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Programme

7th ISEI Symposium, September 15–17, 2005

Thursday, September 15

10:00–16:00	Registration (Monte Carlo Grand Hotel) and poster mounting and viewing	
Opening of the Symposium		(*Salon Grand Prix*)
16:00–16:30	**Introduction: ISEI President and Local Hosts** Jeffrey Woods Stéphane Bermon	
16:30–17:15	***KEYNOTE LECTURE*** Chair: Ryoichi Nagatomi **Inflammatory cell function in muscle injury & disease** James Tidball	
17:30–18:30	***SESSION I:* Interleukin-6 and Exercise** Chair: Mark Febbraio **Interleukin-6 and exercise—an update** Bente Pedersen **Iron and immunity** Cindy Roy	(*Salon Grand Prix*)
18:30–19:00	**Free communications 1–2**	
19:30	***Welcome reception***	

Friday, September 16

8:30–10:00	***SESSION II:* Danger Signals to the Immune System: Does Exercise Protect against Disease via Stress Protein Release?** Co-Chairs: Mark Febbraio and Monika Fleshner **Introduction** Mark Febbraio **How are stress proteins released from cells?** Graeme Lancaster **Stress proteins and initiation of the immune system** Alexzander Asea **Exosomal export of heat shock protein 70 (Hsp70) stimulating the innate immune system** Gabriel Multhoff	(*Salon Grand Prix*)
10:00–10:30	**Free communications 3–4**	
10:30–11:15	***Coffee break and poster session***	(*Salon Lacoste*)

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11:15–11:50	***SESSION III :* ISEI meets IAAF**	(*Salon Grand Prix*)
	Biotechnology in exercise immunology	
	Co-Chairs: Stéphane Bermon and Giuseppe Fischetto	
	Microarray technology and application in exercise immunology	
	Hinnak Northoff	
11:50–12:20	**Free communications 5–6**	
12:30–14:00	***Lunch in Monaco city***	
14:00–15:30	***SESSION IV:* ISEI Meets IAAF**	(*Salon Grand Prix*)
	Immunological management of athletes	
	Co-Chairs: Michael Gleeson and Manikavasagam Jegathesan	
	Exercising when ill	
	Stéphane Bermon	
	Altitude training and immune function	
	Robert Mazzeo	
	Nutritional concerns	
	David Nieman	
	Relevance of salivary IgA to the immunological management of athletes	
	Maree Gleeson	
	Prevention of infectious diseases in an Olympic team	
	Ola Ronsen	
	Sports activity and common cold	
	Ryoichi Nagatomi	
15:30–16:15	***Coffee break and poster session***	(*Salon Lacoste*)
16:15–17:30	***SESSION V:* ISEI Meets IAAF**	(*Salon Grand Prix*)
	Exercise induced asthma	
	Co-Chairs: Ingibjorg Jonsdottir and Birgir Gudjonsson	
	Pathogenesis of EIA	
	Sandra Anderson	
	Neuro-endocrine-immune response and EIA	
	Sergio Bonini	
17:30–18:45	**Free communications 7–11 (ISEI meets IAAF)**	
18:45–19:30	***SESSION VI:* ISEI Meets IAAF Roundtable**	
	What needs to be solved in clinical exercise immunology?	
	Athletes-Juan Manuel Alonso and Harmon Brown-ISEI board in a closing roundtable	
20:00–22:00	***Cocktail***	

Saturday, September 17

8:30–10:30	**Free communications 12–19**	(*Salon Grand Prix*)
10:30–11:15	***Coffee break and poster session***	
11:15–12:30	***SESSION VII: New Trends in Basic and Clinical Exercise Immunology***	
	Chair: Sergio Bonini	
	Probiotics, immunity and sports	
	Claudio De Simone	
	Regulatory T cells	
	Lucienne Chatenoud:	
12:30–13:30	**Free communications 20–23**	
13:30–15:00	***Lunch in Monaco city***	
15:00–16:00	***Closing of the symposium***	(*Salon Grand Prix*)
	Business meeting	
	ISEI Awards	
	Future directions and closing of symposium	

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7th ISEI Symposium Abstracts

1. Inflammatory cell function in muscle injury and disease, James G. Tidball, Departments of Physiological Science and Pathology and Laboratory Medicine, University of California, Los Angeles, CA 90095-1606, USA

Modified muscle use, acute injury, and several forms of muscular dystrophy cause muscle invasion by myeloid cells that can reach concentrations of tens of thousands of myeloid cells in each mm^3 of muscle. Despite these tremendously elevated concentrations of myeloid cells, their roles in promoting muscle injury or repair have only recently begun to be identified. Analyses of several models of modified muscle use or acute injury show that neutrophils that rapidly invade injured muscle are able to increase muscle damage through the production of free radicals. Null mutation of gp^{91phox}, which is necessary for superoxide production by neutrophils, or mutation of myeloperoxidase (MPO), to prevent the conversion of hydrogen peroxide to hypochlorous acid by neutrophils, greatly reduces sarcolemmal damage during modified muscle use. These cytolytic actions of neutrophils can be modulated by muscle-derived factors. Nitric oxide generated by muscle cells greatly reduces myeloid cell invasion of muscle and decreases neutrophil cytotoxicity. On the other hand, muscle loading that is sufficient to cause mechanical damage to muscle causes the release of an unidentified factor that activates MPO and increases damage caused by neutrophils. Macrophages that subsequently invade injured or diseased muscle also affect the course of muscle injury. Early-invading macrophages are a short-lived, phagocytic subpopulation that differentiates to a non-phagocytic phenotype that can persist in damaged muscle for weeks following acute injury, or for years in chronic pathologies. Recent findings implicate this second macrophage population in promoting sarcolemmal repair and muscle regeneration that occur following acute muscle damage. However, the persistent elevation of macrophages in chronically injured muscle, such as in dystrophin-deficiency, is associated with secondary features of muscle pathology, including increased fibrosis. Together, these observations show that interactions between muscle and myeloid cells during muscle injury or disease can contribute significantly to determining the magnitude of muscle damage and the course of muscle repair.

Supported by the NIH and the Muscular Dystrophy Association.

2. Interleukin-6 and exercise—An update, Bente Klarlund Pedersen, Centre of Inflammation and Metabolism, The Department of Infectious Diseases and The Copenhagen Muscle Research Centre, Rigshospitalet, The Faculty of Health Sciences, University of Copenhagen, Denmark

For most of the last century, researchers have searched for a muscle-contraction-induced factor, which mediates some of the exercise effects in other tissues such as the liver and the adipose tissue. It has been called the "work stimulus," the "work factor" or the "exercise factor."

In our search for such a factor, we found a cytokine, IL-6, which is produced by contracting muscles and released into the blood. We have suggested that muscle-derived IL-6 fulfils the criteria of an exercise factor and that such classes of cytokines should be named "myokines."

We have demonstrated that IL-6 has many biological roles such as: (1) Activation/inhibition of metabolic genes; (2) induction of lipolysis; (3) enhancement of insulin sensitivity; (4) stimulation of cortisol production and hence a shift in leukocyte subpopulations; (5) stimulation of anti-inflammatory cytokines IL-10 and IL-1ra; and (6) suppression of TNF-production.

The IL-6 gene is rapidly activated during exercise and the activation of this gene is further enhanced when muscle glycogen content is low. In accordance, training adaptation which leads to elevated muscle glycogen content, reduces the contraction-induced interleukin-6 mRNA expression in human skeletal muscle.

Recent evidence demonstrates that exercise increases IL-6 receptor production in human skeletal muscle. This effect is most prominent in the post-exercise period, suggesting a post-exercise-sensitizing mechanism to IL-6 when plasma IL-6 is concomitantly low. Exercise-induced increases in IL-6 receptor mRNA most likely occur via an IL-6 independent mechanism as shown in IL-6 KO mice.

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3. Iron and immunity, Cindy N. Roy [a,b], Martina U. Muckenthaler [c], Ángel O. Custodio [a,b], Jos de Graaf [c], Susanne Schneider [c], Imo Akpan [a,d], Howard H. Mak [a,b], Lynne K. Montross [a,b,d], Mayka Sanchez [c], Alessandro Gaudino [c], Matthias W. Hentze [c], Nancy C. Andrews [a,b,d], [a]Children's Hospital, Boston, MA 02115, USA, [b]Harvard Medical School, Boston, MA 02115, USA, [c]European Molecular Biology Laboratory, Meyerhofstrasse 1, D-69117 Heidelberg, Germany, [d]Howard Hughes Medical Institute, Boston, MA 02115, USA

The physiological link between immunity and iron balance is illustrated in the anemia of inflammation. Inflammation reduces absorption of dietary iron and hinders iron recycling from senescent red blood cells, resulting in low serum iron concentrations. These events

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are thought to act as part of the host defense against proliferating microorganisms and cancer. However, this ''iron withholding'' also reduces the iron available to maturing red blood cells, restricts hemoglobin synthesis, and eventually contributes to the development of anemia. Hepcidin antimicrobial peptide (HAMP) is a hepatic defensin-like peptide hormone and a part of the type II acute phase response. HAMP is a central mediator of the anemia of inflammation. HAMP likely modulates iron transport from macrophages or enterocytes to red blood cell precursors as a consequence of its interaction with SLC40A1/ferroportin, the only known transporter that facilitates cellular iron egress. Expression of *HAMP* is regulated by at least three types of stimuli including inflammation, anemia or hypoxia, and iron loading. Interleukin-6 is required for induction of *HAMP* expression in response to inflammation, while tumor necrosis factor α down regulates *HAMP* expression. Yet cytokines are not the exclusive immune regulators of *HAMP* expression. The non-classical major histocompatibility complex class I protein, HFE, is also required for appropriate regulation of *HAMP* expression in response to inflammation and increased iron stores. To date, it is unclear how each of these signals at the cell surface of the hepatocyte regulates transcriptional or post-transcriptional control of *HAMP*.

4. How are stress proteins released from cells? Graeme I. Lancaster, Cellular and Molecular Metabolism Laboratory, School of Medical Sciences, RMIT University, Melbourne, Australia

The heat shock proteins (HSPs) are a family of quintessentially intracellular proteins found in all eukaryotes and prokaryotes. HSP families include both constitutive and stress-inducible members whose primary function is to interact with nave and denatured proteins to prevent the aggregation of aberrantly folded proteins, facilitate the folding of nave proteins, facilitate the re-folding of denatured proteins, and to aid intracellular protein trafficking. While these functions underscore the importance of intracellular HSPs in the maintenance of cellular homeostasis and in promoting cell survival in response to stressful cellular conditions, accumulating evidence suggests that HSPs are actively secreted and have important extracellular functions.

The concept that specific HSPs can be actively secreted was suggested $\approx$15 years ago following the demonstration that heat-shocked rat embryo cells rapidly released HSP70 and HSP110. (Hightower and Guidon, 1989). While it has been suggested that cellular HSP70 release may be the result of non-specific processes, such as cell lysis, several lines of evidence argue against this notion (Hightower and Guidon, 1989; Broquet et al., 2003; Febbraio et al., 2002). Interestingly, two recent studies have provided evidence that specialized membrane microdomains, termed lipid rafts, may play a role in HSP70 exocytosis (Broquet et al., 2003; Hunter-Lavin et al., 2004).

Almost 10 years ago, Multhoff and Hightower hypothesized that exosomes, 60–80 nm vesicles secreted following the fusion of multivesicular bodies with the plasma membrane, may provide a secretory pathway allowing cells to actively release specific HSPs. Recently, we (Lancaster and Febbraio, 2005) confirmed the involvement of exosomes in HSP70 release from peripheral blood mononuclear cells in both the basal and stress-induced state.

This presentation will discuss mechanisms of cellular HSP release and the potential mechanisms of HSP release that may contribute to the elevated HSP levels observed following exercise.

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5. Stress proteins and initiation of the immune system, Alexzander Asea, Scott White Clinic and Texas A&M University System Health Science Center, College Station, TX 77843, USA

From its original description as solely an intracellular molecular chaperone, heat shock proteins have now been shown to function as initiators of the host's immune response. Although the exact mechanism by which intracellular heat shock proteins leave cells is still incompletely understood, recent work from several labs suggest that heat shock proteins are released by both passive (necrotic) and active (physiological) mechanisms. Binding to specific surface receptors is a prerequisite for the initiation of an immune response. To date, several cell surface proteins have been described as the receptor for Hsp70 including Toll-like receptors 2 and 4 with their cofactor CD14, the scavenger receptor CD36, the low-density lipoprotein receptor-related protein CD91, the *C-type lectin receptor LOX-1*, and the co-stimulatory molecule, CD40. Binding of Hsp70 to these surface receptors specifically activates intracellular signaling cascades, which in turn exert immunoregulatory effector functions; a process known as the chaperokine activity of Hsp70.

6. Exosomal export of heat shock protein 70 stimulating the innate immune system, Gabriele Multhoff, University Hospital Regensburg, Department Hematology/Oncology, Franz-Josef Strauss Allee 11, 93953 Regensburg, Germany

Heat shock proteins (Hsp) act as danger signals stimulating the adoptive and innate immune system. However, it remained elusive how cytosolic proteins communicate with immunocompetent effector cells. Here, we demonstrated that tumor cells have the capacity for an active release of heat shock protein 70 (Hsp70) in detergent-soluble vesicles. Biophysical properties including floating properties (1.17 g/ml) correlating with a maximum acetylcholine esterase activity characterized them as exosomes. The lumen of tumor-derived exosomes contained a number of cytosolic proteins but lack ER-residing proteins. A specific enrichment of the small GTPase Rab-4 in exosomes documented their intracellular transport route from early endosomes to the plasma membrane. A comparative proteomic analysis of exosomal and plasma membrane localized proteins by flow cytometry and immunoelectron microscopy revealed that exosomal membranes resembled the protein composition and topology of the tumors from which they originated. In line with these findings only exosomes derived from Hsp70 membrane-positive tumors but not that of their negative counterparts stimulated the innate immune system in a similar manner like soluble Hsp70 protein.

In summary, our data provide an explanation how the innate immune system might become activated by tumor-derived, exosomal Hsp70 in vivo.

7. Microarray technology and application in exercise immunology, Hinnak Northoff, Derek Zieker, Elvira Fehrenbach, Department of Transfusions Medicine, University of Tuebingen, Otfried–Mueller–Str. 4/1, 72076 Tuebingen, Germany

Microarray analysis offers a set of analytical platforms that provide rapid, affordable, and substantial information at the DNA, RNA or protein level. It enables the analysis of thousands of genes simultaneously in a parallel manner across samples derived from various

biological sources and treatment regimens. Arrays for analyses of RNA expression will allow gene expression profiling also in exercise physiology. In a recent study, we used a custom-made cDNA microarray focused on inflammation to study gene expression in half-marathon runners. Microarray expression analysis of cell type specific surface molecules reflects the observed individual cellular shifts in peripheral blood cells. Up-regulation of MAPKAP-K2, L-selectin, and IL1-ra after exhaustive exercise was approved. The exercise-associated regulation of three new candidate genes in peripheral blood was revealed: down-regulation of the cellular contact protein CD81; up-regulation of Thioredoxin, which may play an important part in anti-oxidative defence; and, surprisingly, the down-regulation of the anti-carcinogenic gene GSTM3. This pilot study shows that cDNA microarray expression analysis is a reliable tool to complete the list of candidate genes which may play a role in the exercise-modulated of immune system. Using microarrays in exercise physiology will provide new insights into the complex molecular mechanisms of the exercise-induced stress response, adaptation to training and modulation of immune function.

8. Exercising when ill, Stéphane Bermon, Monaco Institute of Sports Medicine, avenue d'Ostende, 98000 Monaco, IAAF Medical and Antidoping Commission, av. Princesse Florestine 98000, Monaco

Upper respiratory tract infections, gastro-intestinal tract and, to a lesser extent, systemic infections are of common concern in athletes. Although these diseases are usually associated with a reduction or a cessation of professional or daily living activities, high-level athletes are generally reluctant to adopt the same behavior.

However keeping on training or competing in such circumstances may worsen the health condition, impair physical performance, and increase the risk of infecting other teammates.

Concerning the athlete's health, several studies pointed out the possibility of a transient immuno-suppression after an intense or lasting exercise, thus worsening the infectious condition of the patient. Moreover, it has been shown that performing intense exercise during the incubation phase of an infection can increase the severity of the illness.

Before making a decision about whether an athlete should train or compete when infected, four different conditions with potential severe harmful effects should be considered by the sports physician:

- The possibility of a viral myocarditis that could lead to severe cardiac arrhythmia on an exercising heart. In spite of complete epidemiological studies, serological and anatomo-pathological fact exist and special attention should be paid to enterovirus infection with systemic symptoms.
- The occurrence or the exacerbation of an asthmatic disease in a context of airways inflammation and increased bronchial secretion.
- The possibility of a splenic rupture in the early phase of a mononucleosis. Special attention will be paid to contact sports.
- The possibility of severe dehydration and electrolytic imbalance in a context of endurance sports and infectious gastro-enteritis.

An infected athlete should also be informed about a likely impairment of its performance level. Indeed, several works reported decreased maximal cardiac output, and hence maximal oxygen consumption, impaired pulmonary flows and exchanges under this condition. Skeletal muscle dysfunctions and impaired thermoregulation have also been reported.

Taking part to team sports, when acutely infected, an athlete may contaminate other persons [sports with physical contacts, and athletes using the same closed playground or facilities (mat, locker rooms, sharing of bottles or towels…)].

Finally, practical advices and discussion about the Eichner's algorithm will be discussed at the end.

9. Altitude training and immune function, Robert S. Mazzeo, Department of Integrative Physiology, University of Colorado, Boulder, CO 80309, USA

A popular training paradigm currently employed by endurance athletes to enhance performance involves living at high altitude while training at low altitude. The concept entails incorporating the physiologic and metabolic adaptations associated with chronic high altitude exposure (increase in RBC, mitochondrial oxidative capacity, capillary density, etc.) while training at a lower altitude allowing for the maintenance of a high absolute training intensity. However, less is known with regard to how such acute and chronic altitude exposure effect immune function. Hypoxia is an environmental stressor that is known to elicit alterations in both the autonomic nervous system and endocrine function. Alterations in these systems can have an immediate as well as a longer lasting impact on immune function. Studies from the summit of Pikes Peak (4300 m) have indicated a strong a- and β-adrenergic component in the regulation of immune function at altitude that can persist weeks after initial exposure. Specifically, interleukin (IL)-6 is elevated with acute altitude exposure primarily mediated via b-adrenergic stimulation and remains elevated for several weeks as a result of α-adrenergic activation. Others have demonstrated that a short-term application (18 days) of the live high-train low paradigm results in suppression of the mucosal immune system as indicated by a cumulative decline in salivary IgA levels. Taken together, the majority of evidence suggest a potential additive effect of combined hypoxia and exercise in transiently suppressing immune function, at least in the short-term. Implications for the athletes will be addressed.

10. Immunological management of athletes: nutritional concerns, David C. Nieman, Department of Health and Exercise Science, Appalachian State University, Boone, NC 28608, USA

Four key principles in nutritional immunology include: (1) almost all nutrients in the diet play a crucial role in maintaining an "optimal" immune response. A balanced, healthy diet provides all the nutrients needed for good immune function in most healthy adults, and nutrient supplementation does not "boost" immune function in these individuals. (2) Deficient intakes of energy and nutrients can have negative consequences on immune status and susceptibility to pathogens. (3) Some nutrients (i.e., glutamine, arginine, fatty acids, and vitamin E) provide additional benefits to immunocompromised persons (i.e., the frail elderly) or patients who suffer from various infections. (4) Advanced supplements may prove useful in countering immune suppression for healthy adults during unusual stress including the physiologic stress of prolonged and intensive exertion. Supplements studied thus far in humans include zinc, iron, ω-3 fatty acids, plant sterols, antioxidants (e.g., vitamins C and E, β-carotene, *N*-acetylcysteine, and butylated hydroxyanisole), glutamine, carbohydrate, and ginseng. Except for carbohydrate beverages, none of these supplements has emerged as an effective countermeasure to exercise-induced immune suppression. Carbohydrate ingestion attenuates exercise-induced increases in stress hormones, plasma cytokines, including IL-6, IL-10, and IL-1ra, and blood neutrophil and monocyte cell counts, but is ineffective in countering decreases in T and natural killer cell function and other aspects of immunity such as salivary IgA output. Vitamins C and E supplements do not consistently counter increases in oxidative stress and alterations in immunity during extreme exertion. Future research may reveal protective effects of advanced supplements including β-glucan, tea extract, flavonoids such as quercetin, and other unique products that have shown utility in animal models.

11. Relevance of salivary IgA to the immunological management of athletes, Maree Gleeson, School of Biomedical Sciences, Faculty of

Health, University of Newcastle and NSW Ministry for Science and Medical Research, Australia

Is salivary IgA relevant? Adequate levels of salivary IgA (sal-IgA) are important to good health. In conjunction with innate immune factors, secretory IgA (s-IgA) antibodies provide protection from infections at external body surfaces. As well as anti-bacterial activities, s-IgA plays a significant anti-viral role at mucosal surfaces. IgA-deficient subjects are susceptible to recurrent infections, particularly in the upper respiratory and gastrointestinal tracts. S-IgA is monitored in saliva due to the ease of collection. Lowered levels of sal-IgA, in response to stressors (psychological and physical), are associated with a higher risk of upper respiratory tract infections (URTI). However, measuring total sal-IgA concentrations provides no information on the specificity of immune protection.

Are changes in salivary IgA relevant to athletes? Intense exercise in highly-trained athletes, particularly over long training periods, can induce lowered levels of sal-IgA. Conversely, sal-IgA can increase in response to moderate intensity exercise and the fitter the individual the greater the increase. In group and individual studies of elite and highly-trained athletes, low levels have been associated with an increased risk of URTI. However, the upper respiratory symptoms (URS) experienced by athletes may not be due to infections but caused by airway inflammation or viral reactivation. Changes in sal-IgA levels mirror the pattern of viral reactivation and may provide an indirect marker for T-cell cytokine regulation at mucosal surfaces.

When is salivary IgA monitoring relevant to individual athletes? Changes in sal-IgA under laboratory-controlled conditions appear to be of little relevance to the individual athlete. Monitoring pre- and post-routine training may be of benefit to pick periods of high risk for infection or viral reactivation. The two groups of athletes who would benefit from monitoring are those at high-risk of recurrent URS and those susceptible to post-viral fatigue associated with viral reactivation. The capacity for sal-IgA to predict athletes at risk of infections appears to be in the early preseason testing and not in periods close to competition. The only current effective intervention for reversing the low levels of sal-IgA in elite athletes is alteration in training programs. Recent studies with probiotic interventions appear to be promising for the post-viral fatigue athlete but sal-IgA is not a good marker for response.

