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Review Lectures

Aging - Cytokines - Exercise

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Strenuous exercise induces increased levels in a number of pro-inflammatory and anti-inflammatory cytokines, natural occurring cytokine inhibitors and chemokines. Thus, increased levels of tumour necrosis factor (TNF)- , interleukin (IL)-1, IL-6, IL-1receptor antagonist (IL-1ra), TNF-receptors (TNF-R), IL-10, IL-8 and macrophage inflammatory protein (MIP)-1 are found after strenuous exercise. Within 30 minutes of running IL-6 is elevated in plasma; heart rate during running was correlated to the peak in IL-6. The latter cytokine can be detected in skeletal muscle biopsies collected after strenuous running. However, the relationship between muscle damage, delayed onset muscle soreness and cell infiltration in skeletal muscle remains unclear at this moment.
Aging is associated with increased inflammatory activity. Increased plasma levels of TNF- were found in centenarians aged 100 years and in individuals aged 80-81 years when compared to a young control group. Plasma-levels of TNF- were linearly correlated to plasma levels of IL-6, TNF-receptors, C reactive protein, and serum amyloid. High levels of TNF- were independently related to dementia and to a low ankle-arm index, indicating arteriosclerosis. In hospitalized patients with Streptococcus pneumonia infection, aging was associated with prolonged inflammatory activity. Similar results were found using an in vivo endotoxin challenge model in old versus young individuals.
Thus, aging is associated with increased and prolonged inflammatory responses. This review will discuss if aging influences the inflammatory response to exercise.

HORMONAL REGULATION

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Aging is associated with a functional decline in several components of the immune system. As a result, the elderly are more vulnerable and are at greater risk for a variety of infectious diseases, tumorigenesis, and autoimmune disorders. Cell-mediated immune function is most adversely affected which is directly related to the

well-documented involution of the thymus. The extent to which regular exercise affects the immune system in older populations is less understood. While it is known that immune function deteriorates with advancing age, the influence of exercise intervention remains unclear. The major questions which need to be addressed are: 1) does participation in regular exercise alter immune function; 2) does this translate into reduced risk for infectious diseases, cancer, and autoimmune disorders and; 3) how does acute bouts of exercise affect immune function in this population. The clinical significance is apparent as the age-related decline in immune function can contribute to increased mortality from various causes. It is clear that the neuroendocrine system plays a critical role in modulating immune function in response to both an acute bout as well as chronic endurance exercise in young populations. However, the aging process is associated with functional declines in many facets of the neuroendocrine system including alterations in receptor number, density and affinity, diminished receptor responsiveness and various components of signal transduction as well as a reduction in the ability to synthesize and release hormones and neurotransmitters. Consequently, elderly individuals are likely to respond differently to both acute and chronic exercise stimulation.
Specifically, age-related changes exist in overall catecholamine metabolism and receptor responsiveness, tissue-specific sympathetic nerve activity, stress-induced corticosteroid production and effectiveness, and sensitivity to PGE2 including T lymphocytes. Additionally, partial restoration of immune function has been observed from administration of thymic hormones. These possible neuroendocrine mechanisms will be discussed in relation to both age and exercise.

NEUROENDOCRINE REGULATION OF ANTIBODY RESPONSES

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Antibody plays an important role in acquired immune responses. The goal of my presentation is to first review the cellular processes involved in the generation and function of antibody in immune responses. Second, I will describe evidence of neuroendocrine regulation of antibody responses. Third, I will discuss the advantages of measuring antigen-specific versus total antibody levels for understanding behavioral (i.e., exercise or stress) regulation of antibody responses. Finally, I will present recent experimental evidence in support of the hypothesis that a physically active lifestyle prevents stress-induced immunosuppression.
Using an established animal model of stress, I have investigated the effect of acute stressor exposure on the development of a specific antibody response to a benign protein, keyhole limpet hemocyanin (KLH). Measurement of specific antibody levels in the blood after challenge with KLH provides an excellent measure of the in vivo immune response and the cellular mechanisms are well understood. Specifically, stressed rats have a reduction in anti-KLH IgM, IgG, IgG2a, but not IgG1. This reduction is still present 4 weeks after stressor termination and KLH immunization. I have previously reported that the reduction in this response is due to suppression in the formation of anti-KLH T helper cells (specifically Th1) and reduction in IFN(, a Th1 cytokine. IFN(is a pluripotent cytokine. One function of IFN(is to stimulate B

cells to make IgG2a (and not IgG1). Current evidence supports the conclusion that stress suppresses the formation of KLH-specific T helper cells, which leads to less IFN(, fewer anti-KLH B cells, and less anti-KLH IgG2a. We have evidence that 4 weeks of freewheel running prior to stressor exposure prevents both the stress-induced suppression in IFN(and anti-KLH antibody, perhaps by blunting sympathetic output. These data add to our understanding of the health benefits of regular, moderate, physical activity.

NEUROENDOCRINE REGULATION OF NK-CELLS

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Natural immunity, including the Natural Killer (NK) cells, is strongly influenced by physical exercise but the physiological significance of the reported changes in NK-cells after exercise training is as yet unclear. The exercise effects includes interaction between the nervous, endocrine and immune systems and central mechanisms including the endogenous opioids are here of great interest. Chronic activation of endogenous opioid systems augments natural cytotoxicity and the possible involvement of opioids in the exercise-induced enhancement of natural immunity is discussed. The pathways by which the central nervous system may communicate with the periphery include neuroendocrine outflow, via the hypothalamic-pituitary-adrenal-axis and the autonomic nervous system (ANS), through direct nerve fiber connections with cells or the organs of the immune system. Thus, the role of various neuroendocrine factors such as growth hormone, catacholamines and glucocorticoids will be discussed as well as the possible role of the ANS, in particular the sympathetic division.

NEUROENDOCRINE REGULATION OF MACROPHAGE FUNCTION

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The overall objective of this review is to introduce to ISEI members current information regarding the role of neuroendocrine factors in mediating stress-induced changes in macrophage function. Under this broad objective this review will: 1) briefly discuss the role of the macrophage in host defense, 2) discuss macrophage cell biology as it pertains the signals that cause macrophage activation, 3) describe the effects of stressors such as exercise on the functions of macrophages, and 4) describe the regulatory influence of various neuroendocrine factors (glucocorticoids, catecholamines, growth hormone, prolactin, substance P, cytokines and others) that potentially mediate these effects. Recent work from our laboratory will highlight the

effects of different doses of exercise on macrophage functions such as activation for tumor cytolysis, antigen presentation and effector molecule production. This review will be of value to exercise immunology researchers in that potential mechanisms as to how exercise/stress affect macrophage function will be highlighted.

VITAMIN E, IMMUNE RESPONSE, AND INFECTIOUS DISEASES: FACT OR FICTION?

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After vitamin C, vitamin E is the vitamin most revered by the public for its capability to preserve health. Health benefits ranging from enhancement of immune function, cancer prevention, and reduction of risk for cardiovascular disease, to prevention of wrinkles and improvement of physical and sexual performance have been attributed to vitamin E. Studies over the years have disproved some of these claims, but have confirmed others.

The role of vitamin E in the maintenance of the immune system has been studied in context of both deficiency and excess using naive and immunologically challenged animal models and humans. Many studies have shown that vitamin E deficiency impairs the immune response, an effect mainly attributed to its function as an antioxidant nutrient, whereby it protects the highly polyunsaturated fatty acids of immune cell membranes against deleterious effects of free radicals. In addition, vitamin E has been shown to regulate signal transduction and gene expression. The vitamin E-induced impairment of immune function is associated with increased mortality from infectious diseases in animals.

An interesting feature of vitamin E and its regulation of the immune function is that supplementation with higher than recommended levels has been shown to enhance certain aspects of the immune response in both animal and human models. Vitamin E supplementation might be particularly effective in enhancing immunity in the aged because free radical formation and lipid peroxidation increase with age. In addition, age-associated dysregulation of the immune response is well documented. Increased production of suppressive factors such as lipid peroxides, eicosanoids, and nitric oxide (NO), contribute to the age-associated dysregulation of the immune response. Vitamin E supplementation of old mice has been shown to significantly improve in vitro and in vivo indices of T cell mediated function while significantly decreasing prostaglandin (PG) E2 and NO production. Co-culture experiments using specific metabolites and inhibitors indicated that vitamin E exerted its immunostimulatory effect mainly through reduction of M(PGE2 production.

Recently completed human studies showed that supplementation with vitamin E significantly improves in vivo indices of T cell-mediated function, including delayed-type hypersensitivity skin response and antibody production in response to vaccine. In addition, a trend toward reduction in incidence of self-reported infections was observed. These prelimiary observations suggest that the immunostimulatory effect of vitamin E in humans might be associated with increased resistance to infectious diseases. Results from animal and epidemiological studies support this notion. Of particular interest is our recent obeservation that supplementation of old mice with

500 ppm/day of vitamin E significantly decreased lung viral titer in influenza-infected mice. Others have reported protection against viral infection as well. Further studies are needed to confirm the protective effect of vitamin E against infectious diseases in humans.
To examine the interactions among vitamin E, exercise, and immunity in elderly persons, we conducted experiments in which young (22-29 y) and old (55-74 y) subjects were supplemented with 400 mg all-rac-(-tocopherol twice daily for 48 days before they underwent eccentric exercise (downhill running). Vitamin E supplementation eliminated the age-associated difference in exercise-induced neutropenia and plasma creatine kinase, prevented the exercise-induced rise in IL-1, and inhibited production of IL-6. Because IL-1 and IL-6 have been implicated in the inflammatory process, the acute phase response, and exercise-induced muscle proteolysis and damage, their inhibition by vitamin E during damaging exercise could have practical implications.
Thus research to date supports an important role for vitamin E in regulation of the immune system. Higher than recommended levels of vitamin E improve the waning immune response of the elderly. Whether this improvement is associated with clinical benefits, such as reduction in incidence of infectious diseases in humans, needs to be determined.

MUCOSAL IMMUNOEPIDEMIOLOGY OF RESPIRATORY ILLNESS IN ELITE ATHLETES

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This review will focus on studies of immunity in elite athletes and specifically address the role of mucosal immunity and respiratory illness. The last ten years of investigations have revealed significant associations between the intensity and duration of exercise, mucosal immunosuppression and their role in respiratory illness. Most studies of mucosal immunity have chosen to study the response to exercise using salivary immunoglobulins, although other mucosal secretions have been studied. Exercise at an elite level causes mucosal immunosuppression. Salivary IgA and IgM concentrations decline immediately after a bout of intense exercise and recover within 12 hours. Longterm training at an elite level can result in a chronic suppression of mucosal immunoglobulin levels and in some endurance sports a decline over the training season has been observed. The degree of suppression is associated with the intensity of the exercise and the duration or volume of the training session. Low levels of salivary IgA and IgM are associated with an increased risk of respiratory illness. The association resides with the lower levels of IgA1 subclass. The nature of the respiratory illnesses is still uncertain. Bacterial pathogens are unlikely. The clinical presentations support viral infections and recent data indicated EBV viral reactivation may be a significant cause. The appearance of EBV-DNA in saliva appears to precede the clinical symptoms and be associated with periods of mucosal immunosuppression reflected by low levels of salivary IgA. Despite mucosal and systemic immunosuppression elite athletes are capable of normal vaccine responses to novel oral and systemic vaccinations.

ELITE ATHLETE IMMUNOLOGY: THE IMPORTANCE OF NUTRITION

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Immunosuppression in athletes involved in heavy training is undoubtedly multi-factorial in origin. Training and competitive surroundings may increase the athlete´s exposure to pathogens and provide optimal conditions for pathogen transmission. Heavy prolonged exertion is associated with numerous hormonal and biochemical changes, many of which have detrimental effects on immune function. Furthermore, improper nutrition can compound the negative influence of heavy exertion on immunocompetence. An athlete exercising in a carbohydrate-depleted state experiences larger increases in circulating stress hormones and a greater perturbation of several immune function indices. The poor nutritional status of some athletes may predispose them to immunosuppression: for example, dietary deficiencies of protein and specific micronutrients have long been associated with immune dysfunction. An adequate intake of iron, zinc and B vitamins is particularly important but the dangers of over-supplementation should also be emphasised; many micronutrients given in quantities beyond a certain threshold will in fact reduce immune responses and may have other toxic effects that are detrimental to health. Although it is impossible to counter the effects of all of the factors that contribute to exercise-induced immunosuppression, it has been shown to be possible to minimise the effects of many factors. Athletes can help themselves by eating a well balanced diet which includes adequate protein and carbohydrate, sufficient to meet their energy requirements. This will ensure a more than adequate intake of trace elements without the need for special supplements. Consuming carbohydrate (but not glutamine) during exercise attenuates rises in stress hormones such as cortisol and appears to limit the degree of exercise-induced immunosuppression. By adopting sound nutritional practise, reducing other life stresses, maintaining good hygiene, obtaining adequate rest and spacing prolonged training sessions and competition as far apart as possible, athletes can reduce their risk of infection.

CLINICAL PERSPECTIVES

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Early medical diagnosis, adequate therapy management and appropriate return-to-play-decisions are important to maintain the elite athletes health and are prerequisites to minimize the loss of performance capacity during infections. The upper respiratory tract (URT) is the most common focus of localized infections and frequently serves as the entry for generalized infections. This review shall provide critical information about the actual situation of medical care of elite athletes in Germany, the most common external and internal reasons of URTI in elite athletes

and about how far immunological lab parameters may help with the return-to-play-decision. In a first trial the exercise immunology literature was looked for the question how far the exercise-induced alterations of immune parameters in elite athletes was associated with clinically relevant illness or disease. Secondly a search for reasons of the sudden death of elite athlets was done. Finally elite athletes consulting the local sports medical care unit for reasons of infections with URT symptoms served as a study collective to answer the question how far immune parameters might help with the diagnosis of the severity of the infection and the return-to-play decision. The majority of studies reporting an enhanced risk of URT infections following single exercise events or intensified training periods fail to show the pathogenic mechanism. Vice versa studies indicating alteration of immune parameters are usually unable to proove an enhanced incidence of URT infections as a result of exercise-induced immune parameter changes. However, under certain circumstances strenuous exercise seems to make the elite athlete more susceptible to infections, but intervention studies are missing. The most common reasons for the sudden death of elite athletes are hypertrophic cardiomyopathy and myocarditis. The diagnosis of the latter is difficult, needs experienced physicians and adequate diagnostic procedures. The exclusion of a myocarditis during URT infections is important and essential for the return-to-play-decision of elite athletes. Immune parameters like monocyte subpopulations, phagocyte function assays, and humoral factors of the acute-phase-response may be helpful with the very early diagnosis of generalized infections starting with URT symptoms. But the return-to-play-decision remains a complex mixture of clinical symptoms, lab parameters, ECG and echocardiography.

