and C-obg) for 10 consecutive days prior to virus inoculation. Following rest or exercise on the last day of training mice were intra-nasally inoculated a standardized dose of herpes simplex virus-1 (HSV-1). They were monitored twice daily for signs of morbidity and mortality for a period of 21 days. Mortality was decreased by 13% ($P<0.05$) in C-obg versus C-h2o. Moderate Exercise decreased morbidity by 46% and mortality by 38% ($P<0.05$). Obg did not further enhance this beneficial effect. Alternatively, Fatiguing Exercise increased morbidity by 25% and mortality by 17% ($P<0.05$), and ingestion of Obg prevented this increase in morbidity and mortality. These data support the hypothesized effects of exercise on susceptibility to infection and suggests that daily ingestion of Obg may offset the increased risk of infection during heavy periods of training.

INFLUENCE OF PHYSICAL FITNESS ON IMMUNE FUNCTION IN CHILDREN

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The purpose of this study was to measure the relationship of aerobic power and body composition with immune function in children. Sixty-five children (N=36 males, N=29 females) ranging in age from 7 to 13 years (mean±SD age, 9.8±1.8 yrs) were recruited, with a special attempt made to include those ranging widely in aerobic fitness and body composition. All children were tested twice during a 2-month period for aerobic power, body composition, and immune function, with data from the 2 measures averaged and then correlated (alpha level, 0.01). Male and female children did not differ significantly in age, aerobic power, sum of two skinfolds, or any of the immune measures, so correlations were conducted for both gender groups combined. The mean sum of 2 skinfolds was 29.5±17.9 mm, and the average VO_{2max} was 45.2±8.3 ml/kg/min. Immune measures included leukocyte and lymphocyte subset counts, delayed-typed hypersensitivity (DTH) to 3 antigens (tetanus, mumps, and candin), global IgG antibody response over 4 weeks to pneumococcal vaccination (pIgG), salivary IgA concentration (sIgA), PHA-stimulated lymphocyte proliferation (PHA-SLP) (at 3 concentrations), natural killer cell activity (NKCA), granulocyte and monocyte phagocytosis and oxidative burst activity, and the number of sick days with upper respiratory tract infection (URTI) during 2 months. Aerobic power and the sum of 2 skinfolds were not significantly correlated with pIgG, sIgA, PHA-SLP, NKCA, or URTI. Aerobic power was negatively correlated with the total leukocyte count ($r=-0.34$, $p=0.008$) and monocyte phagocytosis ($r=-0.35$, $p=0.006$), with the sum of 2 skinfolds positively correlated with the total leukocyte count ($r=0.43$, $p<0.001$), granulocyte count ($r=0.40$, $p=0.001$), monocyte count ($r=0.40$, $p=0.001$), monocyte phagocytosis ($r=0.45$, $p<0.001$), and granulocyte phagocytosis ($r=0.38$, $p=0.002$). Data from this study indicate that obesity and low aerobic fitness are related to elevated leukocyte subset counts and higher monocyte and granulocyte phagocytosis in children, similar to what we have previously found in adults.

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EFFECT OF TIMING AND AMOUNT OF PRE-EXERCISE FEEDING OF CARBOHYDRATE ON BLOOD NEUTROPHIL RESPONSES TO TIME TRIAL CYCLING

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Purpose: It is now well established that ingestion of carbohydrate (CHO) during exercise attenuates some of the immune perturbations including a reduction in the magnitude of the neutrophilia that normally accompanies prolonged strenuous cycling. It is not known if pre-exercise feeding of CHO produces a similar effect. It is possible that any such effect of pre-exercise CHO may depend on the timing and the amount of CHO ingested. Hence, we investigated the effects of feeding 75 g of glucose at 15 and 75 min before exercise; and of feeding a placebo, 25g and 200g of glucose at 45 min before exercise on neutrophil trafficking and function during a cycling time trial.
Methods *Amount Study:* 10 healthy trained male cyclists aged: 27.2 ± 1.7 years, VO_2max: 62.2 ± 2.1 ml/kg/min (mean ± SEM) completed 3 experimental trials consisting of cycling at 65% VO_2max for 20 min followed by a time trial to complete a pre-determined amount of work (~40 min duration). Subjects consumed placebo, low CHO (25 g CHO/500 ml water) or high CHO (200 g CHO/500 ml water) solutions in a randomised design. *Timing Study:* 8 healthy trained male cyclists aged: 28.9 ± 3.2 years, VO_2max: 62.5 ± 3.5 ml/kg/min completed 2 experimental trials using the same exercise protocol as the previous study. Subjects consumed 75 g of CHO in 500 ml water at 15 or 75 min before exercise in a randomised and counter balanced design. In both studies blood samples were obtained at rest pre-CHO ingestion (overnight fasted), pre-exercise and at intervals post-exercise. Differential blood leukocyte counts and lipopolysaccharide-stimulated neutrophil degranulation (elastase release) were measured.
Results: Neither 5% or 40% (w/v) CHO solutions given 45 min pre-exercise had significant effects on neutrophil trafficking compared with placebo at any time point. However, a 15% CHO solution given 15 min pre-exercise resulted in significantly attenuated neutrophil trafficking (4.5 ± 0.7x10^9/l vs 6.5 ±

$0.9x10^9$/l; p<0.05) and a decreased neutrophil:lymphocyte ratio (3.4 ± 0.5 vs 5.9 ± 1.1; p<0.05) at 60 min post-exercise compared to CHO ingestion 75 min pre-exercise. Lipopolysaccharide-stimulated elastase release per neutrophil was unaffected by timing of pre-exercise CHO ingestion.
Conclusions: The timing of a pre-exercise feeding of 75 g glucose significantly affects the blood neutrophil response to exercise. However, a pre-exercise feeding of high or low CHO solutions 45 min before exercise, did not alter blood neutrophil trafficking responses compared with placebo ingestion.

EFFECTS OF MODERATE EXERCISE PROTOCOLS ON PRIMARY AND SECONDARY ANTIBODY RESPONSES IN MICE

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Purpose: Moderate exercise of eight weeks or longer enhances antibody response to a foreign substance. Since changes in hormonal levels occur after each exercise session, we hypothesized that a shorter exercise protocol of two weeks may enhance secondary antibody response. Furthermore, hormonal changes due to moderate exercise prior to the primary injection may have a cumulative effect on the secondary antibody response. The first purpose of this study was to examine the effectiveness of a two week versus eight week moderate exercise protocol in enhancing the secondary antibody response in mice. The second purpose was to examine the effects of moderate exercise prior to the primary injection on the secondary antibody response in mice.
Methods: Young (8-10 weeks), syngeneic, female, C57BL/6 mice were randomly assigned to exercise and sedentary intervention protocols. Mice were immunized with human serum albumin (HSA) to elicit a primary and secondary antibody response. Serum anti-HSA antibody levels were measured in μg/ml using Enzyme-Linked Immunosorbent Assay (ELISA) at day 21 following initial immunization for primary antibody response and at days 10 and 20 following booster immunization for secondary antibody response. Moderate exercise protocol consisted of running the mice on a motorized treadmill at 16 meters/minute for 30 minutes, 5 days per week.
Results: The secondary antibody response at day 10 and 20, following booster immunization, was comparable in mice exercising for two or eight weeks (p> 0.05). Moderate exercise prior to primary injection significantly enhanced secondary antibody response at day 20 when compared to the sedentary group (p=0.02).
Conclusions: A moderate exercise protocol between two and eight weeks may be sufficient to improve secondary antibody production. While we confirmed previous studies showing no effect of moderate exercise training on primary antibody response, moderate exercise prior to the primary injection enhanced secondary antibody response. A shorter moderate exercise protocol may improve compliance and may be used as a strategy to enhance antibody response to vaccinations.

PHYSICAL STRESS INDUCES DIFFERENTIAL ALTERATION IN THE DISTRIBUTION OF MURINE TH1 AND TH2 CELLS IN DIFFERENT COMPARTMENT

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Purpose: Modulation of immune response during or high intensity exercise is known to resemble that induced by physical stress such as restraint or noxious stress. A striking feature of the modulation of immune response, apparently suppressive, however, is in the change in the distribution of immune competent cells. Whether the change in the distribution of the cells is uniform among different cell types and different compartment has not yet been investigated in details as such to assume the biological consequence. By employing a physical stress model of noxious stimuli, the effect of repeated short-term stress on the immune regulatory Th1 and Th2 cells was studied focusing on their distribution and function.
Methods: Male C3H/HeN mice were exposed to 0.5 s foot shock (FS) every 5 s for 30 min a day for five days. Immediately after final FS exposure the FS mice were sacrificed as well as the control mice without FS. Mononuclear cells were obtained from spleen, lymph nodes, and peripheral blood. Isolated

cells were treated with PMA+Ionomycin with Brefeldin A and analyzed for IFN-□ and IL-4 producing cells among CD3+ T cells by FACS.
Results: Th1/Th2 balance tended to be reduced both in peripheral blood and spleen of FS mice with statistical significance detected only in the peripheral blood. The proportion of IFN-□ producing CD3+ T cells significantly decreased in spleen of FS mice, but not in lymph nodes and peripheral blood. The proportion of Th2 cells tended to increase without statistical significance in peripheral blood of FS, but no change was observed in the other compartments.
Conclusion: Our result suggests that physical stress induced alteration in the distribution of regulatory T cell is not uniform among different cell types, namely Th1 and Th2 and also among different compartments. It is possible that the direction of immuno-modulation under stress status may vary among different types of immune response, which largely depend upon the location of cellular components.

EFFECTS OF REPEATED PHYSICAL STRESS ON THE CYTOKINE PRODUCTION OF MURINE INTESTINAL INTRAEPITHELIAL LYMPHOCYTES

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Purpose: Immuno-modulation during or after heavy intensity exercise resembles that under physical stress such as restraint or noxious stress. Intestinal intraepithelial lymphocytes (IEL) are located at the basolateral surfaces of intestinal epithelial cells and are thought to play important roles in the first line of host defense against invasion of microbial pathogens in intestine. Cytokines secreted by IEL are assumed to play a critical role in regulation of the gut associated immune responses, but the effect of stress on the production of cytokines by IEL has not been investigated so far. Thus, we examined the alteration of cytokine production by IEL and its subsets after repeated noxious stress. Furthermore, we determined if glucocorticoid or catecholamine might mediate the stress-induced alteration of the cytokine profile of IEL.
Methods: Male C3H/HeN mice were exposed to 0.5 s foot shock every 5 s for 30 min a day for five days. Immediately after the final shock exposure, IEL were isolated by Percoll density gradient. Purified IEL was stimulated either with immobilized anti-CD3 mAb, or PMA+Ionomycin. IFN-γ and IL-4 producing IEL were analyzed by flow cytometer and the produced cytokines were evaluated by ELISA.
Results: IFN-γ production but not IL-4 production after 48 h culture of IEL stimulated with either PMA+Ionomycin or anti-CD3 mAb was suppressed by foot shock stress. While there were no change in the number and the proportion of each $CD3^+$IEL subsets ($CD4^+CD8^+$, $CD4^+CD8^-$, $CD4^-CD8^+$, $CD4^-CD8^-$, $\alpha\beta TCR^+$ and $\gamma\delta TCR^+$) after repeated FS, the proportion of IFN-γ producing $CD3^+$IEL significantly decreased. The reduction in the proportion of IFN-γ producing cells among $\alpha\beta TCR^+$IEL but not $\gamma\delta TCR^+$IEL may account for the decreased overall production of IFN-γ Both dexamethasone and corticosterone suppressed IFN-γ production of IEL stimulated with immobilized anti-CD3 mAb *in vitro*, and the suppression was blocked by glucocorticoid receptor antagonist RU486. High molar concentrations (10^{-7} M, 10^{-6} M) of NE and EPI could also suppress IFN-γ production of IEL stimulated with immobilized anti-CD3 mAb, and the suppression was blocked by nadolol (α-Adrenergic receptor antagonist) but not phentolamine (α-Adrenergic receptor antagonist).
Conclusions: Physical stress suppresses IFN-γ production of IEL, particularly of $\alpha\beta TCR^+$IEL, possibly by endogenous glucocorticoid. Repeated bout of stress might affect the production of IFN-γ which could facilitate certain bacterial infection.

ABSOLUTE VERSES RELATIVE WORK OUTPUT AND LEUKOCYTE TRAFFICKING

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Purpose: The aim of this investigation was to determine whether the magnitude of leukocyte redistribution increased as absolute, but not relative, work output increased. **Methods:** Previously untrained women underwent six months of resistance training of both upper and lower body (TOTAL,

n=32) or just the upper body (UPPER, n=28). Blood was collected before and immediately following a 6 by 10 repetition maximum (RM) squat resistance exercise before training began (Sep/Oct) and after 3 (Nov/Dec) and 6 (Apr/May) months of training. The leukocyte differential was determined using an automated hematology analyzer; lymphocyte subsets using fluorescently labeled monoclonal antibodies and flow cytometry; serum lactate using a Sport L-Lactate Analyzer; and serum cortisol by radioimmunoassay. **Results:** Squat 1-RM increased (group x time $P<0.001$) to a greater degree in the TOTAL group (+22.4% and +36.4% TOTAL compared to +5.8% and +11.1% UPPER at 3 and 6 months, respectively). Thus, the TOTAL group did a greater volume of absolute work over time during the squat exercise. Post-exercise lactate concentrations increased (group x time $P=0.02$) slightly over time for the TOTAL group, but not for the UPPER group. Both groups had increases in the concentration of cortisol, granulocytes, monocytes, lymphocytes, T, NK, CD8+ and CDRA+ lymphocytes of similar magnitude following each exercise test. For both groups, the pre- to post-exercise increase in concentration of CD3+CD4+ (time $P=0.01$), CD3-CD8+ (time $P<0.001$), CDRO+ (time $P=0.01$), and B (time $P=0.02$) lymphocytes were greater after 3 months of training than at the other time points. **Conclusions:** The magnitude of the redistribution of leukocytes to the circulation was independent of increases in the absolute work performed. However, the pool of cells entering the circulation after 3 months of training was greater for CD4+ T cells, B cells, memory lymphocytes, and CD3-CD8+ lymphocytes (likely to be CD8+ NK cells). Because it was detected both in women who trained their legs and in those who did not, this lymphocyte subset-specific response enhancement after 3 months of training was likely to have been either 1) the result of seasonal changes in immunity, or 2) a temporary (not present at 6 months) systemic adaptation to resistance training.

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INGESTION OF A DIETARY SUPPLEMENT CONTAINING ANDROSTENDIONE AND DEHYDOREPIANDROSTERONE (DHEA) HAS A MINIMAL EFFECT ON IMMUNE RESPONSE

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Dehydroepiandrosterone (DHEA) levels decrease with age and it has been suggested that this age-related decline may result in immunosenescence. Treatment of aged mice with DHEA or androstenedione has been shown to reverse the age-associated decrease in resistance to infection. The purpose of this study was to investigate the effects of 4 weeks of dietary supplementation with a supplement containing DHEA and androstenedione, on lymphocyte function and cytokine production in middle aged men (50-60y). In this double-blind placebo controlled study, subjects consumed either an oral placebo or an oral dose of the dietary supplement for 4 weeks. The supplement contained a total daily dose of 150 mg DHEA, 300 mg androstenedione, 750 mg tribulus terrestris, 625 mg chrysin, 300 mg indole-3-carbinol, and 540 mg saw palmetto. Blood samples were taken prior to supplementation, and once each week during supplementation. In addition, blood samples from middle-aged men were collected to assess the *in vitro* effects of DHEA and androstenedione on immune function. Peripheral blood mononuclear cells were isolated and cultured with the mitogens PHA, ConA and LPS to assess lymphocyte proliferation and cytokine production. The cytokines IL-2, IL-4, IFN-gamma, and IL-10 were assessed in supernatants collected from cells stimulated with PHA and ConA, whereas IL-12 and IL-1beta were measured in supernatants obtained from cells cultured with LPS. Levels of androstenedione, total and free testosterone, dihydrotestosterone (DHT) and estradiol were measured in serum samples. The supplement significantly increased serum levels of androstenedione, free testosterone, estradiol, DHT, but had no effect on total testosterone. The increase in serum levels of these steroids was observed from week 1 through week 4 of supplementation. An increase in PHA-induced lymphocyte proliferation was found in subjects consuming the supplement only at week 1. The supplement did not effect any of the other immune parameters measured (LPS or ConA proliferation, IL-1beta, IL-2, IL-4, IL-10, IL-12, IFN-gamma) at any other time point throughout the study. The addition of the same supplement, or DHEA, or androstenedione alone to lymphocyte cultures in vitro also did not increase lymphocyte proliferation. Our findings, in contrast to the studies with mice, suggest that dietary supplementation with DHEA and androstenedione does not enhance immune function in middle aged men. The dose of DHEA and androstenedione ingested by subjects consuming the dietary supplement was of a sufficient magnitude to increase serum levels of several steroid hormones. However, in spite of the physiological increase in these hormone levels, immune function was not altered, with the exception of PHA-induced proliferation at only one time point. Taken together, these findings do not support the

suggestion that DHEA/androstenedione supplementation reverses some phenomenon typical of immunosenescence.