12. Prevention of infectious diseases in an Olympic team, Ola Ronsen, Norwegian Olympic Sports Centre, 0806 Oslo, Norway, University of Texas Medical Branch and Shriners Hospital, Galveston, TX 77550, USA

Missing a week of training immediately prior to the Olympic Games or the actual day(s) of competition due to an illness is the worst fear of most elite athletes. That is why all means and measures towards illness prevention are of paramount importance before and during such events. Exercise-induced suppression of certain immune functions during hard training periods, increased exposure to foreign pathogens while traveling, as well as close sharing of the same training- and living facilities within a team may put the athlete at increased risk of infections.

Based on a recent survey of 74 Norwegian Olympic athletes, more than 90% of the athletes reported one ore more infectious episodes during a year. Respiratory tract infections and gastroenteritis were the most common diseases reported and the duration of symptoms was mostly within 1 week. However, since many suffered several infectious episodes through a year, the average number of lost training days was 15 per year. Furthermore, on the average one important competition per year was lost due to illness.

Since preventive measures are known to reduce the number and duration of infectious episodes, a program to avoid the occurrence and spread of infections in athletic teams has been implemented among Norwegian Olympic athletes. The importance of the practical guidelines to the teams and individual athletes will be discussed. Also, guidelines for safe return to exercise training and competitions after infectious episodes will be summarized at the end.

13. Sports activity and common cold, Ryoichi Nagatomi, Department of Medicine and Science in Sports and Exercise, Tohoku University, Graduate School of Medicine, Sendai, Japan

The average adult has from one to six common cold episodes each year. URI symptoms not only compromises health-related quality of life of adults, but also can greatly impair athletes' performance and affect the continuation of exercise. Therefore, it is important to seek for factors affecting the risk of URI symptoms and finally to find effective clues for the management of URI symptoms. Regarding the risk of URI symptoms related to exercise, epidemiological evidences based on observation of runners are relevant. A popular working theory regarding exercise and URI symptoms is the J-curve theory. Regular, moderate exercise is considered to decrease risk of URI symptoms as compared to sedentary individuals or strenuous individuals undergoing strenuous or intense exercise. To other categories of sports than endurance-running, however, J-curve theory does not necessarily apply. We demonstrated that higher frequency of sports activity was associated with a significant reduction in the risk of retrospective URI symptoms in a cross sectional questionnaire survey in the young population. Overall sports activity in this population seems beneficial in terms of risk of URI symptoms. For runners, strenuous and prolonged training or competition may increase the risk of URI symptoms. NK cell activity or salivary (secretory) IgA may not be directly responsible for regulating URI symptoms or infection, but may reflect factors regulating disease susceptibility or morbidity, such as autonomic nervous system. Moreover, flu-like symptoms are likely to be elicited without infection as such in the therapeutic administration of IFN-α or other cytokines. Differential immune reactions concomitant with URI symptoms observed under psychological stress may suggest involvement of HPA axis. It is therefore necessary to clarify if URI symptom is due to infection or not to better understand the mechanism by which URI symptoms is elicited.

14. Pathogenesis of exercise-induced asthma, Sandra D. Anderson, Department of Respiratory Medicine, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia

Exercise-induced asthma describes the transient reduction in lung function (e.g., 10% fall in FEV_1) that follows vigorous exercise in people with asthma. EIA occurs as a result of the thermal and osmotic consequences of the water lost, by evaporation, from the airway surface in humidifying the inspired air. Because EIA occurs breathing hot dry air, the osmotic effect of dehydration is thought to be more important than the cooling effect. EIA is associated with airway inflammation because treatment with inhaled steroids universally reduces severity of EIA. EIA is also associated with atopy and presence of IgE. IgE on mast cells makes them more sensitive to an osmotic stimulus. Mast cells release mediators, such as prostaglandins, leukotrienes, and histamine, and their metabolites can be measured. Mast cells are found in the airway epithelium, and in the submucosa, in close proximity to airway smooth muscle (ASM). They are well situated to respond to an increase in osmolarity of the airways. Drugs that inhibit the release of mast cell mediators, or drugs that antagonise their effects, ameliorate or even completely prevent EIA. Transient oedema of the airway sub mucosa can also occur in response to exercise particularly when cold dry air is inspired. This oedema restores normal hydration and may serve to amplify the bronchoconstricting effect of the mediators.

There have been many reports of EIA and airway hyperresponsiveness (AHR) in elite athletes, who perform endurance sports, who

do not have clinically recognised asthma. Healthy people have mast cells and also release mediators in response to a dehydrating stimulus such as exercise. One theory used to explain this 'EIA' is the events associated with dehydration injury of the small airways. We have recently proposed that the contractile properties of ASM are altered as a result of repeated microvascular leak and exposure to bulk plasma (Current Allergy and Asthma Reports 2005; 5, 116–22). In addition the ASM of those athletes who are atopic may become passively sensitised 'in vivo' and thus become responsive to the available mediators. The pathogenesis of EIA and AHR in the elite asthma group may be explained by these events.

15. Neuro-endocrine-immune response and EIA, Sergio Bonini, Professor of Medicine, Second University of Naples and IRCCS San Raffaele Rome, Italy

In healthy individuals, physical exercise induces several changes of immune parameters and functions depending on the type and duration of the exercise as well as on the training and individual response of the subjects. Personal studies in elite athletes suggest that intense and prolonged physical exercise is associated with a preferential Th2-type immune response.

In allergic and atopic subjects, physical exercise may induce allergic symptoms—such as asthma, rhinitis, urticaria-angioedema, and anaphylaxis—also depending on the environmental conditions in which exercise is performed.

But are symptoms induced by exercise in atopics—and particularly exercise-induced asthma—distinct clinical phenotypes or does exercise only represent one of the many triggers of allergic symptoms? And, are immune changes induced by exercise relevant for eliciting symptoms and asthma or does exercise preferentially act on pathophysiological changes of target organs in sensitised individuals?

Accumulating evidence indicates that physical exercise as well as other environmental stimuli induce a complex neuro-endocrine-immune response where several factors play a role. Studies from our research group highlight in particular the relevant role of nerve growth factor in allergic diseases and possibly in causing symptoms in allergic individuals performing physical exercise.

16. Probiotics, immunity and sports, Claudio De Simone, Department of Experimental Medicine, University of L'Aquila, Coppito 2, L'Aquila, Italy

Gastrointestinal (GI) disorders such as abdominal pain, vomiting, diarrhea, nausea, bloating, and cramps occur in athletes during acute strenuous exercise. Symptoms may be so severe to induce gastrointestinal bleeding during training and competition, which may lead to iron deficiency and anaemia in the long term. Mechanisms responsible for GI dysfunction are not known but altered intestinal permeability and local ischemia—due to the reduced blood flow to the gut during intense exercise—have been associated to the symptoms. A compromised barrier function of the gut may produce an inflammatory response and initiate a cytokine cascade that could contribute to GI distress during heavy exercise. GI symptoms can seriously affect athlete's performance and quality of life. Pharmacological treatment to control symptoms is advisable, but unfortunately there are few permitted options. A new and safe approach to target the above described signs and symptoms is the use of high dosage multistrain probiotic products, i.e., VSL#3 (VSL Pharmaceuticals, Gaithersburg, MD) (www.vsl3.com). Experimental studies have shown that treatment of IL-10 gene-deficient mice with VSL#3 results in a normalization of colonic physiologic function and barrier integrity in conjunction with a reduction in mucosal levels of proinflammatory cytokines and a significant improvement in histologic disease (Madsen et al., 2001). Furthermore, in colonic epithelial cells subjected to proinflammatory stimuli, VSL#3 reduced p38 mitogen-activated protein kinase activation, delayed nuclear factor κB activation, stabilized levels of IkB, and inhibited proteasome function and colonic interferon-g secretion (Jijon et al., 2004; Petrof et al., 2004).

Experiments are in progress to verify whether the beneficial effects observed in vitro or ex vivo can be confirmed in athletes suffering from gut problems due to heavy-prolonged training.

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www.vsl3.com

17. Regulatory T cells, Lucienne Chatenoud, INSERM U580, Faculté René Descartes Paris 5, Hôpital Necker 161 Rue de Sèvres, 75015 Paris, France

Over the last few years, there has been a re-emergence of the concept of suppressor/regulatory T cells among the central players of immune mechanisms controlling a wide variety of immune responses from physiological autoreactivity (i.e., response to self-antigens) to responses to transplants, tumors, and infectious antigens. The rejuvenation of the regulatory T cells' field developed with studies, mostly dealing in the beginning with physiologic and pathologic models of autoimmunity, that focused on specific biologic attributes of these cells. These were identified essentially as $CD4^+$ T cells expressing ab T-cell receptors that can potentially recognize known major histocompatibility complex (MHC)-restricted antigenic peptides. These T-cell subsets appear to develop both in the thymus, during the normal process of T-cell maturation, and are defined as ''natural or naturally occurring regulatory T cells,'' while others, termed adaptive regulatory T cells, emerge during normal T-cell responses as a consequence of activation of mature T cells under particular conditions of antigen exposure, co-stimulation, and cytokine milieu.

Regulatory T cells are diverse in their phenotypes, antigen specificity, mode of action, and immunopathological relevance.

Our aim will be to present and discuss studies from different groups, including our own, showing that specialized subsets of regulatory T cells are instrumental in the control of autoimmune diseases and more specifically of type 1 diabetes. In addition, we shall also provide evidence supporting the notion that CD3-specific monoclonal antibodies are representatives of a new category of immunotherapeutic agents that possess the unique capacity to promote immunological tolerance (an antigen-specific unresponsiveness in the absence of long-term generalized immunosuppression) through their ability to induce immunoregulatory T cells.

Free communications (oral presentation)

F-1. Fiber type specific expression of TNF-α, IL-6, and IL-18 in human skeletal muscles, P. Plomgaard [a], M. Penkowa [b], B.K. Pedersen [a], [a]Centre of Inflammation and Metabolism at The Department of Infectious Diseases and The Copenhagen Muscle Research Centre, Rigshospitalet, The Faculty of Health Sciences, University of Copenhagen, Denmark, [b]Centre of Inflammation and Metabolism and Section of Neuroprotection at The Panum Institute, The Faculty of Health Sciences, University of Copenhagen, Denmark

Skeletal muscle has been recognized as an endocrine organ with capacity to produce signal peptides in response to muscle contractions. Here, we demonstrate that resting healthy human muscles express

cytokines in a fiber type specific manner. Human muscle biopsies from seven healthy young males were obtained from *M. triceps*, *M. quadriceps* vastus lateralis and *M. soleus*. Type I fibers contributed (mean ± SE) 24.0 ± 2.5% in triceps of total fibers, 51.3 ± 2.4% in vastus, and 84.9 ± 2.2% in soleus. In accordance, the fiber type composition was reflected by a marked difference between the three muscles with regard to MHC I and MHC IIa mRNA expression.

Immunohistochemistry demonstrated that tumor necrosis factor (TNF)-α and interleukin (IL)-18 were solely expressed by type II fibers, whereas the expression of IL-6 was most prominent in type I compared to type II fibers. The fiber type specificity was found in both triceps, vastus, and soleus indicating that the levels of daily muscle activity did not influence basal cytokine expression. The demonstration of muscle fiber type specific cytokine expression in healthy young males suggests that cytokines may play specific regulatory roles in normal physiology.

F-2. Hypoxia inducible factor-1α is essential for muscle regeneration, Yusuke Ono [a], Hiroomi Sensui [b], Ryoichi Nagatomi [a], [a]Department of Medicine and Science in Sports and Exercise, Graduate School of Medicine, Tohoku University, Sendai, Japan, [b]Division of Cyclotron Nuclear Medicine, Cyclotron and Radioisotope Center, Tohoku University, Sendai, Japan

Hypoxia inducible factor (HIF)-1α is a transcription factor that senses low oxygen availability and enhances activation of hypoxia-inducible genes. Leukocyte HIF-1α is known to sense lower oxygen tension at the site of inflammation and promotes inflammatory activities, thus considered as a target for anti-inflammatory therapy. Recently, however, HIF-1α activity was also shown to be crucial for normal tissue development. Because skeletal muscle is a highly plastic tissue frequently exposed to varying oxygen tension and undergo frequent inflammation, we assumed HIF-1α may be involved in the process of myogenic differentiation.

To clarify the involvement of HIF-1α, we semi-quantified the level of HIF-1α mRNA by RT-PCR and the level of HIF-1α protein and related proteins by Western blotting. The localization of HIF-1α was confirmed by immunostaining in both C2C12 myoblasts and regenerative muscle tissue of C57/BL6 mice at 3 days after muscle damaging eccentric contraction. HIF-1α of C2C12 myoblast was knocked-down by RNA interference, and morphological changes together with changes in muscle specific protein expression was observed.

Although HIF-1α mRNA was constantly expressed under growth condition, HIF-1α protein was undetectable. HIF-1α protein increased after switching to differentiation condition. At the earlier stage of myogenesis, HIF-1α was accumulated in the nuclei of myogenin-positive myoblasts. HIF-1α became positive both in the nuclei and the cytoplasm at the later stage.

Chemical inhibition of proteasome resulted in the accumulation of HIF-1α protein even under growth phase. HSP90 co-localized with HIF-1α, and chemical inhibition of HSP90 abolished HIF-1α accumulation after switching to differentiation condition. Knockdown of HIF-1α effectively blocked myogenesis. HIF-1α expression was confirmed in the regenerative muscle tissue after eccentric muscle contraction.

The present study demonstrated that degradation of HIF-1α by proteasome under growth phase is blocked by HSP90 after switching to differentiation, resulting in the initiation of myogenesis in vitro. Together with the expression of HIF-1α in the regenerative skeletal muscle tissue in vivo, our finding supports the hypothesis that HIF-1α is required for myogenesis. Inactivation of HIF-1α as an anti-inflammatory treatment may attenuate inflammation, but may seriously compromise myogenesis when applied to skeletal muscle tissue.

F-3. Enhanced HSP72 expression in trained humans confers thermal tolerance and cytoprotection against heat induced apoptosis, Shawn G. Rhind [a,b], Teresa Chan [a], Jason Chung [b], Pang N. Shek [a,b], Tom M. McLellan [a,b], [a]Defence Research and Development Canada—Toronto, 1133 Sheppard Avenue W. Toronto, Ont., Canada M3M 3B9, [b]Faculty of Physical Education and Health, University of Toronto, Canada

Background. Improved thermal tolerance of environmental heat stress and heat shock in endurance trained individuals may be linked to enhanced induction of the cellular heat shock protein (HSP) response, which could in turn convey improved cytoprotection against heat-induced apoptosis.

Purpose. This study was designed to compare spontaneous and heat-shock-induced expression of intracellular HSP72 synthesis and rates of apoptosis in peripheral blood leukocytes during in vivo heat-exercise (HE) challenge in trained vs. untrained volunteers.

Methods. Fourteen age-matched men and women were defined as trained [TR: regularly active males ($n = 4$) and females ($n = 4$), = 53.4 and 46.0 mL kg min^{-1}, respectively] or untrained [UT: inactive males ($n = 3$) and females ($n = 3$), = 43.3 and 36.0 mL kg min^{-1}, respectively]. Subjects underwent treadmill exercise (4.0 km h^{-1}) in a heated climatic chamber (40 °C, 30% r.h.) while wearing protective clothing; rectal temperature (T_{re}) was measured at 5 min intervals during the test and subjects exercised until T_{re} reached 39.5 °C or exhaustion. Venous blood was sampled at rest, and at 0.5 °C T_{re} increments (i.e., 38, 38.5, 39, and 39.5 °C) during in vivo HE exposure. Whole blood samples were analyzed flow cytometrically, immediately after blood draw and following in vitro heat shock (42 °C, 2 h) treatment, for detection of spontaneous and heat-shock (HS)-induced HSP72 and Annexin-V immunofluorescence staining by leukocyte subsets.

Results. Induction of spontaneous and HS-induced intracellular HSP72 expression by circulating leukocyte subsets during HE challenge revealed significant T_{re}-dependent upregulation in both fitness groups. Compared to UT subjects, TR individuals exhibited greater and more rapid synthesis (both % and MFI) of intracellular HSP72 by monocytes and neutrophils when exposed to the same level of thermal strain. Moreover, leukocytes from TR subjects were less prone to heat-induced apoptosis than cells from UT subjects.

Conclusions. These data indicate that overexpression of HSP72 in endurance trained individuals is associated with improved thermal tolerance and cytoprotection against heat shock-induced apoptosis. These findings also imply that regular aerobic conditioning confers improved cross-tolerance via adaptations of the cellular heat shock response system, which may be protective against heat-related injuries.

F-4. Changes in plasma cytokines and inflammatory markers following an Ironman triathlon, Katsuhiko Suzuki [a,b], Kazunori Nosaka [c], Paul Laursen [c], Chris Abbiss [c], Mitsuharu Okutsu [b], Jonathan Peake [a], [a]School of Human Sciences, Waseda University, Japan, [b]Consolidated Research Institute for Advanced Science and Medical Care, Waseda University, Japan, [c]School of Exercise, Biomedical and Health Sciences, Edith Cowan University, Australia

Purpose. To investigate the impact of extreme endurance exercise on stress biomarkers, we examined the effects of an Ironman triathlon race on a broad spectrum of cytokines and parameters related to systemic inflammation and skeletal muscle damage.

Methods. Ten well-trained male triathletes completed an Ironman triathlon race, consisting of a 3.8 km swim, followed by a 180 km cycle, and a 42.2 km marathon run. Environmental conditions ranged from 19 to 26 °C and 44 to 87% relative humidity, with ocean temperature at 19.5 °C. Peripheral blood was sampled 2 days prior to the race (pre), within 5 min after the race (post), and between 14 and 20 h post-race (1-day post). Plasma concentrations of IL-1β, IL-1 receptor antagonist (IL-1ra), IL-4, IL-5, IL-6, IL-10, IL-12p40, G-CSF, TNF-α, IFN-γ, heat shock protein 70 (HSP70), C-reactive protein (CRP),

serum amyloid protein A (SAA), myoglobin, and creatine kinase (CK) activity were measured.

Results. The mean (±SD) race time for the 10 triathletes was 611 ± 49 min, and the race was completed at an intensity of 143 ± 9 beats min^{-1} (83 ± 6% HRmax). The race significantly increased the plasma concentrations of IL-1ra, IL-6, IL-10, IL-12p40, and G-CSF at post; among these cytokines, IL-1ra increased most markedly (37-fold). All cytokines had returned towards pre-exercise values at 1-day post. Plasma IL-1β concentration did not change significantly, and TNF-α could not be detected following the race. Plasma HSP70 concentration increased 23-fold at post, and remained slightly but significantly above pre-exercise values (1.6-fold) at 1-day post. Muscle damage markers, including myoglobin and CK, increased significantly by 140-fold and 10-fold at post, and 22-fold and 27-fold at 1-day post, respectively. The acute-phase proteins CRP and SAA were significantly elevated at 1-day post (32-fold and 50-fold, respectively).

Conclusion. Transient increases in cytokines and HSP70 immediately after the race are followed by the release of acute-phase proteins. Despite the very strenuous demands of an Ironman triathlon, pro-inflammatory cytokines remain largely unchanged, whereas anti-inflammatory cytokines increase dramatically. We present new data suggesting that IL-1ra, HSP70, and SAA are integral components of the acute phase response to exhaustive endurance exercise.

Reference

Suzuki et al., 2003. Exerc. Immunol. Rev. 9, 48–57.

F-5. Immune effects of erythropoietin administration in normal and immunosuppressed mice, T.G. Sukhenko, O.P. Kolesnikova, E.P. Mamontova, Institute of Clinical Immunology Siberian Branch Russian Academy of Medical Sciences, Novosibirsk, Russia

Influences of physical activity on the human immune system have been widely studied; generally reporting an immuno-stimulating effect of chronic and low intensity exercises and an immuno-suppressing effect of intense and prolonged exercises.

Moreover, EPO abuse, as well as altitude or hypoxic training, is frequently reported in high-level athletes. Thus, one may hypothesize combined effects of increased EPO concentrations and intense training on the immune system.

The aim of this preliminary animal study was to investigate the influences of different exogenous EPO concentrations on humoral and cellular immune responses, secretory, phagocytic activity of peritoneal macrophages, spontaneous, and mitogen-induced proliferation of splenocytes. In this in vivo and in vitro study, normal and graft vs. host (GVH)-induced immunodeficient (with associated anemia) mice were used.

It was found that injection of small dose (1.2 IU/mouse) of EPO (human recombinant EPO, Recormon, Germany) to normal mice increases the number of plaque-forming cells (PFC) in spleen, but 116 IU/mouse of EPO decreased primary humoral immune response. The number of PFC negatively correlated with the level of hemoglobin. Incubation of peritoneal macrophages with EPO lead to suppression of IL-1 production and phagocytosis. Production of proinflammatory cytokin TNF-α was increased. EPO administration decreased spontaneous, and ConA-induced proliferation of splenocytes, but increased LPS-stimulated proliferation.

In our immunodeficient mice model (GVH + anemia), we report a decreased Ig-M response that was combined with an increased spontaneous and mitogen-induced proliferation of splenocytes. Hypoplastic anemia was accompanied by raised production IL-1 and TNF-α. Subsequent injection of EPO (50 IU/mouse) did not influence the IgM-response, but normalized erythropoietic parameters. EPO decreased spontaneous, mitogen- and antigen-induced proliferations of splenocytes, and reduced IL-1 and TNF-α productions. These results showed that various doses of EPO have various immunomodulating properties both in normal and immunodeficient mice. Hence, further studies on the immune effects of EPO should be conducted in athletes.

F-6. The −511 C/T polymorphism of the interleukin-1β gene in female athletes, G. Casabellata [a], M. Di Santolo [a], S. Cauci [a,b], [a]Department of Biomedical Sciences and Technologies, Gemona (Udine), School of Medicine, University of Udine, Italy, [b]Scienze Motorie, Gemona (Udine), School of Medicine, University of Udine, Italy

Objective. Single nucleotide polymorphism (SNP) in the promoter region of IL-1β gene is associated with differential levels of cytokine expression and may influence the severity of the inflammatory response. Inflammation appears to play an important role in the repair and regeneration of skeletal muscle after an acute bout of resistance exercise. We investigated the frequency of the single nucleotide polymorphism (C/T) at position −511 in the promoter region of the IL-1β gene in female athletes.

Methods. We enrolled a total of 315 Northern Italian women: 27 athletes (>8 h exercise per week) and 288 sedentary controls. Subjects were genotyped for the IL-1β −511 (C/T) SNP using polymerase chain reaction amplification, followed by restriction fragment length polymorphism analysis.