TRAINING STRATEGIES TO MAINTAIN IMMUNOCOMPETENCE IN ATHLETES

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Clinical experience and empirical evidence have lead to the modelling of exercise and training as a form of stress on the immune system. Coaches, athletes and medical personnel are seeking guideless on the ways to reduce the risk of illness compromising training or competition performance. The immune system is influenced by a wide range of physical, psychological, behavioural and environmental factors, which collectively form the basis of the following intervention strategies: i) Training: Careful management of training volume and intensity, variety to overcome training monotony and strain, a periodised approach to increasing loads, and provision of adequate rest and recovery periods; ii) Environmental: Limiting initial exposure when training or competing in adverse environmental conditions (heat, humidity, altitude, air pollution), and acclimatising where appropriate; iii) Psychological: Teaching athletes self-management and coping skills and monitoring of athletes responses to the psychological and psychosocial stresses of high-level training and competition; iv) Behavioural: Adoption of a well-balanced diet with adequate intake of macro-and

micro-nutrients, limiting transmission of contagious illnesses by reducing exposure to common source infections, airborne pathogens and physical contact with infected individuals; and v) Clinical Considerations: medical screening, pathology testing, immunization and prophylaxis, and routine management of illness-prone athletes. Future experimental studies are required to confirm the effectiveness of these strategies in reducing illness in athletes.

COMMENTS ON CLINICAL APPLICATIONS IN ATHLETES

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As presented in the two previous talks, the sports community is interested in preventing illness in athletes and knowing when to allow athletes to return to training after viral illness. At present, there is insufficient empirical evidence to support specific guidelines. There are several key issues which must be resolved before more specific and helpful guidelines are developed. (1) The etiology of URTI symptoms in athletes needs to be understood, whether such symptoms arise from infectious agents or other causes, and if infectious, the particular pathogens involved. Such information is necessary to develop prevention and treatment strategies, and to know when viral myocarditis is of potential concern. (2) Further information is needed about what aspects of training and competition increase risk of URTI symptoms, so that athletes and coaches may know which parts of a training regime to modify to prevent illness. (3) Although risk of URTI may increase in athletes, no clear mechanism has yet been identified to link alterations in immune function with illness; it is likely to be a complex relationship. (4) Recent data suggest that the immune response to exercise may be altered by nutritional supplements such as carbohydrate, vitamin C, and glutamine. It is not yet known whether these can reduce risk of URTI symptoms over the long-term. (5) Finally, it is possible that, in athletes, minor illnesses such as URTI may be protective by providing needed rest from the rigors of training. If URTI can be prevented in athletes, we should be concerned about possible increase in the incidence of overtraining syndrome which has more serious and far-reaching consequences.

FUTURE DIRECTIONS FOR RESEARCH RELATED TO ATHLETES, NUTRITION, AND THE ELDERLY

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Three topics that have received much attention from exercise immunologists will be reviewed in this commentary, with an emphasis on what is currently known and directions for future research. 1) Immune function in athletes and nonathletes, and

acute immune response to heavy exertion; 2) Role of nutritional supplements in attenuating exercise-induced immunosuppression; 3) The influence of moderate exercise training on host protection and immune function in both young and old adults. In general, it will be concluded that the immune system is influenced acutely and to a lesser extent chronically by exercise. Epidemiological and experimental data suggest that moderate exercise enhances immunosurveillance and host protection from upper respiratory tract infection and cancer in young and old adults, while heavy exertion by endurance athletes leads to transient immunosuppression and increased risk of infection. Additional research should provide athletes a clearer understanding of underlying mechanisms, clinical applications, and the development of appropriate countermeasures.

FREE COMMUNICATION

EFFECT OF FITNESS TRAINING ON IN VIVO AND IN VITRO IMMUNE PARAMETERS IN HEALTHY ELDERLY ADULTS (A RANDOMIZED CONTROLLED STUDY)

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The potential ability of fitness training to reverse immune senescence in elderly adults has been suggested based upon observations of in vitro restoration of attenuated immune parameters after physical training. Considering that in vitro examination does not necessarily reflect in vivo immune potential, we performed a randomized controlled study to assess the effect of 6-month fitness training program on in vivo and in vitro immune parameters. Skin reaction to Tuberculin PPD, a Th 1 dependent antigen, was assessed as in vivo immune measure. Th1/Th2 balance was assessed as in vitro immune measure by flow-cytometric analysis of intracellular staining of IFN-gamma (Th1 cytokine) and IL-4 (Th2 cytokine) of PMA+ionomycin stimulated peripheral blood mononuclear cells (PBMC). Sixty-six healthy elderly volunteers (age: 67.2 ± 4.1 y.o.; male/female=30/36) were randomly assigned to either fitness training group (ET) or to sedentary control group (C). All the subjects had no active mycobacterium infection as confirmed by chest X-ray examination and negativity for serum CRP. The subjects in group ET underwent a fitness training program consisted of aerobic exercise on cycle ergometer (50-60% Vo_{2max}) and resistance exercise using rubber band for 6 month (2h day^{-1}, 2-3 days $week^{-1}$). Subjects in group C led normal lives during the 6 month period. Blood sampling and PPD Mantoux skin test was performed on 48 out of 66 subjects (C=19, ET=25) before and after the program. Skin-reaction was recorded after 48hr by a CCD-camera and the area of skin rush was measured. Mean maximal oxygen uptake

(Vo_{2max}) increased significantly in group ET after the program. The area of PPD skin reaction increased significantly in group ET after the program. TH1 population remained unchanged but there was a increase in turn of Th2 population in group ET, resulting in the reduction of Th1/Th2 ratio in group ET after the program. No significant change was observed in neither of the analyzed parameters in group C. These results suggest that fitness training has a potential ability to potentiate immune reactions in vivo in elderly adults. The apparent discrepancy in the results of in vivo and in vitro examination possibly arose from limitation of in vitro non-specific testing using PBMC.

MECHANISMS AND KINETICS OF EXERCISE TRAINING-INDUCED INCREASES IN MACROPHAGE TUMOR KILLING IN YOUNG AND OLD MICE

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Aging is associated with detrimental changes in macrophage (MΦ) function including hyporesponsiveness to activating signals such as IFN-γ and LPS. We sought to determine whether 4 months of treadmill running (EXC: 5 $d.wk^{-1}$, 45 $min.d^{-1}$, 18-22 $m.min^{-1}$) would improve MΦ responsiveness in young (Y, 6 mos.), middle-aged (MA, 12 mos.), and aged (A, 22mos.) male Balb/c mice when compared to sedentary controls (CON). Resident peritoneal MΦ´s (> 90% Mac-3) were obtained 24 hr after the last exercise bout, incubated with various concentrations of IFN-γ and LPS for 18 hrs, and tested for cytolytic ability vs. P815 cells.

We found that aging resulted in a significant reduction in the ability of MΦ´s to respond to the highest doses of IFN-γ and LPS and kill P815 cells (46 ± 4%, 37 ± 6%, 34 ± 2% killing in Y, MA, A mice respectively). Exercise training significantly increased MΦ cytolysis in all age groups (66 ± 7%, 47 ± 5%, 44 ± 2%, in Y, MA, A mice respectively), the magnitude of this effect was dependent on age such that Y > MA > A. MΦ´s from young exercised mice also produced significantly (~50-60%) more NO_2, there was a tendency for higher NO_2 in A exercisers. The inducible nitric oxide synthase (iNOS) inhibitor N^o-mono-methyl-L-arginine (NNMA) significantly reduced MΦ cytolosis and NO_2-production and completely abrogated exercise-induced increases in these measures. RT-PCR and western blot analysis revealed significantly higher iNOS mRNA and protein levels in MΦ´s obtained from EXC vs. CON and significantly lower iNOS mRNA in A when compared to Y mice. Examination of kinetics of the response in Y mice indicated no change in MΦ responsiveness after 1, 2, or 4 wks of training, however, a small change was evident at 8 wks. We conclude that aging reduces, and exercise training increases, the capacity of resident peritoneal MΦ´s to respond to IFN-γ and LPS with increased tumor cytolysis. The development of this effect requires at least 8 wks of training and

is age-dependent. Enhanced iNOS gene and protein expression and NO_2-production are likely the contributing mechanisms of the exercise-induced enhancement of cytolysis in young mice, but can´t entirely explain increased tumor killing in old mice.

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EXERCISE INDUCES RECRUITMENT OF LYMPHOCYTES WITH AN ACTIVATED PHENOTYPE AND SHORT TELOMERES IN YOUNG AND ELDERLY HUMANS

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This study was performed to research the type of T cells recruited to the blood in response to an acute bout of exercise with regard to mean lengths of telomeric terminal restriction fragments (TRF) and surface activation markers and with special emphasis on age-associated differences. Ten elderly and 10 young humans performed maximal bicycle exercise. There was no difference in the number of recruited $CD4^+$ and $CD8^+$ cells between the young and elderly group and in both age groups, the immediate increases could be ascribed to recruitment of $CD28^-$ cells ($CD8^+$ and $CD4^+$ cells) and memory cells (only $CD8^+$ cells). Furthermore, after exercise mean TRF lengths were significantly reduced in blood mononuclear cells and in $CD8^1$ cells from young subjects and in $CD4^1$ cells from elderly subjects compared with lengths pre-exercise. These findings suggest that the mobilization of T lymphocytes during acute exercise is a redistribution of previously activated cells with a longer replicative story than cells isolated from the blood at rest.

THE ROLE OF CATECHOLAMINES IN EXERCISE-INDUCED MODULATION OF SECONDARY ANTIBODY RESPONSE IN AGED MICE

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Studies have shown that chemical sympathectomy leads to enhanced humoral immune responses that are most conspicuous in older animals, suggesting that

catecholamines may be suppressing antibody responses in aged animals. Intense exercise leads to production of catecholamines and we hypothesized that blocking the action of catecholamines may enhance the secondary antibody response in aged mice.
To test this hypothesis, aged (18-24 months old), male, CBA mice immunized to human serum albumin (HSA) were randomly assigned to receive propranolol, placebo, or no pellet implantation. Groups of mice were randomly assigned to receive intense exercise to exhaustion of experience sedentary conditions. Following the exercise/sedentary intervention, all mice were immediately given a booster injection of HSA to elicit the secondary antibody response. Ten days following the booster injection, at the peak of secondary antibody response, serum anti-HSA antibodies were measured by ELISA. Our results indicate that propranolol implanted, exercising mice showed an enhancement of secondary antibody response in comparison to: propranolol implanted sedentary, placebo implanted sedentary and exercising and no pellet implanted sedentary and exercising mice. This suggests that catacholamines may be playing a role in exercise induced modulation of secondary antibody response.

INTRATHYMIC AND INTRASPLENIC OXIDATIVE STRESS FOLLOWING ACUTE EXERCISE IN MICE

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Reactive oxygen species generated through hyperventilation, cellular respiration, or phagocytic respiratory burst activity after intense exercise may contribute to observed apoptosis in lymphoid tissues. Thymic and splenic tissues excised from control mice (C) or mice immediately after (t0) or 24 hours after (t24) a treadmill run-to-exhaustion (RTE) were assayed spectrophotometrically for biochemical indices of oxidative stress (membrane lipid peroxides, superoxide dismutase, catalase, plasma uric acid, and plasma ascorbic acid). There were significant increases in membrane lipid peroxides in thymus ($p<0.001$) and spleen ($p<0.001$) in acutely exercised mice realtive to controls (for example, thymus: t0 = 7.45 ± 0.48 µM; t24 = 9.44 ± 1.41 µM; C = 2.74 ± 0.80 µM). The thymic and splenic tissue antioxidant enzymes concentrations of superoxide dismutase and catalase were significantly lower in samples collected at t0 realtive to C and t24 ($p<0.001$). Plasma uric acid (UA) and ascorbic acid (AA) levels were used to assess the impact of the RTE on the peripheral antioxidant pool. There was non-significant drop in UA levels and a significant reduction in plasma AA at t24 realtive to t0 and C values.
Taken together, these data suggest that oxidative damage occurs in lymphoid tissues after RTE exercise and that such damage may contribute to lymphocyte apoptosis observed after acute exercise.

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ONE-LEG EXERCISE IN RATS INDUCES INTERLEUKIN-6 mRNA PRODUCTION IN THE EXERCISED MUSCLES

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Increased levels of inflammatory cytokines have been found after exercise, predominantly after eccentric exercise. We have recently found that the increase in interleukin (IL-6) was associated with muscle damage (Bruunsgaard et al. (1997) J. Physiol. 499.3, 833-841). Furthermore, using a qualitative PCR-technique, mRNA for IL-6 was found in human skeletal muscle biopsies after strenuous exercise (Ostrowski et al. (1998) J. Physiol. 508.3, 949-953). The present study was performed to further explore the relation between exercise-induced muscle damage and local production of IL-6. A one-legged exercise rat model (Asp et al.(1995) J. Appl. Physiol. 79, 1338-1345) was employed in which male Wistar Kyoto rats were anesthetized with pentobarbiturat sodium (50 mg/kg), the calf muscles on one side were stimulated electrically for eccentric or concentric contractions in 15 minutes, and bilateral calf muscles were obtained 35 minutes postexercise. The soleus muscle and red and white gastrocnemius muscles were recovered and frozen in liquid nitrogen before RNA extraction. A „Quantitative Competitive Reverse Transcription Polymerase Chain Reaction“ (QCRT-PCR) approach (Sun et al. (1996) J. Immunol. Methods 195, 139-148) was employed to measure the amount of IL-6. Total RNA was extracted from the muscle samples and the mRNA converted to cDNA by use of reverse transcription and poly-dT primers. The number of betaactin (internal control) and IL-6 cDNA molecules was measured with competitive PCR and relative IL-6 mRNA levels calculated by correlating to beta-actin. The results show that in the both the eccentric and concentric exercise stimulated muscles the IL-6 mRNA level is highly increased compared to the unstimulated control-leg. The data indicate that the increased blood-level of IL-6 after exercise is, at least in part, due to a production within the exercising muscles. The finding that the IL-6 mRNA level did not increase in the resting control leg indicates that the IL-6 production was not stimulated by systematic factors (metabolic or neuroendocrinological).