COULD AGE-RELATED ALTERATIONS OF HEMATOPOIETIC STEM CELLS AND IMMUNE FUNCTION BE CORRECTED BY LONG-TERM EXERCISE AND ANTIOXIDANTS THERAPY?

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Purpose : Alterations in the properties of hematopoietic stem cells will ultimately affect the lymphocytes of the immune system during aging process. Such kinetic limitations on the stem cell reserve may be in part account for the clinical observations in elderly patients with immune dysregulation. The purpose of this study is to determine the possible immunomodulatory function of exercise and antioxidants both in peripheral lymphocytes and bone marrow stem cells.
Methods : We used 6 to 8 weeks-old C57BL/6 mice and divided into 4 experimental groups ; Control group(G1) did not exercise and fed generally, Exercise group(G2) did exercise 5 times per week for 1 hour with swimming, Antioxidants group(G3) fed Vitamin E, C and β-carotene every day and Combined group (G4) did exercise with swimming as G2 and fed antioxidants as G3 during the whole experimental period (about 15 months). All animals were subjected to aging in a clean air room under the preset condition for 15 months. We sacrificed animals and evaluated their peripheral immune function such as mitogen-induced proliferation response and NK cell activity. And also bone marrow (BM) stem cell function were evaluated using BM transplantation method after sorting the stem cells from each group. Additionally naïve and memory type of lymphocyte subsets were measured in each experimental group. All data were compared with young control animals'.
Results : There were significances between exercise groups(G2,G4) and non exercise group(G1,G3) in the NK cell activity as well as in ConA-stimulated proliferation test. G3 and G4 showed better T cell production after BM stem cell transplantation. Both exercise and antioxidants treatment reduced the rate of shift from naïve to memory type of lymphocytes.
Conclusion : Long-term moderate exercise and antioxidants treatment could reduce the age-related decline and alteration of immune function through keeping the better BM stem cell reserve pool.

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DECLINE IN ENDURANCE EXERCISE INDUCED GLUTATHIONE ALTERATIONS DURING THE 1ST SEMESTER IN SPORT STUDENTS

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Purpose: After strenuous exercise, inducing a high oxidative stress, blood levels of reduced glutathione (GSH) are usually reduced and levels of oxidized glutathione (GSSG) are usually increased. It is not clear, how glutathione system adapts in sport students during the 1st semester at a sport university, and whether female students adapt similar compared to male student.
Methods: 34 sport students (16 female (f) and 18 male (m), age 19 – 25 yrs) of the first semester took part in an incremental run field test (IRT) and a 2 hr endurance run (ER), four times per semester (U1234), every 3rd week. ER was started 24 hrs after IRT. Run velocity in ER remained constant in each run, corresponding to 60-70 % of the individual 4 mmol/l lactate threshold (v4) in U1. Venous blood samples were taken prior to and immediately after ER. GSH and GSSG were determined by HPLC.
Results: V4 increased significantly from U1 to U4 (m: 3.93 ± 0.39 vs. 4.13 ± 0.34 m/s; f: 3.24 ± 0.49 vs. 3.40 ± 0.39 m/s; $p<0.01$ respectively). GSH, GSSG and GSH/GSSG in U1 and U4 before and after ER were: GSH: U1: m: 2.17 ± 0.47 vs 1.59 ± 0.44 mmol/l; f: 1.72 ± 0.54 vs. 1.26 ± 0.45 mmol/l; U4: m: 1.84 ± 0.51 vs. 1.50 ± 0.41 mmol/l; f: 1.40 ± 0.40 vs. 1.23 ± 0.40 mmol/l; GSSG: U1: m: 1.69 ± 0.24 vs. 1.82 ± 0.26 mmol/l; f: 1.50 ± 0.43 vs. 1.84 ± 0.45 mmol/l; U4: m: 1.12 ± 0.31 vs. 1.52 ± 0.52 mmol/l; f: 0.94 ± 0.23 vs. 1.19 ± 0.29 mmol/l; ratio GSH/GSSG: U1: m: 1.30 ± 0.33 vs. 0.87 ± 0.20 mmol/l; f: 1.19 ± 0.32 vs. 0.68 ± 0.20 mmol/l; U4: m: 1.70 ± 0.48 vs. 1.01 ± 0.28 mmol/l; f: 1.49 ± 0.42 vs. 1.02 ± 0.40

mmol/l. Exercise-induced changes were significant for GSH, GSSG and GSH/GSSG ($p<0.01$). GSH but not GSSG or the ratio were lower in f vs. m ($p<0.01$). There were no significant changes in GSH, GSSG, and in GSH/GSSG in U4 vs. U1.
Conclusions: A 2 hr run induces dramatic changes in blood glutathione system, independent of gender, indicating activation of antioxidant defense mechanisms. Female athletes have different resting GSH values compared to males. Activation of glutathione system remains constant, when absolute exercise intensity remains the same, even when endurance capacity increases.

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SUPPLEMENTATION OF BRANCHED CHAIN AMINO ACIDS (BCAA) IN MARATHON RUNNERS FOR ONE MONTH PRIOR TO COMPETITION

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This study investigated the effect of four weeks prior supplementation of BCAA or placebo on some aspects of immune function and central fatigue before, immediately after, and during recovery from acute exercise stress. Ninety-seven marathon runners (81M; 16F) were randomly allocated to consume either BCAA (0.1g/kg body mass) (n=49) or a placebo (n=48) daily for one month prior to the marathon. They were asked to complete a daily training log incorporating modified profile of mood states (POMS), and to report symptoms of illness, during the supplementation period and for one week after the race. Ethical permission was obtained. Blood samples were taken immediately after the race (T0), and one hour later (T1) to measure plasma concentrations of glutamine and BCAA (p[Gln], p[BCAA]), lymphocyte proliferation and neutrophil activity, IL-2 and IL-8, whole-blood counts and lymphocyte and neutrophil differentials. BCAA are precursors for glutamine, which is important for some cells of the immune system. Our laboratory has reported a marked decrease in self-reported illness in >70 marathon runners receiving glutamine compared with a similar number receiving placebo. Similar observations have been made in triathletes receiving BCAA supplementation (Bassitt et al. 1999). Circulating numbers of neutrophils and a decrease in IL-8 were also observed to return to normal more rapidly after a race in those taking glutamine vs. placebo (Castell & Newsholme, 1998). In the present study, compared with the placebo group, the BCAA group had an 18% lower incidence of self-reported illness. A non-significant trend towards a more positive mood state was observed in the BCAA group. At T1 there was a higher p[BCAA] ($p<0.01$) in the BCAA group, but no difference in p[Gln]. IL-8 production was lower at T1 ($p<0.01$) in the BCAA group. In both groups neutrophil activity decreased ($p<0.01$) after the first hour of recovery, possibly due to a higher percentage of immature neutrophils in circulation. The provision of moderate, regular doses of BCAA to marathon runners over several weeks prior to a race, appears to have beneficial effects upon some aspects of the immune system but, despite some trends, there was no effect on mood states overall.
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PHYSICAL ACTIVITY, IMMUNITY AND ESTROGEN

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Purpose: There are many factors, including gender and stress related hormones released during exercise, which are known to influence natural immunity. A major component of natural immunity is the natural killer (NK) cell which are known to be sensitive to physical activity and possess the ability to spontaneously kill a variety of cell types, *i.e.,* tumour cells and virus-infected cells. We have previously shown that *in vivo* cytotoxicity is strongly enhanced in male rats after 5 weeks of voluntary exercise. The purpose of this study was threefold, (1) to assess the effects of exercise and estrogen on *in vivo* cytotoxicity in natural immunity (2) to measure if exercise has the same effects of *in vivo*

cytotoxicity in females compared to males and (3) to determine if estrogen influences the amount of physical activity.
Methods: Female spontaneously hypertensive rats (SHR) were divided into 5 groups: sedentary (n=7), sedentary ovariectomised (OVX) (n=7), runners (n=7), runners OVX (n=5) and runners OVX with implanted estrogen capsule (n=8) with runners exercising for 5 weeks in a voluntary wheel running model. *In vivo* cytotoxicity was measured as the clearance of injected Cr^{51}-marked YAC-1 cells from the lungs.
Results: The preliminary results show that both chronic voluntary exercise and estrogen augments natural cytotoxicity *in vivo*. The effect of exercise on clearance of radiolabelled Cr^{51} YAC-1 cells is the same for males and females. Animals with endogenous estrogen production or implanted estrogen capsules ran 16.2 $\pm$ 1.0 km/24h compared to the animals without endogenous or artificial estrogen which ran 5.9 $\pm$ 1.3 km/24h.
Conclusions: These results suggest that chronic voluntary exercise in female rats as well as estrogen augments natural immunity *in vivo.* The present results also suggest that estrogen can strongly influence activity levels in females and further studies are needed to evaluate the clinical relevance of these results.

This study was supported by grants from the Swedish National Centre for Research in Sports (CIF no. 39/00).

CHANGES IN IL-1 ALPHA AND IFN-GAMMMA DURING INCREMENTAL EXERCISE.

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Purpose: A newly developed and highly sensitive assay was used to determine changes in plasma levels of interleukin-1 alpha (IL-1α) and interferon-gamma (IFN-γ) during incremental exercise.
Methods: Five healthy male subjects (19.8±1.3 yrs, VO_2max = 60.3±6.3 ml•kg^{-1}•min^{-1}) were exercised incrementally on a cycle ergometer for 5 min at 50, 90, 120 and 140% ventilatory threshold (VT)-V_{O2}. Blood samples were collected at rest, immediately after the warm-up exercise and at the end of each of the 5-min exercise stage. Plasma cytokine concentrations were determined by a sandwich-type time-resolved fluoroimuunoassay used a europium chelate (J. Yuan et al., *Anal. Chem*., 1998, 70, 596-601). Results were analyzed using one-factor ANOVA ($p<0.05$), applying Bonferroni correction for multiple comparisons.
Results: Plasma IL-1α concentration increased significantly at 50%VT then returned to the resting value at higher intensities of effort. Plasma IFN-γ remained unchanged throughout incremental exercise.
Discussion: Most previous authors have found no exercise-induced change in IL-1α because of limitations of assay sensitivity. Our results showed an increase of IL-1α but only at the first stage of incremental exercise. It seems that IL-1α may have other physiological functions than mediating inflammation during exercise.

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EXERCISE-INDUCED HUMORAL MODULATION OF PHAGOCYTES BY CYTOKINES AND ANTIOXIDANTS

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Purpose: Endurance exercise causes mobilization and priming of circulating neutrophils and monocytes. We examined whether the increases in circulating cytokines and antioxidants may induce functional modulation of neutrophils and monocytes using in vitro experiments, which can differentiate priming, down-regulation or antioxidant activities of plasma. **Methods:** Blood and urine samples were obtained from 13 healthy male subjects before and after a competitive 42.195-km marathon race. Plasma and urine concentrations of pro- and anti-inflammatory cytokines (IL-1-beta, TNF-alpha, IL-6 and IL-10), chemokines (IL-8, MCP-1 and RANTES), colony-stimulating factors (G-CSF, GM-CSF and M-CSF) and neutrophil activation marker (MPO and lactoferin) were measured by ELISA. Plasma antioxidant levels (albumin, uric acid, transferrin, ceruloplasmin, vitamin B2, C and E, and beta-carotene) were also investigated. In vitro experiments using plasma samples were performed by application of luminol-dependent chemiluminescence responses of standard neutrophils and monocytes.
Results: MPO and lactoferin rose significantly after the race, especially in urine. Plasma concentrations of IL-6, IL-8, IL-10, G-CSF, M-CSF and MCP-1 rose significantly and urine concentrations of IL-1-beta, G-CSF, M-CSF, MCP-1 were increased significantly after the race. However, in vitro experiments of the capacity of plasma on neutrophil and monocyte chemiluminescence responses revealed that there were no priming and down-regulation capacities in plasma but antioxidant activities were observed. Albumin, uric acid and vitamin C rose significantly after the race, but these changes were minor.
Conclusions: In vivo priming phenomena were not reproduced in vitro, suggesting that neutrophils and monocytes with higher priming potentials might be mobilized following exercise. However, even if phagocyte activation occurs, humoral antioxidant activity induced during exercise might prevent oxidative tissue damage. Contributions of other free radical scavengers are under investigation.

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PLASMA INTERLEUKIN-6 DURING STRENUOUS EXERCISE; ROLE OF ADRENALINE

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Purpose: Plasma interleukin (IL)-6 is increased during and after prolonged exercise. The aim of this study was to investigate a possible relationship between the increase in plasma adrenaline seen during exercise and the increase in plasma IL-6.
Methods: This was studied in seven healthy males, who performed one exercise trial and one adrenaline-infusion trial. The exercise consisted of treadmill running at 75% VO2 max for 2.5 hours (h). The infusion trial consisted of 2.5 h of adrenaline infusion calculated to reach the same plasma adrenaline levels seen during the exercise trial.
Results: Peak plasma IL-6 increased 6-fold during adrenaline infusion compared to 29-fold during exercise. The lymphocyte concentration increased to the same extent during exercise and adrenaline infusion. In the post-exercise period the lymphocyte count decreased more than following adrenaline infusion. The neutrophil concentration was elevated 3-fold in response to exercise, whereas only a minor increase was found in response to adrenaline infusion.
Conclusion: In conclusion, exercise-induced increase in plasma IL-6 could not be mimicked by adrenaline infusion. However, adrenaline may partly be responsible for the exercise-induced changes in lymphocyte number, but does not contribute to exercise-induced neutrophilia.

EFFECTS OF GLUTAMINE SUPPLEMENTATION ON EXERCISE PERFORMANCE AND VENOUS PLASMA GLUTAMINE CONCENTRATIONS

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Purpose: Glutamine is the most abundant of all amino acids in plasma and skeletal muscle and accounts for approximately 61% of the total intracellular free amino acid pool. Glutamine is generally believed to be important, if not essential to immunologic function and is utilised as a respiratory fuel for lymphocytes, macrophages and other rapidly dividing cells. However, few studies have assessed the

effects and implications of glutamine supplementation and performance. Therefore, in this randomised cross-over placebo controlled glutamine study, the effects of glutamine supplementation on performance was investigated.
Methods: Six male subjects from the University of Teesside, England participated in the study. Each subject underwent a control (placebo) and glutamine supplementation. Exercise was set at 75% of individuals maximal oxygen uptake (VO_2max) and lasted for 60 minutes. Performance variables measured were heart rate, percentage VO_2max, lactate, glucose and rate of perceived exertion (R.P.E.). Venous plasma samples were collected immediately preexercise, immediately postexercise, 30 minutes post exercise, one hour post exercise and three hours postexercise. In the glutamine trial, 100 $mg.kg^{-1}$ were ingested in carbohydrate free orange juice.
Results: Glutamine supplementation maintained plasma glutamine concentrations above preexercise levels at all time points up to three hours postexercise ($p<0.05$) at immediately postexercise, 30 minutes postexercise and one hour postexercise. In the placebo trial, glutamine concentrations did not decrease significantly ($p>0.05$) from preexercise levels, however, there was a general trend for decrease lasting to three hours postexercise. There were no significant differences in performance variables ($p>0.05$) between conditions and also within trials ($p>0.05$). However, there was a tendency for a decrease in glucose in the glutamine trial and a tendency for increases in heart rate, percentage VO_2max, lactate and R.P.E.
Conclusions: Venous plasma glutamine concentrations can be maintained during exercise and up to three hours postexercise allowing large amounts of glutamine available for rapidly proliferating immune cells. Exercise performance is not improved and there is a general tendency for a decrease in performance via increased heart rate, percentage VO_2max, lactate and R.P.E with glutamine supplementation. There is also a possible glucose ”sparing effect” with glutamine supplementation. Ingestion of glutamine during exercise allows significantly increased availability of glutamine postexercise, which may be utilised by immune cells. Performance may also be decreased when ingesting glutamine during exercise.