Results. In our case-control association study, the frequency of genotype TT of the IL-1β −511 promoter gene tended to be increased in athletes (22.22%) compared to sedentary controls (10.07%); $P = .055$. At variance, CC and CT genotypes revealed not significant trends.

Conclusion. Our data support a role for the −511 C/T polymorphism of the interleukin-1β gene in the athlete phenotype.

F-7. Upper respiratory illness aetiology and symptomatology in elite and recreationally competitive athletes, Luke Spence [a], Michael D. Nissen [b,c,d,e], Theo P. Sloots [b,c,d,e], Joseph G. McCormack [f,g], A. Simon Locke [a,h,i], David B. Pyne [j], Peter A. Fricker [j], Wendy J. Brown [a], [a]School of Human Movement Studies, The University of Queensland, Brisbane, Australia, [b]Clinical Medical Virology Centre, The University of Queensland, Brisbane, Australia, [c]Clinical Virology and Molecular Microbiology Research Units, Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital, Brisbane, Australia, [d]Division of Microbiology, Queensland Health Pathology Service, Royal Brisbane Hospitals Campus, Brisbane, Australia, [e]Department of Paediatrics and Child Health, The University of Queensland, Brisbane, Australia, [f]Department of Medicine and Infectious Diseases, The University of Queensland, Brisbane, Australia, [g]Department of Medicine and Infectious Diseases, Mater Hospital Health Services, Brisbane, Australia, [h]Queensland Academy of Sport, Brisbane, Australia, [i]Sunnybank Medical Clinic, Brisbane, Australia, [j]Centre for Sports Science and Sports Medicine, Australian Institute of Sport, Canberra, Australia

Background. The J-shaped curve hypothesis was proposed to explain the relationship between varying amounts of exercise and risk of upper respiratory tract infections (URTI). However, the strength of evidence supporting this model has been debated, particularly as little evidence presented for or against this hypothesis has quantitatively determined the underlying aetiology of what may more appropriately be termed upper respiratory illness (URI).

Aims and methods. A surveillance study was conducted over a 5-month summer/autumn competition season to identify the pathogenic aetiology and symptomatology of URI in male and female (i) highly trained elite athletes ($n = 32$), (ii) recreationally competitive athletes ($n = 31$), and (iii) untrained sedentary controls ($n = 20$). Nasopharyngeal and throat swabs were collected on subjects presenting with

two or more defined upper respiratory symptoms. Swabs were analysed using microscopy/culture/sensitivity and PCR testing for bacterial, viral, chlamydial, and mycoplasmal respiratory pathogens. Wisconsin Upper Respiratory Symptom Survey (WURSS-44) was administered to assess the daily symptomatology and functional impairment.

Results. A total of 37 URI episodes in 28 subjects were reported (9 controls, 7 recreationally competitive, 21 elite). The overall distribution mimicked the J-shaped curve with rate ratios for illness higher in both the control (1.93, 95% CI:0.72–5.18) and elite (4.50, 95% CI: 1.91–10.59) cohorts than the referent recreationally competitive athlete cohort. However, of these 37 episodes, infectious agents were identified in only 11 (30%) (2 control, 3 recreationally competitive, and 6 elite). The testing confirmed:

(a) two rhinovirus infections in the control cohort;
(b) one mycoplasma pneumoniae, one rhinovirus, and one rhinovirus + adenovirus infections in the recreationally competitive cohort;
(c) one *Staphylococcus aureus* (coagulase positive), one adenovirus + non-typeable haemophilus influenzae, one parainfluenza-3 + group-A β-haemolytic *Streptococcus*, one primary Epstein–Barr virus and two rhinovirus infections in the elite cohort.

No pathogens were identified in 26 episodes. Specific global symptom, total symptom, and functional impairment severity scores were higher in subjects with an infectious URI episode, particularly on illness days 3–5.

Conclusions. These data indicate that URI in elite athletes are seldom infectious and the symptomatology is distinct between infectious and non-infectious episodes. Non-infectious causes of URI should be considered and investigated to identify alternative mechanisms and mediators.

F-8. Effect of a yearlong exercise intervention on natural killer cell cytotoxicity and risk of colds in postmenopausal women, Jessica Chubak [a,b], Cornelia M. Ulrich [a,b], Mark H. Wener [c,d], Bess Sorensen [a], Yutaka Yasui [e], Brent Wood [c], Kristin LaCroix [a], Kumar B. Rajan [a], Catherine M. Wetmore [b], Mariebeth Velasquez [a], John D Potter [a,b], Anne McTiernan [a,b,d], [a]Fred Hutchinson Cancer Research Center, Cancer Prevention Program, Seattle, WA, USA, [b]University of Washington, Department of Epidemiology, Seattle, WA, USA, [c]University of Washington, Department of Laboratory Medicine, Seattle WA, USA, [d]University of Washington, School of Medicine, Seattle WA, USA, [e]Department of Public Health Sciences, Faculty of Medicine and Dentistry, University of Alberta, Alta., Canada

Physical activity is a key component of weight loss and reduces the risk of many diseases. Whereas moderate exercise appears to reduce the risk of upper respiratory tract infections (URIs), effects on natural killer (NK) cell cytotoxicity are less consistent. We addressed this question using a year-long randomized controlled exercise trial in 115 postmenopausal, previously sedentary, overweight (BMI = 25 kg/m^2 or between 24 and 25 kg/m^2 with >33% body fat) women. Participants were randomized to the stretching control or the exercise intervention. Exercisers engaged in 45 min of moderate-intensity exercise, 5 days/week for 12 months, while controls attended, once weekly, 60 min stretching classes for 12 months. We obtained fasting blood samples prior to randomization, and at 3-months and 12-months post-randomization. At each time-point, we measured NK cytotoxicity using flow cytometry at four effector-to-target (E/T) ratios (50:1, 25:1, 12.5:1, and 6.25:1). We assessed the risk of colds by self-administered questionnaires at baseline, 3, 6, 9, and 12 months. Participants reported the number of episodes of URIs in the previous 3-months and identified each episode as cold, flu, other infection, or unknown. We used linear regression to compare the change over time in NK activity between the intervention and control groups. The intervention did not appear to affect NK activity. Percent cytotoxicity decreased 4.8% in exercisers over 12-months compared to a 2.2% decrease in stretchers (for E/T ratio = 25:1); however, this difference was not statistically significant ($P = .36$). We used Poisson regression to compare the risk of colds in each group over the 12-month study period. Exercisers experienced colds approximately half as frequently as stretchers (RR = .57, $CI_{95\%}$: 0.35–0.94). We observed an exercise effect on risk of colds in women who did not use multivitamins ($n = 51$, RR = .27, $CI_{95\%}$: 0.11–0.67), but not in women who did use multivitamins ($n = 57$, RR=1.01; $CI_{95\%}$: 0.53–1.92). The P value for the interaction was .02. This study suggests that exercise can affect risk of colds, although probably through mechanisms other than increased NK activity. The exercise effect may be limited to non-users of multivitamins.

F-9. Exhaustive exercise does not affect serum level of NK cell product granulysin despite large fluctuations in circulating NK cell number, Xiumin Zhang [a], Kaori Matsuo [a], Arta Farmawati [a], Yohei Higashi [a], Kazuyuki Ogawa [b], Kinya Nagata [b], Ryoichi Nagatomi [a], [a]Department of Medicine and Science in Sports and Exercise, Tohoku University Graduate School of Medicine, Sendai, Japan, [b]R&D Center and Laboratory Headquarters, BML, Saitama, Japan

The circulating number of natural killer cells (NK) cells largely changes after acute bout of physical exercise. Granulysin is a cytolytic effector protein with antimicrobial and tumoricidal activities produced by NK cells and cytotoxic T lymphocytes (CTL). Since inactive form of granulysin is spontaneously secreted mainly by NK cells, it may represent the total pool of NK cells as considered from the gradual increase of serum granulysin level proportionately to NK cell activity after bone marrow transplantation to severe immunodeficiency patients defective of NK cell activity. We hypothesized acute bout of exercise may not affect the total pool of NK cells despite large fluctuations of circulating NK cell numbers.

Six healthy men at the age of 22.5 ± 0.6 years participated in the study. Each subject completed two trial: exhaustive graded incremental treadmill exercise and sedentary conditions in a random order with at least 7 days of interval without intensive exercise between two trials. Peripheral blood samples were collected pre-, immediately after, and 1, 3, 6, 12, and 24 h after post-exercise. Serum granulysin levels were measured by ELISA. NK cells were determined by flowcytometry. Exercise duration to exhaustion was 19.6 ± 2.4 min. NK cells increased by 4.81-fold immediately post-exercise and decreased to 0.54-, 0.55-, 0.69-, and 0.76-fold, 1, 3, 6, and 12 h post-exercise, then returned to baseline 24 h post-exercise. Serum granulysin increased immediately after exercise by 1.22-fold and returned to baseline 1 h after exercise, but the increase was abolished after adjustment for the plasma volume change. In sedentary conditions, there were only small fluctuations in the number of NK cells and stable granulysin level throughout the observation period.

The stable level of serum granulysin despite a big fluctuation of NK cells elicited by exhaustive exercise suggests that exhaustive exercise does not affect the total pool of NK cells and the fluctuation in the number of circulating NK cells is a reflection of altered distribution of NK cells among different compartments. We suggest that even intense exhaustive exercise may not significantly affect NK cell immunity.

F-10. Relationship between plasma and salivary cytokine levels before and after a collegiate soccer tournament, Melody D. Phillips [a], Stephen J. Rossi [b], Thomas W. Buford [b], [a]Texas Christian University, Fort Worth, TX, USA, [b]Oklahoma State University, Stillwater, OK, USA

Acute and chronic exercise influences circulating inflammatory-related cytokine concentrations. Previously, we observed elevations in salivary IL-6 and TNF-α from 1 to 15 h after a long-course triathlon, and an elevation in resting salivary IFN-γ after a collegiate football

season. The purpose of this study was to compare inflammatory-related cytokine levels in plasma and saliva in collegiate athletes. A second purpose was to determine the influence of a soccer tournament (two matches on consecutive days) on cytokine levels in male players. Blood and saliva were obtained at three time-points: morning of game 1 (fasting; 8 AM; TP1), within 1 h after game 2 (6 PM; TP2), and 14 h after game 2 (fasting; 8 AM;TP3) from starters(ST) who played 100–180 min during the 2 games (mean = 76 min/game) and from non-starters(NS) who played no more than 30 min in the two games combined (mean = 2.8 min/game). Blood was collected into chilled EDTA tubes. After rinsing their mouths with water, athletes "drooled" into sterile tubes for 10 min. All samples were stored on ice until centrifugation. Clarified saliva and separated plasma were analyzed for IL-6, TNF-α, and IFN-γ concentrations using ELISA. All values are expressed as pg·mL^{-1}. TNF-α was largely undetectable in plasma. There were significant relationships between salivary and plasma cytokine concentrations for IL-6 (TP1: $r = 0.59$, $P = .026$; $n = 14$), IFN-γ (TP1: $r = 0.76$, $P = .001$, $n = 15$; TP3: $r = 0.84$, $P = .003$, $n = 10$) and a tendency for a relationship at TP2 in IL-6 ($r = 0.49$, $P = .087$; $n = 13$). Salivary IL-6 and IFN-γ were consistently greater than plasma levels (sIL-6 vs. pIL-6: TP1=9.27 ± 0.97, 5.43 ± 0.57, respectively, $P = .026$; TP2=17.7 ± 3.1, 8.74 ± 2.0, $p = .006$; sIFN-γ vs. pIFN-γ: TP1 = 123.7 ± 27.4, 10.6 ± 2.8, $P = .001$; TP2 = 166 ± 48, 11.7 ± 3.7, $P = .009$, $n = 10$; TP3 = 114.3 ± 31, 11 ± 3.4, $P = .006$.) There was a significant time × group interaction ($P = .047$) for salivary IFN-γ, however, multiple comparison analyses revealed no statistical differences. It appears that salivary levels of some cytokines mimic those in circulation.

N:(ST/NS)	Starters			Non-starters		
	TP1	TP2	TP3	TP1	TP2	TP3
Saliva Mean ± SE (pg/ml)						
IL-6 (5/4)	6.7 ± 1.4	9.2 ± 3.0	11.4 ± 3.7	6.7 ± 1.6	4.4 ± 3.4	3.1 ± 4.2
IFN-γ (8/6)	115 ± 37	187 ± 45	127 ± 30	78 ± 44	48 ± 52.0	49 ± 34
TNF-α (5/4)	26.8 ± 11	116 ± 43	79.2 ± 37	36.8 ± 12	48.8 ± 48	60 ± 41
Plasma Mean ± SE (pg/ml)						
IL-6 (6/5)	4.4 ± 0.6	6.8 ± 0.9	7.0 ± 3.0	3.9 ± 0.6	3.6 ± 1.0	4.1 ± 3.3
IFN-γ (5/8)	10.7 ± 5.0	13.3 ± 6.0	14.1 ± 5.2	12.9 ± 3.9	11.5 ± 4.5	10.7 ± 4.1

F-11. Changes in mucosal immunity with swim training at altitude, Judith E. Allgrove, Michael Gleeson, School of Sport and Exercise Sciences, Loughborough University, Leics LE11 3TU, UK

Heavy training and competition appear to be associated with a decrease in salivary immunoglobulin A (s-IgA) and an increased risk of upper respiratory tract infection (URTI) in athletes, including swimmers. In contrast, more moderate training may lead to an increase in the levels of this antibody and may reduce the susceptibility to URTI (Gleeson et al., 2003, Int J. Sports Med. 4, 1–14). Furthermore, hypoxia has been associated with alterations in the immune system (Pedersen et al., 1994, Int. J. Sports Med. 3, S116–S121), although its impact on s-IgA is unclear. The present study examined the effect of a swim training camp at altitude and at sea level on mucosal immunity and URTI in elite female youth swimmers. Twelve members of the British Swimming Squad (age ± SEM: 14 ± 1 year) were monitored during 3 weeks training at altitude (1900 m) and 1 week training and competition at sea level (training volumes were 55, 58, 50, and 48 km in weeks 1, 2, 3, and 4, respectively). Unstimulated whole-saliva samples were collected at rest over a 3 min period on seven occasions and analysed for s-IgA and osmolality. In addition, daily symptoms of URTI were recorded. Data were analysed using a one factor repeated measures ANOVA and post hoc t tests with Bonferroni correction applied where appropriate. Significance was accepted at $P < .05$. s-IgA concentration increased throughout the training camp from 141 ± 23 mg L^{-1} to 257 ± 21 mg L^{-1} ($P < .05$). A similar pattern was observed in s-IgA secretion rate: from 108 ± 14 μg min^{-1} to 213 ± 27 μg.min^{-1} ($P < .05$). Saliva osmolality also increased from 68 ± 3 mOsmol kg^{-1} to 102 ± 7 mOsmol kg^{-1} ($P < .05$). The ratio of s-IgA/osmolality did not change significantly suggesting that the increase in s-IgA concentration was at least in part due to dehydration (Walsh et al., 2004, Med. Sci. Sports Exerc. 36, 1535–1542). Nine swimmers exhibited symptoms of URTI in the first 2 weeks of training but these were not associated with lower s-IgA concentrations or secretion rates than those individuals who showed no signs of URTI. These findings suggest that 4 weeks training at altitude and sea level result in an increase in both s-IgA concentration and the secretion rate. However, these changes are not associated with the incidence of URTI.

F-12. Modulation of mucosal innate immunity in fertile women by exercise, S. Cauci [a,b], G. Casabellata [a], M.M. Savonitto [b], S. Driussi [a], L. Polichetti [b], C. Donadel [b], G. Stel [c], F. Gonano [c], [a]Dip. Scienze e Tecnologie Biomediche, University of Udine, Udine, Italy, [b]Corso di Laurea in Scienze Motorie, University of Udine, Udine, Italy, [c]Analisi Cliniche, Facoltà di Medicina e Chirurgia, University of Udine, Udine, Italy

Physical exercise is supposed to have an impact on immunity. No conclusive data are available in the scientific literature on the adverse/protective effects of exercise at mucosal level. Susceptibility to infections at mucosal sites could be affected by physical activity throughout modulation of local innate immunity.

Our aim was to determine levels of innate immunity in vaginal secretions of healthy women according to physical activity.

A total of 79 healthy fertile women aged 18–41 (24 ± 5 years) performing 0–20 (6 ± 5 h) hours exercise per week were enrolled among volley and basket non-professional athletes and matched sedentary women. Women with vaginal infections were excluded. Number of vaginal neutrophils was determined by evaluation of the Gram stained smear of vaginal secretions. IL-1β in vaginal fluid was determined by commercial ELISA assay.

We found that concentrations of IL-1β and the number of vaginal neutrophils were negatively associated with hours of exercise per week: $r = -0.242$, $P = .040$, and $r = -0.252$, $P = .025$, respectively. Athletes demonstrated lower levels of IL-1β, $P = .014$) and number of neutrophils ($P = .017$) than sedentary controls.

Sport exercise has been associated with increased prevalence of yeast urogenital tract infections. In this study, we showed that exercise down regulates vaginal levels of the main proinflammatory cytokine, IL-1β, and of the main white cells, neutrophils, devoted to combat microbial infections at mucosal sites. Our findings could support the hypothesis that exercise favor infections at mucosal sites by dampening local innate defense. However, it cannot be excluded that exercise increases the anti-inflammatory response and thus protects the host by adverse effects of excessive inflammatory reactions.

F-13. Voluntary wheel running exercise enhances antigen specific antibody producing splenic B cell response and prolongs IgG half-life in the blood, Koutarou Suzuki [a], Kazumi Tagami [b], [a]Doctoral Program in Health and Sport Sciences, Postgraduate School of Comprehensive Human Sciences, University of Tsukuba, Japan, [b]Laboratory of Exercise and Environmental Health, Division of Health and Sport Sciences, Postgraduate School of Comprehensive Human Sciences, University of Tsukuba, Japan

Exercise has been recognized to provoke up-regulation of antibodies. However, the mechanism has not been explained. We examined the effects of voluntary wheel running exercise on the number of cells which produce tetanus toxoid (TT)-specific IgG, as well as serum level and clearance of administered ^{125}I-labeled mouse IgG in the blood. Male C57BL/6N mice were randomly divided into a voluntary wheel running exercise group and a sedentary group. Mice were intraperitoneally immunized with 0.375 μg/kg of TT to induce primary and

secondary anti-TT antibody responses. ELISPOT assays that identified TT-specific antibody production were performed on day 0 and 22 after initial immunization (primary response) and on day 32 and 43. To explain why serum TT-specific IgG was elevated in the exercise group, we conducted an ^{125}I-labeled mouse IgG clearance test on day 32. ELISPOT counts of secondary responses to TT immunization were significantly higher in the running group that in the sedentary group ($P < .05$). The serum anti-TT specific IgG concentration was also significantly higher in the running group ($P < .05$) than the sedentary on day 32. The values of both groups were relatively lower on day 43. The ^{125}I-labeled mouse IgG was more rapidly cleared in the non-exercised than the exercised group ($P < .05$). These results show that voluntary wheel running up-regulates the TT-specific humoral immune response. These reactions may be partly explained by accelerated induction of TT-specific IgG producing cells and prolonged serum IgG half-life with voluntary exercise.

F-14. Cell-mediated immune response to influenza vaccination is enhanced by eccentric exercise, Kate M. Edwards [a], Victoria E. Burns [a], Louise M. Allen [a], Jamie S. McPhee [a], Douglas Carroll [a], Mark Drayson [b], Christopher Ring [a], [a]School of Sport and Exercise Sciences, University of Birmingham, UK, [b]Department of Immunology, School of Medicine, University of Birmingham, UK

Animal studies have shown that acute stress can enhance the response to immune challenges, such as immunisations. However, this phenomenon has received little attention in humans. The current study investigated the effect of eccentric exercise on the in vitro cell-mediated immune (CMI) response to influenza vaccination in humans.

Sixty (29 men, 31 women) young, healthy adults were randomised to exercise ($n = 40$) or control ($n = 20$) conditions. Participants completed a 20 min baseline followed by either a 25 min exercise or control (rest) task and returned 6 h later to receive the influenza vaccination. The exercise task comprised 50 repetitions of the eccentric portion of both the bicep curl and lateral raise using 85% of their previously assessed concentric 1-repetition maximum. Limb circumference, as a marker of oedema, was assessed before and immediately after the task. Participants rated the exercise using the Borg scale of perceived exertion immediately after completion of the task. At 8 weeks post-vaccination, a blood sample was taken to assess the CMI response. Briefly, whole blood with the vaccine for 18 h and the plasma harvested; a single-step ELISA was then used to measure interferon-γ (IFN-γ).

Overall, the IFN-γ response tended to be greater in exercise than control [$F(1, 52) = 3.61$, $P = .06$]; separate analysis for men and women revealed a significant effect in men [$F(1, 25) = 6.55$, $P = .02$], with no difference between groups in women [$F(1, 25) = 0.43$, $P = .52$]. Limb circumference increased significantly after exercise compared to control in both upper-arm (+5.5 mm) [$F(1, 58) = 16.13$, $P < .001$] and forearm (+6.4 mm) [$F(1, 58) = 18.22$, $P < .001$]. Within the exercise group, significant positive associations were found between the IFN-γ response to the vaccine and both the change in upper-arm limb circumference after the task ($r = 0.45$, $P = .009$) and the Borg rating of perceived exertion ($r = 0.38$, $p = .025$).

The CMI response to influenza vaccination was increased in the exercise group compared to control. As the increases in limb circumference suggest that local inflammation was induced by exercise and these increases were positively associated with the CMI response, it is possible that local inflammation mediates the observed immunoenhancement by acute stress.

F-15. Exercise induces interleukin-8 receptor (CXCR2) expression in human skeletal muscle, Lone Frydelund Larsen [a], Milena Penkowa [b], Thorbjorn Akerstrom [a], Bente Klarlund Pedersen [a], [a]Centre of Inflammation and Metabolism, Department of Infectious Diseases and The Copenhagen Muscle Research Centre, University Hospital of Copenhagen, Denmark, [b]Section of Neuroprotection, The Panum Institute, University of Copenhagen, Denmark

We have recently shown that concentric exercise induces a marked IL-8 mRNA and protein expression within skeletal muscle fibres (Akerstrom et al., 2005). IL-8 is released from the working muscle, however no increase in the systemic plasma concentration of IL-8 was observed, suggesting that muscle-derived IL-8 may act locally. IL-8 belongs to a subfamily of CXC chemokines containing a Glu-Leu-Arg (ELR) motif. CXC chemokines with ELR motifs are potent angiogenic factors in vivo and IL-8 has been shown to act as an angiogenic factor in human microvascular endothelial cells by binding to the CXC receptor 2 (CXCR2). In the present study, we examined the expression of CXCR2 in human skeletal muscle after concentric exercise.