CD94 EXPRESSION ON NK LYMPHOCYTES – THE INFLUENCE OF EXERCISE

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Exercise induced leukocytosis has been noted for 70 years and more recent studies on mobilized lymphocyte subsets have used phenotype markers and flow cytometry. Our study extends this characterisation pre and post exercise by including the CD94 marker on NK cells. NK cells are involved in the innate immune response. Although they do not have antigen receptors that undergo somatic gene rearrangements, they can respond without prior sensitisation bringing about cytolysis. NK cells can lyse some tumour cells and viral infected cells through as yet unclear mechanisms. What is clear is that MCH class I recognition is important in blocking NK killing. Several molecules are involved in MHC I recognition by NK cells and one of these is a C-type lectin designated CD94. In some systems, CD94 expression levels are related to NK cell function. This study measured CD94 expression on NK cells at rest and after exercise mobilisation. Eleven highly trained male triathletes, 24.2 (3.3) yrs., wt=72.5 (6.5) kg, VO_2max=4.95 (0.40) L/min participated and provided pre and post exercise venous blood samples. The exercise test consisted of a 24 min incremental cycle test to exhaustion on a Lode Excalibur ergometer. FBC with 5 part differential was performed on a Technicon H3^{TM}. A whole blood lysis technique using direct 3 colour staining with IgG_1FITC/IgG_2PE/CD3PerCP, CD94FITC/CD16PE/CD56PE/CD3PerCP and CD94FITC/CD14PE/CD3PerCP was performed on each specimen. Cells were lysed with BD FACSlyse, washed and resuspended in PBS. 20,000 events were acquired using a BDFACcan and Cellquest software. BD generated histograms and sub-sets were further analysed with WinMDI. We observed the expected lymphocytosis seen with exercise however, the CD94 expression levels on NK lymphocytes mobilised into blood stream during exercise did not differ from that which was found at rest. This suggests that the cells do not have a different activation status.

	Pre, mean, sd	**Post, mean**, sd
WBC-, 10^9/L	**5.02**, (1.5)	**8.49***, (1.9)
Lymphocytes, 10^9/L	**1.57**, (0.35)	**3.14***, (0.65)
$CD3^+$, 10^6/L	**998**, (267.9)	**1727***, (428.8)
$CD3^-$, $CD16^-$/$CD56^-$	**338**, (150.7)	**492****, (232.5)
$CD3^-$, $CD16^+$/$CD56^+$	**225**, (81.6)	**911***, (275.6)
NK, $CD94^-$	**104**, (47.9)	**451 ns**, (186.1)
NK, $CD94^+$	**121**, (41.6)	**460 ns**, (117.7)

Mean, (sd)=standard deviation, * P<0.001, ** P=0.01, ns=not significant

INCREASED IMMUNO-ENDOCRINE RESPONSE TO A SECOND BOUT OF INTENSIVE ENDURANCE EXERCISE ON THE SAME DAY

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Our objective was to study the effect of repeated bouts of exercise and specifically compare the immuno-endocrine response of the first bout of exercise with a second and equal bout on the same day.

Methods: Nine elite national team athletes in triathlon (n=4) and speed-skating (n=5), age: 21-27 yrs, weight: 70-83 kg, VO_2max: 65.1-76.8 ml kg^{-1} min^{-1}, participated in three trials of 24h duration: 1) complete bed rest (REST), 2) one bout of exercise (ONE), 3) two bouts of exercise separated by a 3 h rest period (TWO). All exercise bouts consisted of a 10 min warm-up at 50% of VO_2max, followed by 65 min at 75% of VO_2max on a cycle ergometer. The subjects rested in bed at all hours except when exercising. The exercise bouts were performed at 11.00 AM – 12.15 PM (only in TWO) and 3.15 – 4.30 PM (ONE and TWO). Blood was drawn from a venous catheter at 7.30 AM, 12.15 PM, 3.00 PM, 4.30 PM, 4.45 PM, 5.00 PM, 5.30 PM, 6.30 PM, 7.30 PM, 8.30 PM, and 7.30 AM the next day. An ANOVA procedure for repeated measures including 9 measurements of hormones, neutrocytes and lymphocytes and 4 measurements of lymphocyte subsets ($CD4^+$, $CD8^+$, $CD56^+$) were used to compare the exercise and recovery responses from 3.00 PM to 7.30 AM (14.5 h) in the three experiments.

Results: There was an increased response during and after the second bout of exercise on the same day for epinephrine ($F_{8,64}$=6.42; p<0.001), norepinephrine ($F_{8,64}$=8.87; p<0.001). ACTH ($F_{8,64}$=3.42; p=0.002), cortisol ($F_{8,64}$=3.27; p=0.003) and GH ($F_{8,64}$=3.48; p=0.002), as well as for neutrocytes ($F_{8,64}$=6.62; p<0.001), lymphocytes ($F_{8,64}$=8.33; p<0.0005), $CD4^+$ cells ($F_{8,64}$=3.39; p<0.034), $CD8^+$ cells ($F_{8,64}$=8.51; p<0.001) and $CD56^+$ cells ($F_{8,64}$=6.50; p<0.002). All parameters, except neutrocytes (TWO: 6.6 ± 0.5 vs. REST: 3.1 ± 0.2; p<0.001) were at baseline (7.30 AM start of trial) levels before the start of the second bout of exercise, as well as after 14.5 h of recovery (7.30 AM end-trial).

Conclusion: We observed a more pronounced responses in catecholamines, ACTH, cortisol, GH and several leukocyte subsets during and after the second bout of exercise compared to the first bout.

THE IMMEDIATE LEUKOCYTOSIS TO ANAEROBIC EXERCISE: EVIDENCE OF TWO SUBSEQUENT PHASES

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Introduction: Acute physical exercise leads to a biphasic increase of peripheral venous blood leukocytes called the immediate and the delayed leukocytosis, respectively. Prior studies postulated that within the immediate leukocytosis to anaerobic exercise subpopulations were mobilized differently into the circulation. The goal of this study was to test the hypothesis that the immediate leukocytosis contains at least two distinct phases.
Methods: 8 healthy male athletes performed a single maximal (all-out) exercise of 60s on a cycle ergometer (60s-test) [mean exercise intensity: 489 ± 34 W, maximal lactate concentration: 14.1 mmol.l^{-1}] and a control day without exercise. Repeated blood samples were taken before and 1, 2, 4, 8, 16, 32, and 48 min after exercise; leukocyte and in particular monocyte subpopulations were determined flow cytometrically.
Results: Two subsequent phases of the immediate leukocytosis were seen: 1) an early mobilization with maximal increases 1-2 min after the end of exercise of NK cells [+521%, related to preexercise values], $CD8^+$ T cells [+95%] and $CD14^+CD16^+$ regular monocytes [+33%] and 2) a later mobilization of neutrophils [+26%], $CD14^+CD16^+$ mature monocytes (premacrophages) [+372%], $CD4^+$ T cells [+43%] and B cells [+70%], respectively, starting 2-4 min afer the end of the 60s-test and reaching maximal concentrations at 8-16 min postexercise. Monocyte subpopulations showed the most striking differences in mobilization into peripheral blood. Compared to $CD14^+CD16^+$ regular monocytes. $CD14^+CD16^+$ premacrophages were mobilized slower but more than 10-fold stronger.
Conclusions: Assuming a recruitment of adherent leukocytes from the maximal pool of the blood vessels, these results indicate that leukocyte subpopulations follow different mechanisms of mobilization from the endothallum. Related to circulating monocytes at rest the overproportionate increase of $CD14^+CD16^+$ premacrophages suggests a predominant localization of mature monocytes in the marginal pool. Further investigations focussed on the different leukocyte and monocyte adhesion properties are needed to explain different exercise induced mobilization out of the marginal pool.
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APPARENT ENHANCEMENT OF EXERCISE-INDUCED ACUTE-PHASE PROTEIN RESPONSE FOLLOWING VITAMIN C SUPPLEMENTATION IN ATHLETES PARTICIPATING IN AN 88 KM ULTRAMARATHON

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The effect of daily Vitamin C supplementation (2x500mg/d) for 7 days before, on the day of and 2 days following participation in an 88 km ultramarathon running event was investigated in 10 runners (VCS) and compared to 6 runners receiving placebo capsules (P) 18 hrs before, 30 min, 24 and 48 hrs after the race. Total mean intake of Vitamin C in diet and supplements in VCS runners was 1339 (± 401) mg/day during the three days before the race. Pre-race serum Vitamin C levels were significantly ($p<0.01$) elevated in the VCS group when compared to the levels in the P group. Plasma Vitamin A and E levels were not significantly different between VCS and P groups ($p>0.05$). After correction for plasma volume changes, Vitamin C and E levels rose significantly in the P group returning the baseline levels within 24 hrs post-race. In both VCS and P groups mean serum cortisol concentrations rose significantly following participation in the 88 km ultramarathon returning to below baseline levels 24 hrs post-race. Neutrophil:lymphocyte ratio and myeloperoxidase concentration did not reveal a significant effect of the Vitamin C supplementation. The major finding of this study was the significantly higher C-reactive protein concentrations immediately, 24 and 48 hrs post-race in the VCS group ($p<0.05$). This was supported by higher creatine kinase activity in the VCS group 24 hrs post-race. These two markers of proinflammatory response peaked 24 hrs after the 88 km event, but did not correlate significantly. This study provides preliminary evidence of enhanced activation of an exercise-induced acute-phase protein response following Vitamin C supplementation.

EFFECT OF DIETARY LIPIDS AND EXERCISE ON CELLULAR IMMUNE RESPONSES AND PLASMA CYTOKINES AND HORMONES IN RUNNERS

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Training and performing have been shown to stress the immune system particularly if runners are overtrained. Both animal and human studies have reported preserved or increased maximal aerobic power and endurance capacity in response to a high-fat diet. This study compared the cellular immune responses and plasma levels of cytokines and hormones at rest and after maximal exercise in runners after eating diets comprised of increasing levels of fat. The runners (n= 6-8) were 35 years old and weighed 56 kg (women) and 75 kg (men). The diets consisted of low fat diet (LF-17% total calories from diet fat), medium fat diet (MF-32%) and the high fat (HF-

41%). The subjects consumed each diet for 4 wks. $\dot{V}O_{2max}$ and endurance run time increased from 15 to 45% fat diet. The leukocyte numbers significantly increased 82 and 113% after exercise. $CD3^+$ and $CD8^+$ cells were significantly increased by exercise while $CD4^+$ cells were not. The NK cells significantly increased after exercise from 3.9 to 7.9 for women and 4.1 to 9.4 for men with significant effect of increased dietary fat. Increased dietary fat had significant effects on production of IL-2 and IL-1β, IL-6 and TNF-α by PBMN cells. IL-1β and TNF-α levels increased with increasing level of dietary fat. Plasma IL-2 level decreased in men with increased dietary fat. Plasma IL-6 level was significantly lower after the endurance run and decreased with increase in dietary fat. The plasma cortisol level decreased with MF diet compared to LF diet at rest and after exercise. Plasma PGE_2 levels increased after the endurance run and were higher when the runners were on the LF diet. These data support the conclusion that running 40 miles/wk and high % of fat intake (45%) do not compromise the immune system, however, a low fat diet with too few calories is immune suppressive in runners.

THE EFFECTS OF CARBOHYDRATE SUPPLEMENTATION ON NEUTROPHIL DEGRANULATION RESPONSES TO PROLONGED CYCLING

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Carbohydrate (CHO) supplementation during prolonged, strenuous exercise is associated with lower cortisol responses and an attenuated neutrophilia. However, any influence on neutrophil function is uncertain (Smith *et al*., Am. J. Physiol., 270: R838-R845; 1996; Nieman *et al*., J. Appl. Physiol., 84; 1252-1259, 1998). The aim of the present study was to investigate the influence of CHO compared with placebo (PLA) beverage consumption on the plasma cortisol and lipopolysaccharide-stimulated neutrophil degranulation responses to prolonged cycling. In a randomised block design, ten recreationally active men consumed CHO (6% w/v) and PLA beverages before (400 ml) and during (150 ml) two cycling bouts at 60% VO_2max for 2h, separated by one week. Venous blood samples were obtained before and immediately after exercise. Results were analysed using a 2 (treatment) x 2 (times of measurement) repeated measures ANOVA. After exercise, plasma cortisol concentration had increased by 23% on the PLA treatment (P<0.05), but was unchanged on the CHO treatment. At this time, mean (SEM) plasma glucose levels were significantly lower on the PLA treatment compared with the CHO treatment (PLA: 4.95 (0.28) mM; CHO: 6.01 (0.21) mM; P<0.01). Blood neutrophil counts were significantly elevated following both exercise bouts (P<0.05) but those following the PLA treatment were almost double those following the CHO treatment (PLA: 10.9 (1.0) x 10^9 cells.l^{-1}; CHO: 5.5 (1.0) x 10^9 cells.l^{-1}; P<0.01). Lipopolysaccharide-

stimulated elastase release per neutrophil decreased by 40% on the PLA treatment (P<0.05), but did not significantly change on the CHO treatment.
We conclude that carbohydrate supplementation can influence both neutrophil trafficking and degranulation following prolonged, strenuous cycling exercise.

EFFECTS OF GLUTAMINE AND PROTEIN SUPPLEMENTATION ON EXERCISE-INDUCED DECREASE IN LYMPHOCYTE FUNCTION AND SALIVARY IGA

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Post-exercise impaired cellular immune function has been linked to exercise-induced decline in plasma-concentrations (the so-called glutamine hypothesis). Intervention studies in which glutamine supplementation was given post-exercise did not have any effect on immune function in vitro. The purpose of the present study was to examine the effects of glutamine and protein-supplementation during and after the exercise on concentrations of lymphocyte subpopulations, proliferative responses, cytotoxic activity and salivary IgA levels.
Eleven males (mean 38 years; mean VO_2max 59.9 ml/min/kg) participated in a placebo-controlled, cross-over, randomized study. The subjects performed bicycle exercise for 2 hours at 75% of VO_2max on three days separated by at least two weeks.
Supplementations of either glutamine or placebo were given 1 h; 1 h 45 min; 2 h 30 min and 3 h and 15 min after the start of exercise. Due to different absorption kinetics, protein supplementation (casein) was given at times 1; 2; 3; and 4 h after start. The exercise protocol efficiently induced decreases in several lymphocyte subpopulations, as well as decreased lymphocyte proliferative responses, NK and LAK cell activities. Furthermore, decreased salivery IgA was found after the exercise (IgA concentration, IgA relative to total protein and IgA output). The plasma-concentration of glutamine declined post-exercise. This decline was abolished by both glutamine and protein supplementations. In conclusion, neither glutamine, nor casein supplementations were able to abolish exercise-induced immune impairment.
Thus, the present study and previous glutamine intervention studies do not support the glutamine hypothesis.