EFFECT OF CARBOHYDRATE INGESTION ON PLASMA CYTOKINE AND NEUTROPHIL DEGRANULATION RESPONSES TO INTERMITTENT HIGH INTENSITY EXERCISE

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Purpose: Previous work (Bishop *et al*., 1999, *J. Sports Sci*. 17: 787-796) reported that in contrast to findings from studies of prolonged, continuous exercise, ingesting carbohydrate (CHO) during intermittent exercise had negligible effect on immune responses. It appeared that this was due to the overall exercise intensity rather than its intermittent nature. To address this, we examined the effects of CHO ingestion on plasma interleukin-6 (IL-6) and lipopolysaccharide (LPS)-stimulated neutrophil degranulation responses to high-intensity intermittent running.
Methods: Following an overnight fast, 6 healthy, trained male soccer players (mean ± SEM: VO_2max: 50.6 ± 0.4 ml/kg/min) performed two exercise trials, 7 days apart, in a randomised counter-balanced design. On each occasion they completed six 15-min periods of intermittent running consisting of maximal sprinting interspersed with less intense periods of running and walking. During each trial, subjects consumed either a 6.4% (w/v) CHO solution (CHO trial) or a non-carbohydrate placebo (PLA trial) immediately before exercise (5 ml/kg body mass) and every 15 min of exercise thereafter (2 ml/kg body mass). Venous blood samples were obtained pre- and post-exercise, and at 30 min post-exercise.
Results: Mean %VO_2max during the exercise trials was 80.6 ± 0.4%. At 30 min post-exercise, plasma glucose concentration was lower (PLA: 4.1 ± 0.2 mM; CHO: 5.4 ± 0.2 mM; $p<0.01$) and plasma cortisol concentration higher (PLA: 891± 107 nM; CHO: 559± 40 nM; $p<0.02$) on the PLA trial than on the CHO trial. At this time, blood neutrophil counts were markedly higher on the PLA trial compared with the CHO trial (PLA: 13.2 ± 1.8 x 10^9/l; CHO: 7.6 ± 1.3 x 10^9/l; $p<0.05$). Compared with pre-exercise values, post-exercise LPS-stimulated elastase release per-neutrophil fell by 17% on the CHO trial ($p=0.3$) and by 31% on the PLA trial ($p=0.07$). Plasma IL-6 concentration increased significantly on both trials following the exercise ($p<0.01$). At 30 min post-exercise values were significantly greater on the PLA trial than the CHO trial (PLA: 5.5 ± 0.9 pg/ml; CHO: 3.7 ± 0.6 pg/ml, $p<0.02$).

Conclusions: These data suggest that CHO ingestion can influence neutrophil degranulation and IL-6 responses to high-intensity intermittent exercise.

BCAA SUPPLEMENTATION AND THE IMMUNE RESPONSE OF LONG-DISTANCE ATHLETES.

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Purpose: Among different types of exercise, intense long-duration exercise has been associated with immunosuppression. The mechanisms involved, however, are not fully determined, and seem to be multifactorial, including endocrine changes and alteration of plasma glutamine concentration. We have, therefore, presently evaluated the effect of BCAA supplementation upon the immune response of long-distance runners.
Methods: 36 male athletes (24, triathletes and 12 marathoners) were divided in placebo (GP) and supplemented (GS) groups, the last one received 6g of BCAA daily, 15 days prior to a triathlon or a 30km run (2h run). Lymphocyte proliferation, IL-1, 2 and 4, alpha-TNF and gamma-INF production were evaluated, before and after the exercise bout.
Results: BCAA supplementation was effective into keeping plasma glutamine concentration constant after exercise (GP – 29%; GS equal), as well as the proliferative response of lymphocytes to concanavalina A and LPS, reduced in 40% in the GP, and normalized in GS. GP presented, after exercise, a reduction in IL-1, TNF and INF production. BCAA supplementation increased IL-2 and INF production and did not alter that of IL-4, which was reduced after exercise.
Conclusions: Glutamine seems to be an important modulator of the immune response during long-distance intense exercise, and BCAA supplementation diverted the immune response towards a T $_{\text{helper 1}}$ type.

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BRIDGING THE GREAT DIVIDE – NEW METHODS IN LYMPHOCYTE PROLIFERATION

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Purpose: Lymphocyte proliferation assays are used in exercise immunology research to evaluate acute and chronic exercise effects on lymphocyte function. These methods quantify the amount and/or activity of newly-formed lymphocytes following *in vitro* culture. We report a new method enabling more detailed analysis of lymphocyte proliferation.
Methods: Peripheral blood mononuclear cells (PBMC), isolated from resting blood samples from eight healthy male subjects, were labelled with carboxyfluorescein succinimidyl ester (CFSE) by resuspending a cell pellet of 1 x 10^7 cells in a 5ug/ml solution. Labelled cells were cultured (37^{o}C) at a concentration of 8 x 10^5 cells/ml with a range of phytohaemagglutinin (PHA) doses (1.25-20 ug/ml). Following 72 hours of culture, cells were labelled with a cocktail containing anti-CD3 (APC) and anti-CD8 monoclonal antibodies and Viaprobe™ (7-AAD), and placed in a Trucount™ tube. Samples were passed through a 4-colour flow cytometer, with standard gating procedures used to identify viable $CD3^+CD8^+$ and $CD3^+CD4^+$ lymphocyte populations. As CFSE fluorescence intensity halves with mitotic division, each generation of proliferated lymphocytes appears as a distinct peak on a CFSE histogram plot. Using the number of parent cells in an unproliferated sample and the number of cells in each generation of the proliferated sample, iterative processes were used to quantify; expansion rate; proliferation rate of each generation; and apoptosis rate of each cell population.
Results: Following 72 hours of culture, 4 to 5 generations of cells were evident in all samples, with peak expansion rates occurring at 5ug/ml PHA. Proliferation rates of the parent CD4 and CD8 cells were not significantly different (48.0 ± 3.9 vs. 56.7 ± 3.9%), but CD8 proliferation rates were significantly higher for all subsequent generations. The apoptosis rate of $CD4^+$ T-cells was significantly higher than that of $CD8^+$ T-cells (28.5 ± 4.0 vs. 5.3 ± 3.9%). Consequently, the expansion rate of $CD8^+$ T-cells was significantly greater than the expansion rate of $CD4^+$ T-cells (335 ± 40 vs. 131 ± 17%).

Conclusions: These data suggest that previously used lymphocyte proliferation assays have quantified predominantly CD8 T-cell division. Furthermore, this assay technique has the potential to provide a more detailed analysis of the effects of exercise on lymphocyte proliferation, including CD4 and CD8 T-cell proliferation, apoptosis and expansion rates.

GENDER SPECIFIC DIFFERENCES IN RESTING VALUES OF REDUCED GLUTHATIONE (GSH) BUT NOT IN EXERCISE INDUCED ALTERATIONS OF THE GLUTATHIONE SYSTEM IN SPORT STUDENTS

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Purpose: Glutathione peroxidase removes hydrogen peroxide by using it to oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG), thereby participating in antioxidant defense in cells. After strenuous exercise, inducing a high oxidative stress, blood levels of GSH are usually reduced and levels of GSSG are usually increased. It is not clear, whether glutathione system in female athletes reacts differently compared to male athletes.
Methods: 72 sport students (36 female (f) and 36 male (m), age 19 – 25 yrs) of the first semester took part in an incremental run field test and a 2 hr endurance run at a velocity corresponding to 60-70 % of the individual 4 mmol/l lactate threshold. Venous blood samples were taken before and immediately after the endurance run. GSH and GSSG were determined by HPLC.
Results: Velocity at 4 mmol/l lactate acid concentration varied between 2.4 and 4.7 m/s (3.6 ± 0.6 m/s, m: 3.9 ± 0.4 m/s, f: 3.2 ± 0.4 m/s). GSH, GSSG and GSH/GSSG before and after the 2 hr run were: GSH: m: 2.00 ± 0.49 vs. 1.52 ± 0.49 mmol/l, f: 1.74 ± 0.55 vs. 1.31 ± 0.45 mmol/l; GSSG: m: 1.69 ± 0.26 vs. 1.82 ± 0.35 mmol/l, f: 1.48 ± 0.44 vs. 1.78 ± 0.52 mmol/l; ratio GSH/GSSG: 1.20 ± 0.33 vs. 0.84 ± 0.24, f: 1.26 ± 0.60 vs. 0.76 ± 0.25, respectively. 2 hr run induced a significant decline in GSH, an increase in GSSG and a decline in GSH/GSSG in m and f ($p<0.01$, respectively). There was a significantly lower GSH concentration prior to exercise in f compared to m, but no gender specific difference in any other value or exercise effect.
Conclusions: A 2 hr run induces dramatic changes in blood glutathione system, independent of gender, indicating activation of antioxidant defense mechanisms. Resting GSH concentrations, however, were lower in female athletes, which might be due to their lower aerobic endurance capacity or to any other gender specific differences in the antioxidant system.

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LYMPHOCYTE RESPONSIVENESS ASSOCIATED WITH REPEATED BOUTS OF ENDURANCE EXERCISE

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The objective of this study was to study the changes in lymphocyte responsiveness during days of one and two bouts of endurance exercise and compare the changes provoked by a single/first bout of exercise with an identical bout of exercise performed a second time on the same day. Nine elite endurance athletes participated in three 24 h trials:1) complete bed rest (trial REST), 2) one bout of exercise (trial ONE), 3) two bouts of exercise separated by a 3h rest period (trial TWO). All exercise bouts consisted of a 10 min warm-up followed by 65 min at 75% of $V0_2$ max on a cycle ergometer. Exercise was performed between 11.00 AM-12.15 AM (only in TWO) and at 3.15-4.30 PM (both ONE and TWO). Blood samples were drawn at 07:30, 15:00, 16:30, 20:30, and 07:30 the next morning. Lymphocytes in whole blood were stimulated with monoclonal antibodies against CD2 and fluorochrome conjugated anti-CD69 were added to the tubes of each set of aliquots. Lymphocyte responsiveness was assessed by flow cytometry for expression of the early activation molecule CD69. The degree of activation was assessed as the percentage change in CD4+, CD8+, CD56+ cells expressing the CD69+ molecule and the change in mean CD69 fluorescence ratio within these

subsets. A two factor ANOVA procedure for repeated measures and t-test on delta-values were used in the statistical analysis and comparisons resulting in $p<0.05$ was considered significant. Results: The second bout of exercise in trial TWO was associated with significantly increased concentrations of CD4+, CD8+, CD56+ cells, and a significantly decreased percentage of CD56+ cells expressing CD69 when compared to a single bout. Additionally, there was a significantly decreased CD69 fluorescence ratio in CD56+ cells at the end of the second bout of exercise. In conclusion, these differences suggest a "carry-over" effect from a first to a second bout of strenuous endurance exercise in both lymphocyte counts and responsiveness

ARE THE AFFECTIVE CONSEQUENCES OF RUNNING A MARATHON DUE TO IMMUNE RESPONSE?

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PURPOSE: One view of the immune system holds that it operates as a "diffuse sense organ" (Maier & Watkins, 1998, p. 83), providing information to the brain, which directs behavior and mood. Activation of an immune response produces a cluster of adaptive behaviors and physiological changes that are called "sickness," which Maier and Watkins argue has the function of producing energy and conserving its use for processes that fight infection or injury. Among these behavioral and affective consequences of the immune response are reductions in activity, social interaction (Kent, Bluthé, Kelley, & Dantzer, 1992), and depressed mood (Hart, 1988). However, most of the research cited by Maier and Watkins is based on animal studies. The purpose of the present study is to test the linkage between immune response and mood in humans following a major stress event, running a marathon. METHOD: 98 marathon runners completed several measures of mood (4DMS, PANAS, POMS) before and several times after running a marathon race. In addition, these participants also provided blood samples before, immediately after, and 90-minutes after the race. Assays for immune response (IL-1ra, IL-6, IL-8, IL-10, plasma cortisol, plasma glucose) were taken from these samples. Participants were randomly assigned (double-blind) to either a carbohydrate condition or to a no-carbohydrate control condition for the race. Runners drank one-liter of liquid (carbohydrate or placebo) every hour throughout the race. RESULTS: The runners show marked reductions across numerous affect indicators after running the marathon, as anticipated. This relationship is moderated by the carbohydrate condition. Similarly, the runners show marked alterations in immune response following the race, as anticipated, and again with significant differences between carbohydrate and placebo groups. Interestingly, the psychological variables and the immune response variables are not correlated before or after the race. CONCLUSION: The results of this study are rather startling, particularly in light of the theory that has been built to connect mood to immune response and animal research demonstrating the connection. The results are discussed in the context of differences between psychological and physical stressors and their duration. A tentative model is presented that views the affective and immune responses to this stressor (running a marathon) as unassociated outcomes.

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NEW INSIGHTS INTO EXERCISE-INDUCED CHANGES IN T-LYMPHOCYTE PROLIFERATION

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Purpose: Carboxyfluorescein diacetate succinamidyl ester (CFSE) labelling of lymphocyte populations can provide unique insights into cell function at rest and with exercise. This study aimed to characterise the effect of exercise on lymphocyte expansion, proliferation and apoptosis rates. **Methods:** Well-trained endurance runners completed 60 minutes of treadmill running at 95% of individual anaerobic threshold. Blood samples were collected: before exercise, after 30 and 60 minutes of exercise and after 30 and 60 minutes of recovery. Isolated peripheral blood mononuclear cells were labelled with CFSE and cultured for 72 hours with mitogen (PHA). Following culture, cell suspensions were labelled with CD3 (APC), CD8 (PE) and Viaprobe™ (7-AAD) and expansion rates

as well as proliferation rates and apoptosis rates were calculated for samples overall as well as per cell generation.
Results: Exercise was associated with a 60% decrease in cell expansion in both CD4 and CD8 cell types from before exercise to mid-exercise ($p<0.05$). The significant decrease in expansion rate in the mid-exercise samples for both cell types was mirrored by a 65% increase in apoptosis rate ($p<0.05$) in both cell types at that sample point. Exercise had no effect on proliferation rate of either CD4 or CD8 cells in any cell generation (G0-G3). However, there was a significant main effect of cell type ($p<0.05$) in expansion, proliferation and apoptosis rate. CD4 T-lymphocytes had significantly lower expansion and proliferation rates and higher apoptosis rates than CD8 T-lymphocytes at all time points.
Conclusions: This study indicates that one hour of exercise at 95% of individual anaerobic threshold does affect T-lymphocyte function *in vitro.* These data, are able to suggest for the first time that exercise decreases cell expansion via an increase in apoptosis of both CD4 and CD8 T-lymphocytes rather than a decrease in proliferation rate.

EXERCISE AND STRESS PROTEIN EXPRESSION: A CENTRAL SIGNAL IN THE ACTIVATION OF THE IMMUNE SYSTEM?