Healthy volunteers were randomized to either 3 h of ergometer bicycle exercise at 60% of VO_{2max} ($n = 8$) or rest ($n = 8$). Muscle biopsy samples were obtained from the vastus lateralis prior to the exercise (0), immediately after exercise (3 h), and at 4.5, 6, 9, and 24 h.

Skeletal muscle CXCR2 mRNA increased significantly in response to exercise (3 h) when compared to pre-exercise samples, and when compared to resting values ($P < .05$). Expression of the CXCR2 protein was low or absent from skeletal muscle biopsies prior to exercise and at the end of the exercise period (3 h). However, at the time points 9 and 24 h, an increase in CXCR2 protein was seen in the vascular endothelium of blood vessels and also mildly in the muscle fibres, as determined by immunohistochemistry.

The present study demonstrates that bicycle exercise induces CXCR2 mRNA and protein expression in the vascular endothelial cells of the muscle fibres. These findings support the hypothesis that muscle-derived IL-8 may act locally to stimulate angiogenesis through CXCR2 receptor signaling.

F-16. Moderate exercise protects mice from death due to influenza virus infection and alters cytokine gene expression at the site of infection, Jeffrey A. Woods [a], Thomas W. Lowder [a], David Padgett [b], [a]Department of Kinesiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [b]Section of Oral Biology, The Ohio State University, Columbus, OH, USA

The purpose of this experiment was to determine if moderate exercise, performed in the initial days after infection when the host is mounting an immune response, altered mortality, morbidity, and cytokine gene expression in lungs in response to influenza virus infection in mice. Forty hemagglutinating units of influenza virus (A/Puerto Rico/8/34) were administered intranasally to lightly anesthetized mice. Male Balb/cByJ mice were randomized to one of two groups: sedentary control (CON) or moderate exercise (EXC, 20–30 min at 8–12 m/min). Mice exercised on a treadmill 4 h post-infection and for 3 more consecutive days before symptom onset. Mortality, morbidity, bodyweight, and food intake were assessed. In addition, in separate experiments, lung tissue was taken for quantitative real time PCR analysis of interferon-γ, IL-10, and M1 viral protein mRNA expression. EXC had a significantly ($P = .007$) higher survival (18 of 22; 82%) rate when compared to CON (10 of 23; 43%). There was no difference in morbidity, body weight loss or food intake between EXC and CON, despite improved survival. At day 3 post-infection, EXC resulted in a significant reduction in IFN-γ and higher IL-10 mRNA levels. Interestingly, there were no differences in viral M1 protein gene expression at this time point. We demonstrate that moderate exercise, performed in the initial days after influenza virus infection, significantly decreased mortality in mice and reduced interferon-γ gene expression. It may be that moderate exercise, performed after infection, reduces the immunopathology associated with influenza virus infection.

F-17. Long-term exercise training down-regulates caveolin-1 expression in mouse bone marrow and spleen cells during aging process, MiJung Kim [a,b], S.H. Yang [b], K.S. Lee [b], J.Y. Park [b], S.W. Kwon [a], H.S. Chi [a], C.J. Park [a], [a]Department of Laboratory Medicine, University of Ulsan College of Medicine Seoul 138736, Korea, [b]Asan Institiute for Life Sciences, Seoul 138736, Korea

Caveolae are vesicular invaginations of the plasma membrane with a diameter of 50–100 nm, and regulate signal transduction, potocytosis, and transcytosis. Caveolin is a principal structural component of caveolae membranes in vivo. Caveolin is involved in the inactivation of signaling process, caveolin-1 may represent a candidate protein in mediating cellular senescence. However, the expression of caveolin-1 in bone marrow and the correlation between exercise and the expression of caveolin-1 are not known yet elsewhere. One of the hallmarks of normal aging is the decline in several aspects of immune function. Several lines of evidence suggest that moderate exercise may enhance immune responsiveness and improve resistance to infection. Therefore, in this study, we attempted to test the differential expression of caveolin-1 in bone marrow, the primary immune organ and spleen, the secondary immune organ of young (7–15 weeks of age), middle (30–33 weeks) and old (78–115 weeks) mice. We also tried to determine whether declined immune responses from aged immune cells are correlated with caveolin-1 protein expression and long-term exercise (swimming : 5 times /week for 50 min) could affect the caveolin-1-related cellular senescence in immune cells. Through Western blot analysis, we observed age-related upregulation of caveolin-1 expression in sedentary control and long-term exercise group showed relatively lower expression of caveolin-1 protein compared to sedentary control. Through RT-PCR analysis, there are individual variations in caveolin-1gene expression level in bone marrow and spleen. But at certain time point (over 33–35 weeks of age) it start to express caveolin-1 gene and over 60 weeks of age all most all animals express high level of caveolin-1 gene. Exercise group showed significantly lower/or none expression of this gene as well as protein. Therefore, long-term exercise may keep immune cells active in primary and secondary organs by downregulating signal attenuating molecules, such as caveolin-1. The detail mechanism of this observation should be determined further, but it seems to be related with lower cholesterol level in exercise group from our experimental data.

F-18. Effects of physical exercise on the number of colonic preneoplastic lesions in rats treated with a chemical carcinogen, Marcelo Marcos Piva Demarzo, Sérgio Eduardo de Andrade Perez, Patrícia Modiano, Sérgio Britto Garcia, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto-SP, Brazil

Aberrant crypt foci (ACF) have been used for early detection of factors that influence colorectal carcinogenesis in rats, and postulated to be precursor lesions which develop into colorectal cancer. It has been observed that moderate and regular physical activity may prevent colon cancer up to 40% in humans, by mechanisms not completely known. However, exhaustive exercise increases free radical DNA oxidative damage and depresses immune function, events also related to the increased risk for cancer development. Fifteen days after either a single exhaustive swimming bout or a swimming physical training (for 8 weeks), or both, rats were treated with a chemical colon carcinogen (1,2-dimethylhydrazine, 50 mg/kg). We observed a statistically significant ($P < .05$) decreased number of that biomarkers (number of ACF counted per microscopic filed under low power magnification) in rats under training protocol (1.775 ± 0.237), when compared to the non-exercised group (3.550 ± 0.450). A statistically significant increase in the number of ACF was observed in rats under exhaustive protocol (8.375 ± 0.690), when compared to the non-exercised group. The trained rats submitted to the exhaustive protocol presented a statistically significant reduction (3.575 ± 0.528) in the number of ACF when compared to the untrained animals (8.375 ± 0.690). Thus, we concluded that training reduced the development of colonic preneoplastic lesions and exhaustive exercise increased these colorectal carcinogenesis biomarkers in sedentary rats, but this effect was abolished in that group of rats previously trained. From our finding and literature data, we hypothesize that, similarly to the suggested relationship between exercise and infections, physical exercise could be protective against cancer or it could increase the risk for this disease depending on its type, dose and duration.

F-19. Effect of a yearlong exercise intervention on markers of inflammatory response among postmenopausal women, Cornelia M. Ulrich, Mark H. Wener, Bess Sorensen, Brent Wood, Yutaka Yasui, Melinda L. Irwin, Shelley S. Tworoger, John D. Potter, Anne McTiernan, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; University of Washington, Seattle, WA, USA; Yale University, New Haven, CT, USA; Harvard University, Boston, MA, USA

C-reactive protein (CRP) and serum amyloid A (SAA) are markers of inflammatory processes. They are often elevated among overweight individuals and may be predictive of cancer survival. We investigated the effects of a yearlong moderate-intensity exercise intervention on these markers of inflammation.

Methods. One hundred and fourteen postmenopausal, overweight (BMI > 24), sedentary women, age 50–75, were enrolled in a yearlong randomized controlled trial of exercise (4–5 days per week 45 min moderate-intensity activity, $n = 52$) versus stretching control ($n = 62$). Inflammation markers (CRP, SAA) were measured pre-randomization and at 12-months post-randomization. The general estimation equation modification of the linear regression model was used to evaluate the effects of the intervention.

Results. At baseline, CRP and SAA concentrations were positively correlated with body weight, BMI, and percent body fat ($r = 0.27$–0.38, all $P < .01$). Overall, there were no statistically significant differences in the effects of the exercise intervention vs. control for either inflammation marker ($P = .17$–$.83$). However, among obese women (BMI $\geqslant 30$ kg/m^2), the exercise intervention resulted in a statistically significant reduction in CRP and SAA compared to control women ($P = .001$ and $P = .04$, respectively). CRP concentrations declined among obese women randomized to the exercise group [geometric means, 95% CI: baseline 0.40 mg/dL (.29–.54); 3-months 0.37 mg/dL (.26–.51); 12-months 0.32 mg/dL (.24–.42)], whereas a small increase was seen among controls. Similar trends were seen for SAA.

Conclusions. A moderate-intensity exercise intervention can significantly reduce concentrations of serum markers of inflammation in obese women. This finding supports a role of exercise in the prevention of chronic disease, and possibly cancer survival.

F-20. Effects of probiotics supplementation on respiratory infections and immune parameters during intense training, Eve Tiollier [a,b], Mounir Chennaoui [a], Danielle Gomez-Merino [a], Catherine Drogou [a], Edith Filaire [b], Charles Yannick Guezennec [a], [a]Department of Physiology, IMASSA-CERMA, Brétigny-sur-Orge, France, [b]Inter-University Laboratory of Physical Activity Biology, Blaise Pascal University, Clermont-Ferrand, France

The purpose of the present study was to examine the effect of a probiotic supplementation on upper respiratory tract infection (URTI) and mucosal and cellular immune parameters in subjects submitted to a multistressor environment such as the French Commando training. This latter included 3 weeks of physical and psychological conditioning followed by a 5-day combat course with energy restriction, sleep deprivation and psychological stress. Cadets (21 ± 0.4 year) received either a probiotic (*Lactobacillus casei*) ($n = 24$) or a placebo ($n = 23$)

supplementation over the training. Blood leukocyte and lymphocyte subpopulations and salivary IgA (s-IgA) were measured before training and after the combat course. Additionally, s-IgA were assessed after the 3-week training and after 1 week of recovery. Symptoms of URTI were recorded from health logs and medical examinations during the whole study and were classified according to the anatomical location of the infection. We found no difference in the incidence of infections between groups although some elements were suggestive of a lesser severity in the probiotic group. Indeed, analysis of the clinical symptomatology showed that the profile of the probiotic group was characterized by a very large majority (70%) of rhino-pharyngitis whereas in the placebo group the diagnosed symptoms were more evenly distributed. The s-IgA concentration and the natural killer blood concentrations were decreased in the placebo group ($P < .01$, both), but not in the probiotic group, after the combat course compared with before training. This study suggests that the benefits of a probiotic supplementation in a multistressor environment relied mainly on its capacity to maintain the level of immunologic parameters and to lessen the severity of the infection.

F-21. Acute sleep deprivation, cytokines, cognitive function, and glutamine intervention, Liz Gough [a,b], James C. Miller [c], John Allen [a], Rob Ferry [a], Linda M. Castell [a], [a]Cellular Nutrition Research Group, Nuffield Department of Anaesthetics, University of Oxford, USA, [b]School of Sport Science and Education, Brunel University, [c]CASL, Brooks City-Base, San Antonio, TX, USA

Athletes sometimes attribute underperformance in competition to a poor night's sleep beforehand. Sleep disturbances are commonly observed in unexplained underperformance syndrome (UPS), together with some immunodepression. This study investigated the effect of one night's sleep deprivation and the following three days on some factors involved in immune function: (1) cell cytokine production (IL-6, IL-8); concentrations of plasma leptin (p[Lep]), glutamine (p[GLN]), and caffeine (p[Caff]); 2) the effect of GLN supplementation vs. placebo (PLA) on these parameters.

Healthy male subjects gave informed consent ($n = 15$) to a randomized, double blind study for GLN feeding vs. PLA (2×5 g/day). GLN/PLA were administered twice daily for four days. On the night of day 1, the subjects were deprived of sleep, and also completed a battery of cognitive function tasks, questionnaires and vertical jump test. Resting, fasting blood samples were taken daily at 06:30 AM. Cytokines and p[Caff] were measured by ELISA, p[GLN] enzymatically. Independent-samples t test indicated differences between groups; paired-samples t tests indicated changes by day.

After sleep deprivation, IL-6 production tended to be decreased with PLA and significantly ($P < .019$) decreased with GLN. IL-8 production tended to be decreased with PLA and increased with GLN. p[Lep] did not change with Days or groups. p[GLN] was maintained with GLN and decreased with PLA. In the PLA group, p[Caff] significantly decreased on day 2 ($P < .027$) and increased on day 4 ($P < .045$). Jump height was decreased after sleep deprivation. In a separate study in rugby players on tour, sleep quality correlated with motivation.

This is the first study to investigate the effect of one night's sleep deprivation on IL-6 and IL-8 production, p[GLN], and GLN supplementation in humans. After one night's sleep deprivation: IL-6 production decreased the following morning (more markedly with GLN), oral GLN provision increased IL-8 release. Physical performance was decreased by sleep deprivation. The GLN group performed better in cognitive function tasks.

F-22. Protein–carbohydrate intake during moderate cycling and immunological response in elderly individuals, Tokuko Mizuno [a], Keitaro Matsumoto [b], Bo Dilling-Hansen [a], Axel Lahoz [a], Vivian Bertelsen [a], Henrik Münster [a], Henrik Jordening [a], Koichiro Hamada [b], Tatsuya Doi [b], Masao Mizuno[a], [a]Research Unit, Ribe County Hospital, Esbjerg, Denmark, [b]Saga Nutraceuticals Research Institute, Otsuka Pharmaceutical Co., Ltd., Saga, Japan

This study was designed at evaluating the effect of a single oral intake of protein-carbohydrate supplement during moderate cycling on the arterio-venous net balance of lymphocyte (LYMP) and neutrophil (NEUT) across the working muscles in healthy elderly individuals. Nine subjects, 66 (61–86) years, were studied by a randomized double blind placebo-controlled cross-over trial. The subjects performed 3 bouts of 20 min cycling at 50% max. Either a nutrient supplement containing 10.0 g protein, 16.5 g carbohydrate (PROT) or a non-calorie placebo (NONP) was given at 10 min of the 1st exercise bout. Blood samples were taken from arteria radialis and vena femoralis at rest and during exercise. Blood flow in arteria femoralis was determined by ultrasound Doppler technique. Net balance of LYMP, NEUT, and creatine kinase activity (CK) was calculated by the arterio-venous difference method. At rest, no difference in the arterial cell counts of LYMP and NEUT was observed between PROT and NONP, and a similar increase was observed during exercise. At rest, the net balance of LYMP and NEUT across the lower limb was equivalent in both PROT and NONP. During the 2nd and 3rd bout, uptake of LYMP by the lower limb was observed in NONP ($0.18 \pm 0.05 \times 10^9$ min^{-1}, mean ± SEM), whereas PROT resulted in reducing the uptake of LYMP ($0.08 \pm 0.03 \times 10^9$ min^{-1}) as compared to NONP ($P < .05$). An increased uptake in NEUT was observed during the 2nd and 3rd bout with no difference between the two conditions. Arterial plasma CK did not differ throughout the experiments. A greater release of CK was observed during the 2nd and 3rd bout in both PROT (-3.51 ± 1.59 IU ± min^{-1}) and NONP ($-7.16 \pm 3.32 \pm$ IU min^{-1}) as compared to rest (0.05 ± 0.19 vs. $-0.66 \pm 0.31 \pm$ IU ± min^{-1}) ($P < .01$). PROT showed a less CK release from exercising muscles as compared to NONP ($P < .05$). An inverse correlation was observed between the net balance of LYMP and CK ($r_s = -0.415$, $P < .01$), demonstrating that a greater LYMP uptake corresponded to a larger release of CK. These results suggest that protein-carbohydrate intake at onset of exercise modifies immune response to exercise-induced cellular stress even at moderate work intensity in elderly individuals.

F-23. The effect of low-dose tumor necrosis factor-α infusion on the insulin-mediated blood flow in humans, Christian P. Fischer, Peter S. Plomgaard, Rikke Krogh-Madsen, Bente K. Pedersen, Centre of Inflammation and Metabolism, The Department of Infectious Diseases and The Copenhagen Muscle Research Centre, Rigshospitalet and Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

Low-grade inflammation is involved in the pathogenesis of atherosclerosis and insulin-resistance. Infusion of the pro-inflammatory cytokine TNF-α into the brachial artery of humans—causing a high local concentration of TNF-α—reduces the insulin-mediated increase in blood flow and glucose-uptake of the forearm. We hypothesized that an acute low-grade elevation of plasma TNF-α would inhibit the insulin-mediated increase of the femoral blood flow.

Five healthy male subjects, aged 45–60 years, participated in the study. On two separate occasions, subjects received an i.v. infusion of either a low dose of recombinant human TNF-α (TNF) or vehicle (CON) for 6 h. After 1 h of infusion with or without TNF-α, the subjects ingested 0.3 g glucose/kg every 20 min for 3 h (OGTT). Plasma glucose and blood gas concentrations were measured in both the femoral artery and vein. Simultaneously, diameter and blood flow of the femoral artery were measured using ultrasonography.

Plasma TNF-α was ~20 pg/ml during the TNF trial. Of note, no subjects experienced any major discomfort during TNF. In response to the OGTT, there was no difference between CON and TNF trials regarding arterial plasma glucose ($P = .496$) and insulin ($P = .280$) concentrations. Compared to CON, heart rate was higher in TNF ($P = .027$), while MAP was similar ($P = .610$). Femoral blood flow increased from 640 ± 48 ml/min to 802 ± 55 ml/min during OGTT in CON ($P = .004$), while no increase was observed during TNF (612 ± 64 ml–642 ± 75 ml, $P = .600$, difference from CON $P = .023$). During OGTT, femoral vascular resistance decreased 31% ± 6% in CON ($P = .036$), while no change was observed in TNF ($P = .769$). Surprisingly, there was no difference between CON and TNF trials when comparing leg uptake of O_2 ($P = .951$) or glucose ($P = .583$).

Infusion of TNF-α mimicking physiological increases of TNF-α in the circulation completely blunted the insulin-mediated increase of the femoral blood flow during oral glucose intake. The effect of TNF-α infusion was due to loss of the insulin-mediated decrease of leg vascular resistance. Since femoral glucose and O_2 uptake of was similar during the two trials, the vascular endothelia appeared to be more sensitive than skeletal muscle to acute physiological elevation of plasma TNF-α.

Free communications (poster presentation)

P-1. Effect of oral glucose ingestion during endurance training on interleukin-6 mRNA expression in human skeletal muscle, Thorbjorn Akerstrom, Bente Klarlund Pedersen, Centre of Inflammation and Metabolism, The Department of Infectious Diseases and The Copenhagen Muscle Research Centre, Rigshospitalet and Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

Introduction. Human skeletal muscle expresses and releases interleukin-6 (IL-6) during exercise. Muscle-derived IL-6 has been suggested as a regulator of substrate metabolism. In support of this, infusion of IL-6 stimulates lipolytic rate and fat oxidation and lowered muscle glycogen levels augments the IL-6 response to exercise. Furthermore, glucose ingestion during exercise blunts the release of IL-6 from skeletal muscle during exercise, but not the expression of IL-6 mRNA. Exercise training has recently been shown to reduce exercise induced expression of IL-6 mRNA. We hypothesized that oral glucose ingestion during a period of endurance training would result in a lowered exercise induced increase in plasma IL-6, but not IL-6 mRNA expression.

Methods. Nine male subjects volunteered to participate in this study. Before and after training the subjects performed an incremental exercise test to determine the maximal workload (W_{max}) and 3 h one legged-knee-extension exercise bout (3 h-test) at 55% of W_{max} on a modified Krogh ergometer. Subjects with a W_{max} difference of more than 6 W between the right and left leg were excluded. Using a randomized block design the subjects were assigned to train their respective legs on alternate days for 10 weeks 5 days a week for 2 h at a time ingesting a 6% glucose solution (Glc) on one day or sweet placebo (Pla) on the other. Before, immediately following, and 2 h after the 3 h-test muscle biopsies were taken from the vastus lateralis. Blood was sampled from an antecubital vein prior to exercise, during, and in the recovery phase. The muscle biopsies were analyzed for IL-6 mRNA expression and glycogen content and plasma was analyzed for IL-6 concentration.

Results. The 10 weeks of training resulted in a performance improvement in both legs, 11.6 and 12.0% for Glc and Pla legs, respectively. W_{max} did not differ between the two legs before or after training. Training clearly reduced exercise induced IL-6 mRNA expression, but there was no treatment effect. Plasma IL-6 concentration rose in response to exercise. The IL-6 plasma response was similar between treatments, but IL-6 plasma concentration was lower in the recovery phase after 10 weeks of training.

Conclusion/Discussion. We conclude that endurance training has an effect on exercise induced skeletal muscle IL-6 mRNA expression and IL-6 plasma concentration, whereas glucose ingestion during endurance training did not have a detectable effect on the measured parameters.

P-2. The effect of caffeine ingestion on plasma interleukin-6 concentration following prolonged cycling, Gary J. Walker, Catrin E. Sheppard, Nicolette C. Bishop, School of Sport and Exercise Sciences, Loughborough University, Leics LE11 3TU, UK

Prolonged strenuous exercise is known to induce a marked elevation in plasma interleukin-6 (IL-6) concentration (Pedersen et al., 2001, J Physiol. 536, 329–337). Caffeine ingestion is associated with increased sympathetic activity and is commonly consumed by athletes for its ergogenic properties (Graham, 2001, Sports Med. 31, 785–807). Given the apparent relationship between adrenaline and the regulation of IL-6 elevation (Steensberg et al., 2001, Am. J. Physiol. Cell. Physiol. 281, C1001–1004) the aim of the study was to investigate the effect of caffeine ingestion on plasma IL-6 concentration following prolonged cycling.

Following Local Ethics Committee approval eight endurance trained males (mean ± SEM; age 24 ± 1 years; body mass: 72.8 ± 2.8 kg; $VO_{2\,max}$: 65.6 ± 1.9 ml kg^1 min^{-1}) cycled for 90 min on a stationary ergometer at 72.3 ± 0.7% $VO_{2\ max}$. On two occasions, separated by 7 days, subjects arrived at the laboratory following an overnight fast and 60 h abstention from caffeine containing products and were randomly assigned to ingest either 6 mg kg^{-1} body mass of caffeine (CAF) or dextrose powder (PLA) 60 min before exercise. During exercise, participants consumed water only (2 ml kg^{-1} body mass) at 15 min intervals. Venous blood samples were collected at rest, pre-exercise, immediately post-exercise and 1 h post-exercise. Plasma IL-6 and adrenaline concentration were determined using a commercially available ELISA (Diaclone Research, Besencon Cedex, France) and high-performance liquid chromatography, respectively. Serum caffeine and plasma glucose and free fatty acids were determined using commercially available spectrophotometric assays.