MUCOSAL IMMUNITY, ILLNESS AND PERFORMANCE IN SWIMMERS

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The aim of this investigation was to examine the relationships between salivary Immunglobulin concentration, the incidence of respiratory tract illness (RTI) and competition performance in elite swimmers over an 18 week period. Forty one Australian National Team swimmers (21 males, 20 females) aged 15-27 years were monitored during preparations for the 1998 Commonwealth Games in Malaysia. Salivary IgA, IgM and IgG and albumin concentration (mg/l) were measured by rate nephelometry in May 1998 and again in late August 1998, 17 days prior to competition. RTI was verified by the team physician. Subjects categorized as "Ill" (at least 1 RTI) or "healthy". Immunoglobulin concentrations [median (range)] were analysed by student t-test for paired data and Spearmans Rank correlation: a p-value < 0.05 was taken as significant. There were no significant changes in salivary IgA, IgM or IgG concentration in all 41 swimmers between May and August, nor were there any differences between healthy (n=23) and ill (n=18) swimmers. There was a significant positive relationship between IgM and performance in the males (r=0.85, p<0.001) but not for any other parameter. There was a trend (p=0.11) for healthy swimmers to have a higher level of performance at the Commonwealth Games than swimmers who had reported illness. Gold medal winners (n=9) had higher IgM levels than other swimmers (n=32) in May [6.7 (3.3-12.9) to 4.3 (1.0-7.4), p=0.02] and higher IgG in August [23.2 (9.3-35.8) to 9.0 (2.0-27.0), p=0.02]. In conclusion these data indicate the existance of modest associations between: (i) salivary IgM concentration and competition performance, (ii) health status and competition performance, in international-level swimmers.

IMMUNE PARAMETERS AND ACTUAL PERFORMANCE IN SOCCER PLAYERS

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Introduction: Recent studies have shown multiple changes of immunologic parameters after acute exercise. The aim of the present study was to investigate whether parameters, influenced by acute exercise, can be useful to recognize athletes with overreaching.
Subjects and Methods: The performance of 117 soccer players of the german first and second division were rated by two independent experts (coach and sports scientist). The players were classified due to their actual performance in three

categories: Low (1), average (2) and high performance (3). At the same time blood was sampled from the earlobe under conditions of rest. The concentrations of C-reactive protein, soluble interleukin 2-receptor (sIL2-R) and neopterin were determined by standard elisa assays. Subjects with symptoms of an infection were excluded.
Results: The highest levels of the sIL2-R were observed in the subjects with average performance (784 ± 298 IU/ml). In the other groups significantly lower levels were observed (637 ± 177 IU/ml, $p \leq 0.02$ (1), 620 ± 212 IU/ml, $p \leq 0.008$ (3)). About one third of the participating subjects were classified as performing well (group 3), regarding only the subjects with high sIL2-R levels only 15.4% were assigned to this group. No influence of the performance on neopterin and C-reactive protein levels could be observed.
Discussion: A good actual performance was less frequent in subjects with high serum sIL2-R levels. One reason might be that subclinical infections with an activation of the specific immune system affect the performance adversly. Previous studies showed an slight activation of the specific immune system under a high training load, therefore the observations might also indicate an overload in the subjects with high sIL2-R levels.

PRECISION OF URTI DIAGNOSIS IN TOP ATHLETES BY USING CELLULAR IMMUNE PARAMETERS

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Purpose: The diagnostic lab panel in sports medical practice of top athletes has been extended to immunological parameters. Important indications are URTI of athletes and get aids for the return to play decision. The purpose to use self-learning classification programs in this diagnostic process is a) to include all available parameters to find the best diagnosis, b) to find the best parameter panel including common and immunological parameters, c) to establish diagnostic standards enabling direct comparison of diagnosis, d) to be independent on interlab variations and e) last but not least to reduce the diagnostic panel to be the best at minimal effort.
Methods: 70 athletes of local olympic sports centre, 25 with and 45 without URTI, were clinically examined and blood samples for routine lab parameter analysis were taken. In addition direct immunophenotyping (CD3, CD4, CD8, CD14, CD16, CD19, CD45, CD45R0, CD56, HLA-DR) and phagocyte functional assays were done for five-parameter flow cytometry. In a first and retrospective step the CLASSIF-1 program system (Valet et al., Ann. NY Acad. Sci. 20, 677:233, 1993) served to classify the athletes into infected and non-infected groups on the basis of 1554 flow cytometric parameters per blood sample.

Results: The diagnostic sensitivity was 84.0% specificity 91.1%. Positive and negative predictive values were 84.0% and 91.1%, respectively. The calculation process reduced the mass of parameters by 98.5% to a number of only 23. Diagnostic decisive parameters were % grans, % lymphs, surface antigen density of CD45, CD45R0 on monocytes, ratio of CD16/CD56 surface antigen density, % of $CD14^{+}CD16^{+}$ monocytes and CD16 surface antigen density on granulocytes.
Conclusions: Flow cytometric parameters enable the diagnosis of URTI with defined sensitivities and specificities. The panel and therefor costs of immunologically relevant parameters can be reduced to a minimum. Diagnostically most important immune cells are monocytes. The actual process of analysis evaluates different stages and treatments of URTI, comparisons between humoral vs. cellular parameters and prospective approaches with samples of unknown origin.
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LEUKOCYTE INFILTRATION IN HUMAN SKELETAL MUSCLE AFTER ECCENTRIC EXERCISE

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It is well documented that physical exercise induces changes in both the numbers of circulating leukocytes and leukocytes subsets; as well as modifications of the expression of different cell surface antigens. The reasons behind and effects of these changes are largely unknown. In order to investigate the immune systems involvement in muscular events after physical activity, a study was conducted in which blood and muscle tissue was sampled after one 30 min bout of eccentric cycling exerxise. Blood samples were analyzed by 3-way flow cytometry. Muscle samples were frozen in liquid nitrogen, cryostat sectioned and stained with monocional antibodies against cell surface antigens: CD3, CD4, CD8, CD11b, CD15, CD56, CD79∝ and BerMac-3. Stained sections were quantified using light microscope image analysis. In muscle and compared to rest, there was an increased occurence of cells expressing adhesion molecule CD11b, CD3 and CD15 6 to 48 h post exercise. BerMAC-3 increased at 6 h and peaked at 48 h post exercise. There was a decreased expression of CD56 on non-muscle cells 6 h post exercise, and the number of muscle cells expressing CD56 increased 48-168 h post exercise. There was no change in the expression of CD4, CD8 and CD79∝ from before to after the eccentric exercise. In blood, there was a general leukocytosis, neutrophilia and monocytosis 6 h post exercise. The number of $CD62L^{+}CD4^{+}$ monocytes increased 6 h post exercise, and $CD8^{+}CD11b^{+}$ cells increased immediately, 6 and 24 h post exercise while the expression of CD4 on $CD62L^{+}$ monocytes decreased from 6 to 168 h post exercise. It can be concluded that the changes in the number of cells in several leukocyte populations in the blood have a temporal equality in muscle tissue.

One possible interpretation could be that neutrophils and macrophages are important in muscle repair, and consequently adapting muscle tissue to physical exercise.

IMMUNE FUNCTION IN FEMALE ELITE ROWERS AND NONATHLETES

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The immune function of female rowers and controls was compared while in the resting state, and then related to a two-month history of upper respiratory tract infection (URTI). Subjects included 20 elite female rowers located at the Olympic Training Center in California, and 19 nonathletic female controls. PHA-induced lymphocyte proleferation (separated cell method), granulocyte/monocyte phagocytosis and oxidative burst activity, and plasma cytokine concentrations (IL-6, TNF-α, and IL-1ra) did not differ significantly between groups. PHA-induced lymphocyte proliferative responses (adjusted whole blood method) was significantly higher (31% and 36% for optimal and suboptimal concentrations, respectively) in rowers compared to controls. Natural killer cell activity (NKCA) was substantially higher (1.6-fold for total lytic units) in the female rowers compared to controls. Two-month health logs revealed 5.2 ± 1.2 and 3.3 ± 1.1 days with URTI symptoms for the rowers and controls, respectively (P=0.268). For all 39 subjects combined, and for the 20 rowers separately, none of the immune parameters were significantly correlated with number of days URTI symptoms. In this cross-sectional comparison of 20 elite female rowers and 19 female nonathletes, a group difference was found for NKCA and PHA-induced proliferative response (whole blood technique), but not other measures of immune function. The number of days with URTI symptoms during the spring season did not differ between groups, and variances in blood measures of immunity were unrelated to URTI.

PHYSICAL ACTIVITY FACILITATES BACTERIAL INFLAMMATION RESOLUTION PRODUCED BY STRESS

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Regular, moderate, physical activity can modulate many behavioral and physiological consequences of stress. Specifically, voluntary freewheel running modulates many neural and immunological responses to stress. It has been previously reported that

exposure to tail shock (IS) stress alters the development and resolution of the inflammatory response to subcutaneous *E. coli* injection. Specifically, IS exposure decreases the size and the resolution time of the inflammation. Thus we investigated whether freewheel running would modulate the effect stress on this inflammatory process. Adult, male Sprague Dawley rats (8/grp) were housed with either an attached running wheel (Run), or no wheel (Sedentary). Rats were weighed weekly and running distances were continuously recorded via computer. After 8 weeks of voluntary running, rats were either exposed to inescapable tail shock stress (IS) [100 1.6 mA 5-s tail shocks] or remained in their home cages. Immediately after IS termination, all rats were injected with non-replicating *E. coli* (~ 2.5 x 10^8 CFU).
Experiment 1-Size and Grade of Inflammation: Rats were then returned to their home cages (with and without running wheels). Inflammation size (caliper) and grade were monitored daily.
Experiment 2-Histological Exhamination of Inflammation: Rats were sacrificed 2 hrs after IS termination and *E. coli* injection. The inflammatory site was removed, placed in Formalin (24 hrs), and embedded in paraffin. Serial sections (4 microns) were cut and stained with H & E and Brown-Hopps gram stain. The number of neutrophils/mm^2 was measured using the NIH imaging analysis system.
Prior freewheel running altered the stress-induced change in the inflammatory response. In sedentary rats, IS exposure slightly increased the number of subcutaneous neutrophils/mm^2, decreased the initial size of the inflammation (24 hrs), and decreased the time needed to resolve the inflammation (1-2 days earlier then non-stressed control). In freewheel run rats. IS exposure greatly increased the number of subcutaneous neutrophils/mm^2, blocked the early reduction in inflammation (24 hrs), and further decreased the time needed to resolve the inflammation (3-4 days faster than rats exposed to either stress or exercise alone). Freewheel running alone had no effect on inflammation. Thus, daily moderate physical activity facilitates the resolution of bacterial inflammation in the presence of stress.

A 17 FOLD INCREASE IN PLASMA-EPINEPHRINE INDUCES ONLY A 2-3 FOLD INCREASE IN PLASMA CONCENTRATION OF IL-6 AND IL-1ra

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Objectives: Subjects at rest underwent a 1 hour epinephrine infusion and the release of IL-6 and IL-1ra was examined.
Methods: 18 male subjects, 6 HIV seropositive patients receiving antiretroviral therapy, 6 HIV seropositive untreated patients and 6 HIV seronegative control subjects, participated in a 1 hour epinephrine infusion. Blood was sampled before

(t=0 min), during (t=15 min and t=45 min) and 1 hour after the infusion had stopped (t=120 min).

Results: The plasma concentration of epinephrine (p-epinephrine) increased 17 fold (range 1.5 to 87) reaching a plateau after 15 min, whereas p-norepinephrine did not change. In all participants, the epinephrine infusion resulted in the same relative changes in plasma concentrations of IL-6 (pIL-6) and IL-1ra (pIL-1ra). Plasma IL-6 increased at t=45 min and was further increased 1 hour after the epinephrine infusion had stopped, resulting in a 2-3 fold increase. Plasma IL-1ra did not increase until 1 hour after the epinephrine infusion had stopped, where it was 2-3 fold increased. The baseline levels and the absolute increase in pIL-6 and pIL-1ra were higher in the two HIV infected groups as compared to the control group.

Conclusions: A minor increase in pIL-6 and pIL-1ra in response to a super-physiological (17 fold) increase in p-epinephrine were found. This suggests, that the elevated levels of p-epinephrine during exercise may account for the increase in IL-6 observed during concentric exercise. However, the super-physiological concentration of epinephrine obtained in the present study induced only a 2-3 folds increase in pIL-6, which suggests that other factors are involved, causing the large increase in pIL-6 which is observed during strenuous exercise or eccentric exercise. The finding that pIL-6 is elevated after 45 min, but not after 15 min of infusion, despite reaching the plateau-level of epinephrine already after 15 min, suggests that the elevation in pIL-6, is due to *de novo* synthesis and not to exocytosis of intracellular IL-6 stores.

IMMUNE CHANGES IN HUMANS DURING COLD EXPOSURE: EFFECTS OF PRIOR HEATING AND EXERCISE

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This study examined the immunological responses to cold exposure together with the effects of pre-treatment with either passive heating or exercise (with and without a thermal clamp). On four separate occasions 7 healthy males [mean (SE): age = 24.0 (1.9) years, $\dot{V}O_{2}peak$ = 45.7 (2.0) mL.kg.min^{-1}] sat for 2 h in a climatic chamber maintained at 5° C. Prior to exposure, subjects participated in one of four pre-treatment conditions. For the control condition, subjects remained seated in a thermoneutral (35° C) water bath for 1 h. In another pre-treatment, subjects were passively heated in a warm (38° C) water bath for 1 h. In two other pre-treatments, subjects exercised for 1 h at 55% $\dot{V}O_{2}peak$ (once immersed in thermoneutral [35° C] water and once in cold [18° C] water). Core temperature rose by 1° C during passive heating and during exercise in thermoneutral water and remained stable during exercise in the cold water (thermal clamping). Cold exposure induced a leukocytosis and granulocytosis; an increase in NK cell count and activity; and a rise in circulating

levels of IL-6. Pre-treatment with exercise in cold water augmented the leukocyte, granulocyte and monocyte response. These results indicate that acute cold exposure has immunostimulating effects and that pre-treatment with physical exercise can enhance this response. Increases in levels of circulating nor-epinephrine may account for the changes observed during cold exposure and their modification by changes in initial status.
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INCIDENCE OF RESPIRATORY AND GUT INFECTIONS IN ELITE SWIMMERS

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A retrospective study was undertaken of the incidence of upper respiratory tract infections (URTI) and gastrointestinal tract infections (GITI) in the cohorts of elite swimmers holding scholarships at the AIS over the past 11 years (1988-1998). The study entailed a review of the medical records for all swimmers who completed a full year's training program. There were an average of 24 swimmers in each year (62% male and 38% female) representing 117 individual athletes, aged 16-26 years.
Over the 11 years there were 669 episodes of URTI and 134 episodes of GITI in 108 individual athletes. 9 athletes (8%) had no recorded respiratory or gut infection in the study period. The incidence of URTI over the 11 years was 2.5 episodes/year/athlete (range: 0-10). On average the female swimmers had more URTI (mean = 3.0) than male swimmers (mean = 2.2; $p<0.01$). The yearly incidence of URTI decreased over the last 10 years ($p=0.01$). This trend was influenced by the high annual incidence rates and greater decrease in incidence over the last 10 years in female swimmers ($p=0.04$). The incidence of GITI was 0.5 episodes/year/athlete (range: 0-5). The mean episodes/year of GITI for female swimmers (mean = 0.7) was higher than for male swimmers (mean = 0.4; $p<0.01$).
More than 4 URTI episodes/year was considered abnormal. 26% (30/117) of the swimmers had more than 4 URTI episodes during at least one scholarship year. (23% ,16/69 males; 29% ,14/48 females). The most common clinical presentations were inflamed sore throat with either cough, pharyngitis, sinusitis, rhinitis, infected sputum with occasional otitis media or enlarged cervical lymph glands. The GITI symptoms were diarrhoea and abdominal pains.