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Heat shock proteins (HSP) are a group of highly conserved proteins present in cells of all living organisms. Although expressed in low concentrations in the basal state, they are highly inducible by a variety of pathological, physiological and environmental stressors. It has been known for some time that the primary role of the HSP is to act as a molecular chaperone by binding to denatured proteins and acting as a catalyst in the assembly of protein complexes within cells. However, recent evidence suggests that HSP may have another important extracellular role in immune defence. In a recent study, Asea *et al.* (*Nature Med.* 6: 435-442, 2000) demonstrated that exogenous HSP72 (the inducible form of the 70 kDa family of HSP) bound specifically to the cell surface of human monocytes *in vitro*. These authors also demonstrated that the pro-inflammatory cytokine IL-6 was activated by a very small quantity of HSP72. Importantly, these authors demonstrated that the activation of IL-6 was via a CD14 dependent pathway. This glycosylphosphatidylinositol -anchored protein is situated on the plasma membrane. Therefore, these data suggest that in order to activate IL-6 within monocytes, HSP72 must act via binding to the plasma membrane. Studies examining the effect of exercise on monocyte function support this hypothesis. It has been demonstrated that acute exercise increases HSP72 production within monocytes (Fehrenbach *et al., J. Appl. Physiol.* 89: 704-710, 2000). However, we have recently shown that very stressful exercise either does not affect (Starkie *et al., J. Physiol*. 528: 647-655, 2000) or indeed decreases (Starkie *et al., Am. J. Physiol. Cell Physiol.* C769-C774, 2001) intracellular monocyte IL-6 production *in vivo*. It appears, therefore, that in order for HSP72 to activate an IL-6 response within monocytes they must first be released from other cells before adhering to the surface of a monocyte to act via the CD14 dependent pathway. This presentation will focus on recent work from our laboratory that demonstrates that acute exercise can markedly increase HSP72 in the peripheral circulation, suggesting that during exercise HSP may indeed provide a central signal to the immune system.

NITRIC OXIDE IN EXERCISE IMMUNOLOGY - RECENT ADVANCES AND FUTURE PERSPECTIVES

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Nitric oxide (NO) is an important effector molecule that accounts for a variety of functions such as vascular regulation, neurotransmission and modulation of immune function. In immunocompetent cells, NO is generated by the inducible nitric oxide synthase (iNOS) which expression can be induced by a variety of factors such as cytokines, endotoxin, hypoxia and hyperthermia. After a short overview about the various functions of NO in the immune system, this presentation will focus on the potential role of iNOS in exercise immunology. It will summarize data of recent research demonstrating effects

of acute and chronic exercise on expression of iNOS in immunocompetent cells. The final part will discuss future perspectives in this area which may also include aspects of an augmented expression of iNOS in skeletal muscle as observed under pathophysiological conditions.

THE POTENTIAL ROLE OF HEAT SHOCK PROTEINS IN STRESS-INDUCED MODULATION OF INNATE AND ACQUIRED IMMUNITY

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Heat shock proteins (HSP) are a family of molecular chaperone proteins that can increase in response to cellular stress. Increased intracellular HSP is protective against cellular stress, whereas, increased extracelluar HSP following necrotic cell death facilitates functions of innate immunity. Exposure to acute stress (IS, 90 min intermittent tail-shock) both reduces acquired (αKLH Ig) and increases innate (bacterial inflammation resolution) immunity. The effect of acute stress is modulated by activity status. Physical activity prevents the suppressive effect of stress on acquired immunity and facilitates the benefits of stress on innate immunity. Thus we examined whether physical activity also impacts the HSP response to stress, and whether this contributes to the modulation of the stress-induced changes in immunity in physically active organisms. Male, F344 rats (4mos, 6/grp) lived with either mobile (active) or immobile (sedentary) running wheels. After 5 wks, rats were exposed to IS or no stress. Blood, skin, heart, adrenal, liver, spleen, and lymph node were removed 90 min after IS termination. Physically active-stressed rats had reliably increased HSP70 (EIA, StressGen) in every tissue tested. In contrast, sedentary-stressed rats had increased HSP70 only in the blood, spleen, liver, and adrenal, and the increase was smaller than in the physically active-stressed rats. Thus the impact of stress on HSP70 was greater in physically active than sedentary rats. The greater increase in tissue (intracellular) HSP70 could protect lymphocytes from stress-induced immunosuppression and the greater increase in blood (extracellular) HSP70 could facilitate stress-induced immunopotentiation of innate immunity (Supported by NIH-RO3AI45576, NIH-RO1AI48555).

INCREASED MUSCLE-DERIVED IL-6 RELEASE DURING CONCENTRIC ACTIVITY IN ELDERLY PEOPLE

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Introduction: Aging is associated with sarcopenia and decreased ability to maintain glucose homeostasis. Given the fact that IL-6 is produced in large amount by contracting skeletal muscle and that infusion of IL-6 into healthy volunteers induces glucose out-put form the liver, we have previously suggested that muscle-derived IL-6 is to be considered as a glucoregulatory hormone rather than a proinflammatory cytokine. Due to low total muscle mass in elderly people, the total glycogen content in aged muscle is estimated to be low compared to that of young subjects. During exercise therefore, old subjects would be expected to take up relatively more glucose by working muscle than young controls. In order to maintain glucose homeostasis, we hypothesized that elderly people would produce more IL-6 during exercise to ensure a sufficient amount of glucose from the liver.
Methods: Seven elderly healthy males (mean age 70 years) and six healthy young controls (mean age 26 years) performed 3 hours two-legged concentric knee extensor exercise at 50% of W_{max}. Blood samples were obtained by femoral vein and artery. Blood flow was measured by Doppler ultrasound and muscle mass was estimated by DXA.
Results: Glucose uptake by the muscle did not differ between the young and the old subjects despite the fact that the absolute work was at least two times higher in the young controls. Thus the energy consumption of working muscle in elderly vs. young subjects consists of relatively more glucose. The v-af differences of IL-6 from working muscle were increased during the exercise, but were significantly higher in the elderly. When the blood flow was taken into account and net-IL-6 release was calculated, the difference was even more pronounced in the elderly.
Conclusion: Working muscle of elderly individuals takes up more glucose relative to the total energy consumption compared to young subjects. Our data support the hypothesis that in order to maintain

glucose homeostasis during exercise, elderly subjects produce more muscle-derived IL-6 compared to young controls.

TRANSCRIPTIONAL ACTIVATION OF THE IL-6 GENE IN HUMAN CONTRACTING SKELETAL MUSCLE – INFLUENCE OF MUSCLE GLYCOGEN AVAILABILITY

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Introduction: Circulating IL-6 markedly increases during exercise that continues beyond 2-3 h. Measurements across the working limb indicate that skeletal muscle rather than circulating monocytes is the source of IL-6 release. IL-6 mRNA in muscle is nearly undetectable at rest, but is dramatically elevated after 2-5 h of exercise suggesting that the appearance of IL-6 is due to transcriptional activation of the IL-6 gene rather than release of IL-6 from preexisting pools. Given that increases in IL-6 expression appear only with exercise durations in excess of 2-3 h, we hypothesized that IL-6 induction may be related to the depletion of muscle glycogen stores. In the present study, we determined the potential influence of normal versus low pre-exercise muscle glycogen content on muscle IL-6 transcription rate and mRNA content over the course of 3 h of exercise.
Methods: Six healthy male subjects performed two-legged knee-extensor exercise at 60% of maximal workload for 3 h. Two randomized trials were conducted, one with normal glycogen and one in which glycogen levels were lowered by ~50% by exercise the previous day. Muscle samples, obtained from the vastus lateralis at 0, 30, 90 and 180 min of exercise, were used for nuclei isolation or frozen for subsequent RNA analysis. Transcription of the IL-6 and β-actin genes was determined by a RT-PCR based nuclear run-on technique while mRNA was determined by relative RT-PCR analysis.
Results: IL-6 (relative to β-actin) transcription and mRNA were undetectable at rest. In the control trial, IL-6 transcription and mRNA content increased slightly after 3 h of exercise. In the low glycogen trial, IL-6 transcription increased by ~40 fold after 90 min and ~60-fold after180 min of exercise ($P<0.05$ versus control trial). IL-6 mRNA was elevated by >100-fold after 3 h of exercise ($P<0.05$ versus control trial). Both IL-6 transcription and mRNA returned to near control levels within 2 h after exercise.
Conclusions: Exercise activates transcription of the IL-6 gene in working skeletal muscle, a response that is dramatically accelerated and/or enhanced when glycogen levels are low. Given that IL-6 stimulates hepatic glucose production, our results provide further support for the hypothesis that muscle-derived IL-6 may represent a link between working muscles and liver to contribute, in a hormone-like manner, to the maintenance of glucose homeostasis during prolonged exercise.

NO EFFECT OF OLFACTORY BULBECTOMY OR WHEEL RUNNING ON CYTOTOXICITY AND APOPTOSIS IN NATURAL KILLER CELLS

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Purpose: Depression has been shown to negatively affect the immune system, including natural killer (NK) cell activity in human. Exercise has been shown to have an antidepressant-like effect. Olfactory bulbectomy (OBX) is an animal model of depression. This study examined splenic NK cell cytotoxicity *in vitro* and apoptosis after OBX and in response to voluntary activity wheel running in rats. Plasma corticosterone, that has been found to induce apoptotic death in lymphocytes, was also measured.
Methods: A 2 condition (OBX vs. sham) x 2 group (activity wheel vs. sedentary) x 2 treatment (imipramine vs. saline) factorial design was used. Male Long-Evans rats were randomly assigned to groups 24 hours after OBX or sham surgery. After 3 weeks of wheel running and/or imipramine injection, animals were euthanized. Blood and spleens were collected 24-72 hours later for NK cell cytotoxicity against YAC-1 target cells using standard chromium release assay and for plasma corticosterone using radioimmunoassay. Apoptosis of NK cells was analyzed by Annexin-V binding assay using flow cytometry. NK cells were also incubated with dexamethasone (100nM) *in vitro* for 20 hours as a positive control to examine glucocorticoid-induced apoptosis in rat splenic NK cells.

Results: NK cell activity was not affected by OBX, imipramine treatment or 3 weeks of voluntary wheel running. Also, there was no difference in percentage of apoptotic cells between OBX (4.58 3.69 %) and sham (4.93 5.09 %) animals. Incubation with dexamethasone *in vitro* resulted in apoptosis of NK cells (32.07%) as expected. In contrast to expected results, plasma corticosterone level was lower in OBX rats (6.52 6.0 µg/dL) compared to sham surgery animals (10.30 8.70 µg/dL), p = 0.031.
Conclusions: OBX or 3 weeks of voluntary wheel running did not affect cytotoxicity or apoptosis of splenic NK cells in rats. However, glucocorticoid induced apoptosis in NK cells *in* vitro has been shown. Further investigation is needed to examine apoptosis in NK cells as a result of an exposure to stress hormones including glucocorticoids and possible moderating effects of exercise.

FREE RADICAL FORMATION AND NK-CELL SUPPRESSION ARE PARALLELL PHENOMENA DURING CHRONIC PHYSICAL TRAINING IN RATS

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Objective: The aim of this particular study is to elucidate the connection between radical formation and the weakening of natural immunity seen during prolonged periods of physical training.
Methods: Spontaneously hypertensive rats (SHR) were divided into 3 groups allowed free exercise in a running wheel for 3, 5 or 11 weeks, and 3 corresponding sedentary control groups. Half of the animals in the 11-week groups were supplemented with extra vitamin E (approx. 1 mg/day in food). At the end of the respective training period the relative in-vivo cytotoxicity of NK cells was determined by clearance of ^{51}Cr-labelled YAC-1 lymphoma cells from the lung 60 min after i.v. injection of 10^6 cells. Ascorbyl radical (AR), an endogenous general indicator of free radical formation, was quantified with ESR spectroscopy. Malondialdehyde (MDA), an index of lipid peroxidation, was quantitated with HPLC. After 3, 5 or 11 weeks the animals were injected with YAC-1 cells through a tail vein and decapitated 60 min later. Trunk blood and lung tissue were collected for determination of AR, MDA (11 weeks only) and radioactivity retained in the lungs. **Results:** The fraction of YAC-cells trapped in the lungs was decreased almost by half already after 3 weeks of exercise, indicating a significantly increased cytotoxicity of NK cells. This improvement of immunity was completely gone after 11 weeks of running. Measurement of free radicals reflects these results with no increase in AR levels in plasma seen until 11 weeks when they rise to over 150 % of control values. Supplementation of vitamin E throughout the period significantly enhanced the cytotoxicity in the 11 week group back to the level of the 3 - 5 week runners. At the same time the increase of AR seen in the runners at 11 weeks was significantly attenuated in the E group down to about 120 % of that in the sedentary controls supplemented with vitamin E. MDA levels in plasma were unchanged from sedentary controls in the unsupplemented 11 week runners, but decreased by 20 % in the vitamin E group.**Comment:** Loss of enhanced natural immunity after 11 weeks of running coincides with an increase in the level of AR. Furtermore, immunity was re-enhanced and AR were decreased by intervention aimed against free radicals. This lends support to the notion that free radicals are partly responsible for the loss of immune function seen during conditions of overtraining.

EFFECTS OF STRESS ON IMMUNE FUNCTION: CELLULAR & MOLECULAR MECHANISMS EXAMINED FROM AN EVOLUTIONARY PERSPECTIVE

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Most evolutionary selection pressures are stressors, and one of the primary functions of the brain is to perceive stress, warn the body of danger, and enable an organism to respond. We hypothesized that under acute conditions, just as the stress response prepares the cardiovascular and musculoskeletal systems for fight or flight, it may also prepare the immune system for challenges (e.g. wounding) which may be imposed by a stressor (e.g. an aggressor). Initial studies showed that acute (2h) stress induced a significant trafficking of immune cells to the skin. Since the skin is a major protective barrier, we hypothesized that such leukocyte redistribution may serve to enhance skin immunity during acute stress. We tested this hypothesis using the delayed type hypersensitivity (DTH) reaction, which mediates resistance to various infectious agents, as a model for skin immune function. Acute stress

administered immediately before antigen exposure significantly enhanced skin DTH. In contrast, chronic or long term stress significantly suppressed skin DTH. We subsequently showed that changes in leukocyte distribution and adrenal stress hormones are global mediators of the effects of stress on skin immunity and that gamma interferon is a local cytokine mediator of a stress-induced enhancement of skin immune function. Our results suggest that during acute stress the brain sends neural and endocrine warning signals which enhance immune function just as they adaptively enhance the function of other fight/flight systems within the body.

ADAPTATIONAL CHANGE OF THE MUCOSAL IMMUNE SYSTEM UNDER REPEATED EXPOSURE TO PHYSICAL STRESSOR

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Purpose: Immuno-modulation during or after heavy intensity exercise resembles that induced by physical stressors such as restraint or noxious stimuli. Repeated exposure to tolerable intensity of exercise is known to attenuate apparently suppressive change in the immune system. Employing a physical stressor model, we tried to show how an adaptational change could take place in the mucosal immune system under repeated exposure to physical stressors. We focused on the effect of mild electric foot shock given repeatedly as a noxious physical stressor on the major cellular component of intestinal immune system, intraepithelial lymphocyte (IEL), that plays an important role in the first line of host defense against invasion of microbial pathogens by evaluating the proportions of each subset of IEL with flow cytometry.
Methods: Male C3H/HeN mice were exposed to 0.5 s of foot shock (FS) every 5 s for 30 min a day for either once or five consecutive days. Immediately after, 1 days, 2 days after the final shock exposure, mice were sacrificed and IEL were isolated by Percoll density gradient. Purified IEL was analyzed by flow-cytometer for the surface expression of CD3, CD4, CD8, $\alpha\beta$TCR and $\gamma\delta$TCR. Cytokine production of IEL was also evaluated by intracellular staining technique.
Results: 1) Total number of recovered IEL and $CD3^+$ IEL as well as the number of thymocytes significantly decreased 1 day after a single FS and recovered on day2, when there was no change in the number of splenocytes. 2) The major components reduced after single FS were $CD4^+CD8^+$ and $CD4^+CD8^-$ with $\alpha\beta$TCR. 3) There was no significant change in the number of $\gamma\delta TCR^+$IEL. 4) All the observed change after single FS was diminished after repeated FS for 5 consecutive. 5) The proportion of IFN-γ producing $CD3^+\alpha\beta$IEL was significantly reduced after repeated FS.
Conclusions: Physical stressor transiently expels IEL of mainly thymic origin from the intestinal mucosa, leaving majority of T cells of extrathymic origin intact. The alteration in the IEL subsets, however, will quickly be recovered even under repeated exposure to the same stressor, suggesting an adaptational change of mucosal immune system to maintain or restore immune reactivity, even though there still remained a functional defect in cytokine production. Mucosal immune system thus has a potential mechanism to adapt to repeated exposure of stressors, which may in part account for the training effect on the immune system

STRENUOUS EXERCISE INDUCES IMMIGRATION/SUPPRESSION OF TH1/TC1 CYTOKINE PRODUCING CELLS

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Purpose: Long-term, intense exercise induces low number of CD4+ T helper (Th) and CD8+ cytotoxic (Tc) cells. These cells can be divided according to their cytokine profile into Th1/Tc1, which produce interferon(IFN)-γ and interleukin(IL)-2, and into Th2/Tc2, which produce IL-4. This study investigated how the relative balance between the circulating levels of these cytokine producing T cells is regulated by exercise and focuses on the possible mechanistic roles of cortisol, adrenaline and IL-6.