Serum caffeine and plasma adrenaline were significantly higher on CAF than PLA at immediately post-exercise ($P < .01$). At this time, plasma IL-6 concentration was significantly higher on CAF than PLA (CAF: 5.2 ± 0.8 pg ml^{-1}; PLA: 3.3 ± 0.8 pg ml^{-1}, $P < .05$), which corresponded to a 436 ± 81 and 249 ± 43% change from pre-exercise values on the CAF and PLA trials, respectively. Plasma free fatty acid concentration was higher on CAF than PLA (main effect of trial, $P < .05$) however, there were no significant differences for plasma glucose concentration. These findings suggest that caffeine ingestion influenced plasma IL-6 concentration following prolonged, intensive cycling, which may in part be the result of increased sympathetic activity on the CAF trial.

P-3. Enhanced plasma IL-6 responses after a half-marathon race and after prolonged strain with sleep- and energy deprivation, Hilde Grindvik Nielsen [a], Olav Oktedalen [b], Per Kristian [c], Opstad, Torstein Lyberg, [a]Center for Clinical Research, [b]Department of Infectious Diseases, Ulleval University Hospital, Oslo, Norway, [c]Norwegian Defense Research Establishment, Kjeller, Norway

The aim of this study was to examine the effect of a half-marathon race and 8 days with continuous exercise combined with sleep and energy deprivation (ranger-training course) on secretion of pro-inflammatory cytokines TNF-α, IL-1, IL-6, IL-8, and anti-inflammatory cytokine IL-10. Eight women and eight men participating in the Oslo half-marathon race (year 2001), and ten male cadets from the

Norwegian Military Academy participating in a 8 days ranger-training course (2003) as a part of their training program were recruited to this study. The plasma cytokine levels were detected by ELISA techniques. We observed after both the half-marathon (1.1 ± 0.2 to 35.6 ± 5.7 pg/ml, mean ± SEM, $P < .001$) and the ranger-training course (2.2 ± 0.3 to 4.5 ± 1.1 pg/ml, $P < .05$) an increase in IL-6. The IL-10 levels increased after both the half-marathon race (9.7 ± 1.6 to 71.8 ± 11.1 pg/ml, $P < .001$) and the ranger-training course (13.5 ± 3.2 to 25.2 ± 2.9, $P < .05$), while IL-1 only increased after the ranger-training course (0.2 ± 0.03 to 0.4 ± 0.1, $P < .05$), but not after the half-marathon race.

Neither the half-marathon nor the ranger-training course induced changes in TNF-α or IL-8 responses. TNF-α and IL-1 are traditionally being understood to be the main inducer cytokines of acute phase reactions. Several studies have shown that the plasma concentration of these cytokines is either unchanged following exercise or exhibits relatively small, delayed increments. The increase in IL-6 is tightly related to the duration and intensity of the exercise. We observed after the half-marathon race a 33-fold increase in IL-6 levels, while the ranger-training course gave a 2-fold increase. This indicates that intensity rather than duration play a major role. IL-6 is produced in contracting skeletal muscles via a TNF-independent pathway and the net release from the muscle can account for the exercise-induced increase in plasma concentration. In response to exercise larger amounts of IL-6 is produced/released than for any other cytokine. It is known that IL-6 induces hepatic glucose output and lipolysis. These facts indicate that IL-6 may represent an important link between contracting skeletal muscles and exercise-related metabolic changes.

P-4. Comparison between cytokine, metabolic, and hormonal responses between a long distance triathlon and a 100-km run, D. Gomez-Merino [a], C. Drogou [a], C.Y. Guezennec [a], C. Bourrilhon [a], S. Milhau [b], A. Tomaszewski [c], B. Boudjemaa [c], F. Depiesse [c], M. Chennaoui [a], [a]Department of Physiology, IMASSA, Brétigny-sur-Orge, France, [b]Ecole Interarmées des Sports: EIS, Fontainebleau, [c]Fédération Française d'Athlétisme: FFA, France

Aim. The present study was to compare the extent of cytokine, hormone, and metabolic changes between a long distance triathlon and a 100 km endurance run concomitantly with those of physical performance, and evaluate recovery processes.

Methods. Blood samples were collected from 12 triathletes (34.8 ± 1.4 year) and 10 runners (42.4 ± 2.2 year) several months before races (T0), the day before and at the end of races (T1, R1), and 24-h and 7-day post races (R2, R3). Physical performance was assessed through vertical jump tests. Muscle and joint soreness, and muscle flexibility were recorded from medical examinations and the Likert scale.

Results. At R1, magnitudes of increase in levels of interleukin (IL)-6, IL-1ra, and IL-10 were higher in triathletes than in runners ($P < .05$, $P < .05$, $P < .01$, respectively), while the one of IL-8 levels was higher in runners than in triathletes ($P < .001$). At R1, cortisol and prolactin levels significantly increased for the two groups, the magnitudes of increase being higher for triathlete ($P < .001$, respectively). At R1, the magnitude of increase in FFA levels was higher for triathletes ($P < .001$) who were the only one to exhibit a significant increase in urea levels ($P < .001$ for R1 vs. T1). CK levels significantly increased at R1 but only for runners ($P < .001$) who concomitantly exhibited reduction both in muscle flexibility and performance in vertical jump ($P < .01$, respectively). CRP levels increased for the two groups at R2 ($P < .001$, respectively). For the two groups, all parameters recovered pre-race values levels at 7-day of post race. Significant correlations were found between all cytokines and FFA levels for the two athlete groups; the highest being observed for triathletes (IL-6, $r = 0.82$; IL-1ra, $r = 0.86$; IL-10, $r = 0.83$; IL-8, $r = 0.61$).

Conclusion. We observed substantial differences between cytokine, hormone, and metabolic responses to a long-distance triathlon and a 100-km endurance run and suggested that some of them reflected the intensity of the muscle required for substrate availability while others were consequences of inflammation.

P-5. Expression of interleukin-6 in the external zone of the porcine median eminence, Ryan Jankord, James C. Schadt, M. Harold Laughlin, James R. Turk Biomedical Sciences, University of Missouri, Columbia, MO, USA

Interleukin-6 is expressed within the hypothalamic–pituitary–adrenal (HPA) axis and can induce activation of the HPA axis. It was recently shown that IL-6 is expressed within the internal zone of the median eminence, co-localized with vasopressin in the rat. This data provided evidence that IL-6 may be released from the posterior pituitary in response to stress. Based on our interests in understanding the mechanisms of HPA activation during exercise we decided to examine IL-6 expression in the median eminence of our animal model, the pig. We hypothesized that IL-6 would be expressed in the internal zone of the median eminence as was previously shown in rats. To test our hypothesis, five male post-pubertal Yucatan miniature swine were used. Both the brain and the pituitary were removed from the animals after euthanasia and placed in formalin. After the tissue was fixed and processed, slides were made of the median eminence for immunohistochemistry. Antibodies for vasopressin (VP) and corticotropin releasing hormone (CRH) were used to define internal and external zones of the median eminence. A porcine-specific IL-6 antibody was used to determine IL-6 expression. VP expression was seen in both the internal and external zones of the median eminence, while CRH expression was seen in only the external zone of the median eminence. IL-6 was localized to the external zone of the median eminence demonstrating the same staining pattern as CRH. The expression of IL-6 in the external zone was not previously observed in rats, where IL-6 expression was limited to the internal zone. The relevance of this finding is that the axon terminals located in the external zone secrete pituitary hormone releasing and inhibiting factors into portal blood vessels for transport to the anterior pituitary. Therefore, the expression of IL-6 in the external zone of the median eminence provides evidence that IL-6 may be released from the median eminence to affect anterior pituitary hormone secretion.

P-6. Effects of different exercise durations on wound healing in older mice, K. Todd Keylock, Darcie Derricks, J.A.Woods University of Illinois at Champaign-Urbana, Urbana, IL, USA

The purpose of this study was to determine the effect of short term and prolonged exercise on the length of time for wounds to heal in older (18 months) Balb/cByJ mice. We hypothesized that short-term exercise (ST) would decrease wound size over time compared to controls, but mice in the prolonged exercise group (PRO) would have slower healing.

Methods. Twenty-four 18-month-old female Balb/cByJ mice were given two full thickness dermal wounds using a 3.5 mm punch biopsy (Med Plus Medical Supply, Buffalo, NY). Eight mice (ST) were exercised at a moderate intensity (approximately 60% of their $VO_{2\ max}$; at 12 m/min) for 30 min a day for 3 days before wounding, and for 5 days afterwards. Another eight mice (PRO) were exercised at the same intensity but for 2.5 h a day for 3 days before wounding and 5 days afterwards. The remaining eight mice were controls, being exposed to the handling, noise, and vibration of the treadmill, but did not exercise. Wounds were digitally photographed each day at the same time of day, and wound size was quantified using NIH ImageJ software.

Results. There was no difference in wound size between the control group and the PRO group (for example, on Day 3, control mice wounds were 8.1 ± 0.5 mm^2 vs. 7.1 ± 1.2 mm^2 in the PRO group), but wounds in the ST group were smaller (6.3 ± 0.48 mm^2). However, this trend did not reach the 0.05 level of significance, most probably due to small sample size. Previous studies in younger mice (3 months) found that young mice heal faster than older mice but this rate is not affected by short-term or prolonged exercise.

Conclusion. These data suggest that short-term exercise may have a beneficial effect on the wound healing process in older mice, but it appears to be unaffected by prolonged exercise. This study will be repeated to verify the effect and increase sample size. Future studies will evaluate the mechanisms of these findings, including looking at inflammatory cytokines and cellular infiltrate to the wound site.

P-7. Effects of different doses of exercise on the primary antibody response to influenza vaccine in mice, William L. Zelkovich, Thomas Lowder, Jeffrey A. Woods, Department of Kinesiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

The purpose of this study was to determine the effect of different doses of exercise on the primary IgG antibody response to influenza vaccine. Young (14 week old) male Balb/cByJ mice were randomly assigned to moderate exercise ($n = 9$), prolonged exercise ($n = 10$), or home cage control ($n = 9$) groups. Mice were exercised on a motorized treadmill for 25–30 min (moderate) and 150 min (prolonged) at 8–12 m/min, post-injection, for 14 consecutive days after vaccination. Influenza vaccine (100 μL dose) was administered intramuscularly into the right hindquarters immediately before the first exercise bout. Blood was drawn from the right saphenous vein from each mouse pre-vaccine, 2 and 12 week post-vaccination. ELISA was then used to analyze the total IgG response to the vaccine in the collected plasma. The figure below depicts (mean + SEM) the changes in antibody levels among the three groups over time. Repeated measures analysis of variance revealed a significant time, but no group main effect. There was also a non-significant time by group interaction. However, statistical power was low (e.g., 0.25). Our data indicate that prolonged exercise had no effect on the IgG response to flu vaccine. However, moderate exercise tended to increase IgG in response to primary flu vaccination. Future experiments will increase statistical power and verify if moderate exercise is enhancing the primary antibody response to influenza vaccine. Moreover, we will examine the IgM response as well.

P-8. β-Adrenergic receptor blocker attenuates the exercise-induced suppression of TNF-α in response to LPS, Hiromi Kitamura [a], Daisuke Shiva [b], Jeffrey A. Woods [c], Hiromi Yano [b], [a]Department of Health and Nutrition, Wayo Women's University, 2-3-1 Konodai, Ichikawa, Chiba 272-8533, Japan, [b]Department of Health and Sports Science, Kawasaki University of Medical Welfare, 288 Matsushima, Kurashiki, Okayama 701-0193, Japan, [c]Department of Kinesiology, University of Illinois at Urbana-Champaign, 906 S. Goodwin Avenue Urbana, IL 61801, USA

Background. It has previously been shown that stressful exercise reduces proinflammatory cytokine response to lipopolysaccharide (LPS) administration. How exercise attenuates cytokine response to LPS is not known,

Purpose. The purpose of this study was to determine whether exercise-induced glucocorticoid or catecholamine is responsible for the attenuation of tumor necrosis factor (TNF)-α production in response to LPS administration.

Methods. Female F344 rats were randomly assigned to one of five groups: control (vehicle), RU-486 (glucocorticoid receptor antagonist; 30 mg/kg), propranolol (non-selective β-adrenergic receptor blocker; 30 mg/kg), atenolol (β1-adrenergic receptor blocker; 30 mg/kg), and ICI118551 (β2-adrenergic receptor blocker; 30 mg/kg) treated groups. Each group of rats was further sub-divided into sedentary or exercise groups 30 min after each treatment (i.p.). The rats in exercise groups ran until exhaustion on a treadmill at gradually increasing speed from 10 to 36 m/min at 15% grade. The sedentary rats were simply caged for 2 h. All rats were injected with LPS (1 mg/kg, i.v.) immediately after exhaustion or sedentary period of 2 h.

Results. In spite of LPS stimulation, plasma TNF-α concentration of the exhausted rats of either control, RU-486 or ICI118551 treated groups was significantly lower than the sedentary rats of each treated group ($P < .01$, respectively). However, pre-treatment of rats with non-selective β-adrenergic receptor blocker propranolol almost completely reversed the exercise-induced suppression of plasma TNF-α in response to LPS. Furthermore, the selective β1-adrenergic receptor blocker atenolol pre-treatment attenuated the exercise-induced suppression of plasma TNF-α in response to LPS.

Conclusion. These results suggest that catecholamine through β-adrenergic receptors attenuates the exercise-induced suppression of plasma TNF-α in response to LPS. In addition, β1 receptor may have major contribution to this phenomenon after exhaustive exercise in rats.

P-9. Impact of age and exhaustive exercise on intestinal HSP70 production and cellular apoptosis, Ingrid Brenner [a,b], Christianna Hamilton [b], Carolyn Kapron [b], [a]Trent/Fleming School of Nursing, Trent University, Peterborough, Ont., Canada, [b]Department of Biology, Trent University, Peterborough, Ont., Canada

Inducible heat-shock protein 70 (HSP72) has a protective role within physiologically stressed cells. It has been proposed that extracellular HSP72 may alter cytokine signaling by immune cells and increase cellular apoptosis when the inducible HSP72 intracellular repair mechanisms are not successful. Apoptosis has been previously studied in the gastrointestinal (GI) tract. This is the first study to examine the impact of age and exercise on HSP72 expression in the GI tract in relation to cellular apoptosis. HSP72 expression is reduced in the aged organism and this may be related to the increased disease observed in the small and large intestines. This study examines the impact of acute exhaustive exercise on HSP72 expression and cellular apoptosis in the GI tract of young (14–16 weeks) and old (26–28 weeks) C57BL/6 female mice. The control mice (5 young and 5 old) maintained normal cage activity. The exercise mice (5 young and 5 old) underwent a 90 min bout of exhaustive treadmill exercise and were sacrificed 2 h following the exercise. The control mice were sacrificed at the same time of day as the exercise group. Blood samples were obtained by cardiac puncture and the gastrointestinal tract was removed for further processing. Immunohistochemistry was used to determine the extent of exercise-induced HSP72 expression and cellular apoptosis within the gastrointestinal mucosa. Moreover, total HSP72 expression in segments of the small and large intestine was quantified using ELISA techniques. Complete blood counts were assessed using a standard Giemsa staining procedure. Data analysis currently in progress and the results will be discussed. The results of this study will be important in understanding the mechanisms behind exercise-induced gastrointestinal health, altered cell function and rate of apoptosis. Advancing the understanding of HSP72 and its protective roles for the organism and cells under conditions of physiological stress will be beneficial for further development of exercise therapy for disease prevention, and additional treatments related to HSP72 induction and its influence on apoptotic pathways.

P-10. Effects of the combined treatment of indomethacin and pentoxifylline on the LPS-induced reduction in wheel-running activity of mice, Hiromi Yano [a], Yuki Fujinami [a], Takashi Matsumoto [a], Daisuke Shiva [a], Jeffrey A.Woods [b], [a]Department of Health and Sports Science, Kawasaki University of Medical Welfare, 288 Matsushima Kurashiki, Okayama 701-0193, Japan, [b]Department of

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Background. One characteristic of sickness behavior is lethargy, in rodents this is demonstrated by a reduction in voluntary wheel-running activity during infection. Lipopolysaccharide (LPS), also known as endotoxin, is a component of the cell wall of gram-negative bacteria that induces sickness behavior and increases proinflammatory cytokines. However, LPS also activates macrophages to produce prostaglandin (PG) E2, which acts as important mediators of several pathophysiological events (i.e., pain, fever, and hypotension). In contrast, released PGE2 also inhibits the production of proinflammatory cytokines by macrophages as a negative feedback loop. Therefore, it is possible that combined treatment of cyclooxygenase (COX) inhibitor and tumor necrosis factor (TNF)-α inhibitor attenuates the LPS-induced reduction in wheel-running activity, but this treatment influence has not been characterized.

Purpose. The purpose of this study was to determine whether LPS-induced PGE2 production is responsible for reduced spontaneous physical activity.

Methods. We measured LPS-induced changes in voluntary wheel-running activity for 24 h in C3H/HeN mice, which were injected with the cyclooxygenase (COX) inhibitor indomethacin (IM; 0, 1, 10, and 20 mg/kg, i.p.) 30 min before LPS (1 mg/kg) or saline i.v. injection. Furthermore, we examined the effect of IM on LPS-induced TNF-α production in peritoneal macrophages in vitro and that in plasma in vivo. IM plus TNF-α inhibitor pentoxifylline (PF) was given 30 min before LPS injection, and then the mice were monitored voluntary wheel-running activity for 24 h.

Results. Wheel-running activity in mice was reduced by LPS injection (428 ± 79 revolutions/day, $P < .01$), but the activity in IM-treated mice was attenuated to 1.540 ± 257 revolutions/day (1 mg/kg), 2.384 ± 251 revolutions/day (10 mg/kg, $P < .01$), and 2.607 ± 823 revolutions/day (20 mg/kg, $P < .01$). Thus, IM partially, but significantly attenuated the LPS-induced reduction in wheel-running activity in mice. LPS-induced TNF-α production from peritoneal macrophages in vitro was accelerated by IM treatment 12 h and 24 h after incubation ($P < .01$ and $P < .05$, respectively). In LPS stimulation, the running activity of the IM plus PF-treated group was significantly but slightly higher than that in LPS-treated group($P < .05$). However, this level of running activity in IM plus PF-treated mice was significantly lower than that in non-stimulated group ($P < .01$). In addition, the tendency of changes in plasma TNF-α concentration of IM plus LPS-treated group did not consist with the result in in vitro experiment.

Conclusion. Our results suggest that the transient reduction in physical activity after LPS injection is partially mediated by LPS-induced PGE2 production, but the effect of PGE2 on inhibiting the production of proinflammatory cytokines might be few.

P-11. Lipopolysaccharide inhibits to increase IgE and IgG1 production of OVA sensitization but not systemic anaphylaxis in mice, Daisuke Shiva [a], Takashi Matsumoto [b], Yasuko Kato [c], Hiromi Yano [b], [a]Doctoral Program in Health Science, Kawasaki University of Medical Welfare, Okayama, Japan, [b]Department of Health and Sports Science, Kawasaki University of Medical Welfare, Okayama, Japan, [c]Department of Clinical Nutrition, Kawasaki University of Medical Welfare, Okayama, Japan

Background. A number of epidemiological studies has suggested that the increase in the prevalence of allergic disorders that has occurred over the past few decades is attributable to a reduced bacterial endotoxin. Endotoxin (lipopolysaccharide: LPS, cell-wall components of Gram-negative bacteria) exposure might inhibit a reaction of type 1 allergy, which is induced Th2-induced Ig, such as IgE and IgG1, which are considered to be major triggers. Although it was reported that exercise also induced an increase of LPS absorption from the gastrointestine, the relationship between exercise and allergy is unclear. It seems that the most important thing right now is to clear the effect of LPS stimulation.

Objective. In this study, we determine whether or not LPS stimulation inhibits type 1 allergic symptoms by allergen re-exposure.

Methods. C3H/HeJ (tlr4 gene mutated) mice and C3H/HeN (wild type) mice were sensitized systemically with 50 mg chicken OVA emulsified in aluminum hydroxide after LPS stimulation (0.5 mg/kg) i.p. The sensitized mice were measured for total and OVA-specific IgEs, and OVA-specific IgG1 in serum by ELISA. We measured the changes in rectal temperature of all of mice by OVA re-exposure to evaluate systemic anaphylaxis.

Results. Total and OVA-specific IgEs and OVA-specific IgG1 in serum of C3H/HeN mice were strongly inhibited by LPS stimulation. In contrast, those Igs of OVA-sensitized C3H/HeJ mice were inhibited slightly or not at all in spite of LPS stimulation. After OVA re-exposure decreased the rectal temperature in LPS/OVA-sensitized C3H/HeN mice, however, no inhibition was observed.

Conclusions. These results indicate that LPS stimulation via TLR4 strongly inhibited increases of OVA-induced IgE and IgG1 production, but systemic anaphylaxis on OVA re-exposure occurred. We speculate that exercise might hardly contribute the inhibition of the symptom of type 1 allergy via LPS absorption.

P-12. The effect of Salmonella enterica infection on voluntary physical activity and survival rate in C3H/HeJ mice, Takashi Matsumoto [a], Daisuke Shiva [b], Hiromi Yano [c], [a]Master's Programs in Health and Sports Science, Okayama, Japan, [b]Doctoral Program in Health Science, Okayama, Japan, [c]Department of Health and Sports Science, Kawasaki University of Medical Welfare, Okayama, Japan

Background. Salmonella enterica is known as gram-negative bacteria and is the most prevalent food-borne illness, induces sickness behavior such as diarrhea, vomiting, and reduction of physical activity. It is not clear, however, why this induces the decrease in physical activity. On the other hand, it is known that toll-like receptor (TLR) 4 on the outer membrane of immunological cells could recognize lipopolysaccharide (LPS) from gram-negative bacteria. Therefore it is possible that TLR4 regulates physical activity after *Salmonella* infection, but this has not been made clear yet.

Objective. Our objective was to clarify whether TLR4 regulates voluntary physical activity and survival response to *Salmonella* infection or not.