THE IMEX STUDY: STUDY DESIGN AND PRELIMINARY RESULTS ON RECRUITMENT AND ADHERENCE

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Objective: The IMEX Study is a randomized controlled trial investigating the effects of a one-year moderate exercise program, compared to a stretching program, on immune function among postmenopausal women (age 55-75 years), ancillary to an ongoing exercise trial (PATH Study).
Methods: Participating women (n=112) are sedentary, overweight, and do not use sex hormones. The exercise program is a taught, self-monitored endurance and strength program, divided into a 3-month monitored (classes 3x/week) and a 9-month maintenance period (at home with classes 1x/week). The exercise goal is 4-5 times/week for 45-60 minutes each. Immune measures obtained at baseline, 3 months and 12 months after randomization include flow cytometry phenotyping of immune cells (T lymphocytes including helper, cytotoxic/suppressor, naive, memory, B lymphocytes and natural killer (NK) cells), immune function measurements (lymphocyte proliferation and NK cytotoxicity), circulating levels of C-reactive protein and tumor necrosis factor alpha, and frequency of respiratory infections. Other data inculde a panel of serum sex hormones, current diet and dietary supplements, lifetime physical activity, psychosocial data and quality of life, and measurements of body composition, fitness, and bone density. Recruitment tools include mass mailings, as well as targeted advertisements.
Results: Recruitment began in May 1998: as of January 1999, 23 baseline blood draws have been conducted, and 18 women have been randomized to the IMEX part of the exercise trial. Reasons for inellgibility include medical reasons, fitness level exceeding study entry criteria, serious allergies, participation in other research studies, staff concerns regarding compliance, and participation in weight-loss programs. Adherence to date has been excellent (82% of possible exercise sessions completed).
Conclusions: Postmenopausal women can be recruited to participate in a one-year exercise program. This presentation will summarize the methods of IMEX experiences from the recruitment process, and adherence to the exercise program.

THE IMEX STUDY: PRELIMINARY RESULTS ON CORRELATES OF NATURAL KILLER CELL CYTOTOXICITY

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The IMEX Study is a randomized controlled trial investigating the effects of a one-year moderate exercise program, compared to a stretching program, on immune function among postmenopausal women (n=112, age 55-75 years).
Objective: This presentation will describe preliminary results on natural killer cells cytotoxicity in 23 participants.
Methods: NK cytotoxicity was measured by flow cytometry (method by Wener et al.). Age and BMI were verified in clinic and other demographic data were obtained from questionnaries.
Results: As of January 1999, 23 baseline blood draws have been obtained and 18 women have been randomized into the exercise or stretching program. Study participants are mainly Caucasian (81%) with an average age of 59.9 years (range 55-73 yrs), and mean body mass index (BMI) of 30.1 kg/m^2 (25.6-38.9). Most women have attended some college (86%), and 50% are currently employed full time. Many report a family history of cancer (73%), often breast cancer (53%). NK cytotoxicity was 14.0% (mean) ± 9.1% (S.D.) (at a 6.25:1 effector to target ratio), 21.7% ± 13.6% (12.5:1 E:T ratio), 30.0% ± 16.3% (25:1 E:T), and 35,0% ± 14.1% (50:1 E:T). As expected, NK cytotoxicity decreased with age (Pearson correlation coefficients ranging from r= -0.32, p=0.20 (6.25:1 E:T ratio) to r= -0.48, p=0.04 (50:1 E:T)). Average NK cytotoxicity was decreased among separated or divorced women (n=9) compared to married or widowed women (n=14): 8.9% vs. 17.3%, p=0.01 (2-tailed t-test, 6.25:1 E:T ratio); 13.6% vs 26.8%, p=0.01 (12.5:1 E:T ratio); 21.3% vs 35.6%, p=0.02 (25:1 E:T ratio), and 29.1% vs 38.9%, p=0.12 (50:1 E:T ratio), NK cytotoxicity was also lower among former smokers (current smokers were excluded from the study), although these findings were not statistically significant. No differences in NK cytotoxicity were observed based on previous use of hormone replacement therapy, or previous pregnancies.
Conclusions: These preliminary results indicate that NK cytotoxicity may differ depending on demographic and health variables. We will investigate these associations among the growing number of study participants and also evaluate psychosocial variables as predicators of NK function.

COMPARISON OF FLOW CYTOMETRIC AND 51Cr RELEASE ASSAYS OF NATURAL KILLER (NK) CELL CYTOTOXICITY FOR AN EXERCISE IMMUNOLOGY STUDY

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Objective: We are measuring NK cytotoxicity activity (NKA) in the IMEX Study, a randomized controlled trial on exercise and immune function in older women. In preliminary studies, we evaluated a flow cytometric assay (FCA) and compared it to the conventional 51Cr release assay (CRA)of NKA. Methods: NKA was measured in

peripheral blood mononuclear cells (PBMC) after harvesting on Ficoll-Hypaque gradients. K562 target cells in log phase of growth were labeled with 51Cr for the CRA, and with a fluorescent membrane dye for the FCA. After incubation of target cells with different concentrations of PBMC, NKA was assessed by identifying killed target cells by propidium iodide staining for the FCA. Volunteer participants were primarily laboratory personnel, age 45-55, and predominantly female.
Results: Within-run precision of the FCA was 3.4-11.9% for 6 donors assayed by 5-6 replicates each. Between run precision varied from 4.1-22.1% for 4 subjects measured in 3 runs each at 50:1 effector to target (E:T) cell ratio. Results were equivalent when PBMC effector cells were harvested and used immediately or blood was stored overnight at 4 degrees C before harvesting and use. For 13 subjects, NKA was measured using both the CRA and FCA assays at 4 E:T ratios each. For the 52 data points generated, the correlation coefficient was r=0.91, with slope=1.10 and intercept = 9.66 (FCA x-abscissa, CRA ordinate). Individual donors demonstrated similar correlations. In comparison with the CRA, the FCA avoids the use of radioactivity.
Conclusions: The results from CRA and FCA correlate well, although the FCA gives slightly lower values of NKA. Both assays are acceptable measures of NKA.

EFFECT OF FITNESS TRAINING ON THE PERIPHERAL BLOOD TH1/TH2 BALANCE IN THE ELDERLY (A RANDOMIZED CONTROLLED STUDY)

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$CD4^+$T helper cells are subclassified into two subpopulations according to pattern of cytokine production. T helper 1 (Th 1) produces interferon-gamma (IFN-gamma) and interleukin 2 (IL-2), and T helper 2 (Th2) produces IL-4, IL-5, IL-6 and IL-10, the former mainly supports cell-mediated immune responses, while the latter supports humoral immune responses. The ratio of Th1 and Th2 cells, namely Th1/Th2 balance, is supposed to determine the dominant immune reaction to antigens.
The purpose of this study was to investigate whether fitness training could modulate peripheral Th1/Th2 balance in the elderly. Sixty-six healthy elderly volunteers (age: 67.2 ± 4.1 y.o., male=30, female=36) were randomly assigned to either fitness training group (ET) or to sedentary control group (C). Group ET underwent a fitness training program consisted of aerobic exercise on cycle ergometer (50-60% $\dot{V}O_{2\,max}$) and resistance exercise using rubber band (20-repetition maximum (20-RM)) for 6 month ($2h.day^{-1}$, $2\text{-}3days.week^{-1}$). Subjects in group C led normal lives during the 6

month period. Blood samples from 56 out of 66 subjects (C; n=26, ET; n=30) were obtained before and after the training period.
Brefeldin A pretreated peripheral blood mononuclear cells were stimulated with PMA and ionomycin, and stained for CD3, CD8 and intracellular IFN-gamma and IL-4. The percentage of IFN-gamma producing (Th1) cells or IL-4 producing (Th2) cells among $CD3^{+}CD8^{+}$ cells were analyzed by 3-color flow-cytometry.
Mean maximal oxygen uptake ($\dot{V}O_{2\,max}$) increased significantly in group ET after the program, while in group C it remained unchanged. Percentage of Th2 cells increased significantly, while that of Th1 cells remained unchanged in group ET, resulting in the reduction of Th1/Th2 ratio in group ET. No significant change was observed in neither of the analyzed parameters in group C. These results suggest that fitness training could reduce Th1/Th2 balance without any reduction in Th1 population in the elderly population, which potentially could affect the quality of immune responses to antigens.

EFFECTS OF ACUTE AND CHRONIC EXERCISE ON IMMUNE CHANGES

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Objectives: It is well known, that normal and moderate exercise can stimulate the immune system. This stimulating is during certain diseases or reduced immune responsiveness. However maximal acute and long term exhaustive exercise may cause opposite profound changes. The aim of the study was to determine the acute and long term effects of exhaustive exercise on the immune and endocrine system.
Study design: We investigated 20 top sportsmen between the ages 16 to 18 years. Blood samples were obtained before exercise, immediately after exercise on running belt and after 60 minutes of regeneration. To determine the long-term effects, the investigations were performed repeatedly every 3 month during a one year period.
We determined hormons (adrenalin, noradrenalin, growth hormon, cortisol ...), absolute and percentual counts of lymphocytes and their subsets – CD3, CD4, CD8, CD19, CD16/56, receptors of cytokines IL6, IL-1ra and expression of adhesion molecules LFA1 (CD11a/CD18).
Results – acute exercise: Immediately after acute exercise we observed significant increase of absolute counts of T lymphocytes and their subsets – CD3, CD4, CD8 – followed by significant decrease after 60 minutes of regeneration before initial values. The most marked differences after acute exercise were found in the NK cells, which are thought to be responsible for defence against viruses and malignancy cells. The

mean value of the NK cells were 340 cc/ul before exercise, immediately after exercise 1220 cc/ul and after 60 minutes of regeneration were 130 cc/ul.
Results – chronic changes: We saw significant decrease of percentual counts of T lymphocyte subsets CD8 and NK cells CD16/56 after 1 hour of regeneration. To our opinion these changes may be due to the higher training intensity. This changes may be significant markly in cases with daily exhaustive exercise that overlap the open window.
Conclusions: Our results indicate, that exercise may induce redistribution of lymphocytes between the peripheral lymphatic tissue and the circulation. This may be due to muscle damage, increase of IL6 levels, stimulation of neuroendocrine system and increased expression of adhesion molecules. Repeated and exhaustive long term exercise modify the individuals immune response and this may predispose individuals to frequent respiratory viral infections, chronic fatique syndrome or opportune viral (CMV, EBV) and parasital (e.g. toxoplasmosis) infections.
This facts should be taken into consideration during planing at the exercise schedule of top-sportsmen.

DOES VENTILATORY THRESHOLD COINCIDE WITH THE THRESHOLD FOR CHANGES IN NATURAL KILLER CELL CYTOTOXICITY DURING INCREMENTAL EXERCISE?

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To examine the above question, we exercised 9 healthy males on a cycle ergometer at 50, 90, 120 and 140% VT-$\dot{V}O_2$ (Four 5 min stages) incrementally. Exercise at 50 and 90% VT did not increase resting blood lactate values, but exercise at 120 and 140% VT yielded lactate values of 2.7 ± 0.7 and 5.5 ± 1.2 mmol/L respectively. Plasma concentrations of adrenaline (Ad) and noradrenaline (NA) were increased exponentially and the threshold for increase of NA concentration being the same as for ventilatory threshold. Changes in NK cell count followed a similar pattern. Simple regression analysis showed a strong positive correlation between NA concentration and NK cell count ($r=0.803$, $p<0.001$). The intensity of expression of CD44 changed significantly ($p=0.007$) during exercise and a weak negative correlation was found between Ad concentration and the intensity of expression of LECAM-1 ($r=0.309$, $p=0.023$). NKCA increased significantly at 120 and 140% VT ($p<0.001$) without any changes in per cell cytotoxicities. The results suggest that NK cell counts moved in close parallel with NA concentration and that this hormone contributes to changes in NKCA during incremental exercise.

NATURAL KILLER CELL ACTIVITY AFTER SHORT-TERM EXERCISE TRAINING IN RATS

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It has previously been shown that 5 weeks of chronic voluntary exercise in spontaneously hypertensive rats (SHR) improved natural killer (NK) cell activity, measured as *in vivo* cytotoxicity against lymphoma cells. The aim of the present study was to examine if this improved response is seen after short-term exercise training in male SHR. Furthermore, we have studied if there is a correlation between running activity and NK cell activity.

The study was carried out in three groups of rats that had free access to a running wheel for 1, 2, and 3 weeks, respectively, and in one sedentary control group. *In vivo* cytotoxicity was measured as clearance of injected ^{51}Cr labelled YAC-1 lymphoma cells from the lungs. Running data was collected from 4 runners in each group, during the last week of running. Wheel revolutions were automatically registered by a microprocessor, every 30 minute.

It was found that *in vivo* cytotoxicity is enhanced already after one week of training. Similar degree of NK cell avtivity was seen after 1, 2, and 3 weeks of running, thus all groups showed significantly higher NK cell activity than the control group. The runners in the present study did not reach as high levels of *in vivo* cytotoxicity as the runners in the 5 weeks study. No correlation between the mean running activity during the last week of running and NK cell activity was seen in either this study nor in the 5 weeks study.

The results indicate that one week of chronic voluntary exercise in rats is sufficient to augment natural cytotoxicity *in vivo*. Regardless of running activity, *in vivo* cytotoxicity is improved. Further studies are needed to elucidate whether different mechanisms are involved in the rapid positive response of NK cell activity after short-term exercise training compared to enhanced NK cell activity after longer exercise training.