Methods: Nine male runners aged 25-50 years performed treadmill running for 2.5 h at 75% VO2max. Intracellular expression of cytokines was detected following stimulation with ionomycin and PMA in blood obtained before, during and after exercise.
Results: The percentage of IFN-γ and IL-2 producing CD4+ and CD8+ T cells in the circulation was suppressed at the end of exercise and 2 h after ($p<0.05$), whereas no changes were found within percentages of circulating T cells producing IL-4. Plasma adrenaline correlated negatively with the percentage of CD8+ IL-2 producing T cells in the circulation ($r=0.72$, $p < 0.05$), whereas peak IL-6 correlated with the percentage of CD8+ IL-4 producing T cells in the circulation ($r=0.78$, $p <0.02$). Peak plasma IL-6 correlated with plasma cortisol post-running ($r=0.7$, $p<0.05$).
Conclusion: In conclusion, post-exercise decrease in lymphocyte number is accompanied by a relatively more pronounced decrease in the Th1/Tc1 cytokine producing cells. We suggest that exercise favors a relatively higher disappearance of Th1/Tc1 cells from the circulation, which may be mediated by adrenaline or local inflammation. Exercise-induced increase in circulating IL-6 may stimulate more cells to produce IL-4 thereby maintaining a relative unaltered level of these cells. Furthermore, the study demonstrates that exercise-induced increase in plasma IL-6 probably stimulates cortisol production.

EFFECT OF EXERCISE INTENSITY ON MUCOSAL IMMUNITY IN COMPETITIVE ATHLETES SUFFERING FATIGUE AND RECURRENT INFECTIONS

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Purpose: The aim of this study was to compare the mucosal immune responses to exercise at different intensities in competitive athletes experiencing symptoms of fatigue and/or recurrent infections and associated poor performance.
Methods: 19 subjects completed a maximal progressive exercise test (VO2max test) on a cycle ergometer. Subjects then performed sub-maximal cycle exercise tests of 5-minute duration on three separate days, at 50%, 70% and 90% of the subject's VO2max. Saliva samples were collected from each subject immediately prior to, immediately after and one-hour after each exercise test. Salivary IgA (SIgA), IgM and IgG concentrations were analysed by ELISA and albumin concentration by rate nephelometry (Beckmann Immage).
Results: There were no statistically significant changes in SIgA, IgG, IgM or albumin concentrations immediately or one hour after exercise at 50%, 70%, 90% or at VO2max. However, exercise at 70%, 90% and VO2max were associated with a trend for lower SIgA levels immediately post-exercise, while levels increased slightly after exercise at 50% of VO2max. The SIgA levels failed to recover to pre-exercise levels one hour after exercise at VO2max but returned to baseline after the lower intensity exercise tests at 70% and 90% of VO2max.
Conclusions: The median differences in SIgA concentrations between pre-exercise, post-exercise and one-hour recovery samples collected at each exercise intensity indicated a positive association between the degree of immune suppression post-exercise and the exercise intensity level. The results suggest that to induce a significant prolonged suppression of SIgA it is necessary for the athlete to exercise at or near the level of their VO2max and that one hour is insufficient time for the mucosal immune system to recover from the effects of an intense training session. The effect of intense exercise on mucosal immune suppression, and the potential positive effects of moderate exercise, needs to be considered in the design of training programs for athletes suffering fatigue and/or recurrent infections.

SALIVARY IGA CONCENTRATION AS A PREDICTOR OF RESPIRATORY ILLNESS IN ATHLETES

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Purpose: To confirm previous data indicating that lowered salivary immunoglobulin A (IgA) concentration is associated with an increased risk of respiratory illness in highly trained athletes.
Methods: Mucosal immunity in athletes of the Australian Swimming Team (n=49; 26 males, 23 females) was assessed on four occasions over a five month period during training for the 1999 Pan Pacific Championships held in Sydney, Australia. The timing of these measurements defined following study periods during the season as: 'early season', 'taper', 'competition', 'post-competition', and the 'entire-season'. Athletes were categorized as either 'healthy' or 'URTI' on the basis of symptoms of upper respiratory tract illness (URTI) within each period verified by the team physician. Salivary IgA, IgM, IgG and albumin concentrations were measured with an in-house ELISA (CV < 7%) and log-transformed prior to non-parametric analysis. Not all athletes were measured for mucosal immune status at each sampling point.
Results: The number of swimmers (n) and salivary IgA (mg/l) concentrations for the two groups during each of the five study periods were:

Study Period		Healthy Athletes			Athletes with URTI			
Period	Weeks	n	median	range	n	median	range	p-value
'early'	1-3	27	63.5	18-175	16	32.7	20-172	0.03 *
'taper'	14-15	31	47.8	15-134	8	30.0	17-104	0.11
'comp'	16-17	27	78.0	24-164	11	71.0	31-164	0.82
'post-comp'	18	28	44.1	20-148	4	37.3	33-79	0.65
'entire-season'	1-18	17	67.7	20-175	26	39.1	18-172	0.02 *

Conclusions: The early-season salivary IgA level was associated with symptoms of respiratory illness and may be a better indicator than levels measured closer to competiton. These data confirm existing observations that low levels of salivary IgA can be used to assess the risk of respiratory illness in athletes during discrete periods of training for international level competition.

IGG SUBCLASS DEFICIENCIES IN COMPETITIVE ATHLETES

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Purpose: The aim of this study was to compare serum IgG subclass concentrations in a group of high-performance athletes suffering fatigue and/or recurrent infections with an age and fitness-matched healthy control group. The purpose was to determine any associations been recurrent infections and IgG subclass deficiencies.
Methods: Total serum IgG and each IgG subclass concentrations were measured by rate nephelometry (Beckman Immage) for 19 subjects in the fatigued-infected (F/I) group and 21 subjects in the well-control (W/C) group. The median number of infections in the previous 12 months in the F/I group was 4.5 episodes (range: 0-28).
Results: The total IgG level was significantly lower in the F/I group and there were trends for lower concentrations of IgG2 and IgG4. Table indicates median concentration (g/L) and 95% confidence intervals (95% CI).

Subclass	Fatigue/Infected Group	Well/Control Group	Difference (p-value)
IgG1	6.61 (6.22-7.42)	7.17 (6.50-8.26)	0.38
IgG2	2.85 (2.55-3.52)	4.12 (3.25-4.98)	0.07
IgG3	0.42 (0.39-0.61)	0.55 (0.47-0.69)	0.24
IgG4	0.28 (0.28-0.68)	0.46 (0.46-1.07)	0.06
Total IgG	11.0 (9.79-11.3)	14.0 (11.7-15.1)	0.003

IgG3 levels below the age-specific reference range were identified in 8 athletes in the F/I group (42%) and 3 in the W/C group (14%). The differences in the distributions approached statistical significance (p=0.078). The 3 control subjects were high-performance athletes with the highest aerobic fitness levels in the W/C group. After removing these 3 subjects from the control group the median concentration of IgG3 was statistically significantly lower in the F/I group (p=0.038) compared to the adjusted control group (median = 0.62 g/L, 95% CI = 0.53-0.76).

Conclusions: High performance athletes may experience partial suppression of the minor IgG subclasses, particularly IgG3, which have the potential to be associated with recurrent infections during intensive training periods.

INCREASED INFLAMMATORY RESPONSE ASSOCIATED WITH REPEATED BOUTS OF ENDURANCE EXERCISE ON THE SAME DAY

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The objective of this investigation was to study the changes in inflammatory markers released in the blood during days of one and two bouts of endurance exercise. Specifically, we wanted to compare the changes provoked by a single/first bout of exercise with an identical bout of exercise performed a second time on the same day. Nine elite endurance athletes participated in three 24 h trials:1) complete bed rest (trial REST), 2) one bout of exercise (trial ONE), 3) two bouts of exercise separated by a 3h rest period (trial TWO). All exercise bouts consisted of a 10 min warm-up followed by 65 min at 75% of $V0_2$ max on a cycle ergometer. Exercise was performed between 11.00 AM-12.15 AM (only in TWO) and at 3.15-4.30 PM (both ONE and TWO). Blood samples were drawn at 07:30, 12:15, 15:00, 16:30, 17:30, 18:30, 20:30, and 07:30 the next morning. Myeloperoxidase (MPO) and human neutrophil lipokalin (HNL) was analysed in serum with a RIA method. Interleukin-1 receptor antagonist (IL-1ra) and interleukin-6 (IL-6) was analysed in serum with a ELISA method. Wilcoxan's non-parametric sign rank tests were used to compare the pre-to post-exercise changes between the three trials and a p-value <0.05 was considered significant. Compared to the changes in trial REST, the single bout of exercise in the ONE trial resulted in significantly increased concentrations in MPO (REST:3.3±5.0; vs ONE:76.1±7.6) , HNL (REST:1.8±1.3 vs ONE:13.1±1.9), IL-6 (REST:0.2±1.0 vs ONE:5.3±0.9) and IL-1ra (REST:8±7; ONE:174±67). Furthermore, we observed more pronounced changes associated with the second bout of exercise in MPO (TO:136.6±12.7 vs ONE:76.1±7.6) , HNL (TO:21.6±2.1 vs ONE:13.1±1.9), and IL-1ra (TO:910±327 vs ONE:174±67), but not in IL-6 (TO:4.9±1.3; ONE:5.3±0.9) compared to the single bout in trial ONE. In conclusion, a single bout of endurance exercise resulted in increased concentrations of MPO, HNL, IL-1ra and IL-6. When completing a second bout of exercise on the same day, a more pronounced increase in MPO, HNL and IL-1ra, but not in IL-6, was observed compared to a single bout.

COGNITIVE HARDINESS, MOOD STATE, EXHAUSTIVE EXERCISE AND SOME ASPECTS OF THE IMMUNE SYSTEM

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Purpose: Cognitive hardiness (CH) is a sense of control, commitment to the projects and people in one's life, and a tendency to appraise events as challenges (versus threats). CH appears to moderate the relation between stress and both illness and depression. The present study explored these relationships in competitive collegiate athletes training in-season.
Methods: Following ethical review, US Air Force cross-country runners (6F, 21 M) undertaking an 8-week winter training regime were recruited. After eight weeks, they performed a VO_{2max} test. Fasting blood samples were taken at the start of the study; halfway through the study; immediately after the VO_{2max} test; and the morning after the test. Cell counts, some aspects of immune function and mood states (POMS) were measured at these time points; cognitive hardiness (CH) was measured twice (at the start and end of the study).
Results: Immediately after VO_{2max} all subjects displayed an increase in numbers of circulating white blood cells (WBC; p<0.006), a reduction in neutrophil activity (p<0.001), a decrease in the CD4/CD8 ratio, and an increase in IL-6 in CD4 and CD8 cells (p<0.001 and p<0.02, respectively). Psychoimmunological relations were complex and often curvilinear. Correlations were observed between the following: CH with lesser magnitude of change in circulating lymphocyte numbers (p<0.007) and WBC (p<0.02); CH/fatigue with lesser/greater magnitude of change in neutrophil numbers (p<0.002); CH with increased plasma IL-2 (p<0.03); vigor/anger with increased/decreased

intracellular IL-6 ($p<0.02$). CH was robustly related to several mood states. No correlation was observed between CH and cortisol.
Conclusions: The marked decrease observed in neutrophil activity and in the CD4/CD8 ratio immediately after VO_{2max} tests, suggests transient immunodepression resulting from prolonged training followed by this bout of intense exercise. CH and mood were related to immunological reactivity under psychophysiological stress; additional investigation is suggested.

PLASMA CYTOKINES, SALIVA IMMUNOGLOBULIN A AND INDICES OF OVERTRAINING DURING A PERIOD OF INTENSIFIED TRAINING IN TRAINED CYCLISTS

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Purpose: According to the cytokine hypothesis of overtraining (OT), high volume/intensity training, with insufficient rest, will produce tissue trauma stimulating monocytes to produce large quantities of proinflammatory cytokines including IL-6 and TNF-α (Smith, 2000, *Med Sci Sports Exerc* 32: 317-331). These then induce mood and behavior changes, fatigue, upregulation of gluconeogenesis, and immune function changes. We investigated the effects of 2 weeks of intensified training on plasma levels of cytokines, glutamine/glutamate ratio, creatine kinase activity, saliva immunoglobulin A (IgA), psychological mood state and time trial performance in trained cyclists.
Methods: 8 healthy, trained male cyclists aged: 27 $\pm$ 3 years, VO_2max: 67.9 $\pm$ 3.0 ml/kg/min (mean $\pm$ SEM) completed 2 weeks of normal training (NT: 7 $\pm$ 2 h/week), 2 weeks of overtraining (OT: 14 $\pm$ 5 h/week) followed by 2 weeks of recovery training (RT: 3.5 $\pm$ 2.5 h/week). The additional training during OT was predominantly in the form of high intensity interval training. Each week subjects completed an incremental exercise test to exhaustion, a simulated 40-km time trial and 2 x 10-min maximal effort bouts on a cycle ergometer. Blood and whole unstimulated saliva samples were obtained at rest in the morning (overnight fasted) and after the time trial. Subjects also completed daily questionnaires to assess general well-being and psychological state.
Results: Performance on all 3 exercise tests significantly declined following the OT period ($p<0.05$). Subjects demonstrated mood changes with higher scores for fatigue, tension and confusion. Plasma creatine kinase activity was significantly elevated during OT (NT: 55 $\pm$ 8 U/l; OT: 96 $\pm$ 17 U/l) and the plasma glutamine/glutamate ratio fell from 3.28 $\pm$ 0.37 during NT to 1.59 $\pm$ 0.20 during OT ($p<0.01$), recovering to 2.71 $\pm$ 0.28 at the end of RT. Saliva IgA concentration was 121 $\pm$ 14 mg/l during NT and fell during OT to 91 $\pm$ 14 mg/l ($p<0.05$), with some recovery by the end of RT (110 $\pm$ 14 mg/l). Resting plasma IL-6 was unchanged during OT (NT: 0.5 $\pm$ 0.2 pg/ml; OT: 0.7 $\pm$ 0.2 pg/ml; RT: 0.6 $\pm$ 0.2 pg/ml). Resting plasma TNF-α was also unaffected by OT (NT: 7.1 $\pm$ 1.4 pg/ml; OT: 7.4 $\pm$ 1.8 pg/ml; RT: 6.3 $\pm$ 1.8 pg/ml).
Conclusions: These data do not support the cytokine hypothesis of overtraining.

INVESTIGATING CD94 EXPRESSION AND FUNCTIONAL CHANGES OF NK CELLS LONGITUDINALLY AND FOLLOWING ACUTE EXERCISE

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This study undertook the examination of longitudinal changes in circulating NK cell numbers, their responses to acute exercise, their expression levels of CD94 and their cytotoxic capacity. Nine nationally competitive male triathletes volunteered (age 25.9$\pm$4.09 yrs, VO_{2max}5.14$\pm$0.33L.min^{-1}). Athletes came to the lab on four occasions; firstly to undertake a standard 20 min (4x5 min stages) submaximal exercise test. Over three months, 3 full cycle-tests to exhaustion were conducted, with peripheral blood collected pre, mid and post test. Blood was analysed immediately for lactate, cell counts, lymphocyte phenotype and later (after frozen\thawed) for cytolytic activity (^{51} Cr release from K562 cells). Although not reflected in peak performance output, maximum oxygen consumption increased from week 2 values, VO_{2max} of 5.14$\pm$0.33L.min^{-1} to wk 10 values, 5.28 $\pm$0.32L.min^{-1}. Resting NK cell numbers and their expression of the activation marker CD94 were not altered over the time course of this study. Furthermore CD94 positive NK cells were not differentially recruited into the

circulation with acute exercise and cytolytic activity of exercise recruited NKs (lysis per NK cell log transformed) did not differ from those present at rest.
In our triathlete subjects, NK cell numbers (at rest and after exercise) were stable across four months of training. There was also longitudinal stability in CD94 expression on the subjects' NK cells and the cytolytic capacity of those cells.