Methods. In this study, we used C3H/HeJ (tlr4-gene mutated strain) mice and C3H/HeN (wild-type strain) mice. *Salmonella enterica* serovar Dublin was grown for 48 h at 35°C in a brain heart agar medium, and then that was diluted in PBS. The voluntary physical activity of mice was examined by observing their running performance in a cage-adjacent wheel for 2 days after i.p. injection with *Salmonella* 2×102-5 CFU/200 μl/mouse. Furthermore, we also monitored the survival rate for 10 days and the level of infection in the kidney from the mice at 2 days and 10 days after *Salmonella* injection. As an in vitro experiment, the production of tumor necrosis factor (TNF)-α of peritoneal macrophages was measured using an ELISA kit after stimulation with heat-killed *Salmonella* (in 0.1, 1, and 10 μg/wells, respectively).

Results. Although C3H/HeN mice significantly reduced physical activity for 48 h after *Salmonella* injection, C3H/HeJ mice did not. However, the survival rate of C3H/HeJ mice gradually declined from 5 days after *Salmonella* injection, and after 10 days all of these mice had died. The level of kidney infection of C3H/HeJ mice showed persistent and increasing colonization. In contrast, C3H/HeN mice maintained their survival and had cleared infection in the kidney. Isolated peritoneal macrophages from C3H/HeN mice produced a markedly lower level of TNF-α than those from C3H/HeN mice after stimulation with heat-killed *Salmonella* ($P < .01$).

Conclusion. Our findings suggest that TLR4 may well regulate reduction of voluntary physical activity and survival response to *Salmonella* infection.

P-13. CXCR4 expression on EL-4 lymphoma cell lines is augmented at lower temperature in vitro, Mitsuharu Okutsu [a], Kaori Matsuo [b], Ryoichi Nagatomi [b], [a]Consolidated Research Institute for Advanced Science and Medical Care, Waseda University, Tokyo, Japan, [b]Medicine and Science in Sports and Exercise, Tohoku University Graduate School of Medicine, Sendai, Japan

Background. CXCR4 is a chemokine receptor expressed or induced on lymphocytes, lymphoid cells and tumor cells such as mammary gland cancer, and is involved in lymphocyte migration and tumor metastasis. Expression of CXCR4 is partially regulated by glucocorticoids, but factors other than glucocorticoids are still not well understood. During exercise or physical stressors, thermal milieu of the body greatly varies. Since thermal stress is known to influence various aspects of immune system, it may influence immune system through CXCR4 modification.

Purpose. We tested the hypothesis whether different thermal condition would affect CXCR4 expression on lymphoid cell.

Method. Mouse lymphoma cell line EL-4 cells were incubated for 24, 48, or 72 h under either 34 or 37 °C. EL-4 cells were enumerated at each time point. CXCR4 expression was detected by murine SDF-1α fusion protein consisting of human IgG1 and secondary FITC anti-human IgG goat (Fab')2 antibody, and analyzed by a flow-cytometer.

Results. EL-4 growth was greater at 37 °C than at 34 °C. CXCR4 expression on EL-4 cells increased in a time-dependent manner. In contrast to the slower growth, CXCR4 expression was greater at 34 °C than at 37 °C.

Conclusion. Here we demonstrated that cellular temperature may influence CXCR4 expression on lymphoid cells. Lower temperature may thus regulate CXCR4 expression without glucocorticoids. It was suggested that alterations in body temperature during exercise or physical stress may influence immune function through chemokine–chemokine receptor interaction.

P-14. Administration of capsaicin before exercise enhances the intestinal epithelial permeability of an allergen protein, Yoshihiko Kondo [a], Hiromi Yano [b], Yasuko Kato [a], [a]Graduate School of Medical Professions, Kawasaki University of Medical Welfare, Kurashiki, Okayama 701-193, Japan, [b]Department of Health and Sports Sciences, Kawasaki University of Medical Welfare, Kurashiki, Okayama 701-193, Japan

Objective. Capsaicin, which is the main hot spice component in chili pepper, is one of the suspected sensitized factors to allergen, because it has been reported that capsaicin loosens the tight junction of intestinal epithelial cell models. In this study, we studied whether or not the administration of capsaicin before exercise enhances the transportation of allergens across human intestinal epithelial Caco-2 cells or the mucous membrane of the small intestine of a mouse. Before the examination, we would concrete that capsaicin enhances an allergen across the Caco-2 monolayer and mucous membrane of small intestine of a mouse.

Methods. After Caco-2 cells were incubated on an intercell, capsaicin (2–1000 μg) and ovalbumin(OVA, 5 mg) were added to the upper layer, transepithelial electrical resistance (TER) was measured every 30 min, and the lower medium was taken after 180 min incubation. OVA in the lower medium was detected by Immunoblotting and ELISA competitive inhibition analysis using an anti OVA antibody. Evans blue was administered from the orbital vein to the ICR-mouse after oral injection of capsaicin. Absorbance at 620 nm of a formaldehyde neutral buffer solution, in which Evans blue was eluted from the mouse intestine, was measured. The portal blood in mice was collected after orally administration of various amounts or intervals of administration of capsaicin against OVA, and OVA in the portal blood was examined by immunoblotting. Capsaicin and OVA were orally administrated to an ICR-mouse before it was loaded for 30 min treadmill running, and OVA in the blood was detected by immunoblotting.

Results. TER decreased depending on the capsaicin concentration in the upper of Caco-2 cell monolayer. A detectable amount of OVA penetrated into the lower medium and the amount increased after 30 min of capsaicin addition. Evans blue was more absorbed in the intestine of the capsaicin-administered mice than the mice administrated without capsaicin. This result indicates that capsaicin increases permeability of mouse intestinal epithelial monolayers. And further, not only OVA but also low molecular weight peptides were immunoblotted lower medium of Caco-2 layer and in portal blood. Especially, mice loaded for treadmill increased the permeability of OVA through the small intestine mucosal epithelium.

P-15. Why are insoluble wheat proteins major allergens in wheat-dependent exercise-induced anaphylaxis? Hana Kozai [a], Hiromi Yano [b], Tsukasa Matsuda [c], Yasuko Kato [d], [a]Department of Clinical Nutrition, Osaka Prefecture University, Japan, [b]Department of Health and Sports Sciences, Kawasaki University of Medical Welfare, Japan, [c]Graduate School of Applied Molecular Bioagricultural Sciences, Nagoya University, Japan, [d]Graduate School Medical Professions, Kawasaki University of Medical Welfare, Japan

Objective. Wheat proteins are the major proteins induced exercise-induced anaphylaxis and our previous study has shown that the major allergens of wheat-dependent exercise-induced anaphylaxis (WDEIA) were gliadin and glutenin using mouse. In general, proteins recognized as a cause of food allergen are water/salt soluble, such as egg ovomucoid and milk?-lactoglobulin. However, the reason as to why the allergen activities of insoluble proteins like gliadin and glutenin are high is unclear. We postulated that digestion would change insoluble proteins to salt-soluble fragments, and be absorbed into the body. In this study to elucidate the allergenic activity of WDEIA, the digestibility of wheat proteins was compared between raw and heated wheat proteins, in vivo and in vitro digestion.

Methods and results. Wheat proteins were fractionated into three parts, albumins/globulins (water/salt extract), gliadins (70% EtOH extract) and glutenins (alkali extract). Bread was prepared as the heated sample. Each fractionated wheat protein or the bread was digested in pepsin solution. The digestibility at 90 min of salt-soluble protein was 80%, but that of gliadin was only 35% and glutenin 43%. In vivo, four groups (each group, $n = 6$) of ICR mice were treated on a treadmill running after oral injection of each fractionated protein or bread (each protein 25 mg/mouse). After running, the contents of the stomach and small intestine in the mice were sampled, and portal blood was collected. The allergen activity of each digested protein was examined by immunoblotting. In all mice, high molecular weight allergen remained in the stomach and small intestine after allergen ingestion and 30 min exercise, though most of the allergenic proteins were hydrolyzed to small peptides by pepsin. Moreover, it was confirmed that the allergenic activities of the three wheat proteins were detected in the portal blood of mice injected with three raw proteins or bread?

Conclusion. Insoluble wheat proteins changed to soluble in the intestine and were absorbed in the body. We found that heat-coagulated ovalbumin remained in an insoluble form in the gastrointestine and was not detected in portal blood, whereas proteins in bread, which is a heated product, were detectable in portal blood.

P-16. Measurement of voluntary physical activity is good for assessing mouse anaphylaxis, Yasuko Kato [a], Hiromi Yano [b], Tsukasa Matsuda [c], [a]Graduate School of Medical Professions, Kawasaki University of

Medical Welfare, Kurashiki, Okayama 701-193, Japan, [b]Department of Health and Sports Sciences, Kawasaki University of Medical Welfare, Kurashiki, Okayama 701-193, Japan, [c]Graduate School of Bioagricultural Sciences, Nagoya University, Japan

Objective. Immunization studies in mice are an important means of evaluating mechanisms of food allergies. Sensitized mice can easily be judged by monitoring the serum level of specific IgE, but no objective method for the quantitative assessment of anaphylaxis has yet been established. We examined the threshold of allergen development delivered in mice by measuring body temperature, anaphylaxis score, and voluntary physical exercise. Moreover, we looked at how different anaphylaxis symptoms were raised by histamine or leukotriene C4, secreted from mast cells, in mice and discussed their roles on the anaphylaxis.

Methods. Symptoms of systemic anaphylaxis of lysozyme (LY)-sensitized mice after the injection of LY in the orbital veins were observed for 15 min, and were evaluated by scoring on a scale of one to five. Rectal temperatures of mice were measured 15 min after the LY-injection. Allergen-injected mice were housed in cages with rotation wheels and the number of revolutions every 1 h for 15 h from 8PM to 11AM were monitored.

Results. No anaphylaxis symptoms were observed in the LY-sensitized mice injected with 1 μg of LY. When mice were injected with 10 μg of LY, anaphylaxis symptoms were observed, such as scratching and rubbing around the nose, and scored an average of 1 on the scale. High anaphylaxis scores were judged in the sensitized mice injected with 50 μg of LY. The rectal temperature and the number of revolutions of sensitized mice after administration of 50 μg antigen decreased 6.7 °C and about a half revolution against the no-allergen injected mice, respectively. Correlations between each of the three indicators (anaphylaxis score, body temperature, and rotation number) in the sensitized mice by administration of antigen were recognized. However, no correlation between the IgE level and each of the three indicators were observed. Histamine and leukotriene D4, mediated from the mast cells as allergen reaction, decreased the body temperature and anaphylaxis score but not voluntary activity. These results suggest that the voluntary activity was reflected by not only immediate but also delayed allergy reactions.

P-17. Decision of an allergenic activity of heat-coagulated protein by voluntary physical activity, Kana Joo, Yasuko Kato, Graduate School of Medical Professions, Kawasaki University of Medical Welfare, Kurashiki, Okayama 701-193, Japan

Objective. We all eat commonly heat-coagulated food proteins, but in a report on egg allergic patients about 97% had negative responses to rinsed heat-coagulated protein in a double blind test. It is therefore necessary to use animal testing before any human testing. In this study, we examined the digestion and absorption of heat-coagulated protein after oral injection, and the allergenic activity was examined using voluntary exercise as an assessment of exercise-induced anaphylaxis with sensitized mice.

Method. Female BALB/c mice fasted 24 h before the experiments. Five percentage of native ($n = 12$) or heat-coagulated OVA (HC-OVA) ($n = 12$) which was prepared by heating at 100 °C for 15 min, was orally administered to mice, and each group was divided into two groups of 15 min ($n = 6$) or 30 min ($n = 6$) rest in a cage after oral administration. After 15 or 30 min rest, the mice were rapidly celiotomized and portal blood was collected. The remaining contents in the stomach and six parts of the small intestine, each of equal length, were extracted with 1 ml of PBS. The contents were separated to soluble and insoluble fractions. Gastrointestinal digestion and absorption through the intestinal epithelium into portal blood of OVA were investigated with immunoblotting and competitive inhibition ELISA using anti OVA serum. Furthermore, voluntary physical activities of the OVA-sensitized mice were measured for 15 h after HC-OVA administration and the forced 30 min-treadmill running load.

Result. Peptides were not able to react with the anti OVA antibody in a soluble fraction of the gastrointestinal tract of mice administrated HC-OVA, whereas OVA and many peptide bands were immunoblotted in an insoluble fraction of the stomach of HC-OVA and native OVA challenged groups. Moreover, no immunoblotted OVA were detected in the portal blood of mice administrated HC-OVA. Significant differences in wheel-running activities were observed between two groups of native OVA (6039r.n./15 h) and HC-OVA (9837r.n./15 h), respectively ($P < .01$).

Conclusion. These results show that antigenic activity of HC-OVA was not detected in the portal blood of mice administrated HC-OVA and anaphylaxis symptoms were repressed in sensitized mice administrated HC-OVA. This could prove that the allergenic activity of heat-coagulated protein disappears.

P-18. Design, manufacture, and use of a cDNA microarray for analysis of inflammatory status of human peripheral blood or other tissue samples, Derek Zieker [a], Judith Fliegner [a], Elvira Fehrenbach [a], Janko Dietzsch [b], Peter Gebicke [c], Hinnak Northoff [a], [a]Department of Transfusion Medicine, University of Tuebingen, Otfried-Mueller-Street 4/1, D-72076 Tuebingen, Germany, [b]Center for Bioinformatics Tuebingen, Department of Information and Cognitive Sciences, University of Tuebingen, Sand 14, D-72076 Tuebingen, Germany, [c]Central Institute for Mental Health, Department of Psychopharmacology, J5, D-68159 Mannheim, Germany

Microarrays are widely being used for expression profiling studies of tissue or cell culture samples. In contrast to sequential analysis, microarray analyses enable researchers to investigate transcriptional changes of thousands of genes simultaneously in a parallel manner. Several important issues have to be considered to successfully establish the microarray technology in a molecular biology laboratory. They include use of an appropriate combination of coating of the carrier slide, high quality of the spotted PCR products (cDNA), harmonizing temperature and buffer conditions, highly standardized working protocols, and use of high quality mRNA preparations for hybridization. We have established a standardized production, use and analysis procedure for a home made cDNA microarray system designed to give extensive information on the inflammatory status, and some additional immunological information for peripheral blood or other tissue samples in comparison to an appropriate control.

Working procedure and design of our microarray are described in this poster.

P-19. Asthma and sports, K. Bergendiová, M. Drugdová, B. Drugda, M. Brezina, Pneumo-Alergo Centrum, spol.s r.o., Bratislava, Slovakia

Asthma is complex disease process in which both hereditary and environmental factors contribute to the development and expression of the clinical symptoms experienced by the patient. It is a chronic inflammatory disorder of the airways in which many cells play a roll, in particular mast cells, eosinophils, and T lymphocytes.

Exercise-induced asthma (EIB) is present in 40–90% of patients with asthma. Exercise-induced bronchospasm, exercise-induced asthma, and exercise-induced narrowing are synonymous terms that describe a condition in which vigorous physical activity triggers acute airway narrowing in people with heightened airway reactivity.

Wheezing, dyspnea, cought, and chest tightness, in association with hyperinflation, airflow limitation, and hypoxemia are the usual symptomatic and physiological manifestations. Very often cough may be the sole complaint.

Exhaled NO (eNO) levels has been correlated with bronchial hyperresponsiveness to histamine and have correlated with the degree of decrease in FEV1 with exercise. Also in our study, we examine if the eNO could be used as a marker of exertional bronchoconstriction in a population referred specifically for the evaluation of EIB.

When we using an eNO level of <12 ppb, no one demonstrated EIB. eNO measurement may obviate the need for bronchoprovocation testing in patients who complain of exertional dyspnea.

P-20. What is reason of immuno-endocrine response to endurance training? K. Bergendiová [a], E. Tibenská [b], M. Ferenèík [c], [a]Pneumo-Alergo Centrum, sro, Bratislava, Slovakia, [b]Imunologické oddelenie, DFNsP, Bratislava, Slovakia, [c]Ústav imunológie LFUK, Bratislava, Slovakia

Summary. The aim of the study was to determine the acute effects of exhaustive exercise on the immune and endocrine systems in top sportsmen.

Study design. We investigated 50 top sportsmen and 20 obese persons between the ages 16 and 18 years. Blood samples were collected before exercise, immediately after exercise and after 60 min of regeneration. Absolute and percentual counts of leukocytes, granulocytes, lymphocytes, and their subsets were determined by flow cytometer.

Results. Immediately after acute exercise we observed the most significant increase of absolute counts of NK cells and triggers markers of lymphocytes CD38 and CD122.

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.

P-21. Microbicide activity of neutrophils during a single session of moderate exercise performed by sedentary men. Role of norepinephrine and Hsp72, Eduardo Ortega, Esther Giraldo, Mariana Martínez, María Eugenia Collazos, Juan José García, Department of Physiology, Faculty of Sciences, University of Extremadura, Badajoz, Spain

Acute exercise generally stimulates the innate cellular immune response. However, there is some controversy about the immuno-physiological interpretation of the influence of exercise on the neutrophilś microbicidal activity. During the microbicidal phase, there is a major stimulation of cell metabolism, digestive enzymes, and oxygen radical production. Studies using direct techniques such as killing of living antigens have not always shown similar results to those using indirect techniques, such as the production of reactive oxygen species (ROS). In general, it has been concluded that, while intense exercise is potentially immunosuppressive, moderate exercise has variable effects on the ROS production by neutrophils.

The influence of a single session of moderate exercise (45 min at 55% of $VO_{2\ max}$) performed by young sedentary men on the microbicidal capacity of neutrophils has been compared by using both direct (killing of phagocytosed *Candida albicans*) and indirect (superoxide anion production after phagocytosis of inert particles) techniques.

Although no changes in the percentage of destruction of *C. albicans* was found immediately after the moderate exercise, there was an increase in the absolute destruction of this living antigen, which remained higher than basal values one day later. These changes correlated with the variations in the serum concentration of heat-shock protein 72 (Hsp72) during the exercise and the recovery period. However, the increased phagocytosis of inert particles after the exercise was not correlated with a significant higher superoxide anion production.

In previous investigations, it was found that norepinephrine is mediating the increased phagocytosis and chemotaxis of neutrophils induced by this model of exercise. In the present investigation we have found that, although norepinephrine could stimulate the microbicide capacity of neutrophils, it is not mediating the increased killing of *C. albicans* during the exercise. A possible role of Hsp72 as ''stress mediator'' is being investigated.

This investigation is supported by grants from Consejería de Sanidad y Consumo (SCSS04) and Consejería de Educación, Ciencia y Tecnología (2PR04A076) of the Junta de Extremadura and Fondo Social Europeo.

P-22. Role of norepinephrine and Hsp72 on chemotaxis of neutrophils during a moderate exercise performed by sedentary men, Eduardo Ortega [a], Esther Giraldo [a], Mariana Martínez [a], María Dolores Hinchado [a], Juan Pablo Gallardo [b], Fernando Labrador [b], Sergio Ibáñez [c], Antonio Cidoncha [a], Juan José García [a], [a]Department of Physiology, Faculty of Sciences, University of Extremadura, Badajoz, Spain, [b]Medical Service, University of Extremadura, Badajoz, Spain, [c]Faculty of Sport Sciences, University of Extremadura, Cáceres, Spain

In previous studies, we have found that a single session of moderate exercise performed by young sedentary men increases the neutrophilś phagocytic capacity, and that in this stimulation participate ''stress factors'' such as norepinephrine. The mobility of neutrophils towards the focus of infection precedes phagocytosis. Thus, chemotaxis is a good index of the phagocytic cellś activation to carry out their innate immune function. In the present investigation, the influence of a single session of moderate exercise (45 min at 55% maximal oxygen uptake on a cycle ergometer) on the chemotactic capacity of neutrophils is evaluated in sedentary young men. Chemotaxis increased immediately after exercise, and returned to basal values 24 h later. These changes in chemotaxis correlated with changes in the norepinephrine and heat-shock protein 72 (Hsp72; the inducible form of the 70 kDa family of Hsp) concentrations: immediately after exercise, there was a significant increase of NE and Hsp72. However, while NE concentration returned to basal values 24 h later, Hsp72 still remained somewhat higher than basal values. Neutrophils incubated with the post-exercise physiological concentration of norepinephrine also showed stimulated chemotaxis. Norepinephrine is also a chemoattractant/chemokinetic agent for neutrophils at the post-exercise physiological concentrations.

In conclusion, norepinephrine participates in the stimulation of the chemotactic activity of neutrophils from sedentary men during a single session of moderate exercise. Preliminary results also indicate a role for Hsp72 as ''stress mediator'' of stimulated chemotaxis during the moderate exercise; and this role is also discussed.

This investigation is supported by grants from Consejería de Sanidad y Consumo (SCSS04) and Consejería de Educación, Ciencia y Tecnología (2PR04A076) of the Junta de Extremadura and Fondo Social Europeo.

P-23. Intense exercise and innate immune response in sedentary women I: Cytokines and chemotaxis of neutrophils, Role of Hsp 72, Eduardo Ortega [a], Esther Giraldo [a], Mariana Martínez [a], María Dolores Hinchado [a] Juan Pablo Gallardo [b], Fernando Labrador [b], Sergio Ibáñez [c], Antonio Cidoncha [d], Juan José García [a], [a]Department of Physiology, Faculty of Sciences, University of Extremadura, Badajoz, Spain, [b]Medical Service, University of Extremadura, Badajoz, Spain, [c]Faculty of Sport Sciences, University of Extremadura, Cáceres, Spain, [d]Hospital D. Benito-Villanueva, Servicio Extremeño de Salud, Spain

This study is a part of an investigation evaluating the effect of a single session of intense exercise-induced stress on the innate and inflammatory immune response of young sedentary women. Exercise is performed on a cycle ergometer at 70% of maximal oxygen uptake during 60 min. Blood samples are taken before, immediately after exercise and 24 h later.

Here, neutrophils chemotaxis was evaluated on isolated neutrophils in Boyden chambers, and serum cytokines (IL-2, 3, 5, 6, 7, 8, 10, 13, and 15, INF-α, TNF-α and β, TGF-β, MCP-1, 2 and 3, MIG, RANTES, G-CSF, GM-CSF, GRO, GRO-α) through the "array technology". Variations in the blood concentration of heat-shock protein 72 (Hsp72; the inducible form of the 70 kDa family of Hsp) and catecholamines are measured by ELISA and HPLC with electrochemical detection, respectively.

Neutrophil chemotaxis increased immediately after exercise and returned to the basal values 24 h later. These results correlated with the variations in the Hsp72 serum concentrations, which also increased immediately after the exercise and returned to the basal values one day later. An increase after exercise in the serum chemokines GRO, and MCP-1; and a decrease in RANTES were also found. However, we could not detect pro- or anti-inflammatory cytokines in the serum of exercised women with the "array technology".

A possible role of Hsp72 as a mediator of the stimulated neutrophil chemotaxis during the intense exercise, as well as the balance between pro- and anti-inflammatory cytokines (measured now by ELISA) is now being evaluated.