HUMAN NEUTROPHIL DEGRANULATION IS NOT AFFECTED BY THE PLASMA CONCENTRATION OF CORTISOL WITHIN THE PHYSIOLOGICAL RANGE

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The fall in neutrophil bactericidal function following prolonged exercise has been attributed partly to elevated blood cortisol (Fukatsu *et al*., Life Sci., 58: 2337-2343, 1996). Therefore, we wished to investigate the influence of cortisol (within the physiological range) on lipopolysaccharide-stimulated neutrophil degranulation *in vitro* in both fasted resting blood samples and in these samples after the addition of cortisol to give a plasma cortisol concentration similar to that observed following prolonged exercise. Venous blood samples were taken following an overnight fast from eight healthy males. Aliquots of whole blood were incubated for 1 h at 37° C with additional cortisol (resting cortisol concentration plus cortisol added: CORT) or with no additional cortisol (resting cortisol already present in sample: CONT). The *in vitro* neutrophil degranulation response (elastase release) to bacterial lipopolysaccharide was assessed according to Blannin *et al*. (J. Physiol., 495 P, 140P, 1997). A mean (SEM) neutrophil count of 2.78 (0.38) $x10^9$ cells.l^{-1} was recorded. Plasma cortisol concentrations were 284 (26) and 669 (21) nM (P<0.01) for CONT and CORT. There was no effect of cortisol treatment on elastase release from LPS-stimulated neutrophils in whole blood (284 (19) and 300 (21) fg.$cell^{-1}$ for CONT and CORT; P>0.05). These data show that an increase in plasma cortisol from resting fasted levels to typical concentrations measured following prolonged exercise does not affect the *in vitro* neutrophil degranulation response to bacterial lipopolysaccharide.

CHEMOKINES ARE ELEVATED IN PLASMA AFTER STRENUOUS EXERCISE

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The present study investigates to which extent strenuous exercise influence the plasma concentration of the chemokines IL-8, MIP-1α and MIP-1β. Eight male athletes age 24-37 years participated in The Copenhagen Marathon 1997 [median running time 3:21 hour:min (range 2:40 – 3:34)]. Blood samples were obtained prior to, immediately after, and every 30 min in the four hours resting period *post*-running. Plasma was analysed by enzyme-linked immunosorbent assay (ELISA). The plasma concentrations of IL-8 and MIP-1β were markedly elevated immediately after the run, and both peaked 0.5 hours *post* exercise (6.5 and 4.8 fold increase, respectively, as compared to the pre-exercise value. The plasma concentration of MIP-1α was also elevated after the run, but the levels were below the range of the ELISA-kit. Hypothetically, the finding of elevated levels of chemokines in plasma after exercise could have implications for HIV-infected individuals, since high levels of chemokines have been shown to protect against HIV disease progression.

EXERCISE INCREASED NATURAL KILLER RESISTANCE TO IMMUNOSUPPRESSORS, CYCLOSPORINE A, COLD AND FASTING IN RATS

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We proposed a new index, a resistance to artificial immunosuppressor, cyclosporine A, and using rat natural killer activity as an indicator. We named this index increased resistance to immunosuppressor (IRIS), and we demonstrated that consecutive 5 day injections of dexamethasone induced IRIS in natural killer activity in our previous report. The aim of this study is to determine the minimum duration of practical exercise for IRIS onset, and to confirm whether exercise induces IRIS against environmental or behavioral immunosuppressors besides cyclosporine A. In our results, it was found that IRIS in natural killer activity against cyclosporine A was induced by not 2 but 3 weeks of swimming exercise and that exercise also induced IRIS against cold exposure and fasting immunosuppressive factors. Therefore, exercise sustained natural killer activity against cyclosporine A, cold exposure and fasting. Furthermore this finding suggested IRIS in natural killer activity might be a beneficial tool to evaluate immune status against, at least, acute immunosuppressive factors such as chemical, environmental and behavioral ones.

INTENSIVE ENDURANCE EXERCISE IN THE HEAT – ACUTE AND ADAPTATIONAL EFFECTS ON IMMUNOMODULATING STRESS HORMONES AND PERIPHERAL LEUKOCYTE COUNTS

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We questioned whether the response of leukocyte counts, cortisol (CORT) and human growth hormone (hGH) to intensive endurance exercise is additionally affected by heat stress. 12 non-heat-acclimated athletes completed 2 continous runs on the treadmill (C1 and C2) with a 6-day interval between the runs. Duration (60 min) and velocity (90% of the individual anaerobic threshold) were identical in C1 and C2. 6 of the subjects performed C1 at a room temperature/rel. Humidity of 18° C/45% (group N-H) and 6 others at 28° C/45% (group H-H). C2 was completed from both groups at 28° C/45%. Neutrophil (NEU), lympho- (LYM) and monocyte counts, plasma CORT and hGH were assessed at rest, 0, 30 min, 3 and 24 h after C1 and 2. Body core temperature (medians) was 39.56 (H-H)/ 38.35° C (N-H) after C1 and

39.21 (H-H)/ 39.67° C (N-H) after C2, max. lactate was 2.61/1.84 and 2.41/3.83 mmol/l. 3 h after C1 NEU rose more pronounced in H-H (+262%) vs. N-H (+144%). After C2 the rise in NEU of H-H was lower (+160%) compared to C1 and vs. N-H/C2 (+216%). LYM counts exhibited a lower increase in N-H (+33%) vs. H-H (+85%) directly after C1 but did not show differences between the two groups after C2. 30 min after C1 CORT only rose in H-H (666 nmol/l) where at N-H showed no changes (311 nmol/l). Afer C2 the rose of CORT was similar in both groups (594 in H-H and 638 nmol/l in N-H). In group H-H hGH showed a marked increase directly after C1 to 1588 pmol/l, which was different from group N-H (1007 pmol/l). After C2 peak values of hGH in group H-H were lower (883 pmol/l) compared to C1 and showed a tendency to lower values compared to N-H/C2 (1247 pmol/l). In conclusion intensive endurance exercise in the heat induce a more pronounced response of CORT and hGH, NEU and LYMP counts compared to normal thermal conditions. Our results suggest that adaptation to heat stress include a lower exercise-induced rise of NEU counts and plasma hGH at the same relative work intensity.

THE DEVELOPMENT OF IMMUNOLOGIC DISORDERS DURING EXERCISE

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Intensive prolonged exercise can cause changes of various immune parameters. Many aspects of immune disorders development after heavy exertion is unclear now. We studied the influence of intensive physical exertion on induction of chronic graft-versus-host reaction in female mice (C57B1/6xDBA/2) F1 by injection of immunocompetent cells from DBA/2 mice. The induction of chronic GvH reaction in mice causes the development of various immunopathologic states, including immunodeficiency and autoimmune glomerulonethritis at such genetic differences of donor-recipient pair. B-cell immunity is involved in these processes. Previously we demonstrated the influence of physical exertion on the changes of B-link of immune system. Mice were training to swim daily during 2 months before and 1 month after the induction of chronic GvH disease. We studied the frequency of various immune disorders appearance and immune parameters characteristics in exercising and control mice. After training during 2 months (before the induction the chronic GvH reaction) the main parameters of immune system of exercising mice didn´t differ considerably from control animals. There was observed the some enhance of IgG level in serum and spontaneous IgG synthesis in vitro. Despite of the slight changes in immune status the relative frequency of the immunodeficiency and autoimmune disorders differed in the exercising mice and the control group. It´s supposed the influence of physical exertion on Th1:Th2 ratio that can result to change the frequency of various immunopathologic states.

INFLUENCE OF DIFFERENT TYPES OF EXERCISE ON THE EXPRESSION OF HEME OXYGENASE-1 IN LEUKOCYTES

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Heme oxygenase 1 (HO-1) is an antioxidant stress protein, that is mainly induced by reactive oxygen species, inflammatory cytokines and hyperthermia. By using flow cytometry we investigated the influence of different types of exercise on HO-1 expression in mono- (M) and granulocytes (G) of the peripheral blood. An intensive endurance run (half marathon, HM), an exhaustive run above the lactate steady state (ER) and an eccentric exercise (EE) were compared.

Methods: HM: 12 male athletes achieved an intensive endurance run like a competing half marathon (21.1 km, 90.34 ± 12.8 min). ER: Another 15 male subjects performed a graded treadmill test which was followed by a continuous run until exhaustion 15 min later at 110% of the individual anaerobic threshold (10.8 ± 0.7 min, max. lactate 8.9 ± 0,6 mmol.l^{-1}). EE: 12 endurance trained subjects accomplished eccentric muscle exercise at high intensity in a leg pressure test. Blood samples were drawn at rest, directly, 5 and 24 h after exercise for determination of differential counts, plasma CK and cytoplasmic HO-1 expression in M and G by flow cytometry.

Results: Lactate values after exercise were 5.1 ± 2.2 (HM), 9.0 ± 2.1 (ER), 3.8 ± 1.6 (EE) mmol.l^{-1}. Plasma CK reached highest values 24 h after exercise, 289.4 ± 221 (HM), 133.4 ± 91 (ER), 230.9 ± 138.6 (EE) U.l^{-1}. The maximal increase of leucocyte counts after exercise was 14.7 ± 2.1 (HM), 11.5 ± 19.2 (ER), 6.23 ± 1.4 (EE). HM significantly stimulated HO-1 expression in M and G ($p<0.05$) whereas ER as well as EE revealed no significant effects.

Conclusion: Muscle stress or short time heavy exercise alone are not efficient in stimulating the antioxidative stress protein HO-1 in peripheral leukocytes. A systemic inflammatory reaction, hyperthermia and increased oxidative stress in immune competent cells all together caused by a HM may be mainly responsible for the rise of HO-1 expression in leukocytes.

WHITE BLOOD CELL COUNTS AND MOBILISATION AFTER ARM ERGOMETRY IN SUBJECTS WITH PARALYSIS

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Introduction: White blood cell mobilisation after intensive exercise is a well described phenomenon. The aim of the present study was to investigate whether paralysis of big muscle groups has an influence on white blood cell mobilisation.
Subjects and Methods: Eleven subjects participated in the study, all mobilized in wheelchairs. The reasons for the paralysis were paraplegia by accident (6 cases) or poliomyelitis which mainly paralysed the legs (5 cases). As a control served ten healthy subjects (age matched). The subject performed an exhaustive bout of exercise by arm ergometry (beginning with 20 W for 3 minutes and increasing 20 W each 3 minutes until exhaustion). Blood samples were drawn before, immediately after and two hours after exercise. The following parameters were determined: White blood cell count, white blood differential and lymphocyte subpopulations (CD3+, CD4+/CD25+, CD8+, CD20+/CD23+, CD16+/CD56+/CD3-). Subjects with symptoms of an infection were excluded.
Results: Before exercise the patients´ CD4 counts were significantly higher compared to the healthy subjects´ (553 cells/ml^{-1} vs. 472 cells/ml^{-1}, $p \leq 0.027$). The percentage of CD4+ cells expressing the CD25 antigen was significantly higher, too. In the patients´ group significantly more CD20+ cells were mobilized. The other determined parameters were not significantly influenced.
Discussion: Paralysis with denervation of big muscle groups is not followed by major changes of the pattern of white blood cell mobilization, especially NK-mobilization is not impaired. The higher CD4 counts before exercise may be caused by subclinical infections (bladder) in the paralysed subjects.

THE ROLE OF MODERATE EXERCISE TRAINING ON THE METABOLISM OF MACROPHAGES AND LYMPHOCYTES FROM TUMOR BEARING RATS

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There is common belief that moderate intensity exercise can be beneficial to the immune system (IS). The relation between exercise training and IS function is not well established. We tested the effect of a moderate intensity training program upon IS function of male Wistar tumour-bearing rats. The animals trained 5 days/week, 1 hour/day, for 8 weeks, training section beginning at 9:00 am (3 h after the beginning of the dark period). We evaluated the production od the hydrogen peroxide by macrophage, lymphocyte proliferation and glutamine and glucose metabolism in both cell types. Macrophages from Walker-256 tumour-bearing rats (TB) presented increased production of H_2O_2 (12.4 ± 0.7 and 52.3 ± 1.4 nmol/l per mg protein for control and TB rats, respectively). The training protocol caused a decrease of 17.4% by the production of H_2O_2 by macrophages accompanied by a decrease in glucose

consumption (25%) and lactate production (47.1%). Training induced an increase of the production of labelled CO_2 from the oxidation of [U-^{14}C]-glucose (22.6%) in TB-rats. The proliferative response of lymphocytes was reduced (44.8%) in TB rats when compared with controls (544.2 ± 71.3 for TB rats and 987.2 ± 43.9 for normal rats). The training protocol increased the proliferative response of TB-rats lymphocytes (75%), and their response to mitogens. These results show that the training protocol herein adopted was able to play a modulatory effect upon macrophage and lymphocyte metabolism, leading to increased survival time in Walker-256 tumour-bearing rats.
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RESPONSES OF CYTOLYTIC/CYTOTOXIC LYMPHOCYTES TO A SUPRAMAXIMAL EXERCISE: A CARTOGRAPHIC STUDY USING FLOW CYTOMETRY

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Many studies have shown that a single bout of maximal exercise is followed by marked increases in the number of cytolytic and cytotoxic cells. Unfortunately, there are no unique phenotypic profiles for these categories of lymphocyte, leading to a certain confusion in the interpretation of the literature results. The recent onset of quadruple immunostaining coupled to flow cytometry allows a more accurate characterization of such lymphocyte subsets. This study was undertaken to extend the characterization of cytolytic/cytotoxic lymphocytes changes after supramaximal exercise. Nine sedentary male subjects (age 32.2 ± SEM 1.9 yrs) underwent a 30-s Wingate test and blood samples were collected before and 3 minutes following exercise. The different types of lymphocytes were studied on whole blood using a quadruple (CD3, CD8, CD16, CD56) immunostaining. Absolute counts (Flow-count fluorospheres) were simultaneously measured with a Coulter flow cytometry system. The average power mean during the test was 620 ± 6.5 W, and the lymphocytosis increased (+54.6%; $p<0.001$) between the rest and post-exercise periods (2207 ± 84 to 3411 ± 109 $10^6.l^{-1}$). From the seven CD3- possible combinations (excluding CD-CD8-CD16-CD56-, i.e. B cells), 6 of them showed a significant increase ($p<0.05$) in absolute counts. Only the CD3-CD8-CD16-CD56+ phenotype showed no significant change. From the six CD3+ possible combinations (excluding CD3+CD8-CD16-CD56- i.e. CD4+cells, and the chimeric CD3+CD8+CD16+CD56+ combination), only one phenotype (CD3+CD8+CD16-CD56-, suppressor cells) showed a significant ($p<0.01$) increase (602 ± 85 to 940 ± 180 $10^6.l^{-1}$). No significant change was observed for the CD3+CD8-CD16+CD56+ (or-) phenotypes representing the not MHC restricted cytolytic cells. The data indicate that except for the CD3-CD8-CD16-

CD56+ phenotype, all the members of the "Natural Killer cells family" increase their count after exercise. This was not the case for the cytotoxic cells among which only the CD3+CD8+CD16-CD56- phenotype expression increased secondary to exercise.