EXERCISE INDUCED APOPTOSIS IN LYMPHOCYTES

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Purpose: Exercise induced changes in leucocyte count are well documented. Moreover, we could demonstrate that these changes are accompanied by alterations of intracellular calcium signaling processes. Because alterations in calcium signaling could be related to the induction of the programmed cell death (apoptosis) the aim of the present study was to investigate whether apoptosis may account for the post exercise lymphocytopenia and whether there is a dependency on the intensity of exercise or not.
Methods: Healthy volunteers performed two treadmill exercise tests. The first test was performed at 80% of the maximal oxygen uptake (VO_2max) until exhaustion (exhaustive test, ET). One week later a second moderate exercise test was performed at 60% VO_2max while the running time was identical to the first test (moderate test, MT). Blood samples were taken before, immediatally after and 1 hour after the test. Samples were analyzed for lymphocyte subsets using flow cytometry. The lymphocyte fraction which was isolated using density gradient centrifugation was analyzed for the apoptotic and necrotic cells using FITC labelled Annexin V-antibodies and propidium iodide, respectively. Moreover, plasma membrane expression of CD95-receptor and CD95-receptor ligand was investigated.
Results: After the ET the percentage of apoptotic cells increased significantly while the percentage of necrotic cells remained constant. In contrast, the percentage of apoptotic cells was unchanged after the MT. Furthermore we found an upregulation of the CD95-receptor expression after the ET while CD95-receptor ligand showed no difference. Expression of both surface markers was unchanged after the MT.
Conclusions: These results suggest that apoptosis may contribute to the regulation of the immune response after exhaustive exercise. This mechanism can be regarded either as helpful, i.e. deletion of autoreactive cells, or as harmful, i.e. suppression of the immune response, and waits further investigations.

PLASMA CONCENTRATION OF GLUTAMINE IN SPINAL CORD INJURY PATIENTS

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The spinal cord injury is a very important lesion because it carts not only physical and psychological changes but muscular, metabolic and immunological too.
Glutamine, a non-essential amino acid, is a substance of great importance in the macrophages and lymphocytes working, being used by these cells as a substrate in the performance of several functions. Glutamine is synthesized specially by the skeletal muscle being this one its main storage site. The muscle contraction activity during the physical activity would influence the glutamine release and it might change the immunological response, so that the musculature could be considered an integrant part of the immunological system.
The spinal cord injury (SCI) causes its carrier to have a huge muscle mass with no contractile ability, besides a large number of infections. Although many professionals believe that these infections are postural issues, an immunological deficiency cannot be denied.
PURPOSE: to evaluate immunologic changes, specially the plasmatic concentration of glutamine in patients whit SCI.
MATERIAL AND METHODS: patients of the physical rehabilitation program from the Rehabilitation Medicine Division of Hospital das Clínicas of Universidade de São Paulo. Initially, they were submitted to laboratory exams aiming at the exclusion of patients with possible pre-existent infections. Lately, these patients were subject to blood collection (10 ml) before and after the effort exam in which the glutamine dosage was performed, among others.

RESULTS: no hematological changes were noticed in these patients, including in the white series. The differential counting of leucocytes also pointed out no changes. However, the plasmatic concentration of glutamine was extremely reduced in the inactivity period (365,16 ± 65,16 mol/ml), which decreased even more after the effort (235,37 ± 25,23 nmol/ml).
CONCLUSION: Although there is no quantitative change of leucocytes, the low concentration of glutamine may be causing functional damages to these cells, which indicates that a immunodepression in these patients may exist.

GLUTAMINE/GLUTAMATE RATIO AFTER EXHAUSTIVE EXERCISE.

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Purpose: plasma glutamine concentration has been proposed as a means of assessing overtraining. Plasma glutamine concentration reflects tolerance to volume of work and that of glutamate reflects tolerance to high intensity training, so that the glutamine/glutamate (Gm/Ga) ratio would globally represent overall tolerance to training. In this study we determined Gm/Ga ratio in four cyclists during an indoor test, and correlates with performance.
Methods: Four male athletes cycled for 12 hours, in pairs, 1h of activity, 1h rest. At the beginning and after the 3rd and 6th bout, 5 ml of venous blood was collected and plasma prepared for measuring glutamine and glutamate concentration. Both pairs cycled for more than 420km, at 23.4mph, in average.
Results: Gm/Ga ratio before the test were 3.17 ± 0.28, and after the 6th bout, 2.14 ± 0.45. It is interesting to note, however, that the pair that received BCAA during the test presented, at the end of the test, a ratio of 2.84 ± 0.3, and cycled for 450mph, keeping the pace until the last bout. The other pair, on the other hand, finished the test with a ratio of 1.44 ± 0.5, and presented a huge decrease in performance during the last 2hs, when average velocity falls to 20.6mph.
Conclusion: BCAA supplementation keeps high Gm/Ga ratio, allowing the athletes to keep their pace during a twelve hours exhaustive exercise, without symptoms of overreaching or immunesuppression.
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ABSOLUTE VERSES RELATIVE WORK OUTPUT AND LYMPHOCYTE PROLIFERATION

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Purpose: The aim of this investigation was to determine whether the magnitude of change in the lymphocyte proliferation response to mitogen and antigen stimulation increased as absolute, but not relative, work output increased. **Methods:** Previously untrained women underwent six months of resistance training of both upper and lower body (TOTAL, n=32) or just the upper body (UPPER, n=28). Blood was collected before and immediately following a 6 by 10 repetition maximum (RM) squat resistance exercise before training began (Sep/Oct) and after 3 (Nov/Dec) and 6 (Apr/May) months of training. Mononuclear cells were isolated from whole blood and tritiated thymidine uptake was measured in response to stimulation with concanavalin A (ConA), phytohemagglutinin (PHA), pokeweed mitogen (PWM), and *staphylococcus a.* cowans (SAC) at both sub-optimal and optimal concentrations. Lymphocyte subsets were determined using fluorescently labeled monoclonal antibodies and flow cytometry; serum lactate using a Sport L-Lactate Analyzer; and serum cortisol by radioimmunoassay. **Results:** Squat 1-RM increased (group x time P<0.001) to a greater degree in the TOTAL group (+22.4% and +36.4% TOTAL compared to +5.8% and +11.1% UPPER at 3 and 6 months, respectively). Thus, the TOTAL group did a greater volume of absolute work over time during the squat exercise. Additionally, post-exercise lactate concentrations increased (group x time P=0.02) slightly over time for the TOTAL group, but not for the UPPER group. The magnitude of the proliferation decrease was similar over time and between groups for the majority of the stimulation conditions. For PHA at optimal (P=0.05), PWM at sub-optimal (P=0.05), and PWM at optimal (P<0.001) concentrations, the magnitude of the decrease was greater after 3 months of training compared to the other time points. **Conclusions:** Increasing the absolute work output during a 6 by

10 RM squat test did not influence the magnitude of changes in lymphocyte proliferation. Further, the greater post-exercise decrease in proliferation after 3 months of training was not a function of proportionately fewer T or T and B lymphocytes. Thus, this fluctuation may have been either 1) the result of seasonal changes in immunity, or 2) a temporary (not present at 6 months) systemic adaptation to resistance training.

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PARTICIPATING IN A SWIMMING PROGRAM ENHANCED THE NATURAL IMMUNE SYSTEM IN ELDERLY WOMEN

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Purpose: A cross-sectional study was performed to examine the effect of a swimming program on the immune system in elderly women.
Methods: 10 swimming-trained women (mean age 62±1 yrs; mean±standard error), 10 age-matched untrained women (mean age 65±1 yrs), and 9 younger women (mean age 27±1 yrs) participated in this study. The elderly swimmers' group swam an average of 1680 m/week for a year. In a swimming session, the participants swam 1500m for 90min with an average of 75-80% HRmax during the session. We measured VO2max, leg extension power, PHA-stimulated proliferative response, the concentration of CD3+, CD4+, and CD8+, and the natural killer (NK) cell activity at the resting level.
Results: The mean of VO2max and leg extension power of the elderly swimmers were significantly higher than that of the age-matched untrained controls (VO2max ; 36.0±1.0 vs. 31.1±1.0 ml/kg/min, $p<0.01$, leg extension power; 16.8±0.6 vs. 14.5±0.8 watts/kg, $p<0.05$, swimmers vs. untrained controls respectively). NK cell activity was higher in the elderly swimmers than that in the untrained elderly or the young controls. NK cell activity in the elderly women, including swimmers and the untrained group, was strongly correlated to their VO2max ($p<0.01$, $r=0.66$).
Conclusion: This study suggests that the swimming program enhance the NK cell activity in elderly women.

EFFECT OF CHRONIC EXERCISE TRAINING ON INNATE IMMUNITY AND NEUROENDOCRINE HORMONE LEVELS IN NON –OBESE AND OBESE PERSONS

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Purpose:Recent work has clearly shown that various types of exercise may have potentially considerable effects,both positive and negative ,on immune system.The effect of 8 weeks exercise training , either on a bicycle or on a treadmill, on the quantification and phagocytic activity of neutrophils as well as on the serum levels of cortisol and growth hormone (GH) in non-obese and obese healthy subjects was studied.
Methods:Blood samples were obtained from 30 healthy subjects (20 non-obese,10 obese) before and 8 weeks after exercise training.None of the studied subjects was under caloric restricted diet during the study.Neutophil phagocytic function was assessd by cytomorphological method using *Candida albicans* .Serum levels of cortisol and GH were measured using Enzyme Immunoassay technique.
Results: The study revealed a significant increase in the post-exercise band neutrophil count as well as immature/total neutrophil ratio compared to pre-exercise values in all studied groups. A significant increase in the phagocytic index was found after exercise performed by non -obese subjects, whether bicycle or treadmill, whereas a significant increase in the opsonic index was found after exercise performed by obese *subjects.* All studied groups showed a significant rise in the post-exercise serum level of cortisol and GH compared to the pre-exercise values. Correlation analysis revealed a significant negative correlation between the post-exercise serum GH level and lytic index in non-obese subjects ($r = -0.48$, $P<0.05$). A significant positive correlation was found between the post-exercise GH level and the elevation in the segmented neutrophil count ($r = 0.87$, $P<0.05$), whereas a

significant negative correlation was found between post-exercise serum cortisol level and the band neutrophil count (r = -0.83, P<0.05) in obese subjects. Obese persons showed no change in their body weight after 8 weeks exercise training.
Conclusion:It can be concluded that chronic exercise training had a beneficial effect on the innate immunity represented by increase in both count and phagocytic function of neutrophils as well as on the levels of neuroendocrine hormones as cortisol and growth hormone both in obese and non-obese subjects.

CHANGE IN SALIVARY IGA FOLLOWING A COMPETITIVE MARATHON RACE

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The influence of carbohydrate (1 l/h of a 6% carbohydrate beverage), gender, and age on salivary IgA (sIgA) changes and incidence of upper respiratory tract infection (URTI) was studied in 98 runners following two competitive marathon races. The pattern of change in sIgA concentration differed significantly between carbohydrate (C) (N=48) and placebo (P) (N=50) groups, with higher post-race values measured in P. However, when this was adjusted for saliva protein concentration and saliva secretion rate, no difference between groups was measured. For all subjects combined, sIgA concentration, saliva protein IgA (spIgA) concentration, and sIgA secretion rates fell significantly (21%, 31%, and 25%, respectively) below pre-race levels by 1.5-h post-race (p<0.001). The pattern of change in all saliva measures did not differ significantly between the 12 women and 86 men in this study, and between the 23 older (50 yr) and 75 younger (<50 yr) subjects. Ninety-three subjects returned health/sickness logs, and of these, 16 (17%) reported developing URTI during the 15-d period following the race event. The 1.5-h post-race spIgA concentration, but not sIgA concentration or secretion rate, was lower in runners reporting URTI compared to those who did not (254±30 and 388±26 $\mu g \cdot mg^{-1}$, respectively, p=0.002), and this was negatively correlated with the post-race plasma cortisol concentration (r=-0.36, p<0.001). Of the 16 runners, six were in the C group and 10 in the P group (Chi square = 1.11, p=0.293). In conclusion, the output of sIgA decreased in runners following a competitive marathon, and this was not influenced by carbohydrate ingestion, age, or gender. URTI during the 15 days prior to the race was not related to pre-race sIgA levels, and only one of three methods of expressing sIgA was related to URTI during the 15 day period post-race. Thus the relationship between URTI and sIgA does not appear to be clear and consistent, and it is doubtful that sIgA can be used as a reliable indicator of URTI risk in athletes.

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DIURNAL VARIATION IN SALIVA IMMUNOGLOBULIN A CONCENTRATION AND THE EFFECT OF A PREVIOUS DAY OF HEAVY EXERCISE

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Purpose: Both acute psychological stress and exercise can affect saliva immunoglobulin A (IgA) concentration, but it is not altogether clear if there is a delayed effect of such stress or whether significant diurnal variation in saliva IgA exists. We decided to investigate this by examining the diurnal changes in saliva IgA during a day of complete rest and on the day after a day of heavy exercise.
Methods: 10 healthy, recreationally active subjects (5 male, 5 female) aged: 22 ± 1 years, VO_2max: 56.5 ± 2.0 ml/kg/min (mean ± SEM) participated in the study. IgA and cortisol concentrations were measured in saliva samples collected every 2 hours from 08:00 to 20:00 on three consecutive days. Subjects performed no exercise for 2 days prior to the study. The first experimental day involved no exercise; on the second day, subjects performed 90 min of cycling exercise (60% VO_2max) in the morning (starting at 10:00) and again in the afternoon (starting at 16:00); the third day was a resting recovery day with no exercise.
Results: On the first resting day, both saliva IgA and cortisol showed significant diurnal variation. Values for both were highest (89 ± 23 mg/l and 14.3 ± 2.3 nM for IgA and cortisol, respectively) at

08:00. Cortisol declined over the day, reaching its lowest value (3.5 ± 0.6 nM) at 20:00; IgA declined during the morning but was stable (around 57 ± 13 mg/l) from 12:00 onwards. The morning exercise delayed the diurnal fall in cortisol and temporarily increased IgA ($p<0.05$); the afternoon exercise bout was associated with a significant rise in both cortisol and IgA (both $p<0.05$). On the recovery day IgA levels were again highest in the early morning, but throughout the day were significantly lower (on average by 30%) compared with the day before exercise ($p<0.05$).
Conclusions: These data suggest that there is a delayed effect of exercise on IgA. The lower values seen on the day after exercise may be attributable to the delayed effects of the elevated glucocorticoid levels on the exercise day. Studies that measure saliva IgA responses to exercise should take into account both the diurnal variation of IgA and the possible effect of exercise performed the previous day.

VOLUNTARY EXERCISE INDUCES AN INCREASE IN KUPFFER CELL FUNCTION IN THE RAT

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Purpose: Voluntary exercise of rats in freely roading work wheels has been extensively used, but Kupffer cell adaptations have been poorly documented. This study examined the effects of voluntary exercise on Kupffer cell function; including phagocytic capacity, production of tumor necrosis factor-α (TNF-α) and nitric oxide (NO) and tumor cytotoxicity.
Methods: Female Fischer 344 rats were randomly assigned to control (C) and voluntary exercise (VE) groups on wheels for 10 weeks. Immunohistochemical staining of Kupffer cells in the liver was done with anti ED2 monochronal antibody. Isolated Kupffer cells were cultured in 10%FCS DMEM with lipopolysaccharide (LPS), the phagocytosis index (PI) was estimated by latex beads, and the supernatants were analyzed for TNF-α and NO. In addition, AH70B, which is hepatoma cell line, was added to the supernatant, after which cytotoxicity was estimated by the MTT assay.
Results: The number of Kupffer cells in the liver between the two groups did not differ. The PI of the cultured Kupffer cells in the VE rats was significantly greater than that of cells in the C rats. LPS was found to cause concentration-dependent TNF-α and NO releases from the Kupffer cells. Furthermore, the Kupffer cells from the VE rats produced more release of TNF-α and NO than those from the C rats. However, no group differences in the cytotoxicity to AH70B cells were seen in spite of treatment of the supernatant in the primary cultured Kupffer cells.
Conclusions: These results indicate that voluntary exercise can increase the phagocytic capacity and TNF-α and NO production of Kupffer cells. However, exercise may provide little protection against liver cancer.