This investigation is supported by grants from Consejería de Sanidad y Consumo (SCSS04) and Consejería de Educación, Ciencia y Tecnología (2PR04A076) of the Junta de Extremadura and Fondo Social Europeo.

P-24. Intense exercise and innate immune response in sedentary women II: Phagocytosis and microbicide capacities of neutrophils, Role of Hsp 72, Eduardo Ortega [a], Mariana Martínez [a], Esther Giraldo [a], Juan Pablo Gallardo [b], Fernando Labrador [b], Sergio Ibáñez [c], Antonio Cidoncha [d], Juan José García [a], [a]Department of Physiology, Faculty of Sciences, University of Extremadura, Badajoz, Spain, [b]Medical Service, University of Extremadura, Badajoz, Spain, [c]Faculty of Sport Sciences, University of Extremadura, Cáceres, Spain, [d]Hospital Don Benito-Villanueva, Spain

This study is the second part of an investigation evaluating the effect of a single session of intense exercise on the innate immune response of young sedentary women. Exercise was performed on a cycle ergometer at 70% of maximal oxygen uptake during 60 min. Blood samples were collected before, immediately after exercise and 24 h later. Phagocytosis of inert particles and of *Candida albicans* was evaluated on isolated neutrophils. The microbicide capacity of neutrophils was measured by both indirect (superoxide anion production after phagocytosis of latex beads) and direct (candidicide activity by using methylene blue, which stains the dead yeast) techniques. Changes in the blood concentration of heat-shock protein 72 (Hsp72; the inducible form of the 70 kDa family of Hsp) and catecholamines are measured by ELISA and HPLC with electrochemical detection, respectively.

Immediately after exercise, the neutrophilś phagocytic capacity increased, both for inert particles and for living particles, and returned to basal values one day later. This correlated with the variations in the concentration of Hsp 72 during the exercise and the recovery period, suggesting a role as a "stress mediator" of phagocytosis during the exercise for these proteins. However, the intense exercise did not clearly change the microbicide capacity of neutrophils, although a slight increase was observed.

The possible role of Hsp72 as a "stress mediator" of the increased neutrophil phagocytosis during the intense exercise performed by sedentary women will be studied in vitro, and discussed with this role for catecholamines.

This investigation is supported by grants from Consejería de Sanidad y Consumo (SCSS04) and Consejería de Educación, Ciencia y Tecnología (2PR04A076) of the Junta de Extremadura and Fondo Social Europeo.

P-25 Prolonged exercise does not increase lymphocyte DNA damage or apoptosis in welltrained endurance athletes, Edith M. Peters, Michelle Van Eden, Nicholas Tyler, Atishkar Ramautar, Anil Chuturgoon, School of Medical Sciences, Faculty of Health Sciences, University of KwaZulu-Natal, South Africa

Recent research has demonstrated that lymphocyte apoptosis sensitivity appears to be related to training status. This work investigated the effect of prolonged, submaximal treadmill running on percentage (%) apoptosis, % necrosis and DNA strand breaks in lymphocytes and related these to changes in total lymphocyte and blood cortisol concentrations in well trained runners. Venous blood samples ($n = 14$) were taken immediately before (PRE), immediately after (IPE) and 3 h after (3PE) 2.5 h of treadmill running at 75% of VO_{2max} from healthy, well trained male endurance athletes (age: 34.2 ± 2.44 years). Serum was analyzed for cortisol concentrations and full blood counts were performed on EDTA treated blood samples. Lymphocytes were isolated from whole venous blood and apoptotic and necrotic cell percentages were detected by flow cytometry using annexinV-FITC and propidium iodide uptake. DNA strand breaks were measured by single cell gel electrophoresis. Mean circulating lymphocyte counts showed an exercise-induced biphasic response, rising by 1.24 fold in IPE blood samples, but dropping to 0.75 of PRE concentrations in the 3PE samples. Despite a significant ($P < .001$) exercise-induced increase in mean serum cortisol concentrations, the mean % annexin V positive cells (13.3 ± 6.78 in PRE, 11.3 ± 5.51 in IPE and 12.8 ± 6.75 in 3PE samples), was not significantly different at the three time-points ($P < .05$). Mean DNA strand breaks in the lymphocytes did also not change significantly ($P < .05$) rising from 25.7 ± 2.16 to 26.9 ± 1.89 and 27.1 ± 1.38 μm in IPE and 3PE samples, respectively. It was concluded that the exercise-induced increase in cortisol concentrations did not account for the change in % apoptotic lymphocytes or DNA strand breaks and that % lymphocyte apoptosis is not directly related to exercise-induced changes in total blood lymphocyte counts.

P-26. Physical conditioning of endurance athlete and the ratio of the peripheral neutrophils to lymphocytes, K. Matsuo [a], K. Mayumi [b], H. Sasaki [b], R. Nagatomi [a], [a]Department of Medicine and Science in Sports and Exercise, Tohoku University Graduate School of Medicine, Sendai, Japan, [b]Department of Sports Management, Osaka International University, Osaka, Japan

Background. Most of the body functions are know to be influenced or rather dependent on certain rhythms of various cycles. Circadian fluctuation is the most commonly observed cycle of various physical functions including neuro-endocrine and the immune system. Blood leukocyte number is well known to show a circadian cycle. Since neutrophils and lymphocytes show inversed fluctuation patterns, the ratio of lymphocytes and neutrophils when evaluated at a fixed time of the day may indicate disturbance of the rhythm.

Purpose. Therefore we tried to demonstrate whether disturbed neutrophil–lymphocyte ratio affects competitive performance of endurance runners over 8 months period.

Materials and methods. The subjects were 4 female high-school Ekiden road relay (endurance) runners aged 15–8 years who had more than 3 years of experience in Ekiden competition. All runners were in a high-school Ekiden team that participated in All Japan High School Ekiden Championship in November 2000. Blood sampling was performed 3 to 1 week before time trials including the championship and local competition, once or twice per month starting from April until

November. Blood samples were analyzed for the number of leukocytes, lymphocytes, and granulocytes. The time record of 3000 m time trial was employed for the indicator of their performance.

Results. Their height was 156.5 ± 7.2 cm, body weight was 45.7 ± 5.3 kg, % body fat was 14.3 ± 1.1%, and maximal oxygen uptake was 61.1 ± 5.6 ml/kg/min in July 2000. Two subjects failed to exhibit constant improvement in the 3000 m time record while the other two were successful in improving the time record over 8 month period. Those who failed to improve showed higher neutrophil/lymphocyte ratio before the time trials and the Championship, while those who were successful showed lower neutrophil/lymphocyte ratio before the time trials and the Championship.

Conclusion. Despite the preliminary result from a small number of subjects, the ratio of blood neutrophil and lymphocyte possibly reflecting the phase in circadian fluctuation of leukocytes, is in a close relationship with exercise performance of endurance athletes. Increased neutrophil/lymphocyte may correspond to fatigue or exhaustion of the subject.

P-27. The effects of intensive, moderate, and downhill treadmill running on lymphocyte adhesion/activation molecules CD54 (ICAM-1), CD18 (β2-integrin), and CD53, Richard J. Simpson [a], Keith Guy [a], Greg P. Whyte [b], Geraint D. Florida-James [a], [a]Biomedicine and Sport and Exercise Science Research Group, Napier University, Edinburgh, UK, [b]English Institute of Sport, Bisham Abbey NSC, Marlow, Bucks, UK

This study examined the effects of intensive, moderate, and downhill treadmill running on lymphocyte expression of adhesion/activation (AA) molecules. Eight trained male subjects (age: 28 ± 5 years; VO_{2max}: 63 ± 3 ml kg min^{-1}) completed three treadmill running protocols of identical duration: (1) an intensive protocol at 80% VO_{2max} to volitional exhaustion, (2) a moderate protocol at 60% VO_{2max}, and (3) a −10% downhill (eccentric) protocol at 80% VO_{2max}. Blood samples were taken before, immediately after, 1 h and 24 h after exercise. Isolated lymphocytes were assessed for the expression of the AA molecules CD54 (ICAM-1), CD18 (β2 integrin), and CD53 (a tetraspan molecule) by one and two colour flow cytometry using direct and indirect immunofluorescence assays. Total lymphocyte counts increased immediately after all running protocols. Lymphocytopenia was observed 1 h after the intensive and eccentric protocols only. The muscle-damage marker creatine kinase increased in plasma 24 h after the eccentric protocol only. Two lymphocyte populations with strikingly different fluorescent intensities (''dim'' or ''bright'') were found for CD18 and CD53. Increases in the percentage and total number of $CD54^+$, CD18bright and CD53bright cell populations was observed immediately after the intensive and eccentric protocols, with the total numbers falling below pre-exercise values 1 h later. Total numbers of $CD54^+$ and CD53bright lymphocytes were also lower for the moderate protocol at 1 h post-exercise. Lymphocyte phenotypes at 24 h closely resembled those found pre-exercise. No differences in lymphocyte phenotype were found between the intensive protocol and the eccentric protocol at the same relative intensity. Two-colour analysis of lymphocyte subsets showed that the total number of $CD3^+$, $CD4^+$, $CD8^+$, and $CD3^-/CD56^+$ lymphocytes increased after the intensive protocol before falling below pre-exercise values at 1 h post-exercise. The relatively greater lymphocytosis and subsequent lymphocytopenia of $CD3^-/CD56^+$ natural killer (NK) cells and $CD8^+$ T lymphocytes account for the changes in $CD54^+$, CD18bright and CD53bright cell populations. NK cells and $CD8^+$ T lymphocytes that enter and exit the circulation following exercise express high levels of the AA molecules CD54, CD18, and CD53, which may mediate extravasation and post-exercise lymphocytopenia. This effect appears to be influenced by exercise intensity and not muscle-damage.

P-28. Blood lymphocyte apoptosis, expression of CD95 (FAS/APO-1), and the complement regulatory proteins CD55 (DAF) and CD59 (MACIF) after intensive, moderate, and downhill treadmill running, Richard J. Simpson [a], Keith Guy [a], Greg P. Whyte [b], Geraint D. Florida-James [a], [a]Biomedicine and Sport and Exercise Science Research Group, Napier University, Edinburgh, UK, [b]English Institute of Sport, Bisham Abbey NSC, Marlow, Bucks, UK

Apoptosis has been suggested to contribute to post-exercise lymphocytopenia and reduced immunity in athletes. Increased cell surface expression of CD95 (Fas/Apo-1) and a loss of complement regulatory proteins (CRPs) may leave lymphocytes susceptible to apoptosis. Eight trained males (age: 28 ± 5 years; $VO_{2\ max}$: 63 ± 3 ml kg min^{-1}) completed three treadmill running protocols of identical duration: (1) an intensive protocol at 80% $VO_{2\ max}$ to exhaustion, (2) a moderate protocol at 60% $VO_{2\ max}$, and (3) a –10% downhill protocol at 80% $VO_{2\ max}$. Isolated lymphocytes from blood samples taken before exercise, immediately after, 1 h and 24 h later were assessed for markers of apoptosis (Annexin-V+, HSP60+) and necrosis (PI), and expression of CD95 and the CRPs CD55 (DAF) and CD59 (MACIF) by one and two colour flow cytometry.

Lymphocytopenia was observed 1 h after the intensive and downhill protocols only. Only very small percentages of Annexin-V+ and HSP60+ blood-lymphocytes were found and did not change after any of the exercise protocols. No necrotic lymphocytes were found. Two lymphocyte populations with strikingly different fluorescent intensities (''dim'' or ''bright'') were found for CD55 and CD59. A significant increase in the percentage and total number of $CD95^+$, CD55 dim, and CD59 dim lymphocytes were found after the intensive and downhill protocols only, with the total numbers falling below pre-exercise values at 1 h post-exercise. A reduction of CD55 dim and CD59 dim expressing lymphocytes was also found 1 h after the moderate protocol. No differences were found between the intensive and the downhill protocol of the same intensity. Further analysis of lymphocyte subsets after the intensive protocol showed a greater lymphocytosis and subsequent lymphocytopenia of $CD95^+$, CD55 dim, and CD59 dim expressing $CD8^+$ T lymphocytes and $CD3^-/CD56^+$ natural killer (NK) cells relative to $CD4^+$ T lymphocytes and all T cells ($CD3^+$) combined.

Using three different modalities of exercise, it was found that apoptosis does not contribute to post-exercise lymphocytopenia, even when there is an increased expression of CD95 and a diminished expression of CRPs. The changes in CD95 and CRP expressing lymphocytes appear to reflect an altered lymphocyte subset distribution after exercise due to the relatively greater lymphocytosis and subsequent lymphocytopenia of $CD8^+$ T lymphocytes and NK Cells.

P-29. The effects of intensive exercise on lymphocytes expressing markers of senescence in peripheral blood, Keith Guy [a], Richard J. Simpson [a], Scott MacRae [a], Greg P. Whyte [b], Hanspeter Pircher [c], Geraint D. Florida-James [a], [a]Biomedicine and Sport and Exercise Science Research Group, Napier University, Edinburgh, UK, [b]English Institute of Sport, Marlow, SL7 1RT, UK, [c]Institute of Medical Microbiology & Hygiene, University of Freiburg, D-79104, Germany

This study examined the effects of intensive treadmill running on the expression of CD45 and a marker of cellular senescence (KLRG1; Voehringer, Koschella and Pircher. 2002, Blood, 100, 3698–3702) on blood lymphocytes. Trained male subjects ($n = 4$) completed an intensive treadmill running protocol at 80% $VO_{2max}$to volitional exhaustion. Blood samples were taken before, immediately after, and 1 h after exercise. Lymphocytes were examined for expression of KLRG1, CD45RA, CD45RO, and lymphocyte subset-specific markers by 2-colour flow cytometry. Lymphocyte counts increased dramatically in response to exercise, and after cessation of exercise this was followed by a period when lymphocytes left the bloodstream,

Table 1
Mean numbers of lymphocytes before, immediately after and 1 h after exercise analysed with monoclonal antibodies and by flow cytometry

Cell phenotype:	CD4+/ KLRG1+	CD8+/ KLRG1+	CD56+/ KLRG1+
Pre-exercise	0.083 ± 0.025	0.333 ± 0.234	0.202 ± 0.081
Post-exercise	0.111 ± 0.044	0.752 ± 0.046	0.569 ± 1.142
1hr Post-exercise	0.056 ± 0.062	0.094 ± 0.047	0.048 ± 0.012

Cell counts are expressed as cells x 109/L ± SD.

resulting in a lymphopaenia. The total number of KLRG1+ lymphocytes rose from 0.517 ± 0.256 (×109/L; mean ± SD) pre-exercise to 1.120 ± 0.430 immediately after exercise, before falling to 0.184 ± 0.104 at 1 h post-exercise. There were only minor changes in the relative proportions of CD45RA+ and CD45RO+ lymphocytes in response to exercise: a slight increase in the frequency of CD45RA+ cells and a modest decrease in the percentage of CD45RO+ cells. Relatively, small changes in the numbers of CD4+/KLRG1+ lymphocytes were found. In contrast, the numbers of CD8+/KLRG1+ T lymphocytes and CD56+/KLRG1+ NK cells showed considerable exercise-induced changes (Table 1). When sampled in the immediate post-exercise period, there was a large increase in CD8+/KLRG1+ and CD56+/KLRG1+ lymphocytes. One hour later, most of these cells had left the blood and the values for KLRG1+ lymphocytes in both subsets had fallen below pre-exercise levels.

Previous reports suggest that cells mobilized from tissues by exercise have an activated phenotype and shortened telomeres (Bruunsgaard et al., 1999. Life Sci., 65, 2623–2633), and this may be reflected in their expression of KLRG1, and a potentially senescent state.

P-30. The effects of marathon running on red blood cell haemolysis and cell membrane expression of the GPI-anchored complement regulatory proteins CD55 (DAF) and CD59 (MACIF), Keith Guy [a], Richard J. Simpson [a], Greg P.Whyte [b] Natalie Middleton [c], Rob Shave [c] Keith George [d], Geraint D. Florida-James [a], [a]School of Life Sciences, Napier University, Edinburgh, EH10 5DT, UK, [b]English Institute of Sport, Marlow, SL7 1RT, UK, [c]Centre for Sports Medicine and Human Performance, Brunel University, Middlesex, UB8 3PH, UK, [d]Research Institute for Sport and Exercise Science, Liverpool John Moores University, Liverpool, L3 5UX, UK

Various modes of exercise can result in deformity, haemolysis, and a reduced life span of red blood cells (RBCs). The biological mechanisms involved in exercise-induced RBC haemolysis remain elusive, although footstrike during running events is believed to have a significant effect (Telford et al., 2003: J. Appl. Physiol. 94, 38–42). RBCs are potentially susceptible to lysis by complement components in plasma, but are protected from lytic events by complement regulatory proteins (CRPs) bound to the cell membrane via glycosylphosphatidyl (GPI) anchors. CD55 and CD59 are two such CRPs, and their loss can lead to chronic intravascular haemolysis of RBCs and may result in thrombosis. The present study was undertaken to determine if marathon running would result in loss of CRPs from RBCs.

Thirteen male runners of the 2004 London Marathon participated in this study. Total RBC counts, hematocrits and hemoglobin levels did not change significantly after the marathon. Bilirubin concentrations observed post-marathon were significantly greater than the pre-marathon values. No significant changes in RBC membrane expression of CD55 and CD59 were found (Table 1).

Table 1
Mean fluorescent intensities (channel numbers; mean ± SD) of CD55 and CD59 red blood cell expression before, immediately after and 24 h after the 2004 London Marathon CRP expressiojn analysed with monoclonal antibodies and by flow cytometry

CRPs	Pre-marathon	Post-marathon	24 h Post-marathon
CD55 (DAF)	32.9 ± 3.6	32.9 ± 3.6	32.1 ± 4.0
CD59 (MACIF)	203.5 ± 37.4	210.3 ± 51.4	195.2 ± 34.2

Although the total RBC counts did not change in response to the marathon, increased bilirubin concentrations observed post-marathon indicated RBC lysis. The results show that marathon running does not induce a loss of GPI-anchored complement regulatory proteins from RBC membranes, suggesting that other biological mechanisms may be involved in exercise-induced haemolysis of RBCs.

P-31. Repeatability of salivary IgA responses to exercise, Robin Callister, Maree Gleeson, Melanie Dorrington, Amanda Cox, Robert Clancy, School of Biomedical Sciences, Faculty of Health, University of Newcastle, NSW, Australia

Purpose. The aims were to determine the repeatability and sources of variation in salivary IgA (sal-IgA) responses to maximal and moderate intensity exercise.

Methods. All ($n = 32$) subjects performed 3 maximal (VO_{2max}) exercise tests on a cycle ergometer and 19 subjects performed 3 submaximal exercise tests at 50% of VO_{2max} workloads. Saliva samples were collected immediately pre and post-exercise and after 1 h of recovery. sal-IgA concentrations were analyzed by ELISA, albumin concentrations by rate nephelometry (Beckmann IMMAGE) and osmolality determined using a freezing point depression osmometer (Advanced Micro Osmometer, Model 3300).

Results. There were no significant differences for log sal-IgA, sal-IgA/albumin ratio, sal-IgA/osmolality ratio, exercise workloads or durations among the maximal exercise trials or submaximal exercise trials, and there were no significant differences between the means or medians at each stage of the trials. This indicates there was no systematic bias in the salivary marker responses to the exercise intensities examined.

Intraclass correlation coefficients (ICCs) were used as a measure of relative (rank order) reliability. All salivary marker ICCs were significant and ranged between 0.71 and 0.95 indicating good relative reliability; sal-IgA/albumin ratio ICCs were the lowest of the ICCs for salivary markers. Coefficients of variation (CVs) for each individual were used as a measure of absolute reliability and were the greatest source of the variability observed. Mean CVs for sal-IgA concentrations, sal-IgA/albumin, and sal-IgA/osmolality ratios ranged from 19 to 34% whereas mean CVs for log sal-IgA were all below 10%. The values immediately post-exercise had lower ICCs and mean CVs than the other sampling times.

Conclusions. Group sal-IgA responses to both moderate and high intensity exercise are repeatable under controlled laboratory conditions. There was no systematic bias in salivary IgA markers to repeated exercise trials and good relative reliability was demonstrated. Within-subject variability may limit the application of Sal-IgA monitoring of individuals.

P-32. The effects of a 48 h period of fluid, calorie, or fluid and calorie restriction on saliva IgA (s-IgA) responses, Samuel J. Oliver [a], Stewart J. Laing [a], Sally Wilson [a], James L.J. Bilzon [b], Neil P.Walsh [a], [a]University of Wales, Bangor, UK, [b]Headquarters Army Training and Recruiting Agency, Upavon, UK

Table 1
Salive responses

	Trial	0 h	24 h	48 h
sflow	CON	367 ± 62	342 ± 73	371 ± 73
($\mu l.min^{-1}$)	FR	338 ± 70	207 ± 60	123 ± 42^{**,aa,bb}
	FR	366 ± 57	313 ± 57	285 ± 57
	FKR	333 ± 42	218 ± 44**	176 ± 39^{**,aa}
s-IgA	CON	17 ± 2	19 ± 3	21 ± 2
secretion	FR	16 ± 2	14 ± 3	13 ± 3^{aa}
rate	KR	19 ± 2	14 ± 2	15 ± 2
($\mu g.min^{-1}$)	FKR	20 ± 2	12 ± 2^{**,a}	12 ± 2^{**,aa}

$^{**}P < .01$ vs. 0h, ^{a} $P < .05$ and ^{aa} $P < .01$ vs. CON, ^{bb} $P < .01$ vs. KR.

The purpose was to determine the effects of a 48 h period of fluid and/or calorie restriction on s-IgA responses. Eight males participated in four randomised 48 h trials separated by 10 days. During the control trial (CON), participants received sufficient calories (2909 ± 110 kcal d^{-1}: mean ± SEM) and fluids (3790 ± 77 ml d^{-1}) to offset any deficit (0.5 ± 0.1% body mass loss; BML). During the fluid restriction trial (FR), participants received their estimated energy requirements and 25% of their estimated daily fluid requirements (3.2 ± 0.1% BML). In the calorie restriction trial (KR), participants received 10% of their estimated daily calorie requirements (291 ± 11 kcal d^{-1}) and maintained euhydration. The fluid and calorie restriction trial (FKR) was a combination of FR and KR. After 48 h, participants performed a 30 min treadmill time trial (TT) followed by a 2 h rehydration period. Unstimulated saliva samples (dribble) were collected at 0, 24, 48 h, post TT, and 2 h post TT.