EFFECT OF N-3 PUFA SUPPLEMENTATION ON THE CYTOKINE RESPONSE TO STRENUOUS EXERCISE

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N-3 rich PUFAs have been shown to modulate the inflammatory response in in vitro models and in experimental sepsis model. Strenuous exercise induces an acute phase response including increased levels of pro-inflammatory cytokines. The aim of the present study was to investigate whether fish-oil supplementation was able to modulate this response.
Twenty runners were allocated into supplementation with fish-oil (Pikasol) 6.000 mg daily containing 3.600 mg PUFAs (53% EPA and 31% DHA) for 5 weeks or a control group before participating in The Copenhagen Marathon 1998. Median racing time was 3 hours 38 min (range 2:39-4:22), median age was 28 years (range 23-48). Blood samples were collected at rest before race, immediately after and 1.5 and 3 hours post exercise. The plasma-concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1 receptor antagonist (IL-1ra) were measured by ELISA. The level of TNF-α and IL-6 peaked immediately after the exercise, the increase being approximately 3- and 100-folds respectively. The level of IL-1ra peaked 1.5 hours after the exercise, the increase being approximately 90-fold.
We were unable to detect any differences among the two groups regrading exercise-induced increase in the circulating levels of cytokines and leucocytes.

THE EFFECTS OF CARBOHYDRATE SUPPLEMENTATION ON NEUTROPHIL DEGRANULATION RESPONSES TO A SOCCER-SPECIFIC EXERCISE PROTOCOL

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Increasing carbohydrate (CHO) availability has been shown to attentuate some of the immunosuppressive effects of prolonged, continuous, strenuous exercise (Nieman, Exerc. Immunol. Rev., 4: 64-76, 1998). However, the effect of CHO feeding prior to and during intermittent exercise, such as soccer, has not yet been investigated. Therefore we wished to investigate the influence of CHO compared with placebo (PLA) beverage consumption on the immune and plasma cortisol responses to a soccer-specific exercise protocol. In a randomised counter-balanced design, eight University soccer-players consumed CHO (6% w/v) or PLA beverages before (400 ml), during (150 ml every 15 minutes during each half; 400 ml at halftime) and after (400 ml) two 90-min soccer-specific exercise bouts (3 days apart) that were designed to mimic the activity patterns and distance covered during a typical soccer match. Venous blood damples were obtained before, during and after the exercise protocol. Results were analysed using a 2 (treatment) x 4 (times of measurement) repeated measures ANOVA.

Plasma lactate increased to -4.0 mmol l^{-1} at 45 and 90 minutes of exercise on both treatments (P<0.01). After 90 min of exercise, plasma glucose concentration was 17% lower on the PLA treatment compared with the CHO treatment (P<0.01). However, the pattern of change in plasma cortisol and circulating lymphocyte count did not differ between the two treatments. Mean (SEM) blood neutrophil counts were 14% higher 1 h after the PLA treatment compared with the CHO treatment (PLA: 4.8 (0.5) x 10^9 cells.l^{-1}; CHO: 4.2 (0.4) x 10^9 cells.l^{-1}; P<0.05), but there was no effect of treatment on the lipopolysaccharide-stimulated degranulation response of blood neutrophils.

We conclude that carbohydrate supplementation influences neutrophil trafficking, but not degranulation, when the overall exercise intensity is moderate and changes in plasma stress hormones are minimal.

EFFECT OF CARBOHYDRATE BEVERAGE INGESTION ON CYTOKINE AND PHAGOCYTIC RESPONSES TO TWO HOURS OF ROWING IN ELITE FEMALE ATHLETES

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This study examined the influence of carbohydrate (C) versus placebo (P) beverage ingestion on the phagocytic and cytokine responses to normal rowing training by 15 elite female rowers.
Athletes received C or P before, during and after, two, 2-hour bouts of rowing performed on consecutive days. Blood was collected before, and 5-10 minutes and 1.5 hours after rowing. Metabolic measures indicated that training was performed at moderate intensities, with some high intensity intervals interspersed throughout the sessions. Elevations in blood neutrophils and monocytes, phagocytic activity, and plasma IL-1ra were significantly less following C versus P ingestion. No differences were observed for oxidative burst activity, IL-6, IL-8, or TNFα.
Glucose was significantly higher after two hours of rowing with C ingestion, however cortisol, growth hormone, epinephrine, norepinephrine, and CRP were not affected by carbohydrate. In summary, carbohydrate compared to placebo ingestion attenuated the moderate rise in blood neutrophils, monocytes, phagocytosis, and plsama IL-1ra concentrations that followed two-hour bouts of training in elite female rowers. No changes in blood hormone concentrations were found.

DIETARY VITAMIN C INTAKE, MARKERS OF BLOOD ANTI-OXIDANT STATUS, OXIDATIVE STRESS & LOCAL INFLAMMATION IN ENDURANCE EVENTS

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The aim of this study was to ascertain whether a relationship existed between dietary anti-oxidant intake and markers of blood anti-oxidant status, oxidative stress and local inflammation following ultramarathon and marathon running. Ten runners were monitored 18 hrs before and within 1 hr following completion of the 88 km Comrades Marathon and 12 hrs before, 30 min after and 24 hrs following partipating in a standard 42 km marathon.
Pre-race dietary recalls and analysis of intake nutritional supplements revealed a mean total daily Vit C intake of 430 (± 378.4) mg on the day preceding the ultramarathon, while plasma Vit C levels rose significantly ($p<0.05$) from 18.5 (± 2.9) pre-race to 21.9 (± 5.0) $ug.ml^{-1}$ post-race. Pre-race plasma Vit E levels failed to show a consistent rise or fall following completion of the 88 km event, whereas pre-race beta-carotene levels (241.6 ± 75.5) dropped significantly ($p<0.05$). Despite an average 263% increase in serum cortisol concentration and an absolute post-race neutrophilla in each of the subjects, circulating levels of lipid peroxides remained unchanged ($p>0.05$) after completion of the ultramarathon. In participants in the standard marathon total 24 hr dietary intake of Vitamin C was 225.6 (± 275.7) mg and total serum Vit C levels rose from 16.2 (± 4.8) to 19.6 (± 6.7) $ug.ml^{-1}$ 30 min post-

race. The significant ($p<0.05$), but less pronounced neutrophilla, increase in platelet count, rise in neutrophil:lymphocyte ratio and rise in serum cortisol concentrations was accompained by significant rises in serum Vit C, ferritin and creatine kinase.
This study falled to show a significant correlation between reported dietary intake of anti-oxidants and blood anti-oxidant status. In both endurance events the low dietary intake of anti-oxidants was associated with significant, but small rises in C-reactive protein indicating lesser inflammatory response than previously reported in the literature. The results of this study appear to highlight the caution with which blood anti-oxidant status must be interpreted in the light of dietary anti-oxidant intake and markers of oxidative stress.

TRAINING AND NATURAL IMMUNITY - EFFECTS OF DIETS RICH ON FAT OR CARBOHYDRATE

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The findings on chronic exercise training and human natural immunity are controversial. We hypothesized that the increased NK cell activity reported in some groups of athletes was not directly related to training, but dependent on other lifestyle factors, such as the diet. The purpose of the present study was to investigate whether training on a carbohydrate-rich versus fat rich-diet influenced the effect of training on natural immunity. Ten untrained young men ingested a carbohydrate rich diet (65 energy percent (E%) carbohydrate) and ten subjects a fat-rich diet (62 E% fat) while endurance training was performed 3-4 times a week for 7 weeks. Maximal oxygen uptake increased by 11% in both groups. Blood samples for immune monitoring were collected before and in the end of the study. Twenty age matched subjects had blood collected in parallel and data from these subjects were used to eliminate day-to-day variation in the immunological tests.
Training independent of diet increased the percentage of CD3-CD16+CD56+ natural killer (NK) cells ($p=0.05$), whereas the NK cell activity and NK cell count did not change. Furthermore, training did not influence the percentages or concentrations of CD3+, CD4+, CD8+, CD19+ and CD14+ cells. However, when the two diet groups were compared, it was found that the NK cell activity increased in the group on carbohydrate-rich diet compared to the group on fat-rich diet in response to training. The effect of training was significantly different between groups ($p=0.007$). These data indicate that diet manipulation during training may influence natural immunity and suggests that a fat-rich diet is detrimental to the immune system compared to the effect of a carbohydrate-rich diet.

NK AND T CELL RESPONSE TO 2H ROWING IN ELITE FEMALE ROWERS

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The influence of carbohydrate (C) versus placebo (P) beverage consumption on the immune and hormonal responses to normal rowing training sessions was measured in 15 elite female rowers residing at the U.S. Olympic Training Center. In a randomized, counterbalanced design, the athletes received C or P beverages (double-blinded) before, during, and after two 2-hour bouts of rowing (one day apart). Blood samples were collected before, and 5-10 min and 1.5 h after rowing. Metabolic measures indicated that training was performed at moderate intensities, with some high intensity intervals interspersed throughout the sessions. Glucose and insulin were significantly lower after 2 h of rowing with ingestion of P compared to C. The patterns of change in cortisol, growth hormone, epinephrine, and norepinephrine did not differ between C and P rowing trials. Blood neutrophil cell counts and the neutrophil/lymphocyte ratio were significantly higher following P versus C rowing sessions. The patterns of change in blood lymphocyte and lymphocyte subset counts, and lymphocyte proliferate responses did not differ between P and C trials, except for a slight differences in NK cell counts and activity. In summary, minimal changes in blood hormonal and immune measures were found following 2 h bouts of training in elite female rowers. C compared to P ingestion attenuated the moderate rise in blood neutrophil counts, but had slight or no effects on other immune parameters.

BRANCHED CHAIN AMINO ACIDS SUPPLEMENTATION AND THE IMMUNE RESPONSE OF BRAZILIAN TRIATHLETES

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Athletes submitted to intense long duration exercise presented a higher incidence of upper respiratory tract infection (URTI). At least two main factors could be related to this phenomenon, the hormonal changes induced by exercise and the reduction in glutamine plasma levels. To assess the eventual influence of the second mechanism, we have evaluated the effect of branched chain amino acid (BCAA) supplementation upon the immune response of triathletes. Twelve male triathletes (Olympic Triathlon) were suplemented with 3.0 g BCCA daily in the 3 weeks before the São Paulo

International Triathlon, held in April 97 and 98 and on the day of the trial. The carbohydrate intake of the athletes was controlled (during the trial they consumed 60-65 g of carbohydrate). The supplemented group (SG) presented an increased proliferative response to concanavalin A and LPS after the trial, as well as an increased production of IL-1, IL-2, TNF and INF. These athletes showed no changes in glutamine plasma levels, while a reduction of glutamine plasma concentration (22%) was observed in the placebo group. A reduction in URTI (40%) was also reported by the athletes. The data obtained suggest that BCAA supplementation, by avoiding a decrease in glutamine plasma levels, is able to enhance immune function in triathletes.
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EPSTEIN-BARR VIRUS REACTIVATION IN ELITE SWIMMERS

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The aim of the pilot study was to determine whether Epstein-Barr Virus (EBV) reactivation occurs in elite swimmers during episodes of upper respiratory illness. Fourteen elite swimmers (11 males, 3 females, age range: 16-25 years) had EBV serology assessed at the beginning and end of a 6 month training season. Sera was tested for antibodies to EBV capsid antigen IgM, EBV early antigen IgG and EBV nuclear antigen IgG by ELISA. The EBV serology indicated 11 swimmers had past infections (79%), 1 had a recent infection at the commencement of the season (7%) and 2 were negative for exposure to EBV (14%). Saliva was collected at monthly intervals and at the time of each physician verified respiratory illness. At least one episode of respiratory illness occurred in 10 subjects (10/14.71%), including the 2 subjects with negative EBV serology. The saliva samples were stored at –70° C until assayed for EBV-DNA by PCR using primers specific for the DNA polymerase gene of HSV-1, HSV-2, EBV and CMV. The concentrations of IgA was measured simultaneously by a in-house ELISA. Excluding the 2 subjects with negative EBV serology, EBV-DNA was detected in 4 samples (4/81, 5%) from 3 subjects (3/12, 25%). One EBV positive sample was collected during an infection episode. The pattern of appearance of EBV-DNA in saliva in the 3 subjects suggested that viral reactivation may precede the clinical symptoms of respiratory illness and be associated with prior salivary IgA suppression. The study design indicated that testing for viral reactivation during respiratory illness episodes may be un-informative.

SEASONAL VARIATIONS IN TRAINING LOAD DO NOT AFFECT THE IMMUNO-ENDOCRINE RESPONSE TO ACUTE EXHAUSTIVE EXERCISE IN ELITE NORDIC SKIERS

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It is still a scientifically unclear understanding of whether or not chronic alterations in training loads affect the acute exercise-induced response in the endocrine and immune system. Thus, the main purpose of this study was to examine the effect of normal seasonal changes in training- and competition loads on the immuno-endocrine responses to an acute bout of exhaustive endurance exercise along with monitoring mood changes throughout a sport season.

Method: Ten male, international Nordic skiers, age 20-29, maximal oxygen uptake 69-82 $ml.kg^{-1}.min^{-1}$ performed two incremental treadmill tests to exhaustion at the same time of day (± 1 h); one during the competitive season (in-season HI test) and one during their recovery season (off-season LO test). The subjects filled out a training- and competition log (TC-Score) for 3 weeks prior to each test and a 65 item POMS-test at arrival in the lab. Venous blood for haematological, hormonal and IL-6 analysis was drawn before as well as 0, 15, 30, 60, 120 and 240 min after the test.

Results: The TC-Score was more than twice as high during competitive season (16.0 ± 3.9) compared to the off-season period (7.0 ± 4.4). An ANOVA procedure for repeated measures showed no difference between the in-season HI and the off-season LO tests for the exercise-induced changes in neutrocyte, lymphocyte, epinephrine, ACTH or cortisol concentrations, but a small increase in norepinephrine ($F_{1.9}$=8.7, p=0.018) and IL-6 concentrations ($F_{1.9}$=6.36, p=0.007) were found at the in-season HI test compared to the off-season LO test. There was no significant differences in POMS global mood score or subscores between the in-season HI and the off-season LO tests.

Conclusion: In a group of balanced trained elite Nordic skiers we observed that the changes in leukocyte subsets and stress hormones in response to a single bout of exhaustive exercise do not reflect alterations in training and competition load during a sport season.