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Both physically active and sedentary rats have stress-induced increases in brain IL1beta and heat shock protein 70 (HSP70)

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Exposure to stress can elevate brain interleukin 1beta (IL1) protein. Others have proposed that the increase in brain IL1 is responsible for many of the behavioral and immunological consequences of stress. Physical activity status also modulates the behavioral and immunological responses to stress. Physically active rats have less stress-induced behavioral depression and anti-KLH Ig suppression. The purpose of the following study was to test if physical activity also modulates the impact of stress on brain IL1. In addition, we also tested the potential impact of stress on brain heat shock protein (HSP70) expression. Male, F344 rats (4mos, 6/grp) lived with either mobile (active) or immobile (sedentary) running wheels. After 5 wks, rats were exposed to inescapable tailshock (100, 1.6mA, 5-s,

IS) or no stress. Brains were removed, dissected, and flash frozen 90 min after IS termination. Tissues were homogenized. IL1 (R&D) and HSP70 (StressGen) were measured by rat specific ELISAs. Stress reliably elevated both brain IL1 and HSP70 protein levels in hypothalamus, nucleus tractus solitarus (NTS), and pituitary, but not in prefrontal cortex or hippocampus. Physical activity did not alter the impact of stress on brain IL1 and HSP70. These data both replicate and extend previous observations that stress induces brain cytokines in the absence of pathogenic stimulation. Further investigation into the potential impact of physical activity on the duration of the brain IL1 and HSP response, as well as the possible role of brain HSP70 in triggering the IL1 response is currently in progress. (NIH-RO3 AI45576, RO1 AI48555).

METABOLIC ALTERATIONS IN LYMPHOCITES AND MACROPHAGESIN RESPONSE TO A LARGE INCREASE IN TRAINING VOLUME

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Purpose: Overtraining is a chronic fatigue syndrome that causes decreases in performance as well as metabolic and hormonal alterations showing also immunosupressant elements. It's been extensively reported that athletes suffering from the overtrainig sindrome show an immunologic screening weakened therefore increasing the risk of infectious diseases. Among the several possible causes to the imunnosupression concurrent with this sindrome, we can point out the increase on cortisol and the decrese on plasma glutamine. As there's been very little work on wich observations of alterations on important immune system cells as lymphocytes and macrophages were made, it became the focus of this study.
Methods: Lymphocytes from the mesenteric lymphnode and macrophages from the peritoneum were used from rats that were divided between the following groups: MOD, animals trained 1 for a day during 6 weeks; OR, animals trained for 1 a day during 5 weeks and in the last 3 days of the last week were submited to 3 bouts of exercise 2 and a half hours apart each and OT, animals that trained 1h a day for 5 weeks and in the 6^{th} week the training volume was increased to 3 bouts of exercise 2 and a half hours apart each. Training was done in a swimming system with warm water ($32 \pm 1°C$). The animals had a weight equal to 5% of their body weight attached to their tails. After the sacrifice the macrophages and lymphocites were incubed with glucose and glutamine to determine their metabolic behavior.
Results: There was a significant increase in glutamine consumption (9.68 ± 2.76 vs 43.09 ± 11.29) and glutamate production (1.21 ± 0.52 vs 11.24 ± 0.53) from lymphocytes of the OR group in comparison to MOD group. We also found a smaller consumption of glutamine on the OT group (3.07 ± 1.54) in comparison to the MOD group (9.68 ± 2.76) and an increase in the glutamate production of this group in comparison to the MOD group (5.76 ± 1.21 vs 1.21 ± 0.52). Macrophages had an increase on the consumption of glutamine on the OT group in comparison to MOD group (8.09 ± 1.87 vs 13.20 ± 2.64) and there has been an increase in the production of glutamate on the OT group in comparison to the OR group (4.96 ± 1.08 vs 50.19 ± 11.86). There was no significant alterations on the amount of plasma glutamine between the different groups, but there was a significative increase on the corticosterone concentration on OT group versus MOD group (6.05 ± 0.52 vs 2.368 ± 0.216).
Conclusion: We conclued that the protocol used in our study promotted changes in lymphocytes and macrophages metabolism having thus implications on the atlhetes' immune system.
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EFFECT OF STRENGTH TRAINING UPON IMMUNE SYSTEM FROM RATS

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Purpose: Nowadays there is an increasingly interest about the effects of endurance training on the immune system mainly because of its known immunosupressant effect when it's of high intensity. However little attention has been given to anaerobic training or intermittent training as the protocols suggested to increase strength. There were no reports within our knowledge of studies that evaluated the effects of strengh training on macrophages and lymphocytes metabolism, which are two of the

most important cells of the immune system. That's the reason this study wanted to evaluate the effects of a strength training protocol on the metabolism of lymphocytes from the mesenteric lymphonode and macrophages from the peritoneum of rats incubated with glucose and glutamine, two of the main substrates for this type of cells.
Methods: The animals were divided in three groups: TRE submitted to 6 weeks of training that consists of 3 sets of 15 repetitions with 75-80% of 1 RM once a day during 5 days a week stimulated by electric shock. C-Ch group that was not submitted to training but was stimulated by similar electric shocks to those of the trained animals and a COM group that didn't train and was not submitted to electrical stimulation.
Results: There was a significant raise on the glutamine consumption of lymphocytes from the animals of the TRE group in comparison to the controls as well as a increase in the production of glutamate in this group in comparison with the controls. On the contrary, there was no difference on the consumption of glucose and production of lactate as well as on the aspartate production. Regarded to the macrophage metabolism it was observed an increase in the production of glutamate on the control group stimulated by electrical shock in comparison to the controls as in comparison to the trained animals. There were no differences in the other parameters evaluated.
Conclusions: Our results indicate that our protocol can promote alterations on the lymphocyte metabolism and might have implications on the immunity of the studied animals.
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ROLE OF KUPFFER CELLS IN ACUTE MODERATE EXERCISE IN *IN VIVO* FEMALE FISCHER 344 RATS

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Purpose: This study investigated the function of Kupffer cells, and particularly their role as the first immunocompetent cells to come into contact with gut-derived lipopolysaccharides (LPS) in the acute exercise of rats. **Methods**: Female Fischer 344 rats were run on a treadmill at 21 m/min for 60 min on a 15% grade. After being injected with latex particles, exercised and resting rats livers were collected and examined for the phagocytic activity of Kupffer cells. We also measured the plasma endotoxin concentration in the superior mesenteric vein, the CD14 expression of Kupffer cells in liver, the structure of the small intestine, and plasma corticosterone and thyroxine4 (T4) as mediators of macrophage phagocytosis. In addition, we estimated liver damage and the concentration of plasma tumor necrosis factor-α (TNF-α). **Results**: Latex particles were observed in the hepatic sinusoid, and an increase in the number of latex particles phagocytosed by each Kupffer cell was noted. When it was noted that the small intestine was damaged by the exercise, it was found that the plasma endotoxin concentration in the superior mesenteric vein was significantly higher in the exercise group than in the resting rats. In addition, Kupffer cells maintained their expression of CD14. Plasma corticosterone and T4 levels were unchanged. Although plasma aspartate aminotransferase, alanine aminotransferase and guanase activities were slightly increased by acute exercise, plasma TNF-α from the portal vein was not detected.
Conclusion: The results of this study suggest that the increase in phagocytosis by Kupffer cells might be induced by endotoxemia of the portal circulation caused by intestinal mucosal lesions resulting from the acute exercise. In addition, in spite of the increase in Kupffer cell phagocytosis, the TNF-α production level in Kupffer cells was very low in acute exercise.

ACUTE EXERCISE SUPPRESSES TUMOR NECROSIS FACTOR-αEXPRESSION IN THE RAT LIVER

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Purpose: Tumor necrosis factor-α (TNF-α) is an inflammatory cytokine in Kupffer cells, which is induced by an endotoxin (lipopolysaccharide [LPS]). However, this cytokine is not produced during acute exercise in cases in which endotoxemia of the portal circulation has occurred because of

intestinal mucosal lesions. In our study, we investigated the effect of acute exercise on TNF-α expression in the rat liver.
Methods: Female Fischer 344 rats were run on a treadmill at 21m/min for 60 min on a 15% grade. Prior to running and immediately, one, and three hours afterward, the rats were sacrificed and blood samples and livers were collected to determine plasma TNF-α and interferoṇ-γ (IFN-γ) concentrations using the ELISA, and TNF-α mRNA in the liver using the RT-PCR. The plasma endotoxin concentration in the superior mesenteric vein was also analyzed by the endospecic method. In addition, plasma prostaglandin E2 (PGE2), which is an inhibitor of TNF-α release from Kupffer cells, was measured by the EIA. Primered cultured Kupffer cells were stimulated by LPS with or without PGE2, and then were analyzed for TNF-α production.
Results: Plasma TNF-α was not detected, and TNF-α mRNA expression did not significantly increase. However, the plasma endotoxin level in the superior mesentric vein significantly increased immediately after acute exercise. Plasma IFN-γ, which accelerates TNF-α production, was not detected. Plasma PGE2 markedly increased immediately after exercise. In the in vitro experiment, PGE2 inhibited TNF-α release from Kupffer cells in spite of LPS stimulation.
Conclusion: We concluded that the suppression of TNF-α in acute exercise might be due to an increase in PGE2 that inhibits LPS-induced transcription of the TNF-α gene.

INFLUENCE OF EXERCISE ON CELLULAR IMMUNITY IN ATHLETES AND OBESE PERSONS

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The aim of the study was to determine the acute effects of exhaustive exercise on the immune and endocrine systems in top sportsmen and in the group of obese adolescent.
Study design: We investigated 10 top sportsmen and 10 obese adolescent between the ages 16 to 18 years. Blood samples were collected before exercise, immediately after exercise on running belt and after 60 minutes of regeneration. Absolute and percentual counts of leukocytes, granulocytes, lymphocytes and their subsets (CD3, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^-CD16/56^+$, CD19) were determined by flow cytometer.
Results: Immediately after acute exercise we observed significant increase of absolute counts of T lymphocytes and their subsets – CD3, $CD3^+CD4^+$, $CD3^+CD8^+$ and NK cells followed by significant decrease after 60 minutes of regeneration. The same response in the immune system we saw in the group of the obese adolescent. The basal values before exercise were in the group of obese adolescent significant higher for granulocytes, lymphocytes and their subsets, except for cytotoxic/supressor T cells and NK cells. In the obese groups after exercise and after 60 minutes of regeneration the values of NK cells was significantly reduced. The results were compare with methods parametric and also non parametrics.
Conclusion: Our finding in obese subjects probably relate with correlation between higher body mass index and cell counts. These results are in consistent to published epidemiologic data and literature. The lowered values of NK cells are found out in obese subjects may be connected to lowered values observed after the exercise stress. On the other hand in top sportsmen with repeated exercise are very often a more pronounce immuno-endocrine response and higher parameters after exercise.

IMMUNOLOGICAL CHANGES IN HUMAN BLOOD AND SKELETAL MUSCLE IN RESPONSE TO ECCENTRIC AND CONCENTRIC EXERCISE

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Objective. This study was conducted to investigate leukocyte phenotypes (CD3, CD4, CD45, HLA-DR, CD62L, CD11b, CD95, CD14, Ki67), cytokines (IFN-γ, TNF-α, IL-2, IL-4, IL-5, IL-6, IL-10), hormones (testosterone, cortisol) and growth factors (LIF, LIFr, HGF, c-met, IGF-1, IGF-1r, IL-1β, IL-6), in human blood, skeletal muscle and fascia after exercise. **Methods.** One muscle biopsies was taken 48 h after 45 min of either downhill (4° or 8°) or uphill (4°) running and compared to controls. Antigens in skeletal muscle was analysed with immunohistochemistry and in blood with 3-colour flow cytometry. Hormones and serum proteins were analysed by standard methods. **Results.** Different exercise protocols resulted in slightly different expression of CD11b (granulocytes), HGF, LIF and IGF-1r in muscle and HGF and IGF-1r in fascia. Only dowhnill running resulted in muscle soreness. The magnitudes of the immunological changes were not related to eccentric component in the exercise protocol. Granulocyte infiltration was larger ($p<0.01$) in the dowhill 4° group (0.03% stained area) compared to the control group (0.01%) while the downhill 8° and uphill 4° groups did not differ from controls. The dowhill 8° group had significantly higher plasma CK ($p<0.025$) compared to downhill and uphill 4°. Plasma CK was related to running speed and not to protocol. In blood, exercise induced a significant increase (leukocytes, monocytes, CD8+ T-cells) and decreas (T-cells, CD62L on T-cells and CD11b, CD4 and CD45 on monocytes) in several phenotypes and antigen expression on leukocytes. Of the 43 phenotypes/antigens investigated only lymphocytes, monocytes and T-cells (CD4+ and CD8+) were affected differently by different protocols. **Conclusion.** Exercise of mainly eccentric mode did not result in significantly more leukocyte infiltration in human skeletal muscle compared to concentricly exercised or non-exercised muscle tissue. It is concluded that 1) strenous exercise, either concentric or eccentric, does not result in skeletal muscle inflammation 2) correlations between immunological variables in blood and muscle are strong 3) CK is not a reliable marker of muscle inflammation 4) several cytokines and growth factors are expressed in human muscle tissue and the expression is influenced by exercise.

INTERLEUKIN-6: AN EXERCISE-INDUCED GLUCOREGULATORY HORMONE?

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IL-6 is produced in contracting skeletal muscles. The transcription rate for IL-6 in the muscles was shown to be very fast. Arterial-venous differences over an exercising leg and the calculation of the net release of IL-6 demonstrated that IL-6 produced by the muscles more than account for the exercise-induced increase in plasma IL-6. Muscle glycogen availability was further demonstrated to be a determining factor for the rate of IL-6 production and release in contracting skeletal muscle. It is suggested that IL-6 act as a hormone and is involved in glucoregulatory processes by increasing glucose uptake and/or stimulating endogenous production in circumstances where the metabolic demand for glucose is increased such as during prolonged exercise.

INFLUENCE OF CARBOHYDRATE INTAKE ON IMMUNE RESPONSES TO EXERCISE

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To what extent can carbohydrate (CHO) intake alleviate exercise-induced immunosuppression? Some recent studies have examined the effect of CHO status and CHO ingestion before and during prolonged exercise on hormonal, cytokine and immune responses. If exercise is performed in a glycogen-depleted state (induced by prior exercise and several days on a low-CHO diet), the plasma adrenaline, cortisol and cytokine (e.g. IL-6) responses are larger than normal; plasma glutamine falls to lower levels; leukocyte trafficking including the neutrophil/lymphocyte (N/L) ratio is increased; but leukocyte functions, including neutrophil degranulation and lymphocyte proliferation seem unaffected. Ingestion of CHO during exercise also attenuates the rises in catecholamines, cortisol, IL-6, IL-10 and the N/L ratio but does not prevent the post-exercise fall in the plasma glutamine concentration. Consuming CHO during exercise also seems to attenuate some of the immune cell functional changes, including the fall in neutrophil function and mitogen-stimulated T-lymphocyte proliferation, but not Natural Killer Cell cytolytic activity. Regular ingestion of CHO containing drinks during exercise also helps to maintain saliva flow rate and hence IgA secretion rate compared with a restricted fluid

intake. These changes have been observed during prolonged continuous exercise of moderate intensity (~70% VO_2max) and fixed duration. CHO ingestion is less effective in modifying the immune perturbations associated with less demanding exercise of an intermittent nature such as football or rowing training. It is also apparent that CHO feeding is not effective in reducing immune cell trafficking and functional depression when exercise is performed to the point of fatigue. Feeding a bolus amount of CHO at 45 min before exercise does not seem to alter immune responses to prolonged exercise compared with placebo ingestion. In a recent study neither 25 g or 200 g of CHO (consumed as 5% and 40% glucose solutions, respectively) given 45 min pre-exercise had significant effects on the N/L ratio during prolonged cycling exercise compared with placebo fluid ingestion. However, 75 g CHO ingested at 15 min pre-exercise significantly reduced the N/L ratio during and after exercise compared with the same amount of glucose consumed at 75 min pre-exercise. However, neutrophil function was not affected by the timing of pre-exercise CHO ingestion. The conclusion is that CHO status does influence the immune response to exercise and that the biggest benefit appears to be derived from regular CHO intake during prolonged moderate intensity exercise that is not continued to the point of fatigue.