Fluids provided during the 48 h on CON and KR prevented changes in sflow and s-IgA. Conversely, 48 h on FR evoked an increase in s-IgA concentration ($P < .01$) most probably attributable to the decrease in sflow ($P < .01$). Despite a similar decrease in sflow, 48 h on FKR did not increase s-IgA concentration, resulting in a reduced s-IgA secretion rate ($P < .01$). s-IgA secretion rate did not decrease post TT compared with 48 h on any trial. In conclusion, these results show that a 24–48 h period of combined fluid and calorie restriction decreases s-IgA secretion rate.

P-33. Kinetic response of salivary IgA to the Wingate and Luc-Léger test performed by well-trained swimmers, Ana Maria Teixeira [a], Maria do Rosário Cunha [b], Mafalda Martins [b], Luís Rama [a], [a]Centro de Estudos Biocinéticos, Faculdade de Ciências do Desporto e Educação Física da Universidade de Coimbra, Coimbra, Portugal, [b]Laboratório de Patologia Clínica, Hospitais da Universidade de Coimbra, Coimbra, Portugal

The relationship between training load and the immune system response has been the focus of many research projects, however many aspects of the acute mucosal immunity response to exercise remain unanswered (Gleeson et al., 2004). Most papers only analyze one or two time points of the sal-IgA responses to the exercise protocol studied (Borg, 1982; Dimitriou et al., 2002; Fahlman et al., 2001).

In this study, we tried to follow the sal-IgA response to two well-known exercise tests during a 24 h cycle.

Twelve male swimmers of Portuguese national team (17.03 ± 0.89 years old, height 177.10 ± 7, 16 cm, weight 66.45 ± 7, 16 kg, 7.33 ± 0.88 years experience of training, 35 km of mean weekly volume), performed two maximal tests: the Wingate anaerobic test and the Luc-Léger test that was executed 72 h after the Wingate test. The athletes $VO_{2\ max}$ was also estimated using the Luc-Léger protocol.

The results show significant differences in lactate levels, heart rate, and perception of effort (Cr104) between the two tests ($P < .05$), with higher values being found for the Luc Léger test. All sessions were conducted at the same hour (7.00 PM). Saliva samples were colleted for determination of IgA concentration, flow rate, and IgA secretion rate, at several time points: immediately before de exercise; 15 min after, 1.5 and 2.5 h after; the morning after and 24 h after the beginning of the test protocols. The sample showed an identical pattern of sal-IgA concentration and sal-IgA secretion rate values, for both protocols. There was however a major difference between protocols concerning the sal-IgA concentration. After the Wingate test there was a slight rise followed by a decrease until 2.5 h after the test. In the Luc Léger test, there was a significantly decrease 15 min after de test, followed by a recovery in the next 1.5 h and another decrease 2.5 h after. The following morning (wakeup) the athletes showed significantly higher sal-IgA concentrations for both protocols when compared to the values before the tests. Twenty four hours after the exercise protocols, the sal-IgA concentration and secretion rate were similar to the initial ones. Duration and intensity of the exercise protocols seem to differently influence the mucosal immune response.

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P-34. Functional fitness, mood states, and salivary IgA responses to an exercise program in an elderly population, Ana Maria Teixeira [a], Raul Martins [a], Mafalda Martins [b], Maria do Rosário Cunha [b], [a]Centro de Estudos Biocinéticos, Faculdade de Ciências do Desporto e Educação Física da Universidade de Coimbra, Coimbra, Portugal, [b]Laboratório de Patologia Clínica, Hospitais da Universidade de Coimbra, Coimbra, Portugal

This study looked at the effects of an exercise protocol on some physical, immunological, and psychological parameters in 32 elderly subjects with a median age of 81.78 + 5.5 years (range 68–95). Fourteen were submitted to an exercise program that consisted of a 45 min session with an intensity of 50–60% maximal heart rate, 3x/week during 19 weeks. Each session consisted of a 15 min warm-up, 20 min aerobic, strength and flexibility exercises, and 10 min relaxation. The other 18 elderly did not participate in this program and were used as the control group. Both groups were tested for their functional fitness, mood states, salivary IgA (sal-IgA) levels, and blood pressure, before and after the exercise program. Functional fitness was assessed using the Senior Fitness Test battery developed by Rikli and Jones (1999). The mood states were evaluated using the short form of the Profile Mood States questionnaire (POMS-SF) (Viana and Cruz, 1993). Sal-IgA levels were determined by nephelometry. Salivary flux and IgA secretion rate were also determined.

The results show an improvement in the exercising group for all the physical fitness test parameters with statistical significant differences for the upper and lower body strength ($P < .05$ and $P < .006$, respectively) and aerobic endurance ($P < .003$). A decline in all these parameters was shown for the control group, all with statistical significant meaning. Blood pressure values, showed a small reduction in the exercise group while the control group showed an increase in the blood pressure values statistically significant both for the systolic

(139.11 ± 24.79 to 144.0 ± 22.02, $P < .001$) and the diastolic pressure (70.39 ± 11.37 to 74 ± 10.16, $P < .012$). Regarding the POMS-SF questionnaire, statistical significant differences were obtained for the mood states depression, tension, fatigue, irritability (showing lower scores) and vigor for the exercising group and for vigor (showing lower scores) and confusion (showing higher scores) in the control group. IgA secretion rate was significantly higher after the exercise program for the experimental group (53.44 ± 37.90 to 90.02 ± 82.85 μg/min, $P < .018$). Sal-IgA concentration was also higher but did not reach statistical significance ($P < .066$). For the control group no statistical significant differences were found.

These results appear to show a positive effect of exercise on physiological, psychological, and mucosal immunity parameters indicating an improved quality of life.

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P-35. Effect of two bouts exercise compared to one bout exercise in same day on concentration of salivary immunoglobulin A, cortisol, and total protein in elite girl's gymnast, P. Farzanegi [a], M.A. Azarbayjani [b], M.J. Rasaee [c], [a]IAU, Ghaemshahr Branch, Faculty of Physical Education, [b]IAU, Central Tehran branch, Faculty of Physical Education, Tehran, Iran, [c]Tarbiat Modarres University, School of Medical Sciences, Department of Biochemistry, Tehran, Iran

The purpose of this study was to investigate the effect of two bouts exercise compared to one bout exercise in same day on concentration of salivary immunoglobulin A(s-IgA), cortisol (C), and total protein (TP) in elite girl's gymnast. Eleven elite girls gymnast (age: 11 ± 2 years; height: 145 ± 11 cm; body mass: 34 ± 8 kg) performed gymnastic exercise in two separate days. According to a medical examination and running general health questioner (GHQ) test, they were all healthy, and none of them were taking medication or suffered from hormonal disorder. Purpose of the study and its procedure was clearly described to them, their questions were answered, and they were asked to fill a consent form. In first day, each subject exercised only one bout from 6 to 8 PM. In the second day, each subject exercised from 8:30 to 10:30 AM.

And then exercised again from 6 to 8 PM. Salivary samples were collected before, immediately and after 2 h following the exercise in each bout. Saliva was collected after washing the mouth and drinking 100 ml water. Chewing gum or mints, and teeth brushing were prohibited 1 h before sampling. To reduce the effect of diurnal variations on hormone concentrations, saliva samples were obtained from individual subjects at the same time of day. All samples for the s-IgA and hormone determinations were kept frozen at −20 °C until use. The saliva (s-IgA), (C), and (TP) concentrations were measured by neflometry, ELISA, and Bradford method, respectively. Data were analyzed using repeated measures (ANOVA). To determine the correlation among variables, Pearson correlation coefficient was used and value of $P < .05$ was considered as significant. No significant changes were observed in the concentration of (s-IgA) after exercise, but concentration of (C) and (TP) significantly increased after exercise in both days ($P < .05$). Negative linear relationships were found between (s-IgA) and (C) after exercise in first day and between (C) and (TP) after second day. This results indicated that changes in concentration of (s-IgA) influence by volume of exercise, however, the concentrations of (C) and (TP) were shown to be influenced significantly by volume of exercise.

P-36. The effect of intensity of exercise on salivary immunoglobulin A, cortisol, and DHEA in Iranians elite girl swimmers, B. Yazdan parast [a], M.A. Azarbayjani [b], H. Aghaalinejad [c], [a]IAU, Varamin Branch, Faculty of Physical Education, [b]IAU, Central Tehran Branch, Faculty of Physical Education, Tehran, Iran, [c]Tarbiat Modarres University, Faculty of Physical Education, Tehran, Iran

The purpose of this study was to examine the effect of exercise intensity (low, medium, and high) on the salivary concentration of immunoglobulin A (s-IgA), cortisol (C), and DHEA in elite girl swimmers.

The study group comprised 10 elite swimmers of Isfahan Province selected to attend the national championship contests.

The study subjects participated in a 6-week exercise program as follow:

(1) Basic endurance season in the first two weeks (swam up to 60 or 70 percent of their best records).
(2) Special endurance season in the second two weeks (swam up to 70 to 80 percent of their best records).
(3) The hard exercise season in the last two weeks, (swam up to 80 to 90 percent of their best records).

Before saliva sampling, subjects were asked to rinse out their mouths with water to remove any substances that may affect T and C, then 5 ml unstimulated whole saliva was obtained at the end of each two weeks before and immediately after exercise from the study group. To reduce the effect of diurnal variations on hormone concentrations, saliva samples were obtained from individual subjects at the same time of day between 8 and 10 AM. All samples for the hormone determinations were kept frozen at −20 °C until use.

Salivary (s-IgA), (C), and DHEA concentrations were measured in duplicate by Neflometry and enzyme linked immunosorbant assay (ELISA), respectively. Means and standard deviations were calculated for all variables. One-way analyses of variance for repeated measures and Scheffe's post hoc comparison were used to determine significant differences. The level of significance was set at $P = .05$.

The findings of the research are as follow:

(1) Exercise with low medium and high intensity does not have a significant effect on the concentration of (s-IgA), cortisol, DHEA, and the ratio of salivary DHEA: C of elite girl swimmers. There is a negative significant correlation between concentrations with salivary cortisol after exercise with medium intensity. These results suggested that in elite girl swimmers 6-week exercise with different intensities does not influence salivary IGA, cortisol, and DHEA.

P-37. Kinetic response of salivary IgA to two aerobic swimming protocols, Luís Rama [a], Mafalda Martins [b], Maria do Rosário Cunha [b], Ana Maria Teixeira [a], [a]Centro de Estudos Biocinéticos, Faculdade de Ciências do Desporto e Educação Física da Universidade de Coimbra, Coimbra, Portugal, [b]Laboratório de Patologia Clínica, Hospitais da Universidade de Coimbra, Coimbra, Portugal

The relationship between training load and the mucosal immune responses has been the focus of many research projects, however, many research questions remain unanswered, namely defining how exercise influences the acute mucosal immune response (Gleeson et al., 2004; Physiological bases of physical exertion, 1982). The purpose of this study was to monitor the salivary IgA response to different aerobic protocols using several time points. Twelve male swimmers of the Portuguese national team (17.03 ± 0.89 years old, height 177.10 ± 7.16 cm, weight 66.45 ± 7.16 kg, 7.33 ± 0.88 years of training, and a mean week volume of 35 km) performed two different aerobic tasks with a 72 h interval between them. Both tasks — a 20 min (T20) continuous swim and an intermittent 5 × 400 m with 45 rest — were preceded by a normalized warm-up. The exercise intensity of the

two situations was respectively 70.99 ± 2.30% for T20, and 74.20 ± 3.05% for the intermittent load of, the maximal velocity on a maximal test of 15 m (v15). There were no significant differences in lactate levels, heart rate, and perception of effort (Cr102) between the two protocols. All the sessions took place at the same time of the day (7.00 PM). Saliva samples were collected for the determination of IgA concentration, flow rate, and IgA secretion rate. These were collected immediately before the exercise, 15 min, 1.5 h, and 2.5 h after, the next morning at wake-up and 24 h after the beginning of the protocol. The results show an identical pattern of IgA concentration and secretion rate for both protocols. In both protocols, the IgA parameters show a significant rise after the tasks and a decrease at the 1.5 h time point. 2.5 h after the tasks, the sal-IgA seems to recover slightly showing particular high values in the morning after sleep. No significant differences were found between the initial values and the ones from the 24 h time points. However, when comparing the two protocols in terms of sal-IgA concentration and sal-IgA secretion rate values, we found that the continuous exercise task seems to have a stronger negative impact on the immune system response, as significantly lower values were found 24 h after when compared to those obtained with the intermittent exercise task.

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P-38. The effects of walking exercise training on immune response in elderly subjects, Fuminori Kimura [a], Kazuhiro Shimizu [a], Takao Akama [b], Takayuki Akimoto [c], Shinya Kuno [a], Ichiro Kono [a], [a]Department of Comprehensive Human Sciences, Tsukuba University, Ibaraki, Japan, [b]School of Sport Sciences, Waseda University, Saitama, Japan, [c]Medical Center, Duke University, North Carolina, USA

The immune function declines in efficiency with advancing age, making the elderly less resistant to pathogenic microorganisms. The effects of walking exercise training (five 30 min walking sessions/week at 80% VT) on salivary secretory IgA (s-IgA) and plasma lymphocyte subpopulations were studied in elderly subjects. Thirty-one sedentary, elderly subjects (9 men, 22 women; age: 66.7 ± 7.4 years) were preformed walking exercise for 3 months. Aerobic power, body composition, and immune function were examined before and after 3 months. Salivary s-IgA flow rate was measured by enzyme linked immunosorbent assay (ELISA), while lymphocyte subpopulations by flow cytometry. s-IgA flow rate significantly increased at 3 months, especially in 64 years old and under (U-64), 65–74 years old (65–74), and women elderly subjects. Number of total lymphocytes, natural killer (NK) cell, and memory-T helper (Th) cell were significantly decreased at 3 months. We conclude that 3 months of walking provides enhancement of mucosal immune function in elderly subjects, although it is not associated with an improvement in lymphocyte.

P-39. Plasma glutamine concentration and natural killer cell cytotoxicity during intensive training, Masatoshi Suzui [a], Takeshi Kawai [b], Kazuyoshi Takeda [b], Hideo Yagita [b], Ko Okumura [b], Pang N. Shek [c,d], Roy J. Shephard [c], [a]Meiji University, Tokyo, Japan, [b]Juntendo University, Chiba and Tokyo, Japan, [c]University of Toronto, Ont., Canada, [d]Defence R&D Canada—Toronto, Ont., Canada

Our previous research showed decreased lytic units per natural killer (NK) cell at the end of one month of intensive training. This result was partly explained by an increased proportion of the lower cytotoxic NK cell subset. However, the underlying mechanisms remained unclear.

Purpose. We thus investigated plasma levels of glutamine, the essential energy source of lymphocytes, to test the hypothesis that intensive training decreases glutamine levels in peripheral blood.

Methods. Eight college-level female volleyball players undertook one month of heavy pre-season training. Volleyball drills were performed 5 h/day, 6 days/week. After an overnight fast, four morning resting blood samples were taken respectively pre-training, on the 10th day of training, one day before the end of training, and one week after training. Plasma glutamine, glutamic acid, and total amino acid levels were determined by HPLC.

Results. Plasma glutamine, glutamic acid, and total amino acid levels did not change during the experiment. All glutamine levels (46.2–171.1 mmol/L) were below the normal range (420–500 mmol/L). On the other hand, glutamic acid levels were higher than normal. Total amino acid levels remained in the normal range.

Discussion. These results did not explain the observed changes in NK cell cytotoxicity, but low initial plasma glutamine levels may have reduced cytotoxicity values pre-training.

Conclusion. Plasma glutamine levels were not changed during one-month of heavy training. These results suggest that glutamine levels do not link to cytotoxicity per NK cell during training.

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P-40. Effects of a prototype drink, fortified with natural vitamin C, in an intense exercise stress model, Tina Hurst [a], David Bailey [b], Duncan Talbot [a], Jane Rycroft [a], Jan Stocks [a], Clyde Williams [b], Jonathan Powell [a], [a]Unilever Corporate Research, Sharnbrook, Bedfordshire, UK, [b]School of Sport and Exercise Sciences, Loughborough University, Leicestershire, UK

Intense exercise is associated with elevated stress responses and muscle damage. We have developed a prototype drink which is a mix of the Lipton-Fusion ready-to-drink beverage base and acerola cherry. Acerola cherry is found in South America and the Caribbean and naturally contains 17% vitamin C by dry weight. The aim of the current investigation was to determine whether supplementation with a natural source of vitamin C ascorbic acid alleviates muscle damage and aids recovery of muscle function in the days following exercise.

Thirty male participants were divided into three matched groups and assigned to supplementation of a lactose placebo tablet (Pl), vitamin C tablet (Vc) or the Lipton-acerola (Ac) beverage for 7 days pre-exercise and 7 days post-exercise (PE). The vitamin C content of the tablet and drink were matched to be equal to a daily dose of 800 mg. On the seventh day participants completed the 90 min Loughborough Intermittent Shuttle Test (LIST) (Nicholas et al., 2000). Markers of muscle damage, oxidative stress, and immune response were monitored up to 48 h PE. Ratings of perceived soreness and isometric maximal voluntary contraction (MVC) of the leg extensors and flexors were monitored up to 7 days PE. Data were analysed using a repeated-measures mixed model analysis, using the pre-exercise values as a covariate.

Following supplementation with Vc or Ac, there was a significant reduction in the muscle damage markers creatine kinase (1 h, 24 h PE; $P < .05$) and myoglobin (immediately, 1 h PE; $P < .05$). Recovery of muscle function in the leg flexors was greater following either Vc or Ac supplementation, as compared to the placebo group (48, 96 h PE; $P < .05$). There was a significant reduction in subjective muscle soreness in the Vc and Ac groups (24, 48 h PE; $P < .05$).

These data support our previous findings that vitamin C modulates measures of exercise-induced changes in muscle function and self-perceived muscle soreness. Additionally, we have demonstrated these benefits when vitamin C is presented in both a synthetic or natural form.

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P-41. The effect of caffeine ingestion on human neutrophil oxidative burst responses following time-trial cycling, Gary J. Walker, Anneliese Dziubak, Laurence Houghton, S. Laura Lim, Ciaran Prendergast, Nicolette C. Bishop, School of Sport and Exercise Sciences, Loughborough University, Leics, LE11 3TU, UK

Caffeine ingestion has been associated with an attenuation of the exercise induced fall in neutrophil oxidative burst response to formyl-methionyl-leucyl-phenylalanine (fMLP) stimulation following prolonged cycling (Walker et al., 2005, J Physiol (Proceedings), abstract in press). Since caffeine is often consumed by athletes in time-trial events to improve performance, the aim of this study was to investigate the effect of caffeine ingestion on the fMLP-induced neutrophil oxidative burst response following a time-trial after 90 min cycling.

With local ethics committee approval, 9 highly trained male cyclists (mean ± SEM: age 23 ± 1 years; body mass 67.4 ± 1.7 kg; VO_{2max} 71.5 ± 2.3 $ml.kg^{-1}.min^{-1}$) cycled for 90 min on a stationary ergometer at 71.7 ± 2.3% VO_{2max}, followed by a time-trial (TT) ensuring an energy expenditure (kJ) equivalent to cycling at 70% W_{max} for 30 min. In a randomised, counterbalanced design, participants arrived at the laboratory following an overnight fast and 60 h abstention from caffeine containing products and were assigned to ingest either 6 $mg.kg^{-1}$ body mass of caffeine (CAF) or dextrose powder (PLA) 60 min before exercise. The protocol was repeated 7 days later. Venous blood samples were collected at rest, immediately post-90 min of exercise and post-TT and 1 h post-TT. The in vitro neutrophil oxidative burst response to fMLP was assessed using a chemiluminescence (CL) assay (Knight Scientific Limited, Plymouth, UK). Serum caffeine and plasma adrenaline were determined using a spectrophotometric assay and high-performance liquid chromatography, respectively.

Participants completed the TT faster and with greater power output on CAF than PLA ($P < .05$). Serum caffeine ($P < .01$) and plasma adrenaline ($P < .05$) were higher on CAF than PLA at immediately post-90 min and post-TT. Plasma adrenaline at post-TT was 5.2 ± 0.7 $nmol.L^{-1}$ and 3.7 ± 0.5 $nmol.L^{-1}$ on CAF and PLA, respectively. Peak fMLP-induced neutrophil CL fell on both trials (main effect of time, $P < .01$) but there were no significant differences between CAF and PLA. These findings suggest that caffeine ingestion has little effect on neutrophil oxidative burst response following a pre-loaded performance test in contrast to the effect observed following fixed duration exercise.

P-42. Reassessing the acute effect of vitamin C on post-exercise neutrophil oxidative burst activity, Glen Davison, Michael Gleeson, School of Sport and Exercise Sciences, Loughborough University, LE11 3TU, UK

Daily supplementation with antioxidants for several weeks appears to reduce the magnitude of immunodepression following prolonged exercise possibly by reducing the stress hormone and cytokine response or improving antioxidant defense. If auto-oxidative damage to immune cells is a significant factor in exercise-induced immunodepression, then acute antioxidant supplementation may be sufficient to attenuate this. We previously reported that acute vitamin C (VC) supplementation had no effect on neutrophil oxidative burst activity (OBA) (Davison et al. 2004, ECSS Proceedings, p. 213); however, OBA was assessed with a diluted whole blood assay and we have since found, by manipulating VC concentration in vitro, that increasing plasma VC concentration by 50 μM significantly reduces the PMA-stimulated chemiluminescence response (−33%) in this assay. The aim of this study was to reassess the neutrophil OBA results from Davison et al. (2004) using a correction for differences in plasma VC concentration.

With local ethics committee approval, 8 healthy males cycled for 2.5 h at 60% VO_{2max} with placebo (PLA) or VC (500 mg L^{-1}) beverages consumed during exercise (2.5 ml kg^{-1} body mass every 15 min) in addition to a beverage (5 ml kg^{-1} body mass) containing a bolus of 500 and 1000 mg VC, 2 and 14 h pre-exercise, respectively. Venous blood samples were taken before the first drink on the day of exercise (Rest), immediately pre- and post-exercise, and 1 h post-exercise. PMA-stimulated neutrophil OBA was expressed as the integral of the 30 min chemiluminescence response curve per neutrophil.

Results were analysed using a 2-factor repeated measures ANOVA (treatment × time) with post hoc paired t tests.

Plasma VC concentration was significantly higher ($P < .05$) at all times in the VC compared with PLA trial. PMA-stimulated neutrophil OBA was significantly higher post-exercise ($P < .05$) on the VC trial when the values were corrected for plasma VC concentration. However, the significant reduction in markers of oxidative stress but lack of effect on neutrophil trafficking or the cortisol and IL-6 responses (Davison et al., 2004) suggest that auto-oxidation may be a significant contributor to exercise-induced immunodepression, the magnitude of which may be reduced by acute VC ingestion.