SALIVARY LACTOFERRIN AS A MARKER OF MUCOSAL IMMUNOCOMPETENCE IN ELITE SWIMMERS

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This study investigated the impact of endurance training on the innate mucosal immune system and the incidence of upper respiratory tract symptoms (URTS) in elite athletes. Lactoferrin was selected as a marker of innate mucosal immunity because of its documented ability to sequester iron, bind to bacteria, and antimicrobial activities in synergy with secretory IgA and lysozyme. Samples of unstimulated whole saliva were collected from the Australian Institute of Sport (AIS) Swimming Squad. The initial sample was collected after an extended break from training, followed by samples taken during a seven month season of intense endurance training prior to international level competition. The routine samples were collected once each month prior to and immediately after a standard exercise session. Athletes who self presented to the AIS medical facility for treatment of an illness were assessed by a sports physician and if URTS were present an illness sample of saliva was collected. The lactoferrin concentration of the clarified saliva was determined by ELISA, the protocol was developed in house, using commercially available antibodies. The incidence of URTS ranged from 0-4 bouts for individual athletes and the mean for the squad was <2 bouts over the seven months. Preliminary analyses show that in contrast to previous observation of serum lactoferrin[1], there was a reduction in the lactoferrin concentration post exercise. In addition it suggests that salivary lactoferrin may decline over the training season. Further analysis will determine the association between salivary lactoferrin concentration and URTS or training load.

[1] Taylor C, et. al., Hematologic, iron-related, and acute-phase protein responses to sustained strenuous exercise. J Appl Physiol 1987 Feb 62:2 464-9.

SALIVARY IgA IN ELITE FEMALE ROWERS AND CONTROLS

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Salivary IgA (sIgA) and upper respiratory tract infection (URTI) rates were compared and correlated in female groups of 20 elite rowers and 19 nonathletes at the U.S. Olympic Training Center. In phase two of this investigation, the influence of

carbohydrate (C) and placebo (P) beverage consumption on sIgA response to normal rowing training sessions was measured in 15 rowers, and compared to values from five non-exercising rowers. Salivary samples were collected one day before, 5-10 minutes and 1.5 hours after rowing or rest. Pre-exercise sIgA concentration (µg IgA per ml saliva) was 77% higher in the rowers compared to nonathletes ($P<0.001$). The sIgA secretion rate was 34% higher in rowers compared to nonathletes but did not reach statistical significance ($P=0.276$). Two-month logs revealed 5.2 ± 1.2 and 3.3 ± 1.1 days with URTI symptoms for the rowers and controls, respectively ($P=0.268$). For all 39 subjects combined, and for the 20 rowers separately, no significant correlation was found between pre-exercise or exercise-related changes in sIgA concentration or secretion rate with URTI symptoms or hormone levels. The patterns of change in sIgA concentration, saliva protein concentration, saliva protein IgA concentration, saliva secretion rate, and sIgA secretion rate did not differ between the C and P rowing trials, or between exercised and rested athletes. These data indicate an increased sIgA concentration in female elite rowers compared to nonathletes, no association between sIgA and URTI, and no effect of carbohydrate versus placebo on sIgA concentrations or secretion rates following a two hour rowing training session.
Supported by Gatorade Sports Science Institute.

OXIDATIVE BURST ACTIVITY OF BLOOD NEUTROPHILS IS IMPAIRED AFTER FOUR WEEKS OF OVERLOAD TRAINING IN ENDURANCE ATHLETES REVERSIBLY

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The present study was conducted to test the hypothesis that a 4 week period of experimentally induced overload training causes immunosuppressive effects. In randomised order 12 male endurance athletes (age: 27 ± 3 years; $\dot{V}O_{2\,max}$ 59.5 ± 4.9 $ml.min^{-1}.kg^{-1}$) trained for 4 weeks either with their normal (NT) training volume or increased the individual´s volume training by 50% (IT). The weeks following NT or IT, respectively, the athletes reduced their training volume to about 50% of their normal training volume and exercise intensities were kept at moderate levels. At the end of IT, but not at any other time of the study, all athletes complained of typical overload symptoms and a decline of performance was objectified ergometrically. Before, at the end and 2 weeks after NT or IT, respectively, clinical, ergometrical and lab tests were done (2x3 study design). At the end of IT and in comparison to NT the TNFα and/or fMLP induced oxidative burst activity of isolated blood neutrophils showed a significant (ANOVA; $p<0.01$) 46 (TNF), 39 (fMLP) and 62 (TNF+fMLP) % reduction of rhodamine positive neutrophils (flow cytometry) indicating less reactive cells to in vitro stimulation. Also the mean intracellular rhodamine fluorescence intensity as a measure of the neutrophil NADPH-activity on a per cell basis declined by 24-29%

(p<0.01). After 2 weeks of regenerative training all effects were completely reversible. In conclusion overload endurance training leads to an impaired oxygen dependent bactericidal activity of neutrophils.

MODIFICATION OF HEMATOLOGICAL AND IMMUNOLOGICAL PARAMETERS IN TOP-LEVEL ATHLETES DURING ACUTE AND CHRONIC EXERCISE

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We evaluated lymphocyte phenotype and mitogenic response in top-level athletes in baseline condition and during acute (group A, n=50) or chronic (group B, n=25) exercise. Chronic exercise was associated with a reduction of leukocyte and platelet count as compared with acute exercise. Lymphocyte subset counts (CD3, CD19, CD4, CD8, activated T-Lymphocytes and NK cells) increased as compared with control subjects. Lymphocyte mitogenic response significantly decreased in athletes during chronic as compared with acute exercise. Taken together, these findings indicate that: 1) chronic exerxise has an appreciable influence on hematological parameters, determining a reduction of leukocyte and platelet count; 2) lymphocyte subsets increase during chronic exercise if evaluated in absolute numbers, although the relative frequency is unaffected; 3) chronic exercise is associated with a remarkable reduction of lymphocyte mitogenesis, whose clinical significance remains to be determined.

EFFECT OF EIGHT WEEKS OF TRAINING AND EXHAUSTIVE EXERCISE ON SOME ASPECTS OF THE IMMUNE SYSTEM

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Twenty-seven cross-country runners (6F, 21M) were recruited to an eight-week study investigating the effects on some aspects of the immune system of a winter training regime leading up to regional championships, followed by a bout of exhaustive exercise. Ethical permission was obtained for the study. Fasting blood samples were taken several weeks before the study began (S1); at the start of the study (S2); halfway through the study (S3); immediately after performing VO_{2max} tests (S4; these samples were taken in the afternoon) and the morning after these tests (S5).

Measurements included numbers of circulating total white blood cells (WBC), neutrophils and lymphocytes; neutrophil activity, as measured by the oxidative burst technique, and some cytokines.
At S1 there was no difference in WBC or neutrophil numbers between male and female participants but there was a significantly higher number of lymphocytes in females than in males ($p<0.02$). At S2, neutrophil numbers were significantly higher in females than in males. No gender differences were observed at S3, or immediately after VO_{2max} (S4) but males had nearly two-fold higher lymphocyte numbers than females the day after the VO_{2max} tests (S5; $p<0.01$). For all subjects there was a marked increase in numbers of WBC ($p<0.006$) at S4 compared with the other samples. There was a marked reduction in neutrophil activity ($p<0.001$) in all subjects immediately after VO_{2max}, compared with the other samples, particularly S1. There was an increase in CD4IL-6 and CD8IL-6 in all subjects at S4 ($p<0.001$ and 0.02 respectively). A gradual decrease in neutrophil activity during, compared with that observed prior to the study, together with the marked decrease after VO_{2max} tests, might suggest some immunodepression resulting from prolonged training followed by an additional bout of exhaustive exercise.

ALTERATIONS IN INTRACELLULAR CALCIUM SIGNALLING OF LYMPHOCYTES AFTER EXHAUSTIVE EXERCISE

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Exhaustive exercise is accompanied by pronounced quantitative changes of leucocytes. With respect to qualitative changes most studies on lymphocytes have concentrated on their proliferative responses. The concentrations of intracellular free calcium ($[Ca^{2+}]_i$) is an important messenger within the intracellular signalling cascade of the lymphocyte linking extracellular stimuli, e.g. a mitogen, to cellular responses, e.g. proliferation. Therefore, in the present study we investigated the calcium signalling mechanisms in lymphocytes before and after an exhaustive exercise test.
Healthy volunteers underwent a treadmill exercise test at 80% of their maximal oxygen uptake until exhaustion. Blood samples were taken before, immediately after, 1 hour after and 1 day after the test. After isolation lymphocytes were loaded with the calcium sensitive fluorescent dye Fura-2 and emitted fluorescent light was continously monitored both in a cuvette spectrometer and in a flow-cytometer. Compared to results before exercise the Ca^{2+} response after stimulation with anti-CD3 or phytohemaglutinin was unchanged immediately after exercise, but was about 35% and 80% higher 1 h after and 1 day after the test, respectively. Treatment with thapsigargin, an inhibitor of the sacroplasmic Ca^{2+}-ATPase, revealed no significant difference. Similar changes were found in lymphocyte subtypes.

Together, this novel approach demonstrates that exhaustive exercise has profound influence on intracellular calcium signalling of lymphocytes. These effects may explain changes in lymphocyte count and function as previously discribed.

PULMONARY FUNCTIONS IN SPORTSMEN PLAYING DIFFERENT SPORTS IN THE AREA OF CASERTA (ITALY): PRELIMINARY EVIDENCES

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Many evidences suggest that regular exercise has proved to be benefical for the human body and the lungs are no exception. The aims of the present study are to assess the relationship between the quality of the exercise performed and the quantitative effect of this exercise on the lungs. Pulmonary function tests of sportsmen engaged in various sports are compared to each other and to those controls.
Sportsmen practicing football (n=120), karate (n=70), swimming (n=80), basketball (n=100), volleyball (n=70), diving (n=120), riding (n=70), tae kwon do (n=10), tennis (n=30), skate rolley (n=60), water polo (n=90), handball (n=110), gymnastics (n=120), cycling (n=80), athletics (n=90). Young physicians (30) are chosen as controls. The parameters taken into account in this study are: forced vital capacity (FVC), forced expiratory volume (FEV-1), peak expiratory flow rate (PEFR) and idoneity rapid index test (IRI test) a modified test by Montoy from Harwad test.
Prelimary evidences of this study indicate that all the sportsmen have higher values of lung functions and lower values of IRI test compared to the controls. Among the various groups of players chosen for the study, the swimmers appear the maximum increase in their lung functions.

ANTIGEN SPECIFIC TH1/TH2 CYTOKINE PRODUCTION IS DIFFERENTIALLY ALTERED BY ACUTE EXHAUSTIVE EXERCISE AND MODERATE CHRONIC EXERCISE

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The purpose of this study was to examine the antigen-specific cytokine response to herpes simplex type I (HSV-1) viral infection in young mice following either an acute

bout of strenuous exercise or 8 weeks of moderate exercise training. A second purpose was to compare the antigen-specific Th1/Th2 cytokine response following acute exercise in a Th1 dominant strain (C57Bl/6J) to that of a Th2 dominant strain (BALB/cJ). Acute exercise consisted of a treadmill run to fatigue, mice rested for 20 minutes and were then infected with HVS-1 through an intranasal route. Mice were sacrificed 2 and 7 days post infection. Chronic exercise consisted of 8 weeks of moderate treadmill running (~ 45 minutes per day, 5X per week, BALB/cJ mice). Twenty-four hours post-exercise, mice were infected with HSV-1 through an intranasal route. Mice were sacrificed 7 days post infection. Spleen cells were taken for the determination of cytokine production during culture with HSV and sera was collected to assess HSV-specific IgM. The HSV-specific Th1 (IL-2, IFNγ) and Th2 (IL-10) cytokine production from the cell supernatants and HSV-specific IgM from the sera were analyzed by ELISA. Acute exercise resulted in a decrease of HSV-specific Th1 and Th2 cytokine production in both BALB/cJ and C57Bl/6J mice two days following fatiguing exercise and infection. At 7 days post infection, exercise had no effect on Th1 and Th2 cytokine production or HSV-specific IgM titer. In contrast, 8 weeks of exercise training enhanced HSV-specific Th1 cytokine production (IL-2, IFNγ) but failed to alter levels of the Th2 cytokine, IL-10, or the HSV-specific IgM titer. These findings suggest that acute, exhaustive exercise may suppress the early (day 2) development of antigen-specific Th1 and Th2 cytokines, but this suppressive effect is no longer present at 7 days post-exercise. However, the antigen-specific Th1 and Th2 responses to moderate exercise training may be differentially regulated, with an enhancement of the cytokines associated with a cell mediated immune response (Th1), and no effect on humoral immunity (Th2 cytokine and antibody production).

EFFECTS OF INCREASED WEEKLY TRAINING DURATION ON RESTING PLASMA CONCENTRATIONS OF GLUTAMINE, UREA AND IL-6

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Most training programs imply the principle of progressive overload according to which the training stimulus is increased as the body adapts to current stimuli. Occasionally increments in training load surpass the body´s ability to recover and adapt leading to accumulation of fatigue, decrements in performance, and immune suppression. Chronic exercise may induce muscle damage and increased levels of IL-6. Furthermore, chronic exercise may be responsible for a lowering of plasma glutamine and an increase of plasma urea. The purpose of this study was to evaluate effects of increased training duration on resting levels of plasma urea, glutamine, and IL-6. Twenty one male runners were divided into 3 groups: gr. 100 (n=7), gr. 130 (n=7), gr.

160 (n=7). Gr. 130 and 160 increased weekly training duration to 130% or 160% of individual normal training (NORM) for 4 weeks (OVER), and then reduced training duration to 80% for 3 weeks (REC). Gr. 100 (control) did not change amount of training during the study. All bloodsamples were taken after 42 hours without severe physical activity to eliminate the effect of acute exercise and highlight the responses to chronic exercise. In gr. 130 plasma glutamine was increased by 83.6±33.1 mmol/l (p=0.0450) after OVER and by 42.8±17.3 (p=0.0483) after REC compared to NORM. Plasma urea was increased by 0.51±0.24 mmo/l (p=0.0726) after OVER and after REC it had returned to baseline. Plasma IL-6 did not change over time. In gr. 160 plasma glutamine was increased by 24.2±10.7 (p=0.0727) after REC. Plasma urea was increased by 0.61±0.16 (p=0.0084) after OVER and had returned to baseline after REC. Plasma IL-6 increased by 0.190±0.080 (p=0.0642) after REC. In summary, there is indirect evidence of an increased protein breakdown for both groups after OVER. However, plasma urea was not associated with levels of IL-6 or glutamine. Furthermore, the present study found increased level of glutamine in response to increased training.