CD8 AND CD4 T CELL RESPONSES TO LYMPHOCYTIC CHORIOMENINGITIS VIRAL (LCMV) INFECTION FOLLOWING INTENSE EXERCISE IN YOUNG AND OLD MICE

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Purpose: Exhaustive exercise increases susceptibility to and severity of infections. CD8 and CD4 T cells play an important role in resolving a viral infection. We hypothesized that young and old mice undergoing intense exercise to exhaustion following LCMV infection would show suppression of CD8 and CD4 T cell responses.
Methods: One group of young (10-12 weeks) and old (22-24 months) C57BL/6 were exposed to a single bout of intense exercise to exhaustion and immediately infected with $2x10^6$ pfu of LCMV i.p. Eight days later, at the peak of expansion phase of T cell response, we used tetramers of MHC class I molecules containing viral peptides to directly visualize antigen specific CD8 T cells and a sensitive functional assay measuring interferon-γ production at the single cell level to quantitate the CD8 and CD4 T cell response. To evaluate the impact of intense exercise during both the initiation and evolution of the expansion phase of the T cell response; a second group of young and old mice continued their daily bouts of intense exercise to exhaustion over the next 8 days. A control group of young and old mice did not undergo intense exercise.
Results: Control, old mice showed significant decrease in the percentage and number of tetramer specific and intracellular interferon-γ producing CD8 and CD4 T cells when compared to control, young mice. Young mice exposed to single or multiple bouts of intense exercise to exhaustion showed significant decreases in the percentage and number of tetramer specific and intracellular interferon-γ producing CD8 and CD4 T cells when compared to control, young mice. However, old mice exposed to single or multiple bouts of intense exercise did not show a decrease in T cell responses when compared to control, old mice.
Conclusions: Data from young mice supports the hypothesis that mice undergoing intense exercise to exhaustion following LCMV infection would show suppression of CD8 and CD4 T cell responses. However, data from old mice is inconsistent with this hypothesis. We further conclude that while the widely accepted hypothesis that “intense exercise suppresses the immune system” may be true for young mice, the same may not be assumed for old mice.

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EXERCISE ALTERS THE KINETICS OF ANTIGEN-SPECIFIC RESPONSES TO INFLUENZA VACCINE IN THE ELDERLY

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Associated with aging is a decline of immune response, particularly T cell function. The purpose of this study was to determine whether exercise and psychosocial factors influence T-cell mediated

antigen-specific immune response to influenza immunization in adults over age 65. Adults described their exercise practices and those adults that participated in aerobic exercise > 2 times per week, >15 minutes per session were classified as EX whereas adults that did not meet that level of exercise participation were classified as CON. Forty-one older adults were immunized with the 2000-2001 influenza vaccine. Psychosocial surveys were administered at the time of vaccination. Blood samples were taken pre-immunization, 1,4, and 12 weeks post-immunization. Peripheral blood mononuclear cells (PBMC) were isolated and cultured with live viral antigens contained in the vaccine (Influenza A H1N1, Influenza A H3N2, Influenza B). Influenza specific lymphocyte proliferation was assessed in vitro. Influenza-specific Th1 (IL-2) and Th2 (IL-10) cytokine production were measured by ELISA. The results at 1 week post-immunization showed that EX was associated with a shift in the kinetics of IL-2 and IL-10 production in response to Influenza A H1N1 antigen. The production of IL-2 and IL-10 was significantly greater in EX compared to CON at early time points in vitro. The effect of exercise appeared to be antigen-specific, as only a trend towards enhanced IL-2 and IL-10 production was found with Influenza A H3N2 antigen challenge and no effect of exercise was found with Influenza B. Antigen-induced proliferation findings were similar to the cytokines. Again, a greater degree of proliferation was found in cells from EX compared to CON in cultures stimulated with Influenza A H1N1; a trend towards enhanced production was observed in cultures stimulated with Influenza A H3N2, and no effect of EX was found in cultures challenged with influenza B. Results of the psychosocial surveys indicated that greater incidence of hassles measured by the hassles/uplifts scale was associated with reduced IL-10 production. Statistical analyses also suggest that hassles may be a better predictor of IL-10 than exercise; after partialing out the effect of hassles, the effect size of exercise on IL-10 production was decreased. A greater number of hassles was also correlated with reduced proliferation. With respect to IL-2 production, greater scores of depression measured by the Yesavage Aged Depression scale were correlated with reduced IL-2 production to influenza A H3N2. Interestingly, by 4 weeks post-immunization, there no longer was an association between EX and cytokine production or proliferation, yet associations between psychosocial factors and immune parameters were still observed.
These results suggest that exercise may enhance immune response to influenza vaccine, however, the association between exercise and antigen-specific proliferation/cytokine production may be dependent on the antigen as well as the time point that the response is measured. The effects of psychosocial factors may be independent of the relationship between exercise and immunity or in some cases may mediate the association between exercise and the immune response.

THE EFFECT OF BELOW-AVERAGE, AVERAGE, AND ABOVE-AVERAGE PHYSICAL ACTIVITY ON SPECIFIC ANTIBODY PRODUCTION IN OLDER ADULTS

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The purpose of this study was to determine the effect of physical activity on the immune response to a defined antigen, in particular, the hemagglutinin-inhibition response to the H3N2 (A/Sidney/05/97) component of the 1998-99 Influenza virus vaccine. Forty older adults 67-93 years of age (mean 81$\pm$5) participated in the study. Physical activity was assessed using the Physical Activity Scale for the Elderly (PASE) (Washburn, Smith, Jette, & Janney, 1993). Participants were divided into three groups: average (+/- 1 SD from the mean); above (>+ 1SD from the mean) and below average (<+ 1SD from the mean). Plasma samples were collected prior to, one, two, four, and six weeks post vaccination. Titers were normalized by comparison to a standard pooled serum run with each assay and reported as the reciprocal of the highest dilution of serum that completely inhibited hemagglutination. An immune response was calculated as the log-base 2 increase in titer of a serum over the pre-bleed titer for that person. A repeated measures ANOVA was used to evaluate the effect of physical activity on the immune response to the H3N2 component of the vaccine. A significant interaction between group (below-, average-, and above-average physical activity) and time (week zero, one, two, four, and six weeks post vaccination) ($F=2.22$; $df=8$; $p=0.046$) was found. Tukey's post hoc analyses revealed that for weeks one and four post vaccination, the above average physical activity group had significantly higher titers compared to both, the average and below average groups. The results of this study suggest a positive relationship between physical activity and the production of specific antibody within this group of older adults.

THE CYTOKINE RESPONSE TO ECCENTRIC EXERCISE IN YOUNG VERSUS ELDERLY HUMANS

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Objectives: Plasma interleukin (IL)-6 is elevated in aged subjects at rest and it has been shown that plasma IL-6 is increased with exercise. Conflicting results exist regarding a relationship between IL-6 and muscle damage. The questions to be answered in the present study were, whether an age-related impaired IL-6 production exists and whether IL-6 was associated with exercise-induced muscle damage.
Methods: Ten elderly (median age 69, range 67-75) and 10 young (median age 24, range 20-27) subjects completed a high-intensity eccentric exercise program of 60 minutes of eccentric lower limb exercise. The elderly and young groups had the same relative increases in VO2max and pulse rate, but the elderly had a higher relative workload.
Results: The major finding was that the increase in IL-6 in response to eccentric exercise was less pronounced in elderly compared with young subjects. Furthermore, the increase in creatine kinase (CK) and myoglobin was less pronounced in the elderly. A clear association was found between absolute workload on the one hand and peak CK and peak myoglobin on the other. However, there was no correlation between workload and plasma IL-6 or between IL-6 and biochemical markers of muscle damage.
Conclusion: The present study did not support the hypothesis that the exercise-induced increase in IL-6 is caused by muscle damage. However, given that IL-6 is produced in the contracting skeletal muscle and that the elderly had less exercise-induced increase in IL-6, these data suggest that aged skeletal muscles may have an impaired ability to produce IL-6 in response to muscular contractions.

PRO-INFLAMMATORY CYTOKINE RESPONSE TO ACUTE AND CHRONIC RESISTANCE EXERCISE IN WOMEN AGED 65-89 YR.

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To examine the influence of acute and chronic resistance exercise (RE) on cytokine production in post-menopausal women, 35 subjects (age 72±6.2 yr) underwent a moderate- to high-intensity 10-week resistance-training program or served as sedentary controls. The women were assigned to one of 4 groups: no estrogen replacement (NHR, n=8), estrogen replacement (HRT, n=12), selective estrogen receptor modulator (SER, n=8), or control (no estrogen replacement, CON, n=7). After a one-week acclimation to RE, NHR, HRT, and SER performed 3 sets of 10 resistance exercises at 80% of their 1 repetition maximum (1RM) before (BT) and after (AT) the 10-week training period. The CON sat quietly in the lab during these experimental trials. Blood samples were collected pre-exercise (PR), immediately post-exercise (PO), and two hours (2H) post-exercise, or at the same time points for CON. Whole blood was diluted 1:10 in RPMI and incubated (37°C, 5%CO_2) for 24 hours with lipopolysaccharide (LPS, salmonella enteritidis, 25 μg/ml final concentration). Supernatant was analyzed for the inflammatory cytokines IL-6, IL-1β, and TNF-α using ELISA. Resistance training increased 8RM strength by an average of 30% for 10 exercises. Baseline (BT,PR) IL-6 production was greater in SER compared to NHR and CON (11,991, 7608, 7934 pg·ml^{-1}, respectively; p=0.021). There was also a significant training effect with mean BT IL-6 production being greater than AT (9435 vs 8677 pg·ml^{-1}, respectively; p=0.050). Post-exercise IL-6 production was significantly greater than PR and 2H (10,070, 8668, and 8431 pg·ml^{-1}, respectively; p<0.001). Training decreased PRE IL-1β production in HRT (BT=3394, AT=2272 pg/ml; p<0.001), SER (BT=4406, AT=2272 pg·ml^{-1}; p<0.032), and NHR (BT=3034, AT=2163 pg·ml^{-1}, NS p=0.055). Acute exercise increased IL-1β production (PO) (22-87%) in all exercise groups and it remained elevated or was further increased at 2H, depending on the group and training status (BT vs AT). There was also a significant rise in IL-1β production in CON at 2H. TNF-α production tended to be elevated after exercise for all exercise groups BT (PR=652, PO=748, 2H=598 pg·ml^{-1}; p=0.085, NS) and AT (PR=534, PO=750, 2H=670 pg·ml^{-1}; p<0.001). TNF-α production remained elevated at 2H for the AT trial (p=0.03). Training significantly decreased resting TNF-α production (p=0.045). Acute resistance exercise elevated IL-6,

IL1β, and TNF-α production in stimulated whole blood assays, whereas resistance training appeared to decrease resting production of these cytokines. The elevation of IL-1β at 2H in all exercise groups may be partially explained by a diurnal response observed in the non-exercise CON.

INFLUENCE OF AGE ON IMMUNE CHANGES IN RUNNERS FOLLOWING A MARATHON

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Immune changes in 75 younger (age 37.4±0.9 yr) and 23 older (57.0±1.4 yr) runners were compared following a competitive marathon race, with blood samples collected pre-race, immediately post-race, and 1.5-h post-race. The older compared to younger runners were more experienced (38.2±9.5 vs. 13.5±1.9 total marathons raced, p=0.018), trained a similar distance per week (58.8±5.8 vs 52.3±2.4 km/wk), but had a significantly lower aerobic power (45.2±1.7 vs. 51.2±0.6 $ml \cdot kg^{-1}min^{-1}$, p=0.003). Heart rate (HR) and rating of perceived exertion (RPE) were recorded every 3.2 km, and final race time measured in all runners during a competitive marathon race. Race time was slower for the older compared to younger runners (4.7±0.2 vs. 4.3±0.1 h, p=0.015), but both groups performed at a similar intensity (83.4±0.9 vs. 82.9±0.5 % HR_{max}, and 13.4±0.3 vs. 13.8±0.2 RPE, 6-20 scale). The pattern of change in cortisol, epinephrine, growth hormone, and blood neutrophil and natural killer cell counts did not differ significantly between younger and older runners following the marathon race. The pattern of change in blood lymphocyte counts did not differ between the groups, but were 20% to 24% lower in the older compared to younger runners at each time point. This difference was accounted for by a reduced T cell count in the older runners. Following the marathon race, plasma levels for IL-1ra, IL-10, IL-6, and IL-8 rose strongly in all runners, and no group differences in the pattern of change were noted. Saliva protein IgA concentration and sIgA secretion rate were significantly decreased for at least 1.5 h following the competitive marathon race, and age had no significant influence on these changes. We conclude that younger and older runners experience a similar hormonal and immune response to a marathon race when competing at the same relative intensity with the exception of lower blood lymphocyte counts in the older runners.

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CYTOKINE CHANGES AFTER A MARATHON RACE

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The influence of carbohydrate (1 liter/h of a 6% carbohydrate beverage), gender, and age on pro- and anti-inflammatory plasma cytokine and hormone changes was studied in 98 runners for 1.5 h following two competitive marathon races. The marathoners were randomly assigned to carbohydrate (C) (N=48) and placebo (P) (N=50) groups, with beverages administered in a double blind fashion during the races using color codes. Plasma glucose was higher and cortisol lower in C compared to P groups post race (P<0.001). For all subjects combined, plasma levels of IL-10, IL-1ra, IL-6, and IL-8 rose significantly immediately post-race, and were still above pre-race levels 1.5 h later. Post-race plasma levels of IL-1, TNF-, IL-2, IL-4, IL-12, and INF remained near pre-race or at non-detectable levels. The pattern of change in all cytokines did not differ significantly between the 12 women and 86 men in this study, and the n=23 subjects 50 yr and n=75 subjects <50 yr of age. The pattern of change in IL-10, IL-1ra, and IL-8, but not IL-6, differed significantly between C and P groups, with higher post-race values measured for IL-10 (109% higher) and IL-1ra (212%) in P, and IL-8 (42%) in C. In conclusion, plasma levels of IL-10, IL-1ra, IL-6, and IL-8 rose strongly in runners following a competitive marathon, and this was not influenced by age or gender. Carbohydrate ingestion, however, had a major effect in attenuating increases in cortisol and two anti-inflammatory cytokines, IL-10 and IL-1ra.

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INTERLEUKIN-6 EXPRESSION IN TYPE II DIABETICS: INFLUENCE OF ACUTE EXERCISE

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Purpose: We have recently demonstrated that contracting skeletal muscle produces interleukin (IL)-6 during exercise[1,2] and that expression and release of IL-6 during exercise is related to glycogen availability[3]. Since [plasma IL-6] is related to insulin resistance[4], the purpose of this study was to examine IL-6 expression in, and release from, the skeletal muscles of insulin resistant type II diabetics at rest and during exercise.
Method: Nine type II diabetic men (D: 48±1 years, 87±4 kg, 177±2cm, VO_{2max}=2.47±1.48) and 8 control subjects (C: 46±2 years, 87±4 kg, 183±2cm, VO_{2max}=2.37±1.98), underwent 25 min of supine bicycle exercise at 60% VO_{2max}. Blood samples were obtained from a femoral artery and vein from one limb before and after exercise and analyzed for IL-6. Leg blood flow (LBF) was measured by thermodilution in the femoral vein, and net leg IL-6 release was calculated as the product of LBF and femoral arterio-venous (AV) IL-6 difference. In a separate group of subjects (6 D; 4 C), resting muscle biopsies were obtained and analysed for IL-6 mRNA using real-time PCR.
Results: 25 min of exercise increased (P<0.05) IL-6 release in both D and C and although not statistically significant, there was a trend (P=0.11) for augmented exercise-induced IL-6 release in D compared with C [5.6 ± 1.8 vs 30.6 ± 17.2 (mean ± se) ng/min for C and D respectively]. Likewise, although not statistically significant, there was an approximate 10 fold higher IL-6 gene expression in D relative to C.
Conclusions: These data demonstrate that during supine bicycling, 25 min of exercise is sufficient to result in a net release of IL-6 from contracting muscle. In addition, although preliminary, these data suggest that IL-6 expression and release from skeletal muscle may be related to type II diabetes and insulin resistance.